Role of the AcrR Transcriptional Repressor in Directly Regulating metabolism in *Escherichia coli*

Avedis Guedekelian, Jessica Maldonado, Alanis Hernandez, Dana E. Harmon Ph.D., and Cristian Ruiz Rueda Ph.D.

Department of Biology – California State University Northridge

Abstract

Bacteria are equipped with a variety of multidrug efflux pumps. These pumps first gained interest because of their ability to expel antibiotics out of cells, thus conferring resistance to multiple antibiotics. However, recent findings have shown that these pumps have a broad impact on gene expression and cell physiology. A mechanistic understanding of how these pumps control these fundamental processes is still lacking. Based on previous findings, we hypothesize that AcrR, which is the main transcriptional regulator of AcrAB-TolC, is ultimately responsible for many of the gene expression and physiological effects associated to this pump. Using electrophoresis mobility shift assays (EMSA), we have identified direct binding of AcrR to the *mdh* promoter, which encodes for the enzyme malate dehydrogenase, a key enzyme of the TCA cycle and fermentation pathways in *Escherichia coli*. These findings are in agreement with the intra- and extracellular changes in malate concentration previously found in *acrR*-deleted mutants and support a model in which efflux and central metabolism are co-regulated in *Escherichia coli*.

Introduction

Antibiotic resistance is a developing issue in the fight against pathogenic bacteria. More and more bacteria are acquiring methods of resistance to antibiotics, such as by blocking penetration through the cell membrane, target modification, and efflux pumps. Previously we have studied the role of the AcrAB-TolC multidrug efflux pump and its major transcriptional repressor AcrR in efflux and metabolism [1, 2]. We hypothesize that AcrR has many other physiological functions in the cell beyond its role in regulating efflux [1]. By using the known AcrR binding site in the *acrAB* promoter [3] for bioinformatics analysis of the genome of *Escherichia coli*, we found an abundance of gene promoters which have potential binding sites for the AcrR. By finding what genes are directly regulated by the AcrR repressor, we can have a better understanding of the physiological roles of AcrR. In this study, we investigated direct regulation by AcrR of the candidate gene *mdh,* which encodes the enzyme malate dehydrogenase, a key enzyme in central metabolism in *E. coli*.

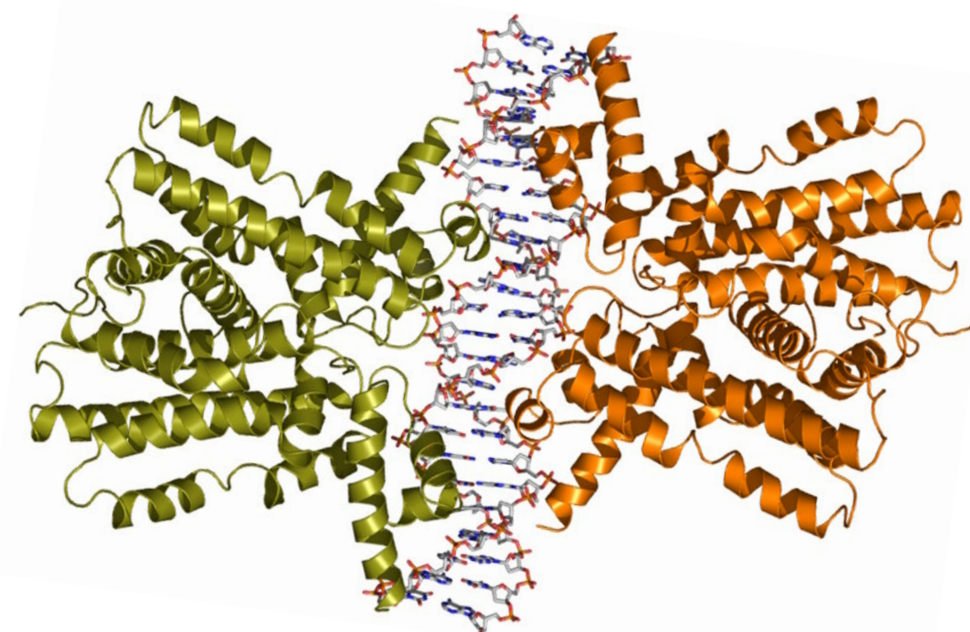

Figure 1. Illustration of the AcrAB-TolC pump.

