Characterizing gene expression kinetics in nutrient-starved *E.coli* cells

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***Short Abstract* — Bacteria are frequently exposed to starvation in their natural environments. Expression of relevant genes during early starvation is essential for their long-term starvation survival or resumption of growth when other nutrients become available. However, it is not clear how starvation limits cells' ability to make gene products and how cells overcome such limitation. Here, using a synthetic biology approach and FISH (fluorescence in situ hybridization), we quantitatively characterized the process of gene expression in carbon starved cells and nitrogen starved cells. Our findings reveal that the different types of starvation limit gene expression differently and cells employ a distinct strategy to overcome the limitation.**

***Keywords* — Starvation response, fluorescence in situ hybridization, transcription and translation coupling, ppGpp**

I. Introduction

MOST bacteria experience nutrient poor conditions for most of their lifetime. Starvation imposes significant limitation on various processes essential for growth and survival in bacteria. Expression of relevant genes under starvation conditions, especially during early starvation, is critical for long-term starvation survival [1] or for rapid switching to other nutrient sources if such nutrient sources are available, e.g., during diauxic shift. However, starvation affects transcriptional and translational machineries, as well as availability of substrates needed to make gene products. As such, cells' ability to express these genes is expected to be significantly limited. Currently, however, it is not clear how starvation limits gene expression and how cells overcome such limitation. In this work, we precisely controlled transcriptional activation and characterized the kinetics of gene expression in carbon-starved cells and nitrogen-starved cells.

II. Approach

To precisely control transcriptional activation, we designed a synthetic construct in which the synthetic promoter drives the expression of a *lacZ* reporter gene. For precise measurement of the low lacZ mRNA levels produced, Fluorescence in situ hybridization, (FISH) was used. Concomitant changes in lacZ protein expression were quantified using a standard β-galactosidase assay.

III. Results and Discussion

We observe that carbon starvation and nitrogen starvation have different effects on the kinetics of gene expression. Nitrogen starvation imposes strong limitation on translation, reducing the protein synthesis rate (per mRNA) as well as the speed of translation by ribosomes (i.e., polypeptide elongation speed). If unchecked, such a reduction would expose naked regions of mRNA, which is known to result in premature termination of transcription and have detrimental effects on gene expression [2]. We find that, cells avoid such effects by slowing down the speed of transcription to match the speed of translation through the stringent response alarmone, ppGpp. By contrast, carbon starvation imposes strong limitation on transcription, reducing mRNA synthesis rate as well as the speed of transcription. In this case, ppGpp is not needed to match the speed of transcription and translation. Our findings show that different types of starvation limit gene expression differently and distinct strategies are employed to overcome such limitation.

References

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