Propagation of signals from the transcription factor MarA to downstream genes

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***Short Abstract* — Bacteria live in rapidly changing, uncertain environments. As such, they need to take advantage of complex control systems to properly anticipate and respond to sudden, potentially dire changes. Here, we examine how signals in the transcription factor MarA are processed by downstream targets, altering the dynamics and distributions of gene expression. These data have implications for understanding bacterial stress response.**

***Keywords* — Signal Processing, Bacteria, Stress-Response, Optogenetics**

I. Purpose

THE purpose of this study is to understand how dynamic expression of MarA impacts the downstream genes it regulates. We selected this transcription factor as it has been shown to upregulate over 40 downstream genes and play a critical role in the general stress-response phenotype [1]. Previous computational results suggest that it may have interesting pulsatile expression dynamics as well [2]. The importance of MarA from a biological point of view, combined with potentially interesting endogenous dynamics make MarA an ideal target for our work. Our goal is to understand how a given MarA signal is interpreted and processed by a number of downstream promoters, providing insight into how general stress response is coordinated, or alternatively how each gene response is specialized through differential processing of a common input signal. In order to analyze the signal processing characteristics of each promoter, we are engineering systems to control the dynamic levels of MarA and simultaneously measure the output activity of downstream targets. First, we are developing constructs that place MarA under the optogenetic control of the CcaS/CcaR light controllable plasmids [2], as well as the IPTG inducible lacUV5 promoter. The combination of these two approaches gives us precise control over the levels of MarA in terms of both single-cell dynamics and population-wide distributions. To measure the output, we are constructing a number of transcriptional fusion reporter plasmids for downstream genes [3], which will allow us to measure the modulation of downstream promoter activity as a function of MarA concentration. By measuring the fluorescence with single-cell, time-lapse microscopy and flow-cytometry, we are able to generate both dynamics and distributions of downstream promoter activity levels. These data will allow us to develop possible models for how promoter activity shapes the phenotype distribution of the population in response to a signal from MarA.

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II. Results

Our preliminary data show correlation between MarA levels and downstream targets given a step input in MarA. By collecting data at the single-cell level we are measuring how MarA alters the shape of downstream protein distributions across populations and the dynamic concentrations within individual cells. Preliminary dynamic data suggest that the downstream gene *micF* pulses and that levels of MarA modulate the frequency and amplitude of these pulses. Initial flow-cytometry data show that the shape of population distributions in two of the transcriptional reporters responds differently to varying levels of MarA, indicating the potential for differential interpretation of MarA signals at the population level

III. Conclusion

The above data represents the first step in developing an understanding of how MarA can dynamically facilitate a multifaceted stress response. Having a distinct input/output relationship for several downstream genes will raise interesting questions about how unified the role of MarA is. Can a single MarA signal produce a multitude of promoter activities – or are all of the downstream promoters coordinated? Furthermore, do endogenous MarA dynamics have advantages over those generated using the synthetic optogenetic system? Additional experiments studying the greater *marRAB* operon will allow us to contrast naturally occurring MarA levels to those present in our synthetic systems.

References

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