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Sec1/Munc18 Proteins: Mediators of Membrane Fusion Moving to Center Stage

Minireview

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Synaptic transmission is mediated by exocytosis of neurotransmitter-filled synapic vesicles. In recent years, we have learned that many of the proteins involved in exocytosis belong to families common to all eukaryotic cells. While neurons usually possess their own particular flavors of these proteins in the form of specific isoforms, the presence of universal components suggests that once the fusion mechanism involving these proteins was invented during evolution, it was robust and versatile enough to allow its adaptation to all intracellular fusion reactions (Bennett and Scheller, 1993; Rothman, 1994; Jahn and Südhof, 1999).

Conserved protein families involved in fusion include the SNAREs, the Rab GTPases, and the Sec1/munc18-related proteins (also referred to as SM proteins) (Jahn and Südhof, 1999). The SNAREs are the best candidates for the "blue-collar" job of executing fusion. SNAREs are small proteins that contain a conserved stretch of about 60 amino acids, referred to as the SNARE motif, and usually possess a membrane anchor domain. The SNARE motifs of appropriate sets of SNAREs spontaneously assemble into core complexes of high stability that are disassembled by the ATPase chaperone NSF in conjunction with cofactors termed SNAPs (Rothman, 1994). The SNAREs operating in exocytosis of synaptic vesicles are amongst the best of the SNARE proteins characterized and include the vesicle protein synaptobrevin/VAMP, the plasma membrane–associated proteins syntaxin 1 and SNAP-25 (no relation to the SNAPs operating as NSF cofactors) (Rothman, 1994). Numerous lines of evidence indicate that SNARE assembly is an essential step in the fusion of synaptic vesicles with the plasma membrane. The specific function of SNARE protein complexes in membrane fusion may be to tie these membranes closely together and thus to facilitate bilayer mixing (reviewed by Bock and Scheller, 1999; Jahn and Südhof, 1999).

SM Proteins—Binding Partners of Syntaxins that Are Essential for Fusion

While the SNARE proteins have enjoyed the limelight of scientific attention for some time, the role of the SM proteins has remained largely undefined. Recent developments, however, appear to be moving them to center stage. Originally identified by genetic screens in *C. elegans* and yeast, these proteins are every bit as essential as the SNAREs for membrane fusion at synapses (reviewed by Halachmi and Lev, 1996; Jahn and Südhof, 1999). When an SM protein loses function, fusion is abolished. One of the most striking examples of this has recently been described in mice lacking the neuron-specific SM protein munc18a (Verhage et al., 2000). As might have been predicted based on studies of the mutants in the *Drosophila* ortholog of munc18, rop (Harrison et al., 1994; Schulze et al., 1994), synaptic transmission is lost in mice lacking munc18. Somewhat surprisingly, the embryonic development of the brain occurs normally in these mice despite complete synaptic silence. Massive apoptotic degeneration occurs, but only becomes apparent after initial synaptogenesis, thus suggesting that synaptic activity is not needed for the initial establishment of synaptic connectivity (Verhage et al., 2000).

Munc18a (also referred to as n-Sec1 or rbSec1) was first identified in brain based on its ability to bind syntaxin with high affinity and was later cloned by homology screening based on its similarity to *Drosophila* rop and *C. elegans* Unc18 (Halachmi and Lev, 1996). Based on these initial binding studies, it was suggested that munc18 may somehow be involved in regulating syntaxin function. Indeed, most if not all of the hitherto known SM proteins appear to interact specifically with syntaxin family members, raising the possibility that perhaps all syntaxin family members may require an SM protein for function. Furthermore, although the interactions with syntaxins are selective, a given SM protein may interact with more than one syntaxin, thus explaining why the yeast genome with its eight known syntaxins contains only four SM proteins (reviewed by Halachmi and Lev, 1996; Jahn and Südhof, 1999).

Structures of Munc18 and Syntaxin Reveal Atomic Details of a Conformational Cycle

How does the munc18/syntaxin 1 interaction relate to the formation of SNARE core complexes? In vitro, binding of syntaxin with munc18 precludes the formation of core complexes. Furthermore, munc18 coprecipitates with syntaxin but not with the other SNAREs from brain extracts, suggesting that these interactions are exclusive (reviewed by Halachmi and Lev, 1996). However, there is also evidence to suggest that munc18 and the SNAREs do not simply compete for the same binding site in syntaxin. Syntaxin possesses an N-terminal domain consisting of an antiparallel three-helix bundle (referred to as the Habc domain) (Fernandez et al., 1998), which is connected via a linker domain to the SNARE motif (also referred to as the H3 domain; see Figure 1). The SNARE motif is both necessary and sufficient for core complex formation. Munc18 binding requires the Habc domain as well as the N-terminal portion of the SNARE motif (the H3 domain), thus suggesting that the binding interactions and conformations of syntaxin in the core complex and in the munc18 complex may be different (for references to the original literature, see Halachmi and Lev, 1996; Jahn and Südhof, 1999).

