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Inter- and intraserotypic variation in the *Streptococcus pyogenes* Rgg regulon

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Abstract

Human isolates of *Streptococcus pyogenes*, a Gram-positive bacterium, are characterized by significant genetic and phenotypic variation. The *rgg* locus, also known as *ropB*, is a global transcriptional regulator of genes associated with metabolism, stress responses, and virulence in *S. pyogenes* strain NZ131 (serotype M49). To assess the breadth of the Rgg regulon, the *rgg* gene was inactivated in three additional strains representing serotypes M1 (strains SF370 and MGAS5005) and M49 (strain CS101). Changes in gene expression were identified in the post-exponential phase of growth using Affymetrix NimbleExpress Arrays. The results identified an Rgg core-regulon consisting of *speB* and adjacent hypothetical protein gene, *spy2040*, and a variable and strain-specific sub-regulon, ranging in size from a single gene (*spy1793*) in strain MGAS5005 to 43 genes in strain SF370. Thus, both interserotypic and intraserotypic variation is characteristic of the Rgg regulon in *S. pyogenes*.

Keywords

Streptococcus pyogenes; Rgg; transcriptional regulation

INTRODUCTION

Streptococcus pyogenes (group A streptococcus) is a Gram-positive pathogen that exhibits significant phenotypic diversity, which is likely to contribute to the wide variety of clinical outcomes associated with human infection. The clinical severity of infection ranges from asymptomatic colonization to severe invasive diseases, such as streptococcal toxic shock syndrome and necrotizing fasciitis. Moreover, post-infection sequelae including rheumatic fever, glomerulonephritis, and neurological disorders contribute substantially to the morbidity and mortality associated with the pathogen (Cunningham, 2002).

Clinical isolates of *S. pyogenes* are differentiated into more than 100 *emm*-types based on variability of the 5′-region of the *emm* gene (http://www.cdc.gov/ncidod/biotech/strep/M-ProteinGene_typing.htm), which encodes the LPXTG-anchored adhesin and antiphagocytic M protein (Fischetti, 1989). More generally, clinical isolates can be differentiated into two classes (Class I and Class II) based on reactivity with antibodies directed against the C repeat region of the M protein and the

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organization and composition of genes proximal to *emm* in the chromosome. For example, class I, but not class II, strains possess the gene encoding the streptococcal inhibitor of complement (SIC), which contributes to virulence in murine models (Lukomski *et al.*, 2000). Class II, but not class I, strains possess the gene encoding serum opacity factor (SOF), which also contributes to virulence and promotes adherence (Timmer *et al.*, 2006). Class I strains are associated with invasive diseases and acute rheumatic fever, while class II strains are associated with pyoderma and acute glomerulonephritis (Cunningham, 2000). Although differences in the host response are clearly important, specific strains of *S. pyogenes* are more likely to cause certain diseases than others, which indicates that strain-variable genetic elements contribute to the disease process. The complete genome sequences of more than 12 strains of *S. pyogenes* have been determined. These include isolates from invasive disease episodes (Ferretti *et al.*, 2001; Beres *et al.*, 2006), rheumatic fever (Smoot *et al.*, 2002; Holden *et al.*, 2007), and puerperal sepsis (Green *et al.*, 2005). The genome content of the strains is heterogeneous, mostly due to various bacteriophages (Banks *et al.*, 2002) and integrated conjugative elements (Beres & Musser, 2007). These strain-variable elements contribute to the so-called pan-genome (Tettelin *et al.*, 2005), or metagenome (Beres & Musser, 2007), which is estimated to consist of approximately 2,500 genes (Lefébure & Stanhope, 2007).

