Expression Variability from a Tet-inducible Positive Feedback Network in Mammalian Cells

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***Short Abstract* — The stochastic nature of gene expression can result in significant cell-to-cell variability in protein levels. Here, we examine gene expression variability from a synthetic gene network in a mammalian system. We have constructed a synthetic mammalian positive feedback circuit in which a tetracycline-regulated transactivator (rtTA) induces its own synthesis in the presence of tetracycline. We analyze interclonal and intraclonal variability in gene expression from several clonal cell lines transduced with the positive feedback network. Finally, we develop a stochastic model of the engineered circuit to investigate the origin of variability in expression from the engineered mammalian gene network.**

***Keywords* — gene regulation, noise, synthetic biology**

I. Introduction

Noise in gene expression can cause variability in genetically identical cell populations that have been exposed to the same environment and have the same history [1]. Several studies have investigated the origins and consequences of gene expression noise in model organisms such as the prokaryote *E. coli* and the eukaryote *S. cerevisiae* [2-4]. Simple synthetic gene regulatory networks have been utilized to enable a quantitative analysis of gene expression noise in *E. coli* [5] and *S. cerevisiae* [6]. The recent development of inducible mammalian transgene control systems has allowed for the construction of synthetic gene circuits in mammalian systems [7]. Thus, it is now possible to design synthetic mammalian gene networks that can facilitate a quantitative analysis of gene expression variability in mammalian systems. For example, a recent study has utilized a tetracycline-regulatable control system to investigate cell-to-cell variation in gene expression in mammalian cells by quantifying mRNA levels in individual cells [8]

Here, we construct a synthetic tetracycline-regulatable positive feedback network in a mammalian system and perform quantitative single-cell gene expression assays to examine intraclonal and interclonal variability for several mammalian cell lines containing the engineered gene network. We develop a stochastic model of the positive feedback circuit to investigate potential sources of the experimentally observed gene expression variability and to generate predictions that can be tested with further quantitative experimentation.

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II. Methods

We have constructed a synthetic gene network consisting of an autoregulatory vector which contains the rtTA gene downstream of the O7-CMVm tetracycline-inducible promoter and a reporter vector [9] containing the sequence for GFP downstream of the O7-CMVm promoter.

We used retroviral infections to introduce the autoregulatory vector and the GFP reporter vector into mouse embryonic fibroblasts (3T3 cells). Following selection, infected cells were cultured in doxycycline and cells with induced GFP expression levels were single-cell sorted and expanded into clonal lines. Flow cytometry was used to measure GFP expression levels from inducible cell lines cultured in a range of doxycycline concentrations.

We have developed a stochastic model of the positive feedback circuit which uses the Gillespie algorithm [10] to simulate the behavior of the biochemical reactions involved in the synthetic positive feedback network.

III. Conclusion

We conclude with testable model predictions for the behavior of the engineered mammalian network.

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