Springs and zippers: coiled coils in SNARE-mediated membrane fusion

Pehr AB Harbury

A conserved molecular machinery based on SNARE proteins catalyzes most, if not all, cellular membrane fusion events. A flurry of recent biophysical studies have established a detailed molecular picture of the core SNARE complex. Structural and biochemical analysis of the SNARE machinery is rapidly advancing our understanding of the specificity, regulation and protein catalysis of membrane fusion.

Address: Department of Biochemistry, Beckman B437, Stanford University, Stanford, CA 94305, USA.

E-mail: harbury@cmgm.stanford.edu

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In eukaryotic cells, membrane bound compartments constantly bud lipid vesicles that travel through the cell cytoplasm and fuse with target membrane surfaces. This vesicle commerce allows the exchange of macromolecules between distinct subcellular compartments, and supports the spatial complexity of cells in higher organisms. After years of genetic and biochemical analysis (primarily studies of vesicle trafficking in yeast [1] and studies of vesicle exocytosis/endocytosis in vertebrate neurons [2]), a structural picture of the molecular transactions underlying vesicle fusion is beginning to emerge.

The fusion of a vesicle with a target membrane occurs in three biochemically defined steps: docking of the vesicle with the membrane, priming of the fusion machinery on both membrane surfaces, and physical merging of the lipid bilayers, allowing mixing of the vesicle contents with the interior of the target organelle [3]. All of these processes revolve around the regulated assembly of a core fusion complex, called the SNARE (soluble NSF attachment protein receptor) complex, which catalyzes the fusion event. A handful of stereotyped molecules are involved (Table 1). In different biological settings, different sequence variants of the same proteins participate, resulting in a formidable literature nomenclature. Where possible in this discussion, the molecular names specific to synaptic exocytosis are used.

Fusion proteins can be divided into five classes. The first class are the components of the SNARE complex itself [2], which (for synaptic exocytosis) consists of three proteins associated in a 1:1:1 stoichiometry. Two of these proteins are donated by the target membrane and are designated t-SNAREs. The first t-SNARE, syntaxin, is an integral membrane protein, whereas the second t-SNARE, SNAP-25 (synaptosome-associated protein of molecular mass 25 kDa), is peripherally attached to the target membrane through two palmitoylated cysteine residues. The third SNARE component, synaptobrevin, is an integral membrane protein donated by the vesicle, and is designated a v-SNARE. A second class of fusion proteins bind

Table 1

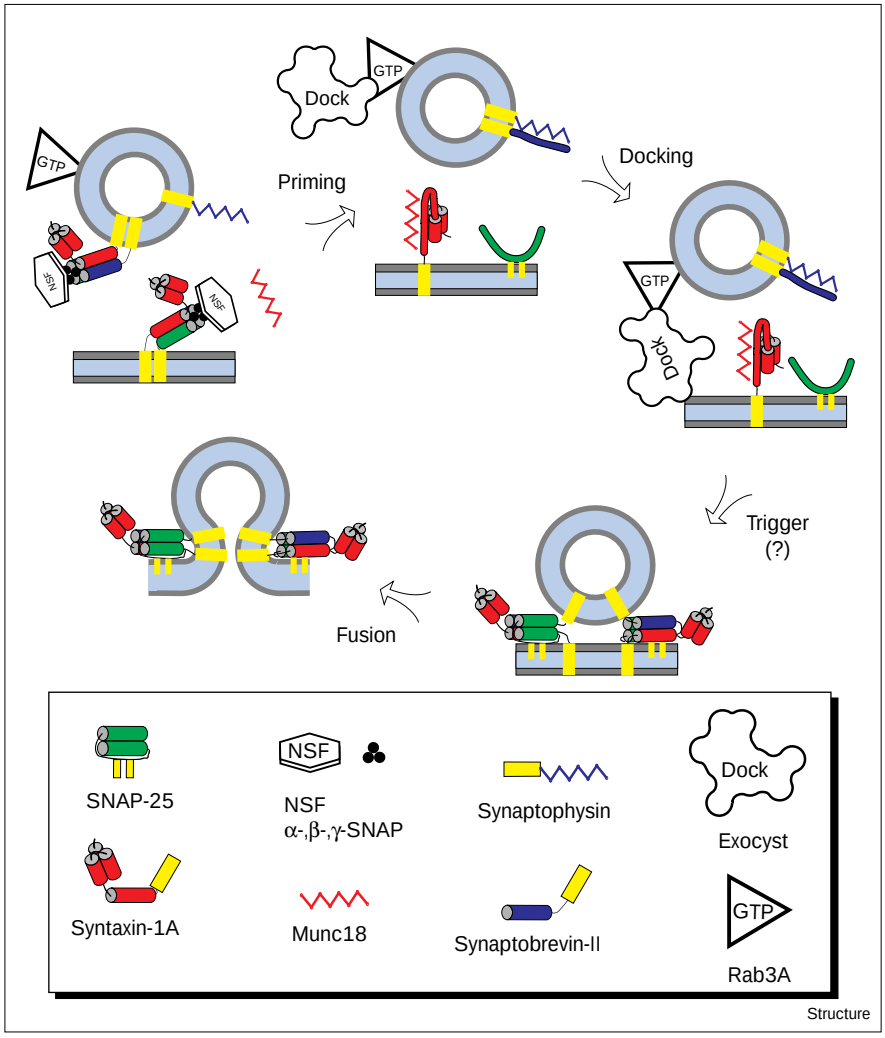
Classification of proteins involved in vesicle trafficking*.

