The fusion of vesicles with target membranes is controlled by a complex network of protein–protein and protein–lipid interactions. Recently determined structures of the SNARE complex, synaptotagmin III, nSec1, domains of the NSF chaperone and its adaptor (SNAP), and Rab3 and some of its effectors provide the framework for developing molecular models of vesicle fusion and for designing experiments to test these models. Ultimately, knowledge of the structures of higher-order complexes and their dynamic behavior will be required to obtain a full understanding of the vesicle fusion protein machinery.

Addresses
The Howard Hughes Medical Institute and Departments of Molecular and Cellular Physiology, Neurology and Neurological Sciences, and Stanford Synchrotron Radiation Laboratory, Stanford University, Stanford, CA 94305, USA; e-mail: axel.brunger@stanford.edu

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Abbreviations
GDI GDP-dissociation inhibitor
GppNHp guanosine-5′-(β,γ)-imidotriphosphate
NSF N-ethylmaleimide-sensitive factor
PEG polyethylene glycol
SNAP soluble NSF-attachment protein
SNAP-25 synaptosomal-associated protein of 25 kDa
SNARE soluble NSF-attachment protein receptor
VAMP vesicle-associated membrane protein

Introduction
Vesicular trafficking in eukaryotic cells is essential for diverse cellular processes, including maintenance of distinct subcellular compartments, protein and hormone secretion, egg fertilization and neurotransmitter release [1–5,6••]. The life cycle of a vesicle generally consists of three stages (Figure 1): endocytosis or formation of the vesicle from specific cellular membranes; exocytosis or fusion of the vesicle with its target membrane; and recycling of the components of the protein machinery after exocytosis. This review focuses on recent structural studies of the key proteins responsible for exocytosis and recycling.

Vesicular exocytosis utilizes a protein machinery that is conserved from yeast to man [7,8]. SNARE (soluble N-ethylmaleimide-sensitive factor [NSF]-attachment protein receptor) proteins are essential components of this machinery [2,4]. In synaptic vesicle exocytosis, three SNARE proteins are involved: the plasma-membrane-associated proteins syntaxin and SNAP-25 (synaptosomal-associated protein of 25 kDa), and the vesicular protein synaptobrevin, also referred to as VAMP (vesicle-associated membrane protein). Other conserved proteins include the ATPase NSF [9] and its adaptor, known as SNAP (soluble NSF-attachment protein) [10], the Rab class of small G proteins and their effectors [11], the synaptotagmin family [12] and the nSec1 (neuronal homolog of the yeast Sec1 protein, also referred to as Munc18) family [13]. Many other factors that interact with SNAREs have been characterized, such as complexin [14], VAP33 [15] and synaptophysin [16].

Figure 2 summarizes some of the key stages involved in synaptic vesicle fusion. Initially, syntaxin is bound to nSec1 and synaptobrevin is probably bound to a factor such as synaptophysin or VAP33. Both syntaxin and synaptobrevin are single-pass membrane proteins. A yet to be identified molecular machinery probably brings the vesicle and plasma membrane into close proximity, allowing SNAREs on opposite membranes to form cis complexes [17••]. Synaptobrevin then binds to syntaxin and SNAP-25. At the priming stage, the system becomes competent to undergo fusion upon an increase in Ca2+ concentration in the micromolar range [18••], possibly involving a Ca2+-binding protein such as synaptotagmin. At the recycling stage, α-SNAP and NSF bind to the SNARE complex, and the SNARE complex is then dissociated upon ATP hydrolysis.

Before docking, vesicles have to be targeted to the correct location at the appropriate time. This process is much less understood than the later stages of vesicle fusion. However, some of the molecular components of this targeting process are beginning to emerge. Among them are the...
sec6/8 complex in mammalian cells [19] and the exocyst in yeast [20]. These are large (> 700 kDa) macromolecular complexes that could be involved in targeting processes before SNAREs become involved.

