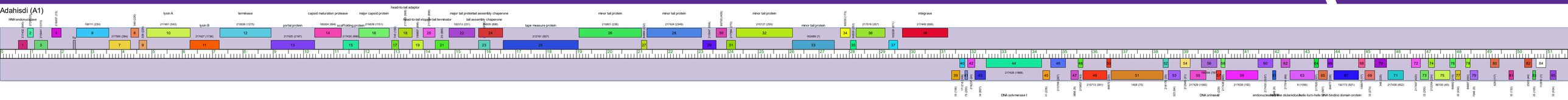
Characterization of the Adahisdi Antirepressor Protein

Ashley Tuhro, Maria D. Gainey, and Jamie R. Wallen

Department of Chemistry and Physics

Western Carolina University



ABSTRACT

The regulation of gene expression in lysogenic bacteriophages is controlled by interactions between the viral repressor protein and DNA. Repressors bind viral DNA to prevent expression of lytic genes so that the virus can lie dormant in the bacterial host cell. When conditions become non ideal for host/virus survival, such as nutrient deprivation, DNA damaging events, etc., the virus will transition to the lytic cycle to generate new viral progeny. For this switch to occur, the repressor protein must release from DNA so that transcription can begin. We have identified a candidate "antirepressor" gene in bacteriophage Adahisdi that could bind to the repressor to promote DNA release. To better understand the antirepressor protein's function, DNA binding studies were conducted to examine how the protein interacts with DNA and the repressor to influence gene expression. Typically, repressor proteins bind tightly to DNA to prevent the initiation of transcription. Our preliminary data shows that when the antirepressor is introduced, it weakens the DNA binding affinity of the repressor. Along with DNA binding studies, we are using a two-hybrid assay to observe if the antirepressor binds to any bacterial host proteins during infection. Furthermore, we are in the process of purifying large amounts of the antirepressor for structural studies. The goal of this project is to provide a deeper understanding of how antirepressors fine-tune gene expression and offer new insight into the dynamic interplay between repressors, antirepressors, and DNA

Objectives

- Understand how the antirepressor protein, repressor protein, and DNA interact
- Determine if our candidate gene is capable of acting as an antirepressor

Goal

Provide a deeper understanding of how antirepressors fine-tune gene expression and offer new insight into the dynamic interplay between repressors, antirepressors, and DNA.

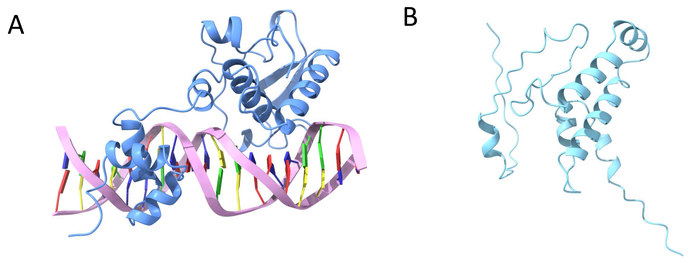


Figure 1: A) X-ray crystal structure of the adahisdi repressor bound to DNA. The repressor is expressed from gene 71 shown in the phamerator map above. **B**): AlphaFold model of the predicted antirepressor made from gene 70. **C)**: Phage RGL3 contains a fusion of the repressor (purple) and antirepressor (orange). We have expressed and purified the RGL3 repressor fusion protein to observe if this protein is capable of binding DNA.

Figure 2: To determine if the adahisdi antirepressor alters repressor DNA binding, a series of DNA binding assays were performed. Protein and DNA were mixed and incubated for 30 minutes at room temperature, then run on a 0.5% agarose gels in 1X TAE buffer at 100V for 15 minutes. **A)**: Samples were set up in the order: repressor, antirepressor, then DNA. Lane 1 contains only DNA. Lanes 2-6 contain a fixed concentration of repressor protein (5 µM). Lane 2 contains no antirepressor, while lanes 3-6 contain increasing concentrations of antirepressor (0.25µM, 0.5µM, 1.0µM, and 5.0µM, respectively).

