Progress/Final Report Template

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Title of Project: The activity dependent regulation of Parkinson's disease associated synuclein in presynaptic plasticity.

Summary:

A hallmark feature of the pathology Parkinson's Disease and related neurodegenerative disorders is the presence of Lewy bodies in neurons. Alpha(α)-synuclein is the predominant component of Lewy bodies, which are made of aggregated proteins. The normal biological function of α-synuclein in neurons is not well understood and this limits the understanding of how α-synuclein contributes to disease. Like most proteins, the function of α-synuclein is regulated by phosphorylation, which is a chemical modification of protein side chains. Phosphorylation is a signalling mechanism within cells that changes the function of proteins in response to environmental stimuli. An example of changed function is when phosphorylation prevents two proteins from interacting, which might stop a particular biological process. The α-synuclein in a healthy brain has very low levels of phosphorylation and is difficult to detect. The α-synuclein in Lewy bodies is highly phosphorylated and so phospho-signalling is relevant to the disease state. We have studied the phosphorylation of α-synuclein in neurons in response to the signals associated with neurotransmission. We discovered a new phosphorylation on α-synuclein. The phosphorylation site is interesting because it is in the part of α-synuclein that likely binds to synaptic vesicles. Synaptic vesicles are the tiny packets of neurotransmitter that fuse with the neuronal membrane to allow neurotransmission. Our aim was to develop tools to study the role of the new phosphorylation site and to determine how phospho-signalling changes α-synuclein function.

The main tool developed was an antibody for the new phosphorylation site on α-synuclein. The antibody was confirmed as being specific for only the phosphorylated α-synuclein. In a continuation of this project, this validated tool is now being used to answer a number of questions about how the phosphorylation affects α-synuclein function. In parallel, we investigated how signalling that occurs during neurotransmission affects which proteins bind to α-synuclein. Previous research has shown that α-synuclein can bind a large number of proteins; however, the methods used don't indicate the dynamic binding that might occur during neurotransmission. We compared the proteins that bind to α-synuclein, before and after a stimulus that normally evokes neurotransmission. Six hundred and twenty three interacting proteins were identified. A small subset of proteins changed their binding to α-synuclein following a stimulus, indicating more clearly that they are involved in the same

biological process as α-synuclein. We are now continuing our investigation of these candidates to shed light on normal α-synuclein function.

Hypothesis vs Findings

We hypothesised that the α-synuclein phosphorylation site we discovered was involved in regulating neurotransmitter release and/or synaptic plasticity. We found that several proteins changed their interaction with α-synuclein following a stimulation that evokes neurotransmitter release. Also, we have generated many tools to further test this hypothesis, including the first phospho-specific antibody to this site, phospho-mimetic and phospho-deficient mutant plasmids for this site to be used in both in vitro binding experiment and cellular assays to directly test the effect of phosphorylation on neurotransmitter release. These experiment are currently being done by a new collaboration arising from this work.

Unanswered Questions

As stated above, the bulk of the project involved tool generation and so these tools are only now being used to properly test the hypothesis. Furthermore, the findings indicate that there are likely a small number of proteins which change their binding characteristics towards α-synuclein following a stimulus. We can now determine the nature of these interactions.

What these research outcomes mean

For the first time there is an antibody and a suite of molecular genetics tools for a particular novel phosphorylation site that can be used to advance α-synuclein and Parkinson's Disease research. As a result of this project, a major collaboration has been initiated to advance knowledge in this area. The tools and data generated are now being used to leverage further funding, answer bigger questions and enable more collaborations.