Recently, work from Misura and colleagues has resolved the the three-dimensional crystal structure of the munc18/syntaxin 1 complex (Misura et al., 2000). The overall structure of munc18 is unique and bears no major similarities to other known proteins. It is composed of three domains that form an arch with a central cavity for holding syntaxin. Domains 1 and 2 consist of β sheets,

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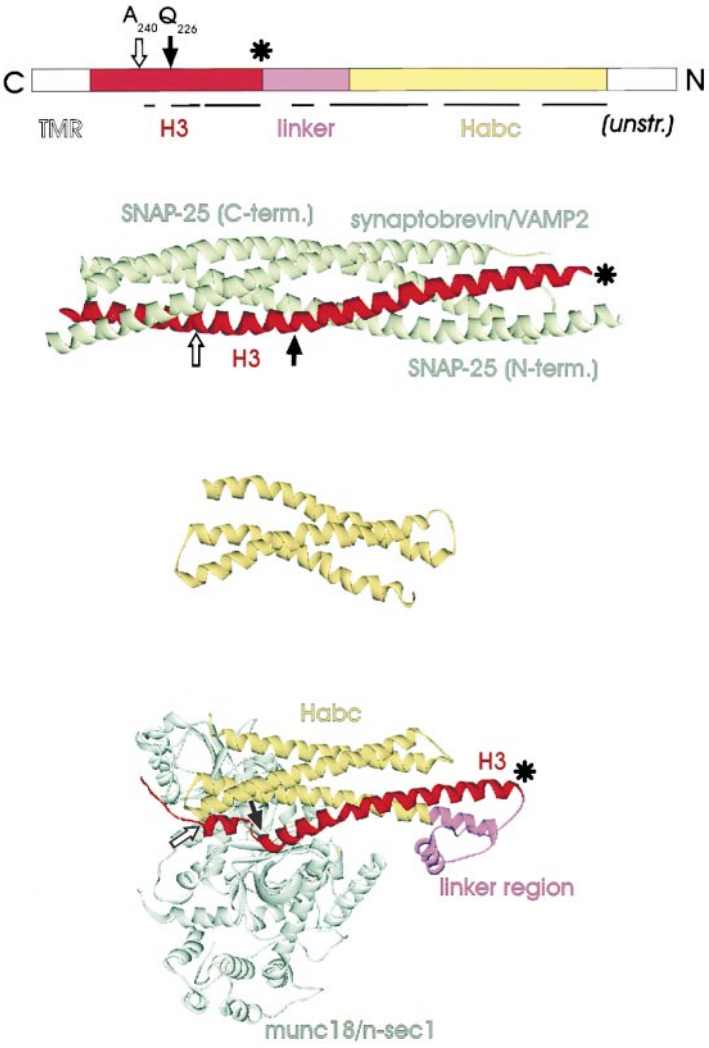

Figure 1. Comparison of Syntaxin 1 Structures

The diagram on top shows the main regions of the molecule (TMR, transmembrane region). The asterisk indicates the N-terminal end of the H3 domain (SNARE motif). Regions that are helical in the munc18/syntaxin complex are underlined. The position of the conserved glutamine ("0" layer) is indicated by a closed arrow; the position of alanine 240 (where syntaxin becomes unstructured in the munc18 complex) is indicated by an open arrow. Ribbon diagrams of the following structures are shown (from top to bottom): neuronal core complex (X-ray structure; adapted from Sutton et al., 1998), Habc domain (NMR structure; adapted from Fernandez et al., 1998), munc18 (n-Sec1)/syntaxin complex (X-ray structure; adapted from Misura et al., 2000). Identical colors for corresponding regions are used in all diagrams.

