Research Briefings

Luke Chamberlain
Regulation of Neurotransmitter Secretion

SYNAPTIC VESICLE FUSION WITH THE NEURONAL PLASMA MEMBRANE UNDERLIES EVERY MOVEMENT, SENSATION AND THOUGHT. SIMILAR VESICLE FUSION EVENTS OUTSIDE THE NERVOUS SYSTEM REGULATE BLOOD INSULIN LEVELS, ALLERGY, FERTILISATION AND THE 'FIGHT OR FLIGHT' RESPONSE. OUR RESEARCH IS FOCUSSED ON DEFINING THE MOLECULAR MECHANISMS THAT MEDIATE VESICLE FUSION AND ON IDENTIFYING PERTURBATIONS THAT OCCUR IN PATHOPHYSIOLOGY.

Specialised cells throughout the body contain secretory vesicles that are filled with specific cargo. Following stimulation, these vesicles fuse with the plasma membrane, releasing their soluble cargo to the cell exterior. This membrane fusion event is termed exocytosis and is the process used by neurons to secrete neurotransmitters, peptides and other important molecules. Exocytosis is also an essential process outside the nervous system and mediates the secretion of molecules such as insulin and adrenaline into the bloodstream. Abnormalities in exocytosis are associated with a range of important clinical conditions, including diabetes, schizophrenia and epilepsy.

Exocytosis is mediated by a family of proteins termed SNAREs. The importance of SNARE proteins is highlighted by the finding that these proteins are the specific targets of clostridial neurotoxins, potent inhibitors of neurotransmitter release.

SNARE PROTEIN FUNCTION IN EXOCYTOSIS

SNAP25 is one of the essential SNARE proteins that mediates neurotransmitter secretion. Our work is investigating how SNAP25 is regulated by post-translational modification (1). Our previous work has shown that the attachment of fatty acids

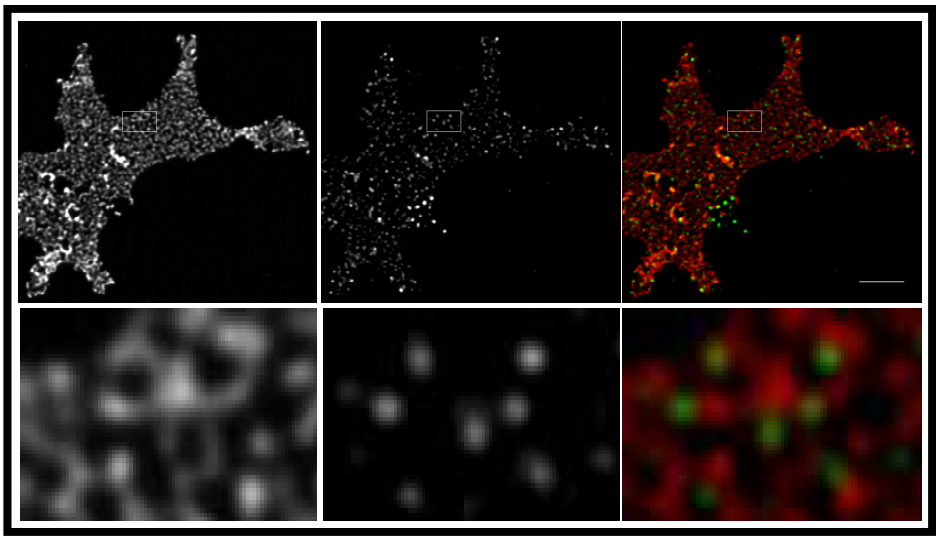

Figure 1. Protein Micro-Localisation at the Plasma Membrane. The image shows plasma membrane sheets generated from neuroendocrine PC12 cells and stained with fluorescently-labelled antibodies against SNAP25 (left panel) and another plasma membrane protein (middle panel). The right panel shows a merge with SNAP25 in red. The lower panels are zoomed images of the top panels. This figure highlights that proteins at the plasma membrane may have distinct, non-overlapping micro-localisations. We are investigating whether the precise micro-localisation of SNAP25 is important for function.

