Membrane fusion: **Structure snared at last**

Frederick M. Hughson

The structure of the core of the neuronal 'SNARE complex', involved in neurotransmitter release, has been determined recently. Its topological similarity to viral fusion proteins suggests how the SNARE complex might facilitate membrane fusion.

Address: Department of Molecular Biology, Princeton University, Princeton, New Jersey 08544, USA.
E-mail: hughson@princeton.edu

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Intracellular transport and secretion are accomplished in eukaryotic cells by vesicles that bud from one cellular membrane and fuse with another. Proteins that mediate these processes have been identified by using genetic and biochemical approaches. A number of recent papers have now reported results that begin to illuminate biophysical and structural principles for the proteins involved in the docking and fusion of carrier vesicles. These results should give a powerful boost to those seeking to understand how these proteins function *in vivo*.

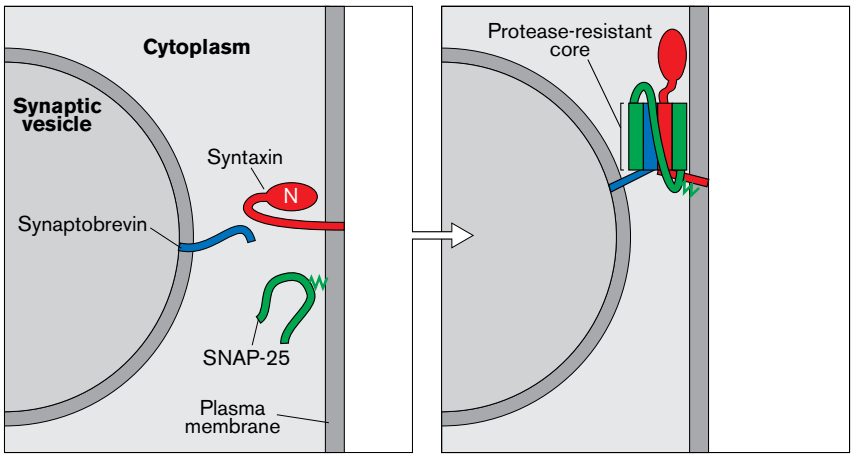
Biophysical studies have proceeded furthest for two sets of proteins. Much work has focused on a set of neuronal proteins required for the fusion of synaptic vesicles with the axon membrane [1]. Concurrently, several groups have been studying the yeast homologs of these neuronal proteins, which act in the fusion of Golgi-derived vesicles with the plasma membrane. In both cases, membrane-associated proteins known as 'SNAREs' seem to play key roles [2]. In neurons, each of the SNAREs is the target of the proteolytic action of at least one neurotoxin that disables neurotransmitter release. Genetic deletion of neuronal SNAREs, or their yeast homologs, severely compromises their function. SNAREs are thus thought to play central roles in vesicle fusion and/or in the still poorly understood docking steps leading up to it. Other intracellular transport pathways — for example, from endoplasmic reticulum to Golgi — require different SNARE family members, suggesting that SNAREs fulfill one or more generally important functions.

Rothman and colleagues [3] coined the terms v-SNARE and t-SNARE for SNAREs localized to vesicle and target membranes, respectively. It seems logical that vesicle docking at a target membrane prior to fusion might be accomplished by the specific pairing of v-SNAREs and t-SNAREs. The founding members of the SNARE protein family — those involved in neurotransmitter release — are synaptobrevin (also called VAMP), syntaxin and SNAP-25, and they do indeed form binary and ternary complexes. Synaptobrevin is a small v-SNARE anchored in the synaptic vesicle membrane by virtue of a carboxy-terminal hydrophobic stretch of amino acids likely to form a single transmembrane helix. Syntaxin is a plasma-membrane-localized t-SNARE with a similar carboxy-terminal anchor. SNAP-25, a t-SNARE, has no obvious transmembrane domain, but near the middle of its sequence are four cysteines, one or more of which are palmitoylated. This lipid modification presumably contributes to SNAP-25's plasma membrane localization. The t-SNAREs syntaxin and SNAP-25 form a binary complex, to which the v-SNARE synaptobrevin binds to form a ternary complex of exceptional stability [4].

What is the structural basis of SNARE complex formation? SNAREs were suggested to interact by forming heteromeric α-helical coiled coils. This conjecture was based on the presence of repeated heptad motifs within many SNARE sequences, in which the first and fourth amino acids within each heptad are generally nonpolar. Consistent with this hypothesis, the α-helix content of binary and ternary SNARE complexes, measured by circular dichroism, is high [4,5]. Furthermore, the ternary complexes visualized by quick-freeze/deep-etch electron microscopy are elongated rods of dimensions approximately 4× 14 nm, compatible with a three-stranded (or four-stranded) coiled coil [6]. Careful analysis of multiple sequence alignments suggested that SNAP-25 might contribute two helical segments to a coiled coil, while syntaxin and synaptobrevin each contribute one (Figure 1) [7].