SNAREs
The SNARE complex can be isolated from neuronal cell extracts [21]. It can also be assembled from recombinantly expressed and purified proteins in vitro [22,23]. The membrane anchors are not required for the assembly of the SNARE complex. The SNARE complex exhibits remarkable thermal and chemical stability [22,23]. Limited proteolysis of the synaptic SNARE complex revealed a core complex with similar biophysical properties to the full-length complex [24,25]. This core complex is sufficient to promote vesicle fusion in vitro [26*,27*,28**].

The SNARE core complex consists of a parallel four-helix bundle [29], whereas the N-terminal domain of syntaxin consists of an antiparallel three-helix bundle [30] (Figure 3). The core of the four-helix bundle of the SNARE complex is composed of layers formed by interacting sidechains from each of the four α helices [29]. These layers are highly conserved across the entire SNARE family. At the center of the core complex, a conserved ionic layer was found, consisting of an arginine and three glutamine residues contributed by each of the four α helices [29]. Interestingly, this ionic layer is sealed off against solvent by adjacent hydrophobic layers. This
energetically somewhat unfavorable configuration presumably has some functional role during SNARE complex assembly or disassembly. A glutamine to leucine mutation in SNAP-25 has been shown to affect exocytosis in adrenal chromaffin cells [31**].

Mutations in these and other layers reduce complex stability and cause defects in membrane trafficking, even in distantly related SNAREs [32]. Based on the conservation of the core of the SNARE complex, SNAREs were reclassified into Q-SNAREs and R-SNAREs, and it was proposed that fusion-competent SNARE complexes generally consist of four-helix bundles comprising three Q-SNAREs and one R-SNARE [32]. A possible exception to this 3Q:1R rule is the homotypic vacuolar fusion system, in which five distinct SNAREs interact [33**]. However, these experiments were carried out with extracts of yeast and analyzed by immunoprecipitation, so it is not clear whether all five vacuolar SNAREs interact quantitatively in a single pentameric complex.

SNAREs have several conformational states [34**–36**] (Figure 4): first, the ‘closed’ conformation of uncomplexed syntaxin and the unstructured or flexible conformations of synaptobrevin and SNAP-25; second, the binary complex of syntaxin and SNAP-25; and third, the ternary complex of syntaxin, SNAP-25, and the cytoplasmic domain of synaptobrevin. The closed conformation of uncomplexed syntaxin contains a four-helix bundle made up of the regulatory N-terminal HAHBHC domain and roughly half of the core complex domain Hcore (Figure 4). The topology of this closed conformation was predicted on the basis of NMR data [34**] and a crystal structure of the yeast syntaxin homolog Sso1 has now been solved [37**]. A similar conformation of syntaxin has recently been observed in the crystal structure of syntaxin-nSec1 complex [38**], suggesting that it is the closed conformation of syntaxin that binds to nSec1.

Syntaxin switches to an ‘open’ state upon binding to SNAP-25 [34**]. In this ‘open’ state, binding to the other SNAREs is mediated by the Hcore domain. Conformational switching of the Hcore domain, mediated by the HAHBHC domain, represents a regulatory mechanism for SNARE complex assembly by affecting the kinetics of ternary complex formation [26•,39]. Formation of binary and ternary complexes is associated with increasing induction of α-helical structure in previously unstructured or flexible regions of SNAREs [22,34**–36**,40]. Because the N-terminal half of the syntaxin Hcore domain is always folded (Figure 4), these data suggest that SNARE complex assembly begins distal to the membrane surfaces and proceeds toward them. This ‘zipper’ model of vesicle fusion was also suggested by experiments using fluorescence resonance energy transfer [41], electron microscopy [4,42,43] and electron spin polarization [44] of labeled SNARE complexes.

