

Testing the SNARE/SM protein model of membrane fusion

Taulant Bacaj^a, Zhiping P. Pang^a, and Thomas C. Südhof^{a,b,1}

^aDepartment of Molecular and Cellular Physiology and ^bHoward Hughes Medical Institute, Stanford University, Stanford, CA 94305-5453

In eukaryotic cells, the budding and fusion of membranes mediates diverse but essential processes, ranging from cell division to organelle biogenesis to neurotransmitter secretion. All intracellular membrane fusion except for mitochondrial fusion is driven by SNARE and SM (Sec1/Munc18-like) proteins (1–3). Membrane fusion has been particularly intensely studied for neurotransmitter secretion. In neurotransmitter secretion, fusion is mediated by the plasma membrane SNARE proteins syntaxin and synaptosomal-associated protein 25 (SNAP-25), the vesicle SNARE protein synaptobrevin/vesicle-associated membrane protein (VAMP), and the SM protein Munc18-1 (Fig. 1 *A* and *B*). Mechanistically, SNARE proteins are thought to fuel fusion by forming a transcomplex between the vesicle and target membranes; in this complex, progressive zippering of a four-helical bundle formed by the SNARE motifs of SNARE proteins forces the fusing phospholipid membranes into close proximity, thereby destabilizing their surfaces (1–3). SM proteins are essential coagonists of SNARE proteins in fusion in that all intracellular SNARE-dependent fusion reactions require an SM protein.

At least for fusion during neurotransmitter secretion, syntaxin constitutes the central organizer that is composed of multiple domains: a conserved N-terminal unstructured peptide, an N-terminal H_{abc} domain, a SNARE motif, and a C-terminal transmembrane region (Fig. 1*A*). Syntaxin assumes two conformations: a closed conformation outside of the SNARE complex in which the H_{abc} domain folds back onto the SNARE motif, and an open conformation in the SNARE complex with a mobile H_{abc} domain (4, 5) (Fig. 1*B*). Both conformations bind to Munc18, but only Munc18 binding to open syntaxin requires the N-terminal syntaxin peptide (6–8). Although both Munc18/syntaxin binding modes are known to be essential for fusion in vivo (9–12), how syntaxin orchestrates fusion remains unclear. Using elegant in vitro liposome fusion and in vivo *Caenorhabditis elegans* experiments, Rathore et al. (13) now address the critical question of whether the N-terminal peptide of syntaxins acts autonomously in fusion, independent of its anchorage to syntaxin, or whether it is required to be coupled to syntaxin.

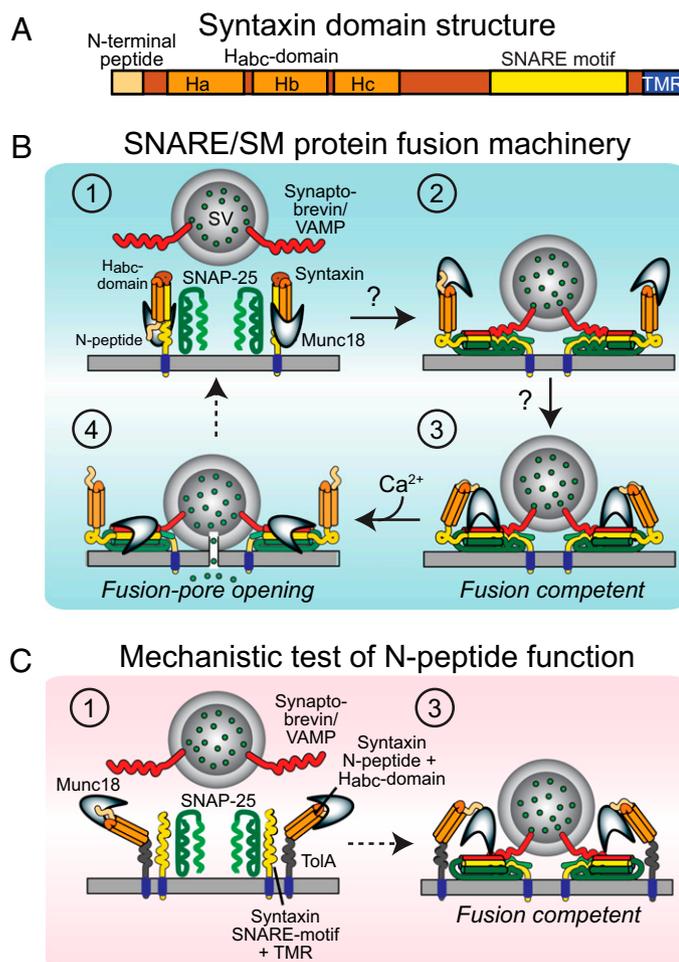


Fig. 1. SNARE/SM protein function in vesicle fusion. (*A*) Domain structure of syntaxins, which are composed of a conserved ≈ 20 -residue N-terminal sequence, an H_{abc} domain containing three α -helices, a ≈ 60 -residue SNARE motif, and a transmembrane region (TMR). (*B*) Working model of membrane fusion mechanism of SNARE/SM proteins, exemplified by synaptobrevin-2 (Syb2/VAMP), syntaxin, and SNAP-25] before SNARE complex assembly, with the SM protein Munc18 bound to the closed conformation of syntaxin; (2) initiation of SNARE complex with opening of syntaxin and continued binding of Munc18 to the syntaxin N-peptide; (3) partial zippering of SNARE complexes; (4) completion of the SNARE complex/Munc18 assembly opens the fusion pore. (*C*) Diagram of the key experiment performed by Rathore et al. (13): an in vitro and in vivo assay in which the functionality of two complementary syntaxin mutants was examined when present alone or expressed simultaneously: in the first syntaxin mutant the SNARE motif was replaced by an unrelated α -helix from the bacterial protein ToIA, whereas in the second syntaxin mutant the N-peptide and H_{abc} domain were deleted. The two syntaxin mutants are individually unable to support fusion but when both mutant proteins are present simultaneously, liposome fusion is restored.

