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Review

Coupling exo- and endocytosis: An essential role for PIP_2 at the synapse ☆

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ABSTRACT

Chemical synapses are specialist points of contact between two neurons, where information transfer takes place. Communication occurs through the release of neurotransmitter substances from small synaptic vesicles in the presynaptic terminal, which fuse with the presynaptic plasma membrane in response to neuronal stimulation. However, as neurons in the central nervous system typically only possess ~200 vesicles, high levels of release would quickly lead to a depletion in the number of vesicles, as well as leading to an increase in the area of the presynaptic plasma membrane (and possible misalignment with postsynaptic structures). Hence, synaptic vesicle fusion is tightly coupled to a local recycling of synaptic vesicles. For a long time, however, the exact molecular mechanisms coupling fusion and subsequent recycling remained unclear. Recent work now indicates a unique role for the plasma membrane lipid phosphatidylinositol 4,5-bisphosphate (PIP_2), acting together with the vesicular protein synaptotagmin, in coupling these two processes. In this work, we review the evidence for such a mechanism and discuss both the possible advantages and disadvantages for vesicle recycling (and hence signal transduction) in the nervous system. This article is part of a Special Issue entitled Lipids and Vesicular Transport.

1. Introduction

1.1. Synaptic vesicle trafficking — an overview

The chemical synapse is the major structure where information is processed and transferred between neurons and their target cells. At the heart of this process is the synaptic vesicle — a small membranous organelle found in the presynaptic terminal, which contains the neurotransmitters that are the basis of neuronal communication. When the presynaptic terminal is depolarized by an action potential, synaptic vesicles fuse with the plasma membrane (exocytosis). Released neurotransmitters then diffuse across the synaptic cleft and attach to receptors on the postsynaptic neuron; depending on the type of neurotransmitter released (excitatory or inhibitory) this may lead to depolarization or hyperpolarization of the postsynaptic neuron, respectively.

A presynaptic terminal in the central nervous system typically contains 200 synaptic vesicles [1], which can be rapidly depleted under conditions of intense stimulation. Given the distance of the presynaptic terminal from the neuronal cell body, replacement of synaptic vesicles by *de novo* synthesis and transport would be too slow to sustain continuous neuronal activity [2]. In addition, unregulated fusion would lead to a dangerous increase in the area of the presynaptic plasma membrane (and possible misalignment with postsynaptic structures). Hence, synaptic vesicle membrane is retrieved (endocytosis), and used to reform synaptic vesicles that are used for subsequent rounds of fusion (see Fig. 1).

Major progress has now been made in understanding the molecular mechanisms underlying exo- and endocytosis. Surprisingly, many of the proteins involved in these processes were found to be part of much larger protein superfamilies that also function in either constitutive fusion of trafficking vesicles or constitutive internalization of receptors [3,4]. However, where synaptic vesicle trafficking differs from constitutive trafficking is that neuronal exo- and endocytosis is a strictly compensatory process — meaning that there must be a cross-talk between the processes that not only matches the number of exocytosed vesicles to those recovered by endocytosis, but also makes sure the vesicles are of the correct size and composition (including the types and copy numbers of proteins).

Two major models have been proposed for how the processes are coupled.

Abbreviations: AP, Adaptor protein; Arf, ADP-ribosylation factor; CALM, clathrin assembly lymphoid myeloid leukemia protein; CAPS, Calcium activated protein for secretion; Cdk, Cyclin-dependent kinase; DAG, Diacylglycerol; EMS, Ethyl methanesulfonate; FCHo1/2, F-BAR domain-containing Fer/Cip4 homology domain-only proteins 1 and 2; GFP, Green fluorescent protein; GST, glutathione S-transferase; HIP, Huntingtin interacting protein; IP_3, Inositol trisphosphate; MARCM, Mosaic analysis with a repressible cell marker; NMJ, Neuromuscular junction; PH, Pleckstrin homology; PIP_2, Phosphatidylinositol 4,5-bisphosphate; PKC, Protein kinase C; PLC, Phospholipase C; PLD, Phospholipase D; PS, Phosphatidylserine; PX, Phox; RIM, Rab3 interacting molecule; SCAMP, Secretory carrier membrane protein; SHD, Stonin homology domain; SNARE, Soluble NSF attachment protein receptor; VAMP, Vesicle associated membrane protein; μHD, mu-homology domain

