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The binding characteristics of IclR transcription factor

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Abstract

Targeting transcriptional regulators in prokaryotes has proven to be a promising antimicrobial therapy. Regulation of the glyoxylate shunt is directly involved in the metabolic processes and survival of pathogenic bacteria and fungi. The IclR transcription factor found in E. coli is responsible for the expression of the aceBAK operon and the activation of the glyoxylate shunt, which is upregulated under low nutrient stress conditions. Three different IclR binding sites are responsible for the regulation of the glyoxylate shunt and are differentially affected by the binding of glyoxylate and pyruvate to IclR. When pyruvate is present, a tetramer is formed which allows IclR to bind to sites one and two upstream of the aceBAK operon, preventing transcription. In contrast, when glyoxylate is present the dimer form is preferred and transcription is allowed. Using gel shift assays, we will determine the binding affinity of the different multimeric states of IclR in the presence of its ligands to its DNA targets.

Introduction

Tuberculosis (TB) is currently the most lethal bacterial infectious agent on the planet, killing millions of people per year. The need for new treatments and vaccines is increasing rapidly, and the current rate of development for vaccines and treatments is not high enough to meet the demand (Schranger, Vekemans, Harris, 2018). Tuberculosis strains have become resistant to current antibiotics, largely due to the misuse of drugs prescribed to the patients. In 2016, over 10 million cases of TB were observed. Over 1.6 million people died from TB, and 250,000 of those were due to rifampin resistant and multi-drug resistant strains of TB. HIV positive individuals were at a much higher risk than the general population. HIV positive individuals alone made up roughly 3.6% of all cases of TB in 2016. Recent projections indicate that if a vaccine with 60% efficacy were to be developed that protected an individual for 10 years, up to 17 million cases of TB could be averted by 2050 (Schranger et al., 2018). The need for a new drug/therapy for TB with high efficacy cannot be overstated.

IclR transcription factor

M.tuberculosis will activate a pathway called the glyoxylate shunt when in the presence of acetate or fatty acids, and no glucose is available. This is done to prevent carbon loss in the citric acid cycle. The carbon dioxide saved by the glyoxylate shunt is used in gluconeogenesis, which produces pyruvate. As glyoxylate and

pyruvate differentially affect the stability of IclR oligomers, one possible explanation for the production of both would be the optimization of the expression and repression of the aceBAK operon. The presence of glyoxylate determines the pathogenicity of *M. tuberculosis* (Lorca G et. al. 2007). If glyoxylate is present *M. tuberculosis* will be pathogenic. The regulation of the aceBAK operon in the glyoxylate shunt appears to be a possible drug/therapy target candidate, as the aceBAK operon is present in *M.tuberculosis* but not humans (Zhou et. al. 2012).

Transcription factors are a type of protein that inhibit or promote the expression of a gene. They do so by binding to a promoter, which is an area where RNA polymerase binds to start transcription. The IclR transcription factor is differentially bound to its promoter depending on the presence of different small effector molecules. When in the presence of glyoxylate, the transcription factor will dimerize (form two subunits) and bind to part of the promoter so that transcription of the aceBAK operon may occur. In the presence of pyruvate, the transcription factor will tetramerize (form four subunits) and bind to the promoter in a way that does not allow transcription of the aceBAK operon to occur (Lorca, G et. al. 2007).

Experimental procedures

Promoter DNA was amplified using standard PCR techniques. The correct DNA has been amplified (figure 1), however, PCR product purification and gel purification have proven challenging. Binding assays are being used to test the differential binding of the IclR transcription factor in the presence of different small effector molecules. It was previously hypothesized that the serine residue at position 147 (S147A) would promote DNA binding. Indeed, the mutant strain had an increased affinity for the aceBAK promoter in the presence of pyruvate. This experiment will examine the affinity for the S147A mutant in the presence of glyoxylate.

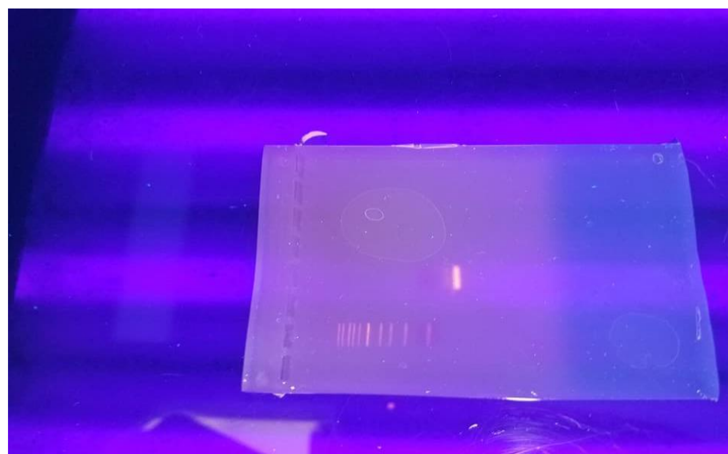

Figure 1. A 1kb molecular ladder next to aceBAK promoter DNA on a 1% agarose gel.

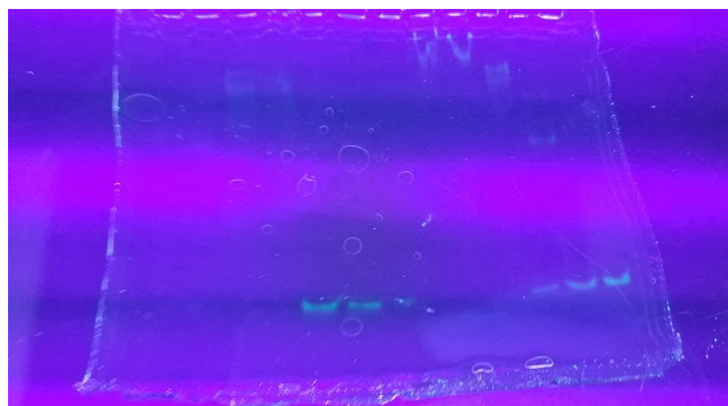

Figure 2. A polyacrylamide gel showing DNA migration in the presence of IclR transcription factor, and different amounts of pyruvate and glyoxylate. From right to left: 0nM IclR + Pyruvate (P), 25 nM wildtype (WT) + P, 100 nM WT + P, 250 nM WT + P, 250 nM WT + G, 250 nM no DNA + P (with some DNA from the next lane), 25 nM S147A + P, 100 nM S147A + P, 250 nM S147A + P, and 250 nM S147A + G.

Conclusion

The glyoxylate shunt appears to be a promising drug target candidate, as it is not

found in humans. The determination of how the IclR transcription factor binds to the aceBAK promoter in the presence of its small effector molecules may provide insight into a novel mechanism which may be exploited for therapeutic purposes.

References

Lorca, G. et. al. (2007) Glyoxylate and Pyruvate Are Antagonistic Effectors of the Escherichia coli IclR Transcriptional Regulator. The Journal of Biological Chemistry. 282(22), 16476–16491

Schrager, L., Vekemans, J., & Harris, R. (2018). Research and development of new tuberculosis vaccines: a review, F100Res, https://doi.org/10.12688/f1000research.16521.1

Zhou, Y et. al.(2012). Molecular mechanisms underlying the function diversity of transcriptional factor IclR family Cellular Signalling. 24. 1270-75

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