

Global Regulation of Gene Expression by ArlRS, a Two-Component Signal Transduction Regulatory System of *Staphylococcus aureus*

Xudong Liang,¹ Li Zheng,¹ Christina Landwehr,¹ Dwayne Lunsford,² David Holmes,²
and Yinduo Ji^{1*}

Department of Veterinary and Biomedical Sciences, College of Veterinary Medicine, University of Minnesota, 1971
Commonwealth Ave., St. Paul, Minnesota 55108,¹ and Anti-Infective Research, GlaxoSmithKline Research
and Development, 1250 S. Collegeville Rd., Collegeville, Pennsylvania 19426²

Received 3 March 2005/Accepted 26 April 2005

***Staphylococcus aureus* expresses various cell wall-associated and extracellular virulence factors, coordinately controlled by different two-component signal transduction systems and transcriptional regulators. In this study, we used microarray technology to identify the genes regulated by ArlR. The microarray data indicate that ArlR functions as a positive regulator and also as a negative repressor to directly and/or indirectly mediate the expression of at least 114 genes involved in different functions, including autolysis, cell division, growth, and pathogenesis.**

Staphylococcus aureus is an important human and animal pathogen that causes a wide range of infections, including life-threatening endocarditis and toxic shock syndrome (22, 26). The ability of this organism to cause a variety of diseases is partly due to the expression of different cell wall-associated and secreted virulence factors which enable the bacteria to adhere to and colonize host cells (13) or cause toxic shock syndrome (26). The expression of virulence factors is coordinately controlled by two-component signal transduction systems, such as *agr* (1), *sae* (16), *arl* (14), and *srrAB* (30), and global regulators, including *sar* (8, 9), *sigB* (3, 31), *rot* (2, 24), and *mgr* (23). Therefore, the elucidation of the regulons of these regulatory systems is important for better understanding molecular mechanisms of pathogenesis. Recently, *S. aureus* regulons of *agr*, *sar*, *sigB*, and *rot* have been revealed by using a microarray-based approach (4, 11, 27). In our studies, we identified target genes controlled by ArlR by a comparison of the transcriptional profile between an *arlR* mutant and the wild-type strain during the mid-exponential phase of growth by using Affymetrix *S. aureus* arrays.

Construction of the *arlR* deletion mutant. The *arlR* deletion mutant (Sa316ko) was constructed by bacteriophage ϕ 11-mediated transduction of a cassette containing the *tetA* gene, flanked by chromosomal fragments upstream and downstream of the *arlR*, from strain RN4220 into a clinical human isolate strain WCUH29 as described previously (12). Selection for tetracycline resistance and screening for the loss of the erythromycin resistance marker carried by the vector indicated that allelic replacement had occurred and resulted in the *arlR* mutant strain Sa316ko. The mutation in *arlR* was verified by PCR and Southern blot analysis (data not shown).

To characterize the *arlR* mutant strain, we examined the

effect of the *arlR* mutation on the bacterial growth rate, CFU, phenotype, and stress responses to different antibacterial agents, including cell wall synthesis inhibitors such as bacitracin, phosphomycin, and vancomycin. No significant effect of the *arlR* mutation on stress response to antibacterial agents was observed (data not shown). However, Sa316ko grew slightly slower than WCUH29 between early log and stationary phases (Fig. 1). Growth curves as measured by optical density (OD) were confirmed by determining the effect of *arlR* mutation on colony size and viable cell counts at intervals during growth. The ArlR mutant strain, Sa316ko, displayed similar-sized colonies on a tryptic soy agar plate and similar morphological features of individual cells under microscopic observation (data not shown) but decreased approximately one log CFU compared to wild-type strain between early log and stationary phases (data not shown). These results suggest that the *arlRS* regulon may be involved in the modulation of expression of genes associated with growth and cell division. Although it is unlikely that the *arlR* allelic gene replacement mutation has a polar effect on *arlRS* downstream gene expression (since *arlR* and *arlS* loci are located in a single operon), we examined the transcription level of *arlRS* downstream gene *odhA*, encoding 2-oxoglutarate dehydrogenase E1. Our real-time reverse transcriptase (RT)-PCR analyses showed that the *arlR* mutation had no significant influence on the expression of *odhA* (see Table 5).

Identification of the *arlRS* regulon using microarray assay.

To better define the *arlRS* regulon, gene expression profiles of the *arlR* mutant and parent cells were analyzed by using Affymetrix *S. aureus* arrays as described previously (11). The *S. aureus* array (Affymetrix) contains probe sets to over 3,300 *S. aureus* open reading frames (ORFs) based on the updated *S. aureus* genomic sequences of N315, Mu50, NCTC 8325, and COL. Total RNA was extracted from *S. aureus* cells grown to mid-log phase (OD at 600 nm, 0.4) by using the RNeasy kit (Qiagen) and treated with a DNA-free kit (Ambion). The RNA (10 μ g) was reverse transcribed to cDNA by using Superscript II reverse transcriptase and random primers (In-

* Corresponding author. Mailing address: Department of Veterinary and Biomedical Sciences, College of Veterinary Medicine, University of Minnesota, 1971 Commonwealth Ave., St. Paul, MN 55108. Phone: (612) 624-2757. Fax: (612) 625-5203. E-mail: jixxx002@umn.edu.

