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## Does CaMKII Regulate Exocytosis Through Its Binding To Syntaxin?

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### Background

Neurons use chemical messengers called neurotransmitters to communicate with muscles and other neurons. In the axon terminal of a neuron, neurotransmitters are stored in synaptic vesicles. Neurotransmitter is released from the neuron through a process called exocytosis. In exocytosis, synaptic vesicles fuse with the cell membrane and neurotransmitter is released from the cell. During exocytosis, important proteins in both the synaptic vesicle membrane and cell membrane called SNARE proteins bind to one another. These proteins are required for exocytosis. Exocytosis and subsequent neurotransmitter release is regulated by  $\text{Ca}^{2+}$ . The mechanism by which  $\text{Ca}^{2+}$  regulates exocytosis is poorly understood.

Recently, a protein called  $\text{Ca}^{2+}$ /Calmodulin-Activated Protein Kinase II (CaMKII) has been suggested as an important component in the regulation of exocytosis (Ohyama et al., *Journal of Neuroscience*, **22**: 3324-3351). When CaMKII binds to  $\text{Ca}^{2+}$ /calmodulin it uses ATP to become activated. Activated CaMKII has been shown to bind to syntaxin, an important SNARE protein in the cell membrane, in a  $\text{Ca}^{2+}$ -dependent manner. This binding has been observed both *in vitro* and *in vivo* and has been shown to affect exocytosis.

I planned to further investigate the binding of CaMKII to syntaxin and its role in exocytosis through this binding. I was in a unique position to do this project because of the excellent resources available to me. Many of the resources I needed were available to me in Dr. Woodbury's lab, where we study the regulation of exocytosis by SNARE proteins. CaMKII was available to me from Dr. Barry Willardson in the Department of Chemistry and Biochemistry.

The goal of my project was to characterize the interaction between syntaxin and CaMKII and to investigate the role of CaMKII in regulating exocytosis through its binding to syntaxin. I hypothesized that if CaMKII binds to syntaxin in a  $\text{Ca}^{2+}$ -dependent manner and increases its affinity for other SNARE proteins it will enhance the rate of fusion in our lab's *in vitro* fusion assay. This result would suggest that CaMKII has an *in vivo* role in regulating exocytosis. To investigate the interaction between CaMKII and syntaxin and the role of CaMKII in exocytosis I planned to conduct three *in vitro* experiments: 1) a protein binding study to determine whether CaMKII binds to syntaxin in a  $\text{Ca}^{2+}$ /ATP-dependent manner, 2) a protein binding study to determine whether the syntaxin-CaMKII complex can bind other SNARE proteins with a higher affinity than syntaxin alone, and 3) time permitting, a fusion assay investigating the role of CaMKII in exocytosis. The fusion assay, used routinely in our lab, measures the rate of fusion between modified synaptic vesicles and a planar lipid bilayer containing syntaxin.

### Results

I was only able to conduct the first of the three proposed experiments. Unfortunately, we discovered that we did not have all the tools necessary to conduct the second experiment and had no success in trying to acquire them. I did not complete the third experiment because we did not

have enough evidence yet to warrant its attempt. After conducting the first experiment three times, I looked closely at the results, which I determined to be inconclusive. There were weak indications that activated CaMKII was binding to syntaxin but it was inconsistent and lacked robustness. I was concerned that the CaMKII I was using may no longer be functional because it was prepared two years ago and has been stored in a freezer since then. I learned of a test commonly used to assess the activity of protein kinases like CaMKII. This test involves incubating a protein kinase with radiolabeled ATP and if the protein is functional it will incorporate  $P^{32}$  from the ATP. I decided to use this test to determine whether the CaMKII I was using was still functional. I attended a course on radiation safety on campus and passed an exam to become authorized to use radiolabeled compounds. I conducted the experiment in Dr. Will Winder's lab and determined that the CaMKII was in fact functional. I then repeated the first proposed experiment and again the results were inconclusive and lacked strong evidence that activated CaMKII was binding to syntaxin. I feel that my failure to observe obvious indications of the binding between CaMKII and syntaxin does not eliminate the possibility of their interaction in real cells. Although I ruled out the possibility that my CaMKII sample is not fully functional, my experimental protocol or reagents may be flawed or nonfunctional.

## **Future Directions**

I feel that the project idea is still valuable and worth pursuing. The protein-protein interaction I proposed to investigate could be a very important component in the regulation of  $Ca^{2+}$ -dependent exocytosis. Recently, another paper has been published that investigates and provides support for this interaction (Nomura et. al., Journal of Neuroscience Research, **72**: 198-202). It is possible that someone from our lab will pursue this project in the future. Had I had more time to work on the project, I would have next tried to experiment with the protocol I designed to optimize conditions for CaMKII-syntaxin binding and perhaps tried several new reagents in place of the ones I was using. I also would have tried the second proposed experiment once I had the tools I needed.

## **Conclusions**

Overall, the entire experience was very valuable for me. This summer I am starting a graduate program in neuroscience at the University of Michigan. The opportunity that the ORCA Scholarship gave me to propose, design, carry out, and report on experiments will prove to be very beneficial.