Cell, Vol. 112, 519–533, February 21, 2003, Copyright ©2003 by Cell Press

Membrane Fusion

Review

Reinhard Jahn,[1,*] Thorsten Lang,[1] and Thomas C. Südhof[2,*]

[1]Department of Neurobiology
Max-Planck-Institute for Biophysical Chemistry
37077 Göttingen
Germany

[2]Center for Basic Neuroscience
Department of Molecular Genetics
Howard Hughes Medical Institute
University of Texas Southwestern Medical Center
Dallas, Texas 75390

Membrane fusion, one of the most fundamental processes in life, occurs when two separate lipid membranes merge into a single continuous bilayer. Fusion reactions share common features, but are catalyzed by diverse proteins. These proteins mediate the initial recognition of the membranes that are destined for fusion and pull the membranes close together to destabilize the lipid/water interface and to initiate mixing of the lipids. A single fusion protein may do everything or assemblies of protein complexes may be required for intracellular fusion reactions to guarantee rigorous regulation in space and time. Cellular fusion machines are adapted to fit the needs of different reactions but operate by similar principles in order to achieve merging of the bilayers.

Introduction

Membrane fusion is a universal reaction that can vary vastly in space and time. When micron-sized organelles such as yeast vacuoles fuse, the area of contact is ~10,000-fold larger than when synaptic vesicles undergo exocytosis, and the time of fusion—minutes for vacuoles, milliseconds for synaptic vesicles—is ~10,000-fold longer. Similarly, extracellular fusion reactions range from the formation of large muscle syncytia to the entry of small viruses into host cells. Despite this diversity, all fusion reactions embody an elementary process that includes membrane contact, membrane merger, and the opening of an aqueous fusion pore (Figure 1). This elementary reaction takes place at a scale of nanometers, well below the resolution of the light microscope, and involves transient non-bilayer intermediates.

Fusion was invented several times during evolution. At least three types can be distinguished: (1) Extra- and intracellular fusion of pathogens with host cells. Of these, fusion of enveloped viruses in which the entire reaction is carried out by a single protein is best characterized. (2) Extracellular fusion of eukaryotic cells. Examples are fusion of sperm with oocytes or formation of syncytia of muscle cells. There is scant knowledge about the underlying molecular mechanisms. (3) Intracellular fusion of organelles. These reactions are mediated by dynamic supramolecular assemblies involving conserved protein families.

In the following, we will attempt to give a "birds-eye" view of the current status of membrane fusion research, focusing as much as possible on common principles rather than details.

*Correspondence: rjahn@gwdg.de (R.J.); thomas.sudhof@utsouthwestern.edu (T.C.S.)

Physics of Bilayer Fusion

Fusion of lipid bilayers in an aqueous environment is a two-step process. First, the membranes are brought into close proximity where counteracting electrostatic forces need to be overcome before the lipids of the proximal leaflets can interact. Second, the boundary between the hydrophilic and hydrophobic portion of the bilayer is destabilized. Non-bilayer transition states are generated that culminate in the formation of an aqueous fusion pore. All transition states are governed by forces that minimize exposure of non-polar surfaces to water. According to the stalk hypothesis, fusion proceeds by an ordered sequence of steps that include the merging of the proximal monolayers, stalk formation, generation of hemifusion intermediates, and fusion pore opening (Kozlov and Markin, 1983; Chernomordik et al., 1987) (Figure 1).

The stalk hypothesis can be described by macroscopic models treating bilayers and monolayers as homogeneous elastic surfaces. However, the structure of the non-bilayer transition states during fusion is unclear. In the elastic models, the high curvature of the non-bilayer intermediates creates void spaces, resulting in unrealistically high activation energies (Siegel, 1993). Allowance for lipid tilting substantially reduced these energies (Kuzmin et al., 2001; Kozlovsky and Kozlov, 2002; Markin and Albanesi, 2002), but the models still involve unphysical discontinuities. Furthermore, they require that fusion proteins bend the membrane in order to overcome the activation energy barrier. Indeed, many fusogenic molecules increase membrane curvature (Epand and Epand, 2000). On the other hand, small molecules (such as polyethylene glycol or small peptides) and even amphiphilic proteins (such as NSF, N-ethyl maleimide-sensitive factor) (Otter-Nilsson et al., 1999) and annexins (Hung et al., 1996) fuse membranes, suggesting that local perturbations of the hydrophilic-hydrophobic boundary or of phospholipid packing suffice to induce fusion (Cevc and Richardsen, 1999). Consequently, local fluctuations and thermal movements of lipid molecules may stabilize the non-bilayer transition states during fusion, and transition states may be substantially more disordered than assumed by the elastic models. This view is supported by recent progress in describing lipid membranes using coarse-grained and atomistic simulations (reviewed by Jahn and Grubmüller, 2002, see also Figure 1, right).

