Quorum sensing in group A Streptococcus

Juan Cristobal Jimenez1 and Michael J. Federle2*

1 Department of Microbiology and Immunology, College of Medicine, University of Illinois at Chicago, Chicago, IL, USA
2 Department of Medicinal Chemistry and Pharmacognosy, Center for Pharmaceutical Biotechnology, College of Pharmacy, University of Illinois at Chicago, Chicago, IL, USA

Quorum sensing (QS) is a widespread phenomenon in the microbial world that has important implications in the coordination of population-wide responses in several bacterial pathogens. In Group A Streptococcus (GAS), many questions surrounding QS systems remain to be solved pertaining to their function and their contribution to the GAS lifestyle in the host. The QS systems of GAS described to date can be categorized into four groups: regulator gene of glucosyltransferase (Rgg), Sil, lantibiotic systems, and LuxS/AI-2. The Rgg family of proteins, a conserved group of transcription factors that modify their activity in response to signaling peptides, has been shown to regulate genes involved in virulence, biofilm formation and competence. The sil locus, whose expression is regulated by the activity of signaling peptides and a putative two-component system (TCS), has been implicated on regulating genes involved with invasive disease in GAS isolates. Lantibiotic regulatory systems are involved in the production of bacteriocins and their autoregulation, and some of these genes have been shown to target both bacterial organisms as well as processes of survival inside the infected host. Finally AI-2 (dihydroxy pentanedione, DPD), synthesized by the LuxS enzyme in several bacteria including GAS, has been proposed to be a universal bacterial communication molecule. In this review we discuss the mechanisms of these four systems, the putative functions of their targets, and pose critical questions for future studies.

Keywords: quorum sensing, pheromones, cell-cell signaling, Streptococcus pyogenes, Rgg, Sil, lantibiotics, AI-2

BACTERIAL COMMUNICATION IN GRAM-POSITIVE BACTERIA

For a long time, bacteria were thought of as organisms carrying out self-sufficient and independent, unicellular lifestyles. During the last 40 years, several studies have demonstrated how, in fact, bacteria interact and establish complex social behaviors with their siblings and with other bacteria in their community to develop beneficial actions for the population, by means of conserved chemical languages. Quorum Sensing (QS) is the communication process in which bacteria produce, secrete and detect chemical signals with the purpose of triggering specific phenotypical responses. QS regulates genes involved in population-wide decisions and behaviors that are beneficial when performed as a synchronous group rather than at the individual level and which include bioluminiscence, sporulation, competence, antibiotic production, biofilm formation, and secretion of virulence factors (Reviewed by Atkinson and Williams, 2009; Ng and Bassler, 2009; Rutherford and Bassler, 2012).

QS signaling in Gram-positive bacteria (Figure 1) operates through the activity of post-translationally modified oligopeptides, named autoinducing peptides or pheromones, which can range from 5 to 34 amino acids in length and can adopt either linear or cyclical conformations (Håvarstein et al., 1995; Li et al., 1995; Kuipers et al., 1995; Solomon et al., 1996; Otto et al., 1998; Mayville et al., 1999; Sturme et al., 2005). These pheromones are initially synthesized as inactive pro- peptides in the ribosome, and then exported from the cell by either the general secretion system (Sec) or by dedicated ABC transporters (Hui and Morrison, 1991; Zhang et al., 2002; Stephenson et al., 2003). During the export event, pro- peptides undergo proteolytic processing (and in some cases additional covalent modification) to generate the active pheromone, and a variety of enzymes have been involved in these maturation processes (Magnuson et al., 1994; Otto et al., 1998; An et al., 1999; Mayville et al., 1999; Zhang et al., 2002; Lanigan-Gerdes et al., 2007; Thoendel and Horswill, 2009). When the pheromones surpass threshold concentrations in the extracellular medium they are efficiently detected by transmembrane receptors of the two-component system (TCS) signal transduction family, leading to differential phosphorylation of a response regulator and consequent change in target gene expression. Alternatively, pheromones can be imported into the cytoplasm via peptide transporter complexes, most commonly the Opp/Ami oligopeptide permease, a promiscuous transporter of peptides involved in the import of nutritional peptides, peptidoglycan recycling components as well as pheromone peptides for other QS systems (Leonard et al., 1996; Lazazzera et al., 1997; Slamti and Lereclus, 2002; Fontaine et al., 2010; Mashburn-Warren et al., 2010; Chang et al., 2011). Once inside the cell, peptide pheromones bind and directly modulate the activity of transcriptional regulators inside the cell. As a result of signaling, target genes change their expression pattern and genes encoding for the pheromone pre-peptides are upregulated, increasing the production of mature pheromone.
and generating a positive-feedback loop (or autoinduction process), that helps strengthen the QS signaling at the individual level and increases pheromone levels in the environment to activate signaling at the population level.

Among the earliest reports describing cell-to-cell communication in bacteria arose from studies of genetic exchange in *Streptococcus pneumoniae*, where it was shown that a “hormone-like cell product” secreted by the bacteria into the culture medium activated their ability to import extracellular DNA and undergo genetic transformation in a process termed competence (Tomasz, 1965). Several years later, the signaling molecule was shown to be a processed peptide named CSP (competence-stimulating peptide). After pheromone detection, the activated response regulator or the pheromone-bound transcription factor induce changes in target gene expression, and genes encoding for pheromone pre-peptides are up-regulated, increasing pheromone production and generating an autoinduction process.

![FIGURE 1 | Quorum sensing signaling in Gram positive bacteria.](image)

In Gram-positive bacteria, two families of conserved transcription factors have been reported to interact with imported peptide pheromones: RNPP and Rgg. The RNPP family, named for its prototypical members Rap, NprR, PlcR, and PrgX, is characterized by the presence of tetratricopeptide repeat domains (TPRs) in their C-terminal domains, defined by motifs that are involved in protein-protein interactions and are involved in peptidic pheromone binding (Blatch and Lässlé, 1999; Core and Perego, 2003). Some members of the RNPP family also possess Helix-Turn-Helix (HTH) motifs for DNA binding and direct regulation of gene expression (Declerck et al., 2007; Rocha-Estrada et al., 2010). RNPP Representatives are found in *Bacillus and Enterococcus* species and have been shown to regulate processes of sporulation, conjugation, biofilm formation and pathogenic responses (Rocha-Estrada et al., 2010). The second conserved group of peptide-binding transcription factor families is the Rgg family. Members of this family possess characteristic HTH motifs in their N-terminal domains and a C-terminal region rich in alpha-helical structures. Rgg members possess a low level of sequence similarity with members of the RNPP family and their C-terminal domains, presumed to bind peptide pheromones, have not been characterized structurally so far, and no crystallographic data of Rgg members is available yet (Fleuchot et al., 2011). The Rgg family members are widespread in the low-G+C Gram-positive bacteria and only absent in the *Clostridiaceae* (Fleuchot et al., 2011). Rgg proteins have been shown to behave like activators or repressors of DNA expression, while some can exhibit simultaneously both regulatory functions (Rawlinson et al., 2002; Samen et al., 2006; Anbalagan et al., 2011). The first Rgg family member was identified in the oral bacterium *Streptococcus gordonii*, in which extracellular glucosyltransferase activity required for tooth surface colonization was shown to be promoted by the activity of the Rgg protein (Sulavik et al., 1992). Since then, several other Rgg members have been characterized in Streptococcal species including *S. oralis* (Fujiwara et al., 2000), *S. thermophilus* (Fernandez et al., 2006; Ibrahim et al., 2007a; Fontaine et al., 2010), *S. salivarius* (Fontaine et al., 2010), *S. pneumoniae* (Bortoni et al., 2009), *S. mutans* (Qi et al., 1999), and *S. gordonii* + C.
to both positively and negatively modulate the expression of several genes (Figure 2A) (Chaussee et al., 2002, 2003, 2004, 2008; Dmitriev et al., 2006, 2008; Hollands et al., 2008; Carroll et al., 2011). RopB was initially discovered in a mutant screen for regulators of the potent cysteine protease SpeB. The streptococcal pyrogenic exotoxin B, SpeB, targets a wide range of proteins from the host, including components of the extracellular matrix and modulators of the immune response, as well as GAS proteins present in its envelope or secreted into the extracellular medium (reviewed by Chiang-Ni and Wu, 2008; Nelson et al., 2011). Hence, SpeB plays an important role in modifying the host response and in reshaping the bacterial surface, a fact that is reflected in the number of factors involved in its regulation (Carroll and Musser, 2011). RopB is essential for speB transcription, and regulates its expression by directly binding the distal P1 promoter in the intergenic region between the ropB and speB genes. As with other genes regulated by activation factors, the speB P1 promoter lacks a canonical −35 promoter sequence, which may result in poor binding by RNA polymerase and low expression in absence of the transcriptional activator. Located upstream of the −35 region of P1 are a two inverted repeat sequences, common recruitment motifs for dimeric DNA-binding proteins, which RopB may interact with in order to bind and recruit polymerase and activate speB expression, consistent with the activity of a type II activator (Neely et al., 2003; Browning and Busby, 2004). How RopB represses the expression of target genes is less understood, but it has been shown to be able to bind two different promoters in the upstream region of the repressed, prophage encoded spd-3 gene, which possess −35 sequences closer to the consensus, suggesting that in this case RopB binding blocks the promoter region, inhibiting polymerase recruitment (Anbalagan and Chaussee, 2013).

