12-24-2007

SarA of Staphylococcus aureus Binds to the sarA Promoter To Regulate Gene Expression

Ambrose L. Cheung
Dartmouth College

Koren Nishina
Dartmouth College

Adhar C. Manna
University of South Dakota

JOURNAL OF BACTERIOLOGY, Mar. 2008, p. 2239–2243 Vol. 190, No. 6
0021-9193/08/$08.00+0 doi:10.1128/JB.01826-07

SarA of *Staphylococcus aureus* Binds to the *sarA* Promoter To Regulate Gene Expression▽

Ambrose L. Cheung,[1]* Koren Nishina, and Adhar C. Manna[2]

Department of Microbiology and Immunology, Dartmouth Medical School, Hanover, New Hampshire 03755,[1] and Division of Basic Biomedical Sciences, Sanford School of Medicine, University of South Dakota, Vermillion, South Dakota 57069[2]

Received 19 November 2007/Accepted 24 December 2007

The 375-bp *sarA* open reading frame is driven by three promoters, P1, P3, and P2. Using gel shift and DNase I footprinting assays, we found that SarA binds to two 26-bp sequences and one 31-bp sequence within the P1 and P3 promoters, respectively. Together with the results of transcription analyses, our data indicate that SarA binds to its own promoter to down-regulate *sarA* expression.

Staphylococcus aureus is an important human pathogen that can cause a wide spectrum of infections ranging from superficial abscesses to deep-seated infections such as pneumonia, endocarditis, and sepsis (14). This versatility may be attributable to an extensive array of extracellular and cell wall-associated virulence determinants that are coordinately expressed during the infection process (4, 19). The temporal regulation of virulence determinants in *S. aureus* has been shown to be under the control of global regulators such as *agr* and SarA (4, 19). SarA is a 14.5-kDa DNA binding protein that modulates the transcription of specific regulatory loci such as *agr* as well as downstream target genes (e.g., *hla* and *spa*). These two modes of regulation have been confirmed by transcriptional profiling studies by Dunman et al. (9). Recognizing that SarA can control over 120 genes both directly and indirectly (9), it is not surprising that expression of *sarA* is carefully controlled during bacterial growth (16).

The *sarA* promoter region is extensive at ~800 bp in length, with three distinct promoters (P2, P3, and P1) that yield three overlapping transcripts (*sarA* P2, P3, and P1 transcripts). The *sarA* P1 and P2 promoters are σ^A dependent, while the P3 promoter, nested between the P1 and P2 promoters (Fig. 1A), is σ^B dependent (17) and is activated postexponentially or during period of metabolic stress (1). Promoter analysis using XylE fusions revealed that the *sarA* P1 promoter is the predominant promoter whereas the P2 and P3 promoters are much weaker (15). Mutation studies indicated that the weaker P2 and P3 promoters contributed to overall SarA expression, since activation of the P1 promoter alone led to lower SarA protein expression compared with expression levels seen with the native P2-P3-P1 promoters, thus resulting in reduced target gene expression (e.g., *agr* and *hla*) (6). Embedded within the *sarA* promoter regions are both direct and indirect repeats (1), suggesting that the promoter region may serve to provide binding sites for regulatory DNA binding proteins. Indeed, promoter trap studies have determined that SarR, a homolog of SarA, binds to the *sarA* P1 promoter to repress SarA expression (16) while SigB binds to the *sarA* P3 promoter (17). Thus, SigB (6, 17) and SarR (16) can affect the *sarA* P3 and P1 promoters, respectively, to modulate SarA expression during growth (16).

Besides SigB and SarR, we have now dissected the contribution of SarA to expression from its own promoter. Our data confirmed the negative regulatory role of SarA in reducing overall expression from the native *sarA* triple promoters.