Fusion Pores

Fusion pores form an aqueous connection across the fusing bilayers (Figure 1). As shown in viral fusion and in exocytosis, fusion pores can open abruptly in micro-

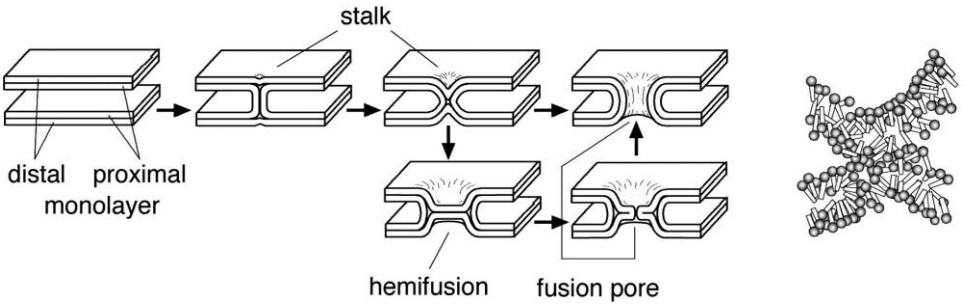

Figure 1. Transition States in Membrane Fusion

On the left, the monolayers are depicted as smooth and bendable sheets as described by the stalk hypothesis. On the right, a snapshot of a non-bilayer intermediate is shown that is derived from a simulation of membrane fusion using coarse-grained lipid models (modified from Noguchi and Takasu, 2001).

seconds with a diameter of ~2 nm. In the next 10–20 ms, fusion pores can increase their conductance or contract, resulting in moderately stable intermediates with variable conductance states (Breckenridge and Almers, 1987). Frequently, irregular rapid pore openings and closures are observed (flickering) that last from a few milliseconds to many seconds (Fernandez et al., 1984). In some cases, the reaction does not proceed further, and the pore closes again. Normally, however, this phase is followed by a gradual expansion of the fusion pore that is irreversible (Lindau and Almers, 1995). The wide variability in the initial conductance of fusion pores and the fusion pore flickering observed upon fusion of protein-free liposomes (Chanturiya et al., 1997) support the notion that even in fusion reactions mediated by proteins, fusion pores are essentially lipidic. Proteins, however, strongly influence the properties of fusion pores, as seen for example during mutagenesis of viral fusion proteins (Chernomordik et al., 1999) or during overexpression of synaptotagmin I and IV (Wang et al., 2001a), cysteine string protein (Graham and Burgoyne, 2000), complexin (Archer et al., 2002), and mutant Munc-18 in PC12 cells (Fisher et al., 2001). It remains to be established whether these proteins directly participate in the transition states or indirectly affect fusion pores, for example by interacting with fusion proteins such as SNAREs (soluble NSF attachment protein receptors).

