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Molecules in focus

Munc18a: Munc-y business in mediating exocytosis

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Abstract

The precise sequence of molecular events underlying release of neurotransmitter in neurons is yet to be fully understood. This process, called exocytosis, is tightly controlled by a number of protein–protein and protein–lipid interactions. One such regulatory factor is Munc18a, a cytosolic protein characterized by its interaction with the molecular machinery of exocytosis, primarily with the target SNARE protein, syntaxin1a. While Munc18a interactions have been extensively investigated for more than a decade, the role of Munc18a in vesicular fusion is still not fully defined. In this review, we discuss: (i) the recent analysis of the role of Munc18a in tethering and docking, (ii) the known structural and (iii) functional data surrounding Munc18a interactions with numerous other proteins of the exocytic machinery. Integration of Munc18a regulation by phosphorylation and lipids and the apparent complexity of its pleiotropic functional interactions is critical to deciphering Munc18a's role in exocytosis.

Keywords: Munc18a; SNARE; Vesicle trafficking; SM protein

1. Introduction

Neuronal communication relies on the fusion of neurotransmitter-containing vesicles with the neuronal plasma membrane in a tightly regulated process called exocytosis. During exocytosis, a number of well-orchestrated protein–protein and protein–lipid interactions occur. This sequence of interactions allows vesicles to approach the plasma membrane (tethering and docking), to undergo priming and, upon Ca^{2+} influx, to trigger vesicle fusion with the plasma membrane thereby releasing neurotransmitter in the synaptic cleft. Understanding the mechanism of vesicular exocytosis has focussed on the role played by the exocytic machinery, namely the soluble N-ethylmaleimide-attachment protein receptor (SNARE) proteins and a cytosolic regulatory protein, Munc18a (reviewed in Rizo & Südhof, 2002). While the function of the SNARE proteins in mediating the latest step of exocytosis is well established, defining the precise role of Munc18a in the process of exocytosis is still under debate.

Munc18a (n-sec1 or Munc18-1) belongs to the Sec1/Munc18 (SM) family of proteins that are involved in mediating membrane trafficking events (Toonen & Verhage, 2003). The first neuronal SM protein, UNC-18, was described in the early 1990s as part of a *Caenorhabditis elegans* screen of uncoordinated phenotypes, and was found to be very similar to the yeast (*Saccharamyces*

Abbreviations: APP, amyloid precursor protein; Cdk5, cyclin-dependent kinase 5; DOC2, double C2-domain; FRET, fluorescence resonance energy transfer; LDCV, large dense core vesicle; Mint, Munc18-interacting protein; PKC, protein kinase C; SM protein, Sec1/Munc18; SNARE, soluble N-ethylmaleimide-sensitive attachment protein receptor; TIRF, total internal reflection fluorescence

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cerevisiae) secretory regulatory protein, Sec1p (Garcia, Gatti, Butler, Burton, & De Camilli, 1994). Subsequent studies identified neuronal homologs in *Drosophila melanogaster* (Rop), a mammalian neuronal homolog, Munc18a (Garcia et al., 1994) and numerous non-neuronal SM proteins also involved in various vesicular trafficking pathways (Toonen & Verhage, 2003).

Munc18a was first described as a mammalian brain-specific protein tightly bound to syntaxin1a, a neuronal plasma membrane t-SNARE protein (Garcia et al., 1994). While the biochemical characterisation of the Munc18a:syntaxin1a interaction points to a negative regulatory role for Munc18a (Misura, Scheller, & Weis, 2000), the functional analysis of Munc18a and of its neuronal counterparts in *D. melanogaster* and *C. elegans* is somewhat conflicting (Rizo & Südhof, 2002). Here, we summarise the current hypotheses surrounding Munc18a regulation of vesicular exocytosis.

