A direct link between carbohydrate utilization and virulence in the major human pathogen group A *Streptococcus*

Samuel A. Shelburne III*, David Keith*, Nicola Horstmann†, Paul Sumby‡, Michael T. Davenport*, Edward A. Graviss§, Richard G. Brennan†, and James M. Musser‡¶

Departments of *Medicine and §Pathology, Baylor College of Medicine, Houston, TX 77030; †Department of Biochemistry and Molecular Biology, M.D. Anderson Cancer Center, University of Texas, Houston, TX 77030; and ‡Center for Molecular and Translational Human Infectious Diseases Research, The Methodist Hospital Research Institute, and Department of Pathology, The Methodist Hospital, Houston, TX 77030

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Although central to pathogenesis, the molecular mechanisms used by microbes to regulate virulence factor production in specific environments during host–pathogen interaction are poorly defined. Several recent *ex vivo* and *in vivo* studies have found that the level of group A *Streptococcus* (GAS) virulence factor gene transcripts is temporally related to altered expression of genes encoding carbohydrate utilization proteins. These findings stimulated us to analyze the role in pathogenesis of catabolite control protein A (CcpA), a GAS ortholog of a key global regulator of carbohydrate metabolism in *Bacillus subtilis*. Inasmuch as the genomewide effects of CcpA in a human pathogen are unknown, we analyzed the transcriptome of a Δ*ccpA* isogenic mutant strain grown in nutrient-rich medium. CcpA influences the transcript levels of many carbohydrate utilization genes and several well characterized GAS virulence factors, including the potent cytolysin streptolysin S. Compared with the wild-type parental strain, the Δ*ccpA* isogenic mutant strain was significantly less virulent in a mouse model of invasive infection. Moreover, the isogenic mutant strain was significantly impaired in ability to colonize the mouse oropharynx. When grown in human saliva, a nutrient-limited environment, CcpA influenced production of several key virulence factors not influenced during growth in nutrient-rich medium. Purified recombinant CcpA bound to the promoter region of the gene encoding streptolysin S. Our discovery that GAS virulence and complex carbohydrate utilization are directly linked through CcpA provides enhanced understanding of a mechanism used by a Gram-positive pathogen to modulate virulence factor production in specific environments.

ccpA | pharyngitis | regulation | streptolysin | transcriptome

Investigations in bacterial pathogens have suggested close links between basic metabolic processes and microbial pathogenesis (1, 2). For example, bacteria alter transcription of carbohydrate utilization genes and virulence factor production in response to changes in environmental conditions encountered during infection in humans (3, 4). Therefore, it is reasonable to speculate that pathogenic bacteria have developed molecular strategies to directly link regulation of carbohydrate utilization and virulence factor production. However, the mechanisms underlying such relationships are largely undefined.

In *Bacillus subtilis*, alterations in gene transcription in response to environmental carbohydrate concentrations are controlled in part by catabolite control protein A (CcpA), which binds to DNA at catabolite response element (*cre*) sites (5). Binding of CcpA to *cre* sites is enhanced by interaction of CcpA with the phosphoprotein HPr-Ser-46-P, the phosphorlyation state of which in turn is affected by uptake of glucose and other readily metabolized carbohydrates by phosphotransferase (PTS) systems (6). Thus, in *Bacillus* spp., CcpA directly links environmental carbohydrate levels with transcriptional regulation of carbohydrate utilization genes. Most studies of CcpA have been conducted in *Bacillus* spp. (5, 7). Several Gram-positive pathogens encode proteins with significant homology to *B. subtilis* CcpA and HPr suggesting that similar molecular processes may occur in other microbes (8–11).

Group A *Streptococcus* (GAS) causes diverse infections in humans ranging from colonization and uncomplicated pharyngeal and skin infections to necrotizing fasciitis and toxic shock syndrome (12). The diversity in routes and manifestations suggests that GAS colonization and infection involve complex regulatory networks that are differentially regulated in distinct environments (13). In fact, recent genomewide investigations of GAS gene expression have demonstrated that GAS responds to different environments by altering the transcription of genes involved in meeting basic metabolic demands and differential transcription of genes encoding major virulence factors (14–17). These studies have resulted in a new understanding of the relationship between metabolism and virulence in GAS. Here, we report the results of studies that extend this understanding to the molecular level.

