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# CcpA Mediates the Catabolite Repression of *tst* in *Staphylococcus aureus*▽

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**Some clinical isolates of *Staphylococcus aureus* produce the superantigenic toxic shock syndrome toxin 1 (TSST-1), encoded by *tst*, located on pathogenicity islands. The expression of *tst* is complex and is influenced by environmental conditions such as pH, $CO_2$, and glucose. We identified a putative catabolite-responsive element (*cre*) in the promoter regions of all known *tst* genes, indicating that *tst* transcription may be regulated by the catabolite control protein CcpA. By introducing *tst* genes under the control of their native promoters or *tst* promoter-reporter gene fusions in wild-type strain Newman, we showed that glucose was able to repress *tst* transcription and TSST-1 production, whereas glucose repression was abolished in the corresponding Δ*ccpA* mutant. Stabilizing the pH ruled out a pH effect due to acid production during glucose catabolism. CcpA thus directly regulates *tst* transcription, linking carbohydrate utilization to virulence gene expression in *S. aureus*.**

Toxic shock syndrome (TSS) is an acute and potentially fatal illness caused by a group of bacterial superantigens, such as toxic shock syndrome toxin 1 (TSST-1), staphylococcal enterotoxins, and streptococcal pyrogenic toxins. Superantigens, unlike conventional peptide antigens, bind to invariant regions of the major histocompatibility complex class II molecules at the surfaces of antigen-presenting cells, outside the classical antigen-binding groove, and also to invariant regions of the T-cell receptor. This leads to activation of T cells at orders of magnitude above that for antigen-specific activation, resulting in massive cytokine release, which in turn leads to capillary leakage and is believed to be responsible for hypotension, shock, and finally, death (19). Especially in the early 1980s, there was a major interest in TSS because there were several staphylococcal TSS cases reported for otherwise healthy young women (7). This rise in TSS cases was associated with high-absorbency tampons, and the disease was named menstrual TSS. In most cases, menstrual TSS is associated with TSST-1, but cases of nonmenstrual TSS have also been associated with this toxin (8, 9), which is encoded by *tst*, a gene found on several staphylococcal pathogenicity islands (SaPIs) (reviewed in reference 25).

The regulation of TSST-1 is known to be complex, and several environmental conditions, including salt, oxygen, carbon dioxide, growth rate, temperature, glucose, and pH, were reported to modulate its production (3, 29, 31, 46). Global regulators shown to be involved in the regulation of TSST-1 are SarA (4, 6, 23), RNAIII of the *agr* system (3, 28), and SrrAB (45) (Fig. 1). However, there is some contradiction about the role of SarA in *tst* expression, since Chan and Foster (4) described that repression of *tst* by SarA was *agr* independent, while Novick (23) found that *tst* repression was dependent on *agr*. SrrAB, which is also a repressor of RNAIII (27), was previously shown to repress *tst* transcription under limited oxygen pressure (45). More recently, Pragman et al. (26) proposed that SrrAB, in addition to its downregulatory function under low-oxygen conditions, enhances the level of *tst* under aerobic conditions. More complexity is added by the fact that *tst* has autoregulatory functions, with TSST-1 acting as a repressor of *tst* transcription (41) (Fig. 1).

We recently analyzed the impact of the carbon catabolite protein A (CcpA) of *Staphylococcus aureus* on carbon metabolism, virulence determinant expression, and biofilm formation (34, 35). CcpA induces or represses the transcription of genes by binding to so-called catabolite-responsive elements (*cre*) (33). These *cis*-acting DNA sequences, which consist of 14 to 18 bp, have been studied extensively in *Bacillus subtilis* (17, 20, 21, 38, 44, 47). Binding of CcpA to the *cre* sites is induced by complex formation with HPr, a component of the phosphoenolpyruvate-dependent phosphotransferase transport system (32, 37, 43). In the presence of glucose or other rapidly metabolized carbon sources, HPr is phosphorylated on serine-46 and binds to CcpA. But CcpA can apparently also regulate gene expression without binding to *cre* sites (12–14, 22). It was proposed that CcpA can directly interact with and inhibit RNA polymerase, in a process that is stimulated by NADP or NADPH, independent of the redox state of the cell (14).

Since TSST-1 production was shown to be repressed by glucose (31) and since our in silico analysis of the sequenced *S. aureus* genomes identified putative *cre* sites in the *tst* promoter regions, we investigated whether CcpA was the mediator of this regulatory phenomenon. We therefore analyzed the effects of glucose on *tst* transcription and TSST-1 production and compared them to the effects in a *ccpA* null mutant. Our results indicate that repression of *tst* by glucose is mediated directly by the carbon catabolite protein CcpA.

