JOURNAL OF BACTERIOLOGY, Apr. 2006, p. 2593–2603 Vol. 188, No. 7
0021-9193/06/$08.00+0 doi:10.1128/JB.188.7.2593–2603.2006

Characterizing the Effect of the *Staphylococcus aureus* Virulence Factor Regulator, SarA, on Log-Phase mRNA Half-Lives†

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Received 7 November 2005/Accepted 17 January 2006

Bacterial pathogens regulate virulence factor expression at both the level of transcription initiation and mRNA processing/turnover. Within *Staphylococcus aureus*, virulence factor transcript synthesis is regulated by a number of two-component regulatory systems, the DNA binding protein SarA, and the SarA family of homologues. However, little is known about the factors that modulate mRNA stability or influence transcript degradation within the organism. As our entree to characterizing these processes, *S. aureus* GeneChips were used to simultaneously determine the mRNA half-lives of all transcripts produced during log-phase growth. It was found that the majority of log-phase transcripts (90%) have a short half-life (<5 min), whereas others are more stable, suggesting that *cis*- and/or *trans*-acting factors influence *S. aureus* mRNA stability. In support of this, it was found that two virulence factor transcripts, *cna* and *spa*, were stabilized in a *sarA*-dependent manner. These results were validated by complementation and real-time PCR and suggest that SarA may regulate target gene expression in a previously unrecognized manner by posttranscriptionally modulating mRNA turnover. Additionally, it was found that *S. aureus* produces a set of stable RNA molecules with no predicted open reading frame. Based on the importance of the *S. aureus agr* RNA molecule, RNAIII, and small stable RNA molecules within other pathogens, it is possible that these RNA molecules influence biological processes within the organism.

Staphylococcus aureus is a gram-positive pathogen that is capable of causing a number of infections, which range in severity from minor skin infections to life-threatening endocarditis and osteomyelitis. Although the organism is part of the normal human flora, it can cause infection when there is a break in the skin or mucous membrane that grants it access to the surrounding tissues (17, 39). *S. aureus* owes its ability to subsequently colonize host tissue and disseminate to other sites to the production of an array of virulence factors. Generally, these factors include accessory cytoplasmic, surface, and secreted components, which are coordinately regulated at the transcriptional level in response to endogenous and environmental cues, i.e., cell density, pH, and subinhibitory concentrations of antibiotics (9, 36, 46, 61). Virulence factor regulation is modulated by at least seven two-component regulatory systems (ArlRS [26], SaeRS [47, 55], AgrAC [35], SrrAB [62], LytRS [14], YycFG [44], and VraRS [37]), the DNA-binding protein SarA (18), and the SarA family of homologues (SarS [19, 57], SarR [40], SarU [42], SarT [51], SarV [43], MgrA [30, 31], and TcaR [45]).

The *S. aureus sarA* locus includes a 1.2-kb DNA fragment that produces three overlapping transcripts (*sarA*, *sarB*, and *sarC*), each of which shares a termination site and encodes SarA protein (5). In the laboratory setting, protein levels remain constant throughout growth phases (12). Yet, *sarA* and *sarB* transcripts are preferentially transcribed during log-phase growth. At higher cell densities, *sarA* and *sarB* transcript titers decrease, whereas *sarC* mRNA levels increase (12). *sarC* transcription is also driven by the alternative sigma factor σ^B (41). The significance of each transcriptional unit's production has not been studied in detail.

SarA is a pleiotropic regulator that negatively effects the protein production of several virulence factors, including protein A (*spa*), collagen-binding protein (*cna*), and serine proteinase (*sspA*) (5, 12, 34). Northern blotting and microarray studies have indicated that SarA's regulatory effects are, at least in part, at the transcriptional level. Electrophoretic gel-mobility shift assays and DNA footprinting have revealed that SarA is capable of binding to a 26-bp and/or 7-bp sequence within the promoter region of these target genes, suggesting that SarA acts as a transcription factor (20, 56). Nonetheless, it remains to be seen whether either of these putative SarA binding sites has any biological relevance. For instance, the 26-bp site cannot be found within 150 bp of the predicted translational start site of ~70% of the genes whose transcript titers were found to be modulated in a *sarA*-dependent manner by microarray analysis (24). Likewise, the *S. aureus* strain N315 sequence contains >2,500 7-bp (ATTTTAT) putative SarA binding sites within its genome (P. M. Dunman and E. Murphy, unpublished data). It is difficult to imagine that a bona fide

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† Supplemental material for this article may be found at http://jb.asm.org/.

TABLE 1. Strains and plasmids used in this study

Strain or plasmid	Relevant genotype or phenotype	Reference
Strains		
UAMS-1	Wild-type osteomyelitis isolate	27
UAMS-929	*sarA*	10
UAMS-969	*sarA* (pSARA)	10
Plasmid pSARA	pLI50::*sarA*	12

transcription factor binds this number of sites. Despite the presence of both putative SarA binding sites (7 bp and 26 bp) within the *spa* promoter region, Arvidson and Tegmark have indicated that protein A production is indirectly regulated by SarA, suggesting that it may regulate target gene expression in a previously unrecognized manner (2). The crystal structure of a SarA-DNA complex has been solved and indicates that SarA mediates DNA supercoiling. Based on this, it was suggested that SarA may act like the *Escherichia coli* proteins Fis, integration host factor, H-NS, and HU and function as a global DNA architectural protein that influences DNA superhelicity and transcription rather than a bona fide transcription factor (52).

Indeed, *E. coli* DNA architectural proteins (also known as histone-like proteins) share several similarities with SarA. They are relatively small (~9 kDa) and abundant proteins, some of which also bind AT-rich regions of the chromosome (59). Histone-like proteins also act as pleiotropic regulatory molecules. Their regulatory functions differ from prototypical transcription factors in that they tend to globally regulate gene expression by binding to and altering the topology of gene promoter regions, which subsequently influences transcript synthesis (3, 25, 58). Histone-like proteins also posttranscriptionally modulate gene expression by binding directly to mRNA molecules and influencing transcript stability and translation (4, 13, 22). Based on the similarities between SarA and the histone-like proteins, we hypothesized that SarA may modulate gene expression on the level of both initiation of transcript synthesis and mRNA turnover.

Most prokaryotic studies of mRNA processing and turnover are limited to a few mRNA species within *E. coli* and, to a lesser extent, *Bacillus subtilis*. Those studies have indicated that bacterial mRNAs are generally unstable and undergo complex degradation processes that differ between *E. coli* and *B. subtilis* (reviewed in reference 21). As our entree to defining the molecular mechanism(s) of *S. aureus* mRNA processing and determining whether SarA influences the stability of target transcripts, we used Affymetrix *S. aureus* GeneChips to define the mRNA turnover properties of both wild-type and *sarA* mutant cells. Results indicate that the majority (90%) of all wild-type log-phase transcripts have an mRNA half-life of less than 5 min, whereas other transcripts are more stable, suggesting that *cis*- and/or *trans*-acting elements modulate mRNA turnover within *S. aureus*. In support of this, we found that two virulence factor transcripts, *spa* and *cna*, were stabilized in a *sarA*-dependent manner. Correlations between *sarA*'s mRNA-stabilizing effects and protein production indicate that *sarA* may posttranscriptionally regulate virulence factor production. It was also found that *S. aureus* produces small stable mRNA molecules, with no obvious open reading frame.