Results

Comparison of GAS Gene Transcript Levels in Saliva and a Nutrient-Rich Medium. Genomewide transcriptome analyses have suggested that differences exist in GAS gene expression during interaction with saliva and the oropharynx compared with growth in laboratory media, but no direct comparison has been done (15, 17). We used real-time TaqMan quantitative reverse transcription (QRT) PCR to test the hypothesis that GAS gene transcript levels differ significantly during growth in human saliva, a major component of innate and acquired immunity in the oropharynx, compared with growth in Todd–Hewitt broth with yeast extract (THY). We measured the transcript levels of 78 GAS genes encoding transcription regulators or proteins with either a known or putative extracellular location because of the likelihood such genes are involved in host–pathogen interaction [see supporting information (SI) Table 1]. Fifty-nine of the 78 genes had at least a twofold significantly different transcript level between the two media for at least one of the time points measured (select genes are shown in SI Fig. 7 with gene functions

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The authors declare no conflict of interest.

Data deposition: Expression microarray data have been deposited in the Gene Expression Omnibus (GEO) database at NCBI, http://www.ncbi.nlm.nih.gov/geo (accession no. GSE10156).

¶To whom correspondence should be addressed. E-mail: jmmusser@tmhs.org.

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available in SI Table 1). Differences in gene transcript levels between saliva and THY media were especially prominent in the early-exponential growth phase, as 50 genes had significantly different transcript levels at the time point studied. A key finding was that, at the early time point, we observed ≈10-fold increase in the *ccpA* transcript level in saliva compared with THY. Together, the data show that GAS markedly alters its transcript profile in response to human saliva and suggest that CcpA mediates some of the observed differences.

Inactivation of the *ccpA* Gene Results in Medium-Specific Growth Defects. To test the hypothesis that CcpA directly mediates some of the observed transcript differences we created isogenic mutant strain Δ*ccpA* from wild-type serotype M1 strain MGAS5005 (confirmatory Southern blot shown in SI Fig. 8). We genetically complemented the Δ*ccpA* isogenic mutant strain by using a plasmid capable of replicating in GAS to make strain comp*ΔccpA*. The growth curves for the three strains in THY were superimposable (SI Table 2 and SI Fig. 9). Conversely, compared with wild-type strain MGAS5005, the Δ*ccpA* mutant strain had a prolonged lag phase in a glucose medium and an increased (slower) doubling time in a maltose medium (Fig. 9 *B* and *C*). Moreover, the Δ*ccpA* strain did not reach as high a cell density or maintain as many viable colony-forming units (CFUs) in human saliva as wild-type strain MGAS5005 (Fig. 9*D*). Gram stain of the Δ*ccpA* isogenic mutant strain grown in human saliva did not reveal enhanced clumping or increased chain length, two possibilities to explain the observed CFU differences (data not shown). There was no significant difference in growth between strain MGAS5005 and the comp*ΔccpA* strain in any of the media tested.

The Δ*ccpA* Isogenic Mutant Strain Has an Altered Colony Morphology Associated with Differential Expression of *pel/sagA* and *hasA* Transcripts. The Δ*ccpA* mutant strain had altered colony morphology and hemolytic phenotype compared with wild-type strain MGAS5005 when grown on blood agar plates (Fig. 1*A*). The colony diameter (1.7 ± 0.1 mm) of the Δ*ccpA* strain was significantly less than that of wild-type strain MGAS5005 (2.6 ± 0.3 mm) or the comp*ΔccpA* strain (2.4 ± 0.4 mm, $P < 0.001$). The observed difference in colony diameter was not caused by a reduced number of bacterial cells, because there was no significant difference in CFUs within colonies of the three strains ($1.45 \pm 0.3 \times 10^9$, $1.21 \pm 0.51 \times 10^9$, $1.33 \pm 0.49 \times 10^9$ for strain MGAS5005, the Δ*ccpA* strain, and comp*ΔccpA* strains, respectively, $P = 0.461$). In addition, the radius of the zone of β-hemolysis surrounding the Δ*ccpA* mutant strain (1.7 ± 0.1 mm) was significantly larger than the zone around wild-type strain MGAS5005 (0.4 ± 0.1 mm) and the comp*ΔccpA* strain (0.6 mm ± 0.2 mm, $P = 0.005$).

The β-hemolytic properties and colony morphology of GAS are determined in part by streptolysin S activity and capsule size, respectively (18, 19). Thus, we used real-time TaqMan QRT-PCR to test the hypothesis that the Δ*ccpA* strain had higher levels of *sagA* transcript, the first gene in a nine-gene operon responsible for streptolysin S production, and lower transcript levels of *hasA*, the first gene in a three-gene operon of capsule synthesis genes. Consistent with the hypothesis, the level of *sagA* transcript was increased ≈20-fold in the Δ*ccpA* mutant strain compared with wild-type strain MGAS5005 (Fig. 1*B*). Moreover, the *hasA* transcript was significantly decreased in the Δ*ccpA* mutant strain compared with its parental strain at both time points. Although not studied in strain MGAS5005, in another GAS strain the *sagA* gene is transcribed as part of an RNA molecule with regulatory activity known as the pleiotropic effect locus (*pel*) (20). Based on QRT-PCR analysis, we found no significant difference in the transcript level of *pel* and *sagA*, consistent with the hypothesis that *sagA* is part of the larger *pel* transcript in strain MGAS5005

