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Identification of Rgg Binding Sites in the *Streptococcus pyogenes* Chromosome▽†

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Streptococcus pyogenes **Rgg is a regulatory protein that controls the transcription of 588 genes in strain NZ131 during the post-exponential phase of growth, including the virulence-associated genes encoding the extracellular SpeB protease, pullulanase A (PulA), and two extracellular nucleases (SdaB and Spd-3). Rgg binds to DNA proximally to the *speB* promoter (P*speB*) to activate transcription; however, it is not known if Rgg binds to the promoters of other genes to influence expression, or if the perturbation of other global regulons accounts for the genome-wide changes in expression associated with the mutant. To address this issue, chromatin immunoprecipitation followed by DNA microarray analysis (ChIP-chip) was used to identify the DNA binding sites of Rgg. Rgg bound to 65 sites in the chromosome. Thirty-five were within noncoding DNA, and 43% of these were adjacent to genes previously identified as regulated by Rgg. Electrophoretic mobility shift assays were used to assess the binding of Rgg to a subset of sites bound *in vivo*, including the noncoding DNA upstream of *speB*, the genes encoding PulA, Spd-3, and a transcriptional regulator (SPY49_1113), and prophage-associated genes encoding a putative integrase (SPY49_0746) and a surface antigen (SPY49_0396). Rgg bound to all target DNAs *in vitro*, consistent with the *in vivo* results. Finally, analyses with a transcriptional reporter system showed that the DNA bound by Rgg contained an active promoter that was regulated by Rgg. Overall, the results indicate that Rgg binds specifically to multiple sites in the chromosome, including prophage DNA, to influence gene expression.**

The Gram-positive respiratory and skin pathogen *Streptococcus pyogenes* (group A streptococcus [GAS]) can be carried asymptomatically and is a common cause of uncomplicated pharyngitis, or "strep throat" (9). *S. pyogenes* also causes several life-threatening diseases, such as streptococcal toxic shock syndrome and necrotizing fasciitis, the so called "flesh-eating" disease (9). Prompt treatment of pharyngitis with antibiotics decreases the likelihood of dissemination and can prevent postinfection autoimmune sequelae such as acute rheumatic fever (ARF) (16). *S. pyogenes* also contributes to secondary bacterial pneumonia following influenza infections (2). During the recent H1N1 influenza A virus pandemic, 29% of deaths were attributed to secondary bacterial pneumonia, and of these, 27% were caused by *S. pyogenes* (2). Moreover, the incidence of invasive *S. pyogenes* disease increased significantly (26%) in December 2010 and January 2011 in England, due, at least in part, to widespread influenza infections (58). Overall, *S. pyogenes* causes more than 500,000 deaths each year worldwide, and no vaccine is available to prevent these deaths (9). Thus, the burden of the microbe on human health is significant.

The determination of the genome sequences of more than a dozen isolates shows that much, but not all, of the genetic variation among isolates is due to differences in the number and types of bacteriophages in the genome (4, 17). All isolates sequenced thus far are polylysogenized, with as many as eight bacteriophages in the chromosome, which can constitute as much as 10% of the genome (20). These lambda-like viruses belong to the family *Siphoviridae* and often encode important virulence factors, including superantigens, hyaluronidases, and secreted nucleases (8, 25, 41, 50). Although some of the bacteriophage-encoded virulence genes were previously shown to be expressed during the course of infection, relatively little is known about the molecular factors that control their expression (54).

Rgg, also known as RopB (11, 31), of *S. pyogenes* belongs to a family of transcriptional regulators (Rgg family; TIGR01716) that are encoded in the chromosomes of several species of low-G+C Gram-positive bacteria (11, 31, 40, 42, 44, 53). The proteins have a helix-turn-helix (HTH)-XRE domain in the amino terminus, which is presumably responsible for DNA binding, and various carboxyl-terminal domains, including a tetratricopeptide repeat (TPR), which may mediate binding to peptide pheromones (34). Rgg is similar to Cro/cI prophage regulators, which has led to speculation that they may be derived from an ancestral prophage (36). Typically, Rgg orthologues control the transcription of adjacent genes (40, 42, 44, 53), which encode secreted proteases (11), nucleases (19), bacteriocins (42, 43), and peptides involved in intraspecies communication (21, 34).

In *S. pyogenes*, Rgg activates the transcription of the adjacent gene encoding streptococcal pyrogenic exotoxin B (SpeB)

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in the post-exponential phase of growth (11). It also represses the transcription of the other adjacent gene, encoding streptodornase B (SdaB; also known as mitogenic factor-1 [MF-1]), which is a secreted nuclease (19). In the post-exponential phase of growth, inactivation of *rgg* in strain NZ131 alters the expression of more than 500 additional genes from that in the parental strain (19). Among these are several virulence and prophage-related genes, including secreted proteases, nucleases, and antigens localized to the cell surface (19). Inactivation of *rgg* in strain NZ131 also altered metabolism. Specifically, the mutant ferments arginine and degrades serine during the exponential phase of growth, in contrast to the wild-type (wt) strain, which is a homolactate fermenter (13). As a result, the pH of the growth medium of the mutant is more neutral due to less excretion of lactic acid and the production of ammonia, and the concentrations of metabolic precursors and end products in the medium differ between the two strains (13), which is likely to indirectly influence gene expression.

Neely et al. (40) showed that Rgg binds to the promoter region of *speB* (P*speB*) to activate transcription. With the exception of *speB*, it is not known if Rgg directly controls the expression of the other Rgg-regulated genes, or if differences in metabolism or the perturbation of other regulatory networks is responsible for the genome-wide changes in expression associated with the mutant strain. The aim of this study was to identify the DNA binding sites of Rgg on a genome scale *in vivo*, as an additional step in defining the regulon. The results indicate that Rgg binds to both coding and noncoding DNA and that the noncoding DNA bound by Rgg is upstream of genes associated with virulence, DNA repair, and metabolism and of prophage-associated genes, suggesting that Rgg directly regulates these genes.

MATERIALS AND METHODS

Bacterial strains, media, and growth conditions. The bacterial strains and plasmids used in this study are described in Table 1. *S. pyogenes* was grown at 37°C under a 5% CO_2 atmosphere without agitation in Todd-Hewitt broth (Becton Dickinson, Sparks, MD) containing 0.2% (wt/vol) yeast extract (THY broth). *Escherichia coli* strain DH5α was obtained from Gibco-BRL (Gaithersburg, MD) and was grown in Luria-Bertani medium at 37°C with agitation. Antibiotics were used at the following concentrations: carbenicillin at 100 μg/ml for *E. coli*, spectinomycin at 100 μg/ml for *E. coli* and *S. pyogenes*, erythromycin at 2.5 μg/ml for *S. pyogenes*, and kanamycin at 50 μg/ml for *E. coli* and 500 μg/ml for *S. pyogenes*.

DNA manipulation. Plasmid DNA was isolated from *E. coli* by alkaline lysis using either the QIAprep Spin Miniprep kit (Qiagen, Valencia, CA) or Maxi/Midi prep purification systems (Qiagen). DNA fragments were isolated from agarose gels using the SpinPrep Gel DNA kit (Novagen, Madison, WI). PCR was carried out with GoTaq DNA polymerase (Promega, Madison, WI). DNA sequencing was performed at the DNA facility at Iowa State University (Ames).

Construction of an *S. pyogenes* strain expressing a Myc-tagged Rgg fusion protein. A series of plasmids were constructed to create an *S. pyogenes* strain expressing an Rgg-Myc fusion protein (Table 1). An 843-bp region containing the *rgg* open reading frame (ORF) was obtained by PCR using genomic DNA (gDNA) from strain NZ131 (Table 1) as the template DNA with primers MycBamHI and MycXbaI (Table 2). The DNA was digested with BamHI and XbaI and was ligated with similarly digested plasmid pTrcHis2B (Invitrogen, Carlsbad, CA), which encodes Myc and 6× histidine epitope tags 3′ of the inserted ORF. The construct was designated pSA1 and was verified by PCR and DNA sequence analysis (data not shown). The *rgg* fusion protein ORF was subsequently subcloned into the streptococcal suicide vector pKK1 to create pSA3 (Table 1). The construct was also confirmed by DNA sequencing (data not shown).

The NZ131 *rgg* mutant (11) was transformed with pSA3 (Table 1), and integration into the native *rgg* locus via homologous recombination was confirmed by PCR using primers RGG-4, which anneals to chromosomal DNA, and VaFwd, which anneals to pSA3 (data not shown) (Table 1). The transformant was designated SA5 and encodes an Rgg-Myc-6×His fusion protein expressed by the native promoter.