TABLE 2. Sequences of oligonucleotides used in PCR, Lightcycler, or Northern blotting reactions in this study

Primer or probe	Oligonucleotide sequence (5′→3′)
Lightcycler	
spa-F	CAGATAACAAATTAGCTGATAAAAACAT
spa-R	CTAAGGCTAATGATAATCCACCAAATAC
cna-F	AACGAACAAGTATACACCAGGAGAG
cna-R	TTTGCTTTTTCATCTAATCCTGTC
16SrRNA-F	ACACAGTCTGAGATGATTGTAGTGTTC
16SrRNA-R	GCTTTCACATCAGACTTAAAAA
SA1278-F	ACACAGTCTGAGATGATTGTAGTGTTC
SA1278-R	ATCGAAAGACTTAGGATATTTCATTGC
gyrA-F	CTGAGCGTAATGGTAATGTTGTATG
gyrA-R	TGCATCTTCTTTTACTTTAGCAACC
dnaA-F	CCAAAAGAAACAACAAAACCTTCTA
dnaA-R	AAACCAACCCCTCCATAGATAAATA
hup-F	CTGGTTCAGCAGTAGATGCTGTATT
hup-R	ATCTTTTAATGCTTTACCAGCTTTG
purH-F	ATCAAGAAGTATTGACGCGATTAAG
purH-R	GATTGTTGTGGATTTTCTCCATATC
PCR	
spa-F	CATACAGGGGGTATTAATTTGAAAA
spa-R	AGTAGAAAGTGTTGAGGCGTTTCAG
16SrRNA-F	TTTTATGGAGAGTTTGATCCTGGCTC
16SrRNA-R	ATATCCTTAGAAAGGAGGTGATCCAG
Northern blotting	
WAN014GIY	CCTGATACACATCTTTCTACGTGTG
cna-F	AACGAACAAGTATACACCAGGAGAG

MATERIALS AND METHODS

Bacterial strains and plasmids. Bacterial strains and plasmids used in this study are listed in Table 1. UAMS-1 is a well-characterized methicillin-susceptible clinical osteomyelitis isolate.

Sampling, RNA isolation, and GeneChip analysis. Overnight cultures of *S. aureus* were diluted 1:100 in 250 ml fresh brain heart infusion (BHI) medium and were incubated at 37°C at 200 rpm with a flask-to-medium volume ratio of 6:1. Once cultures reached mid-log phase (optical density at 600 nm = 0.3 to 0.4), rifampin (200 μg ml^{-1}; Sigma-Aldrich, St. Louis, MO) was added to arrest transcription; Lee and Birkbeck have previously shown that 200 μg ml^{-1} rifampin rapidly and completely blocks *S. aureus* mRNA synthesis (38). Twenty-one milliliters of cells was removed at 0, 5, 15, 30, and 60 min after rifampin treatment. Aliquots were immediately processed for RNA isolation and for monitoring both cell viability and rifampin resistance. More specifically, 20 ml of each aliquot was added to 20 ml ice-cold acetone-ethanol (1:1) and stored at −80°C overnight; 10^{-1} and 10^{-5} dilutions of the remaining 1 ml were plated on BHI-rifampin (200 μg ml^{-1}) and BHI agar, respectively. Plates were incubated overnight at 37°C, and viable numbers of CFU ml^{-1} were calculated to ensure that cell proliferation was halted by the addition of rifampin, suggesting that transcription was arrested. If rifampin-resistant colonies were detected, the experiment was discarded and repeated. For RNA isolation, −80°C suspensions were thawed on ice and centrifuged at 5,000 × *g* at 4°C for 10 min. The supernatant was removed, and the cell pellet was resuspended in 1 ml ice-cold TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) and transferred to a prechilled lysing matrix B tube (Q-BIOgene, Irvine, CA). Cells were lysed by shaking in an FP120 shaker (Q-BIOgene) two times at a setting of 4.5 for 20 s. Suspensions were then centrifuged at 13,000 × *g* at 4°C for 10 min, and the supernatant was used for RNA isolation with an RNeasy Mini column, according to the manufacturer's recommendations for on-the-column DNase treatment and RNA purification (QIAGEN, Valencia, CA). The RNA concentration was determined by spectrophotometry (optical density at 260 nm of 1 = 40 μg RNA ml^{-1}). Any residual DNA contamination was then removed by treatment with 1 U DNase I (Amersham Biosciences, Piscataway, NJ) 10 μg RNA^{-1} at 37°C for 30 min. RNA was then repurified with an RNeasy Mini column, according to the manufacturer's recommendations for RNA clean-up (QIAGEN), and subsequently quantitated by spectrophotometry. In preliminary studies, *S. aureus* rRNA was stable at 60 min post-transcriptional arrest (data not shown). Thus, the integrity of rRNA within each RNA preparation was analyzed by electrophoresis in a 1.2% aga-

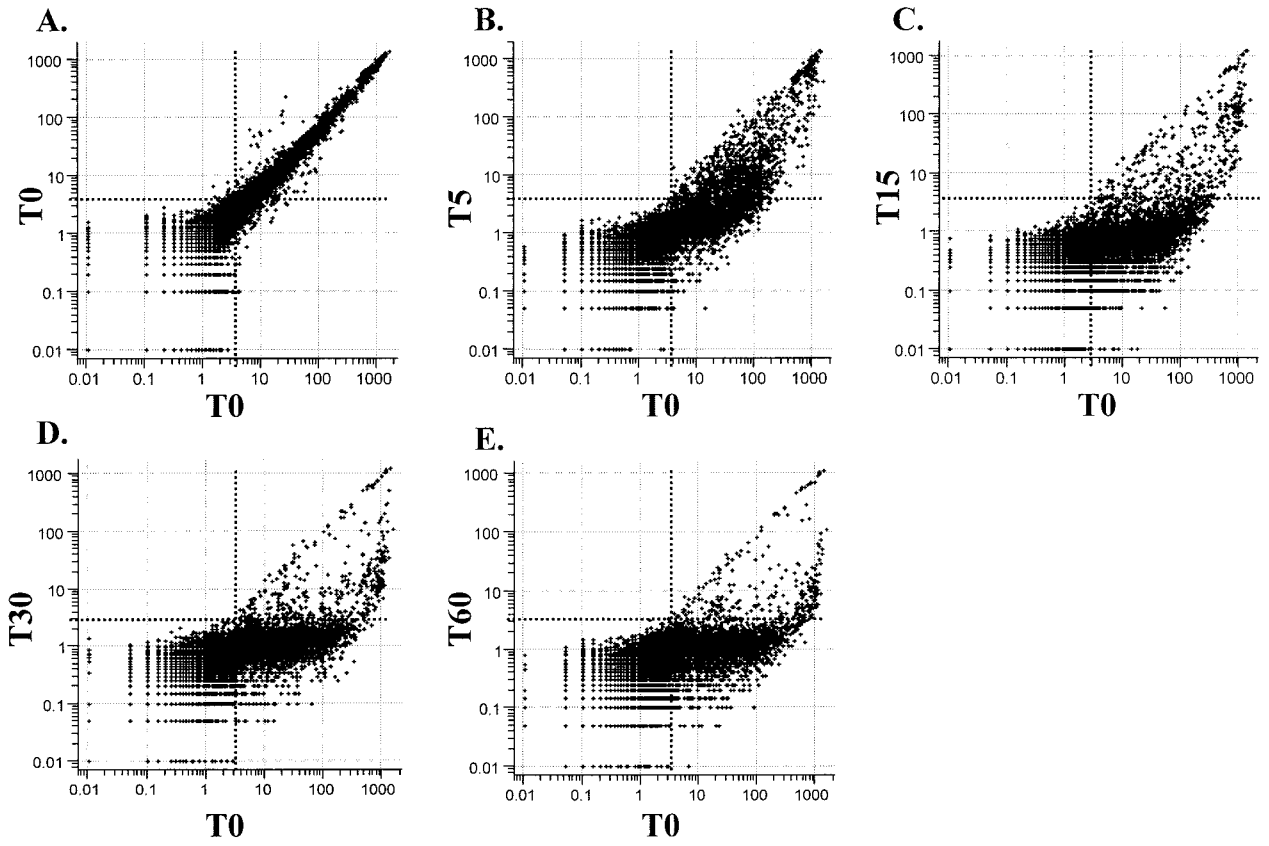

FIG. 1. Degradation profile of *S. aureus* log-phase transcripts. The mRNA signal intensity values for each transcript represented on the GeneChip are plotted (+) at 0 min (T0; *x* axis) and at various time points after rifampin treatment (*y* axis). All measurements are averages of the results from at least two independent experiments. The gray dashed line indicates the level of sensitivity of the system. (A) All GeneChip transcript signals are plotted for T0 samples from two independent experiments, illustrating the reproducibility of the measurements taken. The average transcript signals for each mRNA molecule at T0 are plotted in comparison to the amount of signal detected at 5 min (B), 15 min (C), 30 min (D), and 60 min (E) after rifampin treatment.

rose–0.66 M formaldehyde gel to confirm that RNA preparations were not subjected to contaminating RNase activity during handling. RNA was then reverse transcribed, and cDNA was fragmented, 3′ biotinylated, mixed with exogenous labeled "spike-in" transcripts, and hybridized to *S. aureus* GeneChips by following the manufacturer's recommendations for antisense prokaryotic arrays (Affymetrix, Santa Clara, CA). The *S. aureus* GeneChips (Saur2a) used in this study are second-generation custom-made Affymetrix arrays representing consensus and unique sequences from *S. aureus* strains MRSA252, MSSA476, NCTC 8325, COL, N315, and Mu50 as well as unique GenBank entries and N315 intergenic regions greater than 50 nucleotides (nt) in length (23). GeneChips were washed, stained, and scanned, as previously described (8). Each strain was analyzed at least twice. GeneChip signal intensity values for each qualifier at each time point were then averaged and normalized to spike-in signals using GeneSpring 6.2 software (Silicon Genetics, Redwood City, CA). The half-life of each transcript was calculated as the time point at which the time zero (T0) signal decreased by a factor of 2, as previously described (53).

