INFECTION AND IMMUNITY, Feb. 2002, p. 762–770 Vol. 70, No. 2
0019-9567/02/$04.00+0 DOI: 10.1128/IAI.70.2.762–770.2002

Rgg Influences the Expression of Multiple Regulatory Loci To Coregulate Virulence Factor Expression in *Streptococcus pyogenes*

Michael S. Chaussee,* Gail L. Sylva, Daniel E. Sturdevant, Laura M. Smoot, Morag R. Graham, Robert O. Watson,† and James M. Musser

Laboratory of Human Bacterial Pathogenesis, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, Montana 59840

Received 15 August 2001/Returned for modification 8 October 2001/Accepted 22 October 2001

The human pathogen *Streptococcus pyogenes* secretes many proteins to the cell wall and extracellular environment that contribute to virulence. Rgg regulates the expression of several exoproteins including a cysteine protease (SPE B), a nuclease (MF-1), a putative nuclease (MF-3), and autolysin. The functional heterogeneity of Rgg-regulated exoproteins and the lack of a conserved regulatory motif in the promoter regions of the genes suggested that Rgg interacts with additional regulatory networks to influence gene expression. DNA microarrays were used to test this hypothesis by comparing genomewide transcript profiles of *S. pyogenes* NZ131 and isogenic derivative NZ131 *rgg* during the exponential phase of growth. Transcripts of known and putative virulence-associated genes were more abundant in the *rgg* mutant, including *emm*, *scpA*, *orfX*, *scl1*, *hasAB*, *slo*, *sagA*, *ska*, *speH*, *grab*, *mac*, *mf-1*, and *mf-3*. Increased transcription of *emm*, *scpA*, and *orfX* in the *rgg* mutant was associated with increased production of the corresponding proteins. Differences in the expression of virulence-associated genes were associated with changes in the expression of several regulatory genes, including *mga*, *sagA*, *csrRS*, and *fasBCA*. The results show that Rgg influences the expression of multiple regulatory networks to coregulate virulence factor expression in *S. pyogenes*.

Human infection with *Streptococcus pyogenes* may result in a variety of diseases, including pharyngitis, impetigo, toxic shock syndrome, necrotizing fasciitis, rheumatic fever, and acute glomerulonephritis. *S. pyogenes* secretes many proteins to the cell wall and extracellular environment that directly influence host-pathogen interactions. Although several of these proteins have been studied in detail, the functions of many of them are not known. Insight into the functions of secreted proteins can be gained by identifying coordinately regulated genes, which are likely to have related functions. In addition, identification of virulence-associated regulatory networks may lead to new therapeutic strategies designed to minimize severe disease by inhibiting the expression of virulence-associated genes.

Several transcriptional regulators have been described in *S. pyogenes* that influence the expression of secreted proteins. Among these, Mga is the most thoroughly studied. Mga coordinates expression of colinear genes encoding proteins involved in adherence and the ability to resist phagocytosis. These include the M protein (*emm*), C5a peptidase (*scpA*), OrfX (*orfX*), and in certain serotypes, M-related proteins such as Mrp (*mrp*) and Enn (*enn*; 5, 22, 35, 38, 42, 47). Mga activates transcription by binding to defined response elements in the promoter regions of *mga*, *emm*, and *scpA* (31, 34). Mga also activates transcription of genes located elsewhere in the chromosome, including *sof*, which encodes a fibronectin-binding lipoproteinase designated SOF (35, 44), and *scl1*, which encodes a collagen-like adhesin, Scl1 (28, 45). Mga is considered to be the primary transcriptional activator of these genes; however, other uncharacterized factors also influence expression (34).

Inactivation of regulatory genes that influence expression of secreted proteins often results in a pleiotropic phenotype. For example, inactivation of the two-component regulator designated CsrRS, also known as CovRS, enhanced expression of *hasAB*, which are required for hyaluronic acid capsule biosynthesis and *speB*, *mf-1*, and *sagA* (3, 14, 17, 25). The *speB* gene encodes a secreted cysteine protease (SPE B) that contributes to virulence in mouse models (21, 27, 29), and *mf-1* encodes a secreted nuclease (MF-1) previously described as DNase B (49). The *sagA* locus (also known as *pel*) has regulatory activity and encodes streptolysin S (4, 26). Inactivation of *sagA* decreased expression of *emm*, *speB*, and *ska*, which encodes the secreted plasminogen activator streptokinase (26). Nra influences expression of *prtF2* (encoding a fibronectin binding protein), *cpa* (encoding a collagen binding adhesin), and *mga* (41). Nra has an amino acid sequence similar to that of RofA, which is a positive regulator of the fibronectin binding protein encoded by *prtF1* (16), and also affects expression of *mga*, *emm*, *sagA*, and *speA*, which encodes a secreted superantigen, SPE A (1). A two-component regulator composed of two histidine kinases (FasB and FasC) and a response regulator (FasA) influences expression of *ska* and *sagA* (20). These findings suggest that multiple regulatory networks interact to coordinate expression of virulence-associated genes in *S. pyogenes*.

Complex regulation may result from the influences of multiple regulatory networks on gene expression. For example, transcription of *speB* is abolished in *rgg* (*ropB*) mutants (6, 30), decreased in *mga* (42) and *sagA* (26) mutants, and enhanced in a *csrR* mutant (17). Inactivation of dipeptide and oligopeptide transport operons decreases *speB* expression (39, 40), and SPE B production is influenced by the growth phase and the con-

* Corresponding author. Mailing address: Division of Basic Biomedical Sciences, College of Medicine, University of South Dakota, Lee Medical Building, 414 East Clark St., Vermillion, SD 57069–2390. Phone: (605) 677–6681. Fax: (605) 677–6381. E-mail: mchausse@usd.edu.

† Present address: Section of Microbial Pathogenesis, Yale School of Medicine, New Haven, CT 06536.

centration of glucose in the medium (9). Such intricate regulation of gene expression may be particularly important for proteins, such as SPE B, that can cause significant destruction of host tissues.

Rgg was initially identified in *S. pyogenes* as being required for the expression of *speB* (6, 30). Recently, Rgg was discovered to influence the expression of additional exoproteins, including autolysin, MF-1, and MF-3 (10). The absence of a conserved regulatory motif in the promoter regions of Rgg-regulated genes and the functional diversity of the gene products suggested that Rgg interacts with additional regulatory networks to alter gene expression. DNA microarrays and quantitative RT-PCR were used to identify and quantitate genomewide changes in transcription associated with inactivation of *rgg*. The results demonstrate that Rgg interacts with other regulatory networks to influence the expression of several genes known to mediate interactions of *S. pyogenes* with its human host.

MATERIALS AND METHODS

Strains and media. *S. pyogenes* NZ131 (serotype M49) and the isogenic derivatives NZ131 *speB* and NZ131 *rgg* have been previously described (6, 8). *S. pyogenes* was grown with Trypticase soy agar containing 5% sheep blood agar (Becton Dickinson, Cockeysville, Md.) overnight at 37°C in 5% CO_2 or with Todd-Hewitt broth containing 0.2% (wt/vol) yeast extract (THY; Difco Laboratories, Detroit, Mich.).

RNA isolation. *S. pyogenes* was grown in 10 ml of THY broth in 15-ml tubes (Corning, New York, N.Y.) for 2 to 3 h (A_{600} = 0.2). Cultures were centrifuged, and the bacteria were suspended in 200 μl of diethyl pyrocarbonate (Sigma Chemical Co., St. Louis, Mo.)-treated water and frozen in liquid nitrogen. RNA was isolated with a FastPrep Instrument (Qbiogene, Carlsbad, Calif.) and a FastPrep Blue kit (Qbiogene) as previously described (10).