Rgg, also known as RopB, (Chaussee *et al.*, 1999; Lyon *et al.*, 1998) is one member of a family of transcriptional regulators (TIGR01716) encoded in the genomes of some species of low G+C Gram-positive bacteria. Inactivation of *rgg* in the class II strain NZ131 (serotype M49) is associated with changes in the transcript levels of 706 genes compared to the parental wild-type strain (Dmitriev *et al.*, 2006). Many of these genes encode known or putative virulence factors, such as the hyaluronic acid capsule, C5a peptidase, streptokinase, streptolysins S and O, and mitogenic factor (Chaussee *et al.*, 2001; Dmitriev *et al.*, 2006). Corresponding phenotypic differences identified in the mutant strain include: (i) tolerance to penicillin-mediated killing and thermal and oxidative stressors (Chaussee *et al.*, 2004; Chaussee *et al.*, 2006); (ii) catabolism of arginine during the exponential phase of growth in the presence of glucose (Chaussee *et al.*, 2003); (iii) increased production of DNase, NADase, and SLO; (iv) an inability to grow in chemically defined media (CDM) containing sucrose, fructose or mannose as the primary carbon source; and (v) decreased frequency of prophage NZ131.1 induction (Dmitriev *et al.*, 2006). Thus, Rgg is an important global regulator of genes associated with metabolism, stress response, and virulence in strain NZ131.

The purpose of this study was to assess the breadth of the Rgg regulon in additional isolates of *S. pyogenes* including two M1 serotypes representing class I strains and an additional M49 serotype representing class II strains. DNA microarrays identified an Rgg core-regulon and a variable and strain-specific sub-regulon, which suggests that diversity among regulatory circuits contributes to the phenotypic diversity of *S. pyogenes*.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions

The wild-type *S. pyogenes* strains MGAS5005, SF370, and CS101 have been described (Ferretti *et al.*, 2001; Sumby *et al.*, 2005; Haanes *et al.*, 1992). *S. pyogenes* was grown at 37 °C in a 5% CO_2 atmosphere without agitation in either Todd-Hewitt broth (Becton Dickinson, Sparks, MD) containing 0.2% (wt/vol) yeast extract or CDM (Dmitriev *et al.*, 2006). *Escherichia coli* strain DH5α was purchased from Gibco-BRL (Gaithersburg, MD), and the suicide cloning vector pVA891-2 was kindly provided by H. Malke (Malke *et al.*, 1994).

DNA techniques

Plasmid DNA was isolated from *E. coli* using Plasmid Midi Kit (QIAGEN, Valencia, CA). From agarose gels, DNA was isolated using the QIAquick PCR Purification Kit (QIAGEN). PCR products were purified using DNA Clean & Concentrator (Orange, Calif.). Most of other routine molecular techniques were done as previously described (Sambrook *et al.*, 1989).

Insertional inactivation of *rgg*

The *rgg* gene was insertional inactivated in each strain as previously described (Chaussee *et al.*, 1999). Briefly, the entire *rgg* gene was amplified using the primers RGG+1 (5′-CTG GAG CTG TTG AGA TAA ACT AC-3′) and RGG-4 (5′-GGC TAT TGA CCT TAT GCA CC-3′), and digested with *Eco*RI and *Hin*dIII, which had corresponding restriction sites within *rgg*. The resulting 592 bp fragment was cloned into the vector pVA891-2. Following *E. coli* transformation, the recombinant plasmid was isolated and used to transform *S. pyogenes*. Transformants were selected on agar plates containing 2.5 μg mL^{-1} of erythromycin. Insertional inactivation was confirmed in each strain by nucleotide sequencing and Southern blotting, as previously described (Chaussee *et al.*, 1999). The heterologous DNA inserted into the *rgg* locus was identical in all three *rgg* mutant strains and identical to the previously described NZ131 *rgg* mutant (Chaussee *et al.*, 1999).

Pulsed field gel electrophoresis (PFGE) analysis

Chromosomal DNA for PFGE analysis was isolated and digested with *Sma*I as described (Elliott *et al.*, 1998). Fragments were separated in 1% agarose gel in 0.5 × TBE buffer (GeneLine apparatus, Beckman Instruments, Calif.) using the following conditions: 5 min, 170 V, 5 s pulse time; 16 h, 200 V, 40 s pulse time; and 8 h, 200 V, 8 s pulse time, and visualized with ethidium bromide, 0.5 μg mL^{-1}.

