Characterization of the Rabphilin3A and SNAP25 interaction in PC12 cells.

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Endless eukaryotic cell biological processes rely on membrane fusion, including the synaptic transmission. The liberation of neurotransmitters to the synaptic space is a deeply complex process that needs an accurate control of the recruitment of the many proteins involved. Numerous of these proteins share a common structural motif: the C2 domains, which are regulated by their ability to bind Ca^{2+}, phospholipids and other proteins, providing them with the capability to fine-tune the broad range of vesicle release modes.

Rabphilin3A (Rph3A) is a membrane trafficking protein involved in the Ca^{2+}-dependent regulation of secretory vesicle exocytosis in neurons and neuroendocrine cells. In this work, we have used *in situ* protein ligation assay (PLA) to characterize the molecular determinants driving the Rph3A-SNAP25 interaction in PC12 cells. We observed that these interactions occur both in the cytosol and at the plasma membrane. These signals correspond to populations of transport and synaptic vesicles that might contain Rph3A-SNAP25 and vesicles docking respectively. Furthermore, staining HA-Rph3A and myc-SNAP25 by immunofluorescence demonstrated that both proteins localize as expected. Site-directed mutagenesis of important aminoacidic residues located at the C2B bottom α-helix as well as two regions of the SNAP25-N helix showed that these two motifs are important for the Rabphilin3A-SNAP25 interaction at the plasma membrane and contribute to explain some of the numerous steps and interaction events which take place at the presynaptic neuron.

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