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Munc18-1 in secretion: lonely Munc joins SNARE team and takes control

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SNARE proteins and the Sec1/Munc18 (SM) protein, Munc18-1, are essential components of the mammalian secretion machinery. Until recently, quite divergent working models existed for the central but rather isolated role of Munc18-1 in secretion and its relation to the SNAREs. New studies now solve old discrepancies, bring consensus among SM–SNARE interactions and emphasize how closely these proteins work together. Together, SM and SNARE proteins control each step in the exocytotic pathway as a team. Munc18-1 operates as the chief commander of the exocytotic SNARE team, making teamwork more efficient, working with specific team members on specific jobs, reducing promiscuity with members of noncognate teams, and adjusting team efforts as a function of recent history and environmental cues (presynaptic receptor activation).

Introduction

Neuronal communication requires that a sufficient number of secretory vesicles are loaded with chemical messengers, fusion-ready and adequately replenished, and that exocytosis is fast, properly timed and spatially restricted. A growing number of proteins have been implicated in the organization of these requirements. Among them, only two protein families, SNAREs and Sec1/Munc18 (SM) proteins, are indispensable for fusion in all species, from yeast to mammals, which suggests that they constitute an evolutionary conserved, central aspect of the fusion machinery. For exocytosis in mammals, the vesicular SNARE protein, VAMP2/synaptobrevin2, and the target membrane SNARE proteins, syntaxin-1 and SNAP25, interact in a four helical bundle and thereby bring opposing membranes in close proximity and promote or drive fusion (reviewed in [1–3]). Null mutation of these SNARE genes invariably produces strong fusion and/or secretion defects (reviewed in [1–3]). Null mutation of the gene encoding the SM protein Munc18-1, first identified as a syntaxin-1 interactor (see Box 1), results in an even stronger phenotype [4]. Hence, SM proteins are central factors in membrane fusion, but in contrast to widely accepted models for SNARE function [1–3], working models for the function of Munc18-1 have been divergent and even conflicting [3,5–7]. Recent studies have solved some of the old discrepancies and thus allow a more unifying theory on the central function of Mun18-1 in secretion and its relation to the SNAREs. Although SM proteins are structurally not similar to SNARE proteins, these recent studies provide arguments to conclude that SM proteins should be considered, on functional grounds, as members of the SNARE team: they are always together and without each other they achieve little.

Munc18-1 operates as the chief commander of the exocytotic SNARE team, controlling each step in the exocytotic pathway, making teamwork more efficient, working with specific team members on specific jobs, reducing promiscuity with members of noncognate teams, and adjusting team efforts as a function of second messengers and calcium induced synaptic modulation.

SM–SNARE binding

SM–SNARE binding modes: Munc18-1 joins the SNARE team

Our mechanistic understanding of the function of Munc18-1 has been complicated by the discrepancy between the requirement of Munc18-1 for vesicle fusion (suggesting a positive function) and the crystal structure in which Munc18-1 embraces monomeric closed syntaxin-1, preventing SNARE complex formation [8] (suggesting a negative function; see Boxes 2 and 3). New findings from *in vitro* binding assays and structure studies now solve this discrepancy by providing compelling evidence that Munc18-1, like most other SM proteins, also binds assembled SNARE complexes [9–13]. In addition to its interaction with the coiled-coil domain of syntaxin-1, Munc18-1 interacts with the syntaxin-1 N-terminus, which was not resolved in the crystal structure (Figure 1 and Box 2). This interaction is compatible with SNARE formation and even essential because either deletion of the first 6–24 amino acids or the L8A mutation in syntaxin-1 prevents the interaction between Munc18-1 and the SNARE complex [10,13]. Thus, Munc18-1 is becoming an established component of the SNARE complex and SNARE complex binding, first reported for the yeast ortholog Sec1p [14], is now a general feature of SM proteins (but see Box 2). Binding of Munc18-1 to closed syntaxin-1 appears to be an atypical feature that might have evolved to meet the specific requirements of neuronal exocytosis. These conclusions also help to reconcile the positive and negative roles of Mun18 in vesicle fusion (Box 3).

SM–SNARE binding: Munc18-1 reduces promiscuity

Probably all intracellular pathways use specific (cognate) SNARE proteins (for review see [1]). However, the specificity of SNARE complex assembly is probably not the main

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Box 1. The essential Munc18-1

Munc18-1 belongs to the Sec1/Munc18 (SM) protein family, first identified in seminal forward genetic screens in yeast for secretion deficiency (*sec*) [51] and in nematodes for uncoordinated (*unc*) [52] phenotypes, yielding *sec1* and *unc18*. Munc18-1 was independently identified as a binding partner for syntaxin-1. SM genes have been described in a wide variety of species (for review see [5]). Some of the best studied family members are Sec1p (*S. cerevisiae*), ROP (*D. melanogaster*), UNC-18 (*C. elegans*) and s-Sec1 (*O. japonica*). Mammals express three *munc18-1* genes for plasma membrane fusion reactions (*munc18-1*, *munc18-2* and *munc18-c*) and several additional SM genes for intracellular trafficking (for review see [5]).

Expression

Munc18-1 is predominantly expressed in brain and neuroendocrine tissue where it shows a remarkably high axonal preference and membrane association (see [53] and Figure I) despite having no obvious sorting or hydrophobic motifs. Axonal preference and membrane association can be explained by the interaction with syntaxin-1 (see for instance [13,19]) but other proteins might also contribute (see below and Box 3).