DNA microarray analysis

RNA was isolated from 40 ml cultures of *S. pyogenes* in the post-exponential phase of growth (Fig. 1) with an RNeasy Mini Kit (QIAGEN). Affymetrix NimbleExpress Arrays designed based on the *S. pyogenes* strain SF370 genome sequence (Ferretti *et al.*, 2001) were purchased from Affymetrix (Santa Clara, CA). In addition to the SF370 genome sequence, 660 known streptococcal bacteriophage genes were included in the array platform. Together, the arrays consisted of 3,203 qualifiers representing 2,354 predicted *S. pyogenes* ORFs, 804 intergenic region probes, and 45 control oligos used for spike-ins. Microarray hybridization and analysis of the data was done as recently published (Dmitriev *et al.*, 2006). The average signal intensity value of each gene was transformed to a $\log_2$ (log base 2) value. The change between two experimental conditions (*n*-fold) was calculated by taking the ratio of the signal intensity (difference of the $\log_2$ value) between experimental conditions. Present and absent calls were assessed and statistically significant genes (T-test; P value ≤ 0.05) were considered to be differently expressed. All of the microarray data are available through the Gene Expression Omnibus data repository via accession numbers GSE 7335 and 7341.

Determination of SpeB, NADase, DNase and SLO cytolytic activities

SpeB, NADase, DNase and SLO cytolytic activities were determined as previously described (Chaussee *et al.*, 1999; Bricker *et al.*, 2002; Dmitriev, 2006).

Quantitative reverse transcription (RT)-PCR

Oligonucleotide primers and TaqMan probes used in the study were previously published (Dmitriev *et al.*, 2006). In addition, primers and fluorescent probes for the

phosphoribosylglycinamide formyltransferase *purN* gene (5′-CTT GGC CTA TGA GAG GCG TAT T-3′, 5′-CCG TGG GCA CCT GGA A-3′, and 5′-TCA ATA TTC ACC CAG CCT ACC TGC CTG AA-3′) and the streptolysin S *sagA* gene (5′-TTG CTC CTG GAG GCT GCT-3′, 5′-CTT CCG CTA CCA CCT TGA GAA T-3′, and 5′-ACC ACT TCC AGT AGC AAT TGA GAA GCA ACA AG-3′) were designed with Primer Express 2.0 software (ABI Prism, PE Biosystems, Framingham, Mass.) and purchased from Sigma-Genosys (The Woodlands, TX). Amplification and detection were done with the ABI Prism 7700 Sequence Detection System (PE Applied Biosystems) using TaqMan One-Step RT-PCR Master Mix reagents (Roche, Indianapolis, In.), as previously described (Chaussee *et al.*, 2003).

Sequence analysis

The *rgg* genes from *S. pyogenes* strains MGAS5005, SF370, and CS101 were sequenced using ABI Prism 377 Perkin-Elmer Sequencer and Big Dye Terminator Kit (Applied Biosystems) with the primers RGG+1 and RGG-4. The data were deposited into the GenBank database under accession numbers DQ009036 and DQ176644. The deduced Rgg proteins of all three strains were identical to that of the NZ131 strain. The 498 bp regions upstream the *rgg* genes sequenced with the primers 5′-CGG CAA ATA CTG GGT TAG CAA GA-3′ and 5′-GGA TGC CTA ATG AAT TCA ACG GTT T-3′ were also identical in all the strains.

