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Role of *Staphylococcus aureus* Global Regulators *sae* and σ^B in Virulence Gene Expression during Device-Related Infection

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The ability of *Staphylococcus aureus* to adapt to different environments is due to a regulatory network comprising several loci. Here we present a detailed study of the interaction between the two global regulators *sae* and σ^B of *S. aureus* and their influence on virulence gene expression in vitro, as well as during device-related infection. The expression of *sae*, *asp23*, *hla*, *clfA*, *coa*, and *fnbA* was determined in strain Newman and its isogenic *saeS*/*R* and *sigB* mutants by Northern analysis and LightCycler reverse transcription-PCR. There was no indication of direct cross talk between the two regulators. *sae* had a dominant effect on target gene expression during device-related infection. σ^B seemed to be less active throughout the infection than under induced conditions in vitro.

Staphylococcus aureus causes a variety of local and systemic infections in humans and is one of the most important community-acquired and nosocomial pathogens. Staphylococci are the most frequently implicated etiologic agents in device-related infections, in which the bacteria accumulate locally and often persist until the device is removed. Animal models using tissue cages as devices (37) allow the monitoring of various microbiological and immunological events during the course of infection.

The ability of *S. aureus* to adapt to different environments is probably due to a global regulatory network comprising the loci *agr*, *sar*, *sigB*, *rot*, *arlR*/*S*, *svrA*, and *saeR*/*S* (1, 6–8, 28, 36). Each of these regulators is involved in the control of the expression of virulence factors such as hemolysins (for instance alpha-hemolysin, encoded by *hla*), protein A, fibronectin-binding proteins (FnBPA and FnBPB, encoded by *fnbA* and *fnbB*), or capsular polysaccharide (CP, encoded by the *cap* operon). Knowledge about the impact of these regulatory circuits on virulence gene expression during infection is still limited. In certain infections the central regulator *agr* is not involved in the activation of virulence factors (17, 18, 35). For instance in an experimental infective endocarditis model it was shown that *fnbA* is positively regulated in the absence of *agr* and *sarA* (34), suggesting additional regulatory loci in vivo. We could show that the regulator Sae is essential for the transcription of *hla* during device-related infection in guinea pigs (18). Recently, the importance of *sae* was shown in two whole-genome screens for the identification of genes required for full virulence (2, 3). The *sae* locus consists of four ORFs, two of which (*saeR* and *saeS*) show strong sequence homology to response regulators and histidine kinases of bacterial two-component regulators (12). Two additional ORFs, ORF3 and ORF4, located upstream of *saeR*/*S* are likely to be important for functionality of the *sae* operon (29, 31). Four overlapping *sae*-specific transcripts (T1 to T4) originate from three promoters (P1, P2, and P3): the T1 message (3.1 kb) initiates upstream of ORF4, T2 (2.4 kb) initiates upstream of ORF3, and T3 (2.0 kb) initiates in front of *saeR* (Fig. 1). T4 (0.7 kb) represents a monocistronic mRNA encompassing ORF4 only. The T1, T2, and T3 mRNAs are supposed to terminate at the same stem-loop sequence downstream of *saeS* (31). The similar molecular architecture of the *sae* locus was described by Novick and Jiang (29) using other designations for the components (ORF3 [*saeQ*], ORF4 [*saeP*], etc.). The transcription pattern of the *sae* operon is strongly influenced by other regulators (13, 29), as well as by diverse environmental parameters, such as pH or subinhibitory concentrations of antibiotics (23, 29). The *sae* operon activates the expression of several virulence factors, including serine proteases, nuclease, coagulase (encoded by *coa*), *hla*, and *fnbA*, but it represses the expression of the *cap* operon (14, 15, 29, 31). These effects on target genes are not likely to be mediated by RNAIII or *sarA* activity since the same amounts of these two regulators were transcribed in an *sae*-transposon mutant (13).

The interaction of *sae* with the alternative sigma factor σ^B of *S. aureus* is largely unknown. However, all of the known *sae* target genes were also found to be influenced by σ^B, albeit not always in the same direction. Using a microarray approach it has been demonstrated that *coa*, *fnbA*, *cap*, and also clumping factor A (encoded by *clfA*) are activated by σ^B, whereas *hla*, serine proteases, and nuclease are repressed (4). Interestingly, a typical σ^B consensus promoter could be identified only in front of some of the target genes, indicating that σ^B exerts its effect on virulence factor expression in many cases not by direct activation but via other regulators. In contrast, the transcription of alkaline shock protein 23 (*asp23*) is solely dependent on σ^B (4, 10, 11, 22, 24), making it an ideal target to monitor σ^B activity.