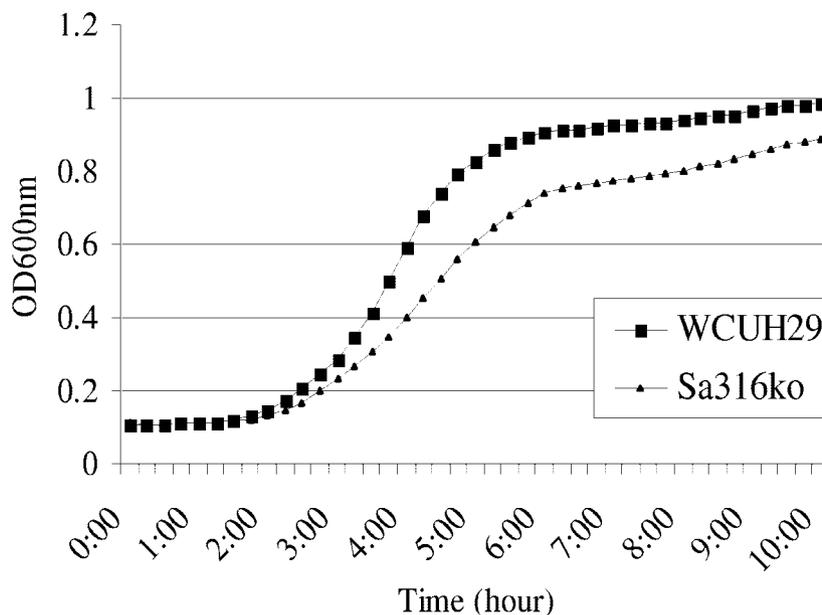


FIG. 1. Growth curve of the *arlR* mutant. The *arlR* mutant strain Sa316ko and parent strain WCUH29 were incubated in TSB at 37°C overnight with shaking; the cultures were inoculated in fresh TSB. The cell growth was monitored at 37°C by a measuring of the OD at 600 nm every 15 min with 1 min of mixing before each reading. These curves represent one of three reproducible experiments.

vitrogen). The cDNA was treated with NaOH, purified by using the QIAquick PCR purification kit (QIAGEN), and digested with DNase I. The fragmented cDNAs were then directly labeled with biotin by using a biotin-ddUTP kit (Affymetrix). Biotinylated cDNA (3 μ g) was hybridized to the GeneChips. The GeneChips were then washed and subjected to a series of staining procedures as described in the manual for the Affymetrix array. Each GeneChip was washed and scanned at a 570-nm wavelength and a 3- μ m resolution in an Affymetrix GeneChip scanner. The Affymetrix Microarray Suite 4.0 algorithms calculated the signal intensities (average differences) and the present or absent determinations for each open reading frame. The GeneChips were then normalized, and their backgrounds were defined by using GeneSpring 4.0 (Silicon Genetics). The GeneSpring software was used to further analyze the transcription patterns of genes. To identify genes with significantly altered expression levels, a series of statistical analyses (filtering) were performed; cutoff values for ratio of expression levels of 1.80 and 0.55 were used to filter genes with expression level changes (*n*-fold) greater than ± 1.8 in all three independent biological samples. Genes with variations (*n*-fold) of >1.5 across the three samples were excluded. Furthermore, a statistical group comparison using the Student *t* test/analysis of variance was conducted to compare the mean expression levels of the control and the *arlR* mutant samples. The genes with significant differential expression levels (*P* value, <0.05) were selected.

The results of three independent experiments demonstrated that on average, transcripts for 73% of all genes on the arrays were detected by the Affymetrix arrays in the mid-log phase of bacterial cells of WCUH29. A comparison analysis of gene expression levels between wild-type WCUH29 and the *arlR* mutant revealed that the expression levels of 114 genes were significantly altered in the *arlR* mutant. Of these, 37 genes

showed a decrease (Table 1) and 77 genes showed an increase (Table 2) in expression level after the mutation of *arlR*.

The array data indicated that ArlR positively regulates a two-component system, *lytR-lytS*, which encodes a response regulator and a sensor histidine kinase and is involved in autolysis (6, 14). The positive regulation of *lytS* expression by ArlR was confirmed by real-time RT-PCR analysis using the Stratagene Mx3000P real-time PCR system. Gene-specific primers were designed to yield ~ 100 bp of specific products (Table 3), and the housekeeping gene 16S rRNA was used as an endogenous control (29). All samples were analyzed in triplicate and normalized against 16S rRNA gene expression. The results were statistically analyzed for correlation to the microarray results. Compared to the wild type, the *arlR* mutant possessed low levels of *lytS* mRNA in the early log, mid-log, and stationary phases of growth (Tables 4 and 5). We also found that the mutation in *arlR* significantly down-regulates the expression of *lrgA* and *lrgB*, encoding different holin-like proteins involved in murein hydrolase transport and inhibition of murein hydrolase activity (7, 17). These enzymes are involved in the cleavage of specific cell wall components, which are important for cell division and growth (7, 17).