DNA microarray analysis. DNA microarrays were prepared and analyzed as previously described (48). Briefly, internal regions (approximately 500 bp) of 1,893 open reading frames identified by WIT2 analysis (wit.mcs.anl.gov/WIT2) of the *S. pyogenes* SF370 (serotype M1) genome (15) were amplified by using open reading frame-specific primers designed with Primer3 software (http://www genome.wi.mit.edu). The PCR products were printed in quadruplicate on CMT-GAPS slides (Corning) with a Chipwriter robotic arrayer (Virtek, Waterloo, Ontario, Canada). Fluorescently labeled cDNA probes were prepared with an ARES DNA labeling kit (Molecular Probes, Eugene, Oreg.). Probes were denatured at 100°C for 5 min and hybridized with microarrays in 100 μl of Perfect-Hyb solution (Sigma) at 55°C for approximately 18 h. The arrays were washed twice in 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)--0.1% sodium dodecyl sulfate for 10 min at room temperature and twice in 0.1× SSC. The slides were scanned with a ScanArray 5000 instrument (Packard Bioscience, Meriden, Conn.), and the data were analyzed with QuantArray software (Packard Bioscience) as previously described (48).

Quantitative reverse transcription (RT)-PCR. Oligonucleotide primers and probes (Table 1) were designed with Primer Express 1.0 software (ABI Prism; PE Biosystems, Framingham, Mass.) and purchased from either MegaBases Inc. (Evanston, Ill.) or PE Biosystems. Amplification and detection were done with the ABI Prism 7700 Sequence Detection System (PE Applied Biosystems) by using TaqMan One-Step RT-PCR Master Mix Reagents (PE Biosystems) as described by the manufacturer. Each assay was done in triplicate with at least two independently isolated RNA samples. Amplification and analysis were done as previously described (10). The quantity of cDNA for each experimental gene was normalized to the quantity of *gyrA* cDNA in each sample, and the mean ± the standard error of the mean of independently isolated RNA samples is reported.

Immunoblot analysis. Immunoblotting was done as previously described (7). Briefly, 10^8 streptococci, determined by dilution plating and enumeration of CFU per milliliter, were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis with a 10% acrylamide resolving gel. Following electrophoresis, proteins were transferred to nitrocellulose membranes (Millipore Corp., Bedford, Mass.) with Towbin's buffer (50). Antisera to M49 (provided by J. B. Dale, Department of Medicine, University of Tennessee, Memphis), C5a peptidase (provided by P. P. Cleary, Department of Microbiology and Immunology, University of Minnesota, Minneapolis), and OrfX (provided by B. Lei, Laboratory of Human Bacterial Pathogenesis, Rocky Mountain Laboratories, Hamilton, Mont.) were used at a dilution of 1:1,000. The antibody-antigen complexes were visualized with the enhanced chemiluminescence Western blotting detection system (Amersham Pharmacia Biotech, Inc., Piscataway, N.J.).

RESULTS

Inactivation of *rgg* increases transcription of several virulence-associated genes. DNA microarrays and cDNA prepared from exponential-phase cultures of *S. pyogenes* NZ131 and NZ131 *rgg* were used to identify genomewide differences in virulence-associated gene transcripts. Transcripts of several genes encoding known or putative virulence factors were more abundant in the *rgg* mutant (Table 2), including well-characterized virulence factors such as M protein, C5a peptidase, streptolysin S, and streptolysin O (*slo*). Transcripts of recently characterized genes likely to contribute to host-pathogen interactions were also more abundant in the mutant, including (i) *scl1*, which encodes an adhesin (28, 45), (ii) *speH*, which encodes a superantigen (43), (iii) *grab*, which encodes a cell wall-associated protein that binds human α_2-macroglobulin to inhibit protease activity (46), and (iv) *mac*, which encodes a secreted protein that inhibits opsonophagocytic killing of *S. pyogenes* (24).

The role of secreted nucleases in the pathogenesis of *S. pyogenes* infection has not been established. However, the toxicity of cytolethal distending toxin of *Campylobacter jejuni* is dependent on its nuclease activity (23). Transcripts of a known and putative extracellular nucleases, including *mf-1*, *mf-3*, and *spy0747*, were more abundant in the *rgg* mutant (Table 2).

Inactivation of *rgg* enhances expression of the Mga regulon. Increased transcripts of the Mga-regulated genes *emm*, *scpA*, and *scl1* in the *rgg* mutant (Table 2) suggested that Rgg represses expression of the Mga regulon. To test this idea, quantitative RT-PCR was used to quantitate Mga-regulated gene transcripts, including *mga*, *emm*, *scpA*, *enn*, *orfX*, *scl1*, and *sof*. Transcripts of each gene were more abundant in the *rgg* mutant than in the isogenic wild-type strain (Fig. 1).

Transcripts of *rgg* are more abundant in NZ131 *speB* than in the isogenic wild-type strain (10). This observation suggested that inactivation of *speB* or the lack of *speB* expression in the mutant could alter the transcription of genes other than *speB*. Because the *rgg* mutant does not express *speB*, it was theoretically possible that differences in expression in the *rgg* mutant resulted from a lack of *speB* expression. To assess this possibility, the quantities of Mga-regulated gene transcripts were determined in strain NZ131 *speB*. The quantities of gene transcripts in NZ131 *speB* were similar to those in the isogenic wild-type strain, albeit slightly elevated (Fig. 1). The results show that increased transcription of Mga-regulated genes in the *rgg* mutant was not due to a lack of *speB* expression (Fig. 1). Importantly, results obtained with DNA microarrays and RT-PCR were qualitatively cognate.

To determine if the observed changes in transcription of the Mga regulon were associated with changes in protein production, immunoblotting was done following whole-cell electrophoresis of wild-type NZ131, *speB* mutant strain NZ131, and *rgg* mutant strain NZ131 harvested in the exponential phase of growth (A_{600} = 0.2). NZ131 *speB* was analyzed to control for potential degradation of antigens by the SPE B protease. The

