Mechanism of sporulation decision in *B. subtilis*

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Short Abstract — **Starving *B. subtilis* cells execute a gene-expression program resulting in formation of stress-resistant spores. But the mechanism that cells use to process environmental information and make this crucial cell-fate decision is still not understood. Using a combination of mathematical modeling and single-cell experiments with both wild-type and synthetic strains, we identified the ultrasensitive mechanism underlying the sporulation decision. Further we showed that cells defer the final sporulation decision to time-average environmental cues.**

I. Introduction

IN response to nutrient deprivation *B. subtilis* cells stop growing and differentiate into stress-resistant spores [1]. Initiation of sporulation is under the control of a phosphorelay that transfer phosphoryl groups from the kinases (KinA-E) to a master transcription regulator Spo0A through two intermediate phospho-transferases [2]. The phosphate flux from KinA to Spo0A is regulated both post-translationally and by multiple feedback loops. The phosphorelay serves as the integration point for a wide variety of environmental signals that are encoded into the Spo0A~P concentration and the decision to sporulate is thought to be based upon whether this concentration exceeds a putative threshold [3,4].

Extensive cell-to-cell variability in starvation conditions has prevented the identification of any robust Spo0A~P threshold. To circumvent this problem, we used a synthetic biology approach and artificially activated Spo0A by inducing the expression of the major kinase KinA in conditions of low extrinsic variability during the log-phase. By tracking the changes of downstream gene expression and eventual cell-fate with increasing Spo0A~P we have identified the decision point in the sporulation program.

II. Results

We externally induced KinA expression in a synthetic strain to show that the fraction of sporulating cells increases ultrasensitively around a threshold level of this kinase. We used a mathematical model to predict how the sporulation network achieves this ultrasensitivity of response to KinA and used single-cell fluorescence microscopy to verify our results.

A. Sporulation decision in a synthetic strain

We found that a model of the phosphorelay based on parameters inferred from experimental results, by itself, could not explain the ultrasensitivity of the cell-fate response despite the presence of multiple positive feedback loops. On the other hand, we predicted that a cascade of coherent AND-type feed-forward loops downstream of Spo0A~P, that is involved in the activation of a compartment-specific sigma factor σ^E, is responsible for the ultrasensitivity of the sporulation network response.

Single-cell fluorescence microscopy confirmed our predictions that σ^E activation is highly ultrasensitive while the phosphorelay response is not ultrasensitive. Moreover we found that only cell-fate predictions based on σ^E activation can explain the ultrasensitive changes in fraction of sporulating cells.

B. Sporulation decisions in wild-type B. subtilis

Similar experiments in wild-type cells showed that while many cells activate Spo0A~P and form asymmetric septa only cells that activate σ^E complete sporulation, thus confirming that σ^E is the cell-fate decision point. We also used mathematical modeling to show that in starvation conditions, slow-down of growth in combination with positive feedback in the phosphorelay leads to a gradual accumulation of KinA and the eventual decision to sporulate.

III. Conclusion

With a combination of single-cell experiments and mathematical modeling we identify the sporulation cell-fate decision point and show that it lies downstream of Spo0A~P. We showed that cells initiate morphological changes even before reaching this decision point. Altogether these results suggest that cells choose to maximally defer their commitment and use a time-averaging strategy to minimize the effects of stochastic fluctuations and make better decisions under uncertain environmental conditions.

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