Methodology

Selection of AcrR-directly regulated candidate genes.

1. Genome wide bioinformatics search of potential AcrR binding sites using the known AcrR-binding site in the *acrAB* promoter.
2. Comparison of hits to genes found by microarray to be upregulated in *acrR*-deleted mutants and/or to belong to metabolic pathways found to be altered in *acrR*-deleted mutants .

Initial Purification and Amplification Steps

1. Purification of AcrR (protein of interest).
2. Primer design for the promoter of interest (*mdh*)
3. PCR amplification of the regions where the potential binding sites are located.
4. Electrophoresis gel to confirm PCR success and sample purity.
5. Purification step (Zymo Kit)
6. Checking of the concentration of DNA using nanodrop.

Figure 2. Electrophoresis Gel for F1/R1, F2/R2 and F1/R2 regions.

EMSA: Tagging and Visualization of the DNA shift

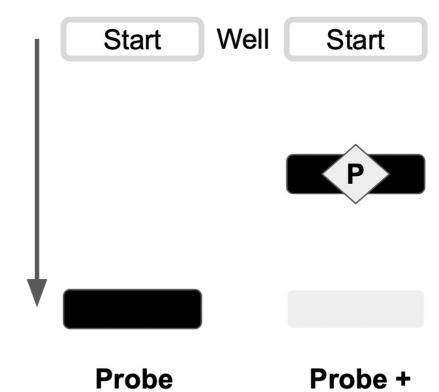

Figure 3. Electrophoretic Motility Shift Assay summary.

1. Tag the purified DNA from step 6 of the initial purification steps with the chemiluminescent tag **Biotin**.
   This step will help us visualize the DNA at later stages.

2. . Conduct the Electrophoretic Motility Shift Assay (**EMSA**) in a gel.
   - Only include the probe (amplified gene) in column one.
   - Combine probe sample and protein sample in column two.

A visible shift will occur if the protein binds to the gene. This is because the complex is now heavier, causing it to move down the gel slower than the column with the probe only.

3. Visualize results by using **film & developer**.
   Biotin exposes the film and turns the area of exposure black.

Results

Figure 4. Illustrative representation of the *mdh* promoter, the 2 potential binding sites along gene, and the placement of the various primers. "F" stands for forward while "R" stands for reverse.

- Mdhp F1/R1 - 376bp
- Mdhp F2/R2 - 226bp
- Mdhp F1/R2 - 584bp

AcrR binding site - *acrAB* promoter

AcrAB	TACATACATTCACAAATGTATGTA
Potential Binding Site 1	TATATTGATAAACTAAGATATGTT
AcrAB	TACATACATTCACAAATGTATGTA
Potential Binding Site 2	TGCATGCCGTGACGCAGGCATGTT

Figure 5. Comparative illustration of AcrR binding site in the *acrAB* promoter (AcrAB) and the predicted binding sites 1 and 2 in the *mdh* promoter. AcrR binds to *acrAB*p in order to repress AcrAB-TolC efflux pump production. The potential AcrR-binding sites in the *mdh* promoter are similar in sequence to the *acrAB*p site. Potential binding site 1 has 9 mismatches (labeled in red) while potential binding site 2 has 10 mismatches. The more mismatches, the less likely for AcrR to bind to the sequence.