The Universalists: Viral Proteins that Do Everything

Research on viral fusion proteins pioneered our understanding of membrane fusion. In enveloped viruses, the nucleocapsid with the genetic material is covered by a host-derived lipid bilayer. During infection, the virus binds to a receptor on the host cell, and the viral and host-cell membranes fuse in order for the nucleocapsid to enter the cytoplasm (Skehel and Wiley, 2000; Lentz et al., 2000). Viral fusion is carried out by single "universalist" proteins that mediate all steps of the fusion reaction, and that operate as "single shot" devices. Best understood are the so-called class I proteins, which are type I transmembrane glycoproteins that are synthesized as single precursor proteins (Skehel and Wiley, 2000; Eckert and Kim, 2001). Before the virus buds from its parental host cells, the fusion proteins are processed by a proteolytic cleavage, resulting in an N-terminal globular and a metastable C-terminal part. The C-terminal part usually contains an amphiphilic fusion peptide of 15–30 amino acids that is adjacent to the cleavage site, and a transmembrane domain that anchors it on the virus. Upon activation of the fusion protein by a shift in pH or binding to a surface receptor, the previously hidden fusion peptide is exposed and triggers fusion (Skehel and Wiley, 2000; Eckert and Kim, 2001). The fusion peptide is essential, altering its amphiphilic nature abolishes fusion. In the case of hemagglutinin (HA), the activated fusion peptide inserts into the hydrophobic interior of the target membrane (Durrer et al., 1996), and isolated fusion peptides are fusogenic when added to liposomes (reviewed by Martin and Ruysschaert, 2000; Tamm and Han, 2000). Thus, these peptides certainly participate in the transition states of fusion reactions.

Crystal structures of fragments of class I fusion proteins from unrelated virus families (influenza HA protein, HIV gp41, and Ebola GP2) revealed striking similarities (for review see Skehel and Wiley, 2000; Eckert and Kim, 2001 and references therein). Most of the structures uncovered elongated trimeric coiled-coil bundles of α helices with the fusion peptides at their tips. The rest of the protein folds back at the base of the helix-bundle, frequently forming additional α helices that bind to the outside grooves of the trimer. The result is an inversely bent structure in which the transmembrane domains are at the same end as the fusion peptides (Eckert and Kim, 2001; see cartoon in Figure 2, right). All of the known structures probably represent the stable endpoint of the conformational change. For influenza HA, a structure is also available for the non-activated conformation. In this state, the fusion peptide of HA is sequestered inside the protein at the base of the molecule, highlighting the magnitude of the conformational transition during the fusion reaction (Bullough et al., 1994).

The conformational transitions of viral fusion proteins proceed via defined intermediates. This is best understood for gp41, the fusion protein of HIV. After activation, gp41 can be arrested in a conformation in which the central trimeric coiled coil has formed, and the fusion peptides have established contact with the target membrane (Figure 2). Peptides corresponding to the C-terminal helices that bind to the outside grooves of the central coiled coil efficiently block fusion, apparently by preventing the alignment of the outer helices (Eckert