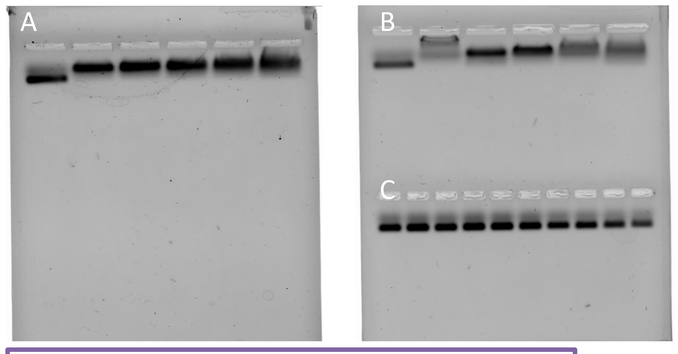
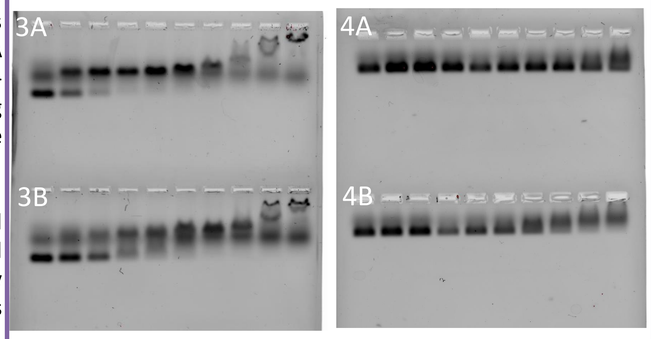


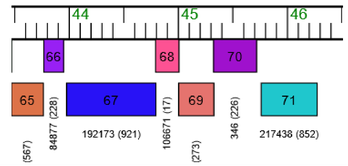
Figure 2B: Assay is identical to 1A, but except that the repressor was allowed to pre-bind DNA before the addition of antirepressor.

Figure 2C: Test of potential DNA + antirepressor interactions. Lane 1 has only DNA, the following lanes have increasing concentrations of antirepressor only to determine if the antirepressor binds DNA.

Figure 3: A) DNA and repressor only. Lane 1 contains labeled DNA only, followed by increasing concentrations of repressor. The band shift indicates protein:DNA complex formation. **B**) Lane 1 contains DNA + antirepressor mixture, followed by increasing concentrations of repressor. Under these conditions, the antirepressor has reduced the repressor DNA affinity.

Figure 4: RGL3 repressor DNA binding assay on a control DNA substrate it should not recognize (**A**)and a predicted DNA substrate (**B**). Lane 1 contains DNA, followed by increasing concentrations of RGL3 repressor. Results show a band shift in panel **B**) not observed in **A**).





Conclusions

- Chosen candidate gene for antirepressor does appear to weaken repressor binding affinity
- Weakened repressor affinity is observed when repressor attempts to bind DNA in the presence of antirepressor
- Antirepressor protein does not bind to DNA
- Preliminary data shows that the RGL3 fusion protein binds DNA. Currently, we do not know if this affinity is weaker than would be observed with the RGL3 repressor domain alone

Future Work

- Continue optimizing assays to calculate changes in DNA binding affinity (KD) in the presence of antirepressor
- Use of two-hybrid to observe if the antirepressor has any interactions with host bacterial proteins
- Purify both the the Adahisdi antirepressor and RGL3 repressor fusion proteins to use in structural studies

References

McGinnis, R.J., et al. Nat. Commun. 2022, 13(1):4105. doi: 10.1038/s41467-022-31678-6.

Duddy, O.P., and Bassler, B.L. PLoS Pathog. 2021, 17(1):4105. doi: 10.1371/journal.ppat.100907410.1371/journal.ppat.1009074

Beggs, G.A., and Bassler, B.L. Curr. Opin. Microbiol.. 2024, 80:102519. doi: 10.1016/j.mib.2024.102519

Acknowledgments

Thank you to Dr. Jamie Wallen and Dr. Maria Gainey, and the WCU department of the Chemistry and Physics