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Exploring A Novel Transcription Factor: Cloning and Purification of the ARF-1 PB1

The Auxin Response Factor (ARF) family of proteins exist in plants to regulate growth and development by repressing or activating transcription. ARF proteins have three domains: the DNA binding domain, the middle region, and the PB1 domain. The PB1 domain that allows ARF proteins to interact with other ARF proteins as well as IAA proteins that regulate the protein based on the presence of the hormone, auxin. ARF-1 is a transcriptional repressor, and while the PB1 domains of the activating proteins ARF-5 and 7 have both been structurally characterized, we cannot assume that repressors such as ARF-1 will act in the same ways that activators do. The focus of our research over the course of this academic year has been on the interactions between the ARF-1 PB1s domain and the PB1 domains of other ARF proteins. We need to compare the isolated domains to determine how they will interact. Our research has had two overarching goals; to clone the gene for the ARF-1 PB1 into E. coli and to purify the protein out of those cells to test for protein-protein association.

The first step in our research was to successfully clone the ARF-1 PB1 into a plasmid, or circular DNA, for expression in E. coli. To do this we used ligation independent cloning which is advantageous as it is a relatively fast process. This consisted of creating primers that corresponded with the PB1 gene, using PCR to amplify that gene from cDNA, preparing the E. coli DNA (or vector), and finally combining the PCR product and vector before transforming them into E. coli cells to be fully combined. Given past success with ARF-5 and 7, we cloned our gene into a vector containing a fusion tag of 6 histidines.

The next step was to express the ARF-1 PB1 in cells. We grew a liter of cells to be able to produce enough protein for later work. E. coli cells used for protein growth are modified in that they have a lac operon that represses the expression of the genes we cloned into them. After the cells grew to a certain concentration we induced protein expression with IPTG which stops the lac operon from restricting expression.

Later, we harvested the cells through centrifuging and lysed them to release the proteins. Then we used a metal affinity resin column to purify out the protein. The metal affinity of the resin functions by capturing the protein by the 6-histidine tag which bonds to it, allowing the other proteins to wash
away in a phosphate buffer. Then the protein is eluted out with a different buffer. We will use gel filtration to detect protein association under the same conditions.

With aims toward future work, we took the first step in designing mutant versions of the ARF-1 PB1. Other research has shown ARF-5 and 7 interact due to charges on their PB1 domains. The mutants we design will remove one or both charges on ARF-1 allowing us to determine if there are other factors that control protein interaction. We have already designed two mutants that correspond to mutant versions of ARF-5.

This poster covers the ligation independent cloning (LIC), protein purification processes, and preliminary tests for association. It explains what procedures were used for both processes and outlines what results were shown. Finally, we present the design of mutants with the potential to disrupt oligomerization. The eventual goal is to develop charge mutants for the ARF-1 PB1 domain that correspond to mutants of other ARFs to see how those mutants interact together.

The LIC was successful, we confirmed this as sample of the product DNA we sent to Cornell University to be sequenced was found to be identical to the ARF-1 PB1. We also know that the protein purification worked as there were results in the protein a280 readings. We will use and acrylamide gel to further confirm the results. This means that we have produced a sample of the ARF-1 proteins PB1 domain.

This research is one step toward having a more complete knowledge about how ARF-1 specifically, but also other ARF repressor proteins function. By being the first to purify the ARF-1 PB1 we set the stage for future researchers to use this protein in even more contexts. In the future, observing how the protein we purified interacts with itself and other ARFs will give greater insight into how transcription activators and repressors interact.

**Bibliography**