These ideas have been borne out strikingly by a landmark X-ray crystal structure of the core of the ternary SNARE complex (Figure 2a) [8]. A structural model, developed on the basis of spin-labeling electron paramagnetic resonance (EPR) spectroscopy measurements, was reported almost simultaneously [9]. This model is remarkably similar to the 2.4 Å crystal structure, suggesting that, at least in special cases, EPR spectroscopy can be an effective structural tool. In each case, expressed proteins that lacked transmembrane domains were combined to form complexes and subjected to limited proteolysis [10,11]. This removed an amino-terminal region of syntaxin, seen by electron microscopy to constitute a separate domain projecting from one end of the elongated rod [6] (Figure 1), as well as a central region of SNAP-25. The X-ray and EPR-based structures thus both have four approximately 70-residue segments, one each from synaptobrevin and syntaxin, and two from SNAP-25. As predicted, the four

Figure 1



A schematic diagram of SNARE complex assembly. The v-SNARE synaptobrevin (blue) pairs with two t-SNAREs, syntaxin (red) and SNAP-25 (green). Synaptobrevin and syntaxin are anchored at their carboxy-terminal ends in the synaptic vesicle and axon plasma membranes, respectively. SNAP-25 associates with the plasma membrane at least in part because of one or more palmitoyl groups attached to cysteine residues near the middle of the polypeptide chain. A central core of the ternary SNARE complex resists proteolytic digestion; the structure of this domain has recently been determined (see text and Figure 2). An amino-terminal domain of syntaxin (labeled N) may control SNARE assembly by preventing the carboxy-terminal domain from interacting with SNAP-25 and synaptobrevin (see text).

segments were seen to form helices in a four-stranded coiled coil (Figures 1,2).

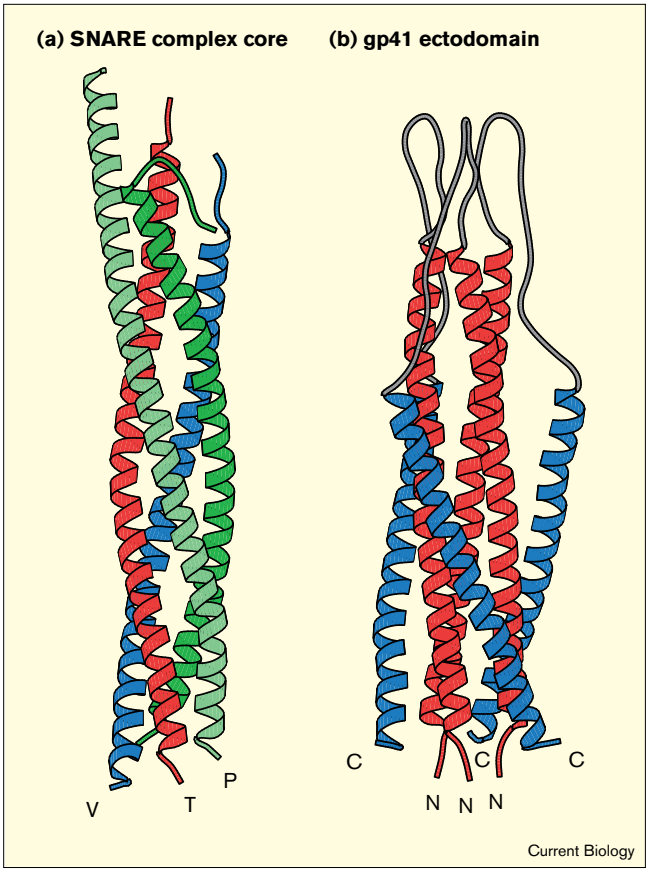
The orientation of the four helices within the bundle holds several surprises that hint at function. In an earlier study, Hanson *et al.* [6] marked the ends of synaptobrevin and syntaxin with tags that could be visualized by electron microscopy, and thereby demonstrated that synaptobrevin and syntaxin are in a parallel orientation in ternary complexes. The carboxyl termini of the two proteins are thus at the same end of the elongated rod, a finding that was initially surprising as the two parallel SNAREs have their carboxyl termini anchored in opposed membranes. This observation provides an appealing mechanism, however, through which the energy of SNARE complex formation might be used to drive the close apposition of the vesicle and target membranes. The newly-determined structures [8,9] confirm that synaptobrevin and syntaxin contribute parallel helices to the four-stranded coiled coil. They further show that the two SNAP-25 helices are parallel to each other and to each of the other helices. The excised linker region of SNAP-25 must therefore traverse the length of the rod in the intact complex.

The structure also provides a likely explanation for the registration of the four helices. Although most of the helix positions that face toward the core of the coiled coil are hydrophobic, one 'layer' of the coiled coil is particularly polar in nature. Here, approximately in the middle of the rod, the four helices contribute an arginine and three glutamine residues to the core [8]. The three glutamine carboxyl groups presumably stabilize the charge on the arginine guanido group that is in an otherwise hydrophobic environment. Alternative structures in which these four residues were not in register with one another would presumably be significantly destabilized.