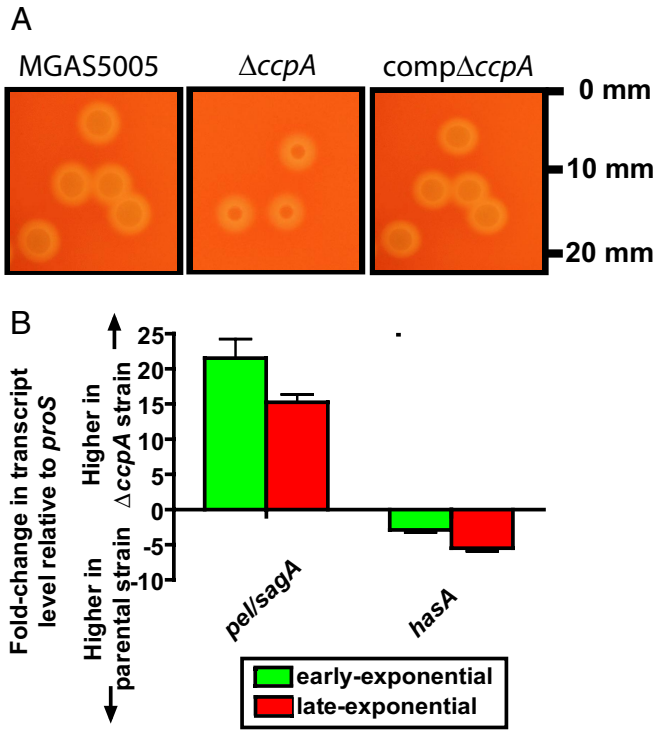

Fig. 1. CcpA influences streptolysins and hyalouronic acid capsule expression. (*A*) Colony phenotype of strain MGAS5005, its Δ*ccpA* mutant derivative strain, and comp*ΔccpA* strain on sheep blood agar plates. The Δ*ccpA* strain has significantly increased hemolysis and decreased colony size compared with strain MGAS5005 and strain comp*ΔccpA*. All three images are of same magnification with size indications at right. (*B*) Changes in colony hemolysis and size were associated with altered *pel/sagA* and *hasA* gene transcript levels. Strain MGAS5005 and Δ*ccpA* were grown in THY to early- and late-logarithmic growth phase, and *pel/sagA* and *hasA* transcript levels were measured by real-time TaqMan QRT-PCR with the $\Delta\Delta C_T$ method. Values above the *x* axis indicate higher gene transcript levels in the Δ*ccpA* strain, whereas values below the *x* axis indicate higher gene transcript levels in strain MGAS5005. Error bars indicate standard deviation among quadruplicate samples done on two separate occasions.

(data not shown). There was no significant difference in *pel/sagA* or *hasA* transcript level between the wild-type and comp*ΔccpA* strains (data not shown). Therefore, we conclude that CcpA directly or indirectly influences the transcript levels of GAS genes encoding critical virulence factors.

Analysis of the CcpA Transcriptome. To test the hypothesis that CcpA influences a broader array of GAS genes, we next compared the genomewide transcriptome of wild-type strain MGAS5005 and the Δ*ccpA* isogenic mutant strain grown in THY. After accounting for multiple comparisons, in the early-exponential growth phase the level of transcripts of ≈20% of all ORFs differed between the wild-type and Δ*ccpA* isogenic mutant strain (Fig. 2*A*, SI Table 3). In the late-exponential phase ≈10% of all ORFs were differentially expressed.

The largest number of transcripts differentially expressed between the two isogenic strains encode proteins known to be or putatively involved in carbohydrate transport and metabolism (Fig. 2*B*, SI Table 3). The differentially expressed genes included ATP-binding cassette (ABC) transporters or PTS operons responsible for glucose, lactose, maltodextrin, mannose, fructose, cellobiose, lactose, galactose, tagatose, and sialic acid transport. The transcript levels of genes in all of the aforementioned operons were increased in the Δ*ccpA* strain except for the maltodextrin operon. The decreased level of the maltodextrin