TABLE 1. Oligonucleotide primers and fluorescent probes used for TaqMan RT-PCR

Accession no.	Designation[a]	Forward primer (5'-3')	Reverse primer (5'-3')	Fluorescent probe[b] (5'-3')
AF057697	*sof49* (SOF)	CACTACAAAGACGATTGGTGCTAAA	CAACACCCCAGTCCCAATCT	TAGAGGAAGTTTGGGAGCAGGCCAAAA
M31789	*emm49* (M49)	GCATCCGTAGCGGTCGC	CAGCAGCCTTAACTTCTGTTTGG	TGGCTGTTTTAGGAGCAGGCTTTGCA
X68501	*mga49* (Mga)	GCTCAATCTCAGCATCACCAATAA	AATGTCTTCTTCGTCTAACACCGA	ACGAAGTTGCCGTAACCCTCCATAACG
X72753	*enn* (Enn)	AGCACCAAAAATTAGAGGCTGATC	GATGCTTCAAGGTCACGGCT	CCAAGTTTCAGAGACTAGCCGCAAGGG
X78055	*scpA49* (C5a peptidase)	AAACGAAAGAACCTTACCGCC	CGACACGCATCAAAAGCAA	AGAAGGTGCGATGCCTGAGGCTCA
X80397	*orfX* (OrfX)	TCTACTGAGTCTACTACTCAGCCAGTTGA	TGTCACCATCGAATCTGAAGCT	CACCCGTGCAGGAGACACAGGC
SPy0167	*slo* (SLO)	GAAATATCCGTATCATGGCTAGAGAGT	CACATCTCTTTCGTCGATCACTTT	GCCACCATTCCCAAGCTAAGCCAGTG
SpyO212	*speG* (SpeG)	TTCTATTGTATCTTTAGGCATTACTGATCA	TTCGAATGTTTTACAATACCACCATAA	CGATGTTTTTGGTCTCCCTTATAATTTTTCCCC
SPy0242	*fasB* (FasB)	TGAGCCGTATAGAGCGTTTAGGT	TCATGAACCGTTTGGGCATA	AAGCCATTAAGAGTGAATCCATTACTCAGATTCAAGA
SPy0244	*fasC* (FasC)	CACAAAAACCAATCGTGTGGATA	CAGTCAAAAAGTGGGCTGAGTTC	AGCCAAACGCTTTGATGCTCAGCAG
SPy0245	*fasA* (FasA)	CGTTGTGGGCTTGAATTAGCT	ATTCTGAATGAGTGGTGACAAACAC	CAGCTATTCGACAAAAAGATCCTAATGCCGTTAT
SPy0336	*csrR* (CsrR)	GGTGCAGACGACTATATTGTTAAACCC	TTGACGGCGGAAAATAGCA	TTTGCCATTGAAGAACTACTTGCCCGTATTC
SPy0337	*csrS* (CsrS)	CCTGGCTTGCATGGTCCTA	CATGGAAAACCCACGATACTGA	CGGTCGTGTGTATCACGACCATATCGA
SPy0528	*vicR* (VicR)	ACGTGACCAAGCCCTTTTCTAA	TTCGGTACGACGCAGATGAG	CACGCGCCAGCAATTCCCG
SPy0529	*vicK* (VicK)	GCGCTTTGCGTTGAATAGG	TCCTGTTCTGTGGTGTCATGGA	CCACAACCAAGCCCGAAATAAAACCACT
SPy0738	*sagA* (SLS)	TTGCTCCTGGAGGCTGCT	CTTCCGCTACCACCTTGAGAAT	ACCACTTCCAGTAGCAATTGAGAAGCAACAAG
SPy0747	None (none)	TTGTGTGCAACCAGTCACTTTTAA	TCTTTCGCAAATTGTGCTAGCA	TCTGAGCAAAGACGTCACGTCTTGGCTA
SPy0861	*mac* (Mac)	ACTTCCGTTTGGACCAAAGGA	CAACATAAGGAGCGTGAAAAACATC	TTACTCCTCCAGCAAACTTCACTCAAGGTGA
SPy0874	*trcR* (TrcR)	TGGCAGATGTCTAATGTTTTGACA	TGCCTCTTGACCGTTAAAAACC	CTGCCATGACTCATCAAGGTTATGACGTTG
SPy0875	None (none)	TTCCGGTATGGTCATCAAACC	ACTCGTGGCCTGCATTAGTAATAA	GCGACGCTGCTTCTCAGAATTTTGGACA
SPy0895	*deoD2* (DeoD2)	TTGCCTTTCTAGCGGAACATG	GCGATTGGTTAAGCCATAATGAAC	CAGAGAATCGGACCATTGAAGACCAGTATCAC
SPy0898	*cpsY* (CpsY)	CACAACTCCTTTTATTTTTCAGAAGAGA	GCACGGTCACTGACCACAAT	ATGTCCCAGATGCCCCACAACAAATC
SPy1008	*speH* (SpeH)	TCACAGGAATTCAAAGATAAAGAAGTAGA	CAAACGCTTCATACCTTTTCCC	ATTTATGCTCTATCTGCACAAGAGGTTTGTGAATGT
SPy1061	*yesN* (YesN)	GTTATCATTGTTGCTAATTGGTCTTTTTAT	GGTTGCAGTGGCATTGATATCA	TCATTAGCGCCACAACAATTAGGCCTCTATC
SPy1062	*yesM* (YesM)	TTGGAGTGGGTACATGCTCATT	AACTCTCACTATACCCTAAGTCATGAGCTA	TCAAGACAAGATCAGCACGAGCCGC
SPy1106	*yufM* (YufM)	GCAATGGGATTCAATTTTTGGA	ATTTGCGGCTGAAATAATAATGACT	AAATGGCGTACTCAGCATATTCCTTGCG
SPy1236	*ciaS* (CiaS)	GGTCCAGGGATAACAGATGAAGAA	CCACCTGTTTGCCGTGTTC	AGCTTGTCAACTCGATAAAAACGATCAAAAATCTTT
SPy1237	*ciaR* (CiaR)	GATCATGACAGCTAAAGAGGGTTTAGA	AATAGAACGGCTTTGTCAGATAATCA	CAGCACCTAATTCAAATCCGTGGCCTT
SPy1357	*grab* (Grab)	GCATCAGTATTAGTCGGTTCAACAGT	CCGCCATTTGGAATAATTCG	TCTGCTGTTGACTCACCTATCGAACAGCC
SPy1436	*mf-3* (MF-3)	TCCTCCTGGCTGGCATAATT	CTAAATGGCCACGGTCCATT	AAATTGACTGACGCTAATGGAAAAACAACTTGG
SPy1556	*zmpS* (ZmpS)	AAAAGAAGTATCAGCAATAGCAATGG	TGCTGAGCCAAGCTTTTTAGG	TCAAATCAGAATCTGCCAACCGCTCA
SPy1587	*lytR* (LytR)	GATTTTACAAGGGCTCACCATGA	TGCCTGATCTTGCTGTTTGAA	ACTTCAAATCCAAGTGCCTGCCAATCA
SPy1588	*lytS* (LytS)	TTTACCTCCGTCTCTTTCATCATTT	AAGCCACCATTTGGCTCCTT	CGAGACAGGGTTTCTTGGTCCATGGC
SPy1801	*isp-2* (Isp-2)	CGTCGCGCACAAATTATGG	GGCTGGAATGGCTGGTGT	CCATGGTCATCAATCCACTTATCCAGGTCT
SPy1908	*salR* (SalR)	TTGATGACCACAGACTATTTGCAA	ACATCGTTAAAATGAGAAGTAATCGTATCT	CCAACTGTTATTCCAACAGTATGATGAAGTAGATGTCA
SPy1909	*salK* (SalK)	GGATTGTACTCATGAAGGTATTAGTTAAAGA	TTGGAGTTTTCTAAATAATAAAAGATGCA	GAAATTACCACTAGACAAAACAACGTGCTTGCGT
SPy1979	*ska* (SKA)	TGCTGACAAAGATGGTTCGG	GCACATGTCCCTTTAACAAAAATTC	ACCTTGCCGACCCAACCTGTCCA
SPy1983	*scl*1 (Scl-1)	CAAACTAGTCTGGCGCTACGG	TTGCCCCGCCTCCAA	CCTCAGCCGCTGCCGTCCTTCT
SPy2025	*isp-1* (Isp-1)	GCAGCGCAAGCCAGGTT	CTTGGCCTGGTGCAAATGA	ACCACCCATAAACCTAAAGTGGGCTA
SPy2026	*ihk* (Ihk)	GGTGACTTTGCAGATCGTGACA	TTGAACCAGTTGGGCTTGC	AAGGACGTCTCGGCACTTTCGCAAATAT
SPy2027	*irr* (Irr)	TCGAAAATGTATTACGCGTCTCTACT	CGCTATGTTCGGTCTCATCAAC	CAGATGAAAAGCGCCAAATCGGAGACTTAC
SPy2043	*mf-1* (MF-1)	CCCAAAATGTAGGAGGTCGTG	CATTCTTGAGCTCTTTGTTCGGT	CCAAAAAGGCGGCATGCGCT
SPy2200	*hasA* (HasA)	ACCGTTCCCTTGTCAATAAAGG	CGTCAGCGTCAGATCTTTCAAA	CGCCATGCTCAAGCGTGGGC
SPy2201	*hasB* (HasB)	GAATCGAGAAAATTAAATAGTCACATGATT	CCTCCATAACCAAATGATGGGT	TTCAAGGAATTTCTTATGATGATCGAATAGGAATGC
SPy2202	*hasC* (HasC)	TGAAACCACTGCCATTAACCTTC	GCTTGTAAGACAGCATCTCCCA	TTTTATCCGTCAAAGCCACCCAAGAGG

[a] Gene designations are shown in italics, and the corresponding protein designations are in parentheses.
[b] Covalently linked at the 5' end to 5-carboxyfluorescein and at the 3' end to *N,N,N*-tetramethyl-6-carboxyrhodamine.

