Research Briefings

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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 ARE INCREASINGLY INTERESTED IN HOW PRESYNAPTIC DYSFUNCTION PRECIPITATES A NUMBER OF NEURODEGENERATIVE AND NEURODEVELOPMENTAL CONDITIONS, AND DISORDERS OF NEURONAL EXCITABILITY.

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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 two major routes by which a SV can be retrieved from the nerve terminal plasma membrane. They are 1) Clathrin-dependent endocytosis (where single SVs are retrieved *de novo*; and 2) 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 will be selectively activated during plastic changes at the synapse.

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 retrieval route.

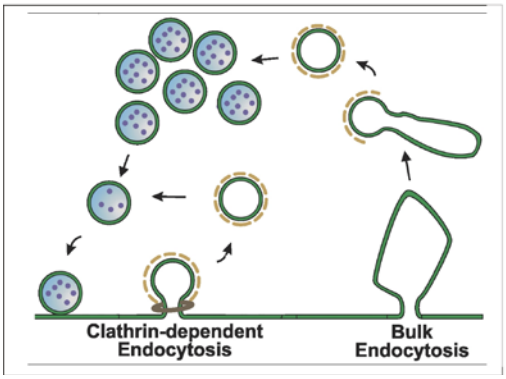

Figure 1: The synaptic vesicle life

CONTROL OF BULK ENDOCYTOSIS VIA SIGNALLING CASCADES

Rephosphorylation of dynamin I is mediated by both cyclin-dependent kinase 5 (cdk5) and glycogen synthase kinase 3 (GSK3). Inhibition of either enzyme results in arrest of ADBE. GSK3 is tightly controlled by signalling cascades activated by extracellular signalling molecules, suggesting these may also impact on ADBE. We have recently shown key roles for both Akt and BDNF in the negative control of both GSK3 and ADBE.

MOLECULAR MECHAISM OF SV GENERATION FROM BULK ENDOCYTOSOMES

We have recently shown that SVs generated by bulk endocytosis selectively refill the reserve pool of SVs within the nerve terminal. However little is known regarding the mechanism of this event.

We have recently shown that the endosomal adaptor protein complexes AP-1 and AP-3 are essential for this event. In addition, fluid phase uptake of calcium also essential, triggering activation of the calcium-dependent phosphatase calcineurin.

SYNAPTOPHYSIN – A SYBTRAP ESSENTIAL FOR SYNAPTOBREVIN RETRIEVAL DURING SV ENDOCYTOSIS

We run a distinct strand of research investigating the functional role of the abundant SV protein synaptophysin. We have shown the synaptophysin's primary role is to retrieve the essential v-SNARE synaptobrevin II from the plasma membrane during endocytosis, in addition to a secondary effect on SV endocytosis kinetics. We have also shown that synaptophysin mutants identified in X-linked intellectual disability do not rescue synaptobrevinII retrieval suggesting dysfunctional retrieval may underlie elements of this neurodevelopmental disorder. Synaptophysin now belongs to a growing family of proteins required for efficient trafficking of synaptobrevin II terms sybtraps (synaptobrevin trafficking partners).

DYSFUNCTIONAL SV RECYCLING IN HUMAN DISEASE

We have a number of projects investigating signatures of presynaptic dysfunction in various preclinical models of human disease. These defective signatures span both SV exocytosis and endocytosis and will be exploited as a tractable system to interrogate disease mechanism.

These disorders include both neurodegenerative and neurodevelopmental disorders and also disorders of uncontrolled excitability such as epilepsy.

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.

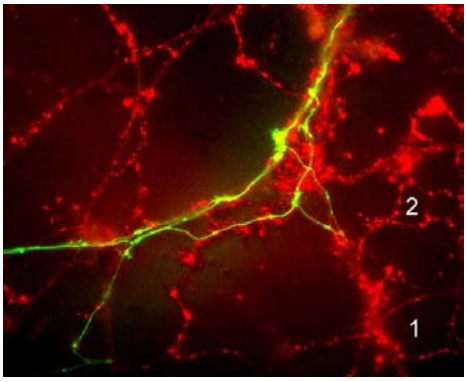

Figure 2 - Imaging of synaptic vesicle turnover using FM-64. The picture shows primary cultures of cerebellar granule neurones loaded with the vital dye FM4-64. Dye is localised to specific puncta corresponding to nerve terminals. The overlaid green neurone has been transfected with a cytosolic EGFP fusion protein

Selected references

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