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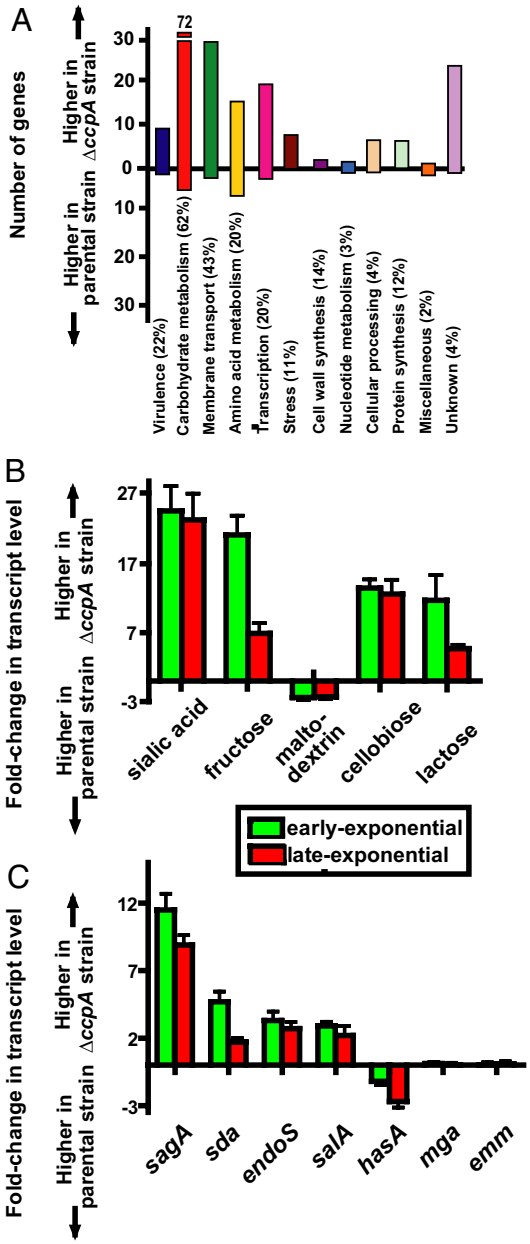

Fig. 2. Analysis of the CcpA transcriptome. Strain MGAS5005 and Δ*ccpA* were grown to early- and late-logarithmic growth phase in THY, and expression microarray analysis was performed as described in *Materials and Methods*. Values above the *x* axis indicate higher gene transcript levels in the Δ*ccpA* strain and values below the *x* axis indicate higher gene transcript levels in wild-type strain MGAS5005. (*A*) Summary of genes with altered transcript levels grouped by functional category. Percentage numbers refer to genes affected by CcpA inactivation/total number of genes in that particular category in the entire GAS genome. (*B*) Genes encoding carbohydrate utilization proteins. Shown are the mean fold changes in transcript levels of genes in an operon known or putatively involved in the transport and/or metabolism of the indicated carbohydrate. The transcript levels of all genes in the indicated operons were similarly affected. (*C*) Mean transcript levels of genes encoding select virulence factors. For *B* and *C*, error bars show standard deviation among four samples.

operon in the Δ*ccpA* strain is in accord with the decreased growth of the Δ*ccpA* strain in a maltodextrin medium (SI Fig. 9*C*). We also observed significant differences in transcript levels of 21 genes encoding known and putative transcriptional regulators. Six of these genes comprise three two-component (TCS) gene regulatory systems, including M5005_spy0784/5, M5005_spy1305/6, and *sptR*/*sptS*. M5005_spy0784/5 positively influences a putative mannose/fructose phosphotransferase system, whereas *sptR*/*sptS* affects carbohydrate metabolism and virulence factor production in human saliva (15, 21).

A third major category of genes affected in the Δ*ccpA* mutant strain included those encoding proteins putatively or known to be involved in GAS virulence. As predicted from QRT-PCR data, the transcript levels of the nine-gene operon encoding streptolysin S were significantly elevated in the Δ*ccpA* strain, whereas the entire *hasABC* operon involved in capsule synthesis was significantly down-regulated in the Δ*ccpA* strain (Fig. 2*C*). Other virulence factors affected by *ccpA* inactivation included *spd*, which encodes an extracellular DNase, and *endoS*, which encodes a protein that cleaves human Ig (22, 23). No significant difference in transcript levels was observed for *emm*, which encodes the anti-opsonophagocytic M protein, or *mga*, which encodes a transcriptional regulator involved in up-regulation of several virulence factors (Fig. 2*C*). Taken together, we conclude that CcpA is a global regulator of carbohydrate metabolism in GAS and has important effects on regulation of genes encoding transcriptional regulators and major virulence factors.

