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Deletion of *ato*R from *Streptococcus pyogenes* Results in Hypervirulence in a Mouse Model of Sepsis and is LuxS Independent

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Abstract

Group A *Streptococcus* (GAS) is a Gram-positive human pathogen that causes a variety of diseases ranging from pharyngitis to life-threatening streptococcal toxic shock syndrome. Recently, several global gene expression analyses have yielded extensive new information regarding the regulation of genes encoding known and putative virulence factors in GAS. A microarray analysis found that transcription of the GAS gene *M5005_Spy_1343* was significantly increased in response to interaction with human polymorphonuclear leukocytes. *M5005_Spy_1343* is predicted to encode a member of the LysR family of transcriptional regulators and is located upstream of a putative operon containing six genes. Five of these genes have sequence similarity to genes involved in short-chain fatty acid metabolism, whereas the sixth gene (*lux*S) is found in many bacterial species and is involved in quorum sensing. Unexpectedly, inactivation of the *M5005_Spy_1343* gene resulted in hypervirulence in an intraperitoneal mouse model of infection. Increased virulence was not due to changes in *lux*S gene expression. We postulate that short-chain fatty acid metabolism is involved in GAS pathogenesis.

Key words: *Streptococcus pyogenes*, *ato*, host-pathogen interactions, short chain fatty acid synthesis, virulence factors

Introduction

Group A *Streptococcus* (GAS) is a Gram-positive human bacterial pathogen that causes a variety of diseases ranging from pharyngitis to life-threatening streptococcal toxic shock syndrome (Sitkiewicz and Hryniewicz, 2010). The molecular mechanisms responsible for these different diseases are an intense area of GAS research. Several studies have examined the global gene expression changes in GAS occurring as it encounters distinct niches within the host (Graham *et al.*, 2002; 2005; Klenk *et al.*, 2005; Shelburne *et al.*, 2005; Sitkiewicz and Musser, 2006; Virtaneva *et al.*, 2005; Voyich *et al.*, 2004; Voyich *et al.*, 2003; Musser and DeLeo, 2005). For example, an expression microarray analysis examined the transcriptional response of GAS to interaction with human polymorphonuclear leukocytes (PMNs) (Voyich *et al.*, 2003). An important discovery was that transcription of the *M5005_Spy_1343* gene (*spy1343*) was up-regulated two-fold 60 min post-PMN contact. *Spy1343* encodes an inferred 298-amino acid protein that belongs to the LysR family of transcriptional regulators.

The *spy1343* open reading frame (ORF) is located downstream and is divergently transcribed from a putative operon containing six genes (Lyon *et al.*, 2001) (Fig. 1). The first three genes *(ato*BDA*)* share sequence similarity with the *ato*BDA operon in *Esherichia coli* (Jenkins and Nunn, 1987a) that encodes enzymes involved in short-chain fatty acid (SCFA) degradation. In *E. coli*, AtoB is an acetoacetyl-CoA acetyltransferase (thiolase II), and AtoA and AtoD form the two subunits of acetate CoA-transferase. These enzymes also can participate in metabolic pathways other than SCFA degradation, such as butanoate metabolism, synthesis and degradation of ketone bodies, and fatty acid biosynthesis *via* pathway 2 (www.brenda.uni-koeln.de). Two other genes present in the putative GAS operon (*M5005_Spy_1347* and *M5005_Spy_1348*) encode proteins with sequence similarity to 3-hydroxybutyrate dehydrogenase and 3-hydroxybutyrate permease, respectively. These enzymes are involved in butanoate metabolism and synthesis and degradation of ketone bodies. Interestingly, upon exposure of GAS to human blood, the transcript level of all 5 genes (*M5005_Spy_1344-1348*)

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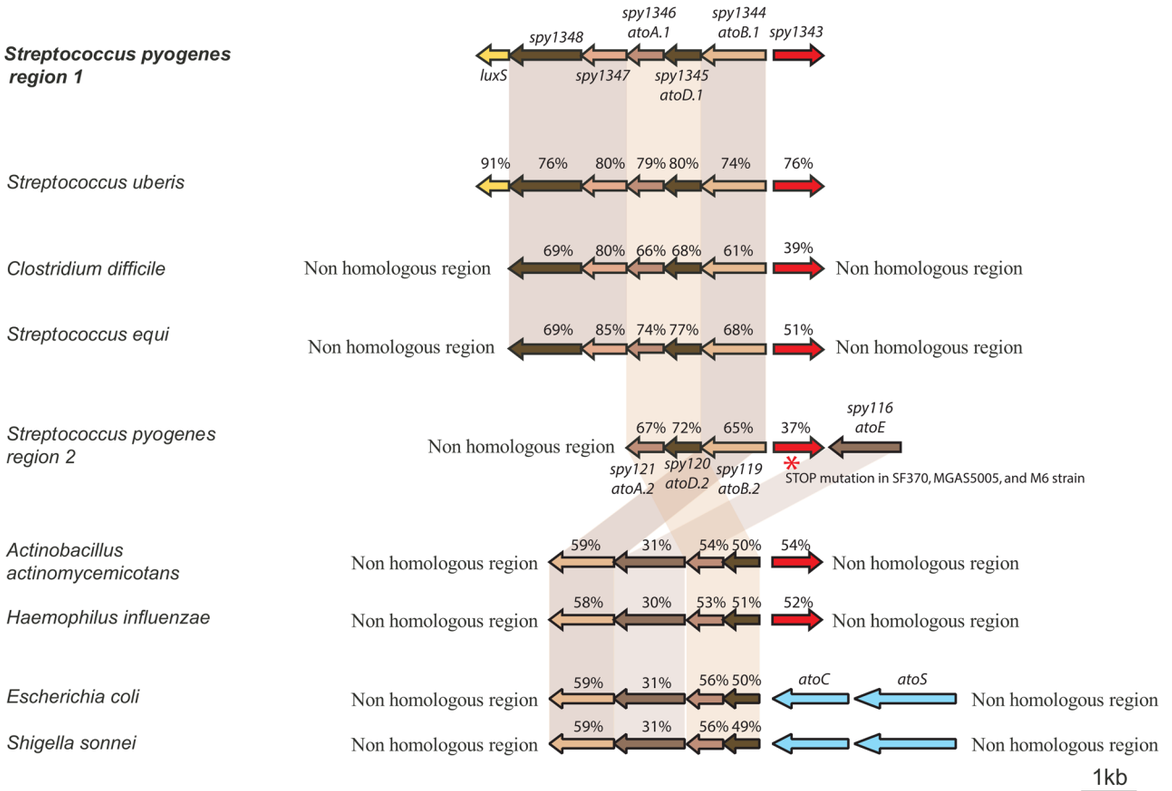