In this report, we present a detailed study of the interaction

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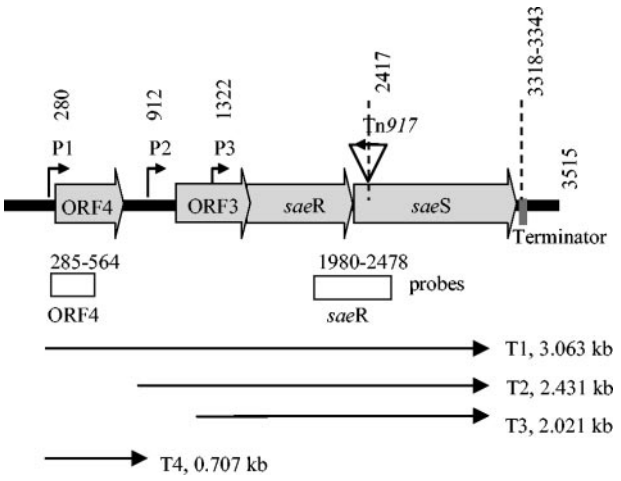

FIG. 1. Schematic drawing of the *sae* operon modified from (31). The hybridization sites of the probes used for Northern analysis (probe ORF4 and *saeR*) are indicated.

between the two regulators *sae* and σ^B and their influence on virulence gene expression both in vitro and in vivo. Transcription analysis was performed with isogenic *sae* and *sigB* mutants of *S. aureus* strain Newman in batch culture and in vivo in a guinea pig model of implant infection.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *S. aureus* strains Newman (9), AS3 (Newman, *sae*::Tn*917*; Em^r) (18), and IK184 (Newman Δ*rsbUVW-sigB*; Em^r) (22) were routinely cultured on sheep blood agar or grown in CYPG medium (27). When included, erythromycin was used in a concentration of 10 μg ml^{-1}. For transcript analysis in vitro, the cells were inoculated from an overnight culture to an initial optical density at 600 nm (OD_{600}) of 0.05 in CYPG and grown to the exponential (OD_{600} = 0.8, 2.5 h) or postexponential (OD_{600} = 8, 8 h) phase.

Animal model of device-related infection. The guinea pig model of implant infection was used (37). Four perforated Teflon tubes were inserted in the flanks of guinea pigs (30). Two weeks after the implantation of the tissue cages, 10^5 CFU of the test strain were inoculated in the tissue cages. Before inoculation, the interstitial fluid that had accumulated in the tissue cages was checked for sterility. The exudate was aspirated 2 and 8 days after infection. One aliquot of the exudate was immediately stored in liquid nitrogen for subsequent RNA preparation. A second aliquot was used for quantitative bacteriology. A third aliquot was mixed with the same volume of 8% paraformaldehyde for immunofluorescence analysis.

RNA isolation and Northern analysis. For RNA preparation from exudates, the frozen samples were thawed rapidly and 200-μl aliquots were used. RNA was isolated and purified as described previously (16). *S. aureus* cells were lysed directly in 1 ml of TRIzol LS reagent (Invitrogen Life Technologies, Karlsruhe, Germany) with 0.5 ml of zirconia/silica beads (0.1 mm in diameter) in a high-speed homogenizer (Savant Instruments, Farmingdale, N.Y.). RNA was isolated as described in the instructions provided by the manufacturer of TRIzol LS. In order to remove PCR inhibitors, the RNA was further purified with the viral nucleic acid kit (Roche Biochemicals, Mannheim, Germany) according to the manufacturer's instructions. Contaminating DNA was degraded by digesting RNA samples with DNase as described elsewhere (17).

RNA preparation from culture and Northern analysis were performed as described previously (17). Briefly, ca. 10^9 *S. aureus* cells were lysed in 1 ml of TRIzol reagent (Invitrogen Life Technologies) in a high-speed homogenizer (Savant Instruments). RNA was isolated as described in the instructions provided by the manufacturer of TRIzol. Several DIG-labeled probes for the detection of specific transcripts were generated by using a DIG-Labeling PCR kit according to the manufacturer's instructions (Roche Biochemicals). Primers are listed in Table 1.