To determine whether the growth defect of the mutant is attributable to increased susceptibility to cell lysis, we examined the effect of ArlR on autolysis induced by Triton X-100 and detected the cell wall murein hydrolase activity by using a zymographic assay as described previously (19). Consistent with a previous report (14), the *arlR* mutant cells displayed increased lysis in the presence of 0.01% Triton and showed enhanced peptidoglycan hydrolase activity compared to the parent control (unpublished data). These results indicated that increased autolysis in the *arlR* mutant may result from the significant down-regulation of the *lytSR* and *lrgAB* operons and, in turn, may partially affect bacterial growth. Although it

TABLE 1. *S. aureus* genes up-regulated by ArlR

N315 ORF	N315 gene	N315 description	Change in expression level (<i>n</i> -fold) ^a	<i>agr</i> , <i>sar</i> , <i>rot</i> , or <i>sigB</i> effect ^b
SA1844	<i>agrA</i>	Response regulator	-2.8	
SA1842	<i>agrB</i>	Accessory gene regulator B	-2.2	
SA1843	<i>agrC</i>	Sensor histidine kinase	-2.4	
SAS066	<i>agrD</i>	AgrD protein	-1.6	
SA1248	<i>arlR</i>	Response regulator	-602.2	
SAS065	<i>hld</i>	Delta-hemolysin	-2.2	<i>agr</i> +
SA0250	<i>lytS</i>	Two-component sensor histidine kinase	-3.1	<i>rot</i> +
SA0251	<i>lytR</i>	Two-component response regulator	-2.2	
SA0144	<i>cap5A</i>	Capsular polysaccharide synthesis enzyme Cap5A	-1.8	σ^B +
SA0147	<i>cap5D</i>	Capsular polysaccharide synthesis enzyme Cap5D	-1.5	σ^B +
SA0211		Putative oxidoreductase	-2.1	
SA0212		Hypothetical protein	-1.9	
SA0220		Hypothetical protein	-2.8	<i>rot</i> +
SA0252	<i>lrgA</i>	Holin-like protein LrgA	-16.5	<i>sar</i> +, <i>sigB</i> -
SA0253	<i>lrgB</i>	Holin-like protein LrgB	-19.7	<i>sar</i> +, <i>sigB</i> -
SA0269		Hypothetical protein	-2.3	
SA0270	<i>ssaA</i>	Secretory antigen precursor SsaA	-2	<i>sigB</i>
SA0271		Hypothetical protein	-5.9	<i>agr</i> +, <i>rot</i> +,
SA0272		Hypothetical protein	-7.4	<i>sigB</i> -
SA0275		Hypothetical protein	-4.2	<i>sigB</i> -
SA0276		Similar to diarrheal toxin	-4.4	
SA0417		Hypothetical protein	-2.5	
SA0519	<i>sdrC</i>	Ser-Asp-rich fibrinogen-binding protein SdrC	-2.3	<i>sar</i> +, <i>rot</i> +
SA0520	<i>sdrD</i>	Ser-Asp-rich fibrinogen-binding protein SdrD	-3.7	
SA0521	<i>sdrE</i>	Ser-Asp-rich fibrinogen-binding protein SdrE	-2.1	
SA0746		Staphylococcal nuclease	-1.9	
SA0893		Hypothetical protein	-1.9	
SA1269		Blt-like protein	-3.1	
SA1270		Similar to amino acid pearmease	-1.8	
SA1271		IlvA threonine deaminase	-3	
SA1272		Alanine dehydrogenase	-2.4	
SA1305		Cell-division initiation protein	-4.9	
SA1583	<i>rot</i>	Repressor of toxin	-1.9	
SA2222		Bicyclomycin-resistant protein TcaB	-2	
SA2303		Hypothetical protein	-11.2	<i>rot</i> +
SA2486		2-Oxoglutarate/malate translocator	-1.8	

^a Normalized values in the wild-type strain over values in the *arlR* mutant.

^b *agr* and *sar* effects are as described by Dunman et al. (13), *rot* effect is as described by Said-Salim et al. (41), and *sigB* effect is as described by Bischoff et al. (4). +, up-regulated; -, down-regulated.

has been reported that the mutation in *rat*, another autolysis regulator, exhibited a growth defect and enhanced autolysis partly due to increased murein hydrolase activity (19), the reason why the mutation in *arlR* led to a slight impact on growth remains undefined, since the *lrgAB* mutation did not show significant impact on the cell shape and growth rate (17).

Also, our microarray data showed that ArlR positively regulates virulence factor genes, such as *sdrC*, *sdrD*, and *sdrE*, encoding different Ser-Asp-rich bone sialoprotein-binding proteins (Table 1) (28). The result for *sdrD* was confirmed by using real-time RT-PCR and demonstrated that the level of *sdrD* mRNA in the *arlR* mutant strain is significantly decreased compared with that in the wild-type strain (Table 4). The up-regulation of *sdrC* by ArlR may indirectly function via up-regulated *rot* expression, since Rot positively regulates the expression of *sdrC* (11). In addition, ArlR positively regulates the *tcaB* gene (which encodes a bicyclomycin-resistant protein), the secretory antigen precursor *ssaA*, and toxin genes, such as *hld* and *SA0276*, encoding delta-hemolysin and diarrheal toxin, respectively (Table 1).