CcpA Contributes to GAS Virulence and Ability to Colonize the Mouse Oropharynx. In light of our findings that CcpA affects the transcript levels of multiple GAS virulence factors, we next tested the hypothesis that the Δ*ccpA* isogenic mutant strain was less virulent for mice than for the wild-type parental strain. Consistent with the hypothesis, significantly more mice inoculated i.p. with wild-type strain MGAS5005 died than with the Δ*ccpA* isogenic mutant strain ($P < 0.001$; Fig. 3*A*). Similarly, the comp*ΔccpA* strain was significantly more virulent than the Δ*ccpA* strain ($P < 0.001$) and as virulent as strain MGAS5005 ($P = 0.072$).

Next, we tested the hypothesis that *ccpA* contributes to the ability of GAS to colonize the mouse oropharynx. As hypothesized, after intranasal inoculation, the percentage of mice colonized over time with strain MGAS5005 was significantly greater than the Δ*ccpA* mutant strain ($P < 0.001$; Fig. 3*B*). Similarly, the average number of CFUs recovered from mice inoculated with strain MGAS5005 was significantly greater than with the Δ*ccpA* mutant strain ($P < 0.001$; data not shown). The comp*ΔccpA* strain was recovered at significantly higher CFUs and from more mice compared with the Δ*ccpA* strain ($P < 0.01$ for both). Taken together, these data indicate that CcpA contributes to GAS virulence and ability to colonize the mouse oropharynx.

CcpA Affects the Transcript Levels of GAS Virulence Factors During Growth in Human Saliva. We next sought to determine whether, under glucose-limiting conditions, CcpA influences the transcript levels of genes encoding virulence factors not influenced by CcpA in glucose-rich conditions (e.g., THY). To this end, we tested the transcript levels of several GAS virulence factors in strain MGAS5005 and the Δ*ccpA* strain during growth in human saliva, a glucose-poor medium. The transcript levels of *speB* (encoding a cysteine protease), *mac* (encoding an Ig-degrading enzyme), and *spd3* (encoding a DNase) were significantly higher in strain MGAS5005 than in the Δ*ccpA* strain during growth in human saliva (Fig. 4). These data demonstrate that CcpA is needed for GAS to respond to human saliva by increasing transcript levels of virulence factors shown to affect the ability of GAS to persist in human saliva (*speB*) and cause pharyngitis in a non-human primate model (*spd3*) (23, 24).

Recombinant GAS CcpA Binds to the Streptolysin S Promoter Region. When complexed with its coeffector HPr-Ser-46-P, CcpA functions by binding to cognate DNA sites (*cre*) in the promoter

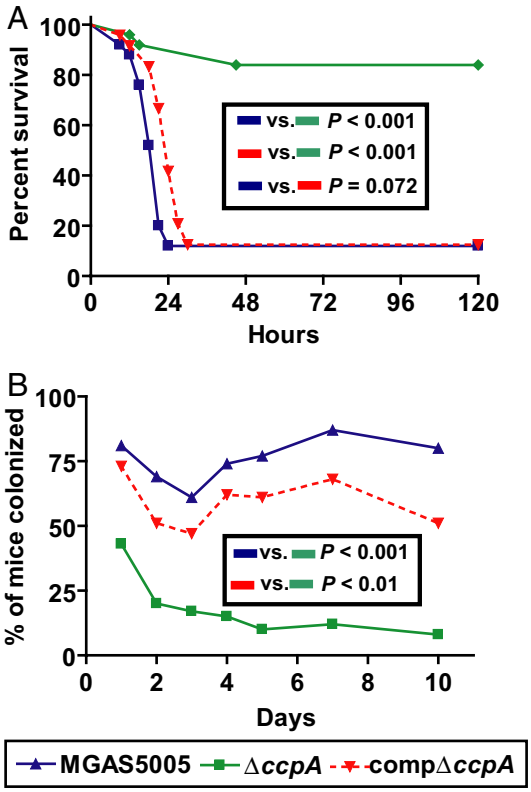

Fig. 3. Inactivation of CcpA significantly decreases GAS virulence. (*A*) Invasive disease model. Twenty-five adult outbred CD-1 mice per group were inoculated i.p. with $\approx 1 \times 10^7$ CFU of the indicated strains. Percent survival is graphed with *P* values for Kaplan–Meier survival analysis. (*B*) Oropharyngeal colonization model. Adult outbred CD-1 mice (35 per group) were inoculated intranasally with $\approx 1.0 \times 10^7$ CFU of the indicated strains. Mice oropharynxes were swabbed daily. Percentage of mice with GAS isolated by day with *P* values shown for repeated measures analysis.