Even though it is a member of the Rgg family, RopB has not been widely characterized as a pheromone binding protein or as a QS component, as it lacks characteristics reported in other Rgg-pheromone systems. No obvious pheromone encoding gene has been described near the ropB gene. Additionally, several studies on SpeB production and mutagenesis screens performed to understand its regulators in GAS have revealed a variety of factors modulating RopB activity, but so far no pre-peptide encoding ORF. Nonetheless, several results have shown that RopB activity is affected by interaction with peptides and proteins: The regulation of speB expression by RopB has been shown to be dependent on cell cycle, with the highest degree of expression beginning in the stationary phase, suggesting a requirement for high cell density conditions for RopB-mediated gene activation. If ropB is expressed from a non-native promoter at high levels during early exponential phase, speB transcription is still not activated, implying that additional factors are required for RopB regulation (Neely et al., 2003). Mutations in Opp or its paralog Dpp (dipeptide permease) generate a drastic decrease in speB expression, suggesting that peptide import into the cell is also required for speB regulation (Podbielski et al., 1996; Podbielski and Leonard, 1998; Wang et al., 2005). Other results have shown the role of proteins that directly interact with RopB to affect its regulatory function: LacD.1 is an aldolase enzyme that has been proposed to link metabolic status of GAS with virulence

S. agalactiae (Samen et al., 2006), and S. suis (Zheng et al., 2011). Some species may even harbor multiple rgg-like genes in their genomes, like the case of Streptococcus thermophilus, predicted to encode in its genome seven different Rgg paralogs (Ibrahim et al., 2007b). After the discovery that the deletion of a small pre-peptide gene inhibited the regulatory activity of an Rgg protein in S. thermophilus, it was recognized that activity of Rgg regulators was modulated by short peptides, constituting putative QS circuits (Ibrahim et al., 2007a). Commonly, Rgg genes are located next to a short open reading frame that encodes the propeptide of their cognate pheromone, short genes which are usually overlooked in genome annotation processes but have been predicted by in silico analysis (Ibrahim et al., 2007b; Fleuchot et al., 2011). Rgg pheromones have been classified in two groups to date, short hydrophobic peptides (SHPs) and peptides involved in competence pathways, termed XIPs (Table 1) (Mashburn-Warren et al., 2010; Fleuchot et al., 2011). Since some of the Rgg/pre-peptide loci show high conservation among different streptococci, it has also been shown that interspecies cross-talk can occur via SHP-pheromones (Cook et al., 2013; Fleuchot et al., 2013). In GAS, four Rgg paralogs can be identified based on sequence homology: RopB (Rgg), Rgg2, Rgg3, and ComR (Rgg4) (Chang et al., 2011; Federle, 2012).

**RopB**

The most studied Rgg member of GAS is the RopB protein. One of the important GAS "stand-alone" regulators, transcription factors whose cognate sensor partners are unknown (Kreikemeyer et al., 2003), RopB, a predicted dimeric protein, has been shown

| Table 1 | GAS Pheromones. |
| --- | --- |
| Pre-peptide (pheromone) | Sequence | References |
| SHP2 (SHP2-C8) | MKKSKFLPILLAMVIDLVGG | Chang et al., 2011 |
| SHP3 (SHP3-C8) | MKKKVNLKFLIIQDLIVVGG | Chang et al., 2011 |
| ComS M1 (XIP) | MLKXYXYFFAALSFKVQ | Mashburn-Warren et al., 2010; Fleuchot et al., 2011 |
| ComS M3 (XIP) | MLKXYXPFLLAANVKAFA | Mashburn-Warren et al., 2010; Fleuchot et al., 2011 |
| SilCR | MNNKKTNNFSTLSEELLK | Hidalgo-Grass et al., 2004 |
| Salivaracin A (SalA1) | MSFMKNSDINTLNAAEVSE | Upton et al., 2001 |
| Streptococcin A | MKKNNEISQEOVSLEE | Jack et al., 1994b |
| Streptococcin A | MKKNTEWAFFLATCSS | Jack et al., 1994b |

Amino-acid sequences of pheromone pre-peptides and predicted cleaved pheromones (in bold). Double-glycine and glycine-alanine cleavage motifs are italicized and underlined. Pheromones residues that undergo dehydration reactions are bold and italicized, and residues believed to form thioether bridges are bold and underlined.
FIGURE 2 | Rgg regulators of Streptococcus pyogenes. (A) RopB directly activates the expression of speB and its associated downstream genes, while directly repressing the prophage encoded spd3 DNase. RopB also affects, by direct and indirect manners, the expression of a varying group of genes in different isolates. Other factors can modulate RopB activity, like LacD.1, the N-terminal peptide of the Vfr protein and unknown factors imported by the Opp and Dpp permeases. (B) The Rgg2/3 system. Left panel: in the absence of SHPs, Rgg3 is bound to the promoter region of the pheromone genes, inhibiting their expression. Addition of exogenous SHP pheromones that bind Rgg3, cause its release from DNA, while allowing Rgg2 to bind the same promoter region and promote expression of pheromone genes. Right panel: activation of the Rgg2/3 system triggers expression of shp2, shp3 and their downstream genes. Translated SHP2 and SHP3 pro-peptides are secreted through an unknown exporter and processed by the activity of Eep and additional extracellular enzymes. The active SHP2-C8 and SHP3-C8 pheromones are imported via Opp to complete the autoinduction loop. (C) Regulation of competence genes by ComR. ComS pro-peptide is produced, secreted and processed to generate the XIP peptide. After being imported through Opp, XIP can bind ComR, which binds to the promoters of comS and sigX to activate their expression. The alternative sigma factor σX together with RNA polymerase bind to Com box sites in target genes and activate the expression of competence related genes. Promoters with open arrows are Rgg-independent. Promoters with filled arrows are activated by Rgg-proteins. Promoters with X symbol are repressed by Rgg-proteins.
binding sites in the NZ131 genome, it was noticed that none of the promoters of these regulators were bound directly by RopB (Anbalagan et al., 2011). These results highlight the fact that pleiotropic effects are not a consequence of direct RopB regulation, and that additional factors, including regulation of uncharacterized putative regulators by RopB (cpsY, Spy49-1761, Spy49-1113) (Anbalagan et al., 2011), or the induction of prophage genes and concomitant phage excision, may play a role in the widespread gene induction events after ropB deletion. When comparing different strains in which the RopB regulon has been analyzed, speB and its related co-transcribed neighboring genes (spi, a putative SpeB inhibitor; prsA peptidyl-prolyl isomerase involved in SpeB processing and Spy2040, a hypothetical gene) appear as the only genetic region consistently regulated (Kagawa et al., 2005; Ma et al., 2006; Dmitriev et al., 2008; Carroll et al., 2011). Overall, this Rgg member has remained most closely tied to virulent phenotypes with a complex regulatory pattern, and the possibility for it to bind and respond to signaling pheromones remains only a theoretical possibility.