Characterization of SarA binding to the native *sarA* promoter. Various studies have shown that SigB and SarR are involved in the modulation of SarA expression (Fig. 1A) (2, 5, 10, 16). Cognizant of the relatively minor role of the P2 promoter in *sarA* expression (6), we proceeded to ascertain whether SarA binds to the P3 and P1 promoters. Promoter fragments were amplified by PCR, gel purified, end labeled, and used in gel shift assays with purified SarA protein. Purification, authenticity, and purity of the purified His_6-tagged SarA protein were reported previously (8, 13). As shown in Fig. 1B, purified SarA protein bound to the P1 and P3 promoters in a dose-dependent fashion. We estimated that the dissociation constants of SarA with respect to the *sarA* P1 and P3 promoters are ~17 and 34 pM, respectively. The onefold difference in dissociation constants for SarA between the two *sarA* promoters is not highly significant and is probably within the experimental error of the gel shift assays. Unlabeled promoter DNA, added in excess (25-fold in molar ratio), was found to reduce the formation of the protein-DNA complex, thus suggesting binding specificity. In contrast to the results seen with the unlabeled *sarA* P3 promoter fragment (Fig. 1B, right panel, lane 6), the reduction in the formation of the protein-DNA complex for the unlabeled *sarA* P1 promoter was only partial (Fig. 1B, left panel, lane 6) in the presence of 3 μg of SarA (the same amount as used for lane 5). As a negative control, we used a 185-bp *mgrA* promoter fragment (in a 33-fold molar ratio) which did not compete as successfully as the unlabeled *sarA* P1 promoter fragment for the binding of SarA (Fig. 1B, left panel, lane 7). The presence of multiple retarded species would suggest the presence of a single binding site with multiple SarA dimers (13); however, we cannot rule out the possibility of multiple binding sites within this 240-bp *sarA* P1 promoter fragment.

The contribution of the *sarA* P2 to SarA expression has been

* Corresponding author. Mailing address: Department of Microbiology and Immunology, Vail 210, Dartmouth Medical School, Hanover, NH 03766. Phone: (603) 650-1340. Fax: (603) 650-1362. E-mail: Ambrose.Cheung@Dartmouth.edu.

▽ Published ahead of print on 4 January 2008.

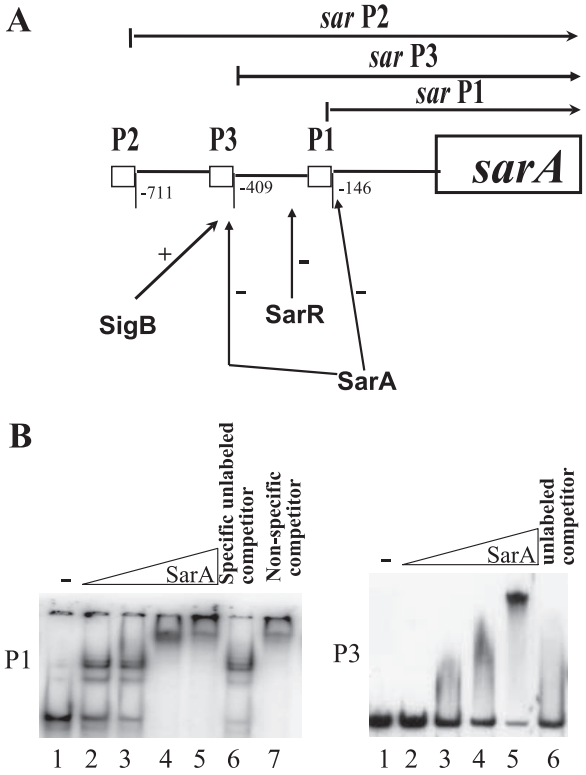

FIG. 1. Binding of SarA to the *sarA* promoters. (A) The structure of the *sarA* locus, showing the triple promoter driving the *sarA* ORF. The nucleotide positions of the transcription starts (upstream of the *sarA* ATG initiation codon) are shown for the three promoters. (B) Gel shift of purified SarA with the γ-^{32}P-labeled *sarA* P1 (nt 620 to 859) and P3 (nt 364 to 525) promoters (based on sequence data from GenBank accession number U46541). Lane 1 represents the labeled DNA probe alone. Increasing amounts of SarA at 0.2, 0.5, 1, and 3 μg, representing 6.8, 17, 34, and 102 pmol, respectively, were added to the probe (lanes 2 to 5). Unlabeled 240-bp P1 *sarA* or 162-bp *sarA* P3 (lane 6) in a 25-molar-ratio excess was added to a lane containing 3 μg of the purified SarA protein as a specific competitor, while an 185-bp *mgrA* promoter fragment with a 33-molar-ratio excess was also added to 3 μg of SarA (lane 7).