## MATERIALS AND METHODS

**Bacterial strains, plasmids, and culture conditions.** The bacterial strains, relevant phenotypes, and plasmids used in this study are listed in Table 1. All strains generated for this study were confirmed by pulsed-field gel electrophore-

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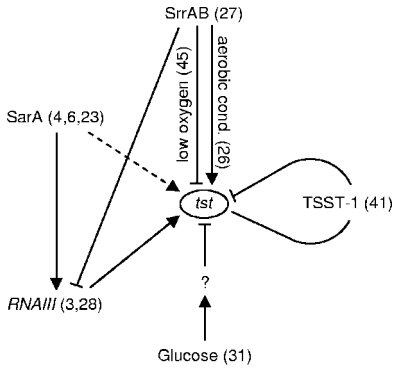


FIG. 1. Regulation of *tst* transcription. Arrows represent upregulation, bars represent downregulation, dashed arrows indicate controversial findings, and numbers in parentheses indicate references. The question mark corresponds to CcpA.

sis of total genomic SmaI digests (42) and by Southern blot analysis according to standard protocols, using CcpA- and RNAIII-specific digoxigenin (DIG)-labeled probes, which were generated using previously published primers (35). *S. aureus* was routinely grown in Luria-Bertani (LB) medium buffered with 50 mM HEPES (pH 7.5), with a flask volume/culture volume ratio of 5:1, at 37°C and 200 rpm. The medium was supplemented with 50 μg $ml^{-1}$ kanamycin and 10 μg $ml^{-1}$ tetracycline, if appropriate.

**Construction of plasmid *tstp*$_{N315}$::*luc*+-pCN34 (pSKA12).** A 0.8-kb fragment containing the *tst* promoter region was amplified by PCR from chromosomal DNA of *S. aureus* N315 by the use of primers TstPAsp718+ (GCGCCATGGTTAATTCTCCTTCATTCAAA), including an Asp718 linker (underlined), and TstPNcoI− (GCGGGTACCTTCGAGAGGCAGATTACTCC), including an NcoI linker (underlined). The PCR product was cloned in front of the luciferase gene of plasmid pSP*luc*+. From this plasmid, a 2.4-kb Asp718-EcoRI fragment, including the *tst* promoter region fused to the luciferase coding region, was cloned into plasmid pCN34, generating plasmid pSKA12. The identity of the construct was confirmed by sequence analysis and comparison to the respective N315 sequence in the NCBI database (accession no. NC_002745). The plasmid was electroporated into strain RN4220 and subsequently transduced into strain Newman and its isogenic Δ*ccpA* mutant (MST14). The plasmid was also introduced into strain Newman Δ*agr* (KS186), which was obtained by transduction of *agr*::*tet*(M) from strain RN6911.

**Construction of plasmids *tst*$_{N315}$-pAW17 (pSKA20) and *tst*$_{RF122}$-pAW17 (pSKA21).** One-kilobase fragments containing *tst* and its promoter region were amplified by PCR from chromosomal DNAs of *S. aureus* N315 and RF122, using primers TstXbaI+ (GTTGTCTAGAACTCACACTTTGTTTTTTGC), including an XbaI linker (underlined), and TstPstI− (GTTGCTGCAGGTTTACTAATTCACCCTAGC), including a PstI linker (underlined). The PCR products were cloned into pAW17, generating pSKA20 and pSKA21, respectively. The identities of the constructs were confirmed by sequence analysis and comparison to the respective sequences in the NCBI database (for N315, accession no. NC_002745; and for RF122, accession no. NC_007622). The plasmids were electroporated into RN4220 and subsequently transduced into strain Newman, its respective Δ*ccpA* mutant (MST14), strain Newman Δ*agr* (KS186), and strain RN450.