**Rgg2 and Rgg3**
The rgg2 and rgg3 genes were identified after a search for additional Rgg orthologs in the GAS genome (Chang et al., 2011). Rgg2 and Rgg3 proteins share a high degree of similarity (55% identical, 76% similar), and are encoded divergently from the genes for the pro-peptide pheromones which are termed SHP2 and SHP3 respectively (Figure 2B). Both SHP2 and SHP3 pro-pheromones are also highly similar (58% identical, 62% similar), 23 and 22 amino acids long, respectively, and contain almost identical C-terminal regions (Table 1). Basal expression of SHP2/3 pheromones is repressed under normal laboratory culturing conditions, suggesting that specific environmental signals are required to activate the QS system endogenously. Addition of synthetic SHP2 or SHP3 full length pro-pheromones are able to generate a small increase in expression from the Pshp2 and Pshp3 promoters, consistent with an autoinducing signaling system, and addition of the last eight C-terminal amino acids of SHP2 or SHP3 (termed SHP2-C8 and SHP3-C8) generate a strong inducing activity (Chang et al., 2011). Further studies have shown that multiple variants of SHP2 and SHP3 pheromones can be found in GAS culture supernatants, corresponding to the C7, C8, C9, and C10 regions of the pheromone pro-peptides, and that the SHP-C8 variants of the pheromones are the most abundant and most biologically active forms (Aggarwal et al., 2014). These results also show that both SHP2 and SHP3 signaling peptides have the same function of activating the Rgg2/3 circuit, in comparison with other dual pheromone systems were one peptide acts as an activator and the other as a repressor of the QS circuit (Nakayama et al., 1994). If a strain that lacks both shp2 and shp3 genes is used, the induction of the system by synthetic pheromones is only transient, suggesting that the autoinduction process is required to generate the full extent of the response (LaSarre et al., 2013). Even though they seem redundant in function, both shp2 and shp3 genes are required for efficient signaling and autoinduction processes, as mutations in either of them affects the timing and breadth of the signaling

### Table 2 | RopB regulated genes.

| Strain (M type) | Genes that change (% genome) | Genes decreasing expression | Genes increasing expression | References |
|----------------|-----------------------------|-----------------------------|-----------------------------|------------|
| NZ131 (M49)a  | 567 (31)                    | 340                         | 227                         | Dmitriev et al., 2006 |
| CS101 (M49)a  | 13                          | 3                           | 10                          | Dmitriev et al., 2008 |
| SF370 (M1)b   | 45 (2.5)                    | 17                          | 28                          | Dmitriev et al., 2008 |
| MGAS5005 (M1)b| 3 (0.15)                    | 2                           | 1                           | Dmitriev et al., 2008 |
| GAS5448 (M1T1)b | 99                     | 47                          | 52                          | Hollands et al., 2008 |
| MGAS10870 (M3)b | 479 (25)                | 27                          | 452                         | Carroll et al., 2011 |
| MGAS9937 (M3)b | 159 (8)                    | 13                          | 146                         | Carroll et al., 2011 |

*Gene expression changes in ΔropB strains compared with wild type strains as shown by microarray analyses.*

*a* Post-exponential/early stationary phase.

*b* In vivo.
response (LaSarre et al., 2013). Interestingly, even though other streptococci carry homologs of the rgg2-shp2 or the rgg3-shp3 loci (discussed below), to date GAS is the only sequenced streptococcal species that carries both rgg2-shp2 and the rgg3-shp3 loci, suggesting that this bacterium has evolved to use both regulators and their two cognate pheromones. As reported for other Rgg systems, an intact Opp system is required in order to import the pheromones and trigger activation of the system. Experiments also revealed that a deletion of the rgg3 gene derepresses expression of the shp2 and shp3 genes, while a double mutant Δrgg2 Δrgg3 lacks nearly all expression of pheromones. A single deletion of rgg2 also renders GAS grossly unresponsive to synthetic SHPC8 pheromones, illustrating how Rgg3 acts as a repressor of gene expression, while Rgg2 activates gene expression in the absence of the repressive effect of Rgg3 (Figure 2B). Deletion of the eep gene, encoding for a metalloprotease involved in pheromone processing in other Gram-positive QS systems, reduces the effectiveness of the autoinduction process, suggesting a role in the processing of SHP2/3 pre-peptides into their mature form. This effect can however be ameliorated if SHP3 is overexpressed, suggesting that additional proteins are involved in SHP2/3 processing (Chang et al., 2011).

Both Rgg2 and Rgg3 have been shown to bind directly to the promoter regions of shp2 and shp3 (Chang et al., 2011; LaSarre et al., 2012). Interestingly, when the precise DNA binding sites of both regulators were mapped by DNase I footprinting, Rgg2, and Rgg3 where shown to bind to the same conserved sequence in the Pshp2 and Pshp3 promoters, hence only one Rgg protein is able to bind per promoter at any given time, indicating that Rgg3 exerts its repressing activity by binding the shared DNA site and generating steric interference that inhibits Rgg2 binding (LaSarre et al., 2012). This is consistent with the fact that the HTH domains of Rgg2 and Rgg3 are distinctively analogous, sharing 71% identity and 94% similarity. A higher degree of variability is seen in the C-terminal regions of the Rgg2 and Rgg3 proteins, which are predicted to be involved in protein oligomerization and interaction with RNA polymerase, and this variability may determine the fundamental differences in the activities of these two regulators (LaSarre et al., 2012). Similar to the RopB interaction with the speB promoter, Rgg2 and Rgg3 bind the Pshp2 and Pshp3 promoters in their -35 regions, which have poor resemblance with consensus sequences, suggestive of a class II activator dependent promoter (LaSarre et al., 2012). Rgg2 and Rgg3 also directly bind mature SHP2 and SHP3 pheromones, exhibiting a higher affinity for the SHPC8 pheromone variants, while not binding full-length pro-peptides (Aggarwal et al., 2014). Using EMSA assays, it was revealed that either Rgg can bind the target DNA sequences in the absence of pheromones. However, when pheromones are added in increasing concentrations, Rgg3 is released from the DNA while Rgg2 remains unaffected. When the experiment is set up with both regulators to mimic the DNA-binding competition event in vitro, addition of synthetic pheromones affects the binding ratio, which becomes skewed in favor of Rgg2-DNA interactions over Rgg3-DNA interactions (LaSarre et al., 2012).