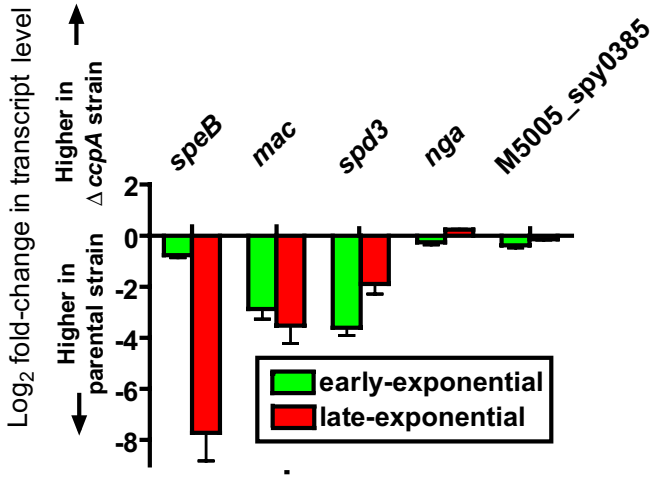

Fig. 4. CcpA affects GAS virulence factor production in glucose-limited medium. Gene transcript levels were measured by TaqMan real-time QRT-PCR in strains MGAS5005 and Δ*ccpA* grown to early- and late-logarithmic growth phase in human saliva. Bars indicate $\log_2$ differences in gene transcript levels between the two strains with error bars showing standard deviation for quadruplicate samples done on two separate occasions. Values above the *x* axis indicate higher gene transcript levels in the Δ*ccpA* strain and values below the *x* axis indicate higher gene transcript levels in strain MGAS5005.

region or within target genes in *B. subtilis* (25, 26). A bioinformatic analysis of the genome of strain MGAS5005 identified 37 sites that contain a *B. subtilis* consensus *cre* sequence (TGWAANCGNTNWCA; see *SI Text* and SI Table 4). To elucidate whether CcpA exerts its regulatory effect on virulence factor production by direct DNA interaction, we analyzed the binding of purified recombinant GAS CcpA (SI Fig. 10) to DNA sequences in the promoter region of three different genes by fluorescence polarization: (*i*) *pel/sagA*, a virulence gene whose expression was repressed by CcpA in our transcriptome analysis; (*ii*) *lctO*, which contains a consensus *cre* sequence and was differentially transcribed between strain MGAS5005 and the Δ*ccpA* mutant strain and thus served as a positive control; and (*iii*) *ftsX*, a gene that does not contain a consensus *cre* sequence, was not differentially expressed in the transcriptome analysis, and therefore was chosen as a negative control (SI Table 5). GAS CcpA bound specifically to the *pel/sagA* DNA (Fig. 5). The binding affinity was specific (K_d = 950 ± 98 nM, assuming a 100% active CcpA) and enhanced ≈65-fold by the presence of 50 μM HPr-Ser-46-P (K_d = 14.5 ± 2.2 nM), further supporting the notion that CcpA binds to the *pel/sagA* promoter *in vivo* and controls its transcription. Nonphosphorylated HPr did not increase the CcpA–DNA interaction (data not shown). Similar results were found for *lctO*, and, as expected, specific binding was not observed for the promoter region of *ftsX* (SI Fig. 11). Thus, binding of CcpA to the promoter region of *pel/sagA* demonstrates a direct mechanism of CcpA regulation of a key GAS virulence factor.

Discussion

Although CcpA orthologs have been investigated in other Gram-positive organisms, before this study the CcpA transcriptome had not been determined in a human pathogen (8, 9, 27, 28). A key discovery was that CcpA influenced the transcript levels of several GAS virulence factors, including the potent cytolysin streptolysin S, the extracellular DNase Spd, and the Ig-degrading EndoS. In *Bacillus* spp., transport of glucose or other readily metabolized carbohydrates through the PTS system leads to phosphorylation of the phosphocarrier protein HPr at residue Ser-46, resulting in HPr-Ser-46-P (6). HPr-Ser-46-P serves as a