ChIP. Overnight cultures of the *S. pyogenes rgg* mutant strain and SA5 were inoculated into 40 ml of THY broth in 50-ml tubes to an A_{600} of 0.08. The cultures were grown to either the exponential (A_{600}, ~0.35) or the post-exponential (A_{600}, ~0.60) phase of growth in the absence of antibiotics. Formaldehyde (1%, wt/vol) was added and incubated at room temperature for 30 min. Glycine (125 mM) was added to quench cross-linking, and cells were washed twice with 40 ml of phosphate-buffered saline (pH 7.3) and were centrifuged at 2,000 rpm for 30 min. The bacterial pellet was resuspended in 1 ml of immunoprecipitation (IP) buffer (50 mM Tris-HCl [pH 8.0], 150 mM NaCl, and 0.5% Triton X-100) supplemented with a mixture of protease inhibitors (Calbiochem, San Diego, CA). The samples were transferred to FastPROTEIN Blue tubes (MP Biomedicals, Solon, OH) and were lysed using a FastPrep FP120 device (Qbiogene, Inc., Carlsbad, CA). The lysates were centrifuged at 4,000 rpm for 10 min at 4°C, and the pellet containing the cross-linked chromatin was suspended in 1 ml IP buffer. The DNA was sheared by sonication, resulting in fragment sizes averaging 0.5 to 1 kb, as determined by agarose gel electrophoresis. Following sonication, DNA bound to Rgg was immunoprecipitated with a monoclonal antibody to Myc (Invitrogen) overnight at 4°C. A 50-μl volume of a 50% protein G-Sepharose slurry was added to the samples and was incubated at 4°C for 3 h, after which the slurry was washed four times with 1 ml of IP buffer and was resuspended in 150 μl of elution buffer (50 mM Tris-HCl [pH 8.0], 10 mM EDTA, and 1% sodium dodecyl sulfate [SDS]). The protein-DNA complexes were first eluted from the slurry by incubation at 65°C for 10 min and then centrifuged at 4,000 rpm for 10 min at 4°C, and the supernatant was collected. The formaldehyde-induced cross-links were reversed by incubation at 65°C overnight. The proteins and RNAs in the samples were degraded by incubation with 150 μl of Tris-EDTA buffer (TE) containing RNase A (10 mg/ml), proteinase K (100 μg/ml), and glycogen (0.27 mg/ml). The DNA was purified by phenol-chloroform extraction, precipitated with isopropanol, and washed with 70% ethanol. Immunoprecipitated DNA was suspended in 25 μl of sterile H_2O. The degree of enrichment of P*speB* and a portion of the *groEL* coding sequence in chromatin immunoprecipitation (ChIP) samples obtained from the *rgg* mutant and SA5 strains was determined with ABsolute SYBR green ROX Mix (ABgene House, Surrey, United Kingdom) using primers for the P*speB* region (speBfwd and speBrev) (Table 2) and the control region (groEL2fwd and groEL2rev) (Table 2). Enrichment of P*speB* was normalized to the amount of nonspecific *groEL* DNA in precipitated samples.

Amplification and labeling of ChIP DNA fragments. Immunoprecipitated DNA was amplified and labeled using the MessageAmp II-Bacteria kit (Ambion, Austin, TX) with some modifications. In brief, the immunoprecipitated DNA was incubated at 95°C for 10 min. The samples were polyadenylated by incubation with 0.5 μl of poly(A) tailing ATP and 0.5 μl of terminal transferase at 37°C for 15 min. First-strand cDNA was synthesized by incubation with 10 μl of reverse transcription master mix for 2 h at 42°C. Second-strand cDNA was synthesized by incubation with 80 μl of second-strand master mix for 2 h at 16°C. The cDNA was purified using a cDNA filter cartridge according to the manufacturer's instructions. To the cDNA, 7.5 μl each of biotin-11-CTP (10 mM) and biotin-11-UTP (10 mM) were added and concentrated to a final volume of 18 μl. Antisense RNA (aRNA) was synthesized by incubation with 22 μl of *in vitro* transcription master mix at 37°C for 14 h. aRNA was purified using an aRNA filter cartridge according to the manufacturer's instructions. The quality of aRNA was checked using Agilent RNA 6000 nanochips (Agilent Technologies, Santa Clara, CA) (data not shown). The concentration of aRNA was determined using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA).

DNA microarray hybridization and data analysis. Custom-designed Gene Chips representing the entire NZ131 genome were made by Affymetrix (Santa Clara, CA). The Gene Chips consist of 3,312 qualifiers representing 1,822 predicted open reading frames and 785 noncoding regions. In addition, 54 control oligonucleotides were used for spike-ins.

Labeled aRNA (10 μg) was hybridized to Gene Chips and was detected according to the manufacturer's instructions for antisense prokaryotic arrays (Affymetrix). The data from three experiments were normalized and analyzed using the Probe Logarithmic Intensity Error (PLIER) estimation algorithm in ArrayStar (DNAStar, Inc., Madison, WI). The regions that showed ≥2-fold enrichment in strain SA5 compared to the *rgg* mutant in all three biological

TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Description[a]	Source or reference
Strains		
E. coli		
DH5α	*hsdR17 recA1 gyrA endA1 relA1*	Invitrogen
Rosetta 2(DE3)	F^- *ompT hsdS*$_B$(r_B^- m_B^-) *gal dcm* (DE3) pRARE2 (Cam^r)	Novagen
S. pyogenes		
NZ131	M49 serotype	D.R. Martin, New Zealand
rgg mutant	NZ131 *rgg* mutant; Em^r	11
SA5	NZ131 *rgg* mutant complemented with pSA3; Em^r Kan^r; expresses an Rgg-Myc fusion protein	This study
wt::*luc* strain	NZ131 transformed with pKSM720; $Spec^r$	This study
wt::P*speB-luc* strain	NZ131 transformed with pSA11; $Spec^r$	This study
wt::P*spd3-luc* strain	NZ131 transformed with pSA12; $Spec^r$	This study
wt::P*pulA-luc* strain	NZ131 transformed with pSA13; $Spec^r$	This study
wt::P*396-luc* strain	NZ131 transformed with pSA14; $Spec^r$	This study
wt::P*746-luc* strain	NZ131 transformed with pSA15; $Spec^r$	This study
wt::P*1113-luc* strain	NZ131 transformed with pSA16; $Spec^r$	This study
rgg::*luc* strain	NZ131 *rgg* mutant transformed with pKSM720; $Spec^r$	This study
rgg::P*speB-luc* strain	NZ131 *rgg* mutant transformed with pSA11; $Spec^r$	This study
rgg::P*spd3-luc* strain	NZ131 *rgg* mutant transformed with pSA12; $Spec^r$	This study
rgg::P*pulA-luc* strain	NZ131 *rgg* mutant transformed with pSA13; $Spec^r$	This study
rgg::P*396-luc* strain	NZ131 *rgg* mutant transformed with pSA14; $Spec^r$	This study
rgg::P*746-luc* strain	NZ131 *rgg* mutant transformed with pSA15; $Spec^r$	This study
rgg::P*1113-luc* strain	NZ131 *rgg* mutant transformed with pSA16; $Spec^r$	This study
Plasmids		
pTrcHis2B	Cloning vector; Amp^r	Invitrogen
pSA1	*rgg* ORF cloned into pTrcHis2B; Amp^r	This study
pKK1	Shuttle vector defective for replication in *S. pyogenes*; Kan^r	26
pSA3	*rgg* ORF in frame with Myc and 6× histidine tags cloned into pKK; Kan^r	This study
pMAL-c4X	Cloning vector; Amp^r	New England BioLabs
pSA4	*rgg* ORF cloned into pMAL-c4X; Amp^r	This study
pSA5	Promoter region of *speB* cloned into pGEM-T-vector; Amp^r	This study
pSA6	Promoter region of *spd3* cloned into pGEM-T-vector; Amp^r	This study
pSA7	Promoter region of *pulA* cloned into pGEM-T-vector; Amp^r	This study
pSA8	Promoter region of *Spy49_0396* cloned into pGEM-T-vector; Amp^r	This study
pSA9	Promoter region of *Spy49_0746* cloned into pGEM-T-vector; Amp^r	This study
pSA10	Promoter region of *Spy49_1113* cloned into pGEM-T-vector; Amp^r	This study
pKSM720	GAS replicating plasmid with firefly luciferase and RBS; $Spec^r$	28
pSA11	Noncoding region upstream of *speB* cloned into pKSM720; $Spec^r$	This study
pSA12	Noncoding region upstream of *spd3* cloned into pKSM720; $Spec^r$	This study
pSA13	Noncoding region upstream of *pulA* cloned into pKSM720; $Spec^r$	This study
pSA14	Noncoding region upstream of *Spy49_0396* cloned into pKSM720; $Spec^r$	This study
pSA15	Noncoding region upstream of *Spy49_0746* cloned into pKSM720; $Spec^r$	This study
pSA16	Noncoding region upstream of *Spy49_1113* cloned into pKSM720; $Spec^r$	This study

[a] RBS, ribosome binding site.

replicates were considered potential areas of the chromosome directly bound by Rgg.

