Population-level control of gene expression

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***Short Abstract* — A current challenge for synthetic biology is to control cell population behavior, which requires controlling gene expression at the cell population level. We focused on controlling independently three "demographic" characteristics of gene expression: the mean, the noise and the non-genetic memory. We developed two chromosomally integrated synthetic gene circuits in yeast that conferred drastically different non-genetic memory to two cell populations while their means and noises remained comparable. Using these gene constructs we found that the genomic sequence and the environment jointly control population-level aspects of gene expression through two interlinked mechanisms, operating at different scales.**

***Keywords* — non-genetic, memory, noise, synthetic biology, gene circuits, selection.**

I. Introduction

GENE expression is the biological process that drives messenger RNA and protein synthesis, and is thought to establish the genotype-phenotype connection in an environment-dependent manner. However, over the last decades it has become increasingly accepted that cells with identical genomes exposed to the same environment can differ dramatically in their gene expression and phenotypes. Therefore, there is an emerging synthetic biology challenge to control cell population rather than single cell behavior. This requires the development of synthetic gene circuits to control various population-level ("demographic") aspects of gene expression.

We focused on independently controlling three relatively well-studied population-level characteristics of gene expression: the *mean*, the *noise* and the *non-genetic memory*. While gene expression noise and mean have been controlled independently before [1,2], and non-genetic memory has been engineered into living cells [3], no current methods exist for decoupling the memory from both the mean and the noise of gene expression.

II. Results

We constructed two chromosomally integrated synthetic gene cascades to decouple non-genetic memory from the noise and the mean. The first, "negative regulatory" (NR) gene circuit employed the TetR transcriptional repressor, to control the expression of the fluorescent reporter - drug resistance gene fusion yEGFP::zeoR. The second, "positive feedback" (PF) gene circuit consisted of a modified version (rtTA-MF) of the rtTA regulator [4] that, upon binding to the inducer ATc, activated its own expression [5] as well the expression as the same target gene yEGFP::zeoR.

By examining the gene expression noise (CV) plotted as a function of the population mean for both the NR and PF constructs, we discovered that their mean-CV characteristics intersected at a well-defined expression level, corresponding to conditions where the means and the noises of the two cell populations were identical, while the memory of the high expression state was drastically higher for the PF construct compared to the NR construct. This allowed us to effectively decouple the memory of target gene expression from its noise and mean.

We developed mathematical models to determine the molecular conditions for bistability. However, a purely molecular model was insufficient to explain the observed numbers of high and low expressors carrying the PF construct. These inconsistencies were resolved by a population-dynamic model that accounted for growth retardation due to activator toxicity at high expression. Moreover, the population-dynamic model predicted population-level gene expression patterns in a selective, drug-containing environment, which were then tested experimentally.

III. Conclusion

Combining mathematical modeling with experimentation we found that engineered genomic sequence (synthetic gene circuits) and the environment jointly control population-level aspects of gene expression through two interlinked mechanisms, operating at different scales: (i) molecular regulatory interactions and (ii) growth rate dependence on various expression states.

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