Construction of RNA standards. Sequence-specific RNA standards for LightCycler reverse transcription-PCR (RT-PCR) were engineered by using the following protocol: PCR was performed with a gene-specific primer with a 5′-extension encompassing the T7 phage promoter sequence, thus generating transcription-competent amplicons. Primers for standard construction are listed in Table 1. T7-driven in vitro transcription was performed by using a standard transcription assay (T7-MEGAShortscript; Ambion, Huntingdon, United Kingdom). The reaction mixture was subjected to DNase I treatment (Roche Biochemicals), and the RNA was recovered with the MEGAclear kit (Ambion). Quantification of the transcripts was done spectrophotometrically and verified by ethidium bromide staining on agarose gels.

Quantification of specific transcripts with LightCycler RT-PCR. LightCycler RT-PCR was carried out by using the LightCycler RNA amplification kit for hybridization probes or with the LightCycler RNA amplification kit SYBR Green I (Roche Biochemicals). Master mixes were prepared according to the manufacturer's instructions with the oligonucleotides shown in Table 1. Specific primers were selected in such a way that they bind to an internal part of the respective RNA standard. Standard curves were generated by using 10-fold serial dilutions (10^4 to 10^8 copies/μl) of the specific RNA standards. The number of copies of each sample transcript was then determined with the aid of the LightCycler software. At least two independent RT-PCR runs were performed for each sample. The specificity of the PCR was verified by ethidium bromide staining on 3% agarose gels. To check for DNA contamination, each sample and RNA standard was subjected to PCR by using the LightCycler DNA amplification kit SYBR Green I (Roche Biochemicals). In none of the cases was an amplification product detectable.

ClfA detection by indirect immunofluorescence in vivo. ClfA production in vivo was determined in exudates that were mixed with the same volume of 8% paraformaldehyde immediately after aspiration and then spotted on poly-L-lysine-coated slides. The slides were washed three times with phosphate-buffered saline (PBS)-Tween and incubated with human serum (1:10 in PBS) for 30 min to prevent unspecific binding of immunoglobulin G by cell-wall-associated protein A. The slides were incubated with a ClfA-specific monoclonal antiserum from the mouse (19) (1:200 in PBS-Tween) for 1 h, followed by incubation of CY2-conjugated anti-mouse $F(ab)_2$ fragment (Dianova, Hamburg, Germany) (1:50 in PBS-Tween) for 1 h. Bacteria were also stained with 2 μg DAPI (4′,6′-diamidino-2-phenylindole)/ml for 5 min, washed three times with water, and air dried. The slides were then mounted with fluorescent mounting medium (DakoCytomation, Hamburg, Germany), and positively stained bacteria were detected by using fluorescence microscopy.

RESULTS AND DISCUSSION

Influence of *sae* and σ^B on target genes during growth in culture. In a first step the interaction between the global regulators *sae* and σ^B was assessed in isogenic mutants of strain Newman (wild type [WT]) by Northern analysis. In addition, selected target genes were analyzed that had already been reported to be influenced by both regulators.

sae-specific transcripts T1 to T4 were discerned by using probes specific for *saeR* to detect T1, T2, and T3 and probes specific for ORF4 to detect T1 and T4. The constitutively expressed T3 transcript is less distinct in strain Newman (31). There was no indication of direct cross talk between the global regulators *sae* and σ^B (Fig. 2). None of the transcripts of the *sae* system are altered with respect to expression in the *sigB* mutant of strain Newman at any time point analyzed. These results were corroborated by LightCycler RT-PCR detecting *saeR* (exponential phase: 1.41 copies of *sae*/copy of *gyr* in the WT, 1.08 copies of *sae*/copy of *gyr* in the *sigB* mutant; postexponential phase: 10.87 copies of *sae*/copy of *gyr* in the WT, 12.65 copies of *sae*/copy of *gyr* in the *sigB* mutant).

These findings are consistent with those of Bischoff et al. (4), who found no influence of σ^B on *sae* expression in the genetically distinct *S. aureus* strains COL, Newman, and GP268. Interestingly, Novick et al. (29) described an inhibitory effect of RsbU and σ^B on *sae* expression in strain 8325-4. This discrepancy in the results is most probably strain dependent. We were able to show in an earlier study that 8325-4 exhibits markedly