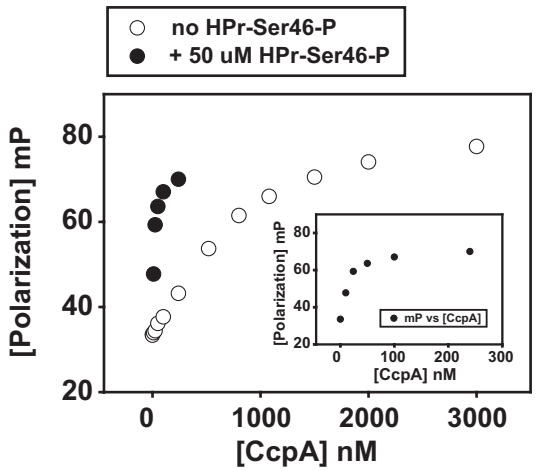

Fig. 5. The CcpA–(HPr-Ser-46-P) complex interacts specifically with the promoter region of *pel/sagA*. Purified GAS CcpA was titrated to 1 nM *pel/sagA cre* in the absence (open circles) and presence (closed circles) of 50 μM HPr-Ser-46-P. Millipolarization units (mP) are plotted against the CcpA concentration. The shift to the left in the binding curve demonstrates stimulation of DNA–CcpA complex formation by HPr-Ser-46P (K_Ds for DNA interaction are 948 ± 89 nM and 14.5 ± 2.2 nM with and without HPr-Ser-46P, respectively). (*Inset*) Shown is relationship between CcpA concentration and mP units in the presence of HPr-Ser-46-P to clarify that saturation is already reached at a CcpA concentration of 240 nM.

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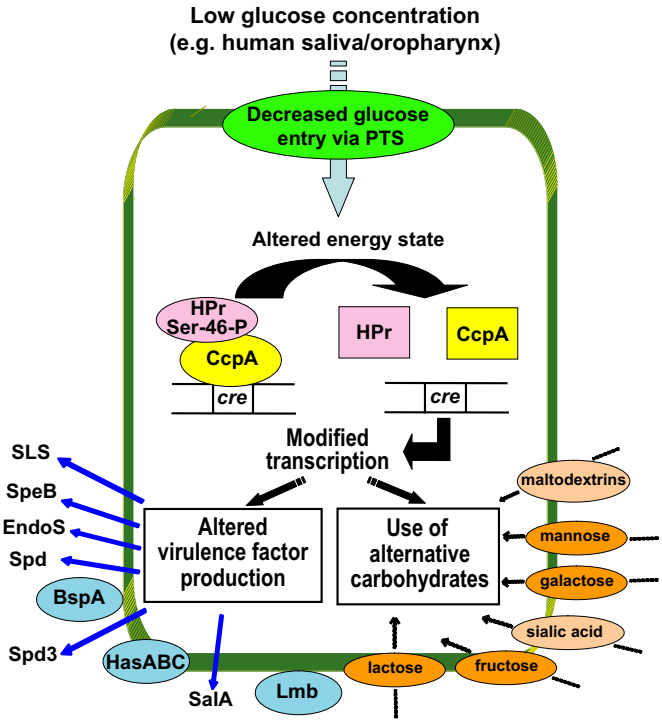

Fig. 6. Hypothesis explaining how CcpA affects GAS carbohydrate utilization and virulence factor production. Decreased extracellular glucose levels result in dephosphorylation of HPr-Ser-46-P leading to dissociation of HPr and CcpA. CcpA/HPr dissociation results in changes in CcpA binding to DNA catabolite response element sites (*cre*) and altered transcription. Systems involved in the uptake and utilization of specific carbohydrates are shown on the right side of the figure. Phosphotransferase systems are shown in orange and ATP-binding cassette transport systems are in tan. On the left side of the figure, in blue, are virulence factors with the relationship of the protein names to the cell surface corresponding to their inferred location (e.g., actively secreted, anchored to the cell wall, or embedded in the cell membrane). Putative or known functions of affected virulence factors: SLS, cytolysin; SpeB, cysteine protease; EndoS, Ig cleavage; Spd and Spd3, DNases; BspA, epithelial cell-binding protein; HasABC, hyaluronic acid capsule production; SalA, salivaricin production; Lmb, laminin binding protein.

protein coeffector for CcpA that mediates CcpA binding to *cre* sites, which ultimately results in altered gene expression (26). Strain MGAS5005 contains a gene (M5005_spy1121) with 66% identity and 82% similarity at the amino acid level to HPr in *Bacillus subtilis* subsp. *subtilis* strain 168, suggesting that similar CcpA pathways are likely to function in GAS. Our GAS data suggest a model that, when environmental glucose levels are low, such as in the human oropharynx and saliva, CcpA-(HPr-Ser-46-P)-*cre* interaction is relieved, thus allowing transcription of carbohydrate utilization genes and virulence factors vital to host–pathogen interaction (Fig. 6).