TABLE 2. Increased transcripts of virulence-associated genes in *rgg* mutant strain NZ131 identified with DNA microarrays

SPy no.	Designation[a]	Mean fold difference ± SEM[b]
0167	*slo* (SLO)	2.1 ± 0.1
0738	*sagA* (SLS)	2.1 ± 0.1
0747	None (none)	1.6 ± 0.1
0861	*mac* (Mac)	1.5 ± 0.1
1008	*speH* (SpeH)	1.5 ± 0.2
1357	*grab* (Grab)	1.6 ± 0.0
1436	*mf-3* (MF-3)	8.8 ± 0.3
1983	*scl1* (Scl-1)	3.3 ± 0.2
2010	*scpA* (C5a peptidase)	1.5 ± 0.1
2018	*emm* (M)	1.9 ± 0.1
2043	*mf-1* (MF-1)	2.8 ± 0.3

[a] Gene designations are in italics, and the corresponding protein designations are in parentheses.

[b] The mean difference ± the standard error of the mean (*rgg* mutant strain NZ131 versus wild-type NZ131) in intensity of quadruplicate spots is shown.

rgg mutant expressed more M49, C5a peptidase, and OrfX than did the isogenic parental strain and the *speB* mutant (Fig. 2). Production of M49 was dramatically influenced by *rgg* inactivation, whereas relatively minor differences in the production of C5a peptidase and OrfX were detected. In addition, faster-migrating species of immunoreactive proteins were repeatedly observed following whole-cell electrophoresis of NZ131 *rgg* compared to the control strains. The molecular basis for the aberrantly migrating proteins is not known. Equal numbers of CFU of each strain were evaluated, and antisera to the group A streptococcal carbohydrate confirmed that similar amounts of immunoreactive proteins were analyzed (Fig. 2).

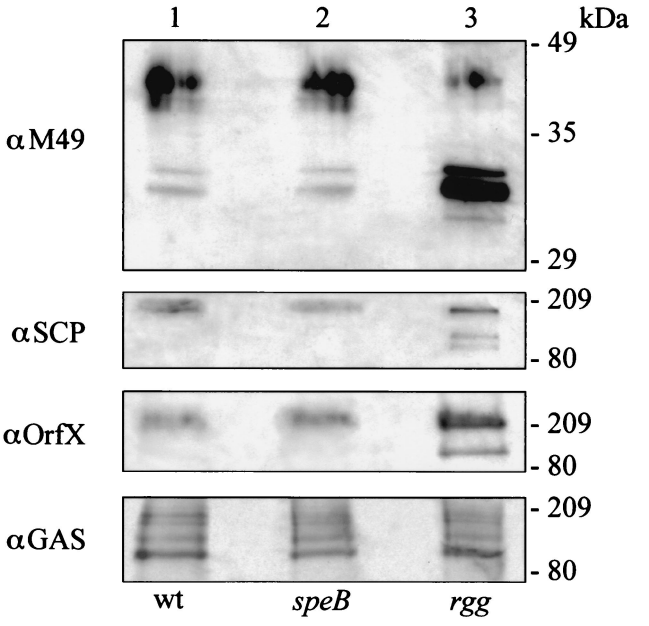

FIG. 2. Increased production of M protein, C5a peptidase (SCP), and OrfX in NZ131 *rgg*. Production of selected Mga-regulated proteins was assessed by immunoblotting following whole-cell electrophoresis of wild-type (wt) strain NZ131 (lane 1), *speB* mutant strain NZ131 (lane 2), and *rgg* mutant strain NZ131 (lane 3). M protein (αM49), C5a peptidase (αSCP), and OrfX (αOrfX) were detected with antisera specific to the polypeptides, as indicated to the left of each panel. The migration and size of the molecular mass standards are shown to the right of each panel. Antisera to the group A streptococcus carbohydrate (αGAS) was used to confirm that similar amounts of cell wall antigens were analyzed. The experiment was repeated twice, and a representative result is shown.

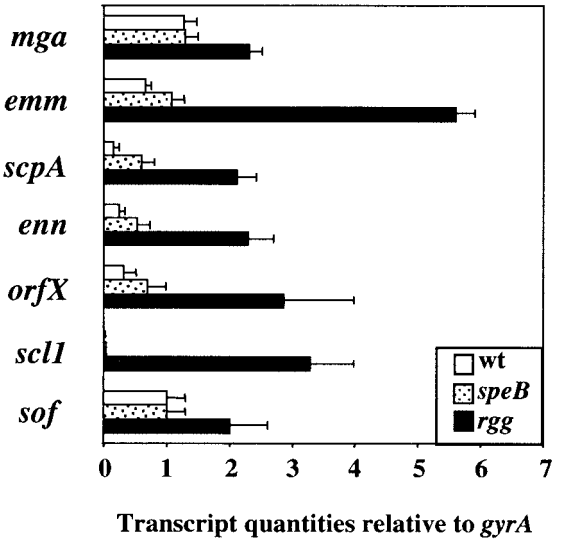

FIG. 1. Increased transcription of the Mga regulon in NZ131 *rgg*. Total RNA was isolated from exponential-phase cultures of wild-type (wt) NZ131, *speB* mutant strain NZ131, and *rgg* mutant strain NZ131, and the quantities of gene-specific transcripts were determined by quantitative RT-PCR. The quantity of cDNA for each gene was normalized to the quantity of *gyrA* cDNA in each RNA sample. The values shown are the mean ± the standard error of the mean of at least two independently isolated RNA preparations analyzed in triplicate.

Rgg influences *csrRS* expression. Transcripts of *mf-1* and *sagA* were more abundant in the *rgg* mutant (Table 2), similar to results previously obtained with *csrR* mutants (14, 17). To determine if the changes in expression of these genes were associated with altered transcription of *csrRS*, the quantities of *csrR*, *csrS*, and CsrRS-regulated gene transcripts were determined in wild-type strain NZ131, *speB* mutant strain NZ131, and *rgg* mutant strain NZ131. Transcripts of *csrR* and *csrS* were less abundant in the *rgg* mutant than in both the isogenic wild-type strain and an *speB* mutant (Table 3). The *hasABC* gene products catalyze hyaluronic acid biosynthesis (13). Expression of *hasAB* is increased in *csrR* mutants (3, 25). Similarly, transcription of *hasA* and *hasB* was enhanced in the *rgg* mutant by 3.3- and 2.3-fold, respectively (Table 3). The *hasABC* genes are contiguous in the chromosome (11). The *hasC* gene is located 192 bp downstream of *hasB* and encodes a UDP-glucose pyrophosphorylase that is not required by all strains of *S. pyogenes* for capsule synthesis (12). In contrast to *hasA* and *hasB*, no difference in the quantity of *hasC* transcripts was detected in the *rgg* mutant (Table 3). Streptokinase is an activator of human plasminogen, and certain alleles, including *ska* from strain NZ131, have been associated with poststreptococcal acute glomerulonephritis in a rabbit model (37). DNA microarray analysis of the *rgg* mutant did not detect a difference in *ska* transcription (data not shown). However,

TABLE 3. Quantitative RT-PCR analysis of CsrRS-regulated genes

SPy no.	Designation[b]	Quantity of transcript[a] (mean ± SEM)			*rgg*/wild-type ratio[c]
		Wild-type NZ131	*speB* mutant NZ131	*rgg* mutant NZ131	
0336	*csrR* (CsrR)	5.95 ± 0.8	7.81 ± 0.6	3.62 ± 0.3	0.6
0337	*csrS* (CsrS)	1.15 ± 0.2	1.23 ± 0.1	0.70 ± 0.0	0.6
0738	*sagA* (SLS)	6.32 ± 0.5	6.38 ± 0.5	26.63 ± 3.2	4.2
1979	*ska* (SKA)	38.39 ± 3.0	47.53 ± 3.9	58.35 ± 19.7	1.5
2043	*mf-1* (MF-1)	1.00 ± 0.1	0.92 ± 0.2	4.48 ± 0.5	4.5
2200	*hasA* (HasA)	0.12 ± 0.0	0.13 ± 0.0	0.39 ± 0.3	3.3
2201	*hasB* (HasB)	0.03 ± 0.0	0.05 ± 0.0	0.07 ± 0.0	2.3
2202	*hasC* (HasC)	0.16 ± 0.0	0.18 ± 0.0	0.16 ± 0.0	1.0

[a] The gene-specific cDNA values were normalized to the quantity of *gyrA* cDNA in each sample. The mean ± the standard error of the mean of two independently isolated RNA samples is given.
[b] Gene designations are in italics, and the corresponding protein designations are in parentheses.
[c] Quantity of *gyrA*-normalized cDNA in *rgg* mutant strain NZ131 ÷ quantity in wild-type NZ131.

ska expression is increased in *csrR* mutants (14). Thus, it was of interest to quantitate *ska* transcripts in wild-type NZ131, *speB* mutant strain NZ131, and *rgg* mutant strain NZ131. In contrast to results obtained with DNA microarrays, *ska* transcripts were more abundant in the *rgg* mutant (Table 3). Quantitative RT-PCR confirmed that the expression of *mf-1* and *sagA* is increased in the *rgg* mutant (Table 3). The results suggest that some of the observed differences in gene transcription in the *rgg* mutant are due to decreased expression of *csrRS*.