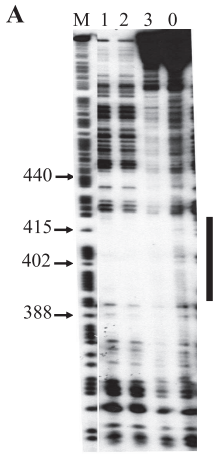

FIG. 2. (A) DNase I footprint of the *sarA* promoter (positions 364 to 525, covering the P3 promoter and part of the P1 promoter) with 1, 2, 3, and 0 μg of purified SarA added. Lane M represents an A/G ladder. (B) Alignment of the protected region (nt position 389 to 419) with the SarA consensus binding sequence derived from an alignment of all the promoter sequences to which SarA binds (8). For the consensus sequence, the capital letters represent highly conserved bases while the lowercase letters represent variable positions. Numbers are from data obtained from GenBank accession number U46541. Please note that the P3 protected region has a 7-bp palindrome (underlined) separated by a 6-bp sequence; this configuration is consistent with the binding site for a dimeric winged helix protein such as SarA (11, 13). Interestingly, the SarA consensus binding site encompasses an imperfect inverted repeat (double underlined), which may also serve as a binding site for winged helix proteins (11).

shown to be relatively minor compared to that of the P3 and P1 promoters (5, 15). For this reason, we decided to analyze the binding site of SarA on the *sarA* P3 and P1 promoter regions by DNase I footprinting assays. We employed a γ-^{32}P-labeled 162-bp *sarA* promoter fragment (nucleotides [nt] 364 to 525) (GenBank accession no. U46541) upstream of P1 that also comprises the *sarA* P3 promoter region. As shown in Fig. 2A, protection from DNase I digestion of the sense strand with SarA was mapped to a 31-bp sequence with positions 389 to 419 (based on GenBank accession no. U46541) (1), approximately seven nucleotides upstream of the −35 promoter box of the P3 promoter. Additional mapping of the nonsense strand with SarA also confirmed this binding site (data not shown). Interestingly, there is a 7-bp palindrome separated by a 6-bp sequence within the protected region. This configuration is consistent with the presence of a binding site of a dimeric winged helix protein (11), and SarA belongs to the winged helix protein family (13). The partially protected region below nt 388 was likely due to nonspecific binding, since this protection was only observed at the highest SarA concentration and was not found in the footprinting assay with the nonsense strand (data not shown). Alignment of this sequence with the consensus SarA binding site (8) disclosed the identities of 15 of 26 residues (Fig. 2B). Notably, the consensus SarA binding site also contains an imperfect 7-bp inverted repeat which may serve as a binding site for winged helix proteins (11). Collectively, these studies confirmed the existence of a SarA binding site upstream of the P3 promoter.

Effect on SarA on the P1 promoter. In contrast to the results seen with the *sarA* P3 promoter, we were not able to obtain a good SarA footprint on the *sarA* P1 promoter despite similar binding affinities of SarA with *sarA* P1 and *sarA* P3 promoters. In examining the *sarA* promoter region, we found two SarA consensus binding sites of 26 bp each (*sarA* P1-I and *sarA* P1-II; Fig. 3A) (8), one overlapping with the −10 promoter boxes and another downstream of the transcription start site. To verify the specificity of these two 26-bp binding sites, two 78-mers, each representing the binding site (i.e., *sarA* P1-I and *sarA* P1-II), together with the native 26-bp flanking sequence on each side, were synthesized and used for gel shift studies with purified SarA (Fig. 3B). We also used a nonspecific 78-bp competitor as a control for each gel; this fragment was similar to the specific competitor except that the central 26-bp sequence (26-bp *sarA* P1-I or 26-bp *sarA* P1-II) was replaced by GAC repeats. The results showed that these two native 78-bp

