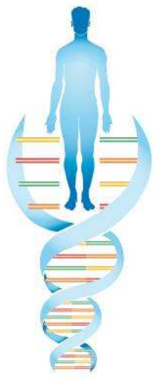

Research Briefings

Mike Cousin
Synaptic Cell Biology

THE RECYCLING OF SYNAPTIC VESICLES IS CENTRAL TO NEUROTRANSMITTER RELEASE, SYNAPTIC FUNCTION AND BRAIN COMMUNICATION. OUR LABORATORY STUDIES THE MOLECULAR MECHANISMS THAT CONTROL BOTH THE ENDOCYTOSIS AND RECYCLING OF SVS. WE ALSO STUDY HOW THESE BASIC PROCESSES ARE REGULATED BY FACTORS SUCH AS NEURONAL ACTIVITY AND PROTEIN / LIPID PHOSPHORYLATION.

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Neurotransmitter release is essential for nerve cell communication. The stimulated fusion of neurotransmitter-containing synaptic vesicles (SVs) with the nerve terminal plasma membrane is essential for this process. The subsequent retrieval and recycling of SVs is equally essential for the maintenance of neuronal communication. There are a number of different routes by which a SV can be retrieved from the nerve terminal plasma membrane. These are 1) Kiss-and-run retrieval (where the SV never fully fuses with the plasma membrane; 2) Clathrin-dependent endocytosis (where single SVs are retrieved de novo; and 3) Activity-dependent bulk endocytosis (where SV membrane is retrieved as large invaginations during intense stimulation). We are particularly interested in the latter SV retrieval route since it is a pathway that may be selectively activated during excessive stimulation such as epileptic seizures.

ACTIVITY-DEPENDENT TRIGGERING OF BULK ENDOCYTOSIS

We have shown that bulk endocytosis is triggered by an activity-dependent protein dephosphorylation cascade. Intense stimulation activates the protein phosphatase calcineurin to dephosphorylate the large GTPase dynamin I. This dephosphorylation event allows an association of dynamin I with the endocytosis protein syndapin. Each step in this cascade is essential for bulk endocytosis, but not any other retrieval route.

We are currently investigating the essential molecular determinants of the syndapin requirement for this

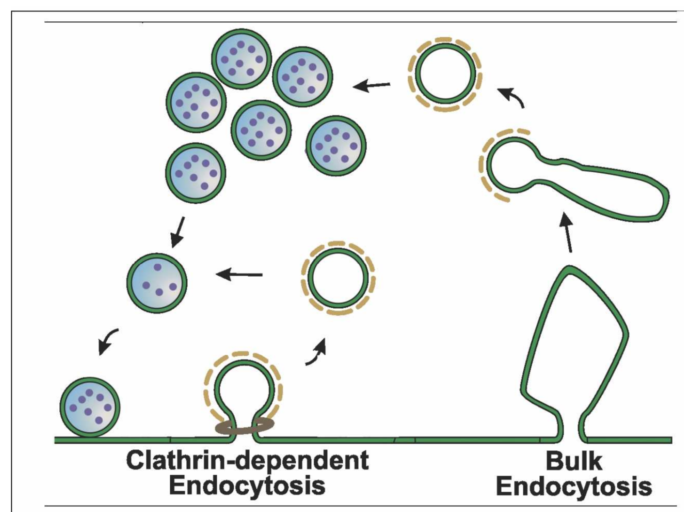


Figure 1: The synaptic vesicle life

retrieval route.

MOLECULAR MECHANISM OF BULK ENDOCYTOSIS

Very little is known regarding the molecules required for bulk endocytosis, apart from its triggering mechanism. We are currently undertaking an unbiased proteomic study to determine the molecules unique to this internalisation route. Generating molecular tools to target key proteins in the mechanism will allow the physiological and pathophysiological role of this retrieval mechanism to be elucidated in more complex experimental systems.

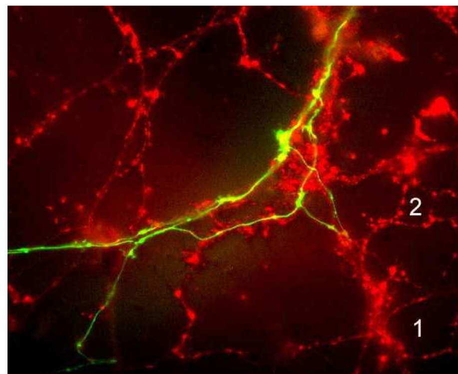
RESERVE POOL REFILLING AFTER BULK ENDOCYTOSIS

We have recently shown that SVs generated by bulk endocytosis selectively refill the reserve pool of SVs within the nerve terminal. The reserve pool of SVs is only mobilised during intense stimulation, providing a possible explanation for its close coupling to bulk endocytosis. We are currently elucidating which molecules are essential for SV budding from endosomes generated by bulk endocytosis. The aim is to generate tools to intervene in the SV life cycle at a novel and key stage that only becomes active during intense stimulation.

TYROSINE PHOSPHORYLATION IN SV RECYCLING

A different line of research concerns the role of tyrosine phosphorylation in nerve terminal function. A number of SV proteins are heavily tyrosine phosphorylated, (such as synaptophysin) however the role of this phosphorylation is undetermined. We have established a research programme that investigates how neuronal src kinases associate with and phosphorylate synaptophysin and how this phosphorylation event controls SV recycling.

All of the projects detailed above use a range of different and complementary techniques. These range from molecular biology, protein biochemistry, cell biology and live cell imaging of neuronal physiology.



Imaging of synaptic vesicle turnover using FM-64. The picture shows primary cultures of cerebellar granule neurones loaded with the vital dye FM4-64. Dye is localised to specific puncta corresponding to nerve terminals. The overlaid green neurone has been transfected with a cytosolic EGFP fusion protein

Selected references

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