flanked by α helices. In contrast, domain 3 consists of clusters of helices, which can be divided into upper and lower parts. The lower portion of domain 3 makes only relatively few contacts with the rest of the molecule and instead lines one side of the binding cavity. A single glycine links the two parts of domain 3 in a 60° bend between two adjacent helices. This linker residue may function as a hinge, suggesting the possibility that the lower part of domain 3 may be mobile and bend away from the binding cavity when syntaxin is not bound. More fascinating, however, is the structure of syntaxin in the complex, which represents the second conformation of syntaxin that is known in atomic detail. In the new structure, the Habc domain is folded back onto the H3 domain, thus presenting a "closed" conformation (see Figure 1). The structure of this bound Habc domain is very similar to the structure of the free Habc domain determined previously by NMR (Fernandez et al., 1998). In addition, the linker region between the Habc domain and the H3 domain is structured. It begins with an extension of the C-terminal α helix of the Habc domain and then connects via loops and a short α helix to the H3 domain. The H3 domain structure is different from that in the core complex where it forms an extended α helix all the way through the C-terminal transmembrane domain (see Figure 1) (Sutton et al., 1998). In the munc18 complex, the N-terminal part of the H3 domain is also α helical and forms an antiparallel four-helix bundle with the Habc domain. However, the H3 helix is bent. Onward from the middle of the SNARE motif (close to the position of a conserved glutamine that forms a conspicuous ionic "0" layer in the core complex [Sutton et al., 1998]), a mix of turns, a short helix, and extended turns follow. This region is in intimate contact with several domains of munc18 and forms a major part of the interacting surface in the complex. The C-terminal remainder of the SNARE motif is unstructured (Misura et al., 2000).

Interestingly, the structure of syntaxin in the complex closely corresponds to the structure assumed by free unbound syntaxin in solution (Dulubova et al., 1999). Although the regions C terminal of the Habc domain could not be resolved to atomic detail in this NMR study, the data show that in free syntaxin, not only is the linker region structured but also that structure extends into the H3 domain up to the conserved glutamine in the middle of the SNARE motif. Apparently, the only change in syntaxin upon munc18 binding involves a folding of the short stretch in the H3 domain that is downstream

of the "0" layer and that makes intimate contact with munc18. Interestingly, a similar closed conformation has also been observed by NMR spectroscopy for free Sso1p, the yeast syntaxin functioning in exocytosis (Fiebig et al., 1999).

Munc18—A Regulator of Conformational Changes in Syntaxin?

Although the recent structural advances have brought considerable insight to the field, it still remains unclear precisely how the munc18/syntaxin complex fits into the conformational cycle of syntaxin and in the sequence of protein–protein interactions leading to membrane fusion. At first glance, it appears obvious that munc18 stabilizes the closed conformation of syntaxin, thus preventing it from forming core complexes. The SM protein may therefore function as a negative regulator that operates upstream of SNARE assembly and controls the pool of available syntaxin. However, the negative regulator model does not explain why the protein is essential for transmitter release (Harrison et al., 1994; Schulze et al., 1994; Broadie et al., 1995; Verhage et al., 2000). Furthermore, structure–function analysis of *Drosophila* syntaxin and rop (the *Drosophila* homolog of munc18) have suggested both activating and inhibiting roles of rop that may be mediated via different mechanisms. Overexpression of either syntaxin 1 or rop reduces neurotransmitter release, whereas no inhibition is observed when both proteins are overexpressed in parallel (Wu et al., 1998). Mutations in both rop and syntaxin that allegedly weaken or even abolish binding lead to an enhancement of transmitter release (Wu et al., 1998, 1999). However, these results too must be interpreted with caution as long as differences in affinity are not quantitatively determined (Matos et al., 2000). Furthermore, overexpression of munc18 in PC12 cells has no effect on secretion (Graham et al., 1997). In PC12 cells, syntaxin overexpression does not inhibit exocytosis when both the closed conformation and munc18 binding are abolished due to amino acid substitutions in the linker region of syntaxin (Dulubova et al., 1999).

If binding to munc18 is an integral part of the conformational cycle of syntaxin, both association and dissociation of the high-affinity complex are probably regulated. The crystal structure provides a snapshot of syntaxin in a state of low potential energy, similar to the crystal structure of the core complex. In both cases it is likely that the crystallized complexes are biologically "inactive" and need energy input through interactions with other proteins to be reactivated. For the core complex, both spontaneous assembly and NSF-mediated disassembly have been studied in detail. In contrast, such reactions are not well understood for the complex of syntaxin with munc18, and the syntaxin conformations preceeding munc18 binding and following its dissociation are unknown. For instance, munc18 may be needed to restore the function of syntaxin after being acted upon by NSF. NSF is known to induce conformational changes on syntaxin even in the absence of its SNARE partners (reviewed by Jahn and Südhof, 1999). Moreover, proteins acting upon SM proteins may influence binding to syntaxins. Candidates for such regulators include, in addition to Rab proteins and Rab effector proteins, tomosyn, a large neuron-specific protein carrying a SNARE motif that is the only protein known to dissociate the munc18/syntaxin complex (Fujita et al., 1998), Munc13, a synaptic protein involved in the regulation of exocytosis (Brose et al., 2000), and protein kinases such as protein kinase C, which phosphorylates munc18 and lowers its affinity for syntaxin (Fujita et al., 1996). In each of these cases, it has been suggested that an interaction of munc18 with syntaxin precedes core complex assembly and fusion. Thus, the interacting proteins might be predicted to promote munc18 to "open" syntaxin and to hand it over to its SNARE partners. However, such models are not fully satisfying since they fail to explain convincingly why munc18 and its relatives are essential for fusion. In membranes, SNAREs spontaneously assemble into core complexes (albeit at a rather slow rate) and still do so when previously disassembled by NSF (reviewed by Jahn and Südhof, 1999). Thus, in terms of making the the H3 domain accessible for binding to the partner SNAREs, there does not appear to be an absolute need for such a chaperone-like function of munc18.