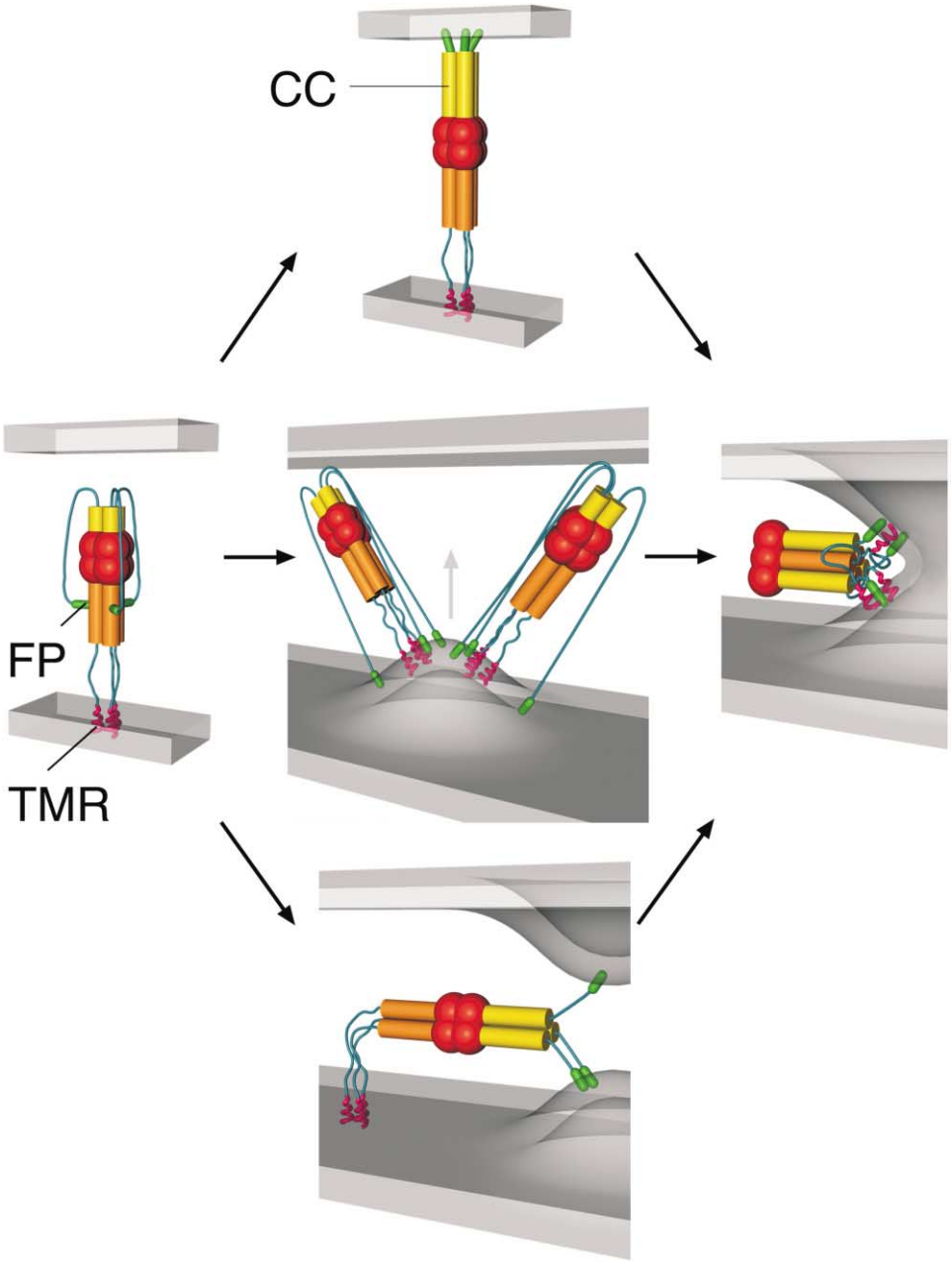

Figure 2. Models Describing the Conformational Changes during Membrane Fusion Catalyzed by Type I Viral Fusion Proteins

TMR, transmembrane domain, FP, fusion peptide, CC, trimeric coiled coil. The models differ in the conformational intermediates. Top: fusion peptides insert into the target membrane, followed by refolding of the C-terminal helices (orange). Middle: fusion peptides insert into the viral membrane, followed by membrane bending during coiled-coil formation. Bottom: fusion peptides insert into both viral and target membrane, followed by membrane apposition and induction of non-bilayer transition states during coiled-coil formation. For clarity, the target membrane binding domains and the membrane receptors are omitted.

and Kim, 2001). Thus, the trimeric helix bundle forms before the surrounding helices bind. Indeed, drugs modeled after peptides that interfere with or stabilize folding intermediates are promising leads for anti-HIV therapeutics (Root et al., 2001). Formation of the central coiled coil is essential for function since inhibiting its formation impedes fusion (Luo et al., 1999; Liu et al., 2002). Intact transmembrane regions are needed for fusion to go to completion. Substitution of the transmembrane regions with glycolipid anchors results in incomplete fusion (Kemble et al., 1994), with the reaction being arrested at, or diverted to, the hemifusion state (Melikyan et al., 1995).

How Exactly Do Viral Fusion Proteins Work?

Despite a wealth of knowledge, no consensus about the fusion mechanisms of viral fusion proteins has been reached. Figure 2 shows three models that illustrate the

range of mechanisms considered. The major difference between the models is whether the fusion peptide is inserted in the target membrane, in the viral membrane, or both. According to the "jackknife" model (Weissenhorn et al., 1997), reviewed by Eckert and Kim (2001), the central trimeric coiled coil forms upon activation, propelling the fusion peptide over a distance of many nanometers toward the tip of the molecule and into the target membrane. In the resulting intermediate conformation, the extended fusion protein is connected with the hydrophobic core of both membranes (Figure 2, top). In the second step, the outer helices fold back on the surface of the coiled coil, dragging the transmembrane regions toward the fusion peptides, thus pulling the two membranes together and initiating lipid mixing. This widely accepted model agrees with most experimental data, but does not explain how bending to one side is achieved in spite of the perfect rotational symmetry of the structures.