The function of the Rgg2/3 circuit and its contribution to the GAS lifestyle has not been completely elucidated. Apart from the shp2 and shp3 genes, other major regulatory targets of the Rgg2/3 pathway are the genes located downstream of the pheromone genes (Figure 2B) (Chang et al., 2011). Downstream of shp3, a putative biosynthetic operon composed of nine genes is encoded, which harbors genes with diverse enzymatic functions, like shikimate dehydrogenase, sugar isomerase, glycosyltransferase, and oxidoreductase. Components from this operon have been identified in mutagenesis screens for genes affecting mucoid colony morphology, virulence in zebra fish, and have also been involved in capsule formation in GAS (Biswas and Scott, 2003; Kizy and Neely, 2009; Cole et al., 2012). A putative locus encoding homologs of rgg3-shp3 and its downstream associated genes are also present in Streptococcus pseudopencinus and Bacillus thuringiensis species. In the latter, an operon that encodes genes with similar enzymatic functions has been implicated in the production of thuringiinsin, a wide-range insecticidal toxin predicted to inhibit RNA polymerase (Liu et al., 2010). Downstream of shp2, Rgg2/3 also controls a highly conserved region in GAS, which encodes a series of hypothetical genes of unknown function. This region however is required for a SHP-dependent increase in biofilm formation seen in NZ131 when the Rgg2/3 system is activated (Jimenez, unpublished results). We are currently working to elucidate the mechanism by which this this system contributes to biofilm formation. Homologous loci encoding rgg2-shp2 with its downstream-associated genes are conserved in species of the pyogenic group streptococci, including S. canis, S. agalactiae, S. dysgalactiae subspecies dysgalactiae and equisimilis and S. iniae. The S. agalactiae Rgg2 homolog, called RovS, has been involved in regulating expression of genes required for epithelial cell attachment and hemolysis activity (Samen et al., 2006), and we have recently shown that this transcription factor is also modulated by a SHP pheromone and that both GAS and S. agalactiae (also called Group B Streptococcus, GBS) can undergo interspecies QS signaling when co-cultured (Cook et al., 2013). Since GAS and GBS can be isolated from the same sites in the human host, it is intriguing to wonder if these common signaling pathways constitute a way for these two organisms to coordinate intraspecies behaviors. Alternatively, and since other pyogenic group species that carry rgg2-shp2 homologs reside in other mammalian species, this system may serve as conserved and efficient way to control gene expression that has evolved to control specific traits in each organism.

ComR

ComR is another Rgg member present in GAS, involved in natural genetic transformation processes in other streptococci through the regulation of early genes in the competence cascade (Figure 2C). Competence, the temporal physiological state of DNA receptivity, relies in the coordinated and sequential expression of a series of genes, encoding proteins responsible for pheromone signaling, DNA binding, uptake, processing and recombination into the chromosome (reviewed by Johnsborg et al., 2007). In the streptococci, the master regulator of competence is SigX/ComX, an alternative sigma factor of RNA polymerase that recognizes a conserved DNA sequence termed cinbox or combox and controls the expression of the competence “late genes” required for DNA binding, uptake and recombination (Lee
and Morrison, 1999; Peterson et al., 2000; Luo and Morrison, 2003). All the genes induced before sigX in the competence cascade and that are involved in the signaling processes that lead to sigX expression are termed “early genes.” In S. pneumoniae, the expression of sigX is under the control of a QS system, composed of the ComDE TCS which senses and responds to the competence inducing pheromone CSP. GAS and other streptococci of the pyogenic, bovis, and salivarius groups lack ComDE but have an alternative circuit, based on ComR activity, to trigger sigX expression and concomitant upregulation of late genes in the bacterial population. ComR was first discovered in Streptococcus thermophilus, a species from the salivarius group that is able to express competence genes and undergo natural transformation in an Opp-dependent manner when grown in peptide-free chemically defined medium (CDM), suggesting that import of self-produced peptides was essential for transformation (Gardan et al., 2009). To identify the regulators of sigX in S. thermophilus, the transcriptome of a mutant unable to express any competence genes (Δopp mutant) was compared with a strain only able to express early competence genes (ΔsigX mutant). One of the identified components was a previously identified rgg-pheromone gene pair which were then shown to be required for sigX expression and natural transformation (Ibrahim et al., 2007a; Gardan et al., 2009). The regulator was named ComR for competence regulator and the pro-peptide gene was called comS for competence signal (Fontaine et al., 2010). The sigX promoter, bound by ComR, lacks a canonical −35 sequence and has an inverted repeat element in this region, suggestive with a Class II activator dependent promoter, similar to the Pshp2/3 promoters activated by Rgg2 and the P1 specB promoter activated by RopB (Mashburn-Warren et al., 2010). In silico screening in streptococcal genomes revealed the presence of comRS orthologs in the genomes of pyogenic, bovis and mutans streptococci (Mashburn-Warren et al., 2010). The predicted ComS pro-peptides from these species had a similar features, being composed by a high degree of hydrophobic residues, possessing a net positive charge and a double tryptophan (WW) motif in their C-terminal region. Characterization of the ComRS system in S. mutans (that interestingly, has both ComDE and ComRS systems) replicated the results seen in S. thermophilus, and additionally showed that a synthetic pheromone consisting of the last 8 C-terminal amino acids of the 17 amino-acid long ComS peptide was able to trigger sigX expression and transformation in an Opp- and ComR-dependent manner, mimicking the activity of the putative mature pheromone. This active pheromone was named XIP for sigX-inducing peptide.

The effect of ComRS signaling in GAS and other pyogenic-group Streptococci, and its effect over transformation have proven to be more cryptic than in non-pyogenic streptococcal counterparts. Even though genome sequencing data has shown that horizontal transfer and genetic exchange between GAS and other streptococcal species is common, the demonstration of natural transformation of GAS in laboratory conditions has proven elusive. Genomic analysis by Mashburn et al. found comRS genes in all sequenced GAS genomes, and two allelic variants named M1 and M3 were found. Between these variants, ComR has a highly conserved N-terminal region, indicating similar DNA-sequence binding, but distinct C-terminal regions, suggesting a differential interaction with pheromones (Mashburn-Warren et al., 2012). Indeed, the sequence of XIPs of M1 and M3 alleles also differ, while both contain WW alleles do differ, while both contain WW motifs (Table 1). In the absence of exogenous XIP, sigX expression is low. Addition of synthetic XIP activates ComR and triggers expression of sigX in GAS strains, in an allele-specific manner. Concentrations as low as 0.5 nM XIP are sufficient to trigger sigX expression, and microarray analysis revealed that XIP sensing by ComR generated the upregulation of 30 different genes, 21 of these containing com boxes, and thus regulated directly by SigX (Mashburn-Warren et al., 2012). Most of the regulated genes corresponded to predicted competence late genes. Two other regulated genes of interest were murM2 and roCA, involved respectively in processes of antimicrobial resistance and upregulation of the CovR TCS component, a major regulator of GAS genes. All gene functions known to be required for genetic transformation in S. pneumoniae and Bacillus subtilis were upregulated in response to XIP in GAS, but nonetheless transformation was not seen in vitro. Further experiments revealed that competence was blocked at the DNA uptake process, suggesting that unknown post-transcriptional regulation events were inhibiting transformation (Mashburn-Warren et al., 2012).

