Mechanisms of Positive Regulation in the *mar/sox/rob* Regulon of *Escherichia coli*

Robert G. Martin[1], David A. Markowitz[2], Emily S. Bartlett[3], Judah L. Rosner[1], and Michael E. Wall[4]

***Short Abstract* — The transcriptional activator MarA enhances the activity of a large number of genes (the *mar/sox/rob* regulon) in *Escherichia coli.* We find that the activation of different members of the regulon requires vastly different concentrations of MarA, and that most promoters do not exhibit a plateau in activity *vs.* MarA. Mathematical modeling of this phenomenon suggests that positive regulation sometimes occurs through an increase in the forward rate of transcription combined with a *decrease* in the affinity of RNA polymerase for the promoter in the presence of activator. Such a mechanism is inconsistent with the regulated recruitment model of transcriptional activation.**

***Keywords* — activation; gene regulation; bacteria; regulated recruitment**

I. Purpose

THE MarA, SoxS and Rob transcription factors activate the same ~40 genes (the *mar/sox/rob* regulon) of the *E. coli* chromosome resulting in different levels of resistance to a wide array of antibiotics and superoxide generating compounds (see [1] for references). In an effort to understand how they differentially stimulate transcription of the genes of the regulon, we initially determined that while some correlation existed between activity and the binding constant of the activator to the different binding regions of the promoters (a 19 bp consensus sequence that varies slightly from one promoter to the next [2]), that correlation was insufficient to explain the different behavior of the activators [3]. To further understand these mechanisms, we have now placed the expression of MarA under the control of the LacI repressor, determined the relationship between IPTG concentration and the intracellular concentration of MarA, and examined the expression of a number of the regulon genes as a function of growth in different concentrations of IPTG. The resulting data were used to develop models that yield insight into mechanisms of activation at different promoters.

Acknowledgements: This work was funded by the National Institutes of Health and the Department of Energy.

[1]Laboratory of Molecular Biology, National Institute of Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892-0560. rgmartin@helix.nih.edu; jlrosner@helix.nih.edu

[2]Department of Molecular Biology, Princeton University, Princeton, NJ 08544. dave@princeton.edu

[3]Cornell University, Ithaca, NY 14853. esb38@cornell.edu

[4]Computer, Computational, and Statistical Sciences Division, Bioscience Division, and Center for Nonlinear Studies, Los Alamos National Laboratory, Los Alamos, NM 87545. mewall@lanl.gov

II. Results

We find that the concentration of MarA required for activation varies by at least 140-fold for different promoters, thus identifying a previously unappreciated form of regulon control in which some genes may remain dormant while other genes are activated. The *mar* promoter is activated at the lowest concentration of MarA, reaching half-maximal stimulation at a concentration of 54±17 molecules/cell. No other promoters exhibit a plateau in activity, even at the highest levels of MarA (about 10,000 copies per cell).

To gain insight into the diversity in stimulation of the regulon, we developed a mathematical model of MarA-dependent promoter activity. In the model, MarA either increases (attraction) or decreases (repulsion) the occupancy of RNA polymerase (RNAP) at the promoter, and either increases (acceleration) or decreases (retardation) the forward rate of transcription by RNAP once bound at the promoter. The best models of *mar* activity combine attraction with acceleration. For other promoters, models that combine *repulsion* with acceleration fit the data best.

III. Conclusion

The results suggest that positive regulation can involve repulsion—a decrease in the occupancy of RNAP at a promoter in the presence of activator—which is an effect commonly associated with negative rather than positive regulation. The results also suggest that acceleration—an increase in the forward rate of transcription in the presence of activator—is an important effect in positive regulation. Both acceleration and repulsion are inconsistent with the regulated recruitment model of transcriptional activation, in which positive regulation occurs solely through an increase in the occupancy of RNAP at the promoter [4, 5].

References

[1] White DG, Alekshun MN, McDermott, PF (2005) *Frontiers in Antimicrobial Resistance*, ASM Press, Washington, DC.

[2] Martin RG, Gillette WK, Rosner JL (1999) Structural requirements for marbox function in transcriptional activation of mar/sox/rob regulon promoters in *Escherichia coli*: sequence, orientation and spatial relationship to the core promoter. *Molec Microbiol*. **34**, 431-441.

[3] Martin RG, Gillette WK, Rosner JL (2000) Promoter discrimination by the related transcriptional activators MarA and SoxS: differential regulation by differential binding. *Molec Microbiol*. **35**, 623-634.

[4] Ptashne M, Gann A (1997) Transcriptional activation by recruitment. *Nature* **386**, 569-577.

[5] Ptashne M (2003) Regulated recruitment and cooperativity in the design of biological regulatory systems. *Philos Transact A Math Phys Eng Sci*. **361**, 1223-1234.