2. Structure

Initial studies of Munc18a-syntaxin1a molecular interactions by Yang and colleagues revealed that Munc18a requires the entire cytoplasmic domain of syntaxin1a for binding (Yang, Steegmaier, Gonzalez, & Scheller, 2000). Syntaxin1a is known to assume two major conformations: (i) open, where the SNARE binding domain is free to interact with the other SNARE proteins, SNAP25 and VAMP2, to form the fusogenic SNARE complex, and (ii) closed, where an intramolecular interaction between the two domains of syntaxin1a prevents SNARE complex assembly (Rizo & Südhof, 2002). Consequently, it was hypothesized that only a closed conformation of syntaxin1a binds to Munc18a, precluding SNARE complex formation (Yang et al., 2000). The crystal structure of the complex between syntaxin1a and Munc18a confirmed that Munc18a interacts with the closed syntaxin1a (Misura et al., 2000) (PDB code 1DN1) (Fig. 1A). Munc18a is a horse-shoe shaped protein consisting of three domains (Fig. 1A–C). Closed-syntaxin1a binds in the cavity created by the three domains and forms contacts with domain 1 and 3a. The crystal structure of the uncomplexed form of neuronal Munc18a (from squid, *Loligo pealei*) has also been determined (Bracher, Perrakis, Dresbach, Betz, & Weissenhorn, 2000) (Fig. 1B) (PDB code 1FVH). Comparison of the crystal structures of monomeric Munc18a and complexed Munc18a:syntaxin1a revealed few changes in Munc18a upon syntaxin1a binding, mainly some secondary structure changes in

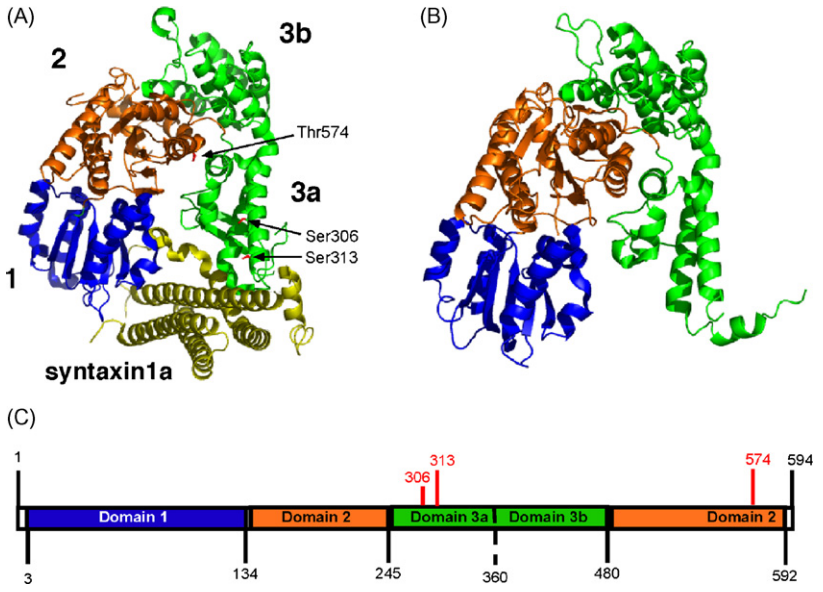

Fig. 1. Structural organization of Munc18a (n-sec1) and the Munc18a:syntaxin1a complex. (A) The crystal structure of the rat (*Rattus norvegicus*) Munc18a:syntaxin1a complex (Misura et al., 2000) (PDB code 1DN1) where Munc18a binds to the closed conformation of syntaxin1a. Domains 1 (blue), 2 (orange), 3a and b (green) of Munc18a and syntaxin1a (yellow) are indicated. Phosphorylation sites for protein kinase C (Ser306 and Ser313) and cyclin-dependent kinase 5 (Thr574) are shown in red and labelled accordingly. (B) The crystal structure of the neuronal squid (*L. pealei*) homolog of Munc18a reveals a conserved structure with few conformational differences from the Munc18a:syntaxin1a complex (Bracher et al., 2000) (PDB code 1FVH). (C) Schematic representation of Munc18a domains, indicating the residues contributing to each domain. Phosphorylation sites are highlighted in red. Domain 2 comprises two segments in the polypeptide sequence but folds as a single domain. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)