Physical Structure of Intracellular Feedback Loop

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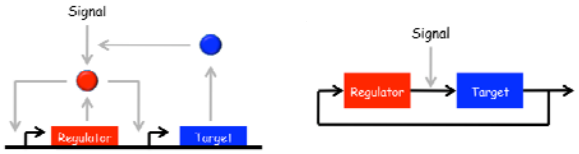
Abstract

The parallels between the feedback control mechanisms used in cells and manufacturing processes have long been noted. Both need to function robustly in response to dynamics changes in supply/demand and also in response to unforeseen disturbances. To deal with these challenges, natural and synthetic systems employ feedback control. As a general control theory for synthetic systems has already been developed, one can imagine that the same tools may be applicable for the analysis and design of natural systems. However, one challenge in applying concepts from synthetic systems to natural one is that with the latter a natural decoupling between the process and control system often does not exist. Moreover, natural systems physical encode their control systems in a number of different ways. Understanding this encoding is often necessary for elucidating the mechanism and associated degrees of freedom. In this work, we explored a simple intracellular control system involved in tetracycline antibiotic resistance, where the relative location and orientation of the regulators encoded within the genome plays a critical role in the feedback mechanism. We demonstrate experimentally that perturbations to the genetic encoding of the feedback dramatically alter its behaviors. These results suggest that the physical instantiation of the feedback needs to be accounted for in any theory of biological control.

Autoregulatory Gene Circuit

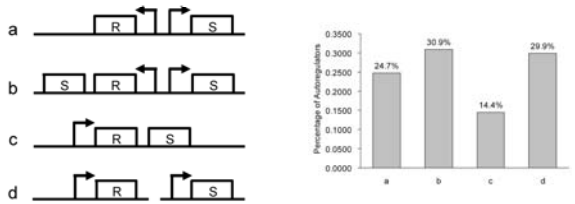
Analysis of gene regulatory circuits in a variety of organisms has identified a number of recurring regulatory mechanisms or so-called network motif. Perhaps the simplest motif found in these circuits is autoregulation, where a given transcription factor regulates its own expression along with the expression of target structural genes. The feedback loops formed in these autoregulatory gene circuits can either be negative or positive, where the transcription factor either represses or activates its own expression. Both forms of control are ubiquitous and have been shown to have various roles.



An example of an autoregulatory gene circuit is shown in the Figure above (**left**), where a regulator control is own expression along with the expression of a target gene in response to some external signal. Often, the target gene product affects the strength of this signal. To a first approximation, we can use the block-diagram formalism as an abstraction for analyzing this circuit (**right**). However, one thing this abstraction does not account for is how the gene circuit is physically encoded within the genome.

Diversity of Gene Circuits in E. coli

We analyzed all known transcription factors in the *E. coli* K12 genome using RegulonDB as the reference. Of the 160 annotated transcription factors, we focused on the 97 thought to regulate their own transcription. Based on the analysis of data from RegulonDB, EcoCyc, and proximal genomic region, we were able to categorize these autoregulators into four types according to their transcriptional organization (left) and determine (right) their relative abundance. An interesting finding was that divergent configurations were utilized by over fifty percent of all autoregulatory gene circuits.



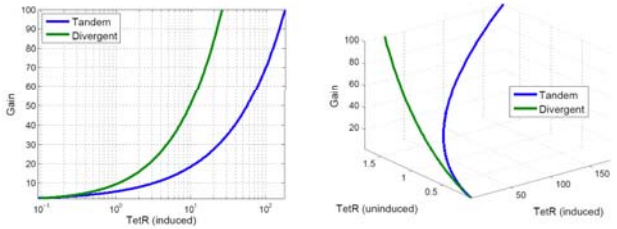
Mathematical Analysis

To explore how the physical encoding affects regulation, we employed the *tetRA* element from transposon Tn10 as our model for an autoregulated circuit involving a divergent promoter. This circuit mediates resistance to the antibiotic tetracycline by regulating the expression of the TetA efflux transporter through the action of the TetR repressor. Based on the known mechanism for gene regulation, we developed the following simplified mathematical model formulated in dimensionless form to analyze the circuit:

$$\frac{da}{dt} = \left(\frac{1}{1+\beta[x]r}\right)\left(\frac{1}{1+\alpha[x]r}\right) - a$$

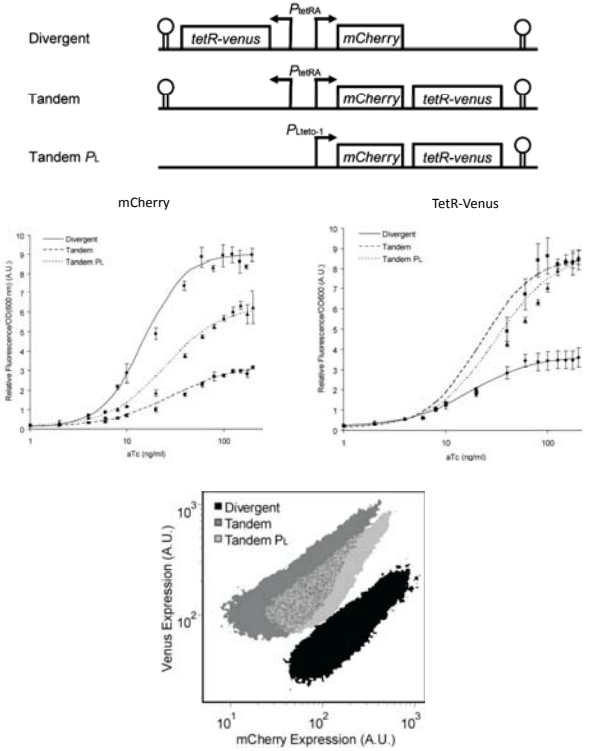
$$\frac{dr}{dt} = k_{r1}\left(\frac{1}{1+\beta[x]r}\right)\left(\frac{1}{1+\alpha[x]r}\right) - k_{r2}\left(\frac{1}{1+\beta[x]r}\right)\left(\frac{\alpha\beta[x]r}{1+\alpha[x]r}\right) - r$$

where a denotes the structural gene and r the regulator. Using the model we compared the behavior of two autoregulatory genes circuits tandem (c) and divergent configuration (a).



Experimental Analysis

Based on our theoretical analysis, we found that autoregulatory circuits involving divergent promoters have reduced control costs relative to those involving a tandem configuration. To validate this hypothesis, we construct a number of synthetic gene circuits and then measured their response to varying levels of inducer.



These experiments validate the key prediction of our model, namely that the divergent configuration minimizes the cost associated with regulating the gene circuit. These results provide a simple example where the physical topology of a gene circuit affects its behavior.

Acknowledgements

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