Slowdown of cell growth acts as the signal triggering cellular differentiation

Jatin Narula1*, Anna Kuchina2*, Masaya Fujita3, Gürol M. Süel2 and Oleg A. Igoshin1

Short Abstract — Gene regulatory networks controlling cellular differentiation need to sense changes in environmental conditions and coordinate gene expression with cell-division and DNA-replication cycles. Here we show how the Bacillus subtilis sporulation network achieves this coordination via pulsatile activation of master-regulator Spo0A in response to slowdown of cell growth.

Keywords — cell-fate decision, sporulation, differentiation.

I. PURPOSE

STARVING B. subtilis cells cease vegetative growth and execute a multistage gene-expression program resulting in formation of stress-resistant spores [1]. This program is triggered by activation the sporulation master regulator, Spo0A (0A) that controls the expression of hundreds of genes [2]. In a recent study we showed that a threshold level of 0A activity commits cells to sporulation [3]. However it remains unclear how the diverse set of environmental and metabolic signals can be simultaneously detected and properly encoded into 0A activity. Recent studies of sporulation raised further questions by showing that cells respond to starvation by producing a pulse of 0A activity every cell cycle [4,5]. Here we use a combination of mathematical modeling, single-cell microscopy and synthetic biology to uncover the mechanisms underlying starvation sensing, the pulsatile response of the sporulation phosphorelay and delayed sporulation commitment.

II. RESULTS

Based on the results of a mathematical model of the phosphorelay, we proposed that the chromosomal positions of two genes controlling the activation of the sporulation master regulator 0A, kinA and 0F, play the pivotal role in pulsed response of 0A activity. Since 0F located close to the origin and kinA near the terminus of the wildtype B. subtilis chromosome, each DNA replication cycle leads to a transient imbalance in their gene dosages. This imbalance is amplified by feedback loops in the sporulation network producing a pulse of 0A activity once per cell-cycle. To test this prediction we have engineered strain, Trans-0F to eliminate the transient gene copy imbalance. In this strain, 0F is translocated to position close to the chromosome terminus without changing its expression level. Single-cell time-lapse microscopy showed that 0A activity pulsing was abolished in the Trans-0F strain and cells were unable to sporulate. Furthermore we found that the addition of an IPTG-inducible copy of 0F close to the chromosome origin in Trans-0F, recovers the 0A activity pulsing and sporulation.

Our model also predicts that as growth slows down over multiple cell-cycles during starvation, pulses of 0A activity increase in amplitude. Thus the cell growth rate can act as the starvation signal and a threshold of 0A activity acts as a growth-rate threshold. Analysis of single-cell data confirms the predicted relationship between cell growth, 0A activity and sporulation. The data indicates that heterogeneity in the sporulation timing of wild-type cells can be explained by heterogeneity in their growth rates. Moreover the gradual decrease in growth rate during starvation defers sporulation for multiple cell-cycles allowing each cell to maximize the number of spores it can produce. This deferral can be eliminated if 0A activity pulses are triggered only after cells sufficiently slowdown their growth.

III. CONCLUSIONS

Thus our results show that gene regulatory networks can be directly coupled to the cell cycle through proper chromosomal arrangement of network genes. Moreover using the cell growth rate as the starvation signal allows the cells to automatically integrate nutritional information without specific metabolite sensing. These simple mechanistic principles may be applicable to wide variety of stress-response and cell-differentiation programs.

REFERENCES


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*Equal Contributions

1Department of Bioengineering, Rice University. E-mails: in3@rice.edu, igoshin@rice.edu

2Division of Biological Sciences, UCSD. E-mails: akuchina@ucsd.edu, gsuel@ucsd.edu

3Department of Biology and Biochemistry, University of Houston, Email: mfujita@uh.edu

gsuel@ucsd.edu

igoshin@rice.edu

Email: mfujita@uh.edu