Cellular Resistance Evolution in Fluctuating Drug Environments

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***Abstract*— Genetically identical cells have different levels of gene expression products due to the randomness in the "noisy" gene expression process. This phenomenon has implications for resistance evolution in constant drug environments. To investigate the effect of gene expression noise on the evolution of resistance in fluctuating drug environments, we experimentally measured growth rates and gene expression mean and noise levels of a drug resistance gene controlled by a synthetic gene circuit. We found the majority of replicates evolved lower mean gene expression levels and higher gene expression noise levels in the fluctuating drug environment compared to a constant drug environment.**

***Index Terms*— antimicrobial resistance, synthetic gene circuit, fluctuating drug environment, gene expression, cellular fitness**

I. Background & Motivation

Life exists and evolves in fluctuating environments. Cells contain gene regulatory networks that enable them to rapidly respond and adapt to changing environmental stressors, such as changes in temperature, antibiotic exposure, or nutrients. Despite this, most studies have focused on gene network function and evolution in constant stress environments [1]. To address this knowledge gap, we experimentally investigate the function, dynamics, and evolution of genetically engineered synthetic gene networks ("circuits')inside of living cells exposed to fluctuating drug conditions.

Genetically identical cells can have different levels of gene expression products (mRNA and protein) due to the randomness inherent in the gene expression process [2]. This variability in gene expression is called gene expression "noise" and can result in a fraction of cells in a genetically identical population having a higher level survival in a given stressful environment [3]. The impact of gene expression noise on growth rate (fitness) has been previously studied using synthetic gene circuits in constant drug environments. For instance, a positive feedback (PF) gene circuit was genetically engineered in the budding yeast *Saccharomyces cerevisiae* to precisely control gene expression mean and noise levels [4]. When these genetically engineered cells are induced (via the chemical inducer Doxycycline) to the high expression state, these cells exhibit high levels of protection against a DNA damaging drug (Zeocine) through the expression of a drug resistance gene (zeoR) controlled by the PF gene circuit. High levels of zeoR expression are not beneficial in all environments, as there is a toxicity cost to the cell when expressing the PF gene circuit. This results in a tradeoff where high expressing cells have lower fitness than low expressing cells in the absence of drug stress [5]. To experimentally investigate the function and evolution of gene circuits in fluctuating drug environments we exposed the PF strains to fluctuating Zeocin conditions and measured growth rates using a cellometer and gene expression levels using a flow cytometer.

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II. Results

After 18 days, the majority of the cells in all conditions of the inducer Doxycycline evolved to have a low expression level of zeoR, despite its expression being beneficial on days where the drug Zeocin was present at a low concentration. Overall, we found that cells evolved towards lower levels zeoR expression levels and that the zeoR expression noise in the fluctuating drug environment was higher than in a constant drug environment.

III. Conclusion

Most replicates evolved to have low zeoR expression which indicated that the Zeocin concentration used was not high enough to create a selective pressure where high zeoR expression would lead to increased cell fitness in a fluctuating drug environment. The higher levels of gene expression noise in the fluctuating environment may have evolved to facilitate adaptation to the fluctuation drug conditions. The next steps are to perform these evolution experiments in higher levels of Zeocin and to obain time series growth rate data using a microplate reader.

References

[1] C. Gonzales, J. Ray, M. Manharat, R. Adams, D. Nevozhay, A. Morozov, and G. Balázsi, "Stress-response balance drives the evolution of a network module and its host genome," *Molecular Systems Biology*, vol. 11, pp. 827–827, 2015.

[2] D. Nevozhay, R. Adams, K. Murphy, K. Josic, and G. Balázsi, "Negative autoregulation linearizes the dose-response and suppresses the heterogeneity of gene expression," *PNAS*, vol. 106, pp. 5123–8, 2009.

[3] D. Fraser and M. Kaern, "A chance at survival: gene expression noise and phenotypic diversification strategies," *Mol Microbiol.*, vol. 71, pp. 1333–40, 2009.

[4] D. Nevozhay, R. Adams, I. E. Van, M. Bennett, and G. Balázsi, "Mapping the environmental fitness landscape of a synthetic gene circuit," *PLOS Computational Biology*, vol. 8, pp. 1–17, 2012.

[5] V. Jan-Willem, W. K. Smits, and O. Kuipers, "Bistability, epigenetics, and bet-hedging in bacteria," *Annu Rev Microbiol.*, vol. 62, pp. 193–210, 2008.