Design Principles of Synthetic Gene Circuits Based on the TetR Family of Regulators

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***Short Abstract* — Gene expression systems using the TetR, tTA and rtTA regulators are useful for studying the effects of gene expression on phenotype. Because these regulators share protein and inducer-binding domain sequences, insights gleaned from the behavior of one can be immediately applied to others. We constructed a template model to describe the dose response of these three gene expression systems with and without autoregulatory feedback. Models derived from this template are being used to predict, optimize, and explain population-wide expression characteristics due to these gene expression systems.**

***Keywords* — synthetic biology, TetR, rtTA, tTA, autoregulation, feedback, dose-response.**

I. Introduction

SYNTHETIC gene circuits have shown a great deal of promise towards engineering cellular behaviors that have generally been associated with electronic circuits, including oscillatory expression [1], toggle switch [2], and linear response to the concentration of an extracellular inducer [3]. Feedback regulation has been instrumental in creating these circuits, indicating its importance in synthetic gene network design.

Gene expression systems based on the TetR family of regulators (including the activators tTA and rtTA) are a particular class of synthetic gene circuits that have been used to control gene expression in a continuous and reversible manner through the concentration of an extracellular inducer molecule. Because these regulators share DNA and inducer binding domains [4], insights into the behaviors of one can immediately be applied to others. Currently it is not well understood how feedback regulation affects the dose-response and noise characteristics of gene expression systems, and whether feedback regulation can be used to improve the performance of synthetic gene circuits.

II. Methods

We use a set of deterministic and stochastic models derived from a common template model with five molecule species (intra- and extracellular inducer, inducer-bound and unbound regulator protein, and a fluorescent reporter protein) to gain insights into the dynamics of gene expression systems based on the TetR family of regulators with and without feedback. Feedback is modeled by making the rate of regulator production dependent on regulator concentration according to a Hill function. Inducer species diffuse in and out of cells and bind to unbound regulator molecules. The active form of the regulator controls reporter synthesis. All proteins in the model degrade at a constant rate. Inducer influx and outflux into the cell play significant roles by controlling the amount of free inducer available for regulator binding, which can result in hypersensitive dose response characteristics.

Using biologically reasonable parameters, our models provide explanations for experimentally observed gene expression characteristics, such as linear dose-response, delayed induction, and bistability even at arbitrarily high levels of induction. For TetR-based expression systems, we have correctly predicted that negative feedback and identical promoters result in a nearly linear dose response, while non-identical promoters will have a curved dose response. For rtTA-based positive feedback systems, we found that bistability can occur even at extremely high levels of induction. These results are being used to design and optimize novel gene expression systems for improving the precision of gene expression control.

III. Conclusions

We have developed a set of mathematical and computational models for describing and explaining the dose response and noise characteristics of gene expression systems based on the TetR family of regulators. Gene expression systems with feedback and the corresponding models will be useful for designing future gene circuits and optimizing controlled cell behavior.

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