Modifications: For *munc18-1* one alternative splice variant is reported [53]. Munc18-1 is an established substrate for PKC (see [24] and references therein) and CDK5 [54], but contains no consensus sites for protein kinase A and Ca^{2+}-calmodulin kinases and no other posttranslational modifications have been reported.

Interactions

Munc18-1 interacts with monomeric syntaxins 1–3 and SNARE complexes (Box 2). Structural data from the Munc18-1–syntaxin-1 dimer reveals a horseshoe shaped molecule surrounding syntaxin-1 in a 'closed' (SNARE complex incompatible) conformation [8]. Other reported interactors are X11/Mint [55], DOC2 [56], granuphilin [28] and phospholipase D [57].

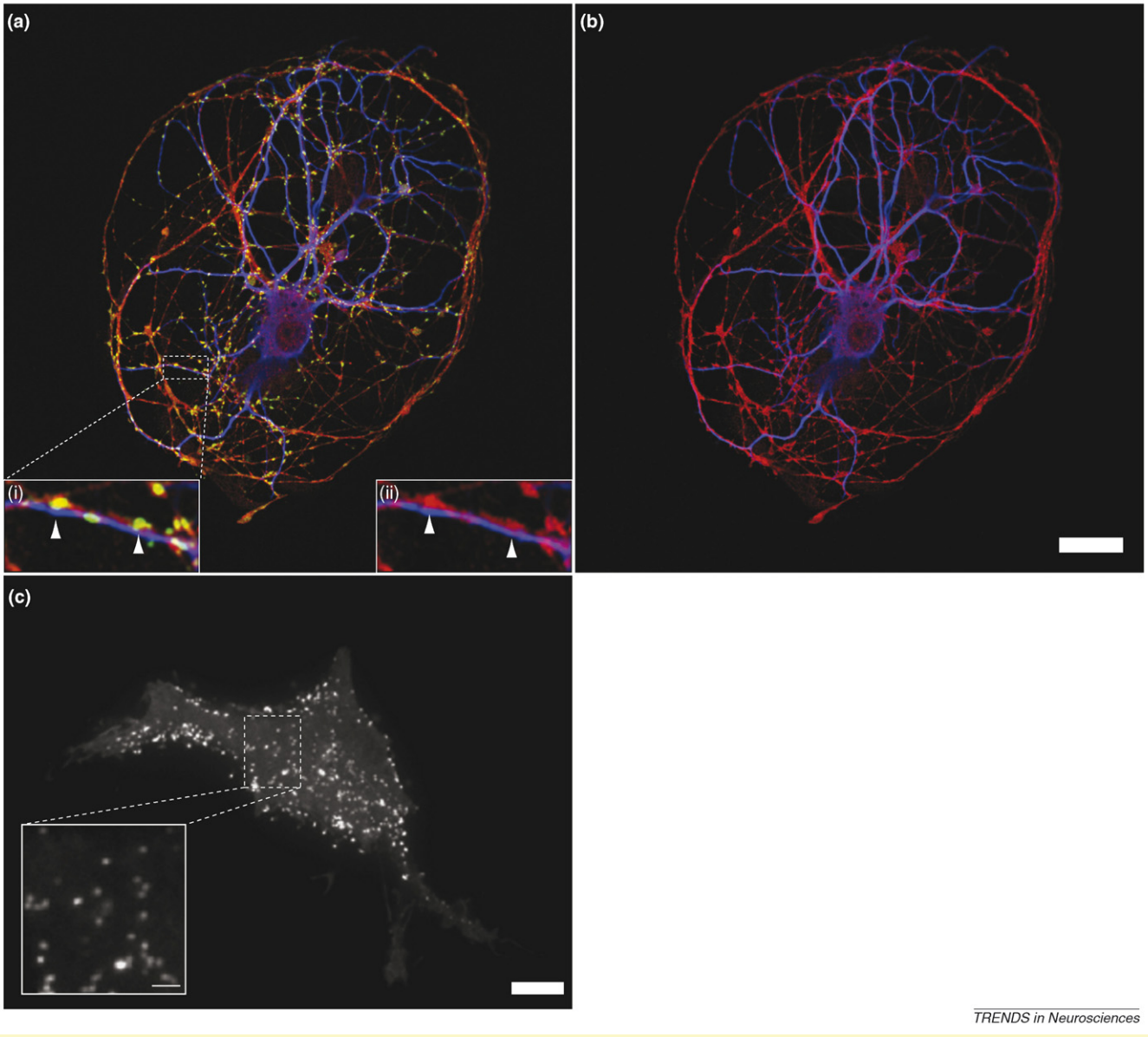

Figure I. Munc18-1 localization in cultured hippocampal neurons and PC12 cells. **(a,b)** Staining of a hippocampal neuron at 16 days *in vitro* with anti-Munc18-1 peptide antibody (directed against the last 15 amino acids of Munc18-1. (a) shows a predominant axonal staining. Endogenous Munc18-1 (red) is present but not particularly enriched in Synapsin positive (green) synaptic terminals [see (a)(i,ii), arrowheads point to Munc18-1 positive terminals] and Munc18-1 is almost absent from MAP2 positive (blue) dendrites. The neuron was cultured on a glia island to promote autaptic synapse formation. (b) Scale bar represents 20 μm. **(c)** EYFP–Munc18-1 expressed in PC12 cells imaged on a total internal reflection fluorescent (TIRF) microscope shows punctate distribution at the plasma membrane similar to endogenous Munc18-1 on membrane sheets [19]. Bar = 2 μm. Inset shows enlargement of boxed area. Scale bar represents 500 nm.