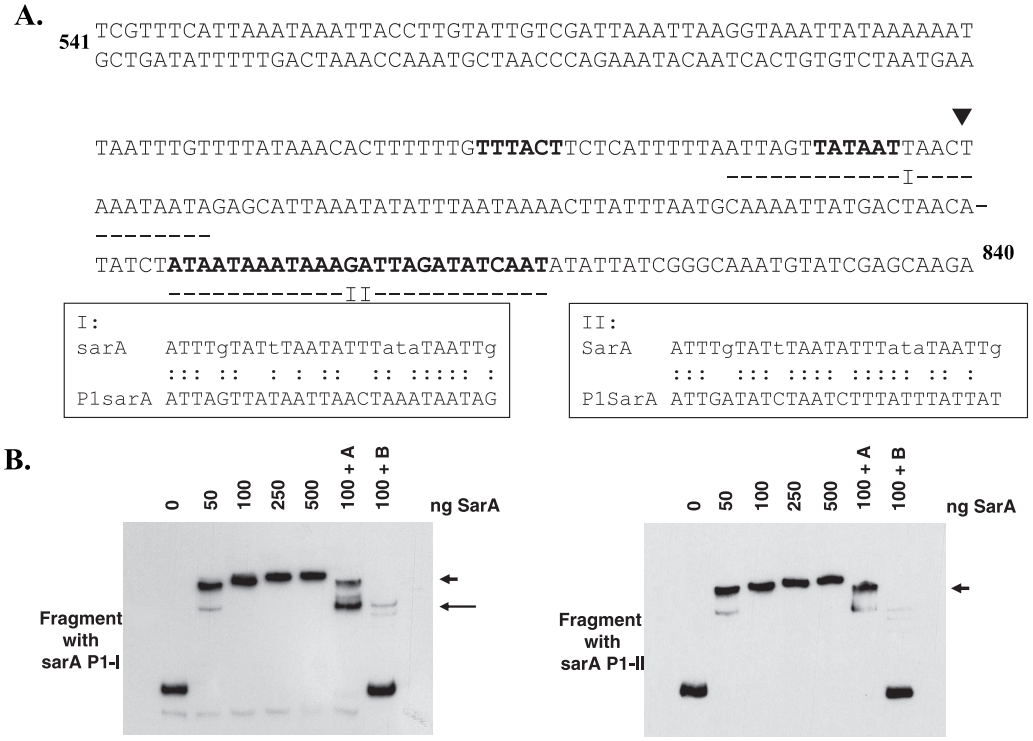

FIG. 3. The binding of SarA to two separate SarA binding sites in the *sarA* P1 promoter region. (A) Sequence of the region, with the −10 and −35 of the P1 promoter boxes represented by boldface characters. The putative SarA binding sites (*sarA* P1-I and *sarA* P1-II) are indicated by dashes below the sequence; the *sarA* P1-II is located in the complementary strand. The transcription start site of the P1 transcript is indicated by a downward-pointing arrowhead. The alignment of the putative SarA binding sites with the SarA consensus sequence is shown. (B) Gel shift studies of the two binding sites with purified SarA. The wild-type oligonucleotides of *sarA* P1-I and P1-II are 78-mers, each consisting of the respective binding region (26 bases underlined in panel A) flanked by 26 bases of native sequence on each side. The wild-type oligonucleotides were biotinylated and exposed to various quantities of purified SarA protein under the following binding conditions: a total of 20 μl consisting of 2× binding buffer (Pierce Chemicals), 5% glycerol, 0.5 μg herring sperm DNA, 0.05% NP-40, 20 fmol of labeled oligonucleotides, and different concentrations of SarA protein. Detection of the bands was performed using a Pierce LightShift chemiluminescent electrophoretic mobility shift assay kit (Pierce Chemicals, Rockford, IL). The specific competitor was the unlabeled 78-bp oligonucleotide, while the nonspecific competitor was also 78 bp in length and was similar to the specific competitor except that the 26-bp binding sequence of *sarA* P1-I or *sarA* P1-II was replaced with GAC repeats while leaving the flanking sequences intact. The "A" in the label "100 + A" represents 50 pmol of the nonspecific competitor; the "B" in the label "100 + B" represents 50 pmol of the unlabeled 78-bp *sarA* P1-I or *sarA* P1-II fragment as the specific competitor (as indicated in the figure panel). The specific complexes are indicated by short arrows, while the long arrow highlights the nonspecific complex.

fragments formed retarded species indicative of protein-DNA complex formation. The binding was likely to be specific, because the unlabeled 78-bp fragment competed successfully for the complex (Fig. 3B) whereas the 78-bp nonspecific competitor fragment was less successful (Fig. 3B). Interestingly, the nonspecific competitor for the *sarA* P1-I fragment formed two complexes, one specific and another nonspecific (3B). Progressive retardation of the complexes with increasing concentrations of SarA suggests that the protein likely forms multiple dimers on target DNA, since modeling studies of the SarA protein with promoter DNA revealed the plausibility of the idea that three SarA dimers bind to the target promoter (13). Additionally, multiple SarA-DNA complexes were also discerned when we used a 26-bp fragment, comprising only the binding site, in place of the 78-bp fragment for the gel shift assay. Given that a 26-bp sequence is probably too short to accommodate multiple binding sites for multiple SarA dimers, we theorize that the SarA dimers likely bind to a single site via dimer-dimer interaction (13).

