Microfluidic Study of a Stochastic Genetic Circuit Carefully Modulated by Environmental Inputs

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***Abstract* — Genetic competence in the bacterial pathogen *Streptococcus mutans* is regulated by many environmental inputs, including two quorum-sensing molecules and pH. We are using microfluidics to control the environmental inputs and examine the output of the competence regulatory circuit at the single cell level. Our studies reveal that the composition and pH of the growth medium are key inputs; different combinations of these inputs can tune an internal feedback circuit to create a stochastic response that ranges from no response to bimodal/unimodal responses. Our findings suggest how cells incorporate environmental inputs to determine their behavior.**

***Keywords* — Stochastic genetic circuit, quorum sensing, bimodal, unimodal, positive feedback, microfluidics, *Streptococcus mutans*, competence, environmental inputs, pH.**

I. Introduction

Genetic competence is a transient physiological state during which a bacterial cell can internalize exogenous DNA from its environment. In the oral pathogen *Streptococcus mutans*, competence is important not only because it contributes to genetic diversity, but also because its regulation is closely intertwined with virulence-related behaviors [1, 2]. Early stages of *S. mutans* competence are governed in part through two secreted quorum-sensing signal molecules: CSP and XIP [1-3]. Interestingly, the activity of these signal molecules depends on environmental cues, including pH and medium composition, through mechanisms that are not well understood [4-6].

Using planktonic or biofilm cultures it is difficult to unravel the separate regulatory effects of signal molecules and environmental conditions, because *S. mutans* continuously modifies its environment during growth, through acid production, generation of other signal molecules, etc. To precisely define environment and inputs to the competence system, we employed a two-layer microfluidic mixer device. Activation of competence genes was then studied at the single cell level using fluorescent protein reporters. We found that the composition and pH of the growth medium modulate activity of the ComR/ComS positive feedback system that controls *S. mutans* competence. This leads to diverse patterns of expression of key competence genes.

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II. Results

A. Bimodal response to CSP vs. Unimodal response to XIP

The two quorum signals CSP and XIP stimulate the competence genes in a very different manner: bimodally vs. unimodally, respectively. The data suggest that the bimodal activation by CSP is due to auto-activation of the ComR/ComS feedback loop.

B. Composition of medium determines which quorum sensing molecules are active

CSP stimulates competence in peptide-rich medium, but not in peptide-free medium. On the other hand, XIP stimulates competence in peptide-free medium, but not in peptide-rich medium. This effect of medium can be understood in terms of control of feedback strength.

C. pH determines onset/offset of the competence phase

Regardless of the growth stage of the cells, both CSP and XIP elicit a response only if the pH of medium is nearly neutral. Experiments and simulation suggest that extreme pH acts to hinder the positive feedback circuit.

III. Conclusion

Although *S. mutans* competence regulation is sensitive to many environmental inputs, its complex behavior can be understood in terms of simple tuning of parameters in the ComR/ComS positive feedback circuit. Our findings propose how *S. mutans* manages competence regulation and other virulence behaviors as it copes with environmental stresses in the human mouth.

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