An alternative model proposes that the fusion peptide is first inserted into the viral rather than the target membrane (Kozlov and Chernomordik, 1998). Such insertion is observed when viral fusion proteins are activated in the absence of a target cell (Weber et al., 1994). The formation of the coiled coil would then exert a strong pulling force on the viral membrane, bending it up toward the target membrane that is attached to the viral fusion protein via its hemagglutinin binding activity (Figure 2, middle). When the fusion proteins are grouped in a ring around a central patch of lipid, a dimple is created that may destabilize the membrane and initiate the fusion reaction, with all proteins contributing to bending toward strong negative curvature.

A third possibility (Figure 2, bottom) is that the fusion peptides insert simultaneously into both the target and the viral membrane (Stegmann et al., 1989). According to this model, the fusion proteins would act as dirty pellet guns that spray fusion peptides into neighboring membranes. They then zipper up the trimeric coiled coil to pull the membranes close together, which may already destabilize the bilayer and cause lipid exchange. Next, the outer helices are thought to align, dragging the transmembrane domains toward the membrane contacts. This results in further destabilization and fusion, perhaps assisted by twisting of the fusion proteins. Like in the previous model, the formation of the trimeric coiled coil rather than the refolding of the outer helices plays a major role in overcoming the energy barrier for fusion. Similar to the first model, it resembles the fusion mechanism discussed for SNARE proteins (see below), but it provides a better explanation for the striking structural similarities between SNARE core complexes and viral fusion proteins.

Other Viral Fusion Proteins

Unlike class I viral fusion proteins, class II fusion proteins such as the E-glycoproteins of flavi- and alphaviruses contain internal fusion peptides. Recent work indicates that class II fusion proteins are radically different from class I proteins (see Heinz and Allison, 2001 for a review). Although the fusion peptides are also exposed at the membrane-distal tip of an elongated structure, the E-glycoproteins are composed of β strands that hold the fusion peptide in place. Coiled coils apparently do not play a role in the conformational changes upon activation. Recent high-resolution studies of entire virus particles have shown that the E-glycoproteins form icosahedral scaffolds on the virus surface and probably interact with each other in a concerted fashion during activation (Kuhn et al., 2002; Lescar et al., 2001; Garoff and Cheng, 2001). During the conformational changes, the fusion peptides are exposed and reorient toward the viral membrane. Insertion into the target membrane may be mediated by β barrels rather than amphiphilic α helices (Kuhn et al., 2002). Apparently, the different classes of viral fusion proteins have no common ancestor and evolved independently.

The Specialists: Proteins Mediating Intracellular Fusion

Intracellular fusion machines are dynamic supramolecular structures that are assembled upon demand and dismantled as soon as fusion is completed to allow them to be easily and quickly reused. Except for the membrane-anchored SNAREs (the presumed fusion catalysts), most components are recruited from the cytoplasm. Some of the proteins are shared among fusion reactions, whereas others are specific for an individual reaction. All intracellular fusion, however, appears to proceed via similar core processes that are controlled by an array of cofactors. An exception to this general mechanism is the fusion of mitochondria and peroxisomes, which are mediated by different proteins operating by unknown mechanisms (Hermann et al., 1998; Sesaki and Jensen, 2001; Titorenko and Rachubinski, 2000).

When an intracellular transport vesicle ("donor") is destined to fuse with an intracellular "acceptor" membrane, it first needs to recognize its partner membrane by physical contact in a specific location. This process provides specificity to fusion reactions and is variably called membrane attachment, tethering, or docking. Attachment requires that the participating membranes are marked, i.e., a site for attachment must be defined in the proteolipid bilayer. Central to membrane attachment are the Rab/Ypt GTPases that shuttle between a soluble GDP bound form that is inactive, and a membrane and GTP bound form that is active. In most fusion reactions, active Rabs on the donor membrane orchestrate the recruitment of effectors (often associated with the assembly of multimeric complexes) that are either present in the acceptor membrane or bind to proteins or lipids on the acceptor membrane, thereby tethering the two membranes together. However, Rab proteins have other functions in membrane traffic, and not all membrane-tethering reactions may require Rab proteins.