Additional environmental signals may therefore be required to unblock the competence machinery, and the first evidence for this notion has been associated with growth in biofilms. As sessile bacterial aggregates, biofilms contain high densities of bacterial cells in close contact and studies have shown that Streptococci that undergo natural transformation do so in higher efficiencies when growing inside biofilms (Marks et al., 2012; Wei and Hävarstein, 2012). Recently, the first report of effective GAS natural transformation in the laboratory was seen in biofilm cultures (Marks et al., 2014). GAS biofilms grown on a substrate of fixed epithelial cells were shown to internalize and incorporate exogenous DNA at a low frequency, in a ComR-dependent manner. Addition of XIP generated a ten-fold increase in efficiency, but was not required for transformation, suggesting that growth on epithelia provides a signal(s) for Com system activation. The transformation phenotype could also be replicated during an in vivo biofilm growth assay, where intranasally colonized mice were also given donor DNA. Again, the addition of the XIP pheromone generated a ten-fold increase in the number of transformed colonies (Marks et al., 2014). These results suggest that GAS are indeed capable of undergoing genetic transformation, but an unidentified regulatory check-point requires an environmental condition that appears to be satisfied through growth on host cells. Also, a number of GAS isolates have reported mutations in the genes that form the DNA uptake complex, indicating the ability to induce the complete competence machinery is naturally lost in some of them, however the conserved maintenance of genes involved in the QS-signaling process suggests that ComR and SigX may have additional beneficial functions in non-competent strains (Woodbury et al., 2006; Mashburn-Warren et al., 2012).

**SIL SIGNALING SYSTEM**

The Sil system was the first QS network characterized in GAS. The sil locus was discovered in a tagged-transposon mutagenesis screen performed on a highly invasive M14 strain (JS95),...
which was isolated from a case of necrotizing fasciitis, and is capable of generating a lethal invasive infection in a murine model (Hidalgo-Grass et al., 2002). The screen was designed to identify mutant clones that lost their ability to spread invasively from a skin infection site to other target organs in mice. One of the isolated mutants lost its ability to reach the spleen, while not affecting its ability to survive in blood, and had an insertion in a novel locus that was termed streptococcal invasion locus (sil).

The core Sil system consists of six genes, silABCD DE and silCR encoded in a putative genomic island of 15–17 Kbps in size, comprising a lower GC content than the average of the GAS genome (Hidalgo-Grass et al., 2002; Eran et al., 2007; Belotserkovsky et al., 2009) (Figure 3). The presence of sil genes in GAS genomes is not widespread, with only four of the 19 sequenced GAS genomes contain this system (MGAS8232, MGAS10750, Alab49, and HSC5) (Kizy and Neely, 2009; Michael-Gayego et al., 2013). Epidemiological studies have shown that the prevalence of Sil ranges from 12% in non-invasive isolates to 16–25% in invasive isolates, and that the locus is restricted to a few emm-types with M4 being the most common sil-harboring isolate (Bidet et al., 2007; Billal et al., 2008; Michael-Gayego et al., 2013). The sequenced strains that do not possess Sil have instead remnants of the genomic island, suggesting that DNA recombination may have been responsible of loss of sil genes (Figure 3B) (Belotserkovsky et al., 2009).

The Sil system has sequence homology with the competence (Com) and the bacteriocin-like peptide (Blp) QS systems of Streptococcus pneumoniae (De Saizieu et al., 2000; Morrison and Lee, 2000). The sil QS locus (Figure 3A) is composed of silAB, a putative TCS, silDE, a putative ABC transporter, and silCR, the pheromone pro-peptide gene. An additional small ORF encoded in the complementary DNA strand and overlapping with silCR is silC, which encodes a 39 amino acid peptide disrupted by the original transposon hit during the mutant screen (Hidalgo-Grass et al., 2002). The promoter of silC has a com box, the conserved sequence found in promoter regions of genes involved in competence establishment in other streptococci (Morrison and Lee, 2000). The silCR gene encodes a 41 amino acid pro-peptide with a glycine-glycine sequence motif, thought to direct cleavage in a manner consistent with Gly-Gly bacteriocin processing, which allows cleavage into a 17 amino acid active mature pheromone (Table 1). In strains carrying a functional copy of silCR, basal pheromone production in standard culture conditions can be quantified by immunoblotting methods and measured in supernatants using fluorescence reporter assays, with a concentration of approximately 24.5 nM being produced by the IB7 GAS isolate (Belotserkovsky et al., 2009). The multiple SilCR-regulated promoters in the sil locus are induced to various levels and the amount of synthetic pheromone required to trigger their expression ranges from low to high nanomolar concentrations (Eran et al., 2007; Belotserkovsky et al., 2009). The silDE and silCR genes are transcribed in a single mRNA whose expression increases in response to synthetic SilCR pheromone. Detection of SilCR is believed to occur through direct interaction of the peptide with the SilB TCS receptor which generates a corresponding phospho-transfer to the SilA and consequent change in DNA expression. The silAB promoter on the other hand, shows a lower degree of response to the peptide pheromone, but increases in response to H₂O₂, and is upregulated upon inoculation of the host. This suggests, that much like production of other pheromones in GAS, environmental conditions or host factors may be key to triggering their expression (Eran et al., 2007; Belotserkovsky et al., 2009; Kizy and Neely, 2009).

SilA is the predicted TCS response regulator and a member of the AlgR/AgrA/LytR family of transcriptional regulators that bind DNA as dimers via a LytTR-type domain (Nikolskaya and Galperin, 2002). SilA was shown to be necessary together with SilB, the putative sensor histidine kinase, to trigger expression of genes in response to SilCR (Belotserkovsky et al., 2009). The binding sequence for SilA was mapped in two SilCR responding promoters, revealing the presence of two direct-repeats of 9 bp that are separated by an 11 bp spacer, both of which are required for SilA activity. As demonstrated by bioinformatic analysis, most of the genes that possess putative SilA binding sites are present in the genomic island surrounding the sil core genes (Figure 3B). These genes increase their expression in a rapid manner after

![FIGURE 3 | Sil signaling system. (A) Model of signaling. SilCR pre-peptide is produced, secreted and processed. Mature SilCR is detected by SilB, activating the response regulator SilA which activates expression from select promoters, including the promoter for the expression of silED-CR genes. Expression of the sil locus is also in an unknown manner by a second two-component system, TrxSR. Asparagine sensing by TrxS alleviates repression of target genes by TrxR, generating an increase in expression of SilCR dependent promoters. (B) sil genomic island. The sil locus plus neighboring genes are located in a putative genomic island in the strain JS96. Chromosomal location is compared with the SF370 strain that does not possess the sil locus.](image-url)
SilCR pheromone addition, and several correspond to predicted bacteriocins and/or bacteriocin maturation components, while the rest of them correspond to transposons or insertion sequences (Belotserkovsky et al., 2009). In *Streptococcus pneumoniae* the Blp bacteriocins were shown to mediate intra- and interspecies competition in cultured conditions, and also provide a competitive advantage during *in vivo* colonization experiments (Dawid et al., 2007). It is unknown whether the Blp homologs found in the *sil* island are able to produce a mature, active bacteriocin, or if they can generate immunity against Blp bacteriocins of *S. pneumoniae*, which have been shown to kill GAS *in vitro* (Lux et al., 2007). Interestingly, addition of SilCR to a culture’s growth medium also increases the expression of several genes that lack SilA binding motifs. As shown by microarray experiments, the early response to SilCR addition consists mainly of genes in the *sil* island. However, 3 h after pheromone addition other genes show a moderate but significant response. Some of these genes include *ptsABCD*, a putative mannose/fructose-specific phosphotransferase system, purine synthesis related genes *purEK* and even the *rrg2* transcriptional regulator, suggesting a possible link in the activation process of these two QS systems (Belotserkovsky et al., 2009). Strains that do not possess the core *sil* signaling genes have also been shown to exhibit differential gene expression in response to SilCR pheromone, in some cases with opposite regulatory effects than those seen in *sil* harboring strains (Salim et al., 2008). If any of the additional TCS of GAS could be also sensing SilCR has yet to be determined.