To confirm the effect of SarA on the *sarA* P1 and P3 promoters, we transformed shuttle plasmid (pSK236) containing the intact P2-P3-P1 promoters into wild-type strain Newman and its isogenic *sarA* mutant. Strain Newman was chosen because it contains an intact *rsbU* gene, which represents part of a regulatory input into the *sigB* operon to generate free SigB under conditions of environmental stresses (20). This is relevant within the context of the SigB dependency of the *sarA* P3 promoter. To improve signal detection, we elected to examine transcription of *gfp* linked to the *sarA* promoter to ascertain *sarA* promoter activity, since this would avoid the problem of protein degradation associated with many reporter fusions (e.g., XylE, β-galactosidase) (3, 6, 15). In strain Newman, the level of *sarA* P3 transcription from the *sarA* P2P3P1:*gfp* construct was lower in the parent than that in the isogenic *sarA* mutant (no detectable units versus 1,340 units in lanes 1 and 2 and 1,002 versus 2,342 units in lanes 3 and 4 for the exponential and postexponential phases, respectively), thus implying a repressive effect by SarA on the P3 promoter (Fig. 4). The *sarA* P1 promoter within the native P2-P3-P1 promoters was also more active in the *sarA* mutant (i.e., in the absence of SarA) than in the parental counterpart Newman (Fig. 4A, lane 2 versus lane 1 and lane 4 versus lane 3). Although our data did not entirely rule out the contributory role of SarA binding to the P2