TABLE 1. Oligonucleotide primers and LightCycler hybridization probes

Target gene	GenBank accession no.	Primer	Primer sequence[a]	Purpose
asp23	S76213	T7-asp	TAATACGACTCACTATAGGGAGAGCATACGACAATCAAACTGG	Standard construction
		asp886	TAAAATGGTGTGCCGAGCTT	Standard construction
		asp493F	AAAATTGCTGGTATCGCTGC	RT-PCR, Northern probe
		asp848R	TGTAAACCTTGTCTTTCTTGGT	RT-PCR, Northern probe
*clf*A	Z18852	T7-clf	TAATACGACTCACTATAGGGAGAGGCGTGGCTTCAGTGCTTGTA	Standard construction
		clf725	CTAATTCCTCCGCATTTGTATTG	Standard construction
		clf382F	CGGTTTTGGACTACTCAGCA	RT-PCR
		clf653R	TAGCTTCACCAGTTACCGGC	RT-PCR
		clfFL1	GGCGCAAAATCCAGCACAACAGGAA-F	Hybridization probe
		clfLC1	Red640-CGACACAATCATCATCAACAAATGCAAC-ph	Hybridization probe
		clfU2	GGCGTGGCTTCAGTGCTTGTA	Northern probe
		clfL2	CACCAGTTACCGGCGTTTCTTC	Northern probe
coa	X17679	T7-coa	TAATACGACTCACTATAGGGAGATAGAAGGTCTTGAAGGTAGC	Standard construction
		coa1624	GGCTTATTGAATCTTGGTCTCGCT	Standard construction
		coaF	AGGTCTTGAAGGTAGCTCAT	RT-PCR
		coaR	GTTGTATTCACGGATACCTGTA	RT-PCR
		coaFL	CTTGATTCTCCTTGAGTACCTTTTAAC-F	Hybridization probe[b]
		coaLC	Red640-TTGATTCAGTACCTTGTGGTTTTATT-ph	Hybridization probe
		coa35F	TAGATTGGGCAATTACATTT	Northern probe
		coa36R	ACCTTTTCCAACAACCTATG	Northern probe
*fnb*A	J04151	fnb1171	ATTGAGACATTTAATAAAGCGA	RT-PCR
		fnb1667	TTTTGAATAATCGGACCATT	RT-PCR
		fnbAF	TGCAAATACGACAGATACTT	Northern probe
		fnbAR	TTGGCCACCTTCATAACCTA	Northern probe
*gyr*B	D10489	T7-gyr	TAATACGACTCACTATAGGGAGATTATGGTGCTGGGCAAATACA	Standard construction
		gyr864	GTACGATTTAATACCGCCCTCATA	Standard construction
		gyr297F	TTAGTGTGGGAAATTGTCGATAAT	RT-PCR
		gyr574R	AGTCTTGTGACAATGCGTTTACA	RT-PCR
		gyrFL1	ATTTTAACTGTTTTACATGCTGGTGGTAA-F	Hybridization probe
		gyrLC1	Red640-TTTGGCGGTGGCGGATACA-ph	Hybridization probe
hla	X01645	T7-hla	TAATACGACTCACTATAGGGAGA AGAAAATGGCATGCACAAAAA	Standard construction
		hla670	TGTAGCGAAGTCTGGTGAAAA	Standard construction
		hla90	AGAAAATGGCATGCACAAAAA	RT-PCR, Northern probe
		hla488	TATCAGTTGGGCTCTCTAAAA	RT-PCR, Northern probe
		hlaFL1	CAGGAAAAATTGGCGGCCTT-F	Hybridization probe
		hlaLC1	Red640-TTGGTGCAAATGTTTCGATTGG-ph	Hybridization probe
*sae*R	AJ556794	T7-sae	TAATACGACTCACTATAGGGAGAAACTGCCAAAACACAAGA	Standard construction
		saeR2	CCATTATCGGCTCCTTTCA	Standard construction
		saeU4	CCATTTACGCCTTAACTTTA	RT-PCR
		saeR4	TAGTCATATCCCCAAACTT	RT-PCR
		saeFL2	GTGACTGTAAATGGTCACGAAGT-F	Hybridization probe
		saeLC2	Red640-CCTATGCGTATTAAGGAATTTGA-ph	Hybridization probe
		sae1980F	TGGTCACGAAGTCCCTATGC	Northern probe
		sae2458R	TGCTTGCGTAATTTCCGTTAG	Northern probe
ORF4	AJ556794	T7-ORF4	TAATACGACTCACTATAGGGAGACAAATTGAAGAAATGAGGAGTTA	Standard construction
		ORF358F	CAAATTGAAGAAATGAGGAGTTA	RT-PCR, Northern probe
		ORF616R	ACCTTTTGATGATTTGTAGTTAG	RT-PCR, standard construction, Northern probe
*sig*B	Y09929	sigB2734	AAAGAACACCAAGAAAATAAG	Northern probe
		sigB3375	TAAACCGATACGCTCACCTGT	Northern probe

[a] F, fluorescein; Red640, LightCycler-Red 640-N-hydroxysuccinimide ester; ph, 3′-phosphate.
[b] LightCycler hybridization probes coaFL and coaLC were designed by TIB Molbiol, Berlin, Germany.

lower overall transcription of *sae* than strain Newman (31). Possibly, the *sigB* effect is not evident in the high-level *sae* producer.

