Precise regulation of gene expression by negative feedback

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***Short Abstract* — We describe how negative feedback can be used to reduce gene expression noise and to linearize the dose response in synthetic gene expression cascades.**

***Keywords* — negative feedback, linearization, gene expression systems.**

Negative autoregulation is prevalent in transcriptional networks, possibly due to evolutionary selection to reduce gene expression heterogeneity [1], metabolic costs of protein production [2], and to induce oscillatory gene expression [3]. While it has also been suggested that negative feedback alters the shape of the dose-response curve in transcriptional and signaling cascades, this effect has not been explored in detail [4,5]. Such knowledge is vital for the rational design and characterization of increasingly complex transcriptional networks in synthetic biology.

Our purpose was to determine the effects of negative feedback on gene expression characteristics in eukaryotes, and whether these effects could be used to improve current synthetic gene expression systems.

We have built a gene expression system (regulatory cascade) in which the constitutively expressed tetracycline repressor TetR controlled the expression of the yEGFP reporter in *Saccharomyces cerevisiae*. *yEGFP* expression was ultimately regulated by the inducer anhydrotetracycline (ATc) introduced in the growth medium that blocks TetR-mediated repression. We measured yEGFP expression over a wide range of ATc concentrations (0-500 ng/ml) by flow cytometry and found a steep (sigmoidal) dose-response with a sharp increase in cell-cell heterogeneity of yEGFP expression at intermediate induction levels. Computational models developed concurrently reproduced the steep dose-response and the heterogeneity of experimental systems, and predicted that negative autoregulation transforms the dose-response from sigmoidal to linear curves. Prompted by these predictions, we constructed a "linearizer" gene circuit by replacing the constitutive promoter upstream of *tetR* with a TetR-repressible promoter, thereby introducing negative autoregulation into the system. As expected, in the negative feedback system the dose-response of the reporter gene was highly linear prior to saturation (R^2=0.99) and the heterogeneity of gene expression in the cell population decreased up to seven-fold. Furthermore, we derived a mathematical model that explained linearization in our constructs by pre-distortion at the repressor level followed by re-distortion at the reporter level, and predicted that negative feedback linearizes the dose-response if the downstream and upstream promoters are identical. We confirmed this mathematical prediction in additional experiments with negative feedback cascades built using different pairs of TetR-repressible promoters.

Based on our results, the incorporation of negative feedback into current gene expression systems will improve their performance by dramatically reducing gene expression heterogeneity and by allowing the experimenter to fine-tune the expression of any gene along a linear dose-response curve. For example, doubling the inducer concentration will exactly double gene expression above the background. This is possible only if the dose-response is truly linear over a wide range of induction, as in our constructs. This seems particularly important considering the nonlinear dose-response of many synthetic transcription activation elements [6], and the wide use of *tet* gene expression systems in bacteria, yeasts, insects, and mammalian cells. Currently, no known alternatives exist to linearize the dose-response in eukaryotic gene expression systems. In summary, the precise control of gene expression accompanied by heterogeneity reduction are attractive features that should make the linearizer gene circuit a highly useful tool in synthetic biology.

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