

Identification of proteins associated with the Slit-Robo GTPase Activating Protein family to examine the mechanisms of filopodial formation
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The human brain contains roughly 100 billion neuronal cells, which send electrical impulses from the axon of one cell to the dendrite of the next to regulate bodily functions, encode memories, and support cognitive processing.¹ The proper formation of neuronal junctions, called synapses, is fundamental to brain function (Figure 1). Disruptions in synapse formation are implicated in a diverse range of mental disabilities and neurocognitive disorders, including autism spectrum disorder.^{2,3} Studying the mechanisms of synapse formation can further illuminate the underpinnings of brain function to better understand these disorders.

One important player in synaptic development is the Slit-Robo GTPase Activating Protein (srGAP). A subfamily of Bin-Amphiphysin-Rvs (BAR) protein, srGAPs bind to and bend the cell membrane to match the protein's curved shape, initiating the outgrowth of long finger-like protrusions called filopodia.⁴ Filopodia play a large role in synapse formation, helping a cell sense the environment and acting as precursors to dendritic spines in a synapse.⁵ As regulators of filopodial growth, srGAPs initiate the growth of dendritic spines and guide a growing axon to the dendrite.^{5,6} The absence of the srGAP gene, therefore, is related to severe mental disability and disruption of long-term memory formation.^{7,8}

Although much is known about the filopodial forming role of srGAPs in neuronal cells, little is understood about the mechanisms behind these processes. My project aims to uncover the specific protein interactions that drive filopodial growth by identifying srGAP-associated proteins in mouse neuronal cells.

I will identify these proteins using a molecular tagging mechanism known as biotin ligase proximity labeling.⁹ This technique utilizes an enzyme called TurboID, which adds a biotin molecule to proteins within a 20 nm radius. By engineering DNA containing adjacent TurboID and srGAP genes, I can create an srGAP protein linked to a TurboID enzyme. The TurboID will thus add biotin to any proteins interacting with srGAP. To ensure that TurboID only biotinylates filopodial proteins, the enzyme will be split into inactive halves, with one half linked to srGAP and the other embedded in the cell membrane. The enzyme is only active when srGAP is at the membrane, where the two halves can refold (Figure 2). Biotin has a high affinity for streptavidin, allowing the use of streptavidin beads to trap and isolate all biotinylated srGAP-associated proteins. I will then use Mass Spectrometry to identify each srGAP associated protein.

I spent this summer designing and amplifying the DNA that will encode for the srGAP-TurboID and TurboID-membrane protein constructs (Figure 3). To start, I mapped out circular DNA, called plasmids, that combine each gene of interest into a single strand (e.g. srGAP and TurboID halves). I then isolated the DNA for each gene from bacteria using DNA purification techniques.

The assembly of both DNA constructs involves annealing multiple fragments of DNA with overlapping homology to each other. I designed this homology with small segments of DNA that were incorporated into the existing genes during polymerase chain reaction (PCR). After PCR, I was left with amplified segments of each gene with the added overlaps. To isolate the DNA and confirm proper amplification, I digested the sample with an enzyme and ran each sample on an agarose gel using electrophoresis, which separated out the template DNA from my genes of interest. I was successful in isolating each gene of interest, which was present on the gel at the expected DNA length (Figure 4). The isolated DNA was then cut out of the gel and purified. For each gene of interest, I succeeded in purifying at least 0.8 picomoles of DNA for future primer construction procedures.

I look forward to the continuation of my project and the construction and expression of my protein constructs in mouse neuronal cells as I begin my Honors Project in the upcoming academic year.

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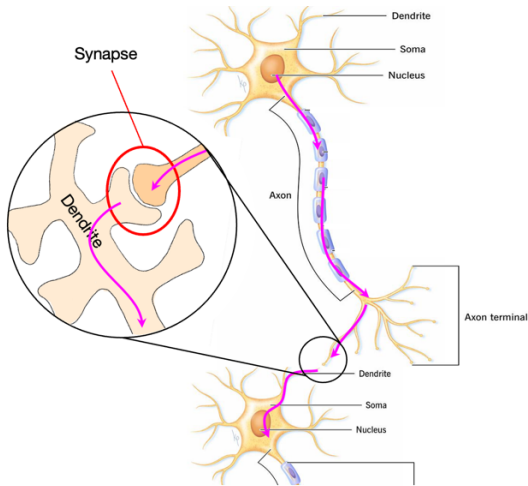


Figure 1. An electrical impulse (pink arrow) traveling through the axon of one neuron into the dendrite of another through the synapse