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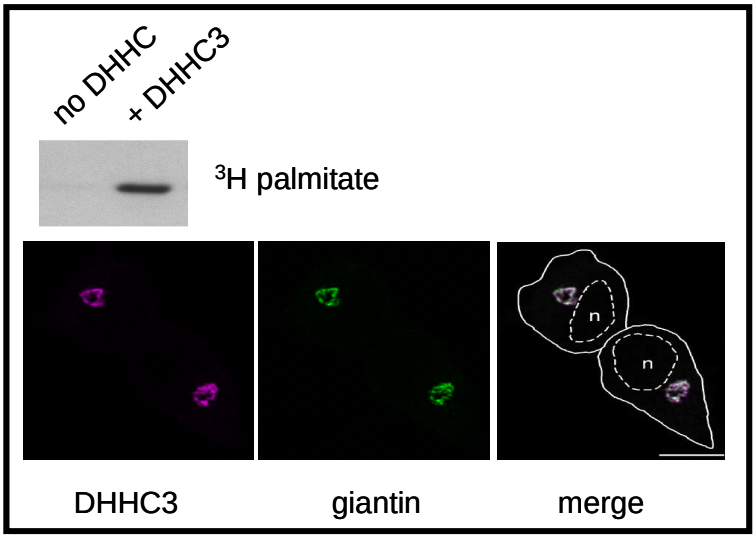

Figure 2. Identification of DHHC proteins that palmitoylate SNAP25. Top panel shows the incorporation of radiolabelled palmitate into GFP-SNAP25 in the presence and absence of DHHC3. Note that DHHC3 promotes a large increase in SNAP25 palmitoylation. The bottom panel shows that DHHC3 is localised to the Golgi complex, exhibiting strong overlap with the Golgi marker, giantin.

('palmitoylation') plays an essential role in mediating plasma membrane targeting of SNAP25 (2) and also regulates the micro-localisation of the protein in cholesterol-rich microdomains (3).

Recent unpublished work from our group suggests that the reversibility of palmitoylation may provide SNAP25 with a dynamic subcellular localisation that allows the protein to function in membrane fusion events away from the plasma membrane. This work is uncovering novel cellular mechanisms that allow proteins to participate in distinct intracellular pathways.

THE IMPORTANCE OF SPLICING: PHYSIOLOGY AND PATHOPHYSIOLOGY

Many important proteins involved in synaptic vesicle exocytosis have variants that are produced by differential splicing of a single gene. Specific proteins often have essential functions that cannot be compensated by closely-related splice variants. We are investigating the importance of gene splicing in synaptic vesicle exocytosis and examining how this process is perturbed in conditions such as schizophrenia and depression.

PALMITOYL TRANSFERASES: SPECIFICITY, TRAFFICKING AND FUNCTION

A large number of presynaptic and postsynaptic proteins are modified by palmitoylation (4). Recent work identified a family of 23 'DHHC' proteins that mediate protein palmitoylation. Genetic studies have linked several of these DHHC proteins with disorders such as schizophrenia, X-linked mental retardation and cancer. We are investigating the mechanisms that mediate substrate specificity (2, 5) of these enzymes and analysing how the specific subcellular localisation of these DHHC proteins regulates their function.

MOLECULAR CHAPERONES AND NEURODEGENERATION

Cysteine-string protein (CSP) is an important neuroprotective chaperone that regulates protein folding and possibly functions to reactivate synaptic proteins. CSP is extensively modified by palmitoylation, and we are investigating how this modification affects trafficking

to synapses where CSP performs its essential function and prevents neurodegeneration (5, 6).

TECHNIQUES

We employ a range of complimentary techniques, such as live-cell confocal imaging (including FRAP and iFRAP), molecular biology (such as RNAi), and biochemistry.

SELECTED REFERENCES

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