Optogenetic investigation of *B. subtilis* sporulation network at the single cell level

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Upon starvation, *Bacillus subtilis* cells will employ a sporulation mechanism to form spores that endure harsh environments. This mechanism is operated by a phosphorelay network, culminating in the activation of a master regulator, Spo0A. Sporulating cells display pulses of Spo0A that gradually increase in amplitude, but the purpose of these specific dynamics is unknown. Using recently developed optogenetic methods from our lab, we apply temporally varied Spo0A inputs to a population of cells. We assess the phenotypic response at the single cell level to determine the role of Spo0A pulsing in sporulation.

Keywords: Sporulation, gene dynamics, optogenetics

I. Introduction

Cellular decision making, the process that governs whether or not a cell will differentiate, is governed by the dynamics of gene networks that process internal and external information [1]. One example of cellular decision making is the sporulation process in *Bacillus subtilis*, in which DNA is preserved in a stress-resistant spore until a favorable environment is found. Under limited nutrient conditions, a phosphorelay network gradually activates a “master regulator”, Spo0A [2]. Sporulation can be artificially triggered by chemical induction of phosphorelay components [2], but very high induction levels decrease sporulation efficiency [3]. Further studies have shown that the chromosomal arrangement of the components in the phosphorelay network results in pulses of Spo0A activation [4]. The amplitude and period of this pulsing have been shown to increase in response to decreases in cellular growth rate (during starvation, for example) [5]. One of the major questions surrounding the sporulation network is if the pulsing dynamics of activated Spo0A are necessary for sporulation or not. The purpose of this work is to probe the sporulation network with synthetically induced Spo0A dynamics to determine which temporal features are required to induce sporulation at the single cell level.

Recently, our lab has developed a model to predict the translational output of a green and red light controlled two component system (TCS). We use the model to achieve a wide variety of synthetic protein dynamics by calculating the light input signal needed for the desired output [6]. We have ported the TCS into *B. subtilis*, allowing us to program custom Spo0A dynamics. The TCS acts as a “biological function generator”, meaning that the gene network is investigated by applying various Spo0A input signals and assessing the overall phenotypic result or specific gene output via fluorescent reporters.

II. Results

A. Single-cell characterization of optogenetic system

We examine the previously characterized optogenetic system again in singular *B. subtilis* cells. The output of the system, measured on a single cell basis, determines the error between individual cell expression and the population mean, which correctly matches the programmed signal.

B. Determining the necessity of Spo0A pulsing for successful sporulation

We are studying the sporulation efficiency for static, ramped, and pulsed Spo0A inputs. In doing so, we determine if linear inputs are sufficient for sporulation, or if pulsing is required. The sporulation failures, such as forespore formation without separation versus no forespore formation at all, provide important information about the extent that each type of signal can activate the network.

III. Conclusion

By studying the output of the optogenetic system at the single cell level, we gain a better understanding of what determines a complete or incomplete sporulation response. This investigation answers the question of which dynamic features of Spo0A input are actually required to induce sporulation and why.

REFERENCES


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