As mentioned earlier, only a fraction of GAS isolates carry the *sil* locus. Interestingly, among the strains that possess Sil, some of them also carry point mutations in components of the system. The pheromone-coding gene, *silCR*, often has a nonsense mutation that changes the ATG start codon for ATA, rendering the strain unable to produce its own peptide, yet is able to respond to exogenous peptide (i.e., strains JS95, HSC5). Other mutations reported are located in the *silD* component of the ABC transporter, where stop codon mutations truncate or divide the gene (strains MGAS8232, MGAS10750) (Michael-Gayego et al., 2013). The strains carrying these *silD* mutations should be able to respond to peptide, but won’t be able to secrete pheromones to generate an autoinduction process. Presence of non-sense mutations in signaling pathways appears to be a recurring theme among GAS QS systems, and also can be seen in the Com and the lantibiotic regulatory systems of GAS (Upton et al., 2001; Wescombe and Tagg, 2003; Mashburn-Warren et al., 2012; Wescombe et al., 2012). Selective pressures leading to such lesions remains unclear.

The clearest understanding of behavior regulated by the Sil system, and the reason for its discovery, is its effect on pathogenicity. After the first study which identified *silC* mutants with deficient invasion of the spleen, *silB* and *silC* mutants were also isolated in an independent signature-tagged mutagenesis screen looking for loss of virulence of the strain HSC5 in a zebra fish model of necrotizing fasciitis (Kizy and Neely, 2009). Interestingly, these screens have been performed in strains possessing the *sil* locus, but contain the sequence ATA in place of a functional start codon in *silCR*, impairing pheromone production. Thus, Sil seems to provide a pathogenic advantage *in vivo* in the absence of the pheromone, and further evidence suggests SilCR pheromone actually represses pathogenicity. Normally, GAS exhibits the ability to degrade the chemokine IL-8 of human origin, and MIP2 and KC of murine origin, by the activity of the ScpC/SpyCEP serine peptidase. Chemokine degradation impairs the process of immune recruitment, resulting in the absence of infiltrating neutrophils in infected mice tissue, thus inhibiting bacterial clearance from infected sites. Preincubation of the strain JS95 with the SilCR pheromone inhibits the chemokine-degrading activity by down-regulating the expression of ScpC in a SilA-dependent SilC-independent manner, and preincubation of bacteria with SilCR reduces skin lesion area and inhibits killing by systemic infection (Hidalgo-Grass et al., 2004, 2006; Eran et al., 2007). For this reason it has been proposed that the Sil system may play a role in controlling invasive disease, and that strains possessing Sil, but have mutations impairing SilCR expression, may exhibit increased virulence phenotypes. The mechanism of ScpC down-regulation has not been elucidated thus far, and its promoter lacks any predicted SilA binding sites. Even though its deletion affects pathogenicity, the exact function and mechanism of SilC remains cryptic. Originally, it was shown that the expression of SilC in *trans* from a plasmid was able to repress SilCR activated promoters (Hidalgo-Grass et al., 2002; Eran et al., 2007), but so far its ability to repress other genes that may affect GAS infection and lifestyle in the host has not been demonstrated. The therapeutic effect of SilCR nonetheless seems to be variable, and may depend on the presence of *sil* genes, emm type and isolate used (Salim et al., 2008), and repression of *scpC* expression by additional regulators like CovRS (Sumby et al., 2008). Recently, it was shown that signaling by Sil is activated *in vivo* during early time-points post infection in a mouse model, and that this activation phenotype and its kinetics can be replicated in culture during infection of cell-culture lines (Baruch et al., 2014). It was shown that activation of the *sil* locus was dependent on the sensing of the amino acid asparagine by the TrxRS TCS of GAS, subsequent to secretion of streptolysin toxins that induce asparagine release by mammalian cells.

Finally, isolates of *Streptococcus dysgalactiae subsp. equisimilis*, known as Group G streptococcus (GGS), also possess the *sil* locus. Prevalence of Sil in invasive isolates of GGS is higher than in GAS isolates, and all analyzed strains had a functional copy of *silCR*, although some of them also carried truncations in the *silD* gene (Belotserkovsky et al., 2009; Michael-Gayego et al., 2013). Both GAS and GGS generated a transcriptional response when incubated with SilCR pheromone produced by one another, supporting the hypothesis that interspecies signaling between these two Streptococci is possible.

**LANTIBIOTIC REGULATORY SYSTEMS**

During the process of colonization and establishment of their niche, bacteria face constant competition for nutrients from other individuals. For this reason, several bacteria have devised systems to give them a competitive advantage against other bacterial organisms. One such way is the production of bacteriocins, ribosomally synthesized antimicrobial peptides (AMP) that can target bacteria in the same species or across genera, with producing bacteria expressing specific immunity proteins to protect themselves
from their cognate peptide. These antimicrobial molecules can act through a variety of mechanisms including membrane pore formation, cell wall synthesis inhibition, and target enzyme inhibition (Quadri, 2002; Cotter et al., 2005). Class I bacteriocins, termed lantibiotics, are post-translationally modified peptides that are produced by Gram-positive bacteria, including *Bacillus, Staphylococcus, Lactococcus* and *Streptococcus* species (Chatterjee et al., 2005). These molecules are synthesized as inactive pro-peptides and then modified through amino acid dehydration and/or thioether bridge formation to generate unusual amino acid residues, and are cleaved during the secretion process to generate a mature active lantibiotic (Kleerebezem, 2004; Chatterjee et al., 2005). The lantibiotic acts not only as an AMP to neighboring bacteria, but also has pheromone properties, as lantibiotic-producing bacteria can detect their own bacteriocin through a TCS. For these reasons, lantibiotic production in several bacteria exhibits a cell-density dependent pattern and is regulated by quorum-sensing like circuits (Kleerebezem, 2004). The machinery required for lantibiotic production is usually encoded in gene clusters of conserved architecture formed by two or more operonic units in which genes are grouped by their function. A model of a prototypical lantibiotic producing system and its mechanism is shown in Figure 4A: The inactive product of the pre-peptide gene is post-translationally modified, cleaved, and exported to give rise to the active lantibiotic, which can exert antimicrobial activity toward sensitive individuals. The mature lantibiotic is also sensed by the TCS, which signals to generate an autoinduction process and increase the production of lantibiotic as well as activates the production of lantibiotic in its siblings. In some cases lantibiotic biosynthetic operons are shared amongst different species, meaning that a bacteriocin can act both as an inter and intraspecies signaling molecule. To remain resistant to the activity of their lantibiotic, bacteria can express membrane bound immunity peptides that bind the bacteriocin and/or ABC transporters that prevent the accumulation of lantibiotics in the bacterial surface (Gebhard, 2012).