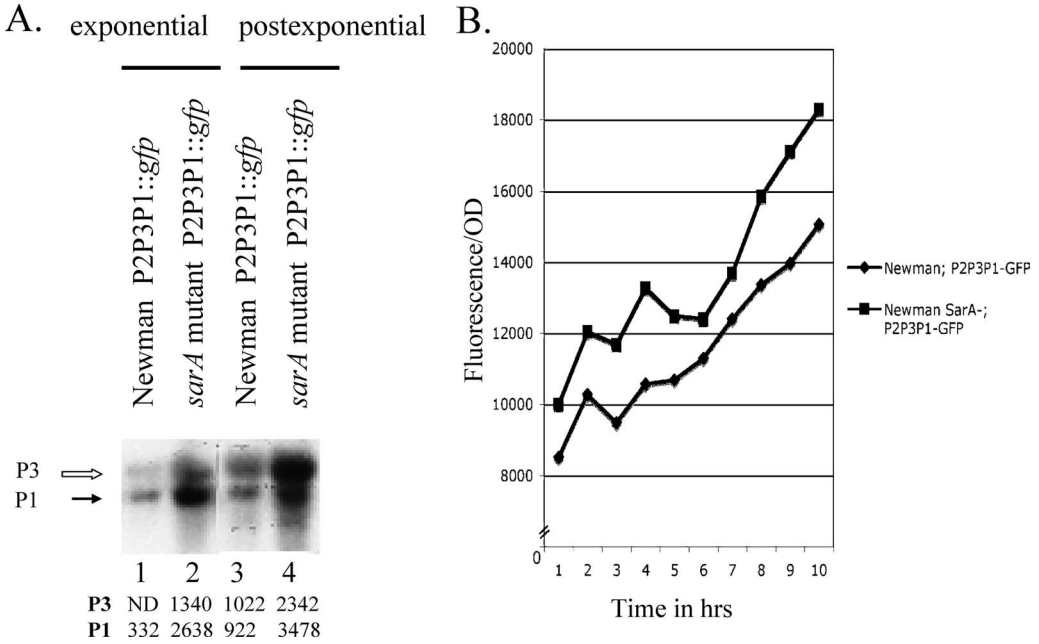

FIG. 4. (A) Northern blots of the *sarA* promoters linked to *gfp* as detected by a *gfp* probe, by use of the coding region of *gfp*, in strain Newman and its isogenic *sarA* mutant. By means of an 18-mm (outer diameter) borosilicate glass tube, RNA was harvested from strain Newman or its isogenic *sarA* mutant at ODs at 650 nm (OD_{650}) of 0.7 and 1.7, corresponding to the exponential and postexponential phases, respectively. Equivalent amounts of total cellular RNA were resolved in denaturing agarose gel, blotted onto a Hybond XL membrane (GE Healthcare), and hybridized with a [^{32}P]dCTP-labeled *gfp* probe as described previously (16). The results of densitometric analyses of signal intensities for the P1 and P3 transcripts, performed using Sigma Gel software from Jandel Scientific, are indicated below the lanes. ND, result was below the minimum level that can be detected with the Sigma Gel software. (B) Promoter fusions of *sarA* P2-P3-P1 promoters driving the gfp_{uvr} reporter gene in isogenic *sarA* Newman strains. *S. aureus* strains containing recombinant shuttle plasmid with the *sarA* P2-P3-P1 promoters driving the GFP_{uvr}-encoding gene were grown in tryptic soy broth overnight, diluted 1:100, and then serially monitored for OD_{650} and fluorescence in microtiter wells (FL600; Biotek, Winooski, VT). About 100 μl each of the samples was added in triplicate to each microtiter well. The data are presented as the numbers of mean fluorescence units/OD in the *y* axis versus time in hours after the initial dilution in the *x* axis. The standard error of the mean was too small to be seen for each data point in the graph.

promoter to alter P1 transcription, the fact that the P2 promoter is very weak (15) and also quite distal to P1 (the two promoters are ~560 bp apart) would suggest minimal influence of the P2 promoter on the proximal P1 promoter (Fig. 1A).

To confirm the effect of SarA on the native *sarA* P2-P3-P1 promoters in overall *sarA* expression, we conducted transcription fusion experiments using the native *sarA* promoter linked to the GFP_{uvr} reporter gene in strain Newman and its isogenic *sarA* mutant. As shown in Fig. 4B, the transcription of the intact *sarA* P2-P3-P1 promoters, as confirmed by the level of green fluorescent protein (GFP)-mediated fluorescence per optical density (OD) unit, was higher in the *sarA* mutant than in the isogenic parental strain Newman throughout the growth cycle. This confirms our Northern blot data (Fig. 4A) showing that expression of the *sarA* P1 and P3 promoters is repressed by SarA and is derepressed in the *sarA* mutant.

The *sarA* promoter region of *S. aureus* is complex, with three promoters (P2, P3, and P1) in an extensive region (~800 bp). Transcriptional analysis revealed that the P1 promoter is most active whereas the *sigB*-dependent P3 promoter also contributes to *sarA* expression (5, 6, 15). Surprisingly, the distal P2 promoter is weak in vitro and in vivo and appears to play a relatively minor role in driving SarA expression (6, 7). We have previously speculated that the region between the P1 and P3 promoters may serve to provide binding sites for regulatory proteins based on the observation that there are both direct and inverted repeats in this region. Indeed, we have subsequently found that SigB and SarR can bind to the *sarA* promoter to activate the P3 promoter and repress the P1 promoter, respectively (Fig. 1) (5, 16). As the *sarA* locus controls over 120 target genes (9), we hypothesize that additional activators or repressors are likely to be present to modulate SarA expression.

In previous *sarA* promoter fusion studies using XylE and GFP in isogenic *sarA* strains (RN6390), we have found inconsistent results with respect to modulation of the *sarA* promoter by SarA (unpublished data). One of the reasons for this inconsistency may have been the presence of reduced SigB expression in strain RN6390, which leads to a partial failure in activating the σ^B-dependent *sarA* P3 promoter. Another plausible reason is that we have previously looked at the *sarA* P3 or P1 promoter activation in isolation but not in the context of the native P2-P3-P1 promoters. To resolve this inconsistency, we decided to examine the effect of SarA on *gfp* transcription from P1 and P3 as driven from the native *sarA* P2-P3-P1 promoters in strain Newman, which carries an intact *sigB* operon with a functional *rsbU* gene. Based on our transcription and fluorescence data (Fig. 4A and 4B), we established that SarA is a negative regulator of overall *sarA* expression by virtue of its repressive effect on the P1 and P3 promoters, which play an important role in driving SarA expression from the native *sarA* P2-P3-P1 promoters (6, 15). This was confirmed by our tran-

scriptional fusion data showing that the native *sarA* promoter was more active in driving GFP_{uvr} expression in the *sarA* mutant than in the parent strain (Fig. 4B). This effect was unlikely to be due to reduced expression of proteases, since a *sarA* mutant is expected to produce more proteases than the parent (4). Gel shift studies revealed that SarA can bind to the *sarA* P3 and P1 promoters. Curiously, we were able to obtain a good DNase I footprint with the *sarA* P3 promoter alone but not with the P1 promoter alone even though SarA seems to bind with similar levels of affinity to these two promoters, as indicated by the comparable amounts of SarA protein required for a complete shift of the labeled probe in gel shift assays. The SarA binding site on the *sarA* P3 promoter lies immediately upstream of the −35 promoter box. This 26-bp binding site shares homology with the 26-bp SarA binding site that we have mapped for the *agr*, *hla*, and other target promoters (8, 23). Additionally, this site also contains a 7-bp palindrome separated by a 6-bp sequence within the SarA protected region. Given that dimeric winged helix proteins typically bind to inverted repeats or palindromic sequences (11), it is highly likely that SarA, as a member of the winged helix family, binds specifically to this site.

