64b Engineering Negative Feedback Regulation in Cells

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Negative feedback is a common mechanism used to regulate transcription. A number of researchers have hypothesized multiple roles for negative feedback in transcriptional regulation including noise attenuation, increased stability, and faster response times. Almost all negative feedback loops in prokaryotes involve autoregulation, where a transcriptional regulator inhibits its own expression. This same transcriptional regulator can also inhibit the transcription of other genes either because they are in the same operon or the transcriptional regulator controls multiple promoters. A detailed understanding of negative feedback in its different forms is a prerequisite for developing comprehensive models of genetic regulation.

As a step towards accomplishing this goal, we have employed a forward engineering approach by designing synthetic, negative feedback loops using the tetracycline repressor (TetR) from Tn10 and the green fluorescent protein (GFP). Similar designs have been proposed by a number of researchers. Rather than focus on one specific design, we have constructed a number of synthetic feedback loops in different configurations. For example, we have constructed feedback loops where TetR and GFP are in the same operon, fused using a flexible linker, under the control of separate TetR regulated promoters, on the same or different plasmids, integrated into the chromosome, and under the control of a divergent promoter. We have also designed synthetic feedback loops where the two proteins have different stabilities through the addition of proteolytic tags and different translational strengths using modified ribosome binding sites.

As molecular noise plays an important role in transcriptional regulation, we have assayed our synthetic feedback loops using flow cytometry under a number of different growth conditions. Flow cytometry enables us to make single cell measurements and, as a result, characterize cellular variability. In conjunction with the experimental systems, we have developed a stochastic model describing the influence of the parameters and architecture on the dynamics of gene expression. The experimental systems and associated model should further our understanding of genetic regulation and also facilitate the design of more complex synthetic gene circuits.