After membrane attachment, fusion is initiated by the concerted action of SNARE and SM proteins. SNAREs are membrane proteins present on both fusion partners, and SM proteins (Sec1/Munc18-like proteins) are soluble proteins often associated with syntaxin-like SNAREs. During initiation, SNAREs engage in *trans*-complexes that bridge the fusing membranes, a reaction that may be controlled by the SM proteins. When the membranes fuse, the SNAREs align with each other to form *cis*-complexes. During or after fusion, Rab proteins are inac-

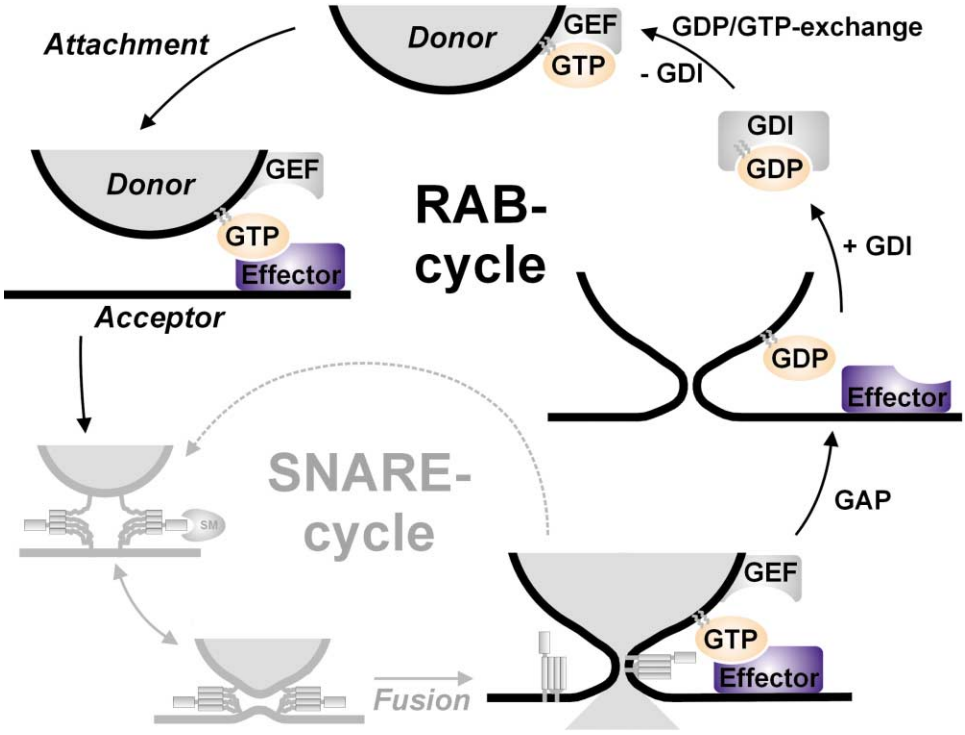

Figure 3. The Rab Cycle and Its Relationship to the SNARE Cycle in Membrane Fusion

GDI, guanine dissociation inhibitor (which removes all GDP-Rab proteins from the membrane by forming a soluble complex in which the hydrophobic geranylgeranyl chains are hidden), GEF guanine nucleotide exchange factor (specific for each Rab protein, mediates GDP-GTP exchange in conjunction with displacement of GDI and membrane binding)

tivated by GTPase-activating proteins (GAPs), and dissociate from the respective membranes to initiate a new cycle of fusion. SNARE complexes, in turn, are dissociated after fusion so that the individual SNARE proteins can be recycled with their resident membranes.