RESULTS

Expression of virulence factors in the *rgg* mutant strains

To assess the Rgg regulon in *S. pyogenes*, three widely studied strains, representing both class I (MGAS5005 and SF370, serotypes M1) and class II (CS101, serotype M49) organisms, were selected for study. Inactivation of *rgg* in strains CS101, MGAS5005, and SF370 as described in the Materials and Methods, abrogated SpeB expression (Fig. 2), similar to previously described mutants created in strains NZ131 and HSC5 (Chaussee *et al.*, 1999;Lyon *et al.*, 1998). Southern blotting showed that the heterologous DNA inserted into only the *rgg* locus (data not shown). These results indicate that Rgg-dependent activation of *speB* expression is conserved among both class I and II clinical isolates of *S. pyogenes*.

The SLO, NADase, and DNase activities, which were elevated in the NZ131 *rgg* mutant strain (Dmitriev *et al*., 2006), were similarly measured in strains MGAS5005, SF370, and CS101 and the corresponding *rgg* mutant derivatives. Despite variation in SLO, NADase, and DNase activities among the wild-type strains, no difference between wild-type and corresponding *rgg* mutant strains was observed (data not shown).

Growth of wild-type and the *rgg* mutant strains in Todd-Hewitt broth and CDM

Inactivation of *rgg* in strain NZ131 is associated with altered growth in Todd-Hewitt broth and an inability to use non-glucose carbohydrates as the primary carbon and energy sources when grown with CDM (Chaussee *et al.*, 2003; Dmitriev *et al*., 2006). To determine if similar changes occurred in strains CS101, MGAS5005, and SF370, the growth of wild-type strains and mutant derivatives was examined. Growth of wild-type CS101 and MGAS5005 strains was identical to the corresponding mutant strains in both Todd-Hewitt broth (Fig. 1) and CDM containing 2% glucose, sucrose or fructose (data not shown). In contrast, the SF370 *rgg* mutant strain had a slightly shorter lag period and different growth yield compared to the parental strain when grown with Todd-Hewitt broth (Fig. 1) or CDM containing various carbohydrates as the primary carbon and energy source (Fig. 1). Thus, while there was a minor effect on growth in strain SF370 following *rgg* inactivation, mutant strains retained the ability to use sucrose, glucose and fructose as the primary carbon and

energy source during growth with CDM, in contrast to results previously obtained with strain NZ131 (Dmitriev *et al.*, 2006).

DNA microarray analysis of the wild-type and *rgg* mutant strains

To identify Rgg-regulated genes, the transcriptomes of CS101, MGAS5005, and SF370 wild-type and *rgg* mutant strains were analyzed. Our initial experiments revealed few differences in the transcriptomes of MGAS5005 *rgg* mutant and CS101 *rgg* mutant compared to corresponding wild-type strains during exponential phase (data not shown). These data are in agreement with earlier observations in strain NZ131, in which *rgg* inactivation alters a larger number of genes during the post-exponential phase of growth compared to the exponential phase (Dmitriev *et al.*, 2006). Therefore, to identify the largest number of Rgg-regulated genes in MGAS5005, CS101, and SF370 strains, RNA was isolated from post-exponential phase cultures and gene transcripts were measured with Affymetrix NimbleExpress Arrays. Inactivation of *rgg* in strains MGAS5005, CS101, and SF370 was associated with a 2-fold or more ($P \leq 0.05$) change in the expression of 3, 13, and 45 loci, respectively (Table 1). In strain MGAS5005, 1 and 2 gene transcripts were more abundant and less abundant, respectively, in the mutant strain compared to the wild-type strain. In strain CS101, 10 and 3 gene transcripts were more and less abundant, respectively, in the mutant strain compared to the wild-type strain. Finally, in strain SF370, 28 and 17 gene transcripts were more abundant and less abundant, respectively, in the mutant strain compared to the wild-type strain (Table 1). The known or putative functions of the majority of Rgg-regulated genes in all of the strains were associated with replication, transcription and translation, and metabolism. Quantitative RT-PCR was used to validate the microarray data (Table 2).