Multiple Em for Motif Elicitation (MEME), version 4.3, was used to search for motifs in the coding and the noncoding regions bound by Rgg *in vivo*. Searches were conducted with either the coding or the noncoding regions, or both, as the input sequence. The program was allowed to identify 6- to 50-bp motifs that occurred between 1 and 10 times per fragment on either strand of DNA. As a control, a similar number of coding and noncoding regions not bound by Rgg was selected, and a similar search was conducted using MEME. In addition, a similar strategy was used with the Gapped Local Alignment of Motifs (GLAM2), version 4.6.

Expression and purification of MBP-tagged Rgg fusion protein. An 843-bp region containing the *rgg* ORF was amplified by PCR from NZ131 gDNA (Table 1) using primers MBP-BamHIfwd and Rgg-stop-PstI (Table 2). The product was digested with BamHI and PstI and was ligated into similarly digested pMALc4x (New England BioLabs, Ipswich, MA) to produce pSA4 (Table 1). Following verification by PCR and DNA sequence analysis (data not shown), pSA4 was transformed into *E. coli* Rosetta 2(DE3) cells (Novagen). Expression of the maltose binding protein (MBP)-Rgg fusion protein was induced with 0.3 mM isopropyl-β-D-thiogalactopyranoside (IPTG) at an A_{600} of 0.5, and the cells were incubated for 3 h. The cultures were centrifuged at 4°C for 30 min at 8,000 rpm. The pellet was resuspended in column buffer (20 mM Tris-HCl, 200 mM NaCl, 1 mM EDTA, and 1 mM dithiothreitol [DTT]) and was stored overnight at −20°C. Bacteria were subsequently lysed by sonication and were centrifuged at 7,500 rpm for 30 min. The supernatant containing the soluble MBP-Rgg fusion protein was collected and purified using amylose resin under nondenaturing conditions according to the manufacturer's instructions (New England BioLabs). The eluants containing MBP-Rgg were concentrated using an Amicon Ultracel 3K centrifuge concentrator (Millipore, Billerica, MA). Glycerol was added to the pure protein to a final concentration of 20%, and the mixture was frozen at −80°C. The protein concentrations were determined using a Bio-Rad protein assay kit, and the purity of the protein was checked by SDS-polyacrylamide gel electrophoresis (PAGE).

Electrophoretic mobility shift assays (EMSA). The previously characterized promoter region of *speB* (40) and noncoding DNA upstream of *spd-3*, *pulA*, *Spy49_0396*, *Spy49_0746*, and *Spy49_1113* were amplified by PCR using NZ131 gDNA as template DNA with the following primer sets: speBfwd and speBrev,

TABLE 2. Primers used in this study

Primer	Sequence[a] (5′–3′)	Reference or source
Cloning		
MycBamHI	TAAACCATGGATCCGGAAATTGGTGAAACCG	This study
MycXbaI	GAGGATCTAGAAGGGACAGTTTATGTTTAATGGC	This study
MBP-BamHIfwd	TCCGGATCCATGGAAATTGGTGAAACCG	This study
Rgg-stop-PstI	AAAACTGCAGCCTCAGGACAGTTTATGT TTAATGG	40
RGG-4	GGCTATTGACCTTATGCACC	This study
VaFwd	CCCCTGATTCTGTGGATAACCGT	This study
EMSA and reporter fusions		
speBfwd	GCGGGCATAGTTTTATCAACTGTCATAT	This study
speBrev	GCGGGCATAGTTTTATCAACTGTCATA T	This study
groEL2fwd	GCTACTCGACGTAACATTGTG	This study
groEL2rev	GGAGCCTTCGTACCCAGCAT	This study
spd3fwd	GGCGTAGCATTTAAATAAACGGAA	This study
spd3rev	GGTGCCTGTAAAATACGAATAAATAAGT	This study
pulAfwd	GCGGATCCGATGCCTTTTGTCTCAAACTG	This study
pulArev	GCCTCGAGCAACTTGATGTGTTAAGATACTAG	This study
42KDa_BamHIfwd	GCGGATCCTAGTCGGTATCTAGAAGA	This study
42KDa_XhoIrev	GCCTCGAGGAGTTCAGACTTGATTTCTG	This study
1113_BamHIfwd	GCGGATCCCGTATCTGGACTGAGCAAAAC	This study
1113_XhoIrev	GCCTCGAGGCGGTTAATATTTTTGTCAC	This study
int_BamHIfwd	GCGGATCCCTACACACTATTACCAC	This study
int_XhoIrev	GCCTCGAGTGGTATTCGTGGCGTG	This study
pspeB_IRfwd	GCGGATCCATGTCAAGCCTTCCTAGTTGATG	This study
pspeB_IRrev	GCCTCGAGTTTTTTTATACCTCTTTCAAAATAAG	This study
pspd3_IRfwd	GCGGATCCGTCGGACTAGTCTATGACAAA	This study
pspd3_IRrev	GCCTCGAGATCCATGTCCTCCTTTTATTATTTAC	This study

[a] Underlining indicates restriction sites incorporated into the primer.

spd3fwd and spd3rev, pulAfwd and pulArev, 42KDa_BamHIfwd and 42KDa_XhoIrev, int_BamHIfwd and int_XhoIrev, and 1113_BamHIfwd and 1113_XhoIrev. The fragments designated as P*speB*, P*spd3*, P*pulA*, P*396*, P*746*, and P*1113* fragments were separated by gel electrophoresis, purified, and cloned into pGEM-T (Promega) to create pSA5, pSA6, pSA7, pSA8, pSA9, and pSA10 (Table 1), respectively. The putative promoter fragments were then excised by digesting the plasmids with BamHI and XhoI, gel purified, dephosphorylated, and end labeled with [γ-^{32}P]ATP using polynucleotide kinase. A similarly sized fragment of the *groEL* ORF was amplified from genomic DNA using groEL2fwd and groEL2rev (Table 2) and was gel purified for use as a nonspecific competing DNA. Various amounts of MBP-Rgg were incubated with the labeled putative promoter fragments in 25 μl of binding buffer (25 mM Tris-Cl [pH 7.5], 0.1 mM EDTA, 75 mM NaCl, 1 mM dithiothreitol, 10% glycerol, and 0.5 μg/ml calf thymus DNA) at room temperature for 20 min. Competition experiments were conducted by including the cold probe prior to protein addition. The reaction mixtures were separated on 6 or 8% nondenaturing polyacrylamide gels. The gels were dried and exposed to an Amersham Biosciences storage phosphor screen, and images were obtained by using a Typhoon 9400 instrument (GE Healthcare, Piscataway, NJ).

Transcription reporter assays. To construct transcriptional fusions, pKSM720, which has a *luc* gene encoding firefly luciferase, was used (28) (Table 1). Noncoding DNAs upstream of *speB*, *spd-3*, *pulA*, *Spy49_0396*, *Spy49_0746*, and *Spy49_1113* were amplified with PCR using NZ131 gDNA as template DNA with primer pairs pspeB_IRfwd and pspeB_IRrev, pspd3_IRfwd and pspd-3_IRrev, pulAfwd and pulArev, 42KDa_BamHIfwd and 42KDa_XhoIrev, int_BamHIfwd and int_XhoIrev, and 1113_BamHIfwd and 1113_XhoIrev, respectively (Table 2). The DNA fragments were gel purified, digested with BamHI and XhoI, and cloned into the BglII and XhoI sites of pKSM720. The P*speB*, P*spd3*, P*pulA*, P*396*, P*746*, and P*1113* DNA fragments were cloned 5′ to *luc* between the BglII and XhoI sites of pKSM720 to create pSA11, pSA12, pSA13, pSA14, pSA15, and pSA16, respectively. NZ131 was transformed with pKSM720, pSA11, pSA12, pSA13, pSA14, pSA15, and pSA16 by electroporation to create the wt::*luc*, wt::P*speB-luc*, wt::P*spd3-luc*, wt::P*pulA-luc*, wt::P*396-luc*, wt::P*746-luc*, and wt::P*1113-luc* strains (Table 1). Similarly, the *rgg* mutant strain NZ131 was transformed with pKSM720, pSA11, pSA12, pSA13, pSA14, pSA15, and pSA16 to create the *rgg*::*luc*, *rgg*::P*speB-luc*, *rgg*::P*spd3-luc*, *rgg*::P*pulA-luc*, *rgg*::P*396-luc*, *rgg*::P*746-luc*, and *rgg*::P*1113-luc* strains (Table 1). PCR was used to confirm the presence of the noncoding DNAs and the *luc* gene. The *S. pyogenes* strains containing the transcriptional fusion plasmids were grown to the post-exponential (A_{600}, ~0.60) phase of growth in THY broth, and luciferase activity was measured according to the manufacturer's instructions (Promega).

