Dynamic Interrogation of the *Bacillus Subtilis* Sporulation Network using an Engineered Light-Switchable Two-Component System

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Short Abstract — **Under starvation, *Bacillus subtilis* differentiates into metabolically-inert spores. This process is triggered by master response regulator Spo0A via activity pulses of increasing amplitude. Simple overexpression of Spo0A does not result in successful spore formation, however, suggesting that Spo0A dynamics are important. To study this further, we develop the first *B. subtilis* optogenetic system based on a cyanobacterial Two-Component System (TCS), and demonstrate its usage in generating time-varying gene expression signals. We use it to express Spo0A under different dynamics and evaluate its effects on sporulation. This novel approach helps us understand the significance of Spo0A dynamics at an unprecedented level.**

Keywords — **Optogenetics, Sporulation, Synthetic Biology, Bacillus subtilis, Two-Component Systems, Dynamical Systems.**

I. INTRODUCTION

COMPLEX, time-varying, heterogeneous gene regulation has been found to occur in several stages of *B. subtilis* endospore formation. Initiation of this process proceeds via increasingly larger pulses of activity of the master response regulator Spo0A [1]. Interestingly, triggering sporulation via overexpression of a constitutively active Spo0A mutant is not possible [2]. It has been proposed that fast Spo0A activation can lead to early repression of essential sporulation genes, thus leading to non-viable spore formation [3]. To fully understand how Spo0A dynamics affect downstream sporulation processes, the ability to manipulate Spo0A dynamics is desirable.

CcaS/R is a photoreversible TCS from *Synechocystis PCC6803*, in which expression of an output gene is controlled by green and red light. We have previously transferred this system into *E. coli*, described its dynamic response using an ODE model, and showed that arbitrary gene expression dynamics can be precisely generated by using the model to calculate a corresponding green light intensity signal [4]. The availability of a similar system in *B. subtilis* would be useful in studying not only sporulation, but other dynamic and heterogeneous processes such as the general stress response and colony formation.

II. RESULTS

Here, we transport the CcaS/R TCS to *B. subtilis*, demonstrate control of gene expression dynamics, and use it to investigate the effect of different Spo0A dynamics on different elements of the sporulation network.

A. *Engineering an optogenetic tool for B. subtilis*

We divide the CcaS/R system into three "modules" – chromosomally integrated DNA sequences encoding the sensor kinase CcaS, the response regulator CcaR, and the enzymes that produce CcaS' chromophore – and separately optimize expression and performance of each one. The resulting system can regulate gene expression in response to light with a 70-fold range. We measure the steady state and dynamic responses, and show that they can be described using our previously developed model [5]. Finally, we use this system to generate complex gene expression dynamics.

B. *Dynamic Interrogation of the Sporulation Network*

We use the CcaS/R system to induce expression of Spo0A under different dynamics, including ramps of different slopes and pulses. We measure the effects of these on the sporulation network, including expression of downstream genes and sporulation efficiency, and identify the mechanism by which Spo0A dynamics are decoded by the network.

III. CONCLUSION

Introducing time-varying perturbations to the sporulation network allows us to elucidate the significance of Spo0A dynamics. Our novel approach based on optogenetics can be directly applied to other *B. subtilis* processes as well.

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