Real-time PCR. Real-time PCR primers are shown in Table 2. For standard real-time PCRs 25 ng of RNA was reverse transcribed, amplified, and measured using a LightCycler RNA Master SYBR green I kit (Roche Applied Science, Indianapolis, IN), according to the manufacturer's recommendations. As an internal control, 25 pg of RNA was used to quantitate rRNA. Transcript concentrations were calculated using LightCycler software and the LightCycler control cytokine RNA (Roche Applied Science) titration kit as a standard and were then normalized to 16S rRNA abundance. Real-time PCR-determined relative (*n*-fold) degradation of the indicated mRNA species was measured as the difference between the transcript titer at T0 and the time point indicated, following the addition of rifampin.

Northern blotting. Ten micrograms of purified total bacterial RNA (as indicated in text) was run in a 1% (wt/vol) formaldehyde-containing agarose gel at 75 V for 1.5 h. RNA samples were transferred to nylon Hybond-N membranes (Amersham Biosciences) by overnight capillary transfer in 20× SSC (0.3 M Na_3 citrate, 3.0 M NaCl; pH 7.0) (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and were immobilized by UV cross-linking. Oligonucleotide probes (Table 2) for *cna* and GeneChip qualifier WAN014GIY transcripts were 3′ digoxigenin labeled using digoxigenin oligonucleotide 3′-end-labeling kits (Roche Applied Science) according to the manufacturer's recommendations for labeling, determining labeling efficiency, hybridization, and detection steps for Northern blot analysis.

RESULTS

Log-phase mRNA half-lives. *S. aureus* strain UAMS-1 is a well-characterized, highly virulent clinical isolate, and its genetic composition has recently been determined (7, 8, 10, 11, 16). The mRNA half-lives of all transcripts produced during log-phase UAMS-1 and isogenic *sarA* mutant cell growth were measured using *S. aureus* Affymetrix GeneChips (Saur2a). Briefly, each strain was grown to mid-log phase in nutrient-rich media and was treated with rifampin to arrest transcription, as previously described (38). Aliquots were removed at 0, 5, 15, 30, and 60 min post-transcriptional arrest and monitored for rifampin resistance. For experiments in which no resistance was detected, total bacterial RNA was labeled and hybridized to Affymetrix *S. aureus* GeneChips.

TABLE 3. Comparison of GeneChip-determined mRNA half-lives and real-time PCR-determined relative degradation

Gene[a]	Half-life (min)[b]	Fold decrease in mRNA titer at time (min)[c]:			
		5	15	30	60
gyrA	≤5	9.8 (±0.8)	ND	ND	ND
purH	≤5	15.3 (±6.5)	ND	ND	ND
hup	30–60	1.2 (±0.5)	1.8 (±0.3)	7.8 (±2.1)	ND
SA1278	>60	1.4 (±0.2)	1.1 (±0.3)	1.3 (±0.2)	1.3 (±0.2)

[a] *S. aureus* strain N315 common gene name or locus.
[b] GeneChip-determined mRNA half-life.
[c] Real-time PCR-determined relative decrease in mRNA titer following 5, 15, 30, and 60 min of transcriptional arrest. ND, not determined.

The GeneChip signal intensity values for all log-phase transcripts at each sampled time point are shown in Fig. 1. All experiments were repeated at least twice. A comparison of T0 samples from two independent experiments confirmed that the methodology used was reproducible; less than 0.3% of all transcripts demonstrated more than a twofold difference in titer (Fig. 1A). Similar levels of reproducibility were observed when comparing all other sampling times among independent experiments (<0.5% variability; data not shown). Results shown in Fig. 1B to E indicate that transcript signals decrease in a time-dependent manner, confirming that the amount of rifampin used was appropriate to rapidly and completely arrest de novo transcript synthesis. Results also suggest that although most transcripts degrade rapidly, several mRNA molecules appear to be less susceptible to degradation. To study transcript degradation rates in more detail, the mRNA half-life of each transcript was determined as the time point at which the amount of signal detected at time point zero decreased by a factor of two and are listed in Table S1 in the supplemental material. This method of measuring mRNA half-lives has been shown to be extremely accurate by Selinger and colleagues (53).

We have previously found that UAMS-1 DNA hybridizes to 2,775 *S. aureus* GeneChip qualifiers, representing known or predicted *S. aureus* open reading frames (ORFs), as well as intergenic regions greater than 50 bp in length (16). A total of 644 (23.2%) of these 2,775 qualifiers demonstrated background levels of signal intensity prior to the addition of rifampin, suggesting that they were not transcribed at an appreciable level during log-phase growth. Thus, their mRNA half-lives could not be measured, and they are not included in Table S1 in the supplemental material. Many of these genes code for factors that are known to be preferentially expressed during late-log-phase growth, such as all of the members of the intercellular adhesion (*ica*), gamma hemolysin (*hlg*), and serine protease (*spl*) operons (24). A total of 1,910 (89.6%) of the 2,131 measurable log-phase transcripts had an mRNA half-life of less than 5 min. The mRNA half-lives of the remaining 221 transcripts were determined to be as follows: 173 (8.1%) between 5 and 15 min; 29 (1.3%) between 15 and 30 min; 3 (0.1%) between 30 and 60 min; and 16 (0.7%) after 60 min. These results suggest that the stability of *S. aureus* transcripts can be influenced by *cis*-acting elements and/or by *trans*-acting factors. In large part, the degradation rates of genes within an operon coincided with one another.

Transcripts with intermediate levels of stability (half-lives of >5 min but <60 min) included members of operons that could be associated with biological functions. These transcripts included the ATP synthase operon (*atpA*, *atpC*, *atpD*, *atpE*, *atpF*, *atpG*, and *atpH*), heat shock proteins (*groES* and *groEL*), members of the urease complex (*ureAB*, *ureC*, *ureF*, and *ureG*), and 33 genes coding for members of the translation apparatus (see Table S1 in the supplemental material). The mRNA half-lives of several transcripts with either short (*gyrA* and *purH*) or long (*hup* and N315 SA1278) half-lives were confirmed by real-time PCR, indicating that the methodology used was appropriate to measure mRNA turnover (Table 3).

Stable RNA molecules. Sixteen RNA species did not demonstrate any appreciable degradation following 60 min of transcriptional arrest (see Table S1 in the supplemental material). Three of these transcripts are expected to encode proteins with no known function. The remaining 13 transcripts do not contain an obvious open reading frame. Among these are five well-defined RNA molecules, including a serine tRNA, 16S and 23S rRNAs, tmRNA (15), and the RNA component of RNase P (54). Indeed, 16S and 23S rRNAs are generally regarded as stable RNA molecules. tmRNA (also known as SsrA) is an RNA molecule involved in protein tagging and rescuing stalled ribosomes that has been defined as a stable RNA species within other organisms (15). Northern blotting analysis demonstrated that the *S. aureus* tmRNA/*ssrA* locus produces at least 3 RNA species during log-phase growth, each of which appears to be stable for at least 2 h post-transcriptional arrest (Fig. 2), further confirming our GeneChip results. Eight transcripts that map to short (75 to 360 nt) N315 intergenic regions, but with no defined function, were also identified (Fig. 3). Two of these molecules (measured by GeneChip qualifiers WAN01CCBZ and WAN01CCFG-rc) were recently characterized to be transcripts without an obvious putative ORF sequence by Pichon and Felden, although their half-lives were not determined in that study (50). Based on the surrounding genomic content and directionality of transcript synthesis, it is likely that some stable RNA molecules are cotranscribed as part of an operon, whereas others are more likely to behave as antisense RNA molecules. For instance, the RNA component of RNase P holoenzyme, a protein/RNA complex that matures tRNA molecules, was measured by GeneChip qualifier WAN01CC66-rc and determined to be stable. Interestingly, a transcript mapping to the opposite strand was also determined to be stable (Fig. 3) (measured by GeneChip qualifier WAN01CC66). One can imagine a scenario whereby the latter RNA molecule may behave as an antisense molecule and regulate RNase P function. Indeed, the importance of the *S. aureus agr*-encoded RNAIII molecule (48) and small stable

