Jeremy Hogaboom

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**A Study of E. Coli RNA polymerase - Template interaction utilizing**

**CRISPR/dCas9 protein**

The goal of FURSCA this summer was to study the interaction between two different RNA polymerase enzymes (RNAP) and a protein blockade bound to a DNA template. RNAP is the enzyme responsible for transcription: copying DNA into RNA. As the enzyme moves along the DNA template, it often runs into proteins bound to the template. I am working to study what happens between the two proteins when that happens. This project was based on the idea of developing a reversible and specific transcriptional control mechanism via the protein dCas9.1 This work, done by Dr. Rohlman and his associates showed that dCas9 could be used to pause transcription by binding a dCas9 protein to a target sequence using guide RNA (sgRNA). The dCas9 protein paused transcription, but not all of the RNAP enzymes behaved similarly. The RNAP from *Escherichia coli* (eco RNAP) remained bound to the template longer than bacteriophage RNAP. My goal for this summer was to pause transcription of both *E. coli* RNAP, as well as T7 RNAP (a model bacteriophage RNA Polymerase) and compare the molecular interactions with the blockade.

Previous work on this project was done by Max King and Khulan Enkhbaatar, so I used their protocols to start my work. First, transcription conditions needed to be set so that we could consistently see RNA products at low time intervals. Then, I could gather the materials needed to pause transcription. This would include amplifying sgRNA and collecting dCas9 proteins. From there I could start exploring pausing conditions and developing a protocol for consistently pausing transcription. This would allow future crosslinking and studying of the protein interactions as well as the interactions of the nucleic acids present.

I was able to continue the T7 work where LuLu left off. I found optimal transcription conditions and was able to see RNA products in as little as 15 seconds. From there I began to synthesize sgRNA using two-step PCR. 2 This is still a work in progress, the product I am getting is not entirely consistent with the literature, but we have yet to try it in a transcription. We had a small amount of dCas9 available from UofM, but I ordered a plasmid that had the dCas9 gene cloned in3. I transformed this plasmid into DH5-α cells and grew them up, but I have not yet harvested the protein. These samples are both frozen away and ready to be used in the fall.

The *E. coli* has been much more difficult. For starters, it is a larger and more complicated protein. The T7 is an extremely efficient and well characterized RNAP, whereas the eco RNAP is not as efficient or commonly used in transcription. I have tried several published procedures with little luck, but there are a wide variety of papers still to read1. We tried the addition of ApC, as well as changing the template DNA that we were transcribing from4. Currently we are working on linearizing a simplified plasmid that includes the necessary promoter to transcribe off of. I did not have time to finish this process, but I have frozen large samples of it away for future use.

I will continue to work at this in the fall while moving forward on the T7 RNAP work. I am looking forward to exploring crosslinking for the first time. When I do finalize the eco conditions, my work on T7 will have given me the necessary experience to proceed with pausing conditions and crosslinking much faster the second go. I will be presenting this work along with any updates at Elkin Isaac, as well as at ASBMB. I am also writing my thesis based on this work.

Last summer, I was modeling this project using a variety of different molecular modeling software. I learned so much through that summer, and it was a great way to use my time stuck at home. This summer I was able to take everything that I had learned and apply it to a laboratory setting. Full-time lab work was a new experience for me, and I absolutely loved it. This confirmed the love for research that I thought I began to experience last year. Living on campus and being able to fully focus on my research was a fantastic experience that I will remember moving on to graduate school in the future.

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References

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