**Luciferase assays.** For glucose impulse experiments, the cultures were inoculated at an optical density at 600 nm ($OD_{600}$) of 0.05 and were grown for 5 h without glucose. At this time point, corresponding to the early stationary growth phase, the cultures were split and different amounts of glucose were added. Samples were taken 0, 30, and 60 min after the glucose impulse. To follow the *tst* promoter-luciferase reporter activity over time, the medium was inoculated with an overnight culture to an $OD_{600}$ of 0.05 and the bacteria were grown for 8 h. The medium contained either 10 mM glucose or no glucose. Sampling was performed on an hourly basis, starting 2 h after inoculation. Luciferase activity was measured as described earlier, using a luciferase assay substrate and a Turner De-

TABLE 1. Bacterial strains and plasmids

| Strain or plasmid | Relevant genotype and/or phenotype[a] | Source or reference |
|---|---|---|
| Strains | | |
| *S. aureus* strains | | |
| RN4220 | NCTC8325-4 $r^-$ $m^+$ (restriction negative, modification positive) | 15 |
| RN6911 | NCTC8325-4 *agr*::*tet*(M) $Tc^r$ | 40 |
| Newman | ATCC 25904; clinical isolate, CP5 producer | 10 |
| MST14 | Newman *ccpA*::*tet*(L) $Tc^r$ | 35 |
| N315 | Multiresistant clinical methicillin-resistant *S. aureus* isolate, TSST-1 producer | 16 |
| RF122 | Wild-type strain from bovine mastitis, TSST-1 producer | 11 |
| RN450 | NCTC8325-4 | 24 |
| KS87 | Newman/pSKA12 (*tstp*$_{N315}$::*luc*$^+$) $Kan^r$ | This study |
| KS64 | Newman *ccpA*::*tet*(L)/pSKA12 (*tstp*$_{N315}$::*luc*$^+$) $Tc^r$ $Kan^r$ | This study |
| KS179 | Newman/pSKA20 (*tst*$_{N315}^+$) $Kan^r$ | This study |
| KS180 | MST14/pSKA20 (*tst*$_{N315}^+$) $Tc^r$ $Kan^r$ | This study |
| KS181 | Newman/pSKA21 (*tst*$_{RF122}^+$) $Kan^r$ | This study |
| KS182 | MST14/pSKA21 (*tst*$_{RF122}^+$) $Tc^r$ $Kan^r$ | This study |
| KS186 | Newman *agr*::*tet*(M) $Tc^r$ | This study |
| KS187 | Newman *agr*::*tet*(M)/pSKA12 (*tstp*$_{N315}$::*luc*$^+$) $Tc^r$ $Kan^r$ | This study |
| KS188 | Newman *agr*::*tet*(M)/pSKA20 (*tst*$_{N315}^+$) $Tc^r$ $Kan^r$ | This study |
| KS189 | Newman *agr*::*tet*(M)/pSKA21 (*tst*$_{RF122}^+$) $Tc^r$ $Kan^r$ | This study |
| KS197 | RN450/pSKA20 (*tst*$_{N315}^+$) $Kan^r$ | This study |
| KS198 | RN450/pSKA21 (*tst*$_{RF122}^+$) $Kan^r$ | This study |
| *E. coli* strain | | |
| DH5α | Restriction-negative strain for cloning | Invitrogen |
| Plasmids | | |
| pSP*luc*+ | Luciferase fusion plasmid, ColE1 replication origin; $Ap^r$ | Promega |
| pCN34 | *E. coli-S. aureus* shuttle plasmid, ColE1 replication origin; pT181 *cop-wt repC*; $Amp^r$ $Kan^r$ | 5 |
| pAW17 | *E. coli-S. aureus* shuttle plasmid, ColE1 and pAMα1 replication origins; $Kan^r$ | 30 |
| pSKA12 | pCN34 containing a 2.5-kb *tstp*$_{N315}$::*luc*$^+$ fusion; $Ap^r$ $Kan^r$ | This study |
| pSKA20 | pAW17 with a 1-kb PCR fragment covering *tst* of strain N315 and its proposed promoter; $Kan^r$ | This study |
| pSKA21 | pAW17 with a 1-kb PCR fragment covering *tst* of strain RF122 and its proposed promoter; $Kan^r$ | This study |

[a] $Tc^r$, tetracycline resistant; $Kan^r$, kanamycin resistant; $Ap^r$, ampicillin resistant.