Role of SNAREs

Although the precise function of the SNAREs is the topic of some debate, there is overwhelming evidence that they play a fundamental role in membrane fusion. First, site-specific cleavage of SNAREs by clostridial neurotoxins inhibits neurotransmission [45,46]. Second, SNAREs represent a minimal fusion machinery: SNAREs reconstituted into artificial liposomes can induce fusion [107**]. Experiment in a permeabilized PC12 cell system also confirmed the importance of SNAREs for fusion in vivo [49**]. Third, the soluble domains of SNAREs spontaneously assemble into an extremely stable four-helical bundle in vitro [22,29]. The α-helical composition and the high thermal and chemical stability are similar to those of proteins involved in viral fusion, possibly indicating a common ancestral mechanism for both fusion systems [50]. Fourth, complex formation probably proceeds in a directed fashion, starting at the membrane-distal end of the complex and proceeding towards the membrane-proximal C terminus [34**] (Figure 4). This directed assembly process may bring membranes into close proximity, thus overcoming the free-energy barrier to stalk formation. The membrane domains of syntaxin and synaptobrevin appear to be essential, as replacing them with a geranylgeranyl group inhibits fusion in yeast [51**]. Recent studies of the transmembrane domain of synaptobrevin indicate that it is α-helical and tilted with respect to the bilayer normal by approximately 50° (ME Bowen, AT Brunger, unpublished data).
The hypothetical model presented in Figure 4 assumes the existence of a partly assembled state of SNAREs docked between two membranes. Although this state has not been directly observed, there is indirect evidence for such an intermediate state. First, the cleavage sites for all clostridial neurotoxin proteases are located in the C-terminal (membrane-proximal) half of the core complex [29,46]. However, SNAREs probably have to bind in an extended conformation to the proteases, as suggested by the co-crystal structure of a fragment of synaptobrevin and the protease domain of the botulinum neurotoxin type B [52**]. As SNAREs are protected against proteolysis in the fully assembled complex [45,46], this suggests that SNAREs must exist in partly assembled or ‘loose’ states for significant periods of time. Recent experiments further support this hypothesis: the C terminus of synaptobrevin is sensitive to toxins in the docked state, but the N terminus is not [53*]. Kinetic studies of chromaffin cell exocytosis revealed a fusion-competent state that is sensitive to the attack of clostridial neurotoxins [54]. Inhibition of SNARE complex assembly by antibody binding differentially affected kinetic components of exocytosis, suggesting the existence of loose and tight SNARE complex states [55**].

Analysis of PEG-induced fusion of artificial liposomes suggested the existence of two intermediate stages of vesicle fusion: a stalk and a hemifusion state [56,57]. Assuming that similar stages exist during the fusion of cellular vesicles with target membranes, one can speculate that SNARE complex formation could lower the free-energy barrier to reaching the stalk intermediate state. In addition, SNARE complex formation could lower the free-energy transition-state barriers between the stalk state, the hemifusion state and the fused state of the system. It is, however, probable that other factors (such as proteins or the lipid composition of synaptic vesicles) are involved in regulating these free-energy barriers, especially in view of the fact that neuronal vesicle fusion is tightly Ca²⁺-regulated and proceeds at a faster timescale (milliseconds) than can be accomplished by in vitro fusion induced by SNAREs (minutes).

Other experiments have been interpreted to cast doubt on the central role of SNAREs in membrane fusion. In vitro experiments on sea urchin cortical vesicles showed a discrepancy between vesicle fusion, as assayed by turbidity measurements, and the time-course of SNARE complex formation [58,59]. Homotypic fusion between cortical vesicles is an extremely unlikely event in vivo. In these experiments, contacts between fusing vesicles therefore had to be induced by applying a centrifugal force. Thus, these experiments do not necessarily dispute the role of SNAREs in the biological context.

In vitro studies of homotypic vacuolar fusion during yeast cell division showed that SNARE complexes can be disassembled before fusion [60]. This observation does not necessarily rule out a role for SNAREs in membrane fusion. Perhaps SNARE complexes could be disassembled without undocking the membranes if the system is committed for fusion at the irreversible hemifusion stage [56,57].