In examining the *sarA* P1 promoter region, we found two 26-bp SarA consensus sequences, one overlapping with the −10 promoter box and another downstream of the transcription start site. Using these two sequences (*sarA* P1-I and P1-II) together with flanking sequence as 78-bp probe fragments, we showed that SarA could bind these two fragments specifically in a dose-dependent fashion, with the unlabeled native 78-bp sequence being a better competitor than the nonspecific 78-bp competitor in which the 26-bp SarA binding site had been replaced by GAC repeats (Fig. 3). We also conducted gel shift assays of purified SarA with only the 26-bp SarA binding sites (*sarA* P1-I and P1-II) lacking any flanking sequence, obtaining essentially similar results (unpublished data). Two interesting themes emerged from these binding studies. First, there were three protein-DNA complex species for each 26-bp sequence, thus hinting at multiple dimer interactions on a single binding site, as has been predicted from the structural studies of SarA (13). Second, the second SarA binding site lies downstream of the transcription start site of the *sarA* P1 transcript. This scenario has also been described for Rns, a global regulator of pilus expression in enterotoxigenic *Escherichia coli* which binds to a region downstream of the transcription initiation site to activate transcription from its own promoter (18).

Cognizant of our recent structural data showing that SarA is a dimeric winged helix protein (13), it is likely the binding site of SarA is greater than 17 bp in size and contains either a palindrome or an inverted repeat (based on our unpublished gel shift data with various sizes of the duplex DNA fragments and purified SarA protein), which is typical of most winged helix proteins with two winged helix motifs (e.g., OhrR, MecI, and BlaI), with each motif capable of binding to the major groove and minor groove of target DNA (11, 12, 21, 22). Accordingly, the data presented here are consistent with a larger binding site for two winged helix motifs.

In sum, our data support the idea of a complex regulatory structure within the triple *sarA* promoter to control SarA expression. In previous studies, we delineated the role of SigB and SarR in modulating SarA expression. We have now added SarA to this list of regulatory proteins. Similar to SarR, SarA also exerts a negative regulatory effect on the P1 and P3 promoters to down-modulate transcription from the native *sarA* P2-P3-P1 promoters to influence SarA expression. Delineation of other factors that may have positive regulatory input with respect to this promoter is currently in progress.

This research was supported in part by National Institutes of Health grant AI37142 to A.L.C.

