Coordinated regulation of multiple-antibiotic resistance in *Escherichia coli*

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***Short Abstract* — Bacterial resistance to antibiotics has focused primarily on the role of acquired genetic elements contained in transposons and plasmids. However, many bacterial species, including *Escherichia coli*, are equipped with intrinsic mechanisms to survive exposure to a wide variety of antimicrobial compounds mediated through activation of efflux pump systems, reducing enzymes, and enzymes in cellular metabolism. Modulating this response in *E. coli* are three homologous, regulatory proteins MarA, SoxS, and Rob. In this work, we examine the degree of cross-talk between these regulatory systems and the cooperative role of these three transcriptional regulators in activating downstream targets.**

***Keywords* — Multiple-antibiotic resisitance, genetic regulatory network, interlocked regulation**

Bacterial resistance to antibiotics has been a persistent problem in clinical and public health situations for decades. While most bacteria acquire antibiotic resistance via genes encoded in mobile genetic elements such as plasmids and transposons, many bacterial species possess intrinsic mechanisms for resistance to antibiotics, organic solvents, oxidative stressors, and household disinfectants [1]. In the enteric bacteria *Escherichia coli* and other closely related bacterial species, such as pathogenic forms of *E. coli* and *Salmonella enterica*, this resistance is mediated by the expression of chromosomally encoded efflux systems, cytoplasmic reducing enzymes, and catabolic enzymes resulting in large-scale changes in cellular metabolism. Governing these cellular responses is three transcriptional regulators of the multiple antibiotic resistance (*marA*), superoxide (*soxS*), and *rob* regulons [2] – collectively known as the *marA/soxS/rob* regulon. Although these networks respond to different environmental queues, *marA*, *soxS*, and *rob* are known to regulate the expression of many downstream genetic targets, as well as regulating the expression of each other [3]. This complex interlocked genetic circuitry is believed to provide the ability to sense and respond to broad classes of toxic chemicals.

In this work, we have employed mixed strategy of experimental genetics coupled with computational modeling to develop a quantitative description of the interlocked *marA/soxS/rob* regulon, a primary determinant of intrinsic antibiotic resistance in *E. coli*. Performing comprehensive deletion and complementation analysis we systematically determined all cross-regulatory and auto-regulatory interactions in the *marA*, *soxS,* and *rob* genetic circuits. In the context of the multiple-antibiotic response, we have focused on the role of Rob on the activation of the *marRAB* operon. Previous studies have suggested that the primary activation of the *marRAB* operon under antibiotic stress is mediated MarA-depedendent auto-activation [4]. However, our results indicate that activation of *marRAB* is largely Rob dependent and that Rob and MarA appear to coordinately activate *marRAB* transcription. In addition, we have examined the role of the interlocked *marA/soxS/rob* genetic circuitry in providing additive and synergistic response to multiple environmental queues. Finally, we have made efforts to develop a quantitative model which captures the behavior of this interlocked network.

Although our current understanding of the *marA/soxS/rob* genetic circuit has largely relied on the use of bacteriostatic antibiotics such as tetracyclines and chloramphenicol, recent genetic and biochemical evidence has suggested that on exposure to bacteriocidal antibiotics superoxide is generated in the cytoplasm of *E. coli* cells [5]. In the context of the *marA/soxS/rob* regulon, superoxides are known activators of the *soxS* regulon providing a critical link between the intrinsic response to bacteriostatic and bacteriocidal antibiotics. Taken together, the results of this work will allow for a more thorough understanding of the mechanism of intrinsic resistance in *E. coli*, and related human and animal pathogens, to clinically relevant antibiotics.

References

[1] Alekshun, M.N., Levy, S.B. (2007) Molecular mechanisms of antibacterial multidrug resistance. *Cell* **128**, 1037-1050.

[2] Alekshun, M.N., Levy, S.B. (1997) Regulation of chromosomally mediated multiple antibiotic resistance: the mar regulon. *Antimicrob Agents Chemother.* **41**, 2067-2075.

[3] Martin, R.G., Rosner, J.H., (2002) Genomics of the marA/soxS/rob regulon of Escherichia coli: identification of directly activated promoters by application of molecular genetics and informatics to microarray data. *Mo. Microbiol.* **44**, 1611-1624.

[4] Martin, R.G., Jair, K., Wolf, R.E., Jr., Rosner, J.L. (1996) Autoactivation of the *marRAB* multiple antibiotic resistance operon by the MarA transcriptional activator in *Escherichia coli*. *J Bacteriol.* **178**, 2216-2223.

[5] Kohanski, M.A., *et al.* (2007) A common mechanism of cellular death induced by bactericidal antibiotics. *Cell* **130**, 797-810.

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