Certain GAS isolates have been reported to produce different lantibiotic molecules that can inhibit growth in other streptococcal species, or in sensitive GAS isolates (Tagg et al., 1973; Simpson and Tagg, 1983; Tagg and Skjold, 1984; Hynes and Tagg, 1985; Karaya et al., 2001; Upton et al., 2001). The ability to produce mature bacteriocins is not widespread amongst all GAS isolates, and in most cases conserved mutations have occurred in biosynthetic loci. This has led authors to hypothesize that some of these circuits may have lost their lantibiotic and QS ability and instead have adapted them to perform other functions (Phipps and Neely, 2007; Namprachan-Frantz et al., 2014). One lantibiotic locus present in GAS is orthologous to Salivaracin A (SalA) synthesis gene clusters. First identified and purified form cultures of *Streptococcus salivarius* strain 20P3, salivaracin A is the result of the processed product of the salA gene, a 51-amino acid propeptide with a Gly-Gly motif, which is modified and cleaved to generate a mature 22-amino acid lantibiotic and autoinducer pheromone (Ross et al., 1993; Upton et al., 2001). Different sal alleles have been described where variations in the sequence of the salA gene give rise to five additional variants of SalA termed SalA1 to SalA5 (Wescombe et al., 2006). The *S. salivarius* salAMTXYKR loci contains all the genes predicted to be required for the processing (salM, previously mistakenly annotated as salBC), export (salT), immunity (salXY), and sensing of the peptide (salKR) by the SalKR TCS (Ross et al., 1993; Wescombe et al., 2006). Salivaracin A from *S. salivarius* was shown to inhibit growth of a wide variety of GAS strains in culture. Surprisingly, the majority of analyzed GAS isolates were also shown to possess a salA1 gene in their genome, while only a couple of M4 isolates were able to produce the active lantibiotic (Simpson et al., 1995; Upton et al., 2001).
immune strain T11 renders it sensitive to SalA. Some GAS strains
production are required for efficient establishment of immunity.
active lantibiotic and the process of self-signaling and autoin-
duction are required for efficient establishment of immunity.
Reinforcing this idea, mutating the SalA maturation genes in the
strain, which has an incomplete sal locus, meaning that this strain
is still able to sense and generate a transcriptional response to
the lantibiotic peptide. Nonetheless, SF370 remains sensitive to
the bacteriocidal effects of SalA, although it carries the genes for
its putative immunity, suggesting that the ability to generate an
active lantibiotic and the process of self-signaling and autoin-
duction are required for efficient establishment of immunity.

Another lantibiotic isolated from GAS cultures is Streptin
(Johnston et al., 2001; Wescombe et al., 2006). Analysis of genomes of these
isolates revealed that most of them carried deletions in the sal
cluster affecting the genes required for maturation and secretion of SalA (Wescombe et al., 2006), while mostly maintaining intact
sequences of the downstream genes required for immunity and
sensing, a phenomenon that seems to be widespread as shown
also in several sequenced strains (Figure 4B). Spent cultures of a
the GAS T11 strain, that is able to produce active SalA1, induces
expression of salA mRNA in S. salivarius 20P3, as well as inducing
low levels of expression of salA1 mRNA in the M1 SF370
strain, which has an incomplete sal locus, meaning that this strain
is still able to sense and generate a transcriptional response to
the lantibiotic peptide. Nonetheless, SF370 remains sensitive to
the bacteriocidal effects of SalA, although it carries the genes for
its putative immunity, suggesting that the ability to generate an
active lantibiotic and the process of self-signaling and autoin-
duction are required for efficient establishment of immunity.

Evidence has suggested however that the sal locus may still play
a role during GAS infection in the host. Analysis of GAS trans-
poson mutants in a zebrafish model of infection detected two
attenuated mutants of the HSC5 strain with insertions in salY
and salK genes. Further inspection showed that the salY mutant
exhibited decreased bacterial loads after zebrafish infection when
compared with is WT counterpart, and the salY mutant was also
attenuated for survival inside macrophages in vitro. The in vivo
survival defect could be rescued if zebrafish were depleted of
macrophages prior to infection (Phelps and Neely, 2007). SalY
forms part of the putative ABC transporter involved in immu-
nity to the SalA lantibiotic. When compared with the salY gene
from S. salivarius, all GAS strains possess conserved nucleotide
changes that generate non-similar changes in several amino acids
of the SalY transporter. Therefore, it has been hypothesized that
GAS SalY may have changed and adapted to bind and/or export
other substrates. It has also been shown that salA expression is
upregulated during murine muscle tissue infection, and recently
it also was shown that human serum increases the expression of the
promoter upstream of the salRK TCS genes, providing a
possible link between host factors and activation of the sal locus
in GAS (Loughman and Caparon, 2006a; Namprachan-Frantz
et al., 2014). In addition, data have suggested that mutations in
the salRK TCS can affect other genes outside the sal cluster of
genes in GAS and other streptococci (Li et al., 2008; Le Breton
et al., 2013). For these reasons, it was hypothesized that while the antimicrobial effect of SalA1 was lost in GAS, it may still
be involved in signaling processes important for host coloniza-
tion, and that this system may have evolved to perform a different
function.

Another lantibiotic isolated from GAS cultures is Streptin
(Karaya et al., 2001; Wescombe and Tagg, 2003). Similar to sali-
varacin A and other lantibiotics, all the required genes are present
in one cluster, formed by the srtIRKATCBBFE genes predicted
to be involved in streptin binding and immunity (srtI), detection
and regulation by TCS (srtRK), pre-peptide (srtA), transport
(srtT), modification (srtCB), and additional immunity through
an ABC-transporter (srtEG). The srtA gene encodes for the
Streptin precursor peptide, a 46 amino acid peptide that is modi-
and cleaved to generate three variants of Streptin (Table 1)
(Wescombe and Tagg, 2003). Levels of srtA mRNA are unde-
tected at early culture time points and increases slightly at later
stages of culture, but can be induced earlier if a streptin extract
is added to the culture, reflecting the autoinducing ability of the
lantibiotic. Similarly, potent streptin production is only obtained
in two-phase cultures, where a lawn of solid media-grown bac-
teria is transferred to liquid culture. Interestingly, presence of
SpeB has been shown to be required to produce active Streptin
and to induce effective immunity in the producer strains, sug-
gest that this protease may be involved in the processing of the
mature lantibiotic (Hynes and Tagg, 1986). Though the srtA
gene is found in several isolates, only a few of these are able to
produce the biologically active lantibiotic, as is seen with SalA.
In some cases lack of production is apparently due to the lack
of genes required for transport and modification, or in other
cases where the srt locus appears intact and autoinduction does
not occur due to probable lack of promoter activity, suggesting
that additional elements are involved in controlling expression of
the lantibiotic genes (Wescombe and Tagg, 2003). One group has
shown that samples of GAS present in throat infection patients
exhibit a high expression of the srtEGI immunity genes (Livezey
et al., 2011), suggesting a requirement of host factors for srt gene
expression and a possible role for streptin immunity-related ABC-
transporters in infection of the host and establishment of GAS in
its niche.

Finally, Streptococccs are another type of lantibiotic shown
to be produced by GAS. There have been two types described
to date, class I type streptococci (chromosomally encoded
Streptococcin A-FF22 and Streptococcin A-M49, named so for
their strains of isolation) and class III type streptococcins (plas-
mid encoded Streptococcin A-M57) (Tagg et al., 1973; Simpson
and Tagg, 1983; Tagg and Skjold, 1984; Jack and Tagg, 1992;
Hynes et al., 1994; Jack et al., 1994b; Heng et al., 2004; Tagg,
2009). The most studied of these is Streptococcin A-FF22 (SA-
FF22) a cationic bactericidal peptide which causes membrane
potential disruption in target cells (Jack et al., 1994a). Similar
to the sal and srt loci, the gene cluster involved in SA-FF22 is
comprised of genes involved in production of the pre-peptides
(scnA and scnA'), regulation (scnRK), modification and transport
(scnMT), and immunity to the lantibiotic (scnFG) (McLaughlin
et al., 1999). The FF22 strain is able to produce an active
Streptococcin that is able to inhibit growth in sensitive strains,
while generating autoinduction in both GAS and S. salivarius
and S. dysgalactiae strains that carry the complete scn gene
cluster. The scnA pre-peptide gene was found only in a small
percentile of analyzed strains, and all of the sequenced strains
to date completely lack the locus, having only a flanking trans-
posase A gene (Wescombe et al., 2012), and studies testing the
streptococcin involvement in the GAS lifestyle have not been
reported.
INTERSPECIES COMMUNICATION: LuxS AND AI-2