Functional family	Canonical example	Membrane association	Proposed role
SNARE components			Catalysis of membrane fusion
t-SNARE	Syntaxin-1A	Integral membrane	
	SNAP-25	Cysteine palmitoylation	
v-SNARE	Synaptobrevin	Integral membrane	
SNARE inhibitors			Regulation of SNARE protein assembly
t-SNARE	Munc18	Protein–protein interaction	
v-SNARE	Synaptophysin	Integral membrane	
Chaperones			Disassembly of SNARE complexes
ATPase	NSF	Protein–protein interaction	
Auxiliary	α-, β-, γ-SNAP	Protein–protein interaction	
Passive	LMA 1 (Yeast)	Protein–protein interaction	
Multisubunit docking complex	Exocyst (Sec6/Sec8)	Protein–protein interaction	Delivery of vesicles to target membrane
Rab GTPase	Rab3A	Geranylgeranylation	Interaction with docking complex and indirect regulation of SNARE inhibition

*Canonical examples are specific to vertebrate synaptic vesicle exocytosis unless otherwise indicated.

Figure 1

Schematic model illustrating the steps in vesicle fusion. During priming, pre-existing SNARE complexes are unfolded by the chaperone ATPase NSF. In the docking step, a multisubunit docking factor, recruited to the vesicle through Rab GTP, guides the vesicle to an appropriate target membrane surface. Finally, inhibitory proteins are released from the SNARE component proteins, the SNARE complex forms and vesicle fusion proceeds.



to SNARE components and inhibit their assembly into the SNARE complex [4]. Inhibitors that bind to transmembrane t-SNAREs (munc18), and to transmembrane v-SNAREs (synaptophysin) have been described. A third class of fusion proteins are chaperones that disassemble pre-existing SNARE oligomers, and maintain them in a primed state ready for subsequent fusion events [1]. The central member of this family of proteins, NSF (N-ethylmaleimide-sensitive factor), hydrolyzes ATP to unfold the highly stable ternary SNARE complex. NSF associates with SNARE components through the adapter proteins α, β- and γ-SNAP (soluble NSF attachment proteins; no relation to SNAP-25). A passive chaperone, LMA1 (low molecular weight activity 1; a heterodimer of thioredoxin and protease B inhibitor IB2 specific to yeast vacuole fusion), maintains disassembled SNARE proteins in the uncomplexed state prior to fusion. A fourth class of fusion proteins comprise the multisubunit docking complexes, large (≥ 800 kDa) protein assemblies that recognize specific vesicles and deliver them to appropriate membrane surfaces [5]. Multisubunit docking complexes in yeast have been described for exocytosis at the plasma membrane (Exocyst), for endosome fusion (the Rabaptin-5 complex) and for endoplasmic reticulum to Golgi transport (TRAPP; transport protein particle). Finally, a fifth class of fusion proteins, the small Rab GTPases, help to regulate the timing and succession of molecular events during fusion [5]. Rab GTPases mediate interactions between vesicles and the multisubunit docking complexes in a manner that depends on the state of the nucleotide cofactor. Rab GTPases also appear to participate indirectly in the relief of inhibition of SNARE components by SNARE inhibitor proteins.