Although CcpA has been shown to influence virulence factor production in other Gram-positive pathogens, there is no information available regarding the mechanism by which this occurs (8, 27, 28). Our finding that CcpA binds to the *pel*/*sagA* promoter region demonstrates a direct link in GAS between environmental carbohydrate concentrations and virulence factor production. In light of the known lytic effect of streptolysin S on neutrophils, the data suggest that the nutritional status of GAS may serve as a mechanism for sensing when key virulence factors are needed to inhibit host defenses (29). Alternatively, streptolysin S may have a unappreciated role in nutrient acquisition. Because *sagA* is transcribed as part of the larger regulatory RNA *pel*, our data demonstrating that CcpA affects transcript levels of an RNA regulatory system are in accordance with a study in *Staphylococcus aureus* in which CcpA influenced transcript levels of *RNAIII*, the RNA effector molecule of the *agr* system (8, 20). Taken together, our findings substantiate the idea that in Gram-positive pathogens CcpA interacts with RNA regulatory systems, the importance of which are being increasingly appreciated in both humans and microbes.

Finally, we also discovered that CcpA positively influences the transcript level of several virulence factors, including *speB*, during growth in the nutrient-limited medium of human saliva. Given that human saliva is the first substance with which GAS interacts in the oropharynx, these data strongly suggest that GAS CcpA is required for the up-regulation of key virulence factors during the initial stages of host–pathogen interaction (24). The absence of increased production of key virulence factors early during pharyngeal infection may explain the relative inability of the Δ*ccpA* mutant strain to colonize the oropharynx. Thus, our data demonstrate the GAS CcpA directly represses production of virulence factors, such as streptolysin S, under nutrient-rich conditions and augments production of other virulence factors, such as SpeB, under nutrient-limited conditions, thereby providing a key mechanism by which GAS responds to changing environments (Fig. 6).

Human pathogenic microbes differentially regulate production of key virulence factors *in vivo*, a hallmark of pathogen–host interaction. We have discovered that the major human pathogen GAS modulates virulence factor production required for survival and infectivity by a CcpA-mediated pathway. Given the highly conserved nature of CcpA, similar genomewide studies of other Gram-positive pathogens may yield enhanced understanding of links between basic metabolic processes and pathogenesis.

Materials and Methods

Bacterial Strains and Culture Media. Serotype M1 strain MGAS5005 is genetically representative of the clone responsible for most contemporary (post-1987) human infections; its genome has been sequenced (30). The Δ*ccpA* isogenic mutant strain was created from parental serotype M1 strain MGAS5005 by nonpolar insertional mutagenesis. We used pDC123, a plasmid capable of replicating in GAS, to genetically complement the Δ*ccpA* strain *in trans* to create the comp*ccpA* strain (*SI Text*). Ultrapure carbohydrates (Sigma) were added at a concentration of 0.5% (wt/vol) to a carbohydrate-free preparation of a commercially available, chemically defined medium (CDM; SAFC Biosciences) to create glucose medium, maltose medium, etc. Growth in human saliva was performed with specimens collected from healthy adult volunteers under a protocol approved by the Baylor College of Medicine Institutional Review Board (24).

RNA Isolation, TaqMan Transcript Level Analysis, and Expression Microarray Analysis. RNA was purified from a minimum of four replicate cultures by using an RNeasy kit (Qiagen). The concentration and quality of RNA were assessed with an Agilent 2100 Bioanalyzer and analysis of the A_{260}/A_{280} ratio. Select gene transcript level analysis was performed with TaqMan real-time QRT-PCR (primers and probes listed in SI Table 6). A custom-made Affymetrix GeneChip that contains 100% of the ORFs of strain MGAS5005 was used for expression microarray (transcriptome) studies. Principal component analysis (PCA) indicated that the data were of high quality and that the two time points provided distinct information regarding GAS gene transcripts differentially expressed between these two strains (SI Fig. 12). To compare gene transcript levels between the wild-type and mutant strain, a two-sample *t* test (unequal variance) was applied, followed by a false discovery rate correction ($Q < 0.05$) to account for multiple testing. Genes were considered differentially transcribed if the *t* test had a corrected *P* value of <0.05 and the difference in mean gene transcript level was at least 2-fold. For further information on assessment of transcript levels see *SI Text*.

Mouse Virulence Studies. Mouse experiments were performed according to protocols approved by the Baylor College of Medicine Institutional Animal Care and Use Committee. For the invasive disease model, 25 female outbred CD-1 Swiss mice (Harlan–Sprague–Dawley) were injected i.p. with $\approx 2.5 \times 10^7$ GAS CFU (31). Throat colonization studies were performed by inoculating 35 mice in each group with 1×10^7 CFU of GAS (32). The throat of each mouse was swabbed before inoculation and then daily thereafter.

Purification and Binding Characteristics of CcpA. CcpA was purified to homogeneity from *Escherichia coli* (SI Fig. 10, *SI Text*). Fluorescence anisotropy was used to determine the binding characteristics of CcpA to 5′-fluorescein-labeled oligonucleotides in the presence and absence of 50 μM HPr-Ser-46-P (33, 34; *SI Text*).

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