The independency of the regulators was further supported by analysis of *sigB* expression in the *sae* mutant. Comparable levels of *sigB* were observed in both the WT and the *sae* mutant under both in vitro conditions analyzed (Fig. 2). The described additional transcripts of the *sigB* operon (21) were not detect-

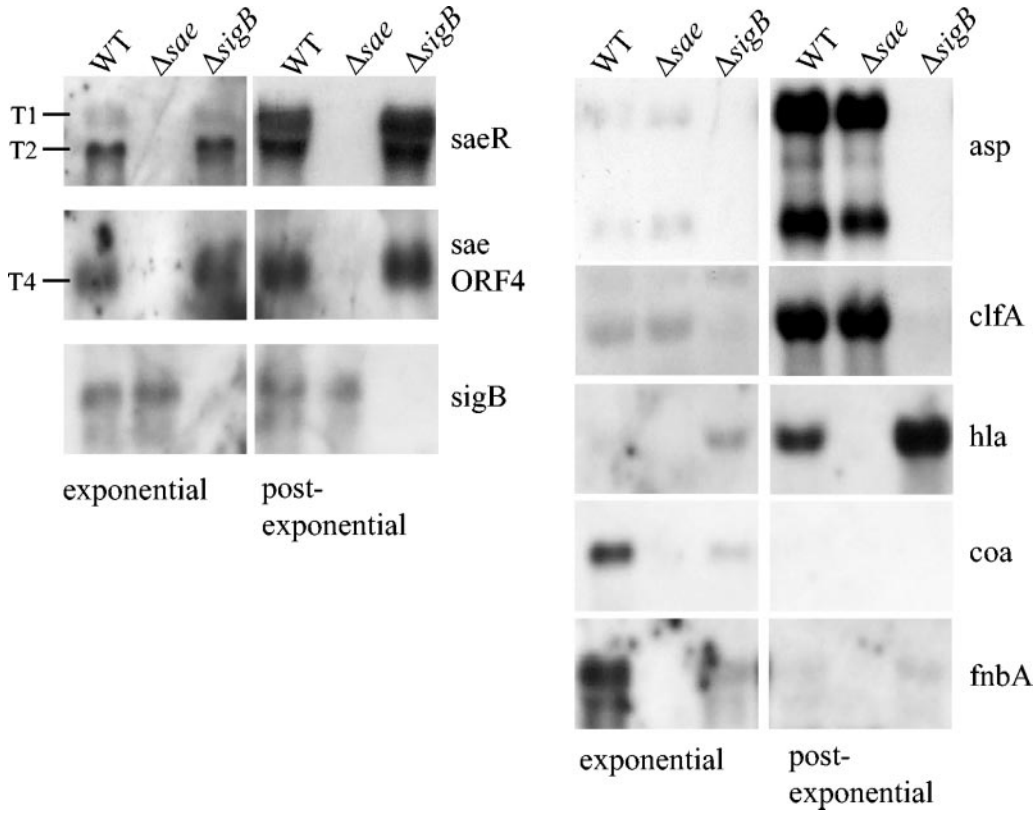

FIG. 2. Northern blot analysis of strain Newman (WT) and its isogenic *sae* and *sigB* mutants grown to exponential and postexponential phases in vitro. The blots were hybridized by using digoxigenin-labeled PCR fragments specific for the indicated transcripts. The different *sae* transcripts (T1, T2, and T4) are specified.

able in our system. In agreement with a recent microarray study (4), σ^B-dependent genes, such as *asp23* and *clfA*, were transcribed mainly during the stationary-growth phase. In addition, our data indicate that *asp23* and *clfA* expression was not affected by *sae*.

The situation is somewhat different for the expression of the cytotoxic *hla*, which is influenced contrarily by both regulators; whereas *hla* transcription is completely abolished in the *sae* mutant, it is clearly elevated in the *sigB* mutant compared to the WT. Thus, *sae* seems to be essential for *hla* activation, whereas the presence of σ^B seems to inhibit *hla*. Since σ^B did apparently not affect *sae* expression, other factors have to be postulated that are responsible for the increase in *hla* expression observed in the *sigB* mutant. A likely candidate for such a scenario might be RNAIII of the *agr* locus, which on the one hand exerts a positive effect on *hla* expression (25) and on the other hand was shown to be influenced negatively by σ^B activity (5, 20).