***mdh* promoter**

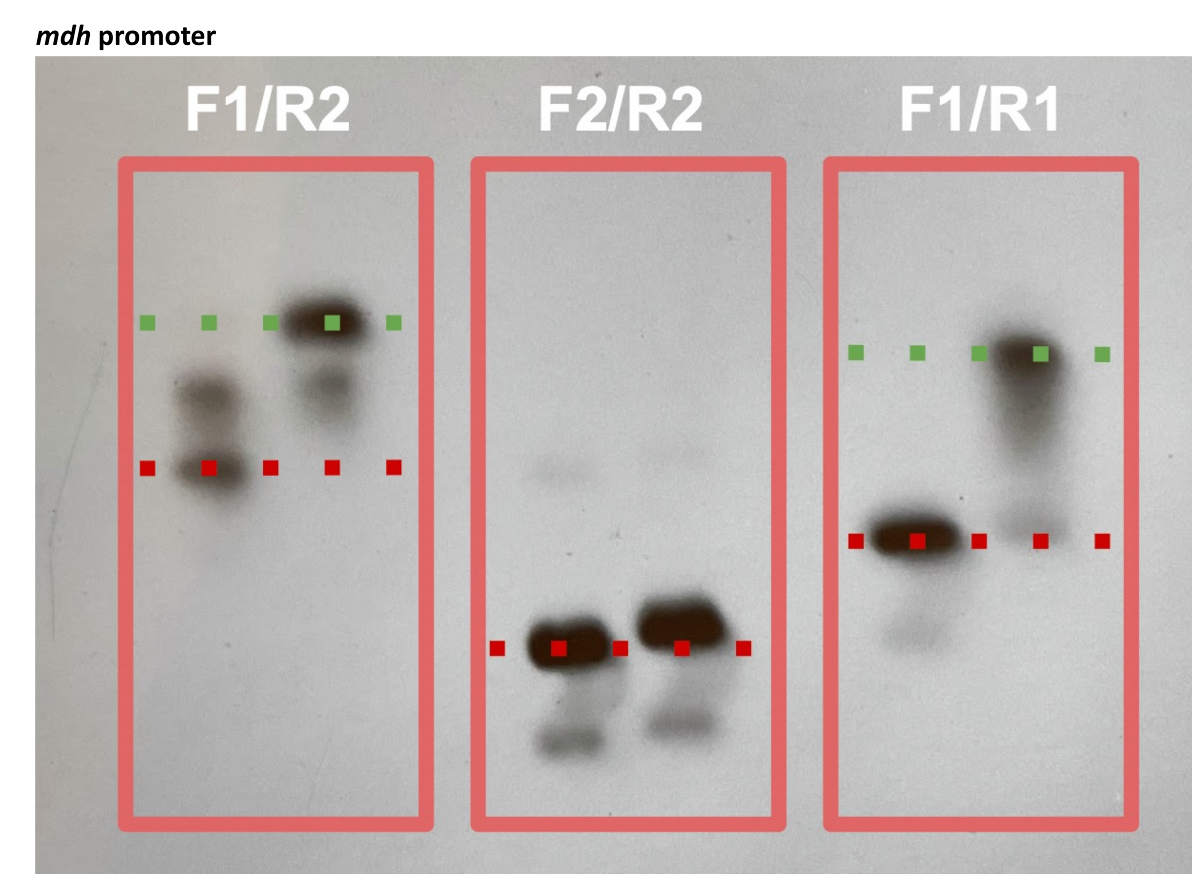

Figure 6. Electrophoretic Motility Shift Assay results for *mdh promoter* F1/R1, F2/R2, and F1/R2 regions. First line for each region includes the *mdh* promoter labeled with biotin. The second line includes both the labeled *mdh* promoter as well as the AcrR protein. Region F1/R1 shows a clear shift going from line 1 to 2. The F2/R2 region does not show any shifts. The F1/R2 region (full promter) also shows a clear shift.

Figure 7. Electrophoretic Motility Shift Assay for AcrAB. Clear shift when we add both the labeled *acrAB* promoter and AcrR protein.