**SNARE interactions are promiscuous**

The primary sequence conservation of the structural core of the SNARE complex casts doubt on the targeting role of SNAREs in vesicle trafficking, as originally proposed by the SNARE hypothesis [1]. Indeed, very similar biophysical and biochemical properties were obtained in vitro for complexes consisting of artificial combinations of SNAREs that are localized to different compartments in vivo [61**,62**]. Furthermore, some SNAREs can function at several different transport steps in vivo [63]. Thus, SNAREs cannot be the sole determinants of vesicle targeting specificity. Rather, the observed SNARE localizations may be important for interactions with other factors, such as nSec1, that interact with nonconserved SNARE residues [38**].
Interactions of syntaxin with nSec1

The partially structured, ‘closed’ state of syntaxin interacts with nSec1 [37**,38**,64**,65**]. The conformation of syntaxin found in the crystal structure of this complex is dramatically different from that in the ternary SNARE complex. C-terminal syntaxin residues that are unstructured or flexible in solution adopt a sequence of small α-helical fragments connected by short loops when complexed with nSec1. In the ternary SNARE complex, these residues form a continuous α helix.

The flexible regions of uncomplexed syntaxin could have local structure that is related to the structure of syntaxin in the nSec1-syntaxin complex. The chemical shift dispersion of α helices is small and the motion produced by flexible hinges may explain the 1H–15N heteronuclear single quantum correlation (HSQC) NMR spectrum that was observed for uncomplexed syntaxin [36**] and its yeast homolog Sso1 [34**]. It is thus probable that nSec1 acts by stabilizing one of the conformations of uncomplexed syntaxin. The conformational transition of syntaxin is a striking example of the role of conformational flexibility in biological function. In contrast to syntaxin, nSec1 does not undergo major conformational changes upon binding to syntaxin [65**].

Experiments in yeast show an interaction between sec1 and the assembled plasma membrane SNARE complex [66**,67**]. This is in contrast with the neuronal case, whereby interactions between syntaxin and nSec1, and between syntaxin, SNAP-25 and synaptobrevin are mutually exclusive [64**]. One could speculate that two distinct conformations exist for the sec1 family of proteins or that a transient interaction exists between sec1 and the partially assembled SNARE complex [38**,64**,67**].

Synaptotagmin

Synaptotagmin is a membrane-associated protein that interacts with SNAREs, phospholipid membranes, Ca2+-channels and proteins involved in endocytosis [12,68–71]. In the cytosolic portion of this protein, a flexible seven α-helical fragment is connected to an extended wing comprising the C2A and C2B domains [72**]. The C2A domain binds to anionic phospholipids [73,74] and other accessory proteins, such as syntaxin [75], in a Ca2+-dependent fashion. No conformational change is observed upon Ca2+ binding [76], except for rotation changes of the Ca2+-coordinating aspartic acid residues. The C2B domain promotes binding to other C2 domains [71], as well as to accessory proteins, independently of Ca2+. The crystal structure of synaptotagmin III, which includes the C2A and C2B domains, exhibits differences in the shape of the Ca2+-binding pocket, the electrostatic surface potential and the stoichiometry of bound divalent cations for the two domains [72**]. The C2A and C2B domains do not directly interact; synaptotagmin, therefore, covalently links two independent C2 domains, each with potentially different binding partners. The C2B domain is involved in oligomerization of synaptotagmin [77*]. Interestingly, neuronal proteins such as rabphilin [78] and Doc2 [79] also possess multiple C2 domains similar to those of synaptotagmin. The structure of the C2B domain of rabphilin is very similar to the C2B domain of synaptotagmin III [72**,80**].

Synaptotagmin and the SNARE complex interact independently of Ca2+, although the interaction is enhanced upon addition of Ca2+ [72**,81*,82]. The Ca2+-binding domains probably interact with the plasma membrane [73], whereas the polybasic regions could interact with the SNARE core complex [29,72**].

Rab3A

Members of the Rab family of small G proteins regulate vesicular membrane traffic in all eukaryotic cells [11,83–86]. Rab3A predominantly localizes to synaptic vesicles and plays an important role in the regulation of neurotransmitter release [87]. Rab proteins were suspected to be determinants of vesicle targeting specificity because distinct isoforms display unique cellular localizations [84]. However, studies of chimeric Rab proteins suggested that Rabs can function at two distinct transport steps — vesicular transport from the endoplasmic reticulum to the Golgi and fusion of post-Golgi secretory vesicles to the plasma membrane [88,89] — suggesting that Rabs cannot be the sole targeting determinant. Like other small G proteins, the Rab family members may function as molecular switches or timers, cycling between the inactive GDP-bound and active GTP-bound forms, regulating their effector proteins and downstream targets accordingly.