Fusion reactions thus include two conserved protein-protein interaction cycles—the Rab cycle (Figure 3) and the SNARE cycle (Figure 5)—that are probably connected by SM proteins, the least well understood of the components of the fusion machinery. Fusion reactions differ in the proteins that mediate these interaction cycles and in the SM protein-dependent mechanisms that couple the cycles. Many transient or stable protein complexes and essential cofactors associated with Rabs, SM proteins, and SNAREs have been described, highlighting the fact that specific adaptations are needed to guarantee the uniqueness of each fusion reaction.

Membrane Attachment

Rab Proteins as Anchors for Attachment

GTP bound Rabs are thought to mediate membrane attachment by interacting with specific effectors on target membranes. Yeast contains 10 classical and one atypical Rabs (called Ypt except for Sec4p), while the mammalian genome encodes more than 60 Rabs (Pereira-Leal and Seabra, 2001). These Rabs are localized to specific intracellular compartments where they may serve as identity tags.

Rab proteins do not have a transmembrane region, but are attached to membranes by a C-terminal modification with hydrophobic geranylgeranyl groups. GDP bound Rabs form soluble cytosolic complexes with GDI (for "GDP dissociation inhibitor"; Araki et al., 1990). GDI solubilizes GDP-Rab proteins by enveloping the hydrophilic geranylgeranyl groups (Wu et al., 1996). A poorly understood process that involves specific guanine-nucleotide exchange factors ("GEFs") targets GDP-Rab proteins to their specific cellular compartments and activates the GDP-Rab proteins by GDP to GTP exchange (Figure 3). At steady state, most Rabs appear to be in the GTP bound, membrane-attached state. When membranes are transported into proximity, GTP bound Rabs form specific effector complexes that connect the two membranes. After fusion, a Rab GTPase activating protein ("GAP") triggers GTP cleavage, and the resulting GDP bound Rab is recognized by GDI, which removes it from the membrane (Figure 3, see also Pfeffer, 2003 [this issue of *Cell*]). Rab effectors are defined as proteins that only interact with a GTP but not a GDP bound Rab. Individual Rabs can interact with multiple effectors that also mediate other functions of Rab proteins and often involve a given Rab protein in sequential steps of the same membrane-trafficking pathway.

The Rab cycle has several implications. First, Rab proteins act directionally, i.e., they are localized on a donor membrane to mediate contact with the target acceptor membrane. Second, there are at least two molecular recognition steps that are specific for a particular Rab protein, namely when a Rab binds to its respective donor membrane, and when the Rab recruits its effector.

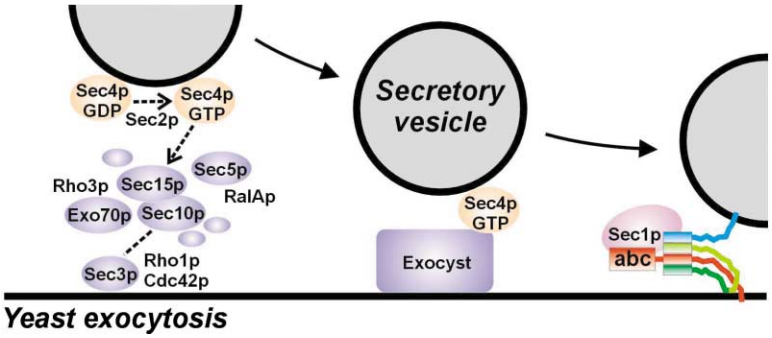

Figure 4. The Exocyst as an Example for Rab Effector Complexes and Their Role in Membrane Tethering

For an assignment of the SNAREs, see Figures 5 and 6. See text for details.

Since the model requires that these two steps are separate and consecutive, the Rab-GEF and the Rab effector—at least for tethering—must be physically distinct, with one being on the donor and the other providing the link to the acceptor membrane. Finally, Rab cycling must be dependent on fusion activity, as shown in the nerve terminal where the association of Rab3 with synaptic vesicles is dramatically changed as a function of exocytosis (Fischer von Mollard et al., 1991).

Rab Effectors Often Assemble into Protein Complexes

An increasing number of protein complexes that contain Rab effectors in fusion have recently been identified (for review see Whyte and Munro, 2002). These large complexes often exhibit multiple binding activities, and membrane attachment mediated by these complexes may serve to organize the sequence, timing, and spatial organization of fusion reactions. Best characterized is the exocyst, a multimeric complex that functions as effector for the Rab protein Sec4p in the fusion of transport vesicles with the plasma membrane in yeast (for review see Novick and Guo, 2002, see also Figure 4).

