Indian Institute of Science
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BSSE Annual Work Presentation

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Heterogeneity in arabinose operon gene expression in bacteria

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ABSTRACT

The arabinose inducible pBAD series of bacterial vectors offers a tuneable and tightly regulated system for gene expression, making them useful for the study of effect of gene products on cell physiology [1]. At the population level, expression from this promoter is graded, with medium levels of expression at intermediate levels of arabinose. However, some studies have shown that the arabinose expression vectors have an all-or-none response at the single cell level [2]. High gene expression noise, a measure of cellular heterogeneity, is particularly pronounced in promoters under low inducer levels and might further be amplified by the architecture of the gene network motifs. Previously, a bimodal distribution of fast degrading version of (fd)GFP expression cloned under the pBAD promoter at intermediate inducer levels has been reported [3]. Interestingly, bulk level mRNA quantification showed similar transcript levels between GFP-positive and GFP-negative cells, possibly indicating post transcriptional regulation of gene expression in the cells. We employed a RNA quantification technique, RNA-FISH, to determine whether the observed protein heterogeneity indeed stems from transcriptional heterogeneity at the single cell level. In this technique, GFP mRNA are labelled with multiple Cy5 tagged probes. Fluorescence microscopy and subsequent processing of the micrographs is done to determine the RNA count per cell. Simultaneous single cell GFP imaging is also done to capture protein level heterogeneity. Combined with live cell studies to capture the dynamics of protein production, we aim to dissect the origin and characteristics of heterogeneous gene expression observed in the arabinose inducible pBAD vectors.

References:

1. Guzman LM, Belin D, Carson MJ, Beckwith J (1995) Tight regulation, modulation, and high-level expression by vectors containing the arabinose PBAD promoter. J Bacteriol 177: 4121-4130

2. Siegele DA, Hu JC (1997) Gene expression from plasmids containing the araBAD promoter at subsaturating inducer concentrations represents mixed populations. Proc Natl Acad Sci U S A 94: 8168-8172

3. Chetana Baliga, Thesis chapter: 'Addressing the issue of heterogeneity in the arabinose inducible PBAD expression system.'