CALIFORNIA STATE SCIENCE FAIR
2011 PROJECT SUMMARY

Name(s)
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Project Number
31848

Project Title
Fluorescent Complexin and Its Role in Cellular Exocytosis

Abstract

Objectives/Goals
Cell secretion (exocytosis) is a cellular process in which vesicles fuse with the cell membrane to release its content to the extracellular environment. The way in which this process is regulated is not yet fully understood yet several proteins have been identified which are believed to play an important role in cell secretion. One of these regulators is complexin (CPX), a protein reported to bind to the SNARE protein complex, a bundle of proteins that mediates vesicle priming and fusion (see image on top right). The goal of this project is to create a genetically encoded fluorescent CPX that I can visualize in living cells.

Methods/Materials
The first step was to create a fluorescent CPX construct using standard molecular biology techniques such as:
#Polymerase Chain Reaction (PCR) - Technique used to amplify DNA
#Restriction Enzymes # Enzymes that cut single or double strands of DNA
#Ligation # The binding of complementary strands of DNA
#Transformation # A process that causes cells to uptake DNA that is not in their genome.
#Miniprep # The extraction of plasmid DNA from cells.

I would use gel electrophoresis to test the functionality of a fluorescent CPX DNA construct and later on TIRFM imaging to test the hypothesis, that complexin localizes near primed vescicles. We would image fluorescent CPX protein within cells (AtT20 and mouse chromaffin cells).

Results
After I introduced two enzymes to the dsRed vector so that they could cut or #digest# the protein at the proper areas. I discovered that these enzymes (Xho1 and BamH1) had not been working the way they should. When I first ran the finished product on a gel to make sure that they cut at the right place, the gel confirmed that. When I tried to introduce the construct to some cells however, it wasn't expressed. By January I had given up on the enzymes and protein, and began trying to bind GFP to the Complexin strand. This attempt resulted in a successful ligation and DNA construct.

Conclusions/Discussion
We will now express the GFP-CPX construct in cells to image and test its function. By determining the localization of CPX in living cells, we can further clarify CPX#s role in secretion. This can provide new targets for pharmaceutical treatments of diseases where secretion is impaired, such as diabetes.

Summary Statement
CPX fused with a fluorescent protein (e.g. dsRed or GFP) can be imaged at the cell surface using TIRFM (total internal reflectance fluorescence microscopy) so that we can confirm that CPX localizes with primed vesicles.

Help Received
Used lab equipment at the University of Southern California; Participant in Engineering for Health Academy; Assisted by Dr. Joyce Rohan, Dr. Rey Dominguez, Dr. Robert Chow, and Rose Citron