Confirmation of changes in virulence-associated gene transcription with RT-PCR. DNA microarray analysis of the *rgg* mutant detected differences in the transcript levels of several genes known or thought to contribute to virulence (Table 2). Quantitative RT-PCR was used to confirm and quantitate differences in these transcript levels in wild-type strain NZ131, *speB* mutant strain NZ131, and *rgg* mutant strain NZ131 (Table 4). Transcripts encoding the potential virulence factors SpeG, Isp-1, and Isp-2 were similarly analyzed. The results confirmed that transcription of *mac* was increased in NZ131 *rgg* compared to both the wild-type and *speB* mutant NZ131 strains (Table 4). However, in contrast to results obtained with DNA microarrays, quantitative RT-PCR showed that *grab* transcripts were less abundant in the *rgg* mutant than in the wild-type and *speB* mutant NZ131 strains (Table 4). Transcription of *speH* was enhanced in the *rgg* mutant independently of *speB*, whereas there was virtually no change in the transcription of *speG*, which also encodes a superantigen (Table 4). The functions of the extracellular proteins Isp-1 and Isp-2 are not known; however, Isp-2 is expressed during human infection with *S. pyogenes* and may be involved in host-pathogen interactions (33). *isp-1*, but not *isp-2*, transcripts were less abundant in the *rgg* mutant (Table 4).

MF-1 is a secreted nuclease (49). Expression of a second putative extracellular nuclease, designated MF-3, was previously discovered to be up-regulated in NZ131 *rgg* in the stationary phase of growth (10). Quantitative RT-PCR confirmed that transcription of *mf-3* and a gene encoding a protein with homology to nuclease H (SPy0747) was increased in the *rgg* mutant (Table 4).

Rgg influences expression of several regulatory genes. Inactivation of *rgg* altered expression of four previously characterized regulatory loci (*mga*, *csrR*, *csrS*, and *sagA*). To identify additional changes in regulatory gene expression, the transcript levels of genes encoding two-component regulators were determined. We focused on two-component regulatory systems because they are important regulators of virulence factor expression in many pathogenic bacteria. FasABC is a three-component system involved in the regulation of fibronectin- and fibrinogen-binding and streptokinase activity (20). Transcription of *fasABC* was decreased by approximately 50% for each gene (Table 5), consistent with the previous finding that transcription of *fasABC* is polycistronic (20). Additional two-

TABLE 4. Quantitative RT-PCR analysis of virulence-associated gene transcripts

SPy no.	Designation[b]	Quantity of transcript[a] (mean ± SEM)			*rgg*/wild-type ratio[c]
		Wild-type NZ131	*speB* mutant NZ131	*rgg* mutant NZ131	
0167[d]	***slo* (SLO)**	**0.09 ± 0.0**	**0.10 ± 0.0**	**1.25 ± 0.1**	**13.9**
0212	*speG* (SpeG)	0.42 ± 0.1	0.56 ± 0.0	0.33 ± 0.0	0.8
0747	**None (none)**	**0.31 ± 0.0**	**0.26 ± 0.0**	**1.00 ± 0.1**	**3.2**
0861	***mac* (Mac)**	**0.03 ± 0.0**	**0.04 ± 0.0**	**0.06 ± 0.0**	**2.0**
1008	***speH* (SpeH)**	**0.090 ± 0.0**	**0.11 ± 0.1**	**0.18 ± 0.1**	**2.0**
1357	***grab* (Grab)**	**1.80 ± 0.2**	**1.71 ± 0.3**	**0.20 ± 0.1**	**0.1**
1436	***mf-3* (MF-3)**	**0.14 ± 0.0**	**0.14 ± 0.0**	**3.47 ± 0.0**	**24.8**
1801	*isp-2* (Isp-2)	3.91 ± 0.9	3.36 ± 0.9	3.63 ± 0.8	0.9
2025	***isp-1* (Isp-1)**	**1.07 ± 0.3**	**0.63 ± 0.1**	**0.46 ± 0.1**	**0.4**

[a] The gene-specific cDNA values were normalized to the quantity of *gyrA* cDNA in each sample. The mean ± the standard error of the mean of two independently isolated RNA samples is given.
[b] Gene designations are in italics, and the corresponding protein designations are in parentheses.
[c] Quantity of *gyrA*-normalized cDNA in *rgg* mutant NZ131 ÷ quantity in wild-type NZ131.
[d] Boldface denotes differences in transcripts between wild-type NZ131 and *rgg* mutant NZ131 of less than 0.5 or greater than 1.5.

TABLE 5. Quantitative RT-PCR analysis of selected regulatory gene transcripts

SPy no.	Designation[b]	Quantity of transcript[a] (mean ± SEM)			*rgg*/wild-type ratio[c]
		Wild-type NZ131	*speB* mutant NZ131	*rgg* mutant NZ131	
0242[d]	***fasB* (FasB)**	**0.50 ± 0.1**	**0.55 ± 0.0**	**0.23 ± 0.0**	**0.5**
0244	***fasC* (FasC)**	**0.69 ± 0.1**	**0.68 ± 0.1**	**0.28 ± 0.0**	**0.4**
0245	***fasA* (FasA)**	**0.28 ± 0.0**	**0.29 ± 0.0**	**0.15 ± 0.0**	**0.5**
0528	*sycF* (SycF)	1.74 ± 0.2	3.55 ± 0.1	2.08 ± 0.0	1.2
0529	*sycG* (SycG)	1.36 ± 0.2	1.65 ± 0.1	1.31 ± 0.2	1.0
0874	*trcR* (TrcR)[e,f]	1.21 ± 0.2	1.36 ± 0.0	1.70 ± 0.2	1.4
0875	**None (none)**	**1.25 ± 0.0**	**1.31 ± 0.2**	**2.28 ± 0.6**	**1.8**
0895	***cpsX* (CpsX)**	**0.92 ± 0.1**	**1.16 ± 0.1**	**1.80 ± 0.2**	**2.0**
0898	*cpsY* (CpsY)	0.34 ± 0.0	0.33 ± 0.0	0.35 ± 0.0	1.0
1061	*yesN* (YesN)	0.67 ± 0.1	0.46 ± 0.0	0.59 ± 0.2	0.9
1062	*yesM* (YesM)	0.39 ± 0.0	0.34 ± 0.1	0.34 ± 0.0	0.9
1106	***yufM* (YufM)**[e,g]	**0.28 ± 0.1**	**0.39 ± 0.0**	**0.53 ± 0.0**	**1.9**
1236	*ciaS* (CiaS)[e,h]	1.33 ± 0.4	1.49 ± 0.4	1.15 ± 0.2	0.9
1237	*ciaR* (CiaR)[e,h]	0.73 ± 0.2	0.92 ± 0.1	0.81 ± 0.2	1.1
1556	*zmpS* (ZmpS)[e,h]	0.48 ± 0.1	0.44 ± 0.1	0.55 ± 0.1	1.1
1587	***lytR* (LytR)**[e,i]	**0.21 ± 0.0**	**0.15 ± 0.0**	**0.41 ± 0.1**	**2.0**
1588	*lytS* (LytS)[e,i]	13.4 ± 2.2	21.7 ± 7.9	16.0 ± 0.9	1.2
1908	*salR* (SalR)[e,j]	0.58 ± 0.0	0.57 ± 0.1	0.58 ± 0.19	1.0
1909	*salK* (SalK)[e,j]	2.95 ± 0.5	3.35 ± 0.1	2.92 ± 0.6	1.0
2026	***ihk* (Ihk)**	**1.23 ± 0.2**	**1.18 ± 0.1**	**0.69 ± 0.0**	**0.6**
2027	***irr* (Irr)**	**0.71 ± 0.2**	**0.64 ± 0.1**	**0.33 ± 0.0**	**0.5**