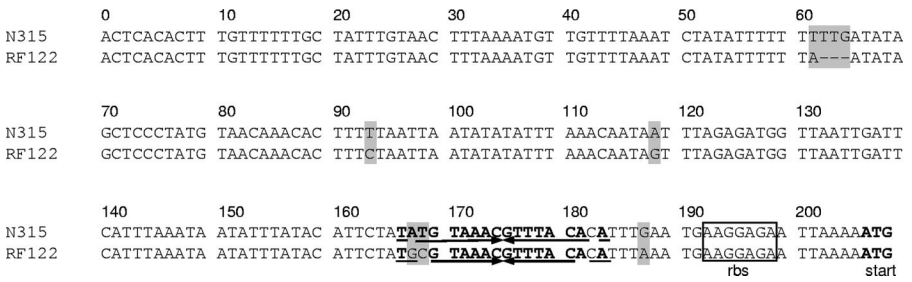


FIG. 2. Alignment of the cloned *tst* promoter regions of strains N315 (GenBank accession no. NC_002745; nucleotides 2,060,871 to 2,061,078) and RF122 (GenBank accession no. NC_007622; nucleotides 400,656 to 400,452) used for the construction of pSKA20 and pSKA21. The ribosome binding site (rbs) suggested by Blomster-Hautamaa et al. (2) is shown. Nucleotides fitting with the *cre* consensus of *B. subtilis* suggested by Miwa et al. (21) are highlighted in bold. Inverted repeats are indicated by arrows. Differences in nucleotides are highlighted by gray boxes.

signs TD-20/20 luminometer (Promega) (1). All luciferase assays were performed at least three times in independent experiments.

**Northern blots.** Sampling for Northern blot analysis was performed basically as described for the glucose impulse experiments in the luciferase assays. Ten millimolar glucose was added to one-half of the culture, while the other half remained without glucose. Samples were centrifuged for 2 min at 12,000 × *g*, and cell sediments were snap-frozen in liquid nitrogen. RNA isolation and Northern blotting were performed as described earlier (18). Primers DIGtst+ (TAAGC CTTTGTTGCTTGCG) and DIGtst− (CACTTTGATATGTGGATCCG) were used to generate DIG-labeled *tst* probes. All Northern blot analyses were performed at least twice with independently isolated RNA samples.

**Exoprotein analysis and Western blots.** For the determination of TSST-1 levels in the supernatants, cells were grown for 3 or 5 h either with or without 10 mM glucose. Supernatants were adjusted to the same volume for all samples, using sterile medium. Identical amounts of bovine serum albumin were added to samples as an internal control for semiquantitative detection of TSST-1. Proteins were precipitated with trichloroacetic acid at a final concentration of 10%, samples were placed on ice for 30 min and centrifuged for 15 min at 16,000 × *g*, and sediments were washed twice with 5 ml acetone. After being air dried, sediments were resuspended in loading buffer and samples corresponding to a final $OD_{600}$ of 0.25 (1.25 for strain N315) were analyzed by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Gels were either stained with Coomassie brilliant blue or blotted on nitrocellulose membranes. TSST-1 detection was performed using rabbit polyclonal anti-TSST-1 antibodies (LucernaChem, Switzerland) after blocking with human immunoglobulin G and visualized using SuperSignal West Pico chemiluminescent substrate (Pierce, Rockford, IL).

For the determination of exoprotein patterns, cells were sampled at an $OD_{600}$ of 3.5 (~5 h). Proteins were precipitated as described above, and samples corresponding to a final $OD_{600}$ of 1.0 were analyzed by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and stained with Coomassie brilliant blue or blotted for TSST-1 detection as described above.

**Determination of glucose levels.** Two-milliliter aliquots of bacterial cultures were harvested at the indicated time points and centrifuged for 2 min at 16,000 × *g*. The supernatants were incubated at 80°C for 15 min and stored at −20°C until use. Glucose levels were determined with kits from R-Biopharm (Darmstadt, Germany) according to the manufacturer's directions.

## RESULTS AND DISCUSSION

**The *tst* promoter region contains a *cre* site.** By screening strain N315 with the consensus sequence proposed by Miwa et al. (21), we identified a putative *cre* site 22 bp upstream of the translational start of *tst* on SaPIn1 (Fig. 2). Because *tst* is located on different pathogenicity islands (SaPIs) in different strains (25), we compared the promoter regions of the *tst* genes of the published SaPIs (SaPIn1 [strain N315], GenBank accession no. NC_002745; SaPm1 [strain Mu50], NP_372535; SaPI1 [strain RN4282], U93688; SaPI2 [strain RN3984], EF010993; and SaPbov1 [strain RF122], NC_007622). We found identical *cre* sites on SaPI1, SaPIm1, SaPIn1, and SaPI2 (data not shown). With the exception of 1 bp, the *cre* sites were palindromic and were located downstream of the transcriptional start site suggested by Vojtov et al. (41). The pathogenicity island SaPIbov1 of the bovine mastitis isolate RF122 had a *cre* site which differed at 2 bp from the *cre* sites of the other *tst* genes. However, it was still palindromic with the exception of 1 bp (Fig. 2).