Figure 1 illustrates a simplified model of vesicle fusion. Newly budded vesicles emerge with Rab–GTP on their surfaces. The Rab–GTP molecules recruit multisubunit docking complexes, which deliver the vesicle to the

appropriate target membrane. Before, during or after the docking process, SNARE components on both the vesicle and target surfaces are primed for fusion. The ATPase NSF disassembles existing intramembrane SNARE complexes, and passive chaperones maintain this open conformation. Independently, a yet undefined triggering process facilitates the dissociation of v- and t-SNAREs from their cognate inhibitory proteins. Finally, intermembrane SNARE complexes form and catalyze the fusion of lipid bilayers. Three key outstanding questions need to be addressed. Which molecules determine the target membrane specificity of vesicles? What regulates the switch between fusion-incompetent and fusion-competent states of membranes? How do SNARE proteins catalyze membrane fusion? A profusion of structural findings reported over the past few months have brought these issues into sharp focus.

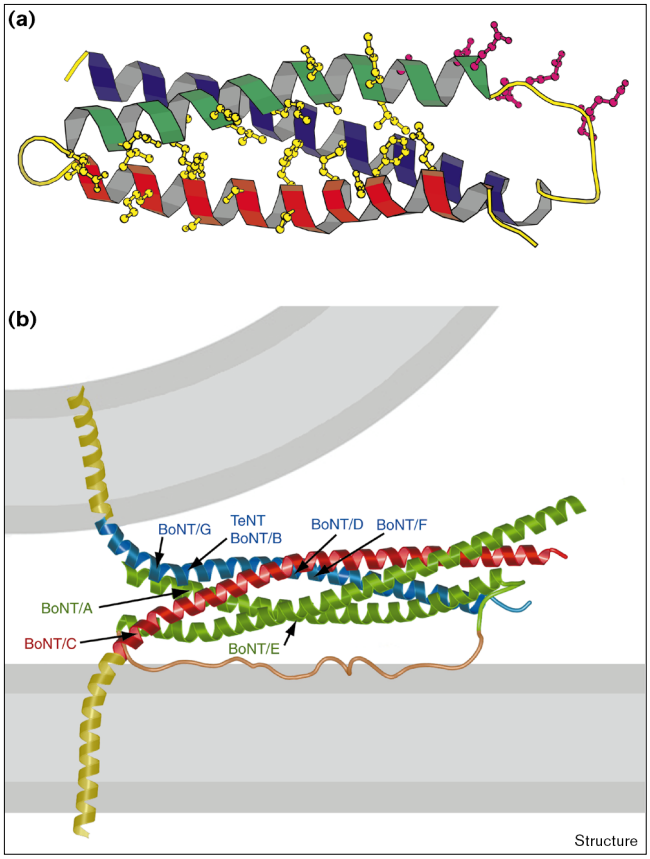
Regulation of fusion competence

The necessity to regulate SNARE complex assembly, in order to prevent uncontrolled or inappropriate association of lipid bilayers, has been recognized for some time [4]. Work from Nicholson *et al.* [6] and Fernandez *et al.* [7], has provided molecular insights into one mechanism for the regulation of t-SNARE accessibility. The biochemical studies of Nicholson and colleagues demonstrate that the N-terminal domain of the t-SNARE Sso1p (the syntaxin homolog that participates in Golgi to plasma membrane transport in yeast) inhibits association of the C-terminal domain with the yeast SNAP-25 homolog. This inhibition of binary SNARE complex formation is demonstrated to