RESULTS

ChIP. To identify Rgg binding sites in the *S. pyogenes* genome, chromatin immunoprecipitation (ChIP) was used to enrich DNA bound by Rgg *in vivo*. For this purpose, an *S. pyogenes* strain expressing an Rgg-Myc fusion protein (SA5) was created by inserting DNA encoding the fusion protein into the chromosome of an *rgg* mutant strain. Thus, the fusion protein is expressed under the regulatory control of the native promoter. In contrast to the parental strain, which does not express *speB* (11), the SA5 strain expressed *speB* at wild-type levels, indicating that the fusion protein functions similarly to the unmodified protein (data not shown).

Strain SA5 was grown to the post-exponential phase of growth, and Rgg-bound DNA was immunoprecipitated with an antibody specific to the Myc epitope. As a negative control, the *rgg* mutant culture was treated similarly. To determine the specificity of the procedure, the enrichment of the *speB* promoter region (P*speB*), which was previously shown to bind Rgg *in vitro* (40), was determined by using quantitative real-time PCR (qPCR), and the values were normalized to the amount of nonspecific DNA in the precipitate (as determined by measuring the amount of an internal portion of the *groEL* ORF). P*speB* was 15-fold more abundant in samples obtained from strain SA5 than in those from the *rgg* mutant strain (Fig. 1),

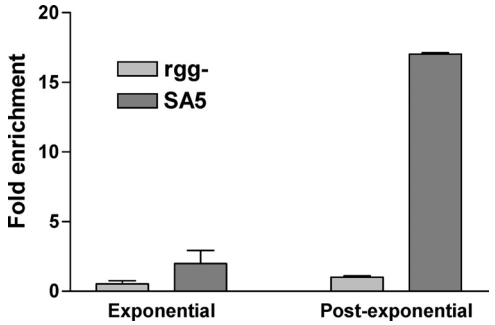

FIG. 1. Rgg binds to PspeB *in vivo* only in the post-exponential phase of growth. Quantitative PCR was used to measure the amounts of P*speB* and, as a negative control, *groEL* in ChIP samples obtained from the *rgg* mutant and strain SA5 in either the exponential or the post-exponential phase of growth.

indicating that the ChIP procedure specifically enriched DNA known to bind Rgg.

Rgg binds to P*speB* in a growth phase-dependent manner. Although Rgg is transcribed in both the exponential and post-exponential phases of growth (14), *speB* is expressed primarily during the post-exponential phase (11–12, 52). In the exponential phase of growth, Rgg interacts with LacD.1, a streptococcal aldolase that has evolved to control gene regulation, in a manner that prevents Rgg-dependent expression of *speB* (30). However, it is not clear if the Rgg-LacD.1 complex is associated with P*speB* in a way that fails to activate expression or if the complex does not bind to P*speB*. To address this issue, Rgg binding to P*speB* was also determined in the exponential phase of growth using ChIP-qPCR. The results showed that Rgg does not bind P*speB* in the exponential phase of growth (Fig. 1), which is consistent with the known pattern of *speB* expression.

Identification of Rgg binding sites *in vivo*. To determine if Rgg binds to chromosomal regions other than P*speB*, DNA enriched by ChIP during the post-exponential phase of growth was hybridized to custom-designed Affymetrix Gene Chips representing the entire NZ131 chromosome. Three independent experiments were conducted using both SA5 and the *rgg* mutant strain. As predicted from the ChIP-qPCR results (Fig. 1), ChIP followed by DNA microarray analysis (ChIP-chip) showed enrichment of the P*speB* DNA in samples obtained from the SA5 cultures but not in the *rgg* mutant controls (Table 3). Sixty-four additional regions of the chromosome were enriched at least 2-fold in all three biological replicates (Fig. 2 and Table 3; see also Table S1 in the supplemental material). Thirty-five (54%) binding sites were in noncoding DNA. Since only 14.7% of the NZ131 genome is noncoding, binding was biased toward noncoding DNA (Table 3; see also Table S1 in the supplemental material). In addition, 43% of the binding sites in noncoding DNA were adjacent to genes previously reported to be regulated by Rgg in the post-exponential phase of growth (Table 3), as determined by expression array analysis (19). Analysis of the Rgg binding regions using MEME and GLAM2 did not identify a unique motif.

Rgg binds to noncoding DNA upstream of several virulence-associated genes. Depending on the isolate, the *S. pyogenes* chromosome can contain as many as four genes encoding extracellular DNases, some of which are associated with virulence (48, 56). For example, DNase Sda1 promotes pathogen dissemination by degrading neutrophil extracellular traps (NETs) (56). Strain NZ131 possesses two genes encoding secreted DNases: *sdaB*, encoding streptodornase B (SdaB), also known as mitogenic factor-1 (MF-1), which is adjacent to *rgg* in the chromosome, and *spd-3*, encoding streptodornase (also known as mitogenic factor-3 [MF-3]), which is carried by a temperate bacteriophage (37). Rgg bound to the noncoding DNA upstream of both *sdaB* and *spd*-3 and upstream of the gene encoding pullulanase (*pulA*), a surface-associated glycan-binding protein involved in virulence (Table 3) (22). Transcriptome and proteome analyses previously showed increases in *sdaB*, *spd-3*, and *pulA* expression in the *rgg* mutant over that in the wild-type strain (14, 19). Taken together, the results suggest that Rgg binds to DNA upstream of the virulence-associated genes *sdaB*, *spd-3*, and *pulA* to directly control expression.

Rgg binds to noncoding DNA upstream of genes involved in metabolism and DNA repair. The majority of ORFs adjacent to Rgg binding sites within noncoding DNA encode proteins either known or predicted to be involved in catabolism and in nucleic acid recombination and repair (Fig. 3; Table 3). For example, Rgg bound to the noncoding DNA upstream of *sdhB* (Table 3), encoding a component of the heterodimeric enzyme serine dehydratase, which converts serine to pyruvate. Previously, we showed that *rgg* inactivation increases both *sdhB* transcript and protein levels and is associated with an increase in the degradation of serine (10, 13, 19).

In addition, Rgg bound adjacent to *rexB*, encoding ATP-dependent exonuclease, and adjacent to genes encoding a photolyase, which removes pyrimidine dimers created by UV radiation (57); RNase HIII, an endoribonuclease that digests double-stranded RNA and RNA/DNA hybrids (15) and regulates phage lysogeny by binding to the 5′ untranslated region (5′ UTR) of bacteriophage λ cIII (1); an RNA helicase; a transcription antiterminator in the BigG family; and NusG, a component of bacteriophage λ antitermination complexes (3, 29, 35, 47) (Table 3). In *S. pyogenes*, *nusG* can be cotranscribed with adjacent genes encoding NADase and streptolysin O, respectively (27), both of which are regulated by Rgg in strain NZ131 (19).