Fig. 1. Homologous regions encoding *lux*S and *ato* genes in GAS and other bacteria.
Red arrows represent *M5005_spy1343* and its homologs. Beige shading indicates regions of high sequence similarity. Percentages denote amino acid identity compared to GAS.

decreased concomitantly with the increase in *spy1343* transcript, suggesting that *spy1343* negatively regulates expression of these five genes (Graham *et al.*, 2005).

The last gene in the putative operon has sequence similarity to *lux*S. LuxS is found in a diverse array of bacterial species and is involved in quorum sensing *via* processing of auto-inducer II (Waters and Bassler, 2005; Jimenez and Federle, 2014). In GAS, LuxS affects the production of at least 3 known virulence factors including streptococcal pyrogenic exotoxin B (SpeB), streptolysin S (SLS) and M protein (Lyon *et al.*, 2001; Marouni and Sela, 2003).

The organization of the *ato* gene region is nearly identical in sequenced GAS genomes, and the inferred homologous proteins have 98% to 100% identity. Additionally, we determined by sequence similarity searches (NCBI BLAST) and operon organization (FGENESB at www.softberry.com) that similar *ato* operons are present in bacteria that are distantly related to GAS, such as *Clostridium difficile*, *Actinobacillus actinomycemicotans*, *Haemophilus influenzae*, and *Shigella sonnei* (Fig. 1). The *ato* operon in *E. coli* and *S. sonnei* is regulated by the *atoSC* two-component gene regulatory system (Jenkins and Nunn, 1987b). However, *A. actinomycemicotans* and *H. influenzae* genomes contain an upstream regulatory gene that encodes a protein with greater than 50% identity to *spy1343*. Additionally, all sequenced GAS genomes contain a second *ato*BDA operon. However in several strains, including MGAS5005, the putative regulator contains a truncation mutation resulting in a stop codon at amino acid 139, suggesting that this protein is not fully functional (Fig. 1).

The studies reported herein were motivated by the observation that the *spy1343* transcript was up-regulated in response to interaction of GAS with human PMNs, the presence in the region of a gene (*lux*S) encoding a known regulator of GAS virulence genes, and the conserved inter-species and cross-genus operon organization. We show that *spy1343* regulates *ato* genes, and that a *spy1343* mutant is hypervirulent in a mouse model of sepsis. We demonstrate that the hypervirulent phenotype is not caused by changes in *lux*S transcription.

Experimental

Materials and Methods

Bacterial strains and culture conditions. Serotype M1 strain MGAS5005 was used in this study. The genome sequence has been published (GenBank acc.

No. CP000017), and MGAS5005 is representative of contemporary clinical M1 isolates (Sumby *et al.*, 2005). GAS were grown in Todd-Hewitt broth (Difco Laboratories) supplemented with 0.2% yeast extract (THY medium) at 37°C in an atmosphere of 5% CO_2, 20% O_2. THY medium or tryptose agar with 5% sheep blood (Becton Dickinson) was used as solid media. THY agar supplemented with spectinomycin (150 µg/ml) was used for selection of the antibiotic-resistant mutant derivative strain. Cloning experiments were performed with *E. coli* DH10B (Invitrogen). Ampicillin (100 µg/ml) or spectinomycin (150 µg/ml) was used for selection of *E. coli* clones when required.

DNA techniques. Restriction and modification enzymes were purchased from New England BioLabs or ThermoFisher. Plasmid DNA from *E. coli* was isolated with a QIAprep Spin Miniprep Kit (Qiagen). Chromosomal DNA was isolated from GAS using the DNeasy Tissue Kit (Qiagen) as described by the manufacturer, with slight modification (Sitkiewicz and Musser, 2006). Southern hybridization was performed with the ECL system according to the manufacturer's instructions (Amersham).

Transformation of GAS. GAS was transformed as described previously (Sitkiewicz and Musser, 2006). Briefly, cells were grown to an OD_{600} of 0.2–0.25 in medium containing L-threonine and sucrose, washed, and frozen in aliquots. The GAS-DNA mixture was subjected to a pulse of 1.8 kV, 25 µF, 400 Ohms, and transformed cells were incubated 2–3 hours at 37°C without antibiotics and plated onto selective media.