In the cytosol, Rab proteins are kept in the inactive, GDP-bound state by the Rab GDI (GDP-dissociation inhibitor), preventing them from nonspecific binding to membranes. Upon binding to a specific donor compartment or vesicle, GDI is displaced by a GDI-displacement factor (GDF). Exchange of GDP for GTP is then catalyzed by GEFs (guanine exchange factors), activating the Rab protein and rendering it resistant to removal from the membrane by Rab GDI. GTP is hydrolyzed by the intrinsic GTPase activity of the Rab protein. The transition-state barrier of the hydrolysis reaction is lowered by GTPase-activating proteins (GAPs). Once vesicle fusion has occurred, GDI can release the GDP-bound form of Rab to the cytoplasm and the cycle begins again.

Gene knockout of Rab3A impairs regulation of neurotransmitter release [86,90]. The GTP-bound form of Rab3A interacts with at least two effector proteins, rabphilin-3A [78] and rim [91], which may interact with, as yet unknown, downstream targets. Activated Rab3A reversibly recruits rabphilin-3A to synaptic vesicles [78]. Rim has sequence similarity to rabphilin-3A, but localizes to the active zone of the presynaptic plasma membrane, instead of to synaptic vesicles [91].

A relatively large number of Rab proteins and their effectors are present in eukaryotic cells. A structural basis for
the specific pairing between these proteins was recently proposed based on the structure of activated Rab3A–GTP–Mg\(^{2+}\) bound to the effector domain of rabphilin-3A [92\textsuperscript{••}]. Rabphilin-3A contacts Rab3A primarily in two distinct areas [92\textsuperscript{**}]; few conformational changes are observed upon complex formation [92\textsuperscript{**},93\textsuperscript{••}]. The first area involves the Rab3A switch I and switch II regions, which are sensitive to the nucleotide-binding state of Rab3A. The second area consists of a hydrophobic surface pocket in Rab3A that interacts with a SGAWFF structural element of rabphilin-3A. Based on sequence analysis and biochemical and structural data, it was proposed that this pocket or ‘Rab complementarity-determining region’ (RabCDR) establishes a specific interaction between each Rab protein and its effectors [92\textsuperscript{••}]. Based on the crystal structure of the Rab3A–rabphilin-3A complex, it was proposed that small G proteins generally can have diverse surface areas for effector recognition. The recent structure of Rho complexed with the effector domain of PKN/PRK1 [94\*] supports this hypothesis: the variable C-terminal α helix of the small G protein Rho is involved in binding to the effector binding domain of PKN/PRK1 in a fashion similar to rabphilin.

The crystal structure of Sec4, a member of the Rab family in the G protein superfamily, was determined in two states: bound to GDP and bound to a nonhydrolyzable GTP analog, guanosine-5’-(β,γ)-imidotriphosphate (GppNHp) [95\textsuperscript{**}]. This represents the first structure of a Rab protein bound to GDP. Sec4 in both states grossly resembles other G proteins bound to GDP and GppNHp. In Sec4–GppNHp, structural features common to active Rab proteins are observed. In Sec4–GDP, the switch I region is highly disordered and displaced relative to the switch I region of Ras–GDP. This structural variability in both the switch I and switch II regions of GDP-bound Sec4 provides a possible explanation for the high off-rate of GDP bound to Sec4 and suggests a mechanism for the regulation of the GTPase cycle of Rab proteins by GDI proteins.

The crystal structure of GDI has also been solved [96], GDI functions in recycling and regulating Rab proteins. Residues have been identified that are probably involved in binding to Rab proteins, but full elucidation of these interactions has to await the determination of the structure of a GDI–Rab complex.