Rgg binds to noncoding DNA upstream of horizontally acquired genes. Strain NZ131 has three prophages constituting 5.6% of the chromosome (37). Two of these (designated NZ131.2 and NZ131.3) are complete prophages, whereas NZ131.1 appears to be a remnant of a unique prophage (37). Rgg bound to six unique DNA sites within the three prophages. Binding to three sites was not associated with changes in the expression of proximal genes, as determined by comparing the transcript levels of the wild-type strain to those of the *rgg* mutant strain (Table 3); however, binding to the other three sites was associated with changes in the expression of the downstream ORFs (19). These binding sites were upstream of *spd-3*, which is carried by prophage NZ131.3; a gene encoding an integrase/excisionase (Spy49_0746) carried by prophage NZ131.2; and a gene encoding a surface antigen (Spy49_0396), which has a motif present in lysins (Table 3) (5, 6, 24, 38) and is carried by prophage NZ131.3. Differences in the expression

TABLE 3. Noncoding DNA bound by Rgg

Category and Spy49 no.[a]	Gene[b]	Description	ChIP-chip fold enrichment[c]	Transcript fold change (*rgg* mutant/wt)[d]
Virulence associated				
1626	*pulA*	Pullulanase	6.0	2
1692–1693	*mf1*	Streptodornase B/mitogenic factor 1	4.90	5
1455	*spd-3*	Streptodornase	3.6	5
1690–1691	*speB*	Streptococcal pyrogenic exotoxin B	2.7	−59
Transcriptional regulator				
1690–1691	*rgg*	Rgg	2.7	3
710–711	*cpsY*	Putative transcriptional regulator	2.8	—
1761–1762	(—)	Putative arginine repressor	2.7	5
1113	(—)	Putative transcription factor	2.4	−2
Prophage related				
1720–1721	(—)	Transposase	8.1	—
355	(—)	Hypothetical protein	7.0	ND
396	(—)	Surface antigen	5.7	6
746	(—)	Integrase	5.6	11
565	(—)	Transposase	5.1	5
1499c	(—)	Phage endodeoxyribonuclease	2.1	—
Metabolism				
612	*rgpAc*	Rhamnosyltransferase	8.7	—
1720–1721	*ahpC*	Alkyl hydroperoxide reductase protein C	8.1	—
1632c	*relA*	(p)ppGpp synthetase	5.7	—
1429–1430	*mutY*	A/G-specific adenine glycosylase	3.4	2
1793–1794	*sdhB*	L-Serine dehydratase, beta subunit	2.5	−3
850–851	(—)	Polysaccharide deacetylase family protein	2.2	—
850–851	*folC1*	Folylpolyglutamate synthase	2.2	−3
Synthesis				
967	*dpfB*	Phosphopantothenate–cysteine ligase	5.0	—
1436–1437	(—)	RNase HIII	4.6	—
143	*nusG*	Transcription antitermination protein	2.9	—
1047	(—)	BigG family transcription antiterminator	2.9	2
1761–1762	*argS*	Arginyl-tRNA synthetase	2.7	2
1793–1794	*mnmA*	tRNA-specific 2-thiouridylase	2.5	—
1091	*deaD2*	Putative RNA helicase	2.3	—
1506	*dnaC*	DNA replication protein C	2.1	—
Repair				
1160	*phr*	Deoxyribodipyrimidine photolyase	9.8	—
603	*rexB*	ATP-dependent exonuclease, subunit B	6.0	—
Hypothetical				
1692–1693	(—)	Hypothetical protein	4.9	—
1436–1437	(—)	Hypothetical protein	4.6	—
1429–1430	(—)	Hypothetical protein	3.4	—
710–711	(—)	Hypothetical protein	2.8	—

[a] Genes are categorized based on KEGG genome annotations. Spy49 numbers are the open reading frames based on the *S. pyogenes* strain NZ131 genome annotation (37). Contiguous genes that are likely to be cotranscribed are separated by a hyphen. Where a Spy49 number is underlined, the description is specific to the underlined Spy49 gene designation.
[b] A dash within parentheses indicates that the gene is not named.
[c] Calculated as a ratio of signal intensity between the SA5 and *rgg* mutant strains.
[d] Determined previously (16). A dash indicates no change in the transcript level. ND, not determined.

of the prophage integrase between the *rgg* mutant and the wild-type strain were previously associated with changes in the frequency of prophage excision from the genome (19). Thus, 50% of the Rgg binding sites in prophage were upstream of known Rgg-regulated genes.

Assessing Rgg binding *in vitro*. The Rgg-regulated promoter region of *speB* (40) and the noncoding DNAs upstream of *spd-3*, *pulA*, *Spy49_0396*, *Spy49_0746*, and *Spy49_1113* that were bound by Rgg *in vivo* (Table 3) were selected for further *in vitro* analysis because of their roles in virulence, lateral gene transfer, and regulation. Moreover, the expression of each gene is altered in an *rgg* mutant strain, consistent with the idea that they are directly regulated by Rgg. To assess binding *in vitro*, the DNAs that bound Rgg *in vivo* were incubated with varying amounts of Rgg in gel shift assays. Rgg bound to all the target DNAs tested (Fig. 4).

Promoter activity assays. To determine whether the noncoding DNAs upstream of *speB*, *spd-3*, *pulA*, *Spy49_0396*, *Spy49_0746*, and *Spy49_1113* that were bound by Rgg *in vivo* contained functional promoters regulated by Rgg, a transcrip-

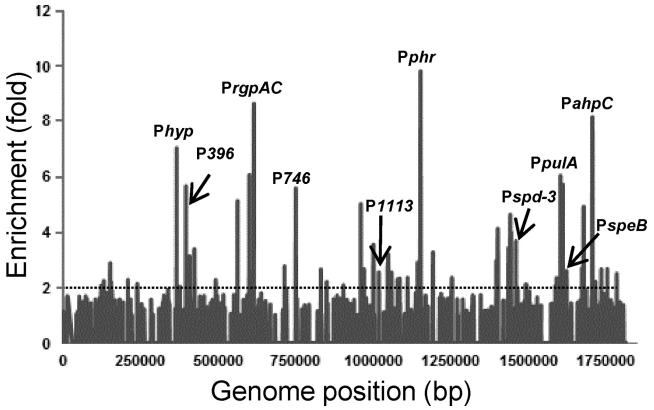

FIG. 2. Genomic Rgg-binding sites. The enrichment of DNA (*y* axis) in ChIP samples is plotted against the location of the DNA in the 1.8-Mb *S. pyogenes* genome (*x* axis).

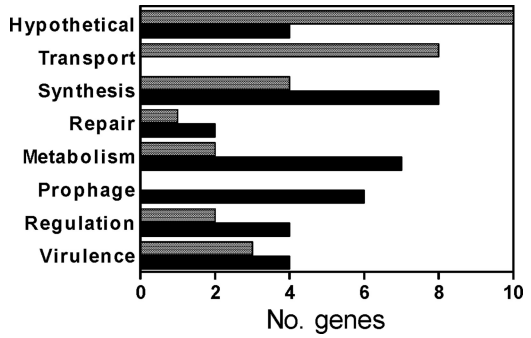

FIG. 3. Functions of genes associated with Rgg binding sites. The *x* axis indicates the number of ORFs (shaded bars) or noncoding DNAs proximal and upstream (filled bars) of genes bound by Rgg and the respective functions (*y* axis).

tional fusion reporter system was used. Each DNA region was cloned adjacent to a firefly luciferase reporter gene (*luc*), and the recombinant plasmids were used to transform both wild-type NZ131 and the *rgg* mutant strain. The transformants were grown to the post-exponential phase of growth, and promoter activity was measured by quantitating luciferase activity. Rgg activated the expression of the promoters associated with *speB* and *Spy49_1113*, whereas it repressed that of *spd-3*, *Spy49_0396*, and *Spy49_0746*, indicating that Rgg can both activate and repress gene expression (19) (Fig. 5). The results also showed that there is a functional promoter in each region of DNA bound by Rgg and that the promoter activity is regulated by Rgg, supporting the idea that Rgg regulates the expression of each of these genes by directly binding to their upstream noncoding regions.

DISCUSSION

Rgg activates *speB* expression by direct binding to the promoter region in the post-exponential phase of growth. In addition to P*speB*, Rgg binding sites were identified on a genome scale by using ChIP coupled to DNA microarrays. The results showed that Rgg bound to 65 regions in the chromosome, which included both coding and noncoding DNA. In many instances, but not all, Rgg bound to DNA upstream of ORFs whose expression is known to be influenced by Rgg, indicating that the genes are probably directly regulated by Rgg (19). Overall, the number of genes bound by Rgg is comparable to that for other global genome regulators, such as *Bacillus subtilis* CodY (a GTP-activated repressor) (39) and *E. coli* FNR (regulator of fumarate and nitrate reduction) (23).