Construction of the Δ*spy1343* mutant strain. The coding sequence of *spy1343* was replaced with a spectinomycin-resistance cassette (spc) by a double crossover strategy. Upstream and downstream sequences of *spy1343* were amplified in PCR reactions with primer pairs 1343 5'F/1343 5'R and 1343 3'F/1343 3'R (Table I). The PCR products were cloned into pUC19 (Fermentas) and pSTblue-1 (Novagen) vectors to generate plasmids p1343-5' and p1343-3', respectively. The spc containing the *add9* gene was excised from plasmid pSL60-2 (Lukomski *et al.*, 2000) with *Sma*I and cloned into the

Table I
Primers and probes used in this study

Primer name	Sequence (5'→3')
1343 5'F	CCCAAACATATGCGGTGCTGAGTTGATACATAG
1343 5'R	CCCAAACCCGGGGGATTCTCCTTGTCTTATCAATTGC
1343 3'F	CCCAAACCTAGGCATGGACGACTTGCTACAGTC
1343 3'R	CCCAAAACCGGTGGAGCGCTCCGCTAAGCGTG
1343L	TCTGTTAAGCCATCATGAACAAG
1343R	ATCGGTCAGACTTTGTTCTTTAC
1348F	GGGTCTTGGTAGGTGTTATTG
1348R	TTTGTGGTTAAGTCCTGTCAATGCTAAG
1347F	ATCATGTTGACAGCACCATTTATTG
1347R	GAACAAGTGGGTATAGCACTTC
spcF	CCCGGGAATACATGTTATAATAACTATAAC
atoA F	CCAACTATATTCCTGAAGGTGTAAC
atoA R	CAGTCAGAGGAAGGGTGCATTTG
1343-taq-F	AGTGGGAAGATTTAACAGATGAACAA
1343-taq-R	GACGCTCGCAAGCTTCAAG
atoB-taq-5'	CAAGACAAGTTGCAGTCCAC
atoB-taq-3'	GCGCAGCTAATTGGATTG
luxS-taq-3'	AAAGGAGAGCAATCAATCATC
luxS-taq-5'	CCGGTTTGCATACCATTG
proS5'	TGAGTTTATTATGAAAGACGGCTATAGTTTC
proS3'	AAT AGC TTC GTA AGC TTG ACG ATA ATC
Probe name	Sequence (5'→3')
luxS	CTTACTTGCCAAGCTCATCCGCCAAC
atoB	TCCAGAACCGCAAACCATACTAATGTGAAAGC
1343	TGCCCTATTTGATCCTAGTTTCATGGTTCACC
proS	TCGTAGGTCACATCTAAATCTTCATAGTTG

*Pml*I site of p1343-3' to generate plasmid p1343-3'spc. The orientation of the spc was determined by PCR with primers spcF/1343 3'R. The *spy1343* fragment and spc from p1343-3'spc were cloned into p1343-5' to generate a plasmid with a DNA cassette containing the upstream and downstream fragments of *spy1343* flanking the *add9* gene. The entire fragment was amplified using PCR primers 1343 5'F and1343 3'R and purified product was transformed into strain MGAS5005. Transformants were selected on THY medium containing spectinomycin. Chromosomal DNA isolated from spectinomycin-resistant colonies was screened by PCR using primers 1343L/1343R, which annealed outside of the targeted integration site, thereby amplifying different-size products in mutant *versus* wild-type clones. Inactivation of the target gene was further confirmed by sequencing of the appropriate chromosomal region, and single integration was confirmed by Southern hybridization using a probe that annealed to the spc.

Mouse infection experiments. GAS strains used for mouse infection studies were grown in THY medium to exponential phase ($OD_{600} \sim 0.5$), harvested, washed twice with cold PBS, and frozen at –70°C in aliquots. The number of CFUs used to inoculate mice was determined by plating GAS on sheep blood agar. Thawed aliquots of wild-type strain MGAS5005 and the isogenic mutant strain were adjusted to the same CFU/ml by diluting with PBS prior to injection. Outbred CD-1 Swiss male mice (4–6 weeks old) (Harlan) were used for intraperitoneal inoculation with a dose of 1×10^7 CFUs. Mortality and morbidity were monitored every 2 h for the first 48 h after infection, and every 6 h for the next 5 days.

TaqMan analysis of gene transcription. MGAS5005 and the Δ*spy1343* mutant strain were grown in THY medium to an OD_{600} of 0.2 (early-exponential), 0.5 (mid-exponential), 1.2 (late-exponential), or 1.8 (stationary phase). Two independent cultures for each strain represented two biological replicates. RNA was isolated as described previously (Sitkiewicz and Musser, 2006). cDNA was generated using SuperScript III reverse transcriptase (RT) and random hexamers (Invitrogen), and TaqMan reactions were performed in quadruplicate using Platinum Quantitative PCR SuperMix (Invitrogen). Primer/probe sets (Table I) used for Taqman reactions were: atoB-taq-5'/atoB-taq-3'/atoB probe for *ato*B (*M5005_Spy_1344*); luxS-taq-5'/luxS-taq-3'/luxS probe for *luxS*; 1343-taq-F/1343-taq-R/1343 probe for *spy1343* and proS5'/proS3'/proS probe for internal *pro*S standard. The transcript level of the genes of interest was normalized to *proS* transcript and compared between the wild-type and Δ*spy1343* mutant strains using $\Delta\Delta C_T$ method (ABI, 2005).

RT-PCR. cDNA was generated using SuperScript III RT (Invitrogen) from RNA collected for TaqMan analysis with primers luxS-taq 3', 1348R and *ato*A-R according to the manufacturer's instructions. First-strand cDNA was purified using S.N.A.P. columns (Invitrogen) and amplified in PCR reactions with primer pairs luxS-taq-3'/luxS-taq-5', luxS-taq-3'/1348F and luxS-taq-3'/1347F (*lux*S transcript); 1348R/1638F, 1348R/1347F and 1347R/1347R (*M5005_Spy_1348* transcript); *ato*AR/*ato*B-taq-5' (*ato*A transcript). MGAS5005 genomic DNA served as a positive control for PCR, while the negative control used template generated in the absence of RT.