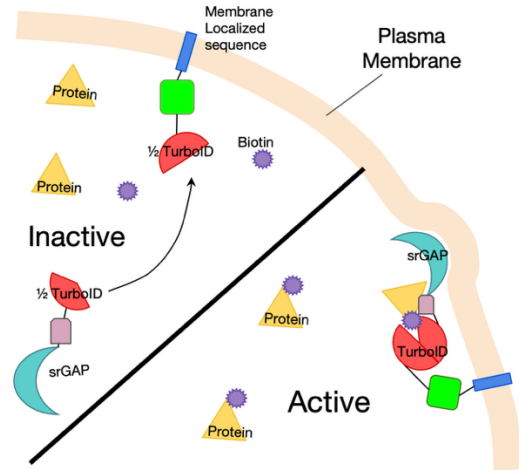


Figure 2. Reformation of TurboID into an active biotinylating enzyme

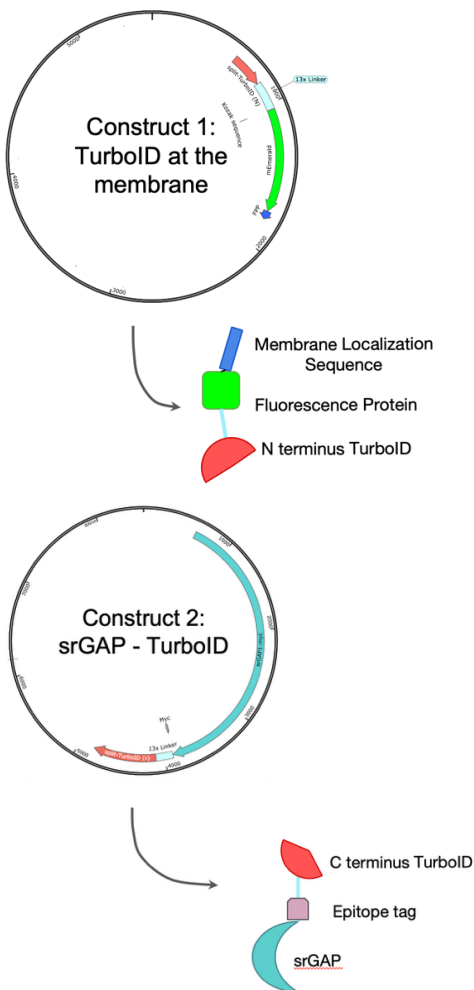


Figure 3. Plasmid DNA encoding my two protein constructs

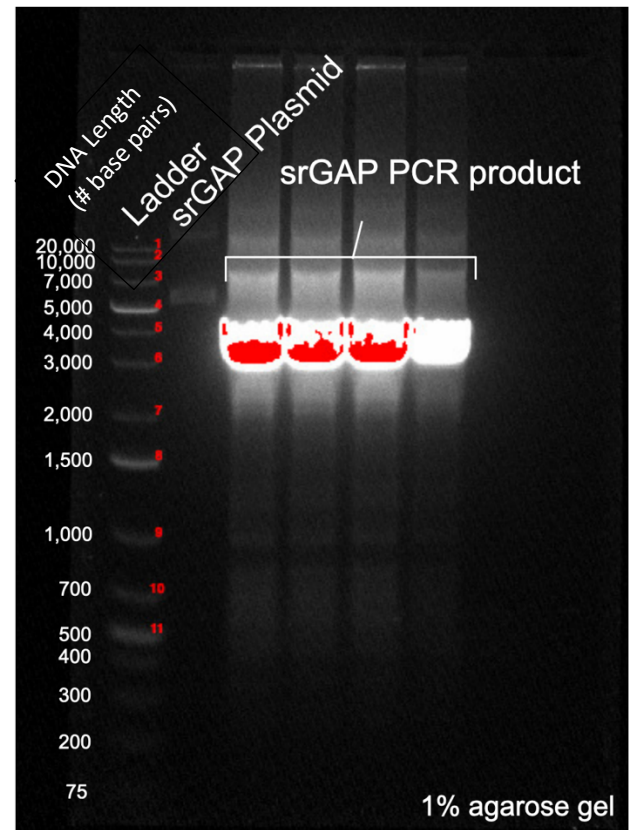


Figure 4. UV image of srGAP DNA amplified with polymerase chain reaction. The ladder acts as a reference for known lengths of DNA. The construct runs slightly above 3,000 base pairs, which is consistent with the expected gene length of 3291 base pairs.

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