Abstract Title:
Elucidation of distinct transcriptional regulatory logic in bacteria: Applications of a constraints-based systems biology knowledgebase

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Project Goals:
This project aims to: (1) create a fully curated, bottom up reconstruction of the transcriptional regulatory network in bacteria, using *Escherichia coli* as a model organism, (2) determine fundamental constraints on the regulatory response via network and sequence level features, (3) develop a non-Boolean constraints based modeling approach for regulation, (4) integrate the transcriptional regulatory network with metabolic and macromolecular synthesis models, and (5) provide a platform for genome scale metabolic engineering and synthetic design.

References:
1. Cho B-K, Federowicz S, Embree M, Park Y-S, Kim D, Palsson BØ. 2011. The PurR regulon in Escherichia coli K-12 MG1655. Nucleic acids research. 39(15):6456-64
2. Cho B-K, Federowicz S, Park Y-S, Zengler K, Palsson BØ. 2011. Deciphering the transcriptional regulatory logic of amino acid metabolism. Nature Chemical Biology. 8(1):65-71

Project Description:
Constructing a systems biology knowledgebase requires the synthesis of a number of critical components into a single platform to allow for diverse computations and analyses. These components include 1) an underlying data model and database 2) tools for integrating, comparing, and analyzing diverse sets of data, and 3) a computational model which mathematically relates the underlying biochemical information. The knowledgebase is built using an iterative workflow: biological experiments are performed to generate data, data is analyzed and its results are integrated into a model, and the model is used to direct future experiments. Here we detail the process of all three steps and display the biological insight gained through the successful utilization of steps one and two.

We first performed an integration and analysis of ChIP-chip, gene expression, and transcription start site (TSS) data obtained at the genome scale for *Escherichia coli.* These specific experiments allow for the elucidation of distinct logical programs and genome scale regulatory mechanisms. These two pieces can then be combined to build a comprehensive model of transcriptional regulation. Logical programs are executed by bacteria in response to common environmental signals or physiological shifts and often include a small molecule signal and associated transcription factor. Initial studies of amino acid metabolism and the transcription factors ArgR and Lrp revealed that arginine and leucine can act as signaling molecules to regulate the transport, biosynthesis, or utilization of 16 amino acids[2]. Similar network motifs governing the flow of an effecter molecule were also shown for purine metabolism and the transcription factor PurR[1]. This has led us to investigate the aerobic-anaerobic shift regulated by ArcA and Fnr, along with the phenomenon of catabolite repression regulated by Crp and Cra. Obtaining a holistic understanding of these systems along with amino acid metabolism allows us to gain an understanding of regulation in response to carbon, nitrogen, and electron acceptor shifts.

In addition to systems level regulation it is possible to gain an understanding of specific regulatory mechanisms at the genome scale in the form of diverse promoter architectures and transcription factor mediated bidirectional transcription. Here we show how promoter architectures occurring in both a unidirectional and bidirectional fashion confer patterns of activation and repression on associated transcription units. Combining this information with systems level principles forges a tie between network and sequence level mechanisms to provide a powerful modeling framework. Utilization of this framework for engineering and synthetic approaches promises to enable a new era of molecular engineering. Overall, the integrated knowledgebase enables a wide range of analysis and is poised to guide future experiments in a model driven fashion towards a comprehensive understanding of transcriptional regulation.