Results

Construction and characterization of the Δ*spy1343* isogenic mutant strain. To determine if *spy1343* is involved in regulation of the putative *ato* operon, we inactivated the gene by allelic replacement using standard methods (Sitkiewicz and Musser, 2006). The mutant strain was confirmed to have the correct construction by PCR and DNA sequence analysis (data not shown) and gene transcript studies (see below). The Δ*spy1343* isogenic mutant strain had no observed phenotypic difference from the parent strain during growth in laboratory media. For example, the growth of the wild-type and mutant strains was identical in THY medium (Fig. 2), and colony morphology was identical on blood agar plates (data not shown).

***spy1343* regulates transcription of the putative *ato* operon.** To test the hypothesis that *spy1343* regulates transcription of the genes in the putative *ato* operon, we compared transcript levels of *spy1343* and the *atoB* gene in the wild-type and Δ*spy1343* mutant strains during early-, mid-, late-exponential, and stationary growth phases. TaqMan analysis indicated that the level of *spy1343* transcript was constant throughout growth in the wild-type strain, and as expected, transcript was not detected in the Δ*spy1343* mutant strain (Fig. 3A). Interestingly, the *atoB* transcript level was decreased two-fold in the Δ*spy1343* mutant compared to wild-type in early-exponential phase, and then gradually attained wild-type transcript levels by stationary phase (Fig. 3B, red bars). This result indicates that *spy1343* is not a direct negative regulator of the *ato* genes.

Deletion of *spy1343* results in hypervirulence in mice. Because the level of *spy1343* transcript increased when GAS interacted with human PMN *ex vivo* (Graham *et al.*, 2005), we hypothesized that this gene played a role in GAS-host interactions. To test this hypothesis, we compared the virulence for mice of the wild-type and Δ*spy1343* mutant strains after intraperitoneal inoculation, as described previously (Sitkiewicz and Musser, 2006). Unexpectedly, mice infected with the Δ*spy1343* mutant strain died significantly more rap-

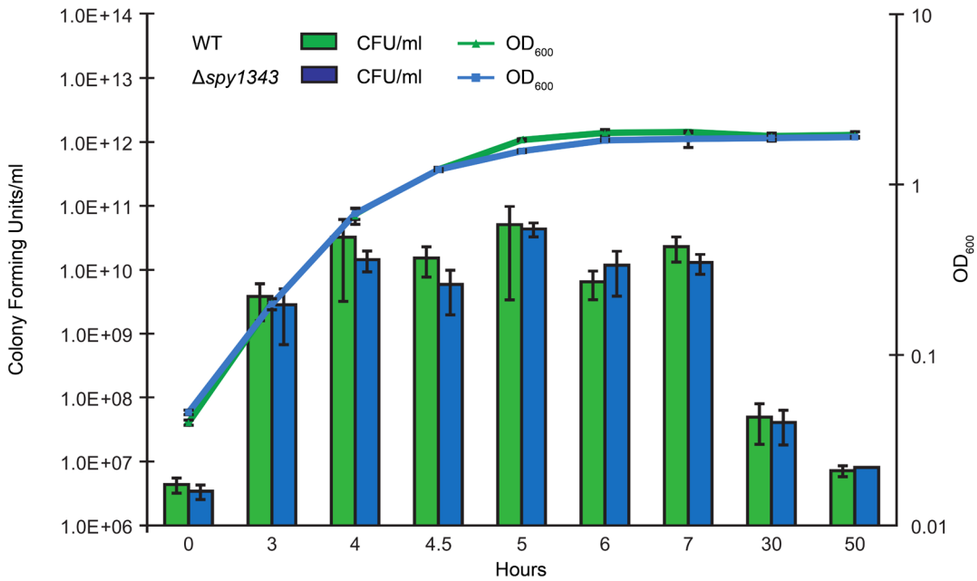

Fig. 2. Growth curve of MGAS5005 (WT) and Δ*spy1343* mutant strains in THY medium. Growth was determined both by OD_{600} measurement (right) and plating aliquots taken at the indicated times on THY agar plates (left).

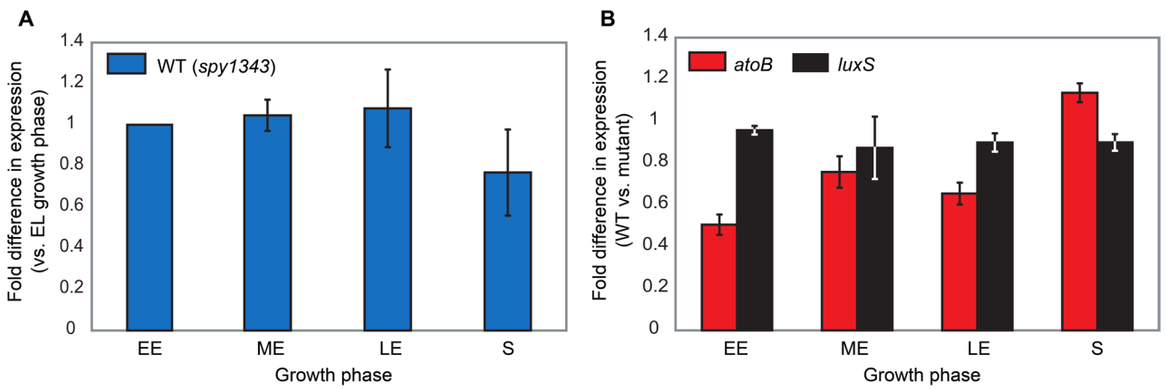

Fig. 3. TaqMan analysis of *spy1343*, *ato*B, and *lux*S expression.