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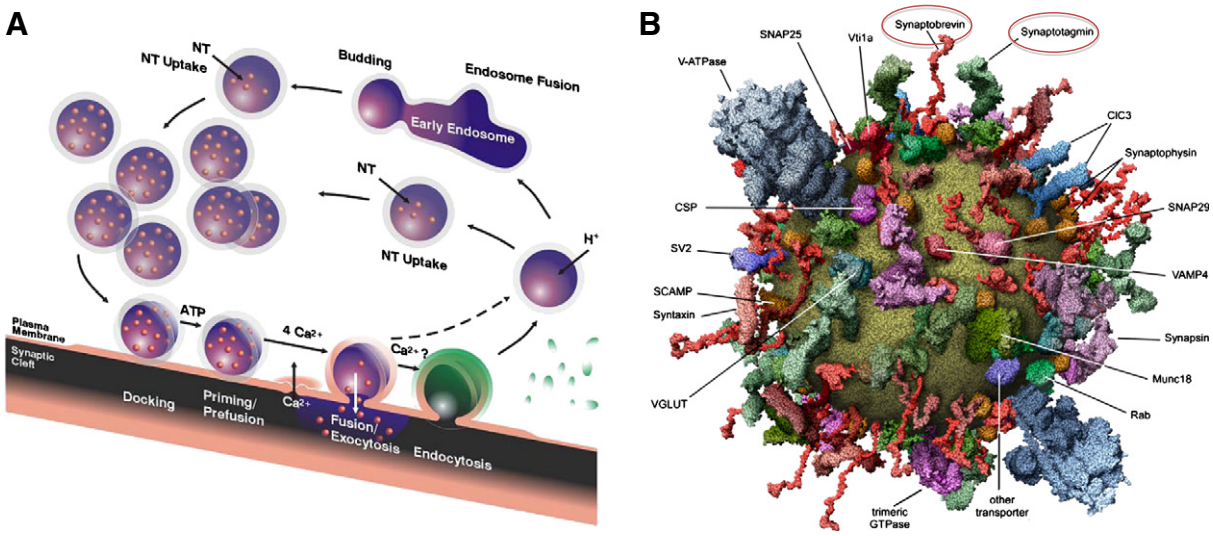

Fig. 1. The synaptic vesicle cycle. (A) Synaptic vesicles are locally recycled in the presynaptic terminal [2,120]. Synaptic vesicles are trafficked to specialist release sites on the plasma membrane - active zones [238]. After docking to release sites, vesicles undergo a complex set of reactions that renders them fusion competent — priming [239]. The arrival of an action potential in the presynaptic terminal leads to the opening of voltage-gated Ca^{2+} channels in the plasma membrane and the subsequent influx of Ca^{2+} — leading to exocytosis [240]. After fusion, vesicle membrane is recovered by endocytosis — a process that may or may not involve clathrin (green) [129]. It is unclear whether endocytosis takes place at the active zone, or occurs at peri-active zones that physically separate the sites of vesicle fusion and retrieval [36,241]. The vesicle lumen is then rapidly acidified by the action of a proton pump [242], which drives the uptake of neurotransmitter into the vesicle [243,244]. It still remains unclear whether recycling vesicles pass through an obligate sorting intermediate (early endosome) that acts as a quality control mechanism, removing damaged vesicle components and allowing their replacement [125–127]. (B) A molecular model of an average synaptic vesicle isolated from rat brain. The model is based on space-filling models of all macromolecules at near atomic scale. As expected, the various trafficking proteins that are known to coordinate key steps in the synaptic vesicle cycle dominate the vesicle surface. All vesicles are thought to share a common set of these proteins, although isoform specificity may vary among synapses. Of particular relevance here are the proteins synaptobrevin and synaptotagmin (highlighted by red rings), which play crucial roles in both exo- and endocytosis (see main text). An average vesicle is thought to contain 70 copies of synaptobrevin and 15 copies of synaptotagmin. Proteins are shown randomly distributed over the vesicle surface, but could also be present in clusters [166,245].
Panel B taken from [52].