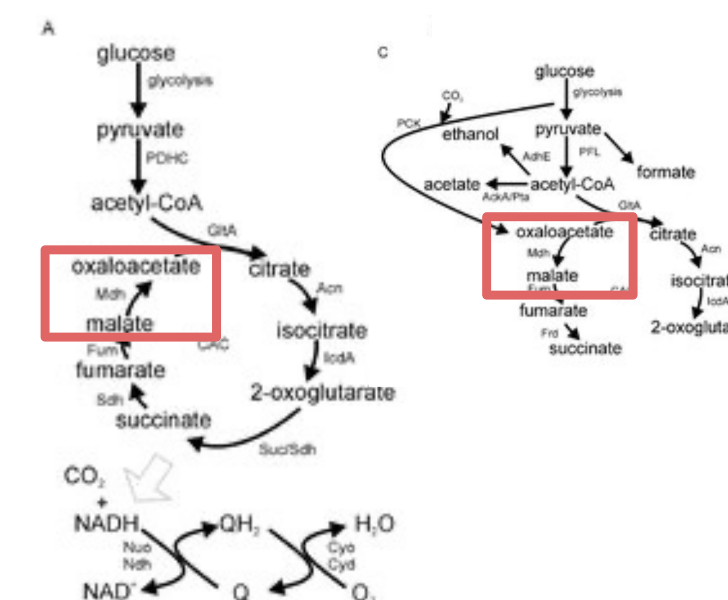

Figure 8. Main metabolic roles of MDH in *E. coli*: TCA cycle (aerobic respiration, left) and fermentation (right). Adapted from [5].

Discussion

In this study, we investigated direct regulation by the AcrR repressor of the candidate gene *mdh,* which encodes the enzyme malate dehydrogenase. MDH catalyzes the reversible oxidation of malate to oxaloacetate, and is a crucial enzyme in the TCA cycle, the pathway of fermentation to succinate (Figure 8), and metabolic pathways for the biosynthesis and transport of amino acids such as arginine and histidine [4]. *mdh* was selected as a candidate because its promoter has two predicted AcrR-binding sites according to our bioinformatics analyses, *mdh* expression was up-regulated in *acrR*-deleted mutants, and because malate is one of the metabolites found to accumulate the most in *acrR*-deleted mutants [2].

Compared to the sequence of known AcrR-binding site in the *acrAB* promoter [3], the AcrR predicted binding site 1 in the *mdh* promoter has 9 mismatches, whereas site 2 has 10 mismatches (Figure 5). In our EMSA assays, we tested direct AcrR binding to the full region of the *mdh* promoter containing both AcrR predicted binding sites, as well as to promoter fragments containing only predicted site 1 or 2 (Figures 4 and 6). Our results clearly show that AcrR directly binds to the *mdh* promoter, and that that binding occurs specifically only at predicted site 1.

These results are significant because they provide for the first time prove that the transcriptional repressor AcrR is a direct regulator of the malate dehydrogenase *mdh* gene and provide a rationale for the role of AcrR regulator in controlling cell physiology and metabolism in *Escherichia coli*.

References

1. Ruiz, C. and Levy, SB. 2014. Regulation of acrAB expression by cellular metabolites in *Escherichia coli*. *J. Antimicrob. Chemother.* 69: 390-399.
2. Cauilan, A., Ramos, K., Harmon DE., and Ruiz, C. 2019. Global effect of the AcrAB-TolC multidrug efflux pump of *Escherichia coli* in cell metabolism revealed by untargeted metabolomics . *Int. J. Antimicrob Agents*. 54(1):105-107.
3. Su, CC., Rutherford, DJ., and Yu, EW. 2007. Characterization of the multidrug efflux regulator AcrR from *Escherichia coli*. *Biochem. Biophys. Res. Commun.* 361:85-90.
4. Park, SJ., Cotter, PA., and Gunsalus, RP. 1995. Regulation of malate dehydrogenase (*mdh*) gene expression in *Escherichia coli* in response to oxygen, carbon, and heme availability. *J. Bacteriol.* 177:6652-6656.
5. Trotter, E. W., Rolfe, M. D., Hounslow, A. M., Craven, C. J., Williamson, M. P., Sanguinetti, G., ... & Green, J. (2011). Reprogramming of Escherichia coli K-12 metabolism during the initial phase of transition from an anaerobic to a micro-aerobic environment. *PloS one*, *6*(9), e25501.

Acknowledgments

1. This work was financially supported by the National Science Foundation (NSF) grant: NSF MCB 2019614
2. A. Guedekelian was supported by the 2020-2021 CSUN presidential scholarship