(A) Transcript levels of *spy1343* in the wild-type (WT) strain during early-exponential (EE), mid-exponential (ME), late-exponential (LE) and stationary (S) growth phases. Results were standardized to an internal control gene (*pro*S) and compared between growth points using the $\Delta\Delta C_T$ method. Transcription in early exponential phase (EE) was used as basal level and differences in transcription during other phases were compared to it. (B) Comparison of transcript levels of *ato*B and *lux*S in wild-type and Δ*spy1343* mutant strains. Transcript levels for each gene at EE, ML, LL and S phases were determined separately for each strain, standardized to *pro*S and compared between strains using the $\Delta\Delta C_T$ method.

idly and in greater numbers than mice infected with the wild-type strain ($P = 0.006$; Fig. 4).

Hypervirulence for mice is not caused by altered *lux*S expression. LuxS in GAS regulates expression of several virulence factors, including SLS, SpeB and M protein (Lyon *et al.*, 2001; Marouni and Sela, 2003). To determine if the mouse hypervirulence phenotype was associated with changes in *lux*S transcript level, we compared the transcript level of *lux*S in the wild-type and Δ*spy1343* mutant strains during growth in THY medium. In contrast to *ato*B, the *lux*S transcript level was constant in all four stages of GAS growth tested and was independent of *spy1343* (Fig. 3B, black bars). This result suggested that *lux*S was not under the same transcriptional regulation as *ato*B. To seek additional support for this hypothesis, we used reverse-transcription polymerase chain reaction (RT-PCR) to characterize the length of transcripts generated from the *ato* promoter. cDNAs were synthesized using purified RNA by RT reactions with primers specific for *luxS*, *M5005_Spy_1348* (*spy1348*), or *atoA* (*M5005_Spy_1346*) (See Fig. 5, legend). When cDNA generated with the *lux*S primer was used as template, only primers that amplified internal fragments of the *lux*S gene yielded a PCR product. That is, use of the *lux*S primer with primers designed to amplify larger transcripts from the *ato* region failed to generate a detectable amplicon (Fig. 5, panels A-C). When cDNA generated with *ato*A or

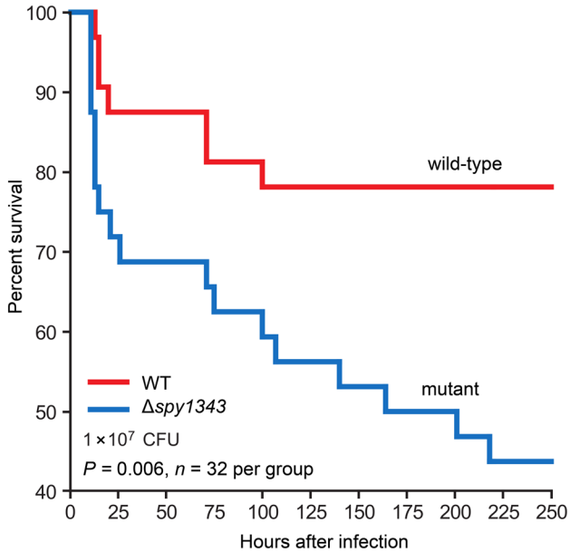

Fig. 4. Survival of mice after intraperitoneal inoculation with wild-type MGAS5005 (WT) or Δ*spy1343* mutant strains.
Kaplan-Meier curves were generated and datasets were compared (logrank test) using GraphPad Prism v. 4.02.

spy1348 primers was used as template, amplification products were obtained with primers that annealed throughout the region (Fig. 5, panels D-G). Taken together, these RT-PCR results demonstrated that *M5005_Spy_1344-1348* yield a multi-gene transcript consistent with an operon organization. These results strongly support the hypothesis that the *luxS* transcript is separate and distinct from this multi-gene transcript.

Discussion

The predicted product of *spy1343* is a member of the LysR family of DNA-binding proteins. These gene regulators have a conserved N-terminal helix-turn-helix domain followed by a less-conserved C-terminal domain which is thought to participate in binding of a co-inducer (see below) (Schell, 1993). In addition to their structural similarities, LysR family members are often transcribed from divergent promoters that are located close to, or overlap, the promoter of their target gene(s). The putative promoter regions of *spy1343*

