Developing a Protein Purification Protocol to Investigate the Non-Canonical Membrane Binding and Deformation of F-BAR Protein Subdomains

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The cell membrane is a crucial piece of cellular architecture, responsible for altering the cell's shape and contributing to key processes such as growth and movement. The exact mechanisms through which protein subsystems regulate membrane deformation remain unclear, however. One group of regulatory proteins, known as BAR proteins, are characterized by their "banana-like" shape and ability to selectively sense membrane curvature[1]. Typically, these proteins pull the membrane inward to create invaginations[2], yet a subclass of BAR proteins containing the F-BAR domain exhibit the opposite behavior and instead push the membrane outward to form protrusions[3]. This non-canonical behavior has been observed in the proteins srGAPs 1, 2, and 3–all of which have been implicated in regulating neuronal cell development[4]. A long term goal of the Henderson Lab is to study the F-BAR domain's regulatory mechanism by interacting the protein subsection with model cell membranes composed of charged phospholipids. My project this summer was a key first step: using recombinant DNA technology to isolate purified F-BAR protein domains of interest from full mammalian srGAP proteins.

To begin, I began by designing a plasmid cloning vector. A plasmid is a circular piece of DNA that has a special region of interest, containing the coding sequence for a desired protein, and a longer vector or "backbone" region designed to aid in the purification and amplification process. My plasmid was designed to contain the F-BAR region of srGAPs 1-3 along with a backbone that contained antibiotic resistance to ensure that only the bacteria who successfully took up the plasmid would survive in culture. Additionally, it contained a histidine tag to eventually purify the protein via affinity chromatography.

First, I transformed E. Coli with the plasmid backbone, injecting the plasmid DNA into their genome. Harnessing the exponential growth of bacteria, the culture was then grown, with each new bacteria copying its DNA along with the inserted plasmid. This method quickly amplified my small starting amount of plasmid to a large, usable stock. A similar method was used to amplify the F-BAR region of interest.

To yield the linearized backbone from a circular plasmid, I employed the use of restriction enzymes, which cut at engineered DNA sites. To isolate the F-BAR region from its circular plasmid, I used the Polymerase Chain Reaction (PCR) technique, a cloning method which is able to extensively amplify a select region of the plasmid. After isolating the backbone fragment and the F-BAR fragment, I used the Gibson Assembly cloning method to combine these fragments back into a circular plasmid that will be used for protein expression. Results from each step of DNA editing were assessed with Agarose Gel Electrophoresis to ensure reaction fidelity.

Once the plasmid sequence is optimized for bacterial expression, next steps will include affinity chromatography, which will enable the purification and isolation of the F-BAR protein domain in preparation for in vivo experiments.

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Faculty Mentor: Professor Michael Henderson

Funded by the Student Faculty Research Grant Fellowship supported by the IDeA Networks of Biomedical Research Excellence (INBRE) National Institutes of Health award