***tst* expression is strongly repressed in the presence of glucose.** The presence of *cre* sites in the different *tst* genes together with previous findings showing that glucose decreased TSST-1 production (31) indicated that CcpA might mediate glucose-induced *tst* repression. To confirm that glucose also had an effect on *tst* transcription, we performed a Northern blot analysis in a glucose impulse experiment. We chose strains N315 and RF122 to compare the two different *cre* variants. Because *tst* is known to be expressed in early stationary growth phase (39), we cultured the cells in buffered medium for 5 h, split the cultures, and added glucose to one-half of each culture. In line with the literature, we found strong *tst* transcription in RF122 during this growth stage, while the addition of glucose led to a clear repression of *tst* (Fig. 3A). Surprisingly, in strain N315, *tst* expression was much weaker and smears appeared instead of a distinct band, impeding an interpretation of the glucose effect (data not shown). To see if the glucose-dependent activation of CcpA was functional in this particular strain, we tested the glucose-mediated repression of *pckA*, a gene previously reported to be under the control of CcpA (35). We observed a strong glucose-dependent repression of *pckA*, indicating that N315 possessed a functional CcpA protein (data not shown).

To determine the influence of the genetic background on *tst* expression in N315, we fused the promoter region of the *tst* gene of this strain to the firefly luciferase gene, yielding plasmid $tstp_{N315}$::$luc^+$-pCN34 (pSKA12), and transduced the construct into strain Newman. The luciferase activity in the transformed Newman derivative reached high values of between 30,000 and 50,000 relative light units. By testing the impact of the glucose concentration on *tst* repression, we observed that the addition of 1 mM (0.018%) glucose reduced the luciferase activity to 66% (±8.5%), 2 mM (0.036%) glucose decreased the activity to 35% (±7.0%), and concentrations of >4 mM decreased the activity to <18% (±8.0%) (Fig. 3B). This indicated that transcription of the *tst* promoter of N315 was highly sensitive to low concentrations of glucose and suggested that the low *tst* expression in N315 was likely due to the genetic background.

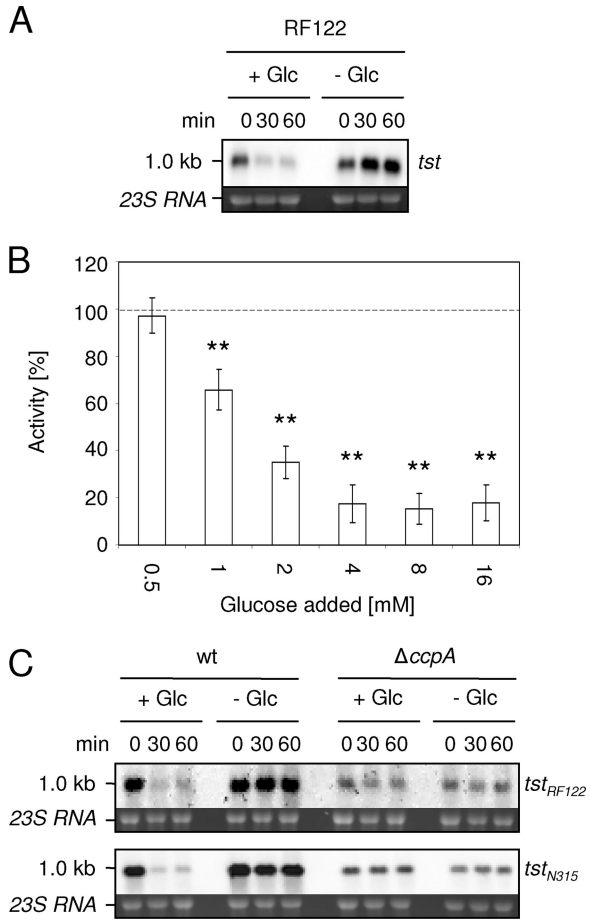


FIG. 3. Influence of glucose on *tst* expression. (A) Northern blot analysis of *tst* expression in response to glucose in strain RF122 after the addition of 10 mM glucose to a culture in the early stationary growth phase. Ethidium bromide-stained 23S rRNA indicates RNA loading. (B) Luciferase activity of strain KS87 in response to different glucose concentrations. The luciferase activity at time zero was set to 100% (dashed line), and relative activity after 60 min was assessed. The averages of three independent measurements are shown. Error bars indicate the standard deviations of the mean activities. **, $P < 0.01$ for supplemented versus unsupplemented cultures. (C) *tst* transcription in response to glucose in strains Newman (wt) and MST14 (Δ*ccpA*) carrying pSKA20 ($tst_{N315}$) or pSKA21 ($tst_{RF122}$) after the addition of 10 mM glucose to a culture in the early stationary growth phase. Ethidium bromide-stained 23S rRNA indicates RNA loading. The exposure time of $tst_{N315}$ had to be adjusted in comparison to that of $tst_{RF122}$.