**NSF**

According to a current model, NSF and members of the SNAP family of proteins act together to disassemble SNARE complexes before and after fusion. SNARE proteins can form both cis (same membrane) and trans (opposing membranes) complexes that are substrates for SNAPs and NSF [21,60]. As discussed above, trans SNARE complexes are important for membrane fusion. Fusion of opposing membranes results in the formation of cis SNARE complexes that are disassembled for recycling and reactivation by the joint action of SNAP and NSF. Interestingly, NSF also interacts with glutamate receptors [97]. An additional function of NSF could be in glutamate receptor cycling in and out of the synaptic postsynaptic membrane through endocytosis and exocytosis [98\*], a process that may be connected to long-term potentiation and long-term depression.

NSF is a hexamer [99] and belongs to the AAA (ATPases associated with cellular activities) family of proteins [100]. Each NSF protomer contains three domains: an N-terminal domain required for SNAP–SNARE binding and two ATPase domains, termed D1 and D2. ATP binding and hydrolysis by D1 are necessary for the SNARE disassembly reaction to occur, and ATP binding, but not hydrolysis, by D2 is necessary for hexamer formation [9,101]. SNAP and NSF bind sequentially to SNARE complexes, forming so-called 20S particles, named after the sedimentation behavior of the supercomplex. Rotationally averaged electron micrographs of 20S particles have been obtained in the presence of ATP\(\mathrm{S}\) [102] and allow one to speculate about the localization of the structurally known components in the 20S complex (Figure 3).

**NSF-D2**

NSF-D2 consists of a nucleotide-binding subdomain and a C-terminal subdomain that is structurally unique among nucleotide-binding proteins [103,104] (Figure 3). There are interactions between the bound ATP moiety and both the neighboring D2 protomer and the C-terminal subdomain that may be important for ATP-dependent oligomerization. Of particular importance are three well-ordered and conserved lysine residues that interact with the ATP β- and γ-phosphates, one of which emerges from a neighboring NSF protomer and probably contributes to the low hydrolytic activity of D2 [103].

**NSF-N**

The N-terminal domain of NSF (NSF-N) is required for SNAP–SNARE binding and disassembly [105]. The structures of the N-terminal domains of NSF [106\textsuperscript{••},107\textsuperscript{••}], of the yeast homolog Sec18p [108\*] and of the homologous VAT protein from the archaeabacterium Thermoplasma acidophilum [109\*] are nearly identical, illustrating the structural conservation of the family of AAA ATPases. The N-terminal domain is composed of two subdomains: a double-ψ-ψ-barrel and an α–β roll [106\textsuperscript{••},107\textsuperscript{••}] (Figure 3). The interface between the two subdomains forms a groove that is a probable site of interaction with the C-terminal portion of α-SNAP [106\textsuperscript{••}]. Unexpectedly, both subdomains are structurally similar to domains of the transcription factor EF-Tu [106\textsuperscript{••}]. Both proteins have an adjacent nucleotide-binding domain, D1 in NSF and domain 1 in EF-Tu, and both proteins couple nucleotide hydrolysis to large conformational changes between domains.

**α-SNAP**

The structure of Sec17, the yeast homolog of α-SNAP, consists of a twisted sheet of α-helical hairpins and a globular
C-terminal domain that is primarily composed of α-helical hairpins [110••]. Sec17 is structurally related to several other α/ε proteins known to mediate protein–protein interactions as part of larger assemblies: tetratricopeptide repeats (TPRs) [111], 14-3-3 [112], HEAT repeats [113•] and clathrin heavy-chain repeats [114•]. The Sec17 twisted sheet has local similarity to the structure of the TPRs from protein phosphatase 5, but the overall twist of the sheet of α-helical hairpins in the two structures is very different.