Surprisingly, the only genes similarly affected by *rgg* inactivation in all three strains were *speB* and *spy2040*, which are co-transcribed (Neely *et al*., 2003; Ma *et al*., 2006). In addition, only the expression of purine metabolism genes (*spy25-spy28*) in strain SF370 and the peptidyl prolyl isomerase gene (*prsA*) in strain CS101 were altered by more than 5-fold following *rgg* inactivation (Table 1). The results indicate that Rgg influences transcription in strains MGAS5005, SF370, and CS101 during the post-exponential phase of growth; however, the magnitude and extent of transcriptional changes is not nearly as great as those previously identified in strain NZ131 (Dmitriev *et al.*, 2006).

Inactivation of *rgg* alters transcription of regulatory genes in strains SF370 and CS101, but not in strain MGAS5005

Inactivation of *rgg* in strain NZ131 altered the transcript levels of 20 genes encoding transcriptional regulatory proteins (Dmitriev *et al.*, 2006). Such perturbation of other regulatory networks presumably contributes to the genomewide changes associated with *rgg* inactivation in this strain (Chaussee *et al.*, 2002; Dmitriev *et al.*, 2006). In contrast, *rgg* inactivation altered the abundance of three regulatory gene transcripts (*sagA*, *regR* and *gntR*) in strain SF370, and only one (*regR*) in CS101. No change in regulatory gene expression was detected in the *rgg* mutant of strain MGAS5005 (Table 1). Thus, the number of differences in the expression of regulatory genes in *rgg* mutant strains correlated with the number of differences in structural gene expression (Table 1). The results indicate that many genes in the Rgg regulon are controlled by strain-specific secondary mechanisms that remain to be elucidated.

DISCUSSION

Rgg core- and sub-regulons

Strain CS101 (serotype M49; class II) was selected to facilitate the identification of intraserotypic variation in the Rgg regulon by comparing the results to those previously obtained with the strain NZ131 (serotype M49; class II) (Dmitriev *et al.*, 2006). In addition, strains MGAS5005 and SF370 (serotypes M1; class I) were selected to identify potential differences within serotype M1 and to identify potential interserotypic and class differences in the regulon by comparing the results to those obtained with M49 strains. The genome sequences of MGAS5005, SF370, and NZ131, but not CS101, have been determined (Ferretti *et al*., 2001; McShan *et al*., 2006; Sumby *et al*., 2005). The M1 strains have similar PFGE patterns of chromosomal DNA, which were different from those of M49 strains (data not shown). Furthermore, MGAS5005 and SF370 belong to the same Multi Locus Sequence Type ST28 (http://www.mlst.net), and their Rgg regulons were expected to be similar. Surprisingly, the Rgg regulon varied significantly not only among the serotypes, but even among strains of the same serotype and Multi Locus Sequence Type. Only *speB* and adjacent *spy2040* (encoding a 56 amino acid hypothetical protein) gene transcripts were less abundant in all of the *rgg* mutant strains (Table 1), indicating that these co-transcribed genes (Neely *et al*., 2003; Ma *et al*., 2006; Dmitriev *et al*.; 2006) comprise the Rgg core-regulon. Rgg binds to DNA in the promoter region of *speB* (Neely *et al.*, 2003), indicating that the core-regulon is directly regulated by Rgg. Other genes, which are influenced by Rgg, varied from a single gene (*spy1793*) in strain MGAS5005 to 43 genes in the strain SF370. These genes can be considered as part of the Rgg sub-regulon, which is strain-variable.

Rgg interacts with other regulons

The strains used in this study were selected, in part, because they encode an identical Rgg polypeptide, have identical *rgg* promoter regions, and have similar levels of *rgg* expression, as determined with TaqMan quantitative RT-PCR (data not shown). Thus, the strain-associated differences in the regulon are not the result of Rgg sequence variation or *rgg* transcript levels, as previously observed for other streptococcal regulatory proteins (Vickerman *et al*., 2003; Vahling & McIver, 2005; Loughman & Caparon, 2007).

