Munc18-1, exocytotic fusion pore regulation and local membrane anisotropy

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The release of hormones and neurotransmitters from vesicles can be modified by the regulation of the fusion pore, an aqueous channel that forms upon the fusion of the vesicle membrane with the plasma membrane. However, the mechanisms are unclear. Munc18-1 protein interacts with Syntaxin1 (Synt1), a member of the SNARE proteins, which plays an important role in exocytosis. It has been shown that Munc18-1 has multiple roles, both in pre- and post-fusion stages of exocytosis. It regulates the traffic of Synt1 to the plasma membrane. By inhibiting the tethering of the vesicle SNARE protein Synaptobrevin 2 (Syb2) solely to Synt1 at the plasma membrane, but favoring the vesicular tethering to the preformed binary cis SNARE complex of Synt1A-SNAP25B, Munc18-1 is tuning vesicle docking and the membrane merger process. Additionally, Munc18-1 affects exocytosis at the post-fusion stage by regulating the fusion pore properties (i.e., dwell-time and fusion pore diameter). Among many possible mechanisms that may regulate the fusion pore, but have never been considered previously, is the influence of Munc18-1 on the membrane anisotropy, which determines the local spontaneous membrane curvature and the architecture of the fusion pore. We here propose that Munc18-1 affects the fusion pore by modulating the dynamic local (re)arrangement of anisotropic membrane components within the highly curved fusion pore nanostructure, to which proteins, lipids or their complexes can participate.

Key words: Munc18-1, fusion pore, membrane anisotropy, atomic force microscopy, membrane capacitance, exocytosis, vesicle fusion, SNAREs

Regulated exocytosis consists of sequential steps, such as secretory vesicle delivery and docking to the plasma membrane, leading to the fusion of the vesicle and the plasma membranes. The proteins which play an important role in the tethering/docking and fusion are members of the SNARE proteins (Soluble NSF Attachment Protein Receptor proteins), which form a complex; Synaptobrevin2 (Syb2) on vesicles and Syntaxin1 (Synt1) and Synaptosome-Associated Protein (SNAP25) on the plasma membrane. It has been proposed, that a small number of SNARE complexes is sufficient for membrane fusion. However, additional proteins are required to fine-tune and increase the speed of this process. Such proteins are Sec/Munc18 (SM) proteins, which are, apart from SNAREs, the only indispensable proteins for exocytosis in all species, from yeast to mammals.

The Role of Munc18-1 in Pre-Fusion Steps of Exocytosis

Munc18-1, which is the neuronal isoform of SM proteins, has multiple roles already in pre-fusion steps. First, Munc18-1 regulates the traffic of vesicles since in Munc18-1 deficient cells fewer vesicles approach the target membrane. Second, it was proposed that Munc18-1, bound to Synt1A, interacts with SNAP25B and Syb2 to initiate the formation of the ternary SNARE complex, which ensures tethering/docking of vesicles. The step after the formation of the ternary SNARE complex is more enigmatic; Munc18-1 is thought to either...
The Role of Munc18-1 in Vesicle Fusion and Post-Fusion Steps of Exocytosis

Tethering/docking of vesicles is followed by the fusion of the vesicle membrane with the plasma membrane. This process results in the formation of the fusion pore, an aqueous channel, through which the vesicle content is released. The fusion pore was considered to be a short-lived meta-stable state, which is quickly subject to widening and eventually leading to the complete merger of vesicle and plasma membranes. Recent studies, however,
have expanded this model. It is now clear, that in the majority of cell types there is an additional mode of vesicle content discharge where the diameter of the fusion pores fluctuates between an open and a virtually closed state.\textsuperscript{11,14-19} Moreover, the diameter and the duration of the open fusion pore state depend on stimulation.\textsuperscript{16,20} Additionally, the stable fusion pore diameter depends also on the vesicle diameter.\textsuperscript{21} However, the molecular events mediating the transitions between discrete open states of a fusion pore are unclear.

A growing body of evidence now points to the Munc18-1 as an important regulator of the fusion pore state transitions.\textsuperscript{1,11-21} It is reasonable to assume that this regulation is mediated through Synt1, the binding partner of Munc18-1 which has the most widely recognized role in exocytosis.\textsuperscript{6} However, other possibilities cannot be excluded. For instance, it was recently shown, that synaptotagmin1 affects the stability of lipid bilayer by phase separating the phosphatidylserine which induces a positive curvature of lipid bilayers.\textsuperscript{24} The region of the fusion pore, on the other hand, is stabilized by anisotropic constituents (lipids, proteins or their complexes), which confer a negative curvature (Fig. 1IV and reviewed in ref. 17). Anisotropic membrane components are, unlike isotropic constituents, characterized by non-axisymmetric structure (see Fig. 2 for comparison of anisotropic and isotropic constituents) which is essential for forming subnanometer diameter fusion pores.\textsuperscript{17} The presence of Munc18-1 may directly or indirectly affect the local membrane anisotropy in the neck of the fusion pore, by modulating the accumulation of anisotropic constituents after the fusion pore is formed (Fig. 1IV). If Munc18-1 would dissociate from the SNARE complex, when this is disassembled following its formation,\textsuperscript{25} this may lead to the rearrangement of anisotropic constituents in the neck of the fusion pore, hence reducing the spontaneous curvature of the fusion pore. Reduced spontaneous curvature of the fusion pore would decrease its stability, and thus promote the widening of the fusion pore diameter (Fig. 1IV).

To conclude, Munc18-1 is a protein ubiquitously participating in practically all exocytotic steps. The role of Munc18-1 has been traditionally evaluated through the prism of its interactions with other proteins, while the interactions of Munc18-1 with lipids remain unexplored. Therefore one of the future goals should be assessing these interactions, which can prove critically important for the overall understanding of Munc18-1 functions in exocytosis.

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