# Growth Rate Effects on Global Regulation of *E. coli* Oxygen Sensing

Dean A. Tolla[1] and Michael A. Savageau[1]

***Short Abstract* — The long-term goal of this work is to characterize the relationship between cyclic regulation and the physiological constraints affecting the fumarate and nitrate reduction (FNR) network in *Escherichia coli.* In this report, we formulate a model of this system, fit its parameters to existing experimental data, and predict results that were not used in the fitting process. The results show excellent agreement between predicted and experimental data. Finally, we predict the effect altering the growth rate has on the dynamics of active FNR protein during the aerobic to anaerobic transition.**

***Keywords* — Fumarate and Nitrate Reduction, FNR, Biochemical Systems Theory, Genetic Regulation.**

## I. Introduction

THE FNR network of *Escherichia coli* regulates the shift between aerobic and anaerobic growth. Regulation of FNR involves a mechanism wherein FNR is produced and cycled through its active and inactive states. Dimeric 4Fe-FNR, a global transcription factor, adapts the cell to oxygen limiting conditions [1-3]. Aerobically, oxygen inactivates FNR, but the cell continues to produce and reactivate it [3]. This results in a constant cycling of FNR between its three states apoFNR, 4Fe-FNR, and 2Fe-FNR. Aerobic cycling is tuned so that the inactive apoFNR predominates [4]. Under anaerobic conditions, the absence of oxygen results in a rapid build up of 4Fe-FNR. The 4Fe-FNR form dimerizes to produce an active transcription factor that regulates hundreds of genes [1,2,5].

Between the synthesis, degradation, and construction of iron-sulfur clusters, *E. coli* pumps a constant stream of material through the FNR pathway and ties up valuable iron resources in the process. Paradoxically, the FNR cycle serves no apparent use under aerobic conditions [6]. Systems with this topology and apparent lack of physiological purpose have been termed futile cycles. There is reason to believe that futile cycles such as FNR, which have all their proteins at the ready, can respond quickly to an environmental signal. A cell that can transition rapidly between aerobiosis and anaerobiosis has a potential growth advantage. The first step toward further understanding for the role of cycling in the FNR system is to develop a model we can analyze that accurately represents the real network.

Acknowledgements: This work was supported in part by a grant to M.A.S. (RO1-GM30054) from the US Public Health Service.

[1]Department of Biomedical Engineering, University of California, Davis, One Shields Ave, Davis, CA 95616. E-mail: datolla@ucdavis.edu, masavageau@ucdavis.edu

## II. Model Characteristics

We formulate a model of the FNR system based on available literature and refine it to satisfy five experimental criteria and a set of pulse-chase data. By employing the technique of compartmental analysis to understand the intricacies of relating the model to a pulse-chase experiment, we are able to tease out a parameter set such that our model correctly mimics two wild-type pulse-chase assays.

We then introduce mutations into our model and predict the related mutant phenotypes. We test our model and its associated parameter set by checking its predictions against experimental data describing those mutant phenotypes. Specifically, we predict the pulse-chase curves of the mutants and show that the literature agrees with our predictions. We then predict the dynamics of the aerobic to anaerobic transition at different growth rates, which has never been experimentally examined.

## III. Conclusion

We formulate a model of FNR regulation and fit its parameters to replicate wild-type behavior. We validate the model by correctly predicting known mutant behavior. We then proceed with predicting the dynamics of the FNR network at different growth rates.

## References

[1] Lazazzera BA, Bates DM and Kiley PJ. (1993) The activity of the Escherichia coli transcription factor FNR is regulated by a change in oligomeric state. *Genes Dev*, **7**, 1993-2005.

[2] Lazazzera BA, Beinert H, Khoroshilova N, Kennedy MC and Kiley PJ. (1996) DNA binding and dimerization of the Fe-S-containing FNR protein from Escherichia coli are regulated by oxygen. *J Biol Chem*, **271**, 2762-2768.

[3] Khoroshilova N, Popescu C, Munck E, Beinert H and Kiley PJ. (1997) Iron-sulfur cluster disassembly in the FNR protein of Escherichia coli by O2: [4Fe-4S] to [2Fe-2S] conversion with loss of biological activity. *Proc Natl Acad Sci U S A*, **94**, 6087-6092.

[4] Sutton VR, Mettert EL, Beinert H and Kiley PJ. (2004) Kinetic analysis of the oxidative conversion of the [4Fe-4S]2+ cluster of FNR to a [2Fe-2S]2+ Cluster. *J Bacteriol*, **186**, 8018-8025.

[5] Kang Y, Weber KD, Qiu Y, Kiley PJ and Blattner FR. (2005) Genome-wide expression analysis indicates that FNR of Escherichia coli K-12 regulates a large number of genes of unknown function. *J Bacteriol*, **187**, 1135-1160.

[6] Lambden PR and Guest JR. (1976) Mutants of Escherichia coli K12 unable to use fumarate as an anaerobic electron acceptor. *J Gen Microbiol*, **97**, 145-160.