The coiled-coil structure is likely to be conserved among different SNARE complexes. In particular, similar segments of the yeast exocytotic SNAREs form elongated rods indistinguishable by electron microscopy from those of their neuronal counterparts [12]. The same types of tagging experiment that proved informative about the orientation of the neuronal proteins were used to demonstrate that four segments from the three yeast proteins form a parallel bundle. The low degree of sequence identity between the yeast and neuronal proteins makes it challenging to build an accurate structural model of the yeast SNARE complex on the basis of the neuronal complex structure. Nonetheless, a parallel four-stranded coiled-coil model is roughly consistent with the locations of a temperature-sensitive mutation in the yeast SNAP-25 homolog and its suppressor mutation in the yeast synaptobrevin homolog [12]. These genetic results further suggest that a parallel coiled-coil structure is functionally relevant *in vivo*.

Do the SNARE proteins themselves mediate membrane fusion? One recent report [13] showed that the neuronal v-SNAREs and t-SNAREs, separately reconstituted into synthetic vesicles, can mediate lipid mixing, and by inference fusion, between these vesicles. Because the observed fusion was much slower than neurotransmitter release, important components may be missing from the reconstituted system or may not be fully active. While further work on this and other *in vitro* systems will undoubtedly shed more light on the precise roles of SNAREs in docking and fusion, the recent structural results provide at least one more good reason for believing that SNAREs play a direct role in fusion: the core structure of the neuronal SNARE complex displays striking parallels with structures of core fragments of viral fusion proteins (Figure 2) [14].

Figure 2



(a) The structure of the SNARE complex core [8]. Four parallel α helices from synaptobrevin (blue), syntaxin (red) and SNAP-25 (green) form the core of the synaptic complex. The carboxy-terminal transmembrane domains of synaptobrevin and syntaxin, a central 'linker' region of SNAP-25 and the amino-terminal regulatory domain of syntaxin are not present. Amino-terminal and carboxy-terminal segments of SNAP-25 (light and dark green, respectively) each contribute one helix separated by a 54-residue loop, a small part of which is visible just prior to the amino-terminal end of the carboxy-terminal helix (top). Membrane attachments are marked as V (vesicle membrane end of synaptobrevin), T ('target' plasma membrane end of syntaxin), and P (the beginning of the SNAP-25 linker, containing the palmitoyl group(s); see Figure 1). **(b)** The structure of a soluble portion of the trimeric SIV gp41 ectodomain [15]. This helical bundle comprises an inner coiled coil of amino-terminal α helices (red) flanked by three carboxy-terminal helices (blue). Beyond the carboxy-terminal ends of this fragment are the transmembrane segments embedded in the viral membrane; prior to the amino termini are the amino-terminal 'fusion peptide' sequences thought to engage the target cell membrane.

To illustrate this point, consider the protease-resistant core of the simian immunodeficiency virus (SIV) protein gp41 (Figure 2b) [15]. Like the neuronal SNARE complex, this structure is a rod-like bundle of α helices in which the hydrophobic segments associated with the opposed membranes are at the same end of the rod. Like the neuronal complex, this structure is thought to be formed in an assembly process that could serve to draw the membranes together. In the case of gp41, this assembly process is intramolecular, involving a significant conformational change. Both structures, once formed, are highly stable, suggesting that the energy of their assembly might be harnessed to do work against the hydration force that hinders close approach of membranes. Other viral fusion proteins share most of these properties [14]. Therefore, the new structure is consistent with the hypothesis that SNAREs can mediate fusion by a mechanism similar to that employed by viral fusion proteins.

What about the amino-terminal domain of syntaxin, which was proteolytically removed from the SNARE complex to promote crystallization (Figure 1)? Electron micrographs of the intact ternary complex showed a separate domain dangling off of one end of the rod-like core [6], and thermal denaturation experiments performed on the yeast complex showed that this domain unfolds independently [16]. A start is being made in elucidating the roles of this domain. The amino-terminal domain of the yeast syntaxin homolog Sso1p can regulate SNARE complex assembly [16]: in the absence of this regulatory domain, binary and ternary SNARE complexes assemble *in vitro* 2,000-fold more rapidly than they do with the domain present. These results imply that activator proteins may be required *in vivo* to allow SNARE complex assembly to proceed at a physiologically plausible rate. Furthermore, isolated amino-terminal and carboxy-terminal regions of Sso1p bind to one another, suggesting a mechanism by which the amino-terminal domain might block SNARE assembly.

The solution structure for the amino-terminal domain of syntaxin, determined by NMR spectroscopy [17], reveals a three-helix bundle with a highly conserved groove. In the intact protein, this helix bundle presumably binds to the carboxy-terminal syntaxin segment and prevents it from pairing with its cognate SNAREs. It seems appealing to suggest that the complex between amino-terminal and carboxy-terminal segments of syntaxin/Sso1p is a four-helix bundle, a suggestion consistent with the available evidence [5,16–18]. It will be interesting to characterize the structure of the intact syntaxin/Sso1p protein at high resolution. Nonetheless, after a long period in which little was known about the detailed structure of any SNARE protein, exciting progress has clearly been made.

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