Moreover, the array data showed that ArlR positively regulates the accessory gene regulator (*agr*) (Table 1). A real-time

RT-PCR was employed to validate this result and demonstrated that the mutation in *arlR* significantly decreased the level of *agrA* expression (Table 4). The expression of *agrA* was constantly up-regulated by ArlR at different times of growth (Table 5). These results are inconsistent with the finding that the mutation in *arlS* leads to the overexpression of Agr (15). This controversy may be due to different sensitivities, culture conditions, and time points of sampling between different assays. In addition, the array results indicated that ArlR also positively regulates the expression of the repressor of toxins (*rot*). This result was confirmed by real-time RT-PCR and demonstrated that the mutation in *arlR* causes a decrease in *rot* expression (Table 4). Our findings are consistent with previous reports that the expression of certain secreted enzymes, toxins, and ureases are repressed by Rot (9, 27). Most virulence factors negatively regulated by ArlR were also repressed by Rot but were up-regulated by Agr (5, 25). Therefore, the down-regulation of these toxins, proteases, and adhesins may be mediated directly by ArlR or indirectly controlled via Rot or Agr (Fig. 2).

To determine the role of the ArlRS regulatory system in pathogenesis, we examined the effect of the *arlR* mutation on

TABLE 2. *S. aureus* genes repressed by ArlR

N315 ORF	N315 gene	N315 description	Change in expression level (<i>n</i> -fold) ^a	<i>agr</i> , <i>sar</i> , <i>rot</i> , or <i>sigB</i> effect ^b
SA0104		Hypothetical protein	1.9	
SA0123		Hypothetical protein	22.1	<i>rot</i> -
SA0124		Hypothetical protein	35	
SA0125		Hypothetical protein	39.6	
SA0126		Hypothetical protein	20.4	
SA0127	<i>cpsM</i>	Capsular polysaccharide repeat, Unit transporter cpsM	4	<i>rot</i> -
SA0135		Hypothetical protein	1.6	
SA0136		Hypothetical protein	2.7	
SA0137		Hypothetical protein	3.6	
SA0138		Hypothetical protein	5.6	
SA0165		Hypothetical protein	1.8	<i>rot</i> -
SA0299		PfkB family carbohydrate kinase	2.5	
SA0304	<i>nanA</i>	<i>N</i> -Acetylneuraminase lyase	2.5	
SA0318		Putative membrane protein	2.5	
SA0319		Hypothetical protein	4.7	
SA0320		Hypothetical protein	5.1	
SA0321		Putative PTS multidomain regulator	5.5	
SA0331		Hypothetical protein	14.2	
SA0332		Hypothetical protein	8	
SA0333		Conserved hypothetical protein	10.6	
SA0395		Hypothetical protein	2.2	
SA0710		Conserved hypothetical protein	7.4	<i>rot</i> -
SA0850		Hypothetical protein	10.7	<i>rot</i> -
SA0851		Oligopeptide ABC transporter, ATP-binding protein	2.2	
SA0899	<i>sspC</i>	Cysteine protease	3.1	<i>agr</i> +, <i>rot</i> -
SA0904		Hypothetical protein	10.7	<i>rot</i> -
SA0956		Hypothetical protein	2.9	
SA0978	<i>isdC</i>	Conserved hypothetical protein	1.9	
SA1090	<i>lytN</i>	LytN protein	10.4	<i>agr</i> +, <i>sar</i> +, <i>rot</i> -, <i>sigB</i> -
SA1091	<i>fmhC</i>	FmhC protein	3.9	<i>rot</i> -
SA1145		Host factor-1 protein	3	
SA1154		Conserved hypothetical protein	9.1	
SA1266		Hypothetical protein	6.4	
SA1267	<i>ebhA</i>	Hypothetical protein	15.1	<i>rot</i> -
SA1268	<i>ebhB</i>	Hypothetical protein	28.6	<i>agr</i> +, <i>rot</i> -
SA1552		Hypothetical protein	3.7	
SA1577		Hypothetical protein	17.2	
SA1630	<i>splB</i>	Serine protease, V8 protease	4.3	<i>agr</i> +, <i>sar</i> +, <i>rot</i> -, <i>sigB</i> -
SA1637	<i>lukD</i>	Leukotoxin, LukD	1.9	
SA1638	<i>lukE</i>	Leukotoxin, LukE	1.6	
SA1752	<i>hlb</i>	Truncated beta-hemolysin	2.5	
SA1848	<i>nrgA</i>	Probable ammonium transporter	1.9	
SA1882	<i>kdpD</i>	Two-component sensor kinase KdpD	2.9	
SA1883	<i>kdpE</i>	Two-component response regulator KdpE	2	
SA1991	<i>lacG</i>	6-Phospho-beta-galactosidase	4.1	
SA1992		PTS system, lactose-specific IIBC component	3.5	
SA1993	<i>lacF</i>	PTS system, lactose-specific IIA component	6.5	
SA1994	<i>lacD</i>	Tagatose 1,6-diphosphate aldolase	4.4	
SA1995	<i>lacC</i>	Tagatose-6-phosphate kinase	4.9	
SA1996	<i>lacB</i>	Galactose-6-phosphate isomerase LacB subunit	7.2	
SA1997	<i>lacA</i>	Galactose-6-phosphate isomerase LacA subunit	5.1	
SA2006		Hypothetical protein	5.5	
SA2007		Hypothetical protein	2.8	
SA2008	<i>alsS</i>	Alpha-acetolactate synthase	2.4	
SA2081		Urea transporter	3.1	
SA2082	<i>ureA</i>	Urease gamma subunit	9.3	<i>rot</i> -
SA2083	<i>ureB</i>	Urease beta subunit	12.1	<i>rot</i> -
SA2084	<i>ureC</i>	Urease alpha subunit	7	<i>rot</i> -
SA2085	<i>ureE</i>	Urease accessory protein UreE	5.2	<i>rot</i> -
SA2086	<i>ureF</i>	Urease accessory protein UreF	4.6	<i>rot</i> -
SA2087	<i>ureG</i>	Urease accessory protein UreG	3.6	<i>rot</i> -
SA2088	<i>ureD</i>	Urease accessory protein UreD	4.5	<i>rot</i> -
SA2091		Hypothetical protein	6.7	
SA2092		Hypothetical protein	4.5	