An association between the number of Rgg-regulated transcription factors and the total number of Rgg-regulated structural genes suggests that many changes in the sub-regulon are due to indirect effects associated with the perturbation of other regulatory circuits. For example, inactivation of *rgg* in strain NZ131 alters the expression of several regulatory genes (Dmitriev *et al*., 2006), each of which is present in the genomes of strains SF370 and MGAS5005; however, only *sagA*, *regR*, and *gntR* expression was altered following inactivation of *rgg* in strain SF370. All chromosomally encoded regulatory proteins present in strain SF370 are also encoded by strain 5005, suggesting that the differences in the sub-regulon between the strains is not due to compositional variation in the set of chromosomally encoded regulatory genes. Of course allelic variation or differential expression of regulatory proteins, including novel regulators such as LacD.1 (Loughman & Caparon, 2006), might contribute to strain-associated differences in the Rgg sub-regulon. In addition, *S. pyogenes* strains are polylysogenic and may differ in the number and types of bacteriophages present in the chromosome. For example, three bacteriophages (370.1, 370.2, and 370.4) are present in the SF370 strain but absent in the MGAS5005 strain. Similarly, two bacteriophages (5005.1 and 5005.3) are present in strain MGAS5005 but absent in the SF370 strain. Each prophage encodes several regulatory proteins involved in structural gene expression and lysogeny. Given that Rgg shares similarity with bacteriophage-encoded regulators, it is possible that the strain-associated differences in bacteriophage-encoded

regulatory proteins influences the Rgg sub-regulon; although, further experiments are clearly necessary to test this hypothesis.

Regulon variation in human bacterial pathogens

The diverse clinical manifestations associated with *S. pyogenes* are due, in part, to variation in the gene content of strains. In addition, strain- or isolate-specific variation in gene expression described in human pathogens significantly contributes to phenotypic diversity and significantly impacts host-pathogen interactions (Kwinn *et al*., 2007). In *Streptococcus pneumoniae*, inactivation of a response regulator gene (*rr09*) altered the transcript levels of 102 and 80 genes in strains D39 and TIGR4, respectively; however, the expression of only 7 of these genes were similarly affected in the two strains (Hendriksen *et al.*, 2007). Similar diversity has been described in the BvgAS regulon of *Bordetella bronchiseptica* and *Bordetella pertussis* (Cummings *et al*., 2006). In *S. pyogenes*, the global transcriptional regulator Mga promotes the expression of a number virulence factors, including M protein (*emm*) and the C5a peptidase (*scpA*) (Hondorp & McIver, 2007). Mga influences the expression of 204, 201, and 37 genes in strains of serotypes serotypes M4, M1, M6, respectively. Notably, only *emm*, *scpA*, and *spy2036* were similarly affected by *mga* inactivation in all three strains (Ribardo & McIver, 2006). The variation in Rgg regulon, in conjunction with variation in the Mga regulon, indicates that significant diversity exists among virulence-associated regulatory circuits of *S. pyogenes*. Additional information related to the molecular basis for such variation is thus necessary to understand the regulation of virulence factor expression in *S. pyogenes*.

In summary, we identified Rgg-regulated genes in strains representing class I and II organisms (M1 and M49 serotypes). The results show both inter- and intraserotypic variation in the Rgg regulon, which was consistent with results of biochemical, microbiological and quantitative real-time PCR assays. Such plasticity in regulatory circuits may provide pathogens with a means to adapt rapidly to changes in host-pathogen interactions.

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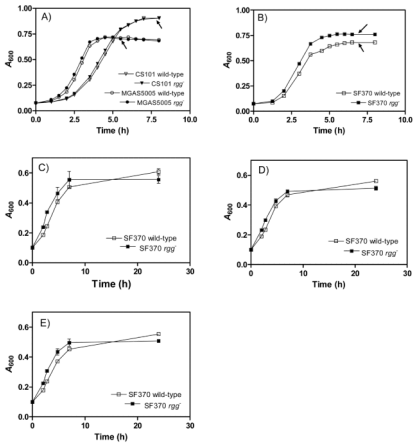

Fig. 1.

