

In GAS, we have identified four Rgg regulators (Rgg1-4) and three short peptide pheromones. We believe each has a separate function. The pheromones are encoded by small open reading frames in the chromosome as inactive pro-peptides. Upon secretion and proteolytic processing, they mature to active pheromones that serve as intercellular signals. Before our discovery, little was understood how or if GAS were capable of quorum sensing.

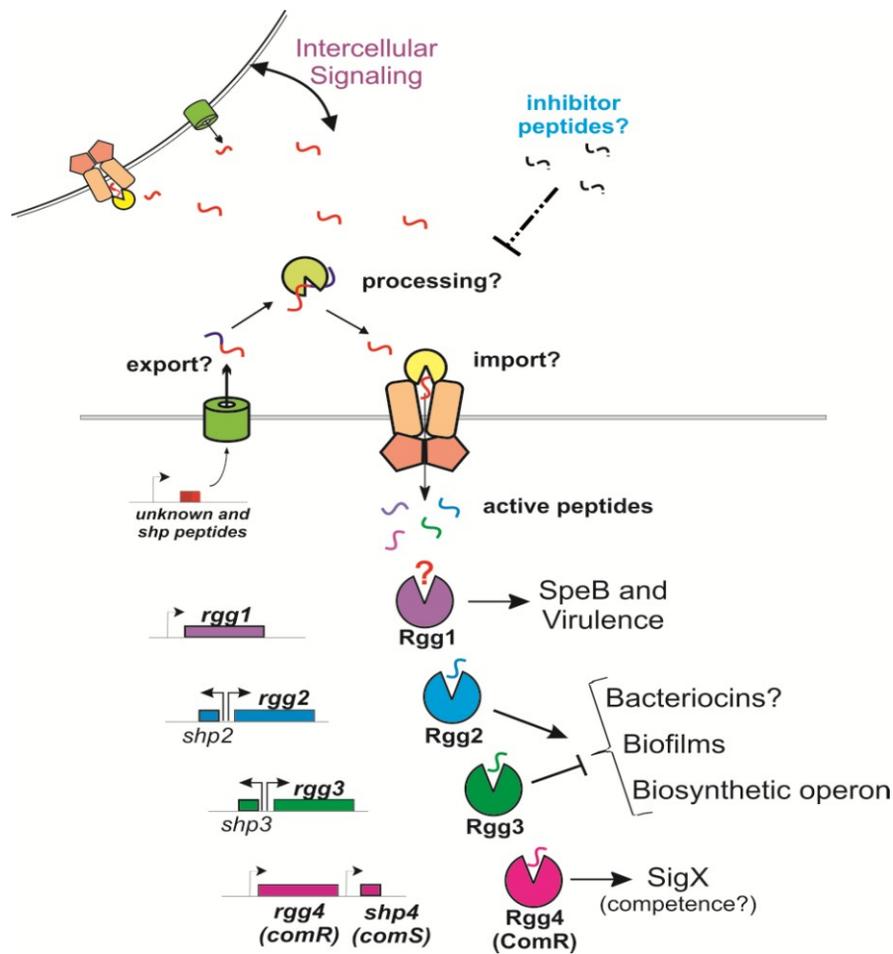


Figure: Pre-peptides are ribosomally produced, secreted by unknown transporters, and processed into mature pheromones. Upon importation by oligopeptide permease, pheromones interact directly with Rgg proteins to control transcriptional activities. Since peptides are actively imported to the cytoplasm, it is feasible to generate peptides that interfere with pheromone-Rgg interactions.

One of the Rggs (Rgg4, renamed ComR) is conserved throughout pyogenic, mutans and bovis species of streptococci. We have determined that a small open reading frame, found adjacent to *comR* in each streptococcal genome, encodes a pro-peptide (ComS) that is secreted from cells. Processing of the propetide to an active form (which we have termed XIP, for *sigX*-inducing peptide) leads to its importation to the cytoplasm through the oligopeptide permease (Opp), where it can directly interact with ComR. The ComR/XIP complex activates transcription of *sigX*, an alternative sigma factor whose function is to activate genes involved in DNA uptake, thus inducing the competent state.

Competence Regulation in Streptococcus mutans

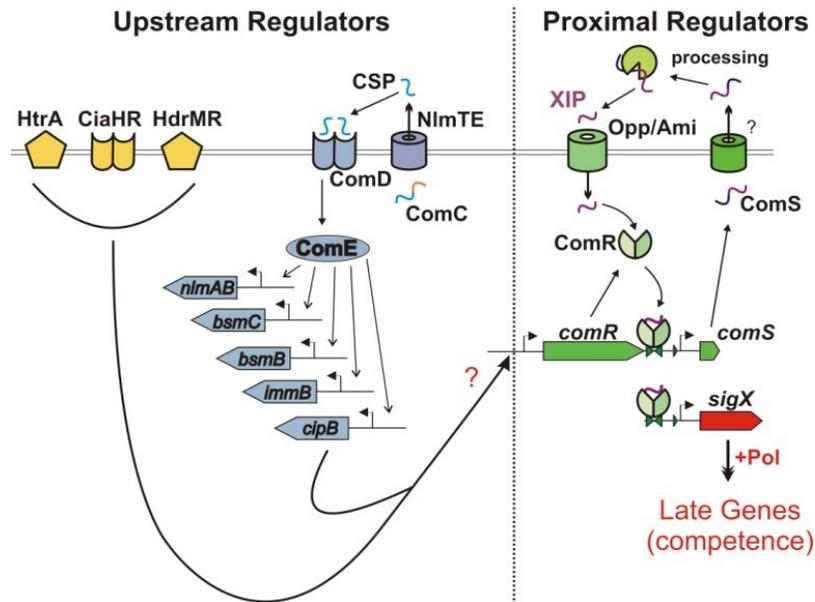


Figure: An Rgg-peptide pair, called ComR-XIP, is found in all sequenced Pyogenic, Bovis and Mutans species of Streptococci. All have the potential to regulate the alternative sigma factor SigX, but whether competence is induced is under investigation. In *Streptococcus mutans*, the development of the competent state requires signaling via ComR-XIP.