be kinetic rather than thermodynamic: the N-terminal domain slows Sso1p–SNAP-25 association by a factor of 2000, but has a negligible effect on the stability of the complex relative to the unfolded state. The second-order rate constant for the binary assembly of intact Sso1p with SNAP-25 (2–3 $M^{-1}s^{-1}$), is too small to account for physiological function. By inference, the intramolecular inhibition must be relieved *in vivo*. Simultaneously, structural studies by Fernandez and colleagues demonstrate that the corresponding N-terminal domain of syntaxin-1A folds into an antiparallel three-helix bundle (Figure 2a). One groove on the bundle surface, formed between helices B and C, is lined with conserved residues. A separate surface is shown to interact with the regulator of Ca^{2+}-induced exocytosis in neurons, synaptotagmin. An attractive hypothesis is that the conserved groove on the N-terminal domain of syntaxin-1A associates with the C-terminal domain (resulting in autoinhibition of SNARE complex formation) or with the t-SNARE inhibitor munc18, and that regulatory factors trigger fusion by disrupting these inhibitory interactions.

Catalysis of membrane fusion

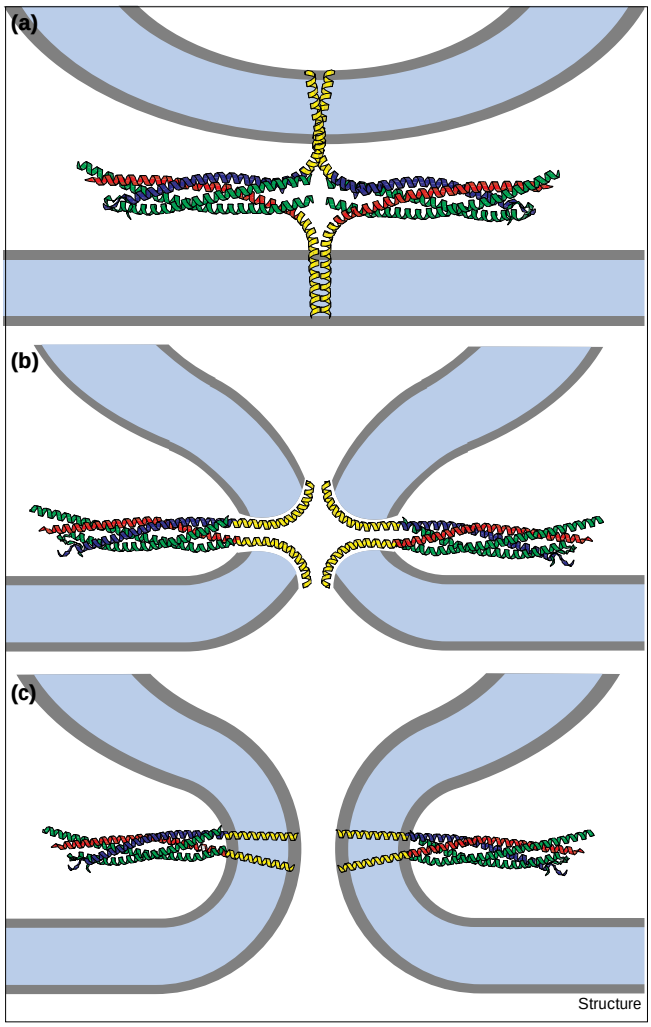
The structure of a core proteolytic fragment of the synaptic SNARE complex, reported by Sutton and coworkers [8], has provided a second breakthrough in the structural biology of vesicle fusion (Figure 2b). The core SNARE complex consists of a four-stranded parallel coiled coil. One helix is contributed by syntaxin-1A, two by SNAP-25B, and the fourth by synaptobrevin-II. The transmembrane regions of syntaxin-1A and synaptobrevin-II, the N-terminal domain of syntaxin-1A (described above), and a 38-residue linker connecting the two SNAP-25 helices were excized from the SNARE complex to facilitate crystallization.