Interestingly, although appearing to act mainly independent of each other, both regulators seem to be important for *coa* and *fnbA* transcription (Fig. 2). The most profound effect on transcription was observed in the *sae* mutant. A σ^B consensus sequence was predicted only in the promoter region of *coa* (24, 26). A comparison of the upstream sequences of *fnbA* and *coa* revealed no obvious common motifs for *sae* activation (31). Thus, additional factors are needed to explain the observed regulatory pattern of *fnbA* and *coa* expression. This is further emphasized by the observation that both genes are strictly repressed during the late growth cycle, which cannot be ascribed to any of the regulatory loci studied thus far.

Influence of *sae* and σ^B on target genes during device-related infection. The impact of *sae* and σ^B on target gene expression was further analyzed during device-related infection by quantitative LightCycler RT-PCR. All strains established infection after inoculation of the tissue cages with 10^5 CFU/ml, reaching a density of ca. 10^9 CFU/ml after 8 days. In general, there were no significant differences between strain Newman and its isogenic *sae* and *sigB* mutants with respect to the densities found in the exudates (data not shown).

In agreement with our in vitro findings, no influence of σ^B on *sae* transcription was discernible. Quantifying either the transcripts T1, T2, or T3 (*saeR*) of the *sae* operon, which are initiated from the promoters P1, P2, and P3, respectively, or the transcripts T1 and T4 (ORF4) initiating from P1 yielded comparable copy numbers in the WT and the *sig*B mutant cells (Fig. 3A and B). Interestingly, in vivo the copy number of ORF4-containing transcripts was significantly lower than that of *saeR*-containing transcripts. The same analysis in vitro revealed nearly identical levels of *saeR* and of ORF4 transcripts in the WT, independent of the growth phase (for instance, 10.9 copies of *saeR*/copy of *gyr* and 14.1 copies of ORF4/copy of *gyr* in postexponential phase). It is conceivable that in vivo P2, P3, or other as-yet-unidentified promoters downstream of ORF4