In a first set of experiments, Rathore et al. (13) use an in vitro liposome fusion assay that monitors lipid mixing to confirm previous data (14) showing that Munc18 is essential for efficient liposome fusion and that the syntaxin N-peptide is required, whereas the H_{abc} domain is dispensable.

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¹To whom correspondence should be addressed. E-mail: tcs1@stanford.edu.

Then, to test whether the N-peptide acts as a modular sequence in fusion, Rathore et al. translocate the N-peptide from syntaxin to the t-SNARE SNAP-25 or to Munc18, or use a soluble version of the N-peptide. They find that only the hybrid protein composed of the syntaxin N-peptide and SNAP-25 supports liposome fusion in the presence of a syntaxin lacking the N-peptide. Furthermore, Rathore et al. (13) create a syntaxin mutant in which the SNARE motif is replaced by an α -helix from the bacterial protein TolA. This mutant or a syntaxin mutant lacking the N-peptide and H_{abc} domain separately are unable to support liposome fusion, but when both mutants are present simultaneously, liposome fusion is restored (Fig. 1C). Finally, Rathore et al. (13) engineer a syntaxin with a cleavable N terminus and incubate this with Munc18 and SNAREs at 4 °C, followed by cleavage of the N-peptide. When incubated at 37 °C, this preparation undergoes fusion, suggesting that the N-peptide is not necessary when the fusion reaction is activated at 37 °C. Together, these experiments show that the syntaxin N-peptide does not need to be on syntaxin or a SNARE protein for liposome fusion but has to be on the target membrane close to the t-SNARE proteins, suggesting that the N-peptide acts independently and autonomously but in conjunction with t-SNARE proteins. Moreover, the N-peptide cleavage experiments suggest that the N-peptide acts before full SNARE complex assembly, although the experiment does not reveal whether the same is true for the SM protein Munc18, whose precise mode of action remains a mystery.

A potential problem with the liposome experiments described by Rathore et al.

(13), as elegant as they are, is that it remains unclear whether the results are transferable to physiological membrane fusion. Even if content-mixing assays of fusion had been included, liposome fusion assays still do not completely test the potential role of the H_{abc} domain in organizing the sites of fusion, let alone analyze

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the function of syntaxin in orchestrating the speed and topology of fusion. Realizing this limitation, Rathore et al. (13) perform a second set of experiments, assaying rescue of neurotransmitter secretion in a syntaxin mutant of *C. elegans*. In a beautiful demonstration of the power of genetics, they demonstrate that the combination of the two complementary syntaxin mutants (the mutant in which the SNARE motif is replaced by an unrelated α -helix, and the mutant in which the N-peptide and H_{abc} domain are deleted) can partially rescue neurotransmission in the neuromuscular junctions of syntaxin-deficient *C. elegans* (Fig. 1C); expression of each syntaxin mutant separately fails to rescue. This important experiment conclusively shows that the syntaxin N-peptide is functionally autonomous in vivo, as long as it is anchored in the target membrane.

The findings of Rathore et al. (13) provide the best evidence to date that the

syntaxin N-peptide functions to recruit SM proteins to the vicinity of assembling SNARE complexes (1–3). The importance of the article lies not only in the persuasiveness of the evidence but also in the approach, which consists of a combination of in vitro liposome fusion with in vivo tests of the resulting conclusions. Like any interesting study, the results of Rathore et al. (13) also raise further questions.

Among many fascinating issues, four stand out. First, why do fusion reactions universally require SM proteins; and what is their function (1)? Second, is the SM protein recruitment mechanism showcased here universally applicable to all SNARE-mediated fusion events, as suggested by the presence of a similar SM protein binding mode in endoplasmic reticulum and endosomal syntaxins in which it was first discovered (15, 16), or do some fusion reactions, such as those involving the HOPS complex (17), use a different mechanism of SM protein recruitment? Third, the H_{abc} domain is highly conserved in syntaxins but dispensable for liposome fusion (13, 14)—does this mean that the H_{abc} domain is not required for fusion in vivo to perform a “mere” regulatory role without participation in the fusion mechanism, or has no function at all? Fourth, at least during neurotransmitter secretion syntaxin does more than fusion—it shapes the kinetics of secretion and interacts with Ca²⁺ channels (18–20). How do the syntaxin domains studied here relate to these significant syntaxin functions? Addressing these questions will require multidisciplinary approaches similar to those reported by Rathore et al. (13) and will be essential for further progress in understanding membrane fusion beyond the identification of its essential components.

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