[a] The gene-specific cDNA values were normalized to the quantity of *gyrA* cDNA in each sample. The mean ± the standard error of the mean of two independently isolated RNA samples is given.
[b] Gene designations are in italics, and the corresponding protein designation are in parentheses.
[c] Quantity of *gyrA*-normalized cDNA in *rgg* mutant NZ131 ÷ quantity in wild-type NZ131.
[d] Boldface denotes differences in transcripts between wild-type NZ131 and *rgg* mutant NZ131 of less than 0.5 or greater than 1.5.
[e] Designation based on homology with a similar gene in indicated bacterium.
[f] *Mycobacterium tuberculosis.*
[g] *Bacillus subtilis.*
[h] *Streptococcus pneumoniae.*
[i] *Staphylococcus aureus.*
[j] *Streptococcus salivarius.*

component regulatory genes that were differentially expressed included *spy0875*, *cpsX*, *yufM*, *lytR*, *ihk*, and *irr* (Table 5).

DISCUSSION

Rgg was initially identified in *S. pyogenes* as a regulatory protein required for the expression of *speB* (6, 30). A recent analysis of culture supernatant proteins showed that Rgg influenced the expression of several additional exoproteins (10). The lack of a conserved regulatory motif in the promoter regions of Rgg-regulated genes and the functional diversity of the gene products indicated that Rgg may influence gene expression indirectly by altering expression of additional regulatory loci. Consistent with this hypothesis, we discovered genomewide differences in transcription in the *rgg* mutant. Differences of 1.5-fold or greater were selected for further study. The biological significance of small differences in transcript levels is likely to vary; however, at least some differences of less than twofold are known to be biologically significant (18). All differentially expressed virulence-associated genes identified by DNA microarrays were further analyzed by quantitative RT-PCR. With the exception of *grab*, the results were qualitatively similar. Transcripts of several previously described regulatory genes, including *csrRS*, *fasBCA*, *mga*, and *sagA*, were altered in the *rgg* mutant. In addition, transcript levels of several uncharacterized two-component regulators such as *ihk*, *irr*, *lytR*, *spy0875*, *cpsX*, and *yufM* were altered. The changes in transcription of regulatory genes were accompanied by changes in expression of known or putative virulence factors, including *emm*, *scpA*, *scl1*, *hasAB*, *slo*, *sagA*, *ska*, *speH*, *grab*, *mac*, *mf-1*, and *mf-3*. The results demonstrate that Rgg is an important regulatory factor that interacts with several regulatory networks to coordinate virulence-associated gene expression in *S. pyogenes* (Fig. 3).

Transcription of *rgg* is elevated in NZ131 *speB* compared to that in the isogenic wild-type strain (10). The observation indicated that the lack of *speB* expression in the *rgg* mutant could influence gene expression independently of Rgg. To control for this possibility, the quantities of selected gene transcripts were determined in wild-type strain NZ131, *speB* mutant strain NZ131, and *rgg* mutant strain NZ131 (Fig. 1; Tables 3 to 5). Levels of transcripts of *emm49*, *enn*, *scpA49*, and *orfX* were slightly higher in the *speB* mutant than in the wild-type strain; however, an increase in the corresponding proteins was not apparent (Fig. 2). In general, the quantities of Rgg-regulated gene transcripts were similar in the wild-type and isogenic *speB* mutant strains. Thus, the observed differences in gene transcription in the *rgg* mutant are associated with inactivation of the *rgg* locus and not with the absence of *speB* expression.

Rgg differentially regulates expression of SPE B and M protein. Rgg represses transcription of *mga* and Mga-regulated genes (Fig. 1). As previously noted, *mga* expression is autoregulated, which implies that a repressor limits transcription (34). Deletion of the region of the chromosome containing the distal *mga* promoter (designated Mga binding site I) increased

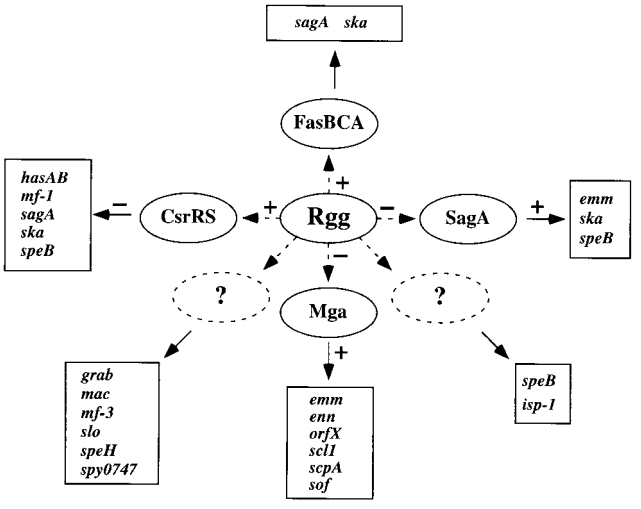

FIG. 3. Influence of Rgg with other regulatory networks. Positive regulation and negative regulation of gene expression are designated by plus and minus signs, respectively. Dashed arrows indicate possible indirect influences on gene expression. Dashed ovals represent putative regulatory intermediates.

transcription of *mga* via the P1 promoter, suggesting that the putative repressor was interacting with *cis* elements contained in the deleted region (34). Our results suggest that Rgg may be the repressor detected by McIver et al. (34). However, it remains to be determined if Rgg acts directly or indirectly to repress *mga* expression.

The discovery that Rgg positively influences *speB* expression and represses *emm49* expression contributes to the idea that the genes encode proteins with contrary functions. Mga and Mga-regulated genes are maximally expressed in the exponential phase of growth (32). The phenotype of exponential-phase *S. pyogenes* is postulated to be associated with adherence and resistance to the host immune response (7). In contrast, SPE B is produced primarily in the stationary phase of growth, which has been suggested to represent a nonadherent-invasiveness phenotype (9, 19). Moreover, the SPE B protease can degrade cell-associated adhesins, including M49, and abrogate interactions (adherence and internalization) of *S. pyogenes* with cultured mammalian cells (2, 7). We speculate that Rgg coordinates the transition between adherent and disseminating phenotypes by repressing genes associated with adherence and activating genes associated with dissemination. Clearly, additional information is needed regarding genomewide transcript profiles of *S. pyogenes* in different stages of growth.

Rgg influences expression of additional regulatory networks. The two-component regulator CsrRS was initially discovered as a repressor of the hyaluronic acid biosynthesis genes *hasAB* (3, 25). The DNA binding site of CsrR in the *hasA* promoter region has been identified (3), which indicates that CsrR directly regulates expression of capsule biosynthesis genes. Phosphorylated CsrR also interacts with the promoter regions of *speB*, *sagA*, *mf-1*, and *ska*, indicating that CsrR directly represses expression of these genes (36). Transcription of *csrR* and *csrS* was decreased in an *rgg* mutant (Table 3), which was associated with increased levels of transcripts of *hasAB*, *ska*, *sagA*, and *mf-1*. Together, the results suggest that increased transcription of *hasAB*, *ska*, *sagA*, and *mf-1* in the *rgg* mutant is due to decreased transcription of *crsRS*. The cellular concentration of CsrR is important in determining the interaction of CsrR with promoter regions (36). Thus, a decrease in *csrR* expression could significantly affect the regulatory activity of CsrR (36). Decreased transcription of *csrR* in the *rgg* mutant was not associated with increased transcription of *speB*, indicating that *speB* expression may require direct activation by Rgg. Nonetheless, we cannot rule out the possibility that Rgg acts directly to influence the expression of *hasAB*, *sagA*, *mf-1*, and *ska*. Similarly, increased expression of *speB* in *csrR* mutants may be associated with increased expression of *rgg*.