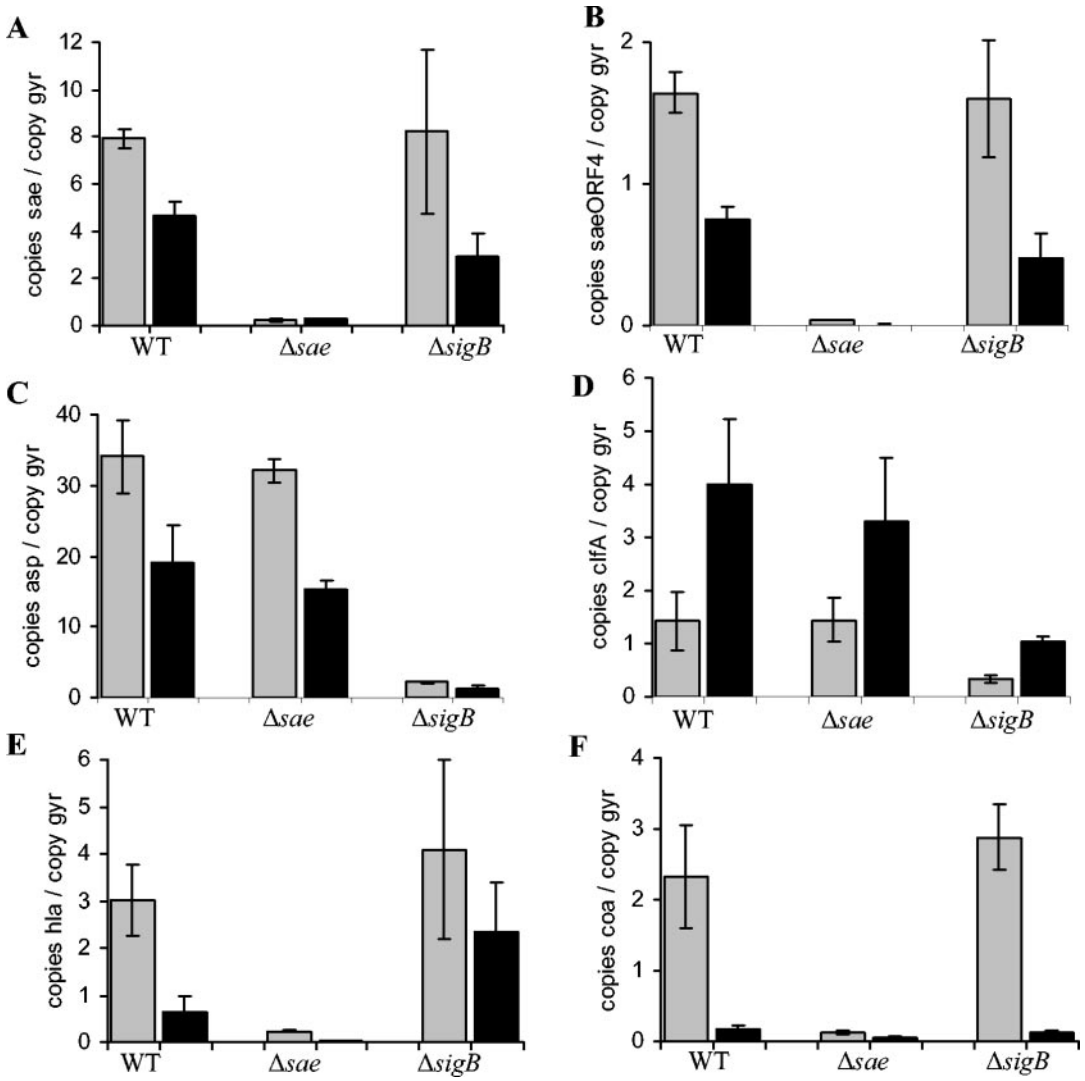

FIG. 3. Quantitative transcript analysis of *S. aureus* strains Newman (WT) and its isogenic *sae* and *sigB* mutants by LightCycler RT-PCR during device-related infection. Transcripts were quantified in reference to the transcription of gyrase (in copies per copy of *gyr*). Expression of *saeR* (A), *sae*ORF4 (B), *asp*23 (C), *clfA* (D), *hla* (E), and *coa* (F) was determined in exudates from infected devices in guinea pigs 2 days (gray columns) and 8 days (black columns) after inoculation. Values from two separate RNA isolations and two independent RT-PCRs each were used to calculate the mean expression (± standard errors of the mean).

are induced. In fact, additional transcripts are detectable under certain growth conditions in vitro (unpublished data).

In order to analyze σ^B effects in vivo, we first focused on the transcription of the target genes *asp*23 and *clfA* with known σ^B consensus promoters. A high copy number of *asp23* transcripts was detectable in Newman WT early in infection (day 2), which seemed to decline in the later infection stage (day 8). The *asp23* in vivo expression pattern was again not influenced by the *sae* mutation, whereas almost no *asp23*-specific transcript was detectable in the *sigB* mutant in both infection stages analyzed (Fig. 3C). This emphasizes the predominant role of σ^B for the regulation of this gene also during infection. However, the finding that *asp23*-specific transcripts were still detectable in a *sigB* mutant was somewhat surprising, since *asp23* transcription is believed to rely exclusively on σ^B activity (4, 10, 11, 22, 24). One explanation for this finding might be that *S. aureus* might produce basal amounts of *asp23*-containing transcripts in a σ^B-independent manner, which are sufficient to be detected by real-time PCR but are not identified by less sensitive methods such as Northern blotting and microarray analysis. Interestingly, transcription levels of *asp23* found in vivo were well below the amounts found in the postexponential phase (data not shown). Thus, σ^B seems to be less active throughout the device-related infection than under induced conditions in vitro.

Unlike *asp23*, *clfA* expression seemed to increase with the infection time. Although 1.4 copies of *clfA*/copy of *gyr* were detectable in exudates from infected devices 2 days after inoculation with the WT, a fourfold increase was observed in exudates from day 8 (Fig. 3D), which is in agreement with previous observations showing that *clfA* expression is induced late during infection (33). In accordance with the in vitro findings, no