Continued on following page

TABLE 2—Continued

N315 ORF	N315 gene	N315 description	Change in expression level (<i>n</i> -fold) ^a	<i>agr</i> , <i>sar</i> , <i>rot</i> , or <i>sigB</i> effect ^b
SA2209	<i>hlgC</i>	Gamma-hemolysin component C precursor	3.2	<i>agr</i> +, <i>rot</i> -
SA2287		Staphylococcal regulator SarH2	7.9	
SA2315		Putative membrane protein	2.6	
SA2319		Putative L-serine dehydratase	3.8	
SA2320		Putative membrane protein	4.7	
SA2329		Murine hydrose exporter	3.8	
SA2337	<i>feoB</i>	Ferrous iron transporter protein	4	
SA2338		Hypothetical protein	7.3	
SA2382		Hypothetical protein	5.4	
SA2455	<i>cap8C</i>	Capsular polysaccharide synthesis enzyme Cap8C	12.7	<i>rot</i> -, <i>sigB</i> +
SA2456	<i>cap8B</i>	Capsular polysaccharide synthesis enzyme Cap8B	18.1	<i>rot</i> -, <i>sigB</i> +
SA2457	<i>capA</i>	Capsular polysaccharide biosynthesis CapA	31.2	<i>rot</i> -, <i>sigB</i> +
SA2482	<i>arcA</i>	Arginine deiminase	2	
SAV0397		Hypothetical protein	12.1	

^a Normalized values in the *arlR* mutant over values in the wild-type strain.
^b *agr* and *sar* effects are as described by Dunman et al. (13), *rot* effect is as described by Said-Salim et al. (41), and *sigB* effect is as described by Bischoff et al. (4). +, up-regulated; -, down-regulated.

virulence by using a murine hematogenous pyelonephritis model as described previously (20). The virulence of the *arlR* mutant was significantly attenuated compared to that of the parent control (unpublished data). Collectively, these findings suggest that ArlRS is a global two-component virulence regulatory system which can interact with other regulators to modulate the expression of virulence factors.

On the other hand, ArlR functions as a repressor of virulence factors. Our microarray data showed that ArlR negatively regulates some toxin genes, *lukD*, *lukE*, *phlC* (*hlb*), and *hlgC*, which encode leukotoxin D, leukotoxin E, beta-hemolysin component C, and gamma-hemolysin component C, respectively (Table 2). Gamma-hemolysin is an *S. aureus* virulence factor that has been shown to play a role in *S. aureus*

endophthalmitis and corneal pathogenesis (10, 21). The microarray result for *lukD* was confirmed by real-time RT-PCR. As shown in Table 4, the *lukD* transcript was present at higher levels in the *arlR* mutant strain than in the wild-type strain. The array results also demonstrated that ArlR negatively regulates *spb* (encoding V8 protease), *sspC* (encoding cysteine protease), and *ebhA* and *ebhB* (encoding adhesins) (Table 2). The negative regulation of *ebhB* expression by ArlR was confirmed by a real-time RT-PCR (Table 4).