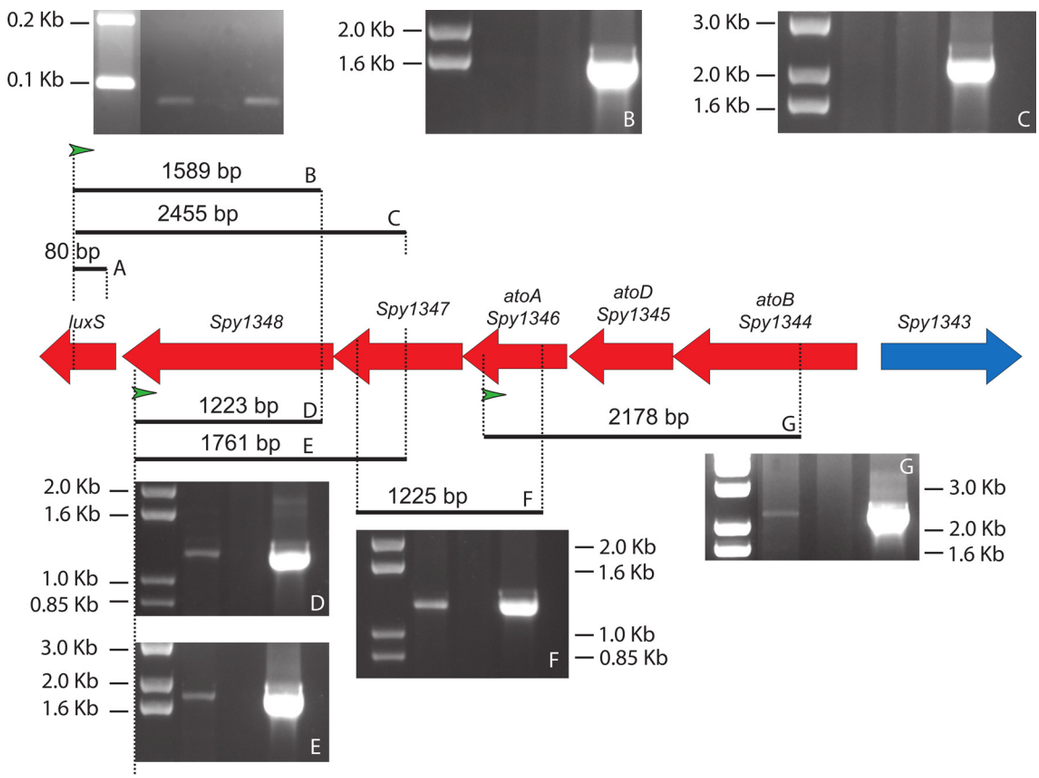

Fig. 5. Analysis of transcripts derived from the *M5005_Spy_1344-luxS* region.
Red and blue arrows represent ORFs; green arrowheads denote location of primer used for cDNA synthesis; thick horizontal black lines represent expected length of products obtained by PCR amplification of cDNAs. cDNA template generated with primer *luxS-taq-3'* was used in PCR reactions with primer pairs *luxS-taq-3'*/*luxS-taq-5'* (A), *luxS-taq-3'*/1348F (B), and *luxS-taq-3'*/*ato*AF (C). cDNA template generated with primer 1348R was used in PCR reaction with primers 1348R/1348F (D), 1348R/1347F (E), and 1348R/atoAF (F). cDNA generated with primer *ato*AR was used in PCR reaction with primers *ato*AR/*ato*B-*taq*-5' (G). Each gel shows four lanes which contain, from left to right: lane 1, DNA ladder (A, 100 bp DNA ladder (Invitrogen); B-G, 1 kb DNA ladder (Invitrogen)), lane 2, PCR with cDNA template, lane 3, negative control (RT was omitted from cDNA synthesis), lane 4, positive control (genomic DNA).

and the *ato* operon overlap, which is consistent with the genes sharing a common regulator (Schell, 1993; Zaim and Kierzek, 2003). Many LysR proteins also bind a co-inducer, such as ions (NhaR), flavonoids (NocR), octopine (OccR), N/O-acetylserine (CysB), or indoglycerol phosphate (TrpI) for optimal activity (Schell, 1993).

Our TaqMan analysis showed that *spy1343* regulates transcription of the *ato* genes. *ato*B transcript levels were decreased in the *spy1343* mutant strain (Fig. 3B), suggesting that *spy1343* acts as an activator rather than a repressor. This result is consistent with the regulation of the *ato* operon in *E. coli*, which is activated by the upstream *atoCS* two-component system(Jenkins and Nunn, 1987b). However, in a previous report by Graham *et al.* (2005), *spy1343* (designated SPy1634) and the *ato* genes (designated SPy1637-41) had an inverse transcriptional profile. That is, the transcript level of *spy1343* increased over time, whereas the level of *ato* gene transcripts decreased (supplemental Table I in Graham *et al.*, 2005). This result suggested that *spy1343* functions as a repressor. The difference in our data could be attributed to several factors. First, the signals encountered by GAS upon contact with human blood, which contains multiple cell types and other factors, are likely different from those present during culture in laboratory media. Second, the microarray data noted changes in transcript level at increasing times (30, 60, and 90 min) after contact with human blood, whereas in our TaqMan analysis, the greatest difference in transcript levels occurred during early-exponential phase, approximately 2 h after sub-culture in THY broth. These qualitative and temporal differences in our methods could have affected the results. Finally, many LysR family members are known to require a co-inducer for full activity. Absence of this co-inducer during culture in broth could influence the *spy1343*-promoter interaction. Despite these differences, our data clearly indicate that *spy1343* regulates transcription of *ato* genes, and we propose that *spy1343* be named *ato*R, for *ato* regulator.

Inspection of the published GAS genome sequences revealed a second *ato*RBDA locus present in GAS strains (region 2 *M5005_Spy_0116-0121*, Fig. 1). The putative regulatory proteins share only 37% identity, and downstream from $atoR_2$ there is an additional gene not present in region 1. The predicted product of this ORF shares sequence similarity to *ato*E from *E. coli,* which encodes a short-chain fatty acid transporter (NCBI BLAST). Region 2 also lacks M5005_Spy_1347 and M5005_Spy_1348, which are involved in butanoate metabolism and transport. Additionally, $atoR_2$ in both sequenced M1 strains and the M6 genome (Sumby *et al.*, 2005; Banks *et al.*, 2004; Ferretti *et al.*, 2001), has a frameshift mutation that introduces a stop codon at amino acid 139. Therefore, a truncated and possibly non-functional regulator is predicted in these strains. And lastly, the microarray data from Graham *et al.* show that there is no clear pattern of transcriptional regulation of the ato_2 genes upon GAS contact with human blood (supplemental Table I in Graham *et al.*, 2005). Unlike the five genes in region 1, which had an identical trend in transcription levels, and decreased with increased exposure times, the transcript level of $atoB_2$ and $atoD_2$ increased at 60 min after culture in blood, whereas $atoA_2$ transcript level was decreased at 60 min. Taken together, these data suggest that the ato_2 locus has a different specificity, function, and regulation than *ato* region 1.