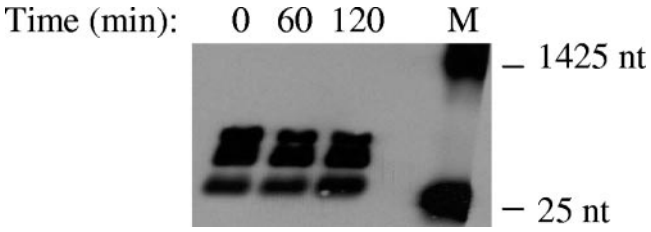

FIG. 2. Transcript degradation profile of small stable RNA molecules. Northern blotting results of small stable RNA molecules at 0, 60, and 120 min following transcriptional arrest (shown across top). M, molecular size markers.

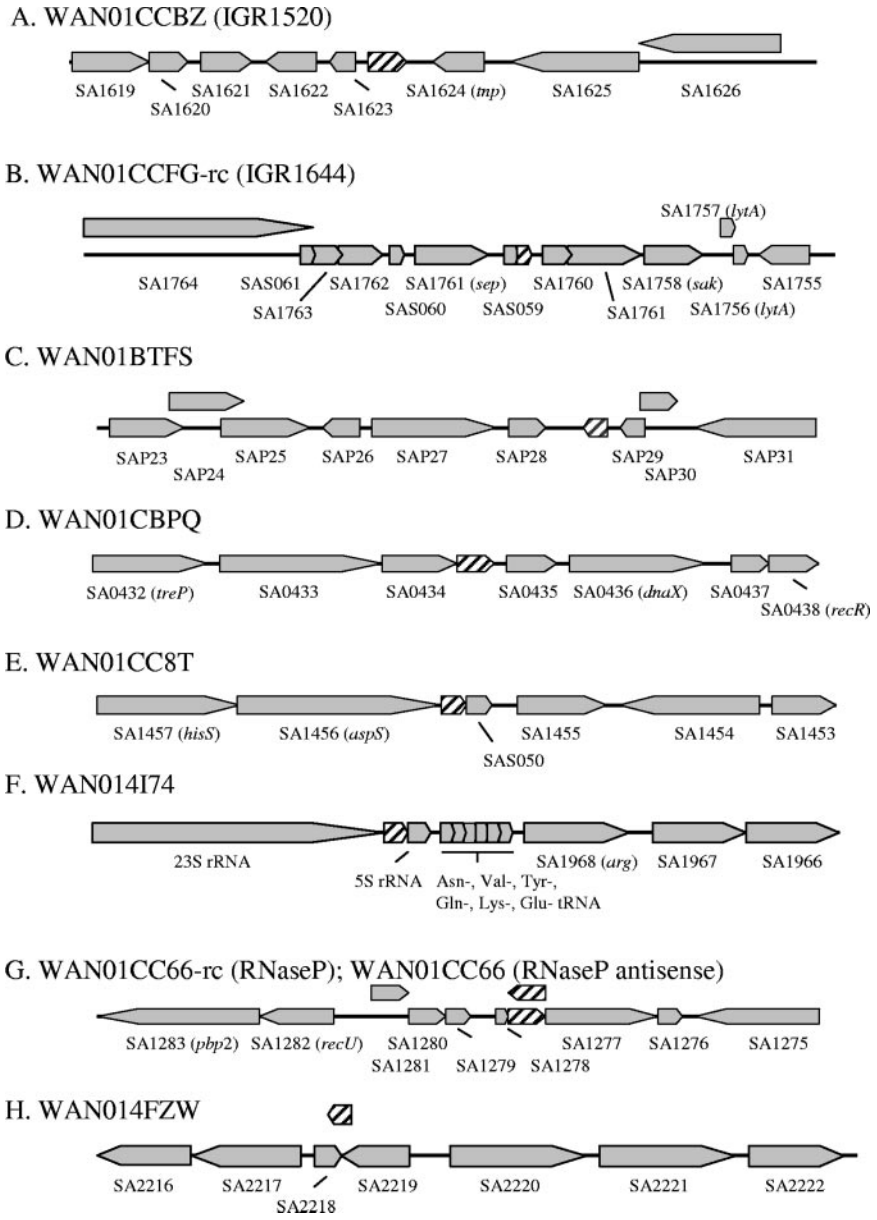

FIG. 3. Chromosomal composition adjacent to UAMS-1 log-phase stable RNA transcripts. Shown is the *S. aureus* strain N315 chromosomal map position of each small stable UAMS-1 RNA locus. The GeneChip qualifier name and corresponding transcript name, if known, are given in parentheses. IGR1520 and IGR1624 correspond to transcripts and corresponding nomenclature previously described by Pichon and Felden (50). Orientations of ORFs (gray arrows) and stable transcript coding regions (striped arrows) are indicated.

RNAs within other pathogens (33) makes it likely that many of the stable transcripts identified here may play an important role(s) in *S. aureus* biological processes.

***sarA* influences *cna* and *spa* transcript stability.** Because SarA is an abundant protein with promiscuous binding characteristics that can alter DNA topology, we hypothesized that the protein may, in part, act as a DNA-structuring protein. Indeed, the *Escherichia coli* histone-like protein integration host factor is an abundant DNA-binding protein which, like SarA, binds to AT-rich regions of the chromosome (59). Moreover, histone-like proteins, such as HU and H-NS, can bind to mRNA molecules in a manner that alters their stability and, in doing so, leads to the posttranscriptional regulation of target genes (4, 13, 22). Accordingly, the mRNA stability of log-phase transcripts produced within UAMS-1 (*sarA*) cells were determined and are included in Table S1 in the supplemental material.

In large part, the mRNA half-lives of transcripts produced in *sarA* cells matched those of wild-type cells, demonstrating the reproducibility of the GeneChip-based measurements. However, 138 mRNA molecules, including collagen-binding protein (*cna*) and protein A (*spa*) virulence factor transcripts, were found to have longer half-lives in wild-type than in *sarA* mutant cells, suggesting that they are stabilized in a *sarA*-dependent manner (Table 4). Nearly the complete set of UAMS-1 transcripts with intermediate level stability was more

TABLE 4. Log-phase transcripts with differential degradation properties within UAMS-1 and isogenic *sarA* mutant cells