Furthermore, the array results showed that ArlR negatively regulates the expression of genes involved in different PTS systems, such as the *lacG*, *lacF*, *lacD*, *lacC*, *lacB*, and *lacA* operons, as well as hypothetical proteins. To validate these results, real-time RT-PCR was performed, and it demonstrated that the mutation of *arlR* increases the expression of hypothetical proteins (SA0123 and SA0319) and constantly

TABLE 3. Primers used in real-time RT-PCR

Primer	Sequence (5'-3')
SA0123for	ATATTACGGCGAACGGACGAC
SA0123rev	TGGCTTGTATGCTCAAATGAATCG
SA0250for	GCATGGTTCTATCGTCGGTACATTG
SA0250rev	ACTTACTTTGCGTTTCGGCTTCAC
SA0252lrgAfor	TGAAACAACAAAAAGACGCATCAAAACCAG
SA0252lrgArev	ACTTCGCCTAACTTAACAGCACCCAG
SA0270ssaAfor	GGCATCCAAGTCAATTAACCAAGATAATG
SA0270ssaArev	CAGTACGGTAGCTGTTTGTGTTGTAAC
SA0319for	GCACCATCTGATATCGAAGTTGAAC
SA0319rev	TAGGCGTTTCGGCATTTCAGC
SA1248for	TGACAAAGTTGCTGGGCTTGATTAC
SA1248rev	TGTGGCTGACGACGTAATAATTGC
SA1583for	TCAGCGAGATTGAAAAGCGAATAC
SA1583rev	CTGTCCATTTCCTTAAGCGTCATAG
SA1637for	TGGGCGGTAAGTATAATGTTTCG
SA1637rev	GATCCATTCAATCCACCTGATAAGC
SA1882for	GAAAGACAAGCTGGTGCAACAAAC
SA1882rev	AACGGCGAGAGAAAGTTCATTTAAC
SA1844agrAfor	GTGAAATTCGTAAGCATGACCCAGTTG
SA1844agrArev	TGTAAGCGTGATGTGCAGTTTCTAAAC
SA1993for	GAAGGAAACAATTGCATTGCTGAAG
SA1993rev	ATATCATCACCTTGGCGCTTCTTTAG
SA1245sodhA1665for	GCAATGAACCAACCCGTAGAAATAGC
SA1245sodhA1833rev	ACGAGAGCAGCACAAGATGATACAC
16S rRNAfor	CTGTGCACATCTTGACGGTA
16S rRNArev	TCAGCGTCAGTTACAGACCA

TABLE 4. Real-time RT-PCR analysis of expression of genes regulated by ArlR

N315 ORF	N315 gene	N315 description	Change in expression level (<i>n</i> -fold) ^a	
			RT-PCR	Microarray
SA0123		Hypothetical protein	42.8	22.1
SA0319		Hypothetical protein	5.1	4.7
SA1637	<i>lukD</i>	Leukotoxin LukD	2	1.9
SA1882	<i>kdpD</i>	Two-component sensor kinase	3.1	2.9
SA1993	<i>lacF</i>	PTS system, lactose-specific IIA component	17	6.5
SA1844	<i>agrA</i>	Two-component response regulator	-5.7	-2.8
SA0250	<i>lytS</i>	Two-component sensor kinase	-2.8	-3.1
SA0252	<i>lrgA</i>	Holin-like protein LrgA	-64.8	-16.5
SA0270	<i>ssaA</i>	Secretory antigen precursor SsaA	-1.7	-2
SA1583	<i>rot</i>	Repressor of toxin	-4	-1.9

^a Normalized values in the *arlR* mutant over values in the wild-type strain. Negative numbers denote up-regulation in the wild-type strain.

TABLE 5. Real-time RT-PCR analysis of expression of genes regulated by ArlR in different growth phases

N315 ORF	N315 gene	N315 description	Change in expression level (<i>n</i> -fold) ^a in RT-PCR by phase		
			Early log	Mid-log	Stationary
SA0250	<i>lytS</i>	Two-component sensor kinase	-4.4	-7	-6.7
SA1844	<i>agrA</i>	Two-component response regulator	-2.7	-2.3	-3.9
SA1882	<i>kdpD</i>	Two-component sensor kinase	-2.6	13.8	1.2
SA1993	<i>lacF</i>	PTS system, lactose specific IIA component	6.3	4	13
SA1245 ^b	<i>odhA</i>	2-Oxoglutarate dehydrogenase E1	NC	NC	NC

^a Normalized values in the *arlR* mutant over values in the wild-type strain. Negative numbers denote up-regulation in the wild-type strain.

^b Downstream gene of *arlRS*. NC, no detectable change.

up-regulates *lacF* expression at different phases of growth (Tables 4 and 5). Also, we found that in the *arlR* mutant, the levels of *kdpD* and *kdpE* transcripts (which encode a two-component sensor kinase and response regulator and involve K⁺ transport [18]) were obviously higher than those in the wild-type strain. To confirm this result, real-time RT-PCR was performed, and the results demonstrated that the mutation of *arlR* increases *kdpD* expression in the mid-log phase of growth but decreases *kdpD* expression in the early log phase of growth (Tables 4 and 5). These results suggest that ArlR differentially regulates *kdpD* expression at different times of growth.