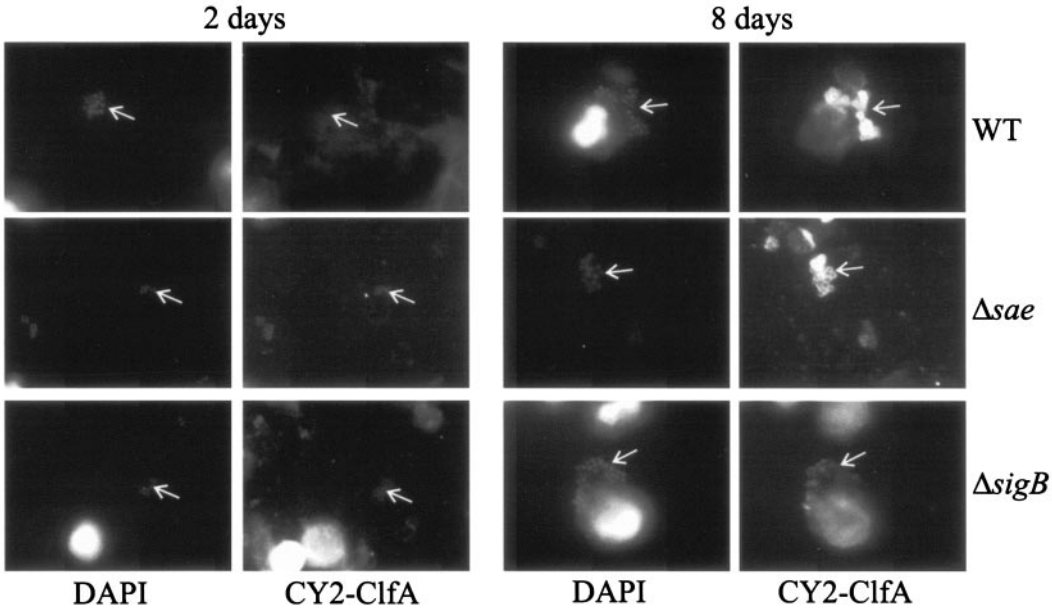

FIG. 4. Immunofluorescence assay for the detection of ClfA in *S. aureus* strain Newman (WT) and its isogenic *sae* and *sigB* mutants in exudates from infected devices in guinea pigs 2 days and 8 days after inoculation. Slides were incubated with a ClfA-specific monoclonal antiserum from the mouse (19) followed by incubation of CY2-conjugated anti-mouse $F(ab)_2$ fragment (right panel). The presence of the corresponding bacteria is shown by DAPI staining (left panel). *S. aureus* cells are indicated by white arrows.

effect of *sae* on *clfA* expression was detectable. Interestingly, transcription of *clfA* was only diminished in the *sigB* mutant compared to the WT, and the temporal expression pattern was still traceable. Additional regulatory pathways are obviously needed for the full expression of ClfA, leading to induction, especially late during infection. However, thus far none of the described regulators have been shown to influence *clfA* expression.

In analyzing genes that are mutually influenced by σ^B and *sae*, it became evident that *sae* had a dominant effect on these target genes. In the *sigB* mutant *hla* transcription was only marginally higher than in the WT in vivo (Fig. 3E), whereas in the *sae* mutant *hla* expression was totally abolished. Surprisingly, the expression of *coa*, a σ^B-activated gene, was not altered in the *sigB* mutant compared to the WT under in vivo conditions (Fig. 3F), which contradicts the in vitro findings, where *coa* expression clearly decreased in the *sigB* mutant (Fig. 2). One possible explanation for this discrepancy might be that *coa* expression is mainly driven by a σ^B-independent mechanism in vivo, which is not that important under in vitro conditions.

When we compared gene expression 2 and 8 days after inoculation it became evident that the sequential activation of virulence genes in vivo was profoundly different from that seen during growth in vitro. It has already been shown that the maximum expression of *hla* occurs early and that of *clfA* late during infection (18, 33). Here, we could also demonstrate that the expression patterns of *hla* and *coa* were closely linked to *sae* expression. Thus, *sae* may be the predominant regulator determining expression of these genes in vivo. The signal leading to downregulation of *sae* during infection remains to be clarified. It has been shown that *sae* reacts to diverse environmental parameters such as pH or subinhibitory concentrations of antibiotics (23, 29, 32).

Correlation between transcript analysis and protein expression. In order to correlate transcript quantification with protein expression, LightCycler data were compared to an immunofluorescence assay for ClfA. The assay was performed with exudates derived from animals infected with strain Newman and its isogenic *sae* and *sigB* mutant 2 and 8 days after inoculation. A clear increase of the fluorescence signal was detected during the course of infection in the WT and in the *sae* mutant (Fig. 4). The *sigB* mutant showed markedly diminished signals at both time points. The results obtained by quantitative transcript analysis of *clfA* (Fig. 3D) correlated well with the detection of ClfA by immunofluorescence staining, indicating that bacterial transcript analysis is an appropriate tool for discerning the phenotype of bacteria in the host.

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