Qualifier[a]	Common	Half-life (min) of[b]: WT[e]	Half-life (min) of[b]: *sarA* mutants	Locus[c]	Description
Amino acid transport and metabolism					
WAN014HF5_at	*gcvH*	15	5	SA0760	Glycine cleavage system protein H
WAN014I0A_at	*glyA*	15	5	SA1915	Serine hydroxymethyl transferase
WAN014G7C_at	*ureAB*	15	5	SA2083	Urease beta subunit
WAN014G7E_at	*ureC*	15	5	SA2084	Urease alpha subunit
Carbohydrate transport and metabolism					
WAN014HDZ_at	*eno*	15	5	SA0731	Enolase
WAN014I16_at	*fba*	15	5	SA1927	Fructose-bisphosphate aldolase
WAN014GEN_at	*fda*	15	5	SA2399	Fructose-bisphosphate aldolase homolog
WAN014HDR_at	*gap*	15	5	SA0727	Glyceraldehyde-3-phosphate dehydrogenase
WAN014HKV_at	*pgi*	15	5	SA0823	Glucose-6-phosphate isomerase A
WAN014HDT_at	*pgk*	15	5	SA0728	Phosphoglycerate kinase
WAN014HDX_at	*pgm*	15	5	SA0730	2, 3-Diphosphoglycerate-independent phosphoglycerate mutase
WAN014HSG_at	*ptsH*	15	5	SA0934	Phophocarrier protein HPR
WAN014HDV_at	*tpiA*	15	5	SA0729	Triosephosphate isomerase
WAN014H3R_at		15	5	SA0528	Similar to hexulose-6-phosphate synthase
WAN014G6N_at		15	5	SA2053	Glucose uptake protein homolog
Energy production and conversion					
WAN014HZV_at	*atpA*	15	5	SA1907	ATP synthase alpha chain
WAN014HZJ_at	*atpC*	15	5	SA1904	F_oF_1-ATP synthase epsilon subunit
WAN014HZO_at	*atpD*	15	5	SA1905	ATP synthase beta chain
WAN014I01_at	*atpE*	15	5	SA1910	ATP synthase C chain
WAN014HZZ_at	*atpF*	15	5	SA1909	ATP synthase B chain
WAN014HZQ_at	*atpG*	15	5	SA1906	ATP synthase gamma chain
WAN014HZX_at	*atpH*	15	5	SA1908	ATP synthase delta chain
WAN014HTB_at	*pdhA*	15	5	MW0976	Pyruvate dehydrogenase complex
WAN014HTC_at	*pdhB*	15	5	SA0944	Pyruvate dehydrogenase E1 component beta subunit
WAN014HTE_at	*pdhC*	15	5	SA0945	Dihydrolipoamide acetyltransferase
WAN014HTG_at	*pdhD*	15	5	SA0946	Dihydrolipoamide dehydrogenase
WAN014GTJ_at	*qoxA*	30	5	SA0913	Similar to quinol oxidase polypeptide II QoxA
WAN014GTH_at	*qoxB*	30	5	SA0912	Quinol oxidase polypeptide I
WAN014GTE_at	*qoxC*	15	5	SA0911	Quinol oxidase polypeptide III
WAN014GVS_at		15	5	SA0367	Similar to nitro/flavin reductase
WAN014GTD_at		15	5	SA0910	Similar to quinol oxidase polypeptide IV QoxD
WAN014G5B_at		15	5	SA2311	Similar to NAD(P)H-flavin oxidoreductase
General transport					
WAN014I5X_at	*adk*	15	5	SA2027	Adenylate kinase
WAN014GDJ_at	*copP*	15	5	SA2345	Similar to mercuric ion-binding protein
WAN014GTL_at	*folD*	30	5	SA0915	FolD bifunctional protein
WAN014I1K_at	*pyn*	15	5	SA1938	Pyrimidine nucleoside phosphorylase
WAN014I5Z_at	*secY*	15	5	SA2028	Preprotein translocase SecY subunit
WAN014H6Q_at	*sodA*	15	5	SA1382	Superoxide dismutase SodA
WAN014I08_at	*upp*	15	5	SA1914	Uracil phosphoribosyl transferase
WAN014H1C_at		15	5	SA0477	Conserved hypothetical protein
WAN014H1E_at		15	5	SA0478	Conserved hypothetical protein
WAN014HF3_at		15	5	SA0759	Similar to arsenate reductase
Miscellaneous					
WAN014I20_at	*acpP*	15	5	SA1075	Acyl carrier protein
WAN01BPWU_x_at	*cspD*	15	5	SA1234	Major cold shock protein CspA
WAN014GCU_at	*ddh*	15	5	SA2312	D-Specific D-2-hydroxyacid dehydrogenase
WAN014GWS_at	*dmpI*	15	5	SAS044	4-Oxalocrotonate tautomerase
WAN014H1M_at	*hup*	60	5	SA1305	DNA-binding protein II (HB)
WAN014I5M_at	*rpoA*	15	5	SA2023	DNA-directed RNA polymerase alpha chain
WAN014GVD_at	*ssb*	15	5	SA0353	Single-stranded DNA-binding protein
WAN014I06_at	*wecB*	15	5	SA1913	UDP-GlcNAc 2-epimerase
WAN014H7P_at		15	5	SA0182	Similar to indole-3-pyruvate decarboxylase
WAN014H3T_at		15	5	SA0529	Conserved hypothetical protein
WAN014FY9_at		30	5	SA1528	Conserved hypothetical protein

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TABLE 4—*Continued*

Qualifier[a]	Common	Half-life (min) of[b]:		Locus[c]	Description
		WT[e]	*sarA* mutants		
WAN014GJ0_at		15	5		tRNA-Ser
WAN014GJ6_at		15	5		tRNA-Gly
WAN014GIY_at		Stable	5		Unknown RNA
Posttranslational modification, protein turnover, chaperones					
WAN014GAE_at	*ahpC*	15	5	SA0366	Alkyl hydroperoxide reductase subunit C
WAN014G9N_at	*ahpF*	15	5	SA0365	Alkyl hydroperoxide reductase subunit F
WAN014HVE_at	*groEL*	15	5	SA1836	GroEL protein
WAN014HVI_at	*groES*	15	5	SA1837	GroES protein
WAN014G7I_at	*ureF*	15	5	SA2086	Urease accessory protein UreF
WAN014G7K_at	*ureG*	15	5	SA2087	Urease accessory protein UreG
WAN014H7X_at		15	5	SA1403	Conserved hypothetical protein
Translation					
WAN014H5H_at	*efp*	15	5	SA1359	Translation elongation factor EF-P
WAN014H2U_at	*fusA*	15	5	SA0505	Translational elongation factor G
WAN014I5V_at	*infA*	15	5	SA2026	Translation initiation factor IF-1
WAN014H2C_at	*rplA*	15	5	SA0496	50S ribosomal protein L1
WAN014I6G_at	*rplE*	15	5	SA2035	50S ribosomal protein L5
WAN014I69_at	*rplF*	15	5	SA2033	50S ribosomal protein L6
WAN014H29_at	*rplK*	15	5	SA0495	50S ribosomal protein L11
WAN014I6K_at	*rplN*	15	5	SA2037	50S ribosomal protein L14
WAN014I61_at	*rplO*	15	5	SA2029	50S ribosomal protein L15
WAN014I6S_at	*rplP*	15	5	SA2040	50S ribosomal protein L16
WAN014I5K_at	*rplQ*	15	5	SA2022	50S ribosomal protein L17
WAN014I67_at	*rplR*	15	5	SA2032	50S ribosomal protein L18
WAN014I2L_at	*rplS*	15	5	SA1084	50S ribosomal protein L19
WAN014I6I_at	*rplX*	15	5	SA2036	50S ribosomal protein L24
WAN014A7W-5_at	*rplY*	15	5	SA0459	50S ribosomal protein L25
WAN014GVM_at	*rpmB*	15	5	SA1067	50S ribosomal protein L28
WAN014I6O_at	*rpmC*	15	5	SA2039	50S ribosomal protein L29
WAN014I63_at	*rpmD*	15	5	SA2030	50S ribosomal protein L30
WAN014FT7_at	*rpmG*[d]	30	5	SAS042	50S ribosomal protein L33
WAN014H6M_at	*rpmG*[d]	15	5	SAS047	50S ribosomal protein L33
WAN014H1V_at	*rpsA*	15	5	SA1308	30S ribosomal protein S1
WAN014I65_at	*rpsE*	15	5	SA2031	30S ribosomal protein S5
WAN014A7X-3_at	*rpsF*	15	5	SA0352	30S ribosomal protein S6
WAN014A7X-5_at	*rpsF*	15	5	SA0352	30S ribosomal protein S6
WAN014H2S_at	*rpsG*	15	5	SA0504	30S ribosomal protein S7
WAN014I6B_at	*rpsH*	15	5	SA2034	30S ribosomal protein S8
WAN014I5O_at	*rpsK*	15	5	SA2024	30S ribosomal protein S11
WAN014H2Q_at	*rpsL*	15	5	SA0503	30S ribosomal protein S12
WAN014I5Q_at	*rpsM*	15	5	SA2025	30S ribosomal protein S13
WAN014I6D_at	*rpsN*	15	5	SAS079	30S ribosomal protein S14
WAN014I6L_at	*rpsQ*	15	5	SA2038	30S ribosomal protein S17
WAN014FXU_at	*rpsT*	15	5	SA1414	30S ribosomal protein S20
WAN014A7V-3_at	*tuf*[d]	15	5	SA0506	Translational elongation factor TU
WAN014A7V-5_at	*tuf*[d]	30	5	SA0506	Translational elongation factor TU
WAN014A7V-M_at	*tuf*[d]	30	5	SA0506	Translational elongation factor TU
WAN014H2O_at		15	5	SA0502	Similar to ribosomal protein
Unknown					
WAN014HTM_at	*ccoS*	15	5	SAS056	Hypothetical protein
WAN014HAL_at	*csbD*	15	5	SA1452	σ^B-controlled gene product
WAN014G0U_at	*hit*	15	5	SA1656	Hit-like protein
WAN014GSG_at		15	5	MW0922	Hypothetical protein
WAN014G09_at		30	5	MWP025	Hypothetical protein
WAN014HY6_at		15	5	SA0269	Hypothetical protein
WAN014GN8_at		60	5	SA0271	Conserved hypothetical protein
WAN014GPI_at		15	5	SA0295	Similar to outer membrane protein precursor
WAN014GVL_at		15	5	SA0359	Conserved hypothetical protein
WAN014H31_at		15	5	SA0509	Conserved hypothetical protein
WAN014IV8_at		15	5	SA0889	Hypothetical protein