Conclusion. The regulon of ArlRS has been identified by employing transcriptome technology using Affymetrix *S. aureus* arrays. The results demonstrate that ArlRS is a global transcriptional regulator which directly and/or indirectly interacts with other regulators in regulatory networks and modulates the expression of genes involved in autolysis, cell growth, and pathogenesis (Fig. 2). Some genes/operons mediated by ArlRS may be missed, due to their lack of stability and/or kinetic regulation as well as low detection sensitivity. Further studies to investigate which genes identified using microarray assays are directly regulated by ArlR and are involved in autolysis and/or pathogenesis are in progress.

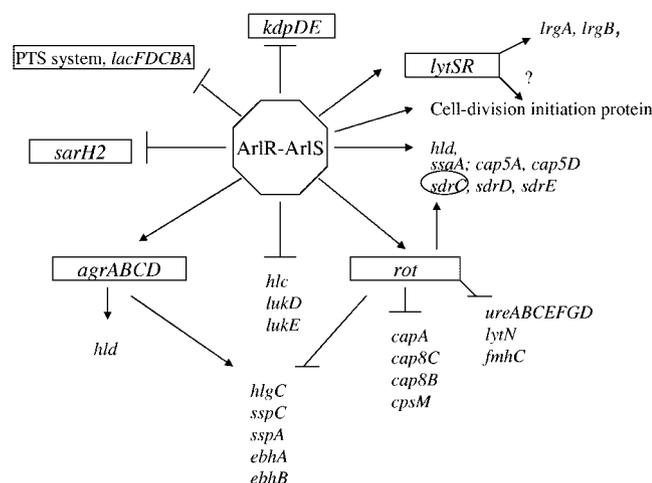


FIG. 2. Schematic figure showing how the staphylococcal two-component signal transduction regulatory system, ArlRS, directly and/or indirectly modulates gene expression. →, positive regulation; ⊥, negative regulation.

We thank Junsong Sun for his assistance in the real-time RT-PCR analysis, Aaron Becker for his assistance in the microarray analysis, and Doug Weiss for his critical reading of the manuscript and for his helpful suggestions.

This work was supported by grant O3-O2 from the Academic Health Center at the University of Minnesota. This research was also supported in part by grant AI057451 from the National Institute of Allergy and Infectious Diseases.

REFERENCES

1. Abdelnour, A., S. Arvidson, T. Bremell, C. Ryden, and A. Tarkowski. 1993. The accessory gene regulator (*agr*) controls *Staphylococcus aureus* virulence in a murine arthritis model. *Infect. Immun.* **61**:3879–3885.
2. Arvidson, S., and K. Tegmark. 2001. Regulation of virulence determinants in *Staphylococcus aureus*. *Int. J. Med. Microbiol.* **291**:159–170.
3. Bischoff, M., J. M. Entenza, and P. Giachino. 2001. Influence of a functional *sigB* operon on the global regulators *sar* and *agr* in *Staphylococcus aureus*. *J. Bacteriol.* **183**:5171–5179.
4. Bischoff, M., P. Dunman, J. Kormanec, D. Macapagal, E. Murphy, W. Mounts, B. Berger-Bächi, and S. Projan. 2004. Microarray-based analysis of the *Staphylococcus aureus* σ^B regulon. *J. Bacteriol.* **186**:4085–4099.
5. Bronner, S., H. Monteil, and G. Prevost. 2004. Regulation of virulence determinants in *Staphylococcus aureus*: complexity and applications. *FEMS Microbiol. Rev.* **28**:183–200.
6. Brunskill, E. W., and K. W. Bayles. 1996. Identification and molecular characterization of a putative regulatory locus that affects autolysis in *Staphylococcus aureus*. *J. Bacteriol.* **178**:611–618.
7. Brunskill, E. W., and K. W. Bayles. 1996. Identification of LytSR-regulated genes from *Staphylococcus aureus*. *J. Bacteriol.* **178**:5810–5812.
8. Cheung, A. L., J. M. Koomey, C. A. Butler, S. J. Projan, and V. A. Fischetti. 1992. Regulation of exoprotein expression in *Staphylococcus aureus* by a locus (*sar*) distinct from *agr*. *Proc. Natl. Acad. Sci. USA* **89**:6462–6466.
9. Cheung, A. L., K. Schmidt, B. Bateman, and A. C. Manna. 2001. SarS, a SarA homolog repressible by *agr*, is an activator of protein A synthesis in *Staphylococcus aureus*. *Infect. Immun.* **69**:2448–2455.
10. Dajcs, J. J., M. S. Austin, G. D. Sloop, J. M. Moreau, E. B. Hume, H. W. Thompson, F. M. McAleese, T. J. Foster, and R. J. O’Callaghan. 2002. Corneal pathogenesis of *Staphylococcus aureus* strain Newman. *Investig. Ophthalmol. Vis. Sci.* **43**:1109–1115.
11. Dunman, P. M., E. Murphy, S. Haney, D. Palacios, G. Tucker-Kellogg, S. Wu, E. L. Brown, R. J. Zagursky, D. Shlaes, and S. J. Projan. 2001. Transcription profiling-based identification of *Staphylococcus aureus* genes regulated by the *agr* and/or *sarA* loci. *J. Bacteriol.* **183**:7341–7353.
12. Fan, F., R. D. Lunsford, D. Sylvester, J. Fan, H. Celesnik, S. Iordanescu, M. Rosenberg, and D. McDevitt. 2001. Regulated ectopic expression and allelic-replacement mutagenesis as a method for gene essentiality testing in *Staphylococcus aureus*. *Plasmid* **46**:71–75.
13. Foster, T. J., and M. Höök. 1998. Surface protein adhesins of *Staphylococcus aureus*. *Trends Microbiol.* **6**:484–488.
14. Fournier, B., and D. C. Hooper. 2000. A new two-component regulatory system involved in adhesion, autolysis, and extracellular proteolytic activity of *Staphylococcus aureus*. *J. Bacteriol.* **182**:3955–3964.
15. Fournier, B., A. Klier, and G. Rapoport. 2001. The two-component system ArlS-ArlR is a regulator of virulence gene expression in *Staphylococcus aureus*. *Mol. Microbiol.* **41**:247–261.
16. Giraudo, A. T., A. L. Cheung, and R. Nagel. 1997. The *sae* locus of *Staphylococcus aureus* controls exoprotein synthesis at the transcriptional level. *Arch. Microbiol.* **168**:53–58.
17. Groicher, K. H., B. A. Firek, D. F. Fujimoto, and K. W. Bayles. 2000. The *Staphylococcus aureus* *lrgAB* operon modulates murein hydrolase activity and penicillin tolerance. *J. Bacteriol.* **182**:1794–1801.
18. Heermann, R., K. Altendorf, and K. Jung. 2003. The N-terminal input