Rgg binding to coding DNA. Prokaryotic transcriptional regulators often bind to noncoding DNA in operator regions located upstream of the target ORF; however, intragenic promoters are also present among prokaryotes (51), and approximately 28% of the *E. coli* promoters are predicted to be within coding DNA (45). Similarly, recent characterization of CodY binding in *Staphylococcus aureus* showed that 42% of the binding sites were in coding DNA (33). More than half (55%) of the Rgg binding sites identified in this study were in coding DNA. Several explanations may account for this finding (32). For example, low-affinity interactions between Rgg and DNA may occur, which do not necessarily influence transcription, perhaps simply because the binding site is incidentally similar to the functional binding sequence. In this regard, the *E. coli* transcriptional repressor LexA binds to both transcriptionally inactive and active regions (55), and the authors speculated that sites in coding regions were lower-affinity sites that had not decayed over time (46, 55). In addition, Rgg binding within coding DNA may influence the expression of distal ORFs by mechanisms involving chromatin conformational changes, such as DNA looping. Another possibility is that a subset of Rgg binding sites may function as a cellular reservoir of the transcription factor. Binding to, and dissociation from, these sites may be responsive to changes in the environment such that the reservoir would function to fine-tune Rgg-mediated regulation. Thus, while many of the Rgg binding sites identified were in noncoding DNA, suggesting a direct role in controlling the expression of adjacent ORFs, the biological significance of the other Rgg binding sites, including those in coding DNA, remains to be determined.

Previously we showed that inactivation of *rgg* in NZ131 significantly ($P < 0.05$) altered the expression of 588 genes in the post-exponential phase of growth, including several regulatory proteins (19). ChIP-Chip analyses did not detect Rgg binding *in vivo* to DNA proximal to known global regulatory genes, which would have provided an explanation for the apparently large degree of indirect changes in gene expression associated with *rgg* inactivation in strain NZ131; however, Rgg did bind to the noncoding DNA regions upstream of three transcriptional regulators (*cpsY*, *spy49_1761*, and *spy49_1113*), the expression of two of which is changed following inactivation of *rgg* (Table 3). The regulators have not been characterized previously in *S. pyogenes*, and thus we cannot rule out the possibility that one or more are global regulators of gene expression and thus contribute to the significant number of indirect changes in expression associated with *rgg* inactivation in strain NZ131. Nonetheless, given the characterized changes in catabolism associated with the *rgg* mutant of strain NZ131 (13) and the information currently available, we favor a model whereby many indirect differences in gene transcription associated with the *rgg* mutant result from differences in metabolism (13). Analysis of Rgg binding sites in the chromosomes of isolates in which *rgg* inactivation is not associated with altered catabolic

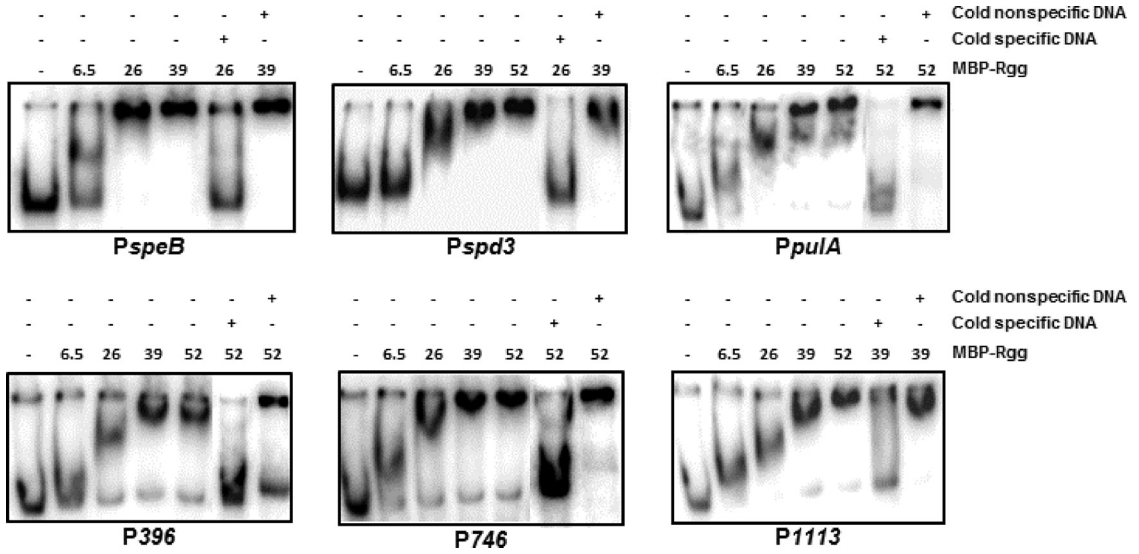

FIG. 4. Gel shift assays of selected target DNAs. The binding of Rgg to P*speB* and the noncoding DNAs upstream of *Spy49_1113*, *Spy49_0746*, *spd-3*, *pulA*, and *Spy49_0396* that bound to Rgg *in vivo* was assessed by incubation without Rgg or with 6.5, 26, 39, and 52 pmol of purified Rgg with radiolabeled DNA. Cold nonspecific DNA refers to unlabeled *groEL*, and the cold specific probe is unlabeled competing DNA.

activity, such as MGAS5005 and SF370, would be useful in testing this model.

Previously, we discovered inter- and intraserotypic variation in the Rgg regulon following inactivation of the *rgg* gene in serotype M49 strains CS101 and NZ131 and in serotype M1 strains 5005 and SF370 (18). The results showed that Rgg has a core regulon consisting of *speB* and the adjacent hypothetical protein gene *spy49_1689c* but that aside from these genes, the regulon differs significantly depending on the isolate under study (18). Rgg bound to noncoding, conserved DNA upstream of at least one gene whose expression is regulated in strain NZ131 but not in the other isolates examined. In strain NZ131, Rgg bound to DNA upstream of the ORF designated *Spy49_0396* both *in vivo* (Table 3) and *in vitro* (Fig. 4). Rgg represses transcription of *Spy49_0396* in the post-exponential phase of growth, as determined both by promoter assays (Fig. 5D) and by DNA microarrays (19). The nucleotide sequence of the Rgg binding site in NZ131 is identical to those in isolates SF370 and MGAS5005; however, inactivation of *rgg* in these strains does not appear to influence expression. It will be of interest to determine if Rgg binds to this site *in vivo* in strains SF370 and MGAS5005, or if strain-specific cofactors influence the specificity of Rgg binding. Such information is likely to provide insight into the molecular basis of the variation of the regulon among isolates of *S. pyogenes*.

Rgg binds directly and specifically to prophage DNA. The genome sequences of more than 13 isolates of *S. pyogenes*, representing 7 serotypes, have been determined. All isolates are polylysogenized (49). Temperate bacteriophages typically encode one or more integrases/excisionases that facilitate phage integration and excision. Coculture of *S. pyogenes* with pharyngeal epithelial cells and the addition of hydrogen peroxide or DNA-damaging agents, such as mitomycin C and UV light, are some of the factors that cause prophage induction (4, 7). Our previous studies showed that *rgg* inactivation decreases the frequency of prophage NZ131.2 excision from the chromosome (previously referred to as NZ131.1) (19, 37). The ChIP-chip results indicate that Rgg binds to at least six sites within prophage DNA, including noncoding DNA adjacent to a gene encoding a putative integrase/excisionase (*Spy49_746*), and binding was recapitulated *in vitro* with gel shift assays (Fig. 3). The expression of *Spy49_746* is 11-fold greater in the *rgg* mutant strain than in the wild-type strain (Table 3). Together, the results suggest that Rgg alters the frequency of prophage excision/insertion by binding directly to prophage DNA to alter the expression of the enzyme.

Significant variation in the Rgg regulon occurs among different isolates of *S. pyogenes* (18). One possible explanation is that Rgg-mediated changes in prophage gene expression may cause secondary, or indirect, changes in chromosomal gene expression. This would seem to be particularly likely if the changes in expression alter the frequency of prophage induction. Thus, we speculate that the variation in the type and number of bacteriophages present in the genomes of various *S. pyogenes* isolates may contribute to strain-associated variation in the Rgg regulon. In this context, a prophage could generate phenotypic diversity in the species by altering chromosomal gene expression. In other words, the coevolution of bacteriophage and *S. pyogenes* regulatory circuits may enhance the ability of the bacterial species to adapt, through natural selection, to various ecological niches, including adaptation to the human host immune response. From the standpoint of the bacteriophage, participation in a global regulatory circuit of the bacterial host, such as Rgg, may increase the likelihood that the prophage will be maintained in the species, since perturbations in regulatory networks associated with the loss of the prophage could be detrimental to the host cell.