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TABLE 4—*Continued*

Qualifier[a]	Common	Half-life (min) of[b]: WT[e]	Half-life (min) of[b]: *sarA* mutants	Locus[c]	Description
WAN014GNH_at		Stable	15	SA1278	Hypothetical protein
WAN014H3P_at		15	5	SA1345	Conserved hypothetical protein
WAN014H3N_at		15	5	SA1344	Conserved hypothetical protein
WAN014H7T_at		15	5	SA1401	Conserved hypothetical protein
WAN014H7V_at		15	5	SA1402	Conserved hypothetical protein
WAN014HGB_x_at		15	5	SA1559	Similar to smooth muscle caldesmon
WAN014G2Y_at		15	5	SA1743	Hypothetical protein
WAN014GKY_at		15	5	SA1754	Hypothetical protein (bacteriophage phiN315)
WAN014HUU_at		30	5	SA1768	Hypothetical protein (bacteriophage phiN315)
WAN014I4W_at		30	5	SA1770	Hypothetical protein (bacteriophage phiN315)
WAN014HUY_at		30	5	SA1771	Hypothetical protein (bacteriophage phiN315)
WAN014HV0_at		30	5	SA1774	Hypothetical protein (bacteriophage phiN315)
WAN014I0C_at		15	5	SA1916	Conserved hypothetical protein
WAN014I3J_at		15	5	SA1985	Hypothetical protein
WAN014G6J_at		15	5	SA2049	Hypothetical protein
WAN014GD4_at		Stable	5	SA2331	Hypothetical protein
WAN014GED_at		15	5	SA2378	Conserved hypothetical protein
WAN014HSP_at		30	5	SAR0694	Hypothetical protein
WAN014IOE_at		15	5	SAR2779	Putative *N*-acetyltransferase
WAN014IVJ_at		Stable	5	SAS059	Hypothetical protein (bacteriophage phiN315)
WAN014IOC_at		15	5	SAV2383	Hypothetical protein SAV2383
WAN014FZW_at		Stable	5		Antisense
Virulence factors					
WAN014HM2_at	*cna*	15	5	MW2612	Collagen adhesin protein
WAN014IPY_at	*fib*	15	5	SAR1130	Fibrinogen-binding protein precursor
WAN014ITO_s_at	*spa*	30	5	SA0107	Immunoglobulin G binding protein A
WAN014G2F_at	*sspB*	15	5	SA1725	Staphopain
WAN014HM5_at		15	5	SA0841	Similar to cell surface protein Map-w
WAN014HGC_at		15	5	SAR1816	Putative membrane protein

[a] Affymetrix *S. aureus* GeneChip (Saur2a) descriptive representing indicated predicted ORF or intergenic region.
[b] Transcript half-life in min; 5 indicates <5 min, 15 indicates between 5 and 15 min, 30 indicates between 15 and 30 min, 60 indicates between 30 and 60 min, and stable indicates >60 min.
[c] *S. aureus* strain N315 loci. Genes not contained within strain N315 but present in other sequenced *S. aureus* strains are indicated. SAV, MW, SAS, SAR, and SACOL preceeding a locus number correspond to Mu50, MW2, MSSA 476, MRSA 252, and COL loci.
[d] Gene regions are represented separately and independently on the GeneChip.
[e] WT, wild type.

rapidly degraded in the *sarA* mutant background. Conversely, seven transcripts were determined to be destabilized in a *sarA*-dependent manner and are highlighted in Table S1 in the supplemental material. Because SarA has been shown to influence Spa and Cna production, we focused on characterizing this phenomenon in more detail.

Using real-time PCR, we confirmed that both *cna* and *spa* transcripts are more stable within wild-type cells than in *sarA* mutant cells. As shown in Table 5, *cna* and *spa* transcript titers decreased 2.6- and 2.7-fold, respectively, following 10 min of transcriptional arrest within wild-type cells. Within isogenic *sarA* mutant cells, the relative decrease in transcript abundance was >2,000- and 66.6-fold for *spa* and *cna* mRNA, respectively. In the case of *spa* mRNA, this relative degradation is not an accurate measurement. The reason for this is that, despite measuring high titers of *spa* transcript at the time of transcriptional arrest (T0), *spa* mRNA was undetectable at 10 min after rifampin treatment. Transcript stability could be restored to *sarA* mutant cells by complementation by a low-copy-number plasmid capable of producing the *sarA* transcriptional unit via its endogenous promoter, confirming that this phenomenon is due to the presence of the *sarA* locus as opposed to another previously unrecognized characteristic of the strain background. Moreover, the observed *sarA*-mediated change in mRNA stability was specific to *spa* and *cna* transcripts; *S. aureus* N315-SA1278 transcripts were unaffected by the presence or absence of the *sarA* locus.

TABLE 5. Relative degradation of *spa* and *cna* transcripts

Strain	Fold mRNA degradation of[a]: *spa*	*cna*	SA1278
UAMS-1	2.7 (±1.1)	2.6 (±1.7)	1.0 (±0.8)
UAMS-929 (*sarA*)	2,405.4 (±486.5)	66.6 (±9.8)	1.0 (±0.7)
UAMS-969 (*sarA*; pSARA)	2.8 (±1.8)	2.6 (±1.5)	ND

[a] Real-time PCR was used to determine the relative decrease in *spa*, *cna*, and strain N315 locus SA1278 transcript titers following 10 min of transcriptional arrest. ND, not determined.

DISCUSSION

Modulating mRNA maturation and degradation are recognized means of regulating bacterial gene expression. Although

these processes have been well characterized in *E. coli*, and to a lesser extent *B. subtilis*, little is known about mRNA processing within staphylococci. In this work, we determined the *S. aureus* mRNA half-lives of log-phase transcripts as a first step toward understanding the organism's transcript turnover properties.

S. aureus mRNA molecules appear to undergo differential rates of degradation, suggesting that there may be previously unrecognized *cis*-acting mRNA elements or *trans*-acting factors that influence mRNA processing. It is intriguing to consider (i) what elements confer stability to these molecules, (ii) whether *trans*-acting factors temporally regulate transcript stability, and if so, (iii) whether the modulation of mRNA stability correlates with a previously unappreciated level of regulation within *S. aureus*. It seems plausible that modulating mRNA turnover is an efficient and dynamic means of posttranscriptional gene regulation that allows *S. aureus* to respond rapidly to endogenous and exogenous signals. Indeed, a number of other bacterial pathogens produce mRNA-binding proteins which influence virulence factor mRNA turnover and protein production. For instance, *E. coli* produces an RNA-binding protein, CsrA, that influences biofilm formation by altering *pgaABCD* transcript stability and, consequently, poly-B-1,6-*N*-acetyl-D-glucosamine production (60). CsrA homologs have been shown to influence virulence factor production in *Salmonella enterica* serovar Typhimurium, *Erwinia carotovora* subsp. *carotovora*, and *Pseudomonas aeruginosa* (1, 32, 49). Although we have not identified a CsrA homolog within *S. aureus*, the finding that at least two virulence factor transcripts, *cna* and *spa*, are stabilized in a *sarA*-dependent manner suggests that *trans*-acting factors do modulate virulence factor mRNA turnover within *S. aureus* and may represent a previously unappreciated mechanism of regulation within this organism. We are currently evaluating whether this change in stability correlates to changes in protein abundance.