Figure 2



Fragments of the core SNARE complex. (a) The antiparallel three-helix bundle structure of the N-terminal regulatory domain of syntaxin-1A [7]. Helices A, B and C are colored blue, green and red, respectively. Conserved residues in the binding groove between helices B and C are colored yellow. Residues that interact with synaptotagmin, the regulator of Ca^{2+} regulated exocytosis in vertebrate neurons, are colored magenta. (b) Hypothetical structure of the SNARE core complex based on the crystal structure of a proteolytic fragment. The four-stranded coiled coil formed by syntaxin-1A (red), synaptobrevin-II (blue), and SNAP-25 (green) derives from the experimental coordinates. The transmembrane helices (yellow) and the extended polypeptide linker connecting the two SNAP-25 helices (orange) have been modeled. Cleavage sites for the botulinum neurotoxin (BoNT) and tetanus neurotoxin (TeNT) proteases are indicated. (The figure was adapted from [8] with permission.)

Figure 3



Speculative transition state for SNARE-mediated membrane fusion. The phospholipid head groups and fatty acids of the lipid bilayer are depicted in gray and light blue, respectively. The crystal structure of syntaxin-1A (red), synaptobrevin-II (blue) and SNAP-25 (green), with hypothetical transmembrane helices (yellow), is shown. The figure is a plane slice through the fusion pore. In three dimensions, multiple SNARE complexes would be assembled in a ring around an axis normal to the membrane (vertical in the page). **(a)** Vesicle and target membranes tethered together by SNARE complexes. **(b)** Speculative transition state. Intramembrane and intermembrane interactions of the transmembrane helices coexist. A small water-filled cavity is shown at the center of the transmembrane helix bundle. **(c)** The transmembrane helices of syntaxin-1A and synaptobrevin-II reside in the same bilayer after membrane fusion. No helix curvature remains.

The first surprise offered by the structure is the parallel orientation of the two SNAP-25 helices, which suggests that the 38-residue linker connecting the helices must adopt an extended conformation. This parallel topology was reported independently by Poirier *et al.* on the basis of spectroscopic studies of spin-labeled SNARE complexes [9]. A second structural surprise was the discovery of an unusual buried polar motif at the center of the SNARE hydrophobic core. This motif consists of three glutamine residues (one from the syntaxin-1A helix and one from each of the SNAP-25 helices) and one arginine residue (from the synaptobrevin-II helix). The sidechain carbonyl groups of the three glutamine residues interact electrostatically with the guanidinium moiety of the arginine sidechain. All of the participant residues are highly conserved across t- and v-SNARE families [10]. The glutamine–arginine contacts in the SNARE complex are reminiscent of buried polar interactions observed in the coiled coils of GCN4 [11] and Myc/Max [12]; these interactions are thought to be important in determining strand number, helix orientation, and specificity of helix association. The core polar residues in the SNARE complex may be important determinants of the heterospecific association of v-SNARE and t-SNARE proteins, and may help to define how regulatory molecules and NSF interact with the SNARE components.

The SNARE complex structure is particularly exciting because purified syntaxin-1A, SNAP-25 and synaptobrevin-II have recently been demonstrated to catalyze the fusion of reconstituted vesicles [13] (albeit at a much slower rate than observed *in vivo*). The synaptic SNARE complex thus represents a minimal fusion machine, and has the potential to resolve the fundamental biological question of how proteins accelerate lipid bilayer association. One clue from the SNARE structure is its topological similarity to the fusion proteins of enveloped viruses [14]. In both vesicle and viral fusion, the formation of a stable helical oligomer pulls two membrane surfaces together. It has been proposed that the free energy derived from the formation of the helical oligomer drives the apposed membranes towards a fusion transition state [2]. But what does the transition state look like? Ultrastructural studies of exocytotic and viral fusion indicate that multiple fusion complexes, anchored in both the source and target membranes, assemble into a circular protein scaffold, promoting formation of a fusion pore. There has been considerable debate as to whether physical proximity alone is sufficient to induce bilayer fusion (through a purely lipidic transition state [15]), or whether the transition state intimately involves the fusion proteins [16].