Inactivation of *ato*R resulted in hypervirulence in our mouse model of GAS sepsis. TaqMan and RT-PCR analyses indicated that transcriptional control of *lux*S is independent of the *ato* genes, and therefore changes in *lux*S transcription likely were not responsible for this phenotype. This result suggests that hypervirulence is due to changes in transcription of the *ato* operon. Although our studies did not directly address the function of the *ato* genes in GAS, based on the conserved operon organization and sequence similarity with *ato* genes of known function, it is likely that proteins encoded by the GAS *ato* genes participate in SCFA metabolism.

Several reports have linked SCFA metabolism to bacterial virulence. For example, exposure of *Salmonella dublin* to SCFAs increased RpoS-dependent transcription of *spv*ABCD, genes involved in pathogenesis (El-Gedaily *et al.*, 1997). Additionally, the presence of SCFAs in the distal ileum has been shown to increase expression of proteins required for *Salmonella typhimurium* epithelial cell invasion (Lawhon *et al.*, 2002), and exogenous SCFAs increased *S. typhimurium* invasion of eukaryotic cells (Durant *et al.*, 1999). SCFAs produced by *Porphyromonas gingivalis* (Kurita-Ochiai *et al.*, 1995) and other anaerobic bacteria can down-regulate host immune responses by inhibiting phagocytosis, cytokine production, and lymphocyte proliferation (Kurita-Ochiai *et al.*, 1995; Eftimiadi *et al.*, 1990). Succinate, a SCFA produced by *Bacteroides* during infection, has been shown to inhibit migration of PMNs and phagocytic killing by inhibiting the respiratory burst (Rotstein *et al.*, 1987). Of note, *Aggregatibacter actinomycetemcomitans*, which also carries a similar *ato* region as GAS, some species of *Bacteroides*, and *Porphyromonas gingivalis*, are oral pathogens that can inhabit the same host niche as GAS. Finally, SCFAs can induce apoptosis in a variety of cell types (Kurita-Ochiai *et al.*, 1995; 2002).

Research on GAS has traditionally focused on virulence factors such as extracellular toxins and adhesins. Our work suggests that manipulation of GAS metabolic properties can influence virulence through the accumulation of certain byproducts during infection. Our data suggest that *ato*R is a positive regulator of the *ato*

operon, and therefore the Δ*ato*R mutant strain could be impaired in degrading SCFA *in vivo*. An increase in the local concentration of SCFA could inhibit the host immune system and account for the observed hypervirulence.

Acknowledgments

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Literature

Banks D.J., S.F. Porcella, K.D. Barbian, S.B. Beres, L.E. Philips, J.M. Voyich, F.R. DeLeo, J.M. Martin, G.A. Somerville and J.M. Musser. 2004. Progress toward characterization of the group A *Streptococcus* metagenome: complete genome sequence of a macrolide-resistant serotype M6 strain. *J. Infect. Dis*. 190: 727–738.

Durant J.A., V.K. Lowry, D.J. Nisbet, L.H. Stanker, D.E. Corrier and S.C. Ricke. 1999. Short-chain fatty acids affect cell-association and invasion of HEp-2 cells by *Salmonella typhimurium*. *J. Environ. Sci. Health. B*. 34: 1083–1099.

Eftimiadi C., M. Tonetti, A. Cavallero, O. Sacco and G.A. Rossi. 1990. Short-chain fatty acids produced by anaerobic bacteria inhibit phagocytosis by human lung phagocytes. *J. Infect. Dis*. 161: 138–142.

El-Gedaily A., G. Paesold, C.Y. Chen, D.G. Guiney and M. Krause. 1997. Plasmid virulence gene expression induced by short-chain fatty acids in *Salmonella dublin*: identification of *rpo*S-dependent and *rpo*-S-independent mechanisms. *J. Bacteriol*. 179: 1409–1412.

Ferretti J.J., W.M. McShan, D. Ajdic, D.J. Savic, G. Savic, K. Lyon, C. Primeaux, S. Sezate, A.N. Suvorov, S. Kenton and others. 2001. Complete genome sequence of an M1 strain of *Streptococcus pyogenes*. *Proc. Natl. Acad. Sci. USA* 98: 4658–4663.

Graham M.R., L.M. Smoot, C.A. Migliaccio, K. Virtaneva, D.E. Sturdevant, S.F. Porcella, M.J. Federle, G.J. Adams, J.R. Scott and J.M. Musser. 2002. Virulence control in group A *Streptococcus* by a two-component gene regulatory system: global expression profiling and *in vivo* infection modeling. *Proc. Natl. Acad. Sci. USA* 99: 13855–13860.

Graham M.R., K. Virtaneva, S.F. Porcella, W.T. Barry, B.B. Gowen, C.R. Johnson, F.A. Wright and J.M. Musser. 2005. Group A *Streptococcus* transcriptome dynamics during growth in human blood reveals bacterial adaptive and survival strategies. *Am. J. Pathol*. 166: 455–465.

Jenkins L.S. and W.D. Nunn. 1987a. Genetic and molecular characterization of the genes involved in short-chain fatty acid degradation in *Escherichia coli*: the ato system. *J. Bacteriol*. 169: 42–52.

