Using microfluidic device based experimentation to unravel the topology of a maltose sub-regulon

Ambhighainath Ganesan[1*], Harsha K. Prabhala[1*], Tao Yu[1], Alex Groisman[2] and Andre Levchenko[1]

***Short Abstract* — Gene regulation networks are the most basic information storage and processing systems of all living cells. Understanding of their function requires the knowledge of connectivity or topology of the underlying genetic circuits or motifs. While the structures of gene regulation networks in *E. coli* and other model organisms have been sufficiently characterized, the required information is a compendium of diverse data frequently coming from different labs, different organisms and variants (strains), and different experimental methods. Here we suggest that one can obtain the structure of a small genetic network such as the maltose sub-regulon comprising *crp*, *malI* and *malX* genes based on a series of experiments, performed in parallel over relatively long time periods within a microfluidic device, using strains carrying GFP under the control of the respective promoters. We argue that this method can be applied to a wide variety of small gene regulation networks.**

***Keywords* — network reconstruction, maltose operon, microfluidics, mathematical model**

I. Introduction

Gene regulatory networks serve as complex and versatile information processing mechanisms employed by living cells in assimilating external signals and in subsequent decision making processes. Inferring regulatory interactions in such networks, therefore, helps us understand the means by which cells process information and respond accordingly.

The maltose regulatory network is a primary metabolite network in *E. coli*. While maltose is the preferred metabolite for *E. coli*, following the consumption of glucose [1], recent work on gene regulatory interactions has primarily been constrained to the lactose operon owing to its simplicity and previous characterization [2]. The maltose regulon, by contrast, is much more complex and certain interactions are still under debate.

The maltose regulatory network comprises ten genes, including those coding for enzymes, transmembrane transporters and transcriptional regulators. While the primary means of regulation is attributed to the *malT* and *crp* transcriptional regulators [3], *malI* and *malXY* are thought to be independent of *malT* regulation. Little is known about the means by which *malI* and *malX* are activated and the roles of these components in maltose regulation. This work attempts to infer the regulatory interactions among *crp*, *malI* and *malX* with a combination of experimental and modeling approaches, while establishing a paradigm for unraveling gene regulation circuit using similar approaches.

II. Results

A microfluidic device was designed based on the devices previously developed and described by our group [4,5], but now capable of supporting the simultaneous growth of up to 4 separate bacterial strains on a single chip, while exposing the colonies to synchronized changes in the medium. *E. coli* strains containing plasmids for *crp*, *malI*, and *malX* gene promoters tagged with GFP were used.

The varying kinetics of gene expression levels obtained in this device for different growth conditions indicated differential activation of *crp*, *malI* and *malX* genes, as would be expected in order to modulate the metabolism of maltose under different starvation scenarios. Regression analyses in conjunction with Bayesian network analyses and Information theoretic approaches applied to this data suggested a ring topography of the *crp*-*malI*-*malX* subgraph, such that *malI* expression is influenced by *crp* and that of *malX* -- by *crp* and *malI*.

Further, it was observed that the system appears to oscillate under specific growth conditions. A representative ODE model was built incorporating the effect of *malX* on *crp* via metabolism of maltose which sets up a negative feedback in the system. Simulations revealed that the negative feedback loop in conjunction with a feedforward loop could likely govern the occurrence of oscillations. The reconstructed structure was supported by a wealth of follow-up biochemical experiments.

III. Conclusion

We propose that the time series-based reconstruction of the inter-connectivity in small gene regulation networks can be facilitated by a combination of a high-throughput microfluidic device experimentation and model analysis.

References

[1] Beg OK, et al. (2007) Intracellular crowding defines the mode and sequence of substrate uptake by Escherichia coli and constrains its metabolic activity. *Proc Natl Acad USA* **104**:12663-12668.

[2] Dekel E, Alon U (2005). Optimality and evolutionary tuning of the expression level of a protein *Nature* **436**: 588-592.

[3] Richet E, Raibauld O (1989). MalT, the regulatory protein of the Escherichia coli maltose system, is an ATP-dependent transcriptional activator. *Embo J* **8**: 981-987.

[4] Cho H, et al. (2007). Self-organization in high-density bacterial colonies: efficient crowd control. *PLoS Biol* **5**, e302.

[5] Groisman, A. et al. (2005). A microfluidic chemostat for experiments with bacterial and yeast cells. *Nat Methods* **2**, 685-689.

[*]These authors contributed equally to this work.

[1]Department of Biomedical Engineering, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

[2]Department of Physics, University of California, San Diego, CA, 92093