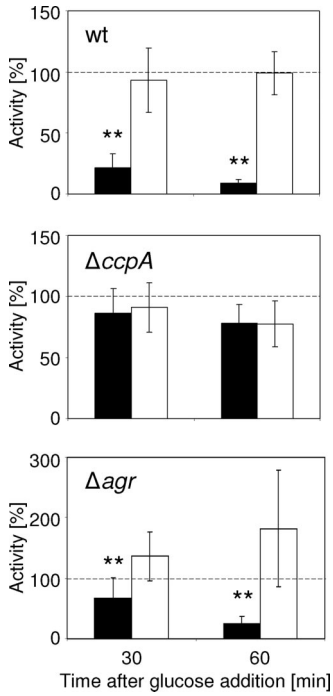


FIG. 4. Relative luciferase activities of strains KS87 (wt), KS64 (Δ*ccpA*), and KS187 (Δ*agr*). Glucose (10 mM) was added to one-half of a culture in the early stationary growth phase (black bars), while the other half remained without glucose (white bars). Activity at time zero was set to 100% (dashed line), and relative activities after 30 and 60 min were assessed. The averages of three independent measurements are shown. Error bars indicate the standard deviations of the mean activities. **, $P < 0.01$ for supplemented versus unsupplemented cultures.

**Glucose-dependent repression of *tst* transcription requires CcpA.** To assess the impact of CcpA on the glucose-dependent repression of *tst* transcription, we transduced the $tstp_{N315}$::$luc^+$-pCN34 construct into the Δ*ccpA* mutant of strain Newman and followed the glucose effect over time in the wild-type and mutant strains. In the wild type, repression of luciferase activity was already clearly detectable 30 min after glucose addition, and activity decreased further until 1 h after glucose addition (Fig. 4). The pH remained stable throughout the time course followed, ruling out a possible pH effect on *tst* expression (31). In the mutant, no changes in activity were observed upon glucose addition, strongly suggesting that CcpA was involved in glucose-mediated *tst* repression (Fig. 4).

To observe the long-term effects of glucose on *tst* promoter activity during growth, the wild-type and Δ*ccpA* mutant strains were grown for 8 h in either the presence or absence of glucose, and luciferase activity was followed at 1-h intervals. Without glucose, activity in the wild type started to rise after 3 h, reached its maximum after 5 h, and remained stable from then on (Fig. 5A). In the presence of glucose, activity remained very low (about 1% of the activity without glucose) and started to rise only slowly after 5 h, when glucose was depleted from the medium, reaching a similar value to that for cells grown without glucose after 8 h (Fig. 5A and B). Despite HEPES buffering, in the presence of glucose, the pH dropped slightly after 5 h (Fig. 5B). For the mutant, luciferase activities were similar in the presence and absence of glucose, and the pH remained stable under both conditions for up to 7 h (Fig. 5A and B). Compared with that of the wild type, luciferase activity in the mutant rose faster and tended to be higher.

The luciferase assays left the question open of whether and how CcpA would repress *tst* transcription in the presence of an intact *tst* gene. Unfortunately, we were not successful in transducing the Δ*ccpA* mutation into strain N315, strain RF122, or other clinical TSST-1-expressing isolates from our strain collection. We therefore introduced the *tst* genes of strains N315 and RF122 ($tst_{N315}$ and $tst_{RF122}$, respectively) in *trans* into strain Newman and its Δ*ccpA* mutant. The plasmid-borne *tst* transcripts were the same size (1 kb) as the chromosomal *tst* transcripts of strains N315 and RF122 (data not shown). Even