Interactions between α-SNAP (Sec17) and SNAREs have been partially mapped using deletion mutagenesis and in vitro binding studies. The SNAP-interacting region of SNAREs overlaps with their core complex-forming regions [115–117]. This, in conjunction with the structure of the synaptic core complex [29] and the observed promiscuity of SNAP–SNARE interactions, suggests that SNAPs recognize general surface features (shape or electrostatic charge distribution) of the parallel four-helix bundle. Indeed, the curvature of the grooves of the four-helix bundle of the SNARE complex is similar to the curvature of the twisted sheet of Sec17 (Figure 3). Electron microscopy and mutagenesis studies of SNAP–SNARE complexes suggest that SNAP ‘coats’ the SNARE complex along most of its length [102]. Residues that are conserved in a variety of homologous SNAP sequences map predominantly to one face and to one ridge of the structure of Sec17. These surfaces are likely to interact with SNAREs and/or NSF (Sec18). SNAP may function as a rigid lever, transmitting the force generated by conformational changes in NSF (Sec18) to drive disassembly of SNARE complexes [110••].

Conclusions
Significant progress has been made in the elucidation of the structures of proteins involved in vesicular exocytosis. One of the most striking properties of the vesicular fusion machinery is the highly dynamic nature of the protein–protein interactions — binding partners frequently change and proteins undergo dramatic conformational changes. Crystal structures can only provide snapshots of the protein machinery. It remains a challenge to connect these snapshots in order to obtain a ‘movie’ of the vesicular fusion machinery and the fusion process itself.

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•• of outstanding interest
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The authors demonstrate that the SNARE core complex (i.e. without the N-terminal domain of syntaxin and the SNAP-25 linker region) is sufficient to promote vesicle fusion in vitro.


Using a liposome fusion assay [47], it was shown that NSF only disrupts cis SNARE complexes at the N-terminus of complex SNAREs that are localized to the same membrane as they are found after vesicle-membrane fusion.


A subset of SNARE complexes was tested for its ability to perform artificial liposome fusion. It was found that cognate SNARE complexes are able to perform fusion as well as complexes between relatively distant members of the SNARE family.


The role of the zero (ionic) layer of the synaptic fusion complex is examined.


A subset of SNARE complexes was tested for its ability to perform artificial liposome fusion. It was found that cognate SNARE complexes are able to perform fusion as well as complexes between relatively distant members of the SNARE family.


The role of the zero (ionic) layer of the synaptic fusion complex is examined.


NMR studies of syntaxin show that extra in a closed conformation in solution. SNARE complex formation requires a conformational change to an open state. nSec1 (Munc18) binds to the closed conformation. This study illustrates that the conformational transition of syntaxin is similar to that observed for the yeast homolog Sso1 [34].


The crystal structure of the entire cytoplasmic domain of Sso1p is described. It is found in a 'closed' conformation. The configuration of Sso1p is very similar to the structure of syntaxin found in the nSec1-syntaplex complex. Differences are found in the linker regions between the N-terminal syntaxin domain and the core complex domain, and at the C-terminal end. Neuronal syntaxin is substantially more flexible in the linker region. Furthermore, several interactions were observed between the unstructured half of the C-terminal core helix and nSec1.


The structure of the nSec1 (Munc18)-syntaxin complex is presented. The conformation of syntaxin is dramatically different from that in the SNARE complex A possible role of Rop proteins in dissociating the stable nSec1(Munc18)-syntaxin complex is proposed.


Experiments in permeabilized PC12 cells suggest that the role of SNAREs in fusion and suggest that SNARE complex formation is completed upon Ca2+ concentration increase.


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In yeast, the assembly of SNARE complexes between the secretory vesicle SNARE Sncp and the plasma membrane SNAREs Sec15p and Sec9p occurs at a late stage of the exocytic reaction. Mutations that block either secretory vesicle delivery or tethering prevent both SNARE complex assembly and the localization of Sec1p yeast homolog of nSec1 [Munc18] to sites of secretion. These results lend further credence to a different role for yeast Sec1 compared with neuronal nSec1.


The authors present the structure of a clathrin fragment that is involved in mediating spontaneous clathrin heavy-chain polymerization and light-chain association. The clathrin fragment has structural similarity to HEAT and TPR repeats, and to Sec17 (α-SNAP homolog).

