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C2 Domain Function in Healthy and Diseased Brain

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English Summary

Imagine the complexity of neuronal processes involved in saying the right thing at the right time. Assessing the situation, recalling previous relevant experiences, estimating the effect your words will produce in the listener, adjusting the tone, volume and facial expression to convey the intended message. Knowing when to start talking. We can do all of this, usually in less than a second. The work going on under the hood is fast, reproducible and seamless (well, most of the time).

If we look at the machinery involved in neuronal function, its complexity is impressive even at the basic level of releasing a certain amount of neurotransmitter at the right time and location. One of the fundamental tools neurons use to regulate their activity is based on calcium signaling, and there are many different proteins sensing and translating this message.

Multiple proteins translate the elevation of local calcium concentration into membrane fusion. Double C2 domain proteins belonging to Synaptotagmin and Doc2 protein families play an important role in the coupling of calcium signal with synaptic vesicle release. Their activity will depend on such factors as precise dosage of calcium in the presynaptic terminal, lipid composition of the cell.

C2 domains were first described in 1980's as calcium regulated binders of acidic phospholipids, and the first crystal structure of a C2 domain was obtained in the 1990's. Many laboratories across the world have worked on C2 domain proteins over the last 30 years, particularly Synaptotagmin-1 has been studied extensively. Searching on pubmed using "C2 domain" as keyword produces over 1000 publications, and more than 2000 results for "Synaptotagmin".

Still, we have a lot to learn about the function and regulation of C2 domains, and describe more of their common features. Identification of "common rules" in the characteristics and behaviour of C2 domains will speed up future characterization of C2 domain function, as well as prediction of patient mutations impact on disease pathogenesis. For example, we know exactly what amino acids are responsible for directly contacting calcium ions in Synaptotagmin-1 and Doc2b, and this helps us predict calcium-binding residues in homologous C2 domains, and identify mutants with defects in calcium binding.

In the work described in this thesis, we have attempted to leverage the common properties of C2 domains in order to study three different proteins: Piccolo, Doc2b and CC2D2A. We have also used Synaptotagmin-1 C2 domains as reference for protein purification method development. We have used different approaches to study

these C2 domains: on one hand, we have studied variations in C2 domains produced by mutations in corresponding genes in patients (chapters 2 and 4). From another perspective, we applied a biophysical tool to investigate C2 domain interaction with membranes (chapter 3). These two approaches can be combined in the future to further deepen our knowledge of C2 domains and their role in health and disease.

In Chapter 2 we describe the effect of a genetic variation in the C2 domain of Piccolo associated with major depressive disorder. We created a knock-in mouse model that carried the variant of the Pclo gene found in patients, and compared it to the mouse carrying the “healthy” version of the same gene. We found that the mice with depression-associated version of Piccolo had slightly higher amounts of Piccolo protein in synapses and increased levels of neurotransmitter release, but the behavioral parameters of these mice was normal.

In Chapter 3 we describe the development of a new method to study the interaction of C2 domains with phospholipid membranes. We used optical tweezers, that allow to measure forces at molecular levels, to detect the effect that Doc2B C2 domains produce on membranes. We created an experimental model representing cellular membranes, in a controlled environment where we could add the desired concentrations of calcium and C2 domains, as well as control the lipid composition of the membranes. This method was also combined with real-time fluorescence microscopy imaging to not only measure the force, but also visualize the membrane configuration at the same time. This allowed us to see and measure double C2 domains of Doc2B induce hemifusion of phospholipid membranes in the presence of calcium and their specific lipid interaction partner phosphatidylserine.

Of the proteins studied in this thesis, CC2D2A (Chapter 4) stands apart since it is not known to be part of the presynaptic release machinery. There were indications that CC2D2A could be important for cilia formation or function, but very little was known about a potential mechanism. Nothing was known about the properties of the CC2D2A C2 domain, except that mutations in the CC2D2A gene resulting in the deletion of its C2 domain were observed in patients with severe neurological disorders. We have studied cellular expression of deletion mutants of CC2D2A, as well as calcium-dependent phospholipid binding by its C2 domain. We did not detect the presence of normal CC2D2A at the cilia in neurons, but found that C2 domain deleted versions of CC2D2A were unstable and present at very low levels in the cells.

Chapter 5 contains a detailed description of the methods for production and purification of recombinant C2 domains, which were indispensable for all the projects described in this thesis, particularly for the highly sensitive force measurements.