- domain of the sensor kinase KdpD of *Escherichia coli* stabilizes the interaction between the cognate response regulator KdpE and the corresponding DNA-binding site. *J. Biol. Chem.* **278**:51277–51284.
19. **Ingavale, S. S., W. Van Wamel, and A. L. Cheung.** 2003. Characterization of Rat, an autolysis regulator in *Staphylococcus aureus*. *Mol. Microbiol.* **48**: 1451–1466.
 20. **Ji, Y., A. Marra, M. Rosenberg, and G. Woodnutt.** 1999. Regulated antisense RNA eliminates alpha-toxin virulence in *Staphylococcus aureus* infection. *J. Bacteriol.* **181**:6585–6590.
 21. **Karlsson, A., P. Saravia-Otten, K. Tegmark, E. Morfeldt, and S. Arvidson.** 2001. Decreased amounts of cell wall-associated protein A and fibronectin-binding proteins in *Staphylococcus aureus sarA* mutants due to up-regulation of extracellular proteases. *Infect. Immun.* **69**:4742–4748.
 22. **Lowy, F. D.** 1998. *Staphylococcus aureus* infections. *N. Engl. J. Med.* **339**: 520–532.
 23. **Luong, T. T., S. W. Newell, and C. Y. Lee.** 2003. *mgr*, a novel global regulator in *Staphylococcus aureus*. *J. Bacteriol.* **185**:3703–3710.
 24. **McNamara, P. J., K. C. Milligan-Monroe, S. Khalili, and R. A. Proctor.** 2000. Identification, cloning, and initial characterization of rot, a locus encoding a regulator of virulence factor expression in *Staphylococcus aureus*. *J. Bacteriol.* **182**:3197–3203.
 25. **Novick, R. P.** 2003. Autoinduction and signal transduction in the regulation of staphylococcal virulence. *Mol. Microbiol.* **48**:1429–1449.
 26. **Projan, S., and R. Novick.** 1997. The molecular basis of pathogenicity, p. 55–81. *In* G. Archer and K. Crossley (ed.), *Staphylococci in human diseases*. Churchill Livingstone, New York, N.Y.
 27. **Said-Salim, B., P. M. Dunman, F. M. McAleese, D. Macapagal, E. Murphy, P. J. McNamara, S. Arvidson, T. J. Foster, S. J. Projan, and B. N. Kreiswirth.** 2003. Global regulation of *Staphylococcus aureus* genes by Rot. *J. Bacteriol.* **185**:610–619.
 28. **Tung, H., B. Guss, U. Hellman, L. Persson, K. Rubin, and C. Ryden.** 2000. A bone sialoprotein-binding protein from *Staphylococcus aureus*: a member of staphylococcal Sdr family. *Biochem. J.* **345**:611–619.
 29. **Yarwood, J. M., J. K. McCormick, M. L. Paustian, V. Kapur, and P. M. Schlievert.** 2002. Repression of the *Staphylococcus aureus* accessory gene regulator in serum and in vivo. *J. Bacteriol.* **184**:1095–1101.
 30. **Yarwood, J. M., J. K. McCormick, and P. M. Schlievert.** 2001. Identification of a novel two-component regulatory system that acts in global regulation of virulence factors of *Staphylococcus aureus*. *J. Bacteriol.* **183**:1113–1123.
 31. **Ziebandt, A. K., H. Weber, J. Rudolph, R. Schmid, D. Hoper, S. Engelmann, and M. Hecker.** 2001. Extracellular proteins of *Staphylococcus aureus* and the role of SarA and sigma B. *Proteomics* **1**:480–493.