The effect of negative feedback strength on gene expression noise

Dmitry Nevozhay[1], Kevin F. Murphy[2], and Gábor Balázsi[1]

We studied experimentally and computationally the effect of negative feedback regulation strength on gene expression noise in eukaryotic gene regulatory cascades. We found, measured and computationally modeled noise reduction at varying strengths of negative feedback regulation.

***Keywords* — Gene expression noise, negative feedback regulation, gene networks.**

I. Purpose

TRADITIONALLY, changes in DNA sequence were considered to be the sole cause of population diversity. However, many studies have shown that genetically identical cells exposed to the same environment can exhibit distinct phenotypes due to non-genetic inter-cell variation of protein levels (*"gene expression noise"*) [1]. While it was demonstrated that negative feedback regulation reduces noise [2-4], the effect of feedback strength on gene expression noise has not yet been tested experimentally. Our aim was to determine how the strength of negative feedback affects the expression noise of a downstream target gene.

II. Experimental design and results

We created two yeast strains bearing negative regulatory cascades, consisting of separate reporter and regulatory parts. In both of these strains, the reporter yEGFP was placed under the control of a modified GAL1-D12 promoter, which can be repressed by the TetR protein binding to two tetO sites introduced into the promoter [5]. The regulatory part of the cascades consisted of the tetR gene under the control of either the native GAL1 promoter (strain with no feedback, NR) or the modified GAL1-D12 promoter (strain with negative feedback, NF). The repression strength can be controlled by altering the extracellular concentration of anhydrotetracycline (ATc) that prevents TetR from binding to DNA [6]. Both strains were grown in the synthetic drop-out medium with 2% galactose to ensure induction of GAL1 promoters.

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[1]Department of Systems Biology, UT M. D. Anderson Cancer Center, Houston. E-mail: dnevozhay@mdanderson.org, gbalazsi@mdanderson.org

[2]Department of Biomedical Engineering, Center for BioDynamics and Center for Advanced Biotechnology, Boston University, Boston. E-mail: kfmurphy@bu.edu

To establish the relationship between negative feedback strength and noise, we measured the mean and noise (defined as coefficient of variation = standard deviation/mean) of reporter gene expression at different levels of repression, depending on the extracellular ATc concentration (0-500 ng/ml). We observed a different ATc-dependence of reporter mean and noise in the strain with feedback compared to the one with no feedback. Namely, yEGFP expression in the strain with negative feedback was higher even at ATc=0 ng/ml, followed by a gradual (nearly linear) dose-response curve at ATc concentrations between 10 and 100 ng/ml. Gene expression reached saturation earlier in the NF strain. The distribution of yEGFP expression in the NF strain was narrower throughout the ATc range tested, maintaining lower noise. By contrast, yEGFP distribution in the NR strain was wider, especially at intermediate concentrations of ATc, revealing higher noise at all induction levels. This agrees with earlier findings that negative feedback reduces noise [3], but also shows that noise reduction is efficient at wide range of feedback strength.

We have also developed computational models to better understand the response of gene expression mean and noise to varying inducer concentrations, and to predict the mean and noise in the NF strain based on the NR strain.

III. Conclusion

In the present work we demonstrated that negative feedback regulation is able to linearize the dose-response curves in regulatory cascades and decrease noise of gene expression regardless of the negative feedback strength. This work tests the feasibility of a bottom-up approach in synthetic biology by aiming to predict the behavior of more complex regulatory networks from their simpler constituents.

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