The first model treats exocytosis and endocytosis as two mechanistically distinct processes — requiring separate triggers. An obvious candidate for such a trigger is Ca^{2+}. Exocytosis is strictly dependent on high levels of local Ca^{2+}, which are generated at release sites due to an influx through voltage gated Ca^{2+} channels which open during an action potential. Ca^{2+} has also been proposed to positively regulate endocytosis [5–7], as some of the key endocytic proteins are known to be dephosphorylated by the Ca^{2+} activated phosphatase calcineurin [8]. However, whether Ca^{2} acts as a universal trigger remains unclear, as several studies report an actual inhibition of endocytosis by Ca^{2+} [9–11]. There remains the possibility, however, that these discrepancies arise from the use of different synaptic preparations and differing stimulus levels which may invoke different recycling mechanisms [12–14].

The second model envisages that the fusion of the vesicle with the plasma membrane causes a detectable change in the membrane structure – possibly deposition of synaptic vesicle proteins into the membrane – that acts as a signal for membrane to be endocytosed [15].

Interestingly, these two models may not be mutually exclusive: different aspects of exo-endocytic coupling (such as the initiation event and extent of coupling) may be mediated by different mechanisms, such as the local cytoplasmic Ca^{2+} concentration.

Kinetically, the coupling between exo- and endocytosis is fast — with evidence that endocytosis can actually begin immediately after exocytosis [16,17]. Thus, there have been severe technical difficulties to exploring the coupling mechanism — and physically isolating the individual processes. Traditional biochemical/cell biological methods, such as the addition of small molecular weight compounds [18], or the overexpression of dominant negative proteins to interfere with endocytosis [19], take in the order of minutes to hours to act and, together with the high concentrations typically used, can produce many "off-target" effects [20].

To date the "cleanest" approaches have involved the genetic manipulation of exo- and endocytosis. The vesicular Ca^{2+} sensor synaptotagmin has long been recognized as an essential component of the exocytic machinery in neurons [21]. However, recent work using chronic genetic ablation of synaptotagmin in mouse [22], and acute inactivation of the protein in *Drosophila melanogaster* [23], has indicated that not only is this protein involved in neurotransmitter release but it also has a potential role in endocytosis.

Given that exo- and endocytosis are mechanistically very different (see Section 3), how does a single protein like synaptotagmin work to couple them? Is synaptotagmin the principal player? Has our ability to selectively manipulate proteins at the synapse, but not lipids, obscured 'the hub of the wheel'? Is synaptotagmin the focal point of the coupling process, or should our attention actually be diverted elsewhere?

In this review, we will argue that the plasma membrane lipid PIP_2 forms the 'hub' of this essential coupling process, with synaptotagmin acting as an effector protein. We base our case on three main arguments. First, the unique properties of PIP_2 make it an ideal molecule to locally recruit the machinery needed for both exo- and endocytosis [24,25]. Second, there is already substantial biochemical and cell biological evidence that the plasma membrane lipid PIP_2 is actively involved in exocytosis, primarily through its interactions with synaptotagmin [21]. Third, there is substantial evidence for a role of PIP_2 in recruiting essential endocytic proteins following fusion [4], including some which bind synaptotagmin [26]. Finally, we will attempt to illustrate not only the benefits but also the disadvantages of employing such a system.

2. Phosphatidylinositol 4,5-bisphosphate at the presynaptic terminal

2.1. Structure, localization and synthesis

PIP_2 is a member of the phosphoinositide family of membrane lipids, which all share the same basic structure; a long fatty-acyl