Growth of the wild-type strains and corresponding *rgg* mutants in Todd-Hewitt broth (A, B) and CDM containing 2% glucose (C), fructose (D) or sucrose (E) as the primary carbon and energy source. Arrows designate points of RNA isolation for microarray analysis. The results are shown as the means and standard errors of the means from two independent experiments.

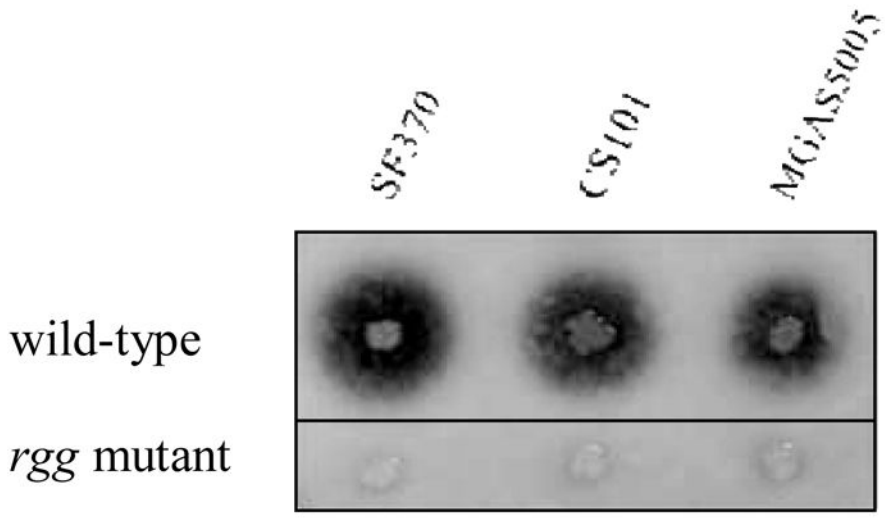

Fig. 2.
Abrogation of SpeB expression in the strains SF370, CS101, and MGAS5005 following *rgg* inactivation.

Table 1

Transcriptome changes (2-fold or greater; *P* values ≤ 0.05) associated with *rgg* nactivation during post-exponential growth

			Fold changes (*rgg*/wt)[b]		
SPy no.[a]	**Gene**	**Description**	**SF370**	**CS101**	**MGAS 5005**
More abundant genes in *rgg* mutants					
25		phosphoribosylformylglycinamidine synthase II	6	-[c]	-
26	*purF*	phosphoribosylpyrophosphate amidotransferase	5	-	-
27	*purM*	phosphoribosylformylglycinamide cyclo-ligase	6	-	-
28	*purN*	phosphoribosylglycinamide formyltransferase	7	-	-
32	*purD*	phosphoribosylamine-glycine ligase	3	-	-
34	*purK*	phosphoribosylaminoimidazole carboxylase II	4	-	-
35	*abiR*	abortive infection phage resistance protein	2	-	-
36	*purB*	adenylosuccinate lyase	2	-	-
37*[d]		hypothetical protein	3	-	-
39		protein-tyrosine phosphatase	2	-	-
663		hypothetical protein	2	-	-
670		hypothetical protein	2	-	-
673		hypothetical protein	2	-	-
728		hypothetical protein	-	2	-
732*		hypothetical protein	3	-	-
733*		hypothetical protein	2	-	-
738*	*sagA*	streptolysin S associated protein	2	-	-
739*	*sagB*	streptolysin S associated ORF	4	-	-
742*		hypothetical protein	4	-	-
744*		ABC transporter	3	-	-
746*		ABC-2 type transport system permease protein	3	-	-
1037	*ybbR*	hypothetical protein	-	3	-
1125*		GTP pyrophosphokinase	-	3	-
1126*		hypothetical protein	-	3	-
1154		hypothetical protein	-	2	-
1243	*pstC*	phosphate ABC transporter (permease protein)	-	3	-