Although it was found that the *sarA* locus stabilizes *spa* and *cna* virulence factor transcripts, it is possible that this phenomenon is more global than initially observed. There are two main reasons for this. First, we measured the mRNA stability of log-phase cells. Many UAMS-1 virulence factors (and other genes) are preferentially transcribed during late-log-phase growth; thus, they were not expressed at our initial sampling time and could not be evaluated. Second, most virulence factors that were transcribed during log-phase growth decayed before the earliest post-transcriptional arrest measurement (5 min), making it difficult to discern whether *sarA* influenced the stability of those transcripts (a transcript's half-life might be 30 s in a *sarA* mutant but 3 min in wild-type cells).

The finding that *spa* and *cna* transcripts are stabilized in a *sarA*-dependent manner was unexpected. We anticipated that *sarA*'s effect, if any, would be to accelerate the degradation rate of these two transcripts. The reason for this is that both collagen-binding protein and protein A production are repressed in a *sarA*-dependent manner. Accordingly, we hypothesized that SarA may lower the pool of target mRNA molecules available for translation via two mechanisms, repressing transcript synthesis and accelerating mRNA degradation of any basally produced transcripts. However, we found the opposite to be the case; these transcripts were stabilized by *sarA*. Although our initial hypothesis was incorrect, our observations could be explained by other scenarios. Because *sarA* is a negative regulator of Spa and Cna production, it is quite possible that it may act to both repress transcript synthesis and stabilize any transcripts that are basally produced in a manner that interferes with translation and delays degradation (degradosome accessibility). Indeed, within *B. subtilis* the 5′ end of a transcript not only serves as a potential entry point for the degradosome but also is the entry point for ribosomes during translation. Moreover, the binding of regulatory proteins and ribosome stalling within this region has been shown to increase *B. subtilis* mRNA stability (6, 28, 29). Accordingly, it is easy to imagine that SarA or a SarA-regulated molecule might bind this region of a transcript in a manner that simultaneously influences translation and degradation. Thus, there may be multiple layers by which *sarA* modulates gene expression. Alternatively, *sarA* may alter the transcriptional start site of affected transcripts in a manner that provides a 5′ stabilizing structure. Admittedly, we are in the initial stages of testing this hypothesis; nonetheless, these studies may have expanded significance in that SarA represents a prototypical regulatory molecule with a multitude of homologues within *S. aureus* and other bacterial pathogens.

Our results also indicate that *S. aureus* is capable of producing a set of extremely stable RNA molecules (half-life > 60 min) with no predicted open reading frame during log-phase growth. These transcripts were detected by GeneChip features representing intergenic regions of the *S. aureus* N315 genome and, based on size predictions, are expected to be ~75 to 300 nt in length. Further analysis indicates that none of these transcripts maps to an ORF within any of the publicly available *S. aureus* genomes. Thus, many, if not all, of these RNA species may represent short, noncoding RNAs (also known as microRNAs and short interfering RNAs), which regulate essential processes within eukaryotic cells as well as stress responses and pathogenicity factors within bacteria. Because the GeneChip used in these studies contains oligonucleotides representing segments of these stable RNA molecules, we cannot accurately determine the full sequence of each species. Studies are under way to better characterize each of these molecules and determine whether they influence biological processes within *S. aureus*.

ACKNOWLEDGMENTS

We thank Christine Reekie for technical assistance with tables and figures.

This work is partially supported by American Heart Association grant 0535037N to P.M.D. K.L.A. was supported by a University of Nebraska Medical Center Assistantship award.

REFERENCES

1. **Altier, C., M. Suyemoto, and S. D. Lawhon.** 2000. Regulation of *Salmonella enterica* serovar Typhimurium invasion genes by *csrA*. Infect. Immun. **68:** 6790–6797.
2. **Arvidson, S., and K. Tegmark.** 2001. Regulation of virulence determinants in Staphylococcus aureus. Int. J. Med. Microbiol. **291:**159–170.
3. **Auner, H., M. Buckle, A. Deufel, T. Kutateladze, L. Lazarus, R. Mavathur, G. Muskhelishvili, I. Pemberton, R. Schneider, and A. Travers.** 2003. Mechanism of transcriptional activation by FIS: role of core promoter structure and DNA topology. J. Mol. Biol. **331:**331–344.
4. **Balandina, A., L. Claret, R. Hengge-Aronis, and J. Rouviere-Yaniv.** 2001. The Escherichia coli histone-like protein HU regulates rpoS translation. Mol. Microbiol. **39:**1069–1079.
5. **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.
6. **Bechhofer, D. H., and D. Dubnau.** 1987. Induced mRNA stability in Bacillus subtilis. Proc. Natl. Acad. Sci. USA **84:**498–502.