Sutton and colleagues make the intriguing suggestion that the helices of the SNARE coiled coil could propagate directly into the transmembrane helices of syntaxin-1A and synaptobrevin-II. This proposal raises the possibility that intermembrane helix association extends from the coiled coil into the transmembrane regions, giving rise to the speculative transition state illustrated in Figure 3. It seems likely that future study of SNARE proteins will answer definitively such questions, and provide fundamental insights into the basis of protein-catalyzed membrane fusion in the year to come.

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References

1. Gotte, M. & von Mollard, G.F. (1998). A new beat for the SNARE drum. *Trends Cell Biol.* **8**, 215-218.
2. Hanson, P.I., Heuser, J.E. & Jahn, R. (1997). Neurotransmitter release – four years of SNARE complexes. *Curr. Opin. Neurobiol.* **7**, 310-315.
3. Nichols, B.J., Ungermann, C., Pelham, H.R., Wickner, W.T. & Haas, A. (1997). Homotypic vacuolar fusion mediated by t- and v-SNAREs. *Nature* **387**, 199-202.
4. Pfeffer, S.R. (1996). Transport vesicle docking: SNAREs and associates. *Annu. Rev. Cell Dev. Biol.* **12**, 441-461.
5. Schimmoller, F., Simon, I. & Pfeffer, S.R. (1998). Rab GTPases, directors of vesicle docking. *J. Biol. Chem.* **273**, 22161-22164.
6. Nicholson, K.L., Munson, M., Miller, R.B., Filip, T.J., Fairman, R. & Hughson, F.M. (1998). Regulation of SNARE complex assembly by an N-terminal domain of the t-SNARE Sso1p. *Nat. Struct. Biol.* **5**, 793-802.
7. Fernandez, I., Ubach, J., Dulubova, I., Zhang, X., Sudhof, T.C. & Rizo, J. (1998). Three-dimensional structure of an evolutionarily conserved N-terminal domain of syntaxin 1A. *Cell* **94**, 841-849.
8. Sutton, R.B., Fasshauer, D., Jahn, R. & Brünger, A.T. (1998). Crystal structure of a SNARE complex involved in synaptic exocytosis at 2.4 Å resolution. *Nature* **395**, 347-353.
9. Poirier, M.A., Xiao, W., Macosko, J.C., Chan, C., Shin, Y.K. & Bennett, M.K. (1998). The synaptic SNARE complex is a parallel four-stranded helical bundle. *Nat. Struct. Biol.* **5**, 765-769.
10. Weimbs, T., Mostov, K., Low, S.H. & Hofmann, K. (1998). A model for structural similarity between different SNARE complexes based on sequence relationships. *Trends Cell Biol.* **8**, 260-262.
11. O'Shea, E.K., Klemm, J.D., Kim, P.S. & Alber, T. (1991). X-ray structure of the GCN4 leucine zipper, a two-stranded, parallel coiled coil. *Science* **254**, 539-544.
12. Lavigne, P., *et al.*, & Kay, C.M. (1995). Preferential heterodimeric parallel coiled-coil formation by synthetic Max and c-Myc leucine zippers: a description of putative electrostatic interactions responsible for the specificity of heterodimerization. *J. Mol. Biol.* **254**, 505-520.
13. Weber, T., *et al.*, & Rothman, J.E. (1998). SNAREpins: minimal machinery for membrane fusion. *Cell* **92**, 759-772.
14. Chan, D.C. & Kim, P.S. (1998). HIV entry and its inhibition. *Cell* **93**, 681-684.
15. Monck, J.R. & Fernandez, J.M. (1996). The fusion pore and mechanisms of biological membrane fusion. *Curr. Opin. Cell Biol.* **8**, 524-533. [Published erratum in *Curr. Opin. Cell Biol.* (1996) **8**, 890].
16. Lindau, M. & Almers, W. 1995. Structure and function of fusion pores in exocytosis and ectoplasmic membrane fusion. *Curr. Opin. Cell Biol.* **7**, 509-517.