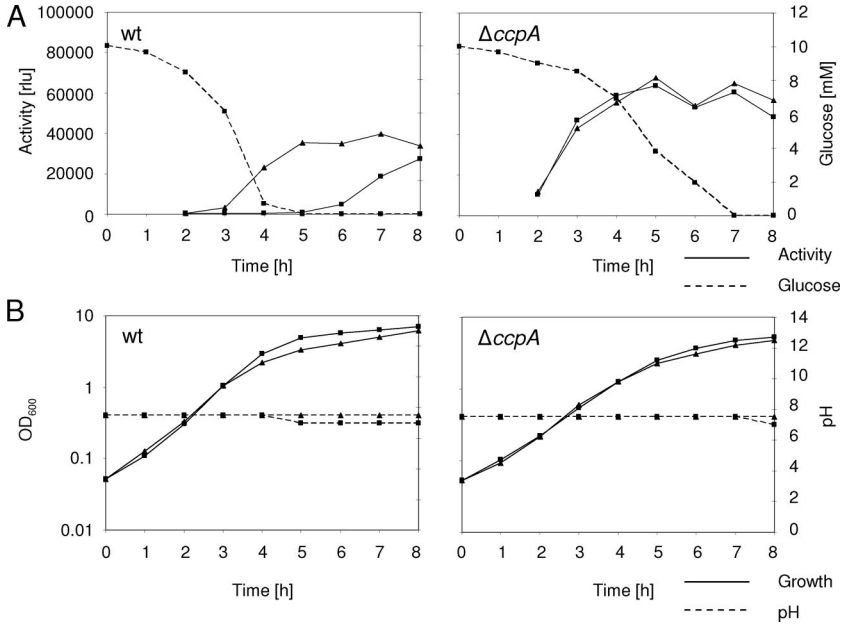


FIG. 5. *tst* promoter activity and growth characteristics of KS87 (wt) and KS64 (Δ*ccpA*) in the presence (squares) and absence (triangles) of glucose. (A) Luciferase activity and glucose consumption. rlu, relative light units. (B) Growth and pH. Data are representative of three independent measurements.

though the higher copy number of the plasmid-carried $tst_{N315}$ and $tst_{RF122}$ genes may blur a CcpA effect in strains KS181 and KS179, *tst* transcription in the respective Newman Δ*ccpA* mutants KS182 and KS180 was lower than that in the wild type, indicating that CcpA might exert some regulatory function on *tst* expression even in the absence of glucose (Fig. 3C). The overall apparently stronger $tst_{N315}$ transcription, although it was cloned into the same plasmid backbone as $tst_{RF122}$ and expressed in the same Newman background, may be due to small sequence differences in the operator region (Fig. 2) or possibly also within the coding or terminator region (not shown).

In performing glucose impulse experiments with the strains carrying the *tst*-expressing plasmids, we found strong repression of *tst* transcription by glucose in strain Newman, independent of whether it carried the N315 or RF122 *tst* variant. However, no such effect was observed in the Δ*ccpA* mutant (Fig. 3C). The finding that both *tst* genes were repressed by glucose in a CcpA-dependent manner suggests that the minor difference in their *cre* sites did not affect CcpA activity.

***tst* does not affect exoprotein patterns in LB medium.** To assess TSST-1 production and the impact of TSST-1 on the exoprotein patterns of the different strains, we analyzed supernatants of post-exponential-growth-phase cultures. Consistent with its low *tst* transcription, strain N315 produced significantly smaller amounts of TSST-1 than those in RF122 or Newman carrying the *tst* plasmids after 5 h of growth (data not shown). The production of larger TSST-1 amounts in KS179 (Newman $tst_{N315}$) than in KS181 (Newman $tst_{RF122}$) (Fig. 6A) was in line with the stronger $tst_{N315}$ transcription in this strain.

Unlike Vojtov et al. (41), who found a strong difference in exoprotein production between TSST-1-producing isolates and their isogenic non-TSST-1-producing strains in CYGP medium (Casamino Acids [10 g/liter]; yeast extract [10 g/liter], NaCl [5 g/liter], 20% glucose, 1.5 M phosphoglycerate), we did not observe such differences between the TSST-1-producing derivatives of strain Newman and the parental strain in LB medium (Fig. 6A). Transducing the *tst*-expressing plasmids into strain RN450 (NCTC8325-4 $agr^+$), a background similar to the one used by Vojtov et al. (41), did not change the

