Intracellular bistable signaling in *Streptococcus mutans* competence regulation

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The regulation of genetic competence in the bacterium *Streptococcus mutans* is sensitive to quorum sensing signals, environmental factors, and stochastic gene expression. The master competence regulator ComX is directly regulated by the *comRS* system, which is viewed as a novel type of Gram-positive quorum sensing system based on a diffusible signal derived from ComS. However it has also been posited that intracellular autofeedback in *comS* is a source of bistability in competence. We combine experiments and modeling to show that the *comRS* mechanism provides both intercellular and intracellular signaling, so that its quorum sensing is enhanced by positive feedback amplification.

***Keywords* – stochasticity, microfluidics, quorum sensing**

I. Background

Bistability in gene expression, which causes a population of cells to form two subpopulations of different phenotype, is often a consequence of positive transcriptional feedback. In the bacterial pathogen *Streptococcus mutans*, entry into the state of genetic competence (transformability) has a bimodal character under certain environmental conditions. Stimulation by exogenous CSP (the 18-residue competence stimulating peptide) causes a subpopulation of cells to activate expression of *comX* (also called *sigX*), which encodes a master regulator for genetic transformability (1). Alternativ[1]ely, homogenous or unimodal expression of *comX* can be induced by providing a different exogenous peptide, XIP (comX/sigX-inducing peptide). The 7-residue XIP is the intercellular signal of a novel Gram positive quorum sensing system known as *comRS*: The XIP peptide, which is derived from ComS, binds with the cytosolic receptor ComR to form a transcriptional activator for both *comX* and *comS*. Mechanisms that process ComS and export it as extracellular XIP remain unknown. Therefore the relation between the bimodal and unimodal modes of *comX* activation, the ComRS system, and intercellular XIP signaling have been unclear.

We have argued that bimodal *comX* expression can be understood as resulting from positive intracellular transcriptional feedback via *comS*: If ComR binds endogenously produced ComS (or XIP) to activate both *comX* and *comS*, then each individual cell can autoactivate (or not) *comX*, depending on its intracellular ComS level (*comX* bimodality). By contrast, extracellular XIP gives unimodal *comX* expression because it readily enters the cell, interacts with ComR, and drives all cells in a population to express *comX* at roughly similar levels (2,3).

Here we have combined microfluidic and single cell methods with quantitative modeling to test the relationship between intercellular XIP signaling, *comS*, and *comX* activation. We used signaling mutants and reporter strains of *S. mutans* in co-cultures and under microfluidic flow to test the efficacy of intercellular signaling and its dependence on environment and on the *comS* gene.

II. Results

Our data show that possession of the *comS* gene under native control has a distinct effect on the behavior of *comX*, over a range of different environmental conditions and modes of circuit stimulation. Although extracellular XIP can stimulate *comX*, the presence of *comS* boosts the *comX* response of individual cells to the XIP signal. Further, deletion of *comS* impairs *comX* response in ways that cannot be fully corrected either by addition of exogenous XIP or by overexpression of ComS from a plasmid. These data indicate that the cell's own control of endogenous ComS synthesis always plays a role in the control of *comX*. Our data also show that neither export nor import of extracellular XIP necessarily accompanies *comX* activation, so that intracellular signaling can be more important than *comRS* quorum sensing in competence.

III. CONCLUSIONS

Our data show that a cell's own *comS* generates an intracellular feedback signal that boosts quorum sensing response in the competence circuit of *S. mutans*. The dual role of ComS as an internal signal and a quorum signal provides positive feedback amplification (4) in the competence pathway.

References

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