In summary, this study identified the genome-wide DNA binding characteristics of Rgg in the post-exponential phase of growth. The results indicate that Rgg binds to both coding and noncoding DNA, although binding is biased toward noncoding DNA and in several instances was shown to be in the promoter

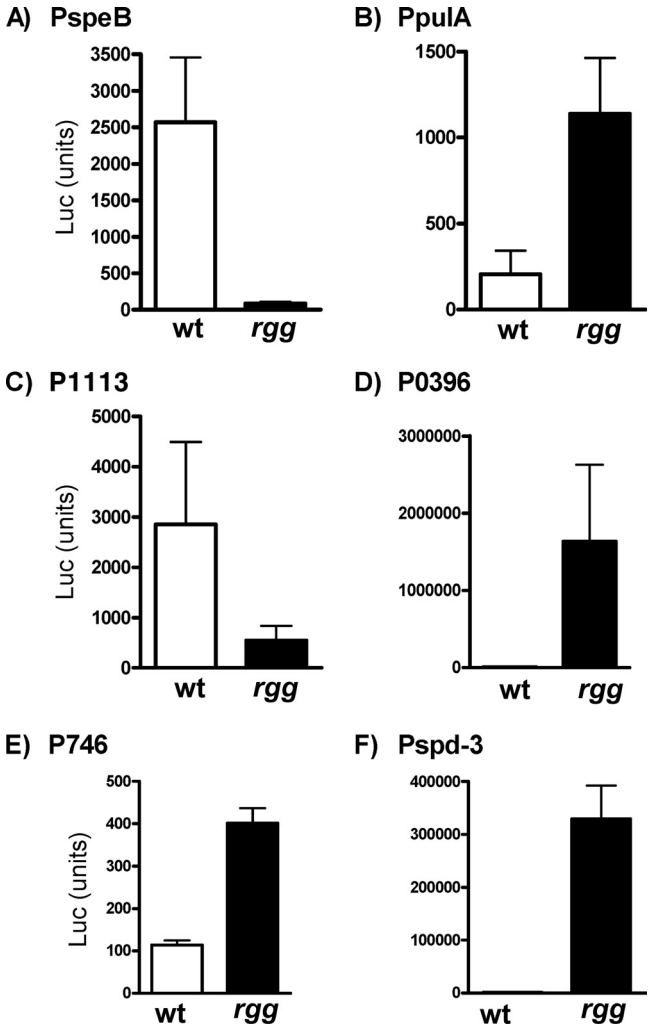

FIG. 5. Promoter activity of DNA bound by Rgg. Transcriptional fusions were constructed between the DNA upstream of *speB* (A), *pulA* (B), *spy49_1113* (C), *spy49_396* (D), *spy49_746* (E), and *spd-3* (F) and the firefly luciferase gene (*luc*). The plasmids were transformed into the wild type (wt) and the *rgg* mutant, which were then grown to the post-exponential phase of growth, and promoter activity was determined by measuring luciferase. The data shown are representative results from three independent biological replicates.

regions of Rgg-mediated genes. Of particular importance was the discovery that Rgg binds directly to specific prophage DNA and influences prophage gene expression, which may reflect the ongoing coevolution of the pathogen and temperate bacteriophage.

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REFERENCES

1. **Altuvia, S., H. Locker-Giladi, S. Koby, O. Ben-Nun, and A. B. Oppenheim.** 1987. RNase III stimulates the translation of the cIII gene of bacteriophage lambda. Proc. Natl. Acad. Sci. U. S. A. **84:**6511–6515.
2. **Anonymous.** 2009. Bacterial coinfections in lung tissue specimens from fatal cases of 2009 pandemic influenza A (H1N1)—United States, May-August 2009. MMWR Morb. Mortal. Wkly. Rep. **58:**1071–1074.
3. **Artsimovitch, I., and R. Landick.** 2000. Pausing by bacterial RNA polymerase is mediated by mechanistically distinct classes of signals. Proc. Natl. Acad. Sci. U. S. A. **97:**7090–7095.
4. **Banks, D. J., B. Lei, and J. M. Musser.** 2003. Prophage induction and expression of prophage-encoded virulence factors in group A Streptococcus serotype M3 strain MGAS315. Infect. Immun. **71:**7079–7086.
5. **Bateman, A., and M. Bycroft.** 2000. The structure of a LysM domain from *E. coli* membrane-bound lytic murein transglycosylase D (MltD). J. Mol. Biol. **299:**1113–1119.
6. **Birkeland, N. K.** 1994. Cloning, molecular characterization, and expression of the genes encoding the lytic functions of lactococcal bacteriophage ϕLC3: a dual lysis system of modular design. Can. J. Microbiol. **40:**658–665.
7. **Broudy, T. B., V. Pancholi, and V. A. Fischetti.** 2001. Induction of lysogenic bacteriophage and phage-associated toxin from group A streptococci during coculture with human pharyngeal cells. Infect. Immun. **69:**1440–1443.
8. **Brussow, H., C. Canchaya, and W. D. Hardt.** 2004. Phages and the evolution of bacterial pathogens: from genomic rearrangements to lysogenic conversion. Microbiol. Mol. Biol. Rev. **68:**560–602.
9. **Carapetis, J. R., A. C. Steer, E. K. Mulholland, and M. Weber.** 2005. The global burden of group A streptococcal diseases. Lancet Infect. Dis. **5:**685–694.
10. **Chaussee, M. A., E. A. Callegari, and M. S. Chaussee.** 2004. Rgg regulates growth phase-dependent expression of proteins associated with secondary metabolism and stress in *Streptococcus pyogenes*. J. Bacteriol. **186:**7091–7099.
11. **Chaussee, M. S., D. Ajdic, and J. J. Ferretti.** 1999. The *rgg* gene of *Streptococcus pyogenes* NZ131 positively influences extracellular SPEB production. Infect. Immun. **67:**1715–1722.
12. **Chaussee, M. S., D. Phillips, and J. J. Ferretti.** 1997. Temporal production of streptococcal erythrogenic toxin B (streptococcal cysteine proteinase) in response to nutrient depletion. Infect. Immun. **65:**1956–1959.
13. **Chaussee, M. S., G. A. Somerville, L. Reitzer, and J. M. Musser.** 2003. Rgg coordinates virulence factor synthesis and metabolism in *Streptococcus pyogenes*. J. Bacteriol. **185:**6016–6024.
14. **Chaussee, M. S., R. O. Watson, J. C. Smoot, and J. M. Musser.** 2001. Identification of Rgg-regulated exoproteins of *Streptococcus pyogenes*. Infect. Immun. **69:**822–831.
15. **Condon, C.** 2003. RNA processing and degradation in *Bacillus subtilis*. Microbiol. Mol. Biol. Rev. **67:**157–174.
16. **Cunningham, M. W.** 2000. Pathogenesis of group A streptococcal infections. Clin. Microbiol. Rev. **13:**470–511.
17. **Desiere, F., W. M. McShan, D. Van Sinderen, D. Ferretti, and H. Brussow.** 2001. Comparative genomics reveals close genetic relationships between phages from dairy bacteria and pathogenic streptococci: evolutionary implications for prophage-host interactions. Virology **288:**325–341.
18. **Dmitriev, A. V., E. J. McDowell, and M. S. Chaussee.** 2008. Inter- and intraserotypic variation in the *Streptococcus pyogenes* Rgg regulon. FEMS Microbiol. Lett. **284:**43–51.
19. **Dmitriev, A. V., et al.** 2006. The Rgg regulator of *Streptococcus pyogenes* influences utilization of nonglucose carbohydrates, prophage induction, and expression of the NAD-glycohydrolase virulence operon. J. Bacteriol. **188:**7230–7241.
20. **Ferretti, J. J., et al.** 2001. Complete genome sequence of an M1 strain of *Streptococcus pyogenes*. Proc. Natl. Acad. Sci. U. S. A. **98:**4658–4663.
21. **Fontaine, L., et al.** 2010. A novel pheromone quorum-sensing system controls the development of natural competence in *Streptococcus thermophilus* and *Streptococcus salivarius*. J. Bacteriol. **192:**1444–1454.
22. **Gourlay, L. J., et al.** 2009. Group B streptococcus pullulanase crystal structures in the context of a novel strategy for vaccine development. J. Bacteriol. **191:**3544–3552.
23. **Grainger, D. C., H. Aiba, D. Hurd, D. F. Browning, and S. J. Busby.** 2007. Transcription factor distribution in *Escherichia coli*: studies with FNR protein. Nucleic Acids Res. **35:**269–278.
24. **Hu, S., J. Kong, W. Kong, J. Guo, and M. Ji.** 2010. Characterization of a novel LysM domain from *Lactobacillus fermentum* bacteriophage endolysin and its use as an anchor to display heterologous proteins on the surfaces of lactic acid bacteria. Appl. Environ. Microbiol. **76:**2410–2418.
25. **Hynes, W. L., L. Hancock, and J. J. Ferretti.** 1995. Analysis of a second bacteriophage hyaluronidase gene from *Streptococcus pyogenes*: evidence for a third hyaluronidase involved in extracellular enzymatic activity. Infect. Immun. **63:**3015–3020.
26. **Kappeler, K. V., et al.** 2009. A naturally occurring Rgg variant in serotype M3 *Streptococcus pyogenes* does not activate *speB* expression due to altered specificity of DNA binding. Infect. Immun. **77:**5411–5417.
27. **Kimoto, H., Y. Fujii, Y. Yokota, and A. Taketo.** 2005. Molecular characterization of NADase-streptolysin O operon of hemolytic streptococci. Biochim. Biophys. Acta **1681:**134–149.
28. **Kinkel, T. L., and K. S. McIver.** 2008. CcpA-mediated repression of streptolysin S expression and virulence in the group A streptococcus. Infect. Immun. **76:**3451–3463.
29. **Li, J., R. Horwitz, S. McCracken, and J. Greenblatt.** 1992. NusG, a new