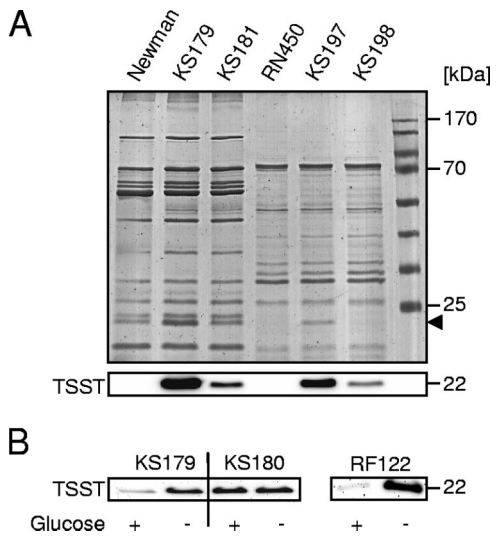


FIG. 6. Influence of TSST-1 production on exoprotein patterns and influence of *ccpA* deletion on TSST-1 production in response to glucose. (A) Exoprotein patterns and TSST-1 production of strains Newman, KS179 (Newman $tst_{N315}$), KS181 (Newman $tst_{RF122}$), RN450, KS197 (RN450/pSKA20 $tst_{N315}$), and KS198 (RN450/pSKA21$tst_{RF122}$) at an $OD_{600}$ of 3.5. The arrow indicates the position of TSST-1. (B) Comparison of TSST-1 production in response to glucose in strains KS179 (Newman $tst_{N315}$), KS180 (Newman Δ*ccpA* $tst_{N315}$), and RF122 after 3 h of growth.

exoprotein pattern in comparison to that of the wild type (Fig. 6A), suggesting that this effect may be medium dependent. As already found in the Newman background, *tst* transcription and TSST-1 production (Fig. 6A) were stronger when RN450 carried the $tst_{N315}$ gene than when it carried $tst_{RF122}$.

**Glucose affects TSST-1 production in a CcpA-dependent manner.** To confirm that the observed glucose-mediated repression of *tst* transcription also affected TSST-1 production, we determined the TSST-1 contents in the supernatants of KS179 (Newman $tst_{N315}$) and KS180 (Newman Δ*ccpA* $tst_{N315}$) cell cultures after 3 h of growth in the presence and absence of 10 mM glucose. In the presence of glucose, KS179 reached a higher $OD_{600}$ than that in the absence of glucose, while no such difference in $OD_{600}$ was found for strain KS180. In line with the transcriptional data, we found a clear decrease in TSST-1 in the supernatant of strain KS179 grown in the presence of glucose, while the TSST-1 levels in the supernatants of the Δ*ccpA* mutant KS180 were almost identical in the presence and absence of glucose (Fig. 6B). Glucose also led to a higher $OD_{600}$ in strain RF122 and repressed TSST-1 production (Fig. 6B).

**Repression of *tst* expression by CcpA in an *agr*-null mutant.** Strain N315 was previously reported to have defective RNAIII production (36). Since RNAIII is a known inducer of *tst* transcription (3, 28), this may explain the low *tst* transcription levels in N315. We confirmed that *agr* inactivation in strain Newman strongly reduced $tst_{N315}$ and $tst_{RF122}$ transcription (data not shown). The low level of *tst* transcription observed in N315, which harbors a functional CcpA system, might therefore be due, at least in part, to the lack of RNAIII. Nevertheless, by luciferase assays, we observed glucose-mediated repression of *tst* promoter-reporter gene activity in the Δ*agr* mutant of strain Newman (Fig. 4), indicating that the glucose-mediated repression by CcpA remained functional in the Δ*agr* mutant. This suggests that expression of *tst* depends on dual, direct RNAIII stimulation and glucose-mediated CcpA repression and on indirect CcpA-mediated control of RNAIII levels. However, we cannot rule out that additional factors may influence *tst* transcription, as N315 differs in terms of metabolism from strain Newman by being unable to catabolize acetate in the post-exponential growth phase (36). This loss of secondary metabolite catabolism might have consequences on *tst* expression. Also, regulatory circuits which may be present in strain N315 but not in the other strains could be the reason for the low *tst* expression in N315.

**Conclusion.** The expression of *S. aureus* virulence genes is regulated by complex networks in which global regulators such as the *agr* system and the *sarA* family play a central role. We recently showed that CcpA is also involved in the regulation of major virulence determinants by influencing the expression of *hla*, *spa*, and RNAIII (35). By demonstrating that the expression of *tst* is affected by CcpA as well, we add another important virulence factor to the group of CcpA-regulated genes/operons. The significance of glucose-mediated TSST-1 expression in vivo is unknown and remains to be evaluated using appropriate *S. aureus* constructs in wild-type and diabetic animal pathogenesis models, with the latter elaborating elevated blood glucose levels.

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