REFERENCES

1. **Bayer, M. G., J. H. Heinrichs, and A. L. Cheung.** 1996. The molecular architecture of the *sar* locus in *Staphylococcus aureus*. J. Bacteriol. **178:**4563–4570.
2. **Bischoff, M., J. M. Entenza, and P. Giachino.** 2001. Influence of a functional *sig*B operon on the global regulators *sar* and *agr* in *Staphylococcus aureus*. J. Bacteriol. **183:**5171–5179.
3. **Chan, P. F., and S. J. Foster.** 1998. Role of SarA in virulence determinant production and environmental signal transduction in *Staphylococcus aureus*. J. Bacteriol. **180:**6232–6241.
4. **Cheung, A. L., A. S. Bayer, G. Zhang, H. Gresham, and Y.-Q. Xiong.** 2004. Regulation of virulence determinants *in vitro* and *in vivo* in *Staphylococcus aureus*. FEMS Microbiol. Lett. **1649:**1–9.
5. **Cheung, A. L., Y. T. Chien, and A. S. Bayer.** 1999. Hyperproduction of alpha-hemolysin in a *sigB* mutant is associated with elevated SarA expression in *Staphylococcus aureus*. Infect. Immun. **67:**1331–1337.
6. **Cheung, A. L., and A. C. Manna.** 2005. Role of the distal *sarA* promoters in SarA expression in *Staphylococcus aureus*. Infect. Immun. **73:**4391–4394.
7. **Cheung, A. L., C. C. Nast, and A. S. Bayer.** 1998. Selective activation of *sar* promoters with the use of green fluorescent protein transcriptional fusions as the detection system in the rabbit endocarditis model. Infect. Immun. **66:**5988–5993.
8. **Chien, C.-T., A. C. Manna, S. J. Projan, and A. L. Cheung.** 1999. SarA, a global regulator of virulence determinants in *Staphylococcus aureus*, binds to a conserved motif essential for *sar*-dependent gene regulation. J. Biol. Chem. **274:**37169–37176.
9. **Dunman, P. M., E. Murphy, S. Haney, D. Palacios, Tucker-Kellogg, S. Wu, E. L. Brown, R. J. Zagursky, D. Shlaes, and S. J. Projan.** 2001. Transcriptional profiling-based identification of *Staphylococcus aureus* genes regulated by the *agr* and/or *sarA* loci. J. Bacteriol. **183:**7341–7353.
10. **Giachino, P., S. Engelmann, and M. Bischoff.** 2001. σ^B activity depends on RsbU in *Staphylococcus aureus*. J. Bacteriol. **183:**1843–1852.
11. **Hong, M., M. Fuangthong, J. D. Helmann, and R. G. Brennan.** 2005. Structure of an OhrR-ohrA operator complex reveals the DNA binding mechanism of the MarR family. Mol. Cell **20:**131–141.
12. **Liu, Y., A. C. Manna, R. Li, W. E. Martin, A. L. Cheung, and G. Zhang.** 2001. Crystal structure of the SarR protein from *Staphylococcus aureus*. Proc. Natl. Acad. Sci. USA **98:**6877–6882.
13. **Liu, Y., A. C. Manna, C. H. Pan, I. A. Kriksunov, D. J. Thiel, A. L. Cheung, and G. Zhang.** 2006. Structural and function analyses of the global regulatory protein SarA from *Staphylococcus aureus*. Proc. Natl. Acad. Sci. USA **103:**2392–2397.
14. **Lowy, F.** 1998. *Staphylococcus aureus* infections. N. Engl. J. Med. **339:**520–532.
15. **Manna, A. C., M. G. Bayer, and A. L. Cheung.** 1998. Transcriptional analysis of different promoters in the *sar* locus in *Staphylococcus aureus*. J. Bacteriol. **180:**3828–3836.
16. **Manna, A. C., and A. L. Cheung.** 2001. Characterization of *sarR*, a modulator of *sar* expression in *Staphylococcus aureus*. Infect. Immun. **69:**885–896.
17. **Miyazaki, E., J.-M. Chen, C. Ko, and W. R. Bishai.** 1999. The *Staphylococcus aureus rsbW* (*orf159*) gene encodes an anti-sigma factor of SigB. J. Bacteriol. **181:**2846–2851.
18. **Munson, G. P., and J. R. Scott.** 2000. Rns, a virulence regulator within the AraC family, requires binding sites upstream and downstream of its own promoter to function as an activator. Mol. Microbiol. **36:**1391–1402.
19. **Novick, R. P.** 2003. Autoinduction and signal transduction in the regulation of staphylococcal virulence. Mol. Microbiol. **48:**1429–1449.
20. **Palma, M., and A. L. Cheung.** 2001. SigB activity in *Staphylococcus aureus* is controlled by RsbU and additional factors during growth. Infect. Immun. **69:**7858–7865.
21. **Safo, M. K., T. P. Ko, F. N. Musayev, Q. Zhao, A. H. Wang, and G. L. Archer.** 2006. Structure of the MecI repressor from *Staphylococcus aureus* in complex with the cognate DNA operator of mec. Acta Crystallograph. Sect. F Struct. Biol. Cryst. Commun. **62:**320–324.
22. **Safo, M. K., Q. Zhao, T. P. Ko, F. N. Musayev, H. Robinson, N. Scarsdale, A. H. Wang, and G. L. Archer.** 2005. Crystal structures of the BlaI repressor from *Staphylococcus aureus* and its complex with DNA: insights into transcriptional regulation of the *bla* and *mec* operons. J. Bacteriol. **187:**1833–1844.
23. **Wolz, C., A. Steinhuber, P. Pohlmann-Dietze, Y. T. Chien, A. C. Manna, W. Van Wamel, and A. L. Cheung.** 2000. Agr-independent regulation of fibronectin binding protein(s) by the regulatory locus *sar* in *Staphylococcus aureus*. Mol. Microbiol. **36:**230–243.