Given such inconsistencies, it is important to remain open to alternative possibilities for munc18 function. In yeast, interactions between syntaxins and SM proteins have recently been reported that differ from those of their neuronal counterparts. For instance, binding between the SM protein Sly1p and the syntaxin homolog Sed5p does not appear to involve the H3 domain of Sed5p (Kosodo et al., 1998). Furthermore, Sec1p appears to bind preferentially to the core complex rather than to isolated syntaxin Sso1p (Carr et al., 1999). Results from these studies cannot be readily reconciled with the crystal structure of the munc18/syntaxin complex, in which the H3 domain forms a major and essential part of the binding interface and which precludes core complex formation (see above). However, perhaps under appropriate circumstances an interaction of lower affinity may (also?) occur with an open conformation of syntaxin. Such a model would place an SM protein–dependent step in the fusion process somewhere after the formation of trans-SNARE complexes (Carr et al., 1999). For instance, such SM protein–syntaxin interaction may prevent trans-SNARE complexes from being attacked by NSF or else may directly promote fusion. In addition, it cannot be excluded that individual SM proteins might differ somewhat in their mechanisms of action.

It should be noted that munc18 has also been shown to interact with a number of other proteins whose role in the syntaxin conformational cycle is unclear. Unfortunately, the proteins identified so far have little in common, making it difficult to evaluate how they relate to function (Jahn and Südhof, 1999). For instance, the munc18 family of proteins termed mints were recently identified as binding partners for munc18. Mints contain phosphotyrosine binding domains that associate with phosphatidylinositol phosphates and two PDZ domains. In addition, mints also bind to CASK, a junctional synaptic protein that may be involved in the organization of the exocytotic site. Munc18 also interacts with Doc2a and Doc2b, two synaptic proteins of unknown function that contain C2-like domains and thus have been proposed to bind calcium. It is not yet known whether these proteins operate by controlling the interactions of SM proteins with syntaxins or whether their binding to

SM proteins involves separate pathways that are linked to fusion in a still-unknown manner (for references to the original literature, see Jahn and Südhof, 1999). Such complexities illustrate that despite high-resolution structures of two different syntaxin conformations, our understanding of the conformational cycle of syntaxin and its link to membrane fusion remains far from clear.

In summary, much needs to be done before the role of SM proteins in membrane fusion will be fully understood. Although the evidence favors the view that they operate through syntaxins, large pieces of the puzzle are still missing. Fusion of biological membranes involves highly complex and ordered assemblies of thousands of molecules that operate at the boundary of two phases, the hydrophobic interior of the bilayer and the hydrophilic cytoplasm. It seems likely that the proteins involved in fusion need somehow to be spatially arranged with respect to the fusing membranes in a very specific and controlled manner. In light of munc18's interactions with mints and Doc2, munc18 may well play a role in regulating the formation of superstructures required for fusion. Recent advances—most notably, the solution of the three-dimensional structure of the munc18/syntaxin complex—should allow for more highly refined experimental approaches that will hopefully begin to shed new light on the way these proteins operate. As exciting as these structural insights are, they teach us with sobering clarity that despite major efforts and numerous appealingly simplistic cartoons, we have only begun to understand the principles governing biological membrane fusion, let alone the principles underlying the highly sophisticated and regulated process of exocytosis of synaptic vesicles.

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Note Added in Proof

After completion of this article, Bracher and colleagues reported the crystal structure of s-Sec1, the squid homolog of munc-18, in its syntaxin-free form. Although only 66% identical at the amino acid level, the two structures are remarkably similar, including the V-shaped binding pocket for syntaxin. The differences between the syntaxin-free and the syntaxin-bound structures are only local. The most conspicuous changes are seen in the syntaxin-binding region localized in the lower portion of domain III. Here, a binding loop is unstructured, and a contacting loop is converted into a helix in the free form. Thus, syntaxin binding causes local rearrangements but no major conformational changes in munc-18 (Bracher, A., Perrakis, A., Dresbach, T., Betz, H., and Weissenhorn, W. [2000]. Structure *8*, 685–694).