Jenkins L.S. and W.D. Nunn. 1987b. Regulation of the *ato* operon by the *ato*C gene in *Escherichia coli*. *J. Bacteriol*. 169: 2096–2102.

Jimenez J.C. and M.J. Federle. 2014. Quorum sensing in group A *Streptococcus*. *Front. Cell Infect. Microbiol*. 4: 127.

Klenk M., D. Koczan, R. Guthke, M. Nakata, H.J. Thiesen, A. Podbielski and B. Kreikemeyer. 2005. Global epithelial cell transcriptional responses reveal *Streptococcus pyogenes* Fas regulator activity association with bacterial aggressiveness. *Cell Microbiol*. 7: 1237–1250.

Kurita-Ochiai T., K. Fukushima and K. Ochiai. 1995. Volatile fatty acids, metabolic by-products of periodontopathic bacteria, inhibit lymphocyte proliferation and cytokine production. *J. Dent. Res*. 74: 1367–1373.

Kurita-Ochiai T., K. Ochiai, N. Suzuki, K. Otsuka and K. Fukushima. 2002. Human gingival fibroblasts rescue butyric acid-induced T-cell apoptosis. *Infect. Immun*. 70: 2361–2367.

Lawhon S.D., R. Maurer, M. Suyemoto and C. Altier. 2002. Intestinal short-chain fatty acids alter *Salmonella typhimurium* invasion gene expression and virulence through BarA/SirA. *Mol. Microbiol*. 46: 1451–1464.

Lukomski S., N.P. Hoe, I. Abdi, J. Rurangirwa, P. Kordari, M. Liu, S.J. Dou, G.G. Adams and J.M. Musser. 2000. Nonpolar inactivation of the hypervariable streptococcal inhibitor of complement gene (*sic*) in serotype M1 *Streptococcus pyogenes* significantly decreases mouse mucosal colonization. *Infect. Immun*. 68: 535–542.

Lyon W.R., J.C. Madden, J.C. Levin, J.L. Stein and M.G. Caparon. 2001. Mutation of *lux*S affects growth and virulence factor expression in *Streptococcus pyogenes*. *Mol. Microbiol*. 42: 145–157.

Marouni M.J. and S. Sela. 2003. The *lux*S gene of *Streptococcus pyogenes* regulates expression of genes that affect internalization by epithelial cells. *Infect. Immun*. 71: 5633–5639.

Musser J.M. and F.R. DeLeo. 2005. Toward a genome-wide systems biology analysis of host-pathogen interactions in group A *Streptococcus*. *Am. J. Pathol*. 167: 1461–1472.

Rotstein O.D., P.E. Nasmith and S. Grinstein. 1987. The Bacteroides by-product succinic acid inhibits neutrophil respiratory burst by reducing intracellular pH. *Infect. Immun*. 55: 864–870.

Schell M.A. 1993. Molecular biology of the LysR family of transcriptional regulators. *Annu. Rev. Microbiol*. 47: 597–626.

Shelburne S.A., P. Sumby, I. Sitkiewicz, C. Granville, F.R. DeLeo and J.M. Musser. 2005. Central role of a bacterial two-component gene regulatory system of previously unknown function in pathogen persistence in human saliva. *Proc. Natl. Acad. Sci. USA* 102: 16037–16042.

Sitkiewicz I. and J.M. Musser. 2006. Expression microarray and mouse virulence analysis of four conserved two-component gene regulatory systems in group A *Streptococcus*. *Infect. Immun*. 74: 1339–1351.

Sitkiewicz I. and W. Hryniewicz. 2010. *Pyogenic streptococci* – danger of re-emerging pathogens. *Pol. J. Microbiol*. 59: 219–226.

Sumby P., S.F. Porcella, A.G. Madrigal, K.D. Barbian, K. Virtaneva, S.M. Ricklefs, D.E. Sturdevant, M.R. Graham, J. Vuopio-Varkila, N.P. Hoe and others. 2005. Evolutionary origin and emergence of a highly successful clone of serotype M1 group A *Streptococcus* involved multiple horizontal gene transfer events. *J. Infect. Dis*. 192: 771–782.

Waters C.M. and B.L. Bassler. 2005. Quorum sensing: cell-to-cell communication in bacteria. *Annu. Rev. Cell Dev. Biol*. 21: 319–346.

Virtaneva K., S.F. Porcella, M.R. Graham, R.M. Ireland, C.A. Johnson, S.M. Ricklefs, I. Babar, L.D. Parkins, R.A. Romero, G.J. Corn and others. 2005. Longitudinal analysis of the group A *Streptococcus* transcriptome in experimental pharyngitis in cynomolgus macaques. *Proc. Natl. Acad. Sci. USA* 102: 9014–9019.

Voyich J.M., D.E. Sturdevant, K.R. Braughton, S.D. Kobayashi, B. Lei, K. Virtaneva, D.W. Dorward, J.M. Musser and F.R. DeLeo. 2003. Genome-wide protective response used by group A *Streptococcus* to evade destruction by human polymorphonuclear leukocytes. *Proc. Natl. Acad. Sci. USA* 100: 1996–2001.

Voyich J.M., K.R. Braughton, D.E. Sturdevant, C. Vuong, S.D. Kobayashi, S.F. Porcella, M. Otto, J.M. Musser and F.R. DeLeo. 2004. Engagement of the pathogen survival response used by group A *Streptococcus* to avert destruction by innate host defense. *J. Immunol*. 173: 1194–1201.

Zaim J. and A.M. Kierzek. 2003. The structure of full-length LysR-type transcriptional regulators. Modeling of the full-length OxyR transcription factor dimer. *Nucleic Acids Res*. 31: 1444–1454.