Pathogenic streptococci speak, but what are they saying?

Michael J. Federle
Center for Pharmaceutical Biotechnology; Department of Medicinal Chemistry and Pharmacognosy; University of Illinois at Chicago; Chicago, IL USA

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Correspondence to: Michael J. Federle; Email: mfederle@uic.edu

Currently, intercellular chemical signaling in bacteria, known as quorum sensing, is described for several species of bacteria; however, for many clinically important pathogens this significant sensory mechanism remains unknown. Among such pathogens are the pyogenic streptococci that include groups A and B streptococcus (GAS, GBS). Evidence now points to a family of transcription factors, known as Rgg/GadR/MutR, can serve as receptors for secreted pheromones. Within the genome of Streptococcus pyogenes four Rgg paralogs can be identified, two of which (Rgg2 and Rgg3) were shown to rely on short hydrophobic peptides (SHPs) to control transcription of their target promoters. SHPs were found to promote biofilm development and could offset biofilm-dispersion effects caused by Rgg1. Since Rgg homologs are present in genomes throughout Firmicute species, their newfound ability to serve as quorum-sensing mediators offers a potential opportunity to manipulate bacterial behaviors by interfering with communication networks.

Three decades of efforts have established the paradigm that communication between bacterial cells occurs via chemical signaling. In a process termed quorum sensing, bacteria secrete small molecules into their environment to tell their siblings and neighbors of their presence and willingness to work together. These communications coordinate a wide variety of bacterial behavior that include the development of biofilms, the triggering of pathogenic attacks, the assault on neighbors to acquire new genetic material, and several other complex actions. While considerable knowledge of how quorum sensing operates and of the benefits it provides to communities has been gleaned from model organisms and a few pathogenic species, many important bacterial taxa seem virtually silent when it comes to intercellular communication due to lack of recognizable orthologous quorum-sensing signals or receptors. Such has been the case for many members of the large group of Gram-positive bacteria known as streptococci. Many species of this phylum, including groups A and B streptococci (GAS, S. pyogenes and GBS, S. agalactiae, respectively) largely lack proteins that provide these features.

Recent findings, however, reveal a new purpose for a widespread family of proteins among Gram-positive bacteria enabling the cellular equivalent of social networking. The family of proteins known as Rgg/GadR/MutR is found throughout most Firmicute species, and though its members are known to serve as transcription factors, clearly containing a recognizable DNA-binding helix-turn-helix motif and being necessary for transcriptional activation of many genes among the streptococci, little has been explained for how they differentially control target gene expression. Despite lacking recognizable primary-sequence similarity to any other quorum-sensing components, structural prediction algorithms reveal potentially similar secondary and tertiary structure to PlcR and PrgX, two prototypical members of the Rap/NprR/PrgX/PlcR (RNPP) protein family, also found throughout Gram-positive bacteria. Each member of this family serves as a receptor for imported signaling peptides. Upon ligand interaction, RNPP proteins respond with changes in their regulatory activity. All currently available genome sequences of S. pyogenes (all coming from clinical
isolates attributable to various diseases) each contain four rgg paralogs. The best studied is RopB (Rgg1), a transcription factor known for its requirement as an activator of the secreted cysteine protease SpeB. SpeB is among the most important GAS virulence factors. Another Rgg protein is ComR, found recently to control competence development in Streptococcus mutans and Streptococcus gordonii. Adjacent to comR in these genomes is comS, a small gene encoding a peptide pheromone that interacts directly with ComR. In S. mutans, the pheromone, called XIP, converts ComR into an active transcription factor, thereby inducing expression of the alternative sigma factor, SigX. Our unpublished results indicate ComR and ComS are capable of inducing sigX expression in GAS as well.

These findings provided the first proof of principle that an Rgg protein could respond to a peptide pheromone and led to the question: are all Rgg proteins peptide receptors? Two uncharacterized paralogs, named rgg2 and rgg3, numbered in order to their similarity to RopB, offered an exceptional choice to test this hypothesis, as both rgg genes lie adjacent to small open reading frames encoding short hydrophobic peptides (SHPs, with gene names shp2 and shp3), thus providing a direct target for mutagenesis. Indeed, it turns out that differential transcriptional activity provided by both Rgg2 and Rgg3, as well as by synthetic peptides designed to block peptide signaling or if RopB causes disruption of SHP signaling via other regulatory pathways. Therefore, it appears that the Rgg2/3 circuit promotes biofilm development while a separate Rgg (RopB) prevents or disassembles biofilms. Of course the fact that RopB has served as the oligopeptide permease, Opp. Interestingly, the response to SHPs was greatly enhanced when RopB (Rgg1), the activator of SpeB protease, was deleted. SpeB is known to act upon the extracellular matrix of GAS biofilms and may facilitate cellular dispersion, but it remains to be seen if the protease also interferes with peptide signaling or if RopB causes disruption of SHP signaling via other regulatory pathways. Therefore, it appears that the Rgg2/3 circuit promotes biofilm development.

What function does the Rgg2/3 path-
keep streptococci in a state that is susceptible to antimicrobial drugs, thus improving success in treating recurrent infections. Two attributes of these signaling systems that could substantially contribute to the feasibility in developing therapeutics rest on the basic findings that the peptides are unmodified and linear and are actively imported to the cytoplasm where they interact with the Rgg receptors. Since streptococcal pheromones appear to have a simple linear form, unlike the cyclical pheromones of Staphylococcus, and do not possess modified amino acids seen in many other bioactive bacterial peptides, developing peptide molecules that compete with pheromone-Rgg interactions appears to be a straightforward challenge. Technologies like peptide arrays and phage display provide libraries of molecules that could be screened for abilities to interact, and possibly interfere, with pheromone-Rgg interactions. Second, since peptides are transported into the cytoplasm indiscriminately by an oligopeptide transporter, designed inhibitors, if peptides, would not face a cell-permeability issue that is of concern when designing drugs. In this case, the inhibitory peptide would gain access to the cytoplasm in the same way other peptides are brought into the cell, unbeknownst to serve as a Trojan horse.

Throughout the Firmicute phylum, homologs of Rgg proteins are present in all species of Streptococcus as well as many of the Lactobacillus, Listeria, and Enterococcus families. Interestingly, some isolates of Streptococcus pneumoniae, a species that resides in the nasopharynx along with S. pyogenes, contains nearly identical copies of rgg3 and shp3 genes. The genetic neighborhood surrounding these genes on the S. pneumoniae chromosome, however, is unlike the one nearby rgg2/shp2 in GAS, possibly suggesting that different sets of genes are influenced by SHP3 among the two organisms. Likewise, nearly identical copies of rgg2 and shp2 are found in Streptococcus agalactiae. Recognition that Rgg proteins serve as pheromone receptors not only reveal a new quorum sensing pathway in Gram-positive bacteria, but also provokes questions of whether these bacteria, commonly the most abundant in the human microbiome, take part in interspecies conversations that lead to beneficial or detrimental health outcomes of the host.