The quantities of 23 gene transcripts associated with 12 two-component regulatory systems were determined in wild-type strain NZ131, *speB* mutant strain NZ131, and *rgg* mutant strain NZ131 (Table 5). Transcripts of 11 loci were altered by at least 0.5- or 1.5-fold in the *rgg* mutant compared to those in the isogenic parental strain NZ131 and the *speB* mutant. Although the majority of two-component regulatory systems have not been characterized in *S. pyogenes*, the changes in transcription are likely to contribute to the pleiotropic phenotype of the *rgg* mutant.

Initial characterization of NZ131 *rgg* did not identify a general defect in the secretion apparatus of the mutant (6). Consistent with this, no significant changes in hemolytic activity, DNase activity, and streptokinase activity were observed in the mutant compared to those in the wild-type strain (6). Similar results were reported for an *rgg* (*ropB*) mutant of *S. pyogenes* strain HSC5 (30). The previous results differ with the findings of the present study, which showed elevated transcripts of genes encoding known and putative extracellular nucleases (*mf-1*, *mf-3*, and *spy0747*), hemolysins (*slo* and *sagA*), and *ska* in *rgg* mutant strain NZ131. In addition, the nuclease MF-1 and the putative nuclease MF-3 were more abundant in culture supernatant proteins from stationary-phase *rgg* mutant strain NZ131 cultures than in those from the control strain (10). Several explanations may account for these discrepancies. In the present study, transcript levels were determined at a single time point early in the exponential phase of growth. In contrast, the plate assays previously used to assess changes in hemolytic, nuclease, and streptokinase activities represented the accumulation of exoproteins throughout the exponential and stationary phases of growth. In addition, production of the SPE B protease in the wild-type strains may have diminished protein activity in plate assays. Future studies aimed at determining the influence of *rgg* inactivation on transcription during additional growth stages and under different culture conditions are clearly important.

To summarize, inactivation of *rgg* has genomewide effects on virulence-associated gene transcription. Many of the changes are likely the result of changes in the expression of known and putative regulatory loci. Further genome scale analyses of additional regulatory gene mutants are likely to reveal similar interactions among regulatory networks. Such analysis will result in a better understanding of how bacteria coordinate gene expression and may lead to new strategies for the control of virulence by inhibiting global regulators of virulence-associated genes.

ACKNOWLEDGMENTS

We thank P. Cleary, J. Dale, and B. Lei for providing antiserum, J. R. Fitzgerald, B. J. Hinnebusch, and N. P. Hoe for critical review of the manuscript, and the University of Oklahoma Genome Center for release of serotype M1 genome sequence data prior to completion of the project.