SPy no.[a]	Gene	Description	Fold changes (*rgg*/wt)[b] SF370	CS101	MGAS 5005
1267	*pcrA*	ATP-dependent DNA helicase	3	-	-
1326		hypothetical protein	-	2	-
1600		hyaluronidase	2	-	-
1718		esterase	2	-	-
1793		ABC transporter	-	-	2
1815*	*scrA*	sucrose-specific PTS permease, enzyme II	2	-	-
1858	*pepXP*	X-Pro dipeptidyl-peptidase IV	-	3	-
1884		similar to several eukaryotic hypersensitive-induced response proteins	2	-	-
1916	*lacG*	phospho-beta-D-galactosidase	2	-	-
2032		ATP-binding cassette transporter-like protein	-	3	-
2107		oxidoreductase	2	-	-
2148	*mutS*	DNA mismatch repair protein	2	-	-
Less abundant genes in *rgg* mutants					
246	*rnpA*	ribonuclease P protein component	−3	-	-
600		hypothetical protein	−4	-	-
609	*ftsW*	cell division protein	−2	-	-
627	*regR*	transcriptional regulator (LacI family)	−2	2	-
1016		hypothetical protein	−3	-	-
1017		hypothetical protein	−3	-	-
1140	*tdk2*	thymidine kinase	−3	-	-
1141	*prfA*	peptide chain release factor 1	−2	-	-
1201	*ylxM*	DNA-binding protein	−3	-	-
1202	*gntR*	transcription regulator, GntR family	−4	-	-
1210		hypothetical protein	−3	-	-
1233	*coaA*	pantothenate kinase	−2	-	-
1632	*gmk*	guanylate kinase	−2	-	-
1741		hypothetical protein	−3	-	-
2037*	*prsA*	peptidyl prolyl isomerase	-	−11	-

SPy no.[a]	Gene	Description	Fold changes (*rgg*/wt)[b]		
			SF370	CS101	MGAS 5005
2039*	*speB*	pyrogenic exotoxin B	−37	−71	−35
2040*		hypothetical protein	−146	−335	−69
2207	*trsA*	tryptophanyl-tRNA synthetase	−3	-	-

[a] SPy numbers designate open reading frames based on the SF370 *S. pyogenes* genome annotation (Ferretti *et al.*, 2001).

[b] Change in transcript level of *rgg* mutant compared to that of the wild-type.

[c] *rgg* inactivation doesn't affect transcript level.

[d] Asterisks indicate genes similarly regulated in NZ131 strain (Dmitriev *et al.*, 2006).

Table 2

Correlation between results obtained with DNA microarrays and quantitative TaqMan RT-PCR

SPy no.[a]	Designation [b]	Strain	Fold changes (*rgg*/wt) [c]	
			RT-PCR (Log10)	Microarrays (Log10)
0028	*purN* (phosphoribosylglycinamide formyltransferase)	SF370	1.72	0.87
0165	*spn* (NAD-glycohydrolase)	CS101	0.33	0.46
0165	*spn* (NAD-glycohydrolase)	MGAS5005	0.21	0.24
0738	*sagA* (streptolysin S)	SF370	0.76	0.32
0738	*sagA* (streptolysin S)	MGAS5005	−0.06	−0.16
1547	*arcA* (arginine deiminase)	SF370	0.35	0.12
2039	*speB* (pyrogenic exotoxin B)	CS101	−3.5	−1.8
2039	*speB* (pyrogenic exotoxin B)	SF370	−2.28	−1.6
2039	*speB* (pyrogenic exotoxin B)	MGAS5005	−1.7	−1.5
2043	*mf* (mitogenic factor)	CS101	−0.02	−0.16
2043	*mf* (mitogenic factor)	SF370	0.45	0.14

[a] SPy numbers designate open reading frames based on the SF370 *S. pyogenes* genome annotation (Ferretti *et al.*, 2001).

[b] Gene designations are in italics, and the corresponding protein function is in parentheses.

[c] Change in transcript level of *rgg* mutant compared to that of the wild-type.