Two other Rgg proteins in GAS (Rgg2 and Rgg3) work together to control a set of target genes that are separate from the ComR/ComS pathway. Adjacent to *rgg2* and *rgg3* are small ORFs encoding short hydrophobic peptides (SHPs), whose names, *shp2* and *shp3*, reflect their locations adjacent to each *rgg*. By a mechanism that parallels ComR/ComS, the interaction of SHPs with Rgg2 and Rgg3 results in differential gene regulation. Rgg2 and Rgg3 regulate growth in biofilms, a lifestyle whose purpose for GAS is in early stages of understanding.

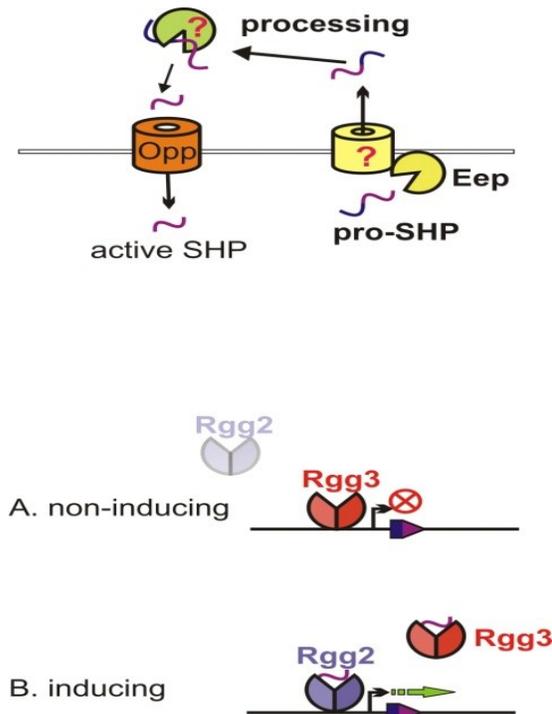


Figure: Rgg2 and Rgg3 proteins respond to Short Hydrophobic Peptides (SHPs) differently. In the absence of SHP (non-inducing conditions) Rgg3 binds target-promoter DNA and represses transcription. When SHPs are present (inducing conditions), Rgg proteins bind to the peptides, causing Rgg3 to release DNA, thus de-repressing transcription of target promoters, whereas Rgg2-SHP complexes have enhanced DNA-binding abilities and function as transcriptional activators. Why GAS relies on two Rgg proteins to control target promoters, and the function of genes controlled by Rgg2/Rgg3 is currently under investigation.

The remaining Rgg of GAS, Rgg1, is a well-established regulator of virulence, but has yet to be implicated in quorum sensing. Identification of the Rgg1-dependent pheromone is a priority in our lab. Taken together, we have proven the concept that Rgg proteins are central to a previously unidentified quorum sensing paradigm. We expect this paradigm to be true for all other Gram-positive bacteria that contain Rgg proteins (nearly all sequenced genomes from these bacteria contain *rgg* genes).

The major questions we are currently addressing are to: (1) Identify and define the active structures for each GAS pheromone. This requires an understanding how the pheromones are secreted and processed during maturation. To do this, we are taking a genetic approach to identify the components of the pheromone biosynthetic pathway, by identifying mutants that are unable to generate pheromones. We are also using mass spectrometry to characterize peptides produced by GAS. (2) Define the interactions between pheromones and Rgg proteins, and the interaction of *apo*- and *holo*-Rgg with DNA at the promoters of target genes. We are currently purifying Rgg proteins with expectations that we will be able to study peptide-Rgg-DNA interactions using a variety of technologies, including phage-display, electrophoretic mobility shift, crystallography, surface plasmon resonance, and fluorescence polarization anisotropy. (3) Identify the target genes of each Rgg regulator and determine their effects during infection. Using microarray and proteomic approaches, we are uniquely positioned to identify and categorize responses to individual and combinatorial application of pheromone to cells. We will also develop mutant strains of GAS that are unresponsive to pheromone, or that have constitutive Rgg activity, and test their effects in animal models of infection. (4) Identify small molecule inhibitors of Rgg signaling. Using phage display technology, we expect to identify short peptides that competitively inhibit response to endogenous pheromones. Alternatively, we wish to screen UIC's small molecule library using the High Throughput Screening (HTS) facility to identify molecules that specifically interfere with Rgg signaling. Our whole-cell luminescence reporter assays are ideal for HTS, by simply monitoring luminescence activity in response to pheromone. Identification of inhibitors will provide us a foundation on which to develop therapeutics that interfere with bacterial gene regulation.

It has been stated the most important discovery of the 20th Century was penicillin; unfortunately, the effectiveness of every antibiotic is vanishing as resistant bacteria emerge. Our alternative idea is to develop therapeutics that control behaviors of bacteria--to manipulate expression of toxins and virulence factors without bacterial death. Our studies will determine how streptococci use cell-cell communication to coordinate virulence factors, and we will develop mechanisms to interfere with this coordination. We foresee application of our studies not only influencing bacterial pathogenesis, but also advancing technologies that develop probiotic (beneficial) therapies, and those that use bacteria for industrial applications, such as producing medicines and alternative energy sources.