The autoinducer-2 molecule (AI-2) was initially discovered in the Gram-negative bacterium *Vibrio harveyi*, in which it forms part of the QS-dependent regulation of luciferase production. It was later found that the LuxS enzyme was required for AI-2 production, and since the luxS gene is present in the genomes of a wide variety of Gram-negative and Gram-positive bacteria, has led to the proposal of AI-2 as a universal signal for interspecies cell-to-cell communication (Miller and Bassler, 2001; Xavier and Bassler, 2003). Differing from other Gram-positive QS signals, AI-2 signaling is not peptide-based. Also, unlike the biosynthetic processes of the other QS molecules of GAS, the AI-2 synthesis pathway is tightly coupled with a metabolic cycle termed activated methyl cycle (AMC), which is involved in the utilization of S-adenosylmethionine (SAM) and the degradation of its toxic derivatives. SAM is an essential donor of methyl groups for processes of DNA, RNA, fatty acid, and protein synthesis. Its utilization generates the toxic intermediate S-adenosylhomocysteine (SAH), which is then hydrolyzed by the Pfs nucleosidase to produce S-ribosylhomocysteine (SRH) and adenine. LuxS catalyzes the cleavage of SRH to homocysteine and 4,5-dihydroxy 2,3-pentanedione (DPD). DPD then spontaneously cyclizes to form pro-AI-2 molecules capable of reacting with borate to generate the signal detected by Vibrios (Figure 5A) (Schauder et al., 2001).

Two different families of receptors have been reported to sense AI-2 molecules in different bacteria (Figure 5B). The LuxPQ family, only found in *Vibrio* spp., consists of the LuxP periplasmic protein that binds AI-2 and in consequence modulates the activity LuxQ, a transmembrane two-component histidine kinase. Binding of AI-2 switches the activity of LuxQ from kinase to phosphatase, affecting the activity of downstream DNA-binding response regulators. The other family of receptors is the Lsr family, found in *E. coli*, *Salmonella* and members of the *Rhizobiacaeae* and *Bacilliacaeae* families (Pereira et al., 2009). LsrB binds a non-borated derivative of the AI-2 molecule, while the LuxPQ signal transduction system, LsrB delivers AI-2 to the Lsr ABC transporter, which imports the molecule into the cytoplasm. The transport of AI-2 it’s coupled with its phosphorylation, which both serve to its sequestration inside the cell and to enhance AI-2 binding to the LsrR transcriptional repressor protein, deactivating its repressive function (Miller et al., 2004; Xavier and Bassler, 2005).

LuxS and AI-2 have been shown to influence expression of virulence factors, motility, and biofilm formation in several bacteria, including Streptococcal species (Reviewed by Pereira et al., 2013). In GAS, LuxS is required for AI-2 synthesis, and luxS deletion resulted in increased expression of the haemolysin streptolysin S and Emm protein, decreased production of hyaluronic acid capsule and diminished activity of the SpeB protease due to abnormal processing (Lyon et al., 2001; Marouni and Sela, 2003). These changes resulted in increased invasiveness toward HEp-2 epithelial cells. Additionally, Siller et al. showed that the deletion of the luxS gene resulted in increased tolerance to acidic conditions, affected the expression of fasX (an sRNA involved in regulation of virulence factor expression), (Kreikemeyer et al., 2001) and upregulated expression of sibA, a gene encoding a secreted immunoglobulin binding protein (Siller et al., 2008).

One of the central debates in the topic of LuxS function is how to differentiate its QS effects from its metabolic role. Inactivation of luxS not only inhibits AI-2 formation, but it also could result in gene expression changes generated by defective recycling of homocysteine or accumulation of intermediates of SAM metabolism. Recently, Redanz et al. have shown that in Streptococcus sanguinis close to 96% of the genes that exhibit a change in expression in the ΔluxS mutant are due to accumulation of homocysteine in the cell (Redanz et al., 2012). Additionally, genomic analysis has revealed that several species possessing the luxS gene lack any homologs to the AI-2 receptors LuxPQ or Lsr, including the Streptococci (Rezzonico and Duffy, 2008). Thus, it has been
proposed that to clearly establish the role and the effects of the AI-2 system, the ΔluxS mutant phenotypes should be complemented by the addition of synthetic DPD to generate AI-2 in the culture medium (Pereira et al., 2013). No group has yet demonstrated directly the effects of exogenous AI-2 on GAS gene expression, so the nature of the LuxS-promoted phenotypes is unknown. Even though Siller et al. have suggested that luxS has a limited role in the AMC cycle in GAS (Siller et al., 2008), the overall effect of this deletion over the bacterial metabolism is unknown. Nonetheless, AI-2 has been shown to directly influence biofilm formation in other oral streptococci like S. intermedius and S. gordonii (Ahmed et al., 2008; Cuadra-Saenz et al., 2012), meaning that these bacteria may have an as yet uncharacterized AI-2 sensing machinery, and that even if GAS does not directly respond to its own AI-2, could influence the behaviors of related species in its surroundings.

CONCLUDING REMARKS AND PERSPECTIVES

The Streptococcus pyogenes pan-genome harbors several varieties of QS circuits, and GAS has the ability to produce an assortment of dedicated signaling molecules that affect expression of several target genes. While there is increasing information regarding the mechanisms of these QS networks, our current understanding of these systems is still in its infancy, and despite data regarding regulated genes and observed phenotypes resulting of QS signaling, we do not fully comprehend yet when and where these communication systems are triggered in vivo, and how GAS benefits from these processes of population-wide coordination of gene expression. Apart from the SIl system, the direct contribution of QS to GAS pathogenesis is unclear. However, its putative involvement in the control of DNA exchange (ComR) and in the sensing and production of antimicrobial substances (SalA and Srt) suggest that QS may play roles involved in the less understood process of GAS interaction with other bacteria in its milieu. Recently, Marks et al. have shown that GAS growing inside biofilms exhibit lower expression of genes involved in localized and invasive-disease, and bacteria originating from biofilm cultures had reduced virulence in a septic infection model, while showing an increased ability to asymptptomatically colonize nasal associated lymphoid tissue of mice, when compared with planktonic cultured bacteria (Marks et al., 2014). There is evidence that both Rgg2/3 and Sil QS systems can affect biofilm formation (Lembke et al., 2006; Chang et al., 2011; Cook et al., 2013), suggesting a possible link between QS and the asymptomatic, commensal lifestyle of GAS inside the human host. Future efforts in these areas may be able to reveal the role of QS signaling in GAS population behavior.

With the increasing emergence of antibiotic resistance amongst infectious bacteria, there is a dire need for alternative therapeutic strategies to control pathogens. One of these strategies is the control of virulence gene expression, mainly through the manipulation of QS in a process of quorum-sensing inhibition (also referred to as “quorum quenching,” reviewed by LaSarre and Federle, 2013), with successful results of QS-inhibition during in vivo infection models with pathogens Vibrio cholerae and Staphylococcus aureus (Mayville et al., 1999; Wright et al., 2005; Duan and March, 2010). We think the multiple QS systems of GAS offer interesting molecular targets to block or interfere in order to modulate the behavior of this pathogen as a way of future treatment.

ACKNOWLEDGMENTS

Support for this work was provided by CONICYT Becas-Chile Scholarship, NIH grant AI091779, and the Burroughs Wellcome Fund Investigators of Infectious Diseases.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 28 May 2014; paper pending published: 03 July 2014; accepted: 26 August 2014; published online: 12 September 2014.

Citation: Jimenez JC and Federle MJ (2014) Quorum sensing in group A Streptococcus. Front. Cell. Infect. Microbiol. 4:127. doi: 10.3389/fcimb.2014.00127
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