REFERENCES

1. **Beckert, S., B. Kreikemeyer, and A. Podbielski.** 2001. Group A streptococcal *rofA* gene is involved in the control of several virulence genes and eukaryotic cell attachment and internalization. Infect. Immun. **69:**534–537.
2. **Berge, A., and L. Björck.** 1995. Streptococcal cysteine proteinase releases biologically active fragments of streptococcal surface proteins. J. Biol. Chem. **270:**9862–9867.
3. **Bernish, B., and I. van de Rijn.** 1999. Characterization of a two-component system in *Streptococcus pyogenes* which is involved in regulation of hyaluronic acid production. J. Biol. Chem. **274:**4786–4793.
4. **Betschel, S. D., S. M. Borgia, N. L. Barg, D. E. Low, and J. C. S. De Azavedo.** 1998. Reduced virulence of group A streptococcal Tn *916* mutants that do not produce streptolysin S. Infect. Immun. **66:**1671–1679.
5. **Caparon, M. G., and J. R. Scott.** 1987. Identification of a gene that regulates expression of M protein, the major virulence determinant of group A streptococci. Proc. Natl. Acad. Sci. USA **84:**8677–8681.
6. **Chaussee, M. S., D. Ajdic, and J. J. Ferretti.** 1999. The *rgg* gene of *Streptococcus pyogenes* NZ131 positively influences extracellular SPE B production. Infect. Immun. **67:**1715–1722.
7. **Chaussee, M. S., R. L. Cole, and J. P. M. van Putten.** 2000. Streptococcal erythrogenic toxin B abrogates fibronectin-dependent internalization of *Streptococcus pyogenes* by cultured mammalian cells. Infect. Immun. **68:** 3226–3232.
8. **Chaussee, M. S., D. Gerlach, C.-E. Yu, and J. J. Ferretti.** 1993. Inactivation of the streptococcal erythrogenic toxin B gene (*speB*) in *Streptococcus pyogenes*. Infect. Immun. **61:**3719–3723.
9. **Chaussee, M. S., E. R. 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.
10. **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.
11. **Crater, D. L., and I. van de Rijn.** 1995. Hyaluronic acid synthesis operon (*has*) expression in group A streptococci. J. Biol. Chem. **270:**18452–18458.
12. **DeAngelis, P. L., J. Papaconstantinou, and P. H. Weigel.** 1993. Isolation of a *Streptococcus pyogenes* gene locus that directs hyaluronan biosynthesis in acapsular mutants and in heterologous bacteria. J. Biol. Chem. **268:**14568–14571.
13. **Dougherty, B. A., and I. van de Rijn.** 1992. Molecular characterization of a locus required for hyaluronic acid capsule production in group A streptococci. J. Exp. Med. **175:**1291–1299.
14. **Federle, M. J., K. S. McIver, and J. R. Scott.** 1999. A response regulator that represses transcription of several virulence operons in the group A streptococcus. J. Bacteriol. **181:**3649–3657.
15. **Ferretti, J. J., W. M. McShan, D. Ajdic, D. J. Savic, G. Savic, K. Lyon, C. Primeaux, S. Sezate, A. N. Suvorov, S. Kenton, H. S. Lai, S. P. Lin, Y. Qian, H. G. Jia, F. Z. Najar, Q. Ren, H. Zhu, L. Song, J. White, X. Yuan, S. W. Clifton, B. A. Roe, and R. McLaughlin.** 2001. Complete genome sequence of an M1 strain of *Streptococcus pyogenes*. Proc. Natl. Acad. Sci. USA **98:**4658–4663.
16. **Fogg, G. C., C. M. Gibson, and M. G. Caparon.** 1994. The identification of *rofA*, a positive-acting regulatory component of *prtF* expression: use of an mγδbased shuttle mutagenesis strategy in *Streptococcus pyogenes*. Mol. Microbiol. **11:**671–684.
17. **Heath, A., V. J. DiRita, N. L. Barg, and N. C. Engleberg.** 1999. A two-component regulatory system, CsrR-CsrS, represses expression of three *Streptococcus pyogenes* virulence factors, hyaluronic acid capsule, streptolysin S, and pyrogenic exotoxin B. Infect. Immun. **67:**5298–5305.
18. **Hughes, T. R., M. J. Marton, A. R. Jones, C. J. Roberts, R. Stoughton, C. D. Armour, H. A. Bennett, E. Coffey, H. Dai, Y. D. He, M. J. Kidd, A. M. King, M. R. Meyer, D. Slade, P. Y. Lum, S. B. Stepaniants, D. D. Shoemaker, D. Gachotte, K. Chakraburtty, J. Simon, M. Bard, and S. H. Friend.** 2000. Functional discovery via a compendium of expression profiles. Cell **102:**109–126.
19. **Kapur, V., S. Topouzis, M. W. Majesky, L.-L. Li, M. R. Hamrick, R. J. Hamill, J. M. Patti, and J. M. Musser.** 1993. A conserved *Streptococcus pyogenes* extracellular cysteine protease cleaves human fibronectin and degrades vitronectin. Microb. Pathog. **15:**327–346.
20. **Kreikemeyer, B., M. D. P. Boyle, B. A. Buttaro, M. Heinemann, and A. Podbielski.** 2001. Group A streptococcal growth phase-associated virulence factor regulation by a novel operon (Fas) with homologies to two-component-type regulators requires a small RNA molecule. Mol. Microbiol. **39:** 392–406.
21. **Kuo, C. F., J. J. Wu, K. Y. Lin, P. J. Tsai, S. C. Lee, Y. T. Jin, H. Y. Lei, and Y. S. Lin.** 1998. Role of streptococcal pyrogenic exotoxin B in the mouse model of group A streptococcal infection. Infect. Immun. **66:**3931–3935.
22. **La Penta, D., X. P. Zhang, and P. P. Cleary.** 1994. *Streptococcus pyogenes* type IIa IgG Fc receptor expression is co-ordinately regulated with M protein and streptococcal C5a peptidase. Mol. Microbiol. **12:**873–879.
23. **Lara-Tejero, M., and J. E. Galán.** 2000. A bacterial toxin that controls cell cycle progression as a deoxyribonuclease I-like protein. Science **290:**354–357.
24. **Lei, B., F. R. DeLeo, N. P. Hoe, M. R. Graham, S. M. Mackie, R. L. Cole, M. Liu, H. R. Hill, D. E. Low, M. J. Federle, J. R. Scott, and J. M. Musser.** 2001. Evasion of human innate and acquired immunity by a bacterial homologue of CD11b that inhibits opsonophagocytosis. Nat. Med. **7:**1298–1305.
25. **Levin, J. C., and M. R. Wessels.** 1998. Identification of *csrR*/*csrS*, a genetic locus that regulates hyaluronic acid capsule synthesis in group A streptococcus. Mol. Microbiol. **30:**209–219.
26. **Li, Z., D. D. Sledjeski, B. Kreikemeyer, A. Podbielski, and M. D. P. Boyle.** 1999. Identification of *pel*, a *Streptococcus pyogenes* locus that affects both surface and secreted proteins. J. Bacteriol. **181:**6019–6027.
27. **Lukomski, S., C. A. Montgomery, J. Rurangirwa, R. S. Geske, J. P. Barrish, G. J. Adams, and J. M. Musser.** 1999. Extracellular cysteine protease produced by *Streptococcus pyogenes* participates in the pathogenesis of invasive skin infection and dissemination in mice. Infect. Immun. **67:**1779–1788.
28. **Lukomski, S., K. Nakashima, I. Abdi, V. J. Cipriano, R. M. Ireland, S. D. Reid, G. G. Adams, and J. M. Musser.** 2000. Identification and characterization of the *scl* gene encoding a group A streptococcus extracellular protein virulence factor with similarity to human collagen. Infect. Immun. **68:**6542–6553.
29. **Lukomski, S., S. Sreevatsan, C. Amberg, W. Reichardt, M. Woischnik, A. Podbielski, and J. M. Musser.** 1997. Inactivation of *Streptococcus pyogenes* extracellular cysteine protease significantly decreases mouse lethality of serotype M3 and M49 strains. J. Clin. Investig. **99:**2574–2580.
30. **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.
31. **McIver, K. S., A. S. Heath, B. D. Green, and J. R. Scott.** 1995. Specific binding of the activator Mga to promoter sequences of the *emm* and *scpA* genes in the group A streptococcus. J. Bacteriol. **177:**6619–6624.
32. **McIver, K. S., and J. R. Scott.** 1997. Role of *mga* in growth phase regulation of virulence genes of the group A streptococcus. J. Bacteriol. **179:**5178–5187.
33. **McIver, K. S., S. Subbarao, E. M. Kellner, A. S. Heath, and J. R. Scott.** 1996. Identification of *isp*, a locus encoding an immunogenic secreted protein conserved among group A streptococci. Infect. Immun. **64:**2548–2555.
34. **McIver, K. S., A. S. Thurman, and J. R. Scott.** 1999. Regulation of *mga* transcription in the group A streptococcus: specific binding of Mga within its own promoter and evidence for a negative regulator. J. Bacteriol. **181:**5373–5383.
35. **McLandsborough, L. A., and P. P. Cleary.** 1995. Insertional inactivation of *virR* in *Streptococcus pyogenes* M49 demonstrates that VirR functions as a positive regulator of ScpA, FcRA, OF, and M protein. FEMS Microbiol. Lett. **128:**45–51.
36. **Miller, A. A., N. C. Engleberg, and V. J. DiRita.** 2001. Repression of virulence genes by phosphorylation-dependent oligomerization of CsrR at target promoters in *S. pyogenes*. Mol. Microbiol. **40:**976–990.
37. **Nordstrand, A., W. M. McShan, J. J. Ferretti, S. E. Holm, and M. Norgren.** 2000. Allele substitution of the streptokinase gene reduces the nephritogenic capacity of group A streptococcal strain NZ131. Infect. Immun. **68:**1019–1025.
38. **Podbielski, A., A. Flosdorff, and J. Weber-Heynemann.** 1995. The group A streptococcal *virR49* gene controls expression of four structural *vir* regulon genes. Infect. Immun. **63:**9–20.
39. **Podbielski, A., and B. A. B. Leonard.** 1998. The group A streptococcal dipeptide permease (Dpp) is involved in the uptake of essential amino acids and affects the expression of cysteine protease. Mol. Microbiol. **28:**1323–1334.
40. **Podbielski, A., B. Pohl, M. Woischnik, C. Körner, K.-H. Schmidt, E. Rozdzinski, and B. A. B. Leonard.** 1996. Molecular characterization of group A streptococcal (GAS) oligopeptide permease (Opp) and its effect on cysteine protease production. Mol. Microbiol. **21:**1087–1099.
41. **Podbielski, A., M. Woischnik, B. A. Leonard, and K. H. Schmidt.** 1999. Characterization of *nra*, a global negative regulator gene in group A streptococci. Mol. Microbiol. **31:**1051–1064.
42. **Podbielski, A., M. Woischnik, B. Pohl, and K. H. Schmidt.** 1996. What is the size of the group A streptococcal *vir* regulon? The Mga regulator affects expression of secreted and surface virulence factors. Med. Microbiol. Immunol. **185:**171–181.
43. **Proft, T., S. L. Moffatt, C. J. Berkahn, and J. D. Fraser.** 1999. Identification and characterization of novel superantigens from *Streptococcus pyogenes*. J. Exp. Med. **189:**89–102.
44. **Rakonjac, J. V., J. C. Robbins, and V. A. Fischetti.** 1995. DNA sequence of the serum opacity factor of group A streptococci: identification of a fibronectin-binding repeat domain. Infect. Immun. **63:**622–631.
45. **Rasmussen, M., A. Edén, and L. Björck.** 2000. SclA, a novel collagen-like

surface protein of *Streptococcus pyogenes*. Infect. Immun. **68:**6370–6377.
46. **Rasmussen, M., H.-P. Müller, and L. Björck.** 1999. Protein GRAB of *Streptococcus pyogenes* regulates proteolysis at the bacterial surface by binding α_2-macroglobulin. J. Biol. Chem. **274:**15336–15344.
47. **Simpson, W. J., D. LaPenta, C. Chen, and P. P. Cleary.** 1990. Coregulation of type 12 M protein and streptococcal C5a peptidase genes in group A streptococci: evidence for a virulence regulon controlled by the *virR* locus. J. Bacteriol. **172:**696–700.
48. **Smoot, L. M., J. C. Smoot, M. R. Graham, G. A. Somerville, D. E. Sturdevant, C. A. Lux-Migliaccio, and J. M. Musser.** 2001. Global differential gene expression in response to growth temperature alteration in group A streptococcus. Proc. Natl. Acad. Sci. USA **98:**10416–10421.
49. **Sriskandan, S., M. Unnikrishnan, T. Krausz, and J. Cohen.** 2000. Mitogenic factor (MF) is the major DNase of serotype M89 *Streptococcus pyogenes*. Microbiology **146:**2785–2792.
50. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76:**4350–4354.

Editor: E. I. Tuomanen