Escherichia coli elongation factor involved in transcriptional antitermination by the N protein of phage lambda. J. Biol. Chem. **267:**6012–6019.
30. **Loughman, J. A., and M. G. Caparon.** 2006. A novel adaptation of aldolase regulates virulence in *Streptococcus pyogenes*. EMBO J. **25:**5414–5422.
31. **Lyon, W. R., C. M. Gibson, and M. G. Caparon.** 1998. A role for trigger factor and an rgg-like regulator in the transcription, secretion and processing of the cysteine proteinase of *Streptococcus pyogenes*. EMBO J. **17:**6263–6275.
32. **Macquarrie, K. L., A. P. Fong, R. H. Morse, and S. J. Tapscott.** 2011. Genome-wide transcription factor binding: beyond direct target regulation. Trends Genet. **27:**141–148.
33. **Majerczyk, C. D., et al.** 2010. Direct targets of CodY in *Staphylococcus aureus*. J. Bacteriol. **192:**2861–2877.
34. **Mashburn-Warren, L., D. A. Morrison, and M. J. Federle.** 2010. A novel double-tryptophan peptide pheromone controls competence in *Streptococcus* spp. via an Rgg regulator. Mol. Microbiol. **78:**589–606.
35. **Mason, S. W., J. Li, and J. Greenblatt.** 1992. Host factor requirements for processive antitermination of transcription and suppression of pausing by the N protein of bacteriophage lambda. J. Biol. Chem. **267:**19418–19426.
36. **McDonnell, G. E., H. Wood, K. M. Devine, and D. J. McConnell.** 1994. Genetic control of bacterial suicide: regulation of the induction of PBSX in *Bacillus subtilis*. J. Bacteriol. **176:**5820–5830.
37. **McShan, W. M., et al.** 2008. Genome sequence of a nephritogenic and highly transformable M49 strain of *Streptococcus pyogenes*. J. Bacteriol. **190:**7773–7785.
38. **Mesnage, S., F. Chau, L. Dubost, and M. Arthur.** 2008. Role of *N*-acetylglucosaminidase and *N*-acetylmuramidase activities in *Enterococcus faecalis* peptidoglycan metabolism. J. Biol. Chem. **283:**19845–19853.
39. **Molle, V., et al.** 2003. Additional targets of the *Bacillus subtilis* global regulator CodY identified by chromatin immunoprecipitation and genome-wide transcript analysis. J. Bacteriol. **185:**1911–1922.
40. **Neely, M. N., W. R. Lyon, D. L. Runft, and M. Caparon.** 2003. Role of RopB in growth phase expression of the SpeB cysteine protease of *Streptococcus pyogenes*. J. Bacteriol. **185:**5166–5174.
41. **Niemann, H., A. Birch-Andersen, E. Kjems, B. Mansa, and S. Stirm.** 1976. Streptococcal bacteriophage 12/12-borne hyaluronidase and its characterization as a lyase (EC 4.2.99.1) by means of streptococcal hyaluronic acid and purified bacteriophage suspensions. Acta Pathol. Microbiol. Scand. B **84:**145–153.
42. **Rawlinson, E. L., I. F. Nes, and M. Skaugen.** 2005. Identification of the DNA-binding site of the Rgg-like regulator LasX within the lactocin S promoter region. Microbiology **151:**813–823.
43. **Rawlinson, E. L., I. F. Nes, and M. Skaugen.** 2002. LasX, a transcriptional regulator of the lactocin S biosynthetic genes in *Lactobacillus sakei* L45, acts both as an activator and a repressor. Biochimie **84:**559–567.
44. **Sanders, J. W., G. Venema, J. Kok, and K. Leenhout.** 1998. Identification of a sodium chloride-regulated promoter in *Lactococcus lactis* by single-copy chromosomal fusion with a reporter gene. Mol. Gen. Genet. **257:**681–685.
45. **Shavkunov, K. S., I. S. Masulis, M. N. Tutukina, A. A. Deev, and O. N. Ozoline.** 2009. Gains and unexpected lessons from genome-scale promoter mapping. Nucleic Acids Res. **3:**4919–4931.
46. **Shimada, T., A. Ishihama, S. J. Busby, and D. C. Grainger.** 2008. The *Escherichia coli* RutR transcription factor binds at targets within genes as well as intergenic regions. Nucleic Acids Res. **36:**3950–3955.
47. **Sullivan, S. L., and M. E. Gottesman.** 1992. Requirement for *E. coli* NusG protein in factor-dependent transcription termination. Cell **68:**989–994.
48. **Sumby, P., et al.** 2005. Extracellular DNase made by group A streptococcus assists pathogenesis by enhancing evasion of the innate immune response. Proc. Natl. Acad. Sci. U. S. A. **102:**1679–1684.
49. **Sumby, P., A. R. Whitney, E. A. Graviss, F. R. DeLeo, and J. M. Musser.** 2006. Genome-wide analysis of group A streptococci reveals a mutation that modulates global phenotype and disease specificity. PLoS Pathog. **2:**e5.
50. **Suvorov, A. N., E. M. Polyakova, W. M. McShan, and J. J. Ferretti.** 2009. Bacteriophage content of M49 strains of *Streptococcus pyogenes*. FEMS Microbiol. Lett. **294:**9–15.
51. **Tutukina, M. N., K. S. Shavkunov, I. S. Masulis, and O. N. Ozoline.** 2007. Intragenic promoter-like sites in the genome of *Escherichia coli*: discovery and functional implication. J. Bioinform. Comput. Biol. **5:**549–560.
52. **Unnikrishnan, M., J. Cohen, and S. Sriskandan.** 1999. Growth-phase-dependent expression of virulence factors in an M1T1 clinical isolate of *Streptococcus pyogenes*. Infect. Immun. **67:**5495–5499.
53. **Vickerman, M. M., M. C. Sulavik, and D. B. Clewell.** 1995. Oral streptococci with genetic determinants similar to the glucosyltransferase regulatory gene, *rgg*. Infect. Immun. **63:**4524–4527.
54. **Virtaneva, K., et al.** 2005. Longitudinal analysis of the group A streptococcus transcriptome in experimental pharyngitis in cynomolgus macaques. Proc. Natl. Acad. Sci. U. S. A. **102:**9014–9019.
55. **Wade, J. T., N. B. Reppas, G. M. Church, and K. Struhl.** 2005. Genomic analysis of LexA binding reveals the permissive nature of the *Escherichia coli* genome and identifies unconventional target sites. Genes Dev. **19:**2619–2630.
56. **Walker, M. J., et al.** 2007. DNase Sda1 provides selection pressure for a switch to invasive group A streptococcal infection. Nat. Med. **13:**981–985.
57. **Weber, S.** 2005. Light-driven enzymatic catalysis of DNA repair: a review of recent biophysical studies on photolyase. Biochim. Biophys. Acta **1707:**1–23.
58. **Zakikhany, K., et al.** 2011. Increase in invasive *Streptococcus pyogenes* and *Streptococcus pneumoniae* infections in England, December 2010 to January 2011. Euro Surveill. **16:**pii:19785.