7. **Beenken, K. E., J. S. Blevins, and M. S. Smeltzer.** 2003. Mutation of *sarA* in *Staphylococcus aureus* limits biofilm formation. Infect. Immun. **71:**4206–4211.
8. **Beenken, K. E., P. M. Dunman, F. McAleese, D. Macapagal, E. Murphy, S. J. Projan, J. S. Blevins, and M. S. Smeltzer.** 2004. Global gene expression in *Staphylococcus aureus* biofilms. J. Bacteriol. **186:**4665–4684.
9. **Bernardo, K., N. Pakulat, S. Fleer, A. Schnaith, O. Utermohlen, O. Krut, S. Muller, and M. Kronke.** 2004. Subinhibitory concentrations of linezolid reduce *Staphylococcus aureus* virulence factor expression. Antimicrob. Agents Chemother. **48:**546–555.
10. **Blevins, J. S., K. E. Beenken, M. O. Elasri, B. K. Hurlburt, and M. S. Smeltzer.** 2002. Strain-dependent differences in the regulatory roles of *sarA* and *agr* in *Staphylococcus aureus*. Infect. Immun. **70:**470–480.
11. **Blevins, J. S., M. O. Elasri, S. D. Allmendinger, K. E. Beenken, R. A. Skinner, J. R. Thomas, and M. S. Smeltzer.** 2003. Role of *sarA* in the pathogenesis of *Staphylococcus aureus* musculoskeletal infection. Infect. Immun. **71:**516–523.
12. **Blevins, J. S., A. F. Gillaspy, T. M. Rechtin, B. K. Hurlburt, and M. S. Smeltzer.** 1999. The Staphylococcal accessory regulator (sar) represses transcription of the Staphylococcus aureus collagen adhesin gene (cna) in an agr-independent manner. Mol. Microbiol. **33:**317–326.
13. **Brescia, C. C., M. K. Kaw, and D. D. Sledjeski.** 2004. The DNA binding protein H-NS binds to and alters the stability of RNA in vitro and in vivo. J. Mol. Biol. **339:**505–514.
14. **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.
15. **Cairrao, F., A. Cruz, H. Mori, and C. M. Arraiano.** 2003. Cold shock induction of RNase R and its role in the maturation of the quality control mediator SsrA/tmRNA. Mol. Microbiol. **50:**1349–1360.
16. **Cassat, J. E., P. M. Dunman, F. McAleese, E. Murphy, S. J. Projan, and M. S. Smeltzer.** 2005. Comparative genomics of *Staphylococcus aureus* musculoskeletal isolates. J. Bacteriol. **187:**576–592.
17. **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 Immunol. Med. Microbiol. **40:**1–9.
18. **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.
19. **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.
20. **Chien, Y., 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.
21. **Condon, C.** 2003. RNA processing and degradation in *Bacillus subtilis*. Microbiol. Mol. Biol Rev. **67:**157–174.
22. **Deighan, P., A. Free, and C. J. Dorman.** 2000. A role for the Escherichia coli H-NS-like protein StpA in OmpF porin expression through modulation of micF RNA stability. Mol. Microbiol. **38:**126–139.
23. **Dunman, P. M., W. Mounts, F. McAleese, F. Immermann, D. Macapagal, E. Marsilio, L. McDougal, F. C. Tenover, P. A. Bradford, P. J. Petersen, S. J. Projan, and E. Murphy.** 2004. Uses of *Staphylococcus aureus* GeneChips in genotyping and genetic composition analysis. J. Clin. Microbiol. **42:**4275–4283.
24. **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.
25. **Dworkin, J., A. J. Ninfa, and P. Model.** 1998. A protein-induced DNA bend increases the specificity of a prokaryotic enhancer-binding protein. Genes Dev. **12:**894–900.
26. **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.
27. **Gillaspy, A. F., S. G. Hickmon, R. A. Skinner, J. R. Thomas, C. L. Nelson, and M. S. Smeltzer.** 1995. Role of the accessory gene regulator (*agr*) in pathogenesis of staphylococcal osteomyelitis. Infect. Immun. **63:**3373–3380.
28. **Glatz, E., R. P. Nilsson, L. Rutberg, and B. Rutberg.** 1996. A dual role for the Bacillus subtilis glpD leader and the GlpP protein in the regulated expression of glpD: antitermination and control of mRNA stability. Mol. Microbiol. **19:**319–328.
29. **Glatz, E., M. Persson, and B. Rutberg.** 1998. Antiterminator protein GlpP of Bacillus subtilis binds to glpD leader mRNA. Microbiology **144:**449–456.
30. **Ingavale, S., W. van Wamel, T. T. Luong, C. Y. Lee, and A. L. Cheung.** 2005. Rat/MgrA, a regulator of autolysis, is a regulator of virulence genes in *Staphylococcus aureus*. Infect. Immun. **73:**1423–1431.
31. **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.
32. **Johansson, J., and P. Cossart.** 2003. RNA-mediated control of virulence gene expression in bacterial pathogens. Trends Microbiol. **11:**280–285.
33. **Julio, S. M., D. M. Heithoff, and M. J. Mahan.** 2000. *ssrA* (tmRNA) plays a role in *Salmonella enterica* serovar Typhimurium pathogenesis. J. Bacteriol. **182:**1558–1563.
34. **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.
35. **Kornblum, J., B. N. Kreiswirth, S. J. Projan, H. Ross, and R. P. Novick.** 1990. Agr: a polycistronic locus regulating exoprotein synthesis in Staphylococcus aureus, p. 373–402. *In* R. P. Novick (ed.), Molecular biology of the staphylococci. VCH Publishers, New York, N.Y.
36. **Kupferwasser, L. I., M. R. Yeaman, C. C. Nast, D. Kupferwasser, Y. Q. Xiong, M. Palma, A. L. Cheung, and A. S. Bayer.** 2003. Salicylic acid attenuates virulence in endovascular infections by targeting global regulatory pathways in Staphylococcus aureus. J. Clin. Investig. **112:**222–233.
37. **Kuroda, M., H. Kuroda, T. Oshima, F. Takeuchi, H. Mori, and K. Hiramatsu.** 2003. Two-component system VraSR positively modulates the regulation of cell-wall biosynthesis pathway in Staphylococcus aureus. Mol. Microbiol. **49:**807–821.
38. **Lee, K. Y., and T. H. Birkbeck.** 1984. In vitro synthesis of the delta-lysin of *Staphylococcus aureus*. Infect. Immun. **44:**434–438.
39. **Lindsay, J. A., and M. T. Holden.** 2004. Staphylococcus aureus: superbug, super genome? Trends Microbiol. **12:**378–385.
40. **Manna, A., and A. L. Cheung.** 2001. Characterization of *sarR*, a modulator of *sar* expression in *Staphylococcus aureus*. Infect. Immun. **69:**885–896.
41. **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.
42. **Manna, A. C., and A. L. Cheung.** 2003. *sarU*, a *sarA* homolog, is repressed by SarT and regulates virulence genes in *Staphylococcus aureus*. Infect. Immun. **71:**343–353.
43. **Manna, A. C., S. S. Ingavale, M. Maloney, W. van Wamel, and A. L. Cheung.** 2004. Identification of *sarV* (SA2062), a new transcriptional regulator, is repressed by SarA and MgrA (SA0641) and involved in the regulation of autolysis in *Staphylococcus aureus*. J. Bacteriol. **186:**5267–5280.
44. **Martin, P. K., T. Li, D. Sun, D. P. Biek, and M. B. Schmid.** 1999. Role in cell permeability of an essential two-component system in *Staphylococcus aureus*. J. Bacteriol. **181:**3666–3673.
45. **McCallum, N., M. Bischoff, H. Maki, A. Wada, and B. Berger-Bachi.** 2004. TcaR, a putative MarR-like regulator of *sarS* expression. J. Bacteriol. **186:**2966–2972.
46. **Novick, R. P.** 2000. Pathogenicity factors and their regulation, p. 392–407. *In* V. A. Fischetti, R. P. Novick, J. J. Ferretti, D. A. Portnoy, and J. I. Rood (ed.), Gram-positive pathogens. American Society for Microbiology, Washington, D.C.
47. **Novick, R. P., and D. Jiang.** 2003. The staphylococcal saeRS system coordinates environmental signals with agr quorum sensing. Microbiology **149:**2709–2717.
48. **Novick, R. P., H. F. Ross, S. J. Projan, J. Kornblum, B. Kreiswirth, and S. Moghazeh.** 1993. Synthesis of staphylococcal virulence factors is controlled by a regulatory RNA molecule. EMBO J. **12:**3967–3975.
49. **Pessi, G., F. Williams, Z. Hindle, K. Heurlier, M. T. Holden, M. Camara, D. Haas, and P. Williams.** 2001. The global posttranscriptional regulator RsmA modulates production of virulence determinants and *N*-acylhomoserine lactones in *Pseudomonas aeruginosa*. J. Bacteriol. **183:**6676–6683.
50. **Pichon, C., and B. Felden.** 2005. Small RNA genes expressed from Staphylococcus aureusgenomic and pathogenicity islands with specific expression among pathogenic strains. Proc. Natl. Acad. Sci. USA **102:**14249–14254.
51. **Schmidt, K. A., A. C. Manna, S. Gill, and A. L. Cheung.** 2001. SarT, a repressor of alpha-hemolysin in *Staphylococcus aureus*. Infect. Immun. **69:**4749–4758.
52. **Schumacher, M. A., B. K. Hurlburt, and R. G. Brennan.** 2001. Crystal structures of SarA, a pleiotropic regulator of virulence genes in S. aureus. Nature **409:**215–219.
53. **Selinger, D. W., R. M. Saxena, K. J. Cheung, G. M. Church, and C. Rosenow.** 2003. Global RNA half-life analysis in Escherichia coli reveals positional patterns of transcript degradation. Genome Res. **13:**216–223.
54. **Spitzfaden, C., N. Nicholson, J. J. Jones, S. Guth, R. Lehr, C. D. Prescott, L. A. Hegg, and D. S. Eggleston.** 2000. The structure of ribonuclease P protein from Staphylococcus aureus reveals a unique binding site for single-stranded RNA. J. Mol. Biol. **295:**105–115.
55. **Steinhuber, A., C. Goerke, M. G. Bayer, G. Doring, and C. Wolz.** 2003. Molecular architecture of the regulatory locus *sae* of *Staphylococcus aureus* and its impact on expression of virulence factors. J. Bacteriol. **185:**6278–6286.
56. **Sterba, K. M., S. G. Mackintosh, J. S. Blevins, B. K. Hurlburt, and M. S. Smeltzer.** 2003. Characterization of *Staphylococcus aureus* SarA binding sites. J. Bacteriol. **185:**4410–4417.
57. **Tegmark, K., A. Karlsson, and S. Arvidson.** 2000. Identification and characterization of SarH1, a new global regulator of virulence gene expression in Staphylococcus aureus. Mol. Microbiol. **37:**398–409.

58. **Tupper, A. E., T. A. Owen-Hughes, D. W. Ussery, D. S. Santos, D. J. Ferguson, J. M. Sidebotham, J. C. Hinton, and C. F. Higgins.** 1994. The chromatin-associated protein H-NS alters DNA topology in vitro. EMBO J. **13:**258–268.
59. **Ussery, D., T. S. Larsen, K. T. Wilkes, C. Friis, P. Worning, A. Krogh, and S. Brunak.** 2001. Genome organisation and chromatin structure in Escherichia coli. Biochimie **83:**201–212.
60. **Wang, X., A. K. Dubey, K. Suzuki, C. S. Baker, P. Babitzke, and T. Romeo.** 2005. CsrA post-transcriptionally represses pgaABCD, responsible for synthesis of a biofilm polysaccharide adhesin of Escherichia coli. Mol. Microbiol. **56:**1648–1663.
61. **Weinrick, B., P. M. Dunman, F. McAleese, E. Murphy, S. J. Projan, Y. Fang, and R. P. Novick.** 2004. Effect of mild acid on gene expression in *Staphylococcus aureus*. J. Bacteriol. **186:**8407–8423.
62. **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.