The exocyst contains eight subunits (Sec3p, Sec5p, Sec6p, Sec8p, Sec10p, Sec15p, Exo70p, and Exo84p). At the beginning of the assembly sequence, GDP bound Sec4p is recruited to exocytic vesicles by the GEF Sec2p that produces Sec4p-GTP. Sec4p-GTP in turn interacts with the exocyst, probably in a two-stage reaction whereby Sec4p first recruits a subcomplex composed of Sec15p (the direct GTP-Sec4p-effector protein) and Sec10p to vesicles. This subcomplex then interacts with other exocyst subunits on the plasma membrane. The assembly site of the exocyst on the membrane is defined by Sec3p, which specifically localizes to future sites of exocytosis. The exocyst mediates vesicle attachment to the plasma membrane prior to the function of SNAREs and the SM protein Sec1p, although it is unknown how the vesicles proceed from the Sec4p/exocyst tether to SNARE complex assembly (Novick and Guo, 2002). In addition to Sec4p, the yeast exocyst interacts with the GTP binding proteins Cdc42p and Rho1p (which bind to the N terminus of Sec3p), and with the GTP binding protein Rho3p (which binds to the Exo70p subunit; reviewed in Novick and Guo, 2002). A mammalian exocyst has been characterized that closely resembles the yeast complex (Hsu et al., 1999). However, the Rab target has not been identified since no true Sec4p homolog exists in mammals (Pereira-Leal and Seabra, 2001). Furthermore, the mamalian exocyst binds to the GTP binding protein ral (which does not exist in yeast), but not to Cdc42 and Rho1.

Most Rab effectors mediating vesicle attachment are not intrinsic membrane proteins. How is the acceptor membrane for these effectors chosen? Little is known about potential mechanisms. In the endosomal/vacuolar system, phosphatidylinositol 3-phosphate and phosphatidylinositol 3,5-bisphosphate are bound by specific domains in the fusion machinery and may be important for attachment (e.g., the FYVE domains of Vac1p and Rabenosyn, or the PX domain of the SNARE protein Vam7p which binds to phosphatidylinositol-3-phosphate; reviewed in Odorizzi et al., 2000; Misra et al., 2001; see also Figure 7). However, the function of these lipids is unclear; for example, it is unknown whether these lipids are on the acceptor membranes or colocalized with the Rab proteins on the donor membranes.

From Membrane Attachment to Fusion: SNAREs and SM Proteins

After membrane attachment, fusion is initiated when the membranes are forced into close proximity by the actions of SM and SNARE proteins that are probably essential for all fusion reactions. It is likely that SM proteins link Rab effectors and tethering complexes to SNARE assembly.

SNARE Proteins: Workhorses for Fusion

SNAREs are a superfamily of small proteins with 24 known members in yeast and more than 35 in mammals (Bock et al., 2001). The synaptic SNARE proteins synaptobrevin/VAMP (on the vesicles) and syntaxin 1 and SNAP-25 (on the plasma membrane) are best characterized and have served as paradigms for many of our ideas about SNAREs (for reviews, see Jahn and Südhof, 1999; Chen and Scheller, 2001; Rizo and Südhof, 2002). SNAREs vary widely in size and structure, and share only one homologous sequence, the SNARE motif that serves as their defining feature (Bock et al., 2001). The SNARE motif contains 60–70 amino acids that include eight heptad repeats typical for coiled coils. Most SNAREs contain a single, C-terminal transmembrane domain adjacent to the SNARE motif, although some SNAREs lack membrane anchors (e.g., yeast Vam7p and mammalian SNAP-29), and others feature hydrophobic posttranslational modifications instead of a transmembrane domain (such as neuronal SNAP-25).

SNARE proteins associate into core complexes during fusion and are dissociated again afterward (Söllner et al., 1993a, 1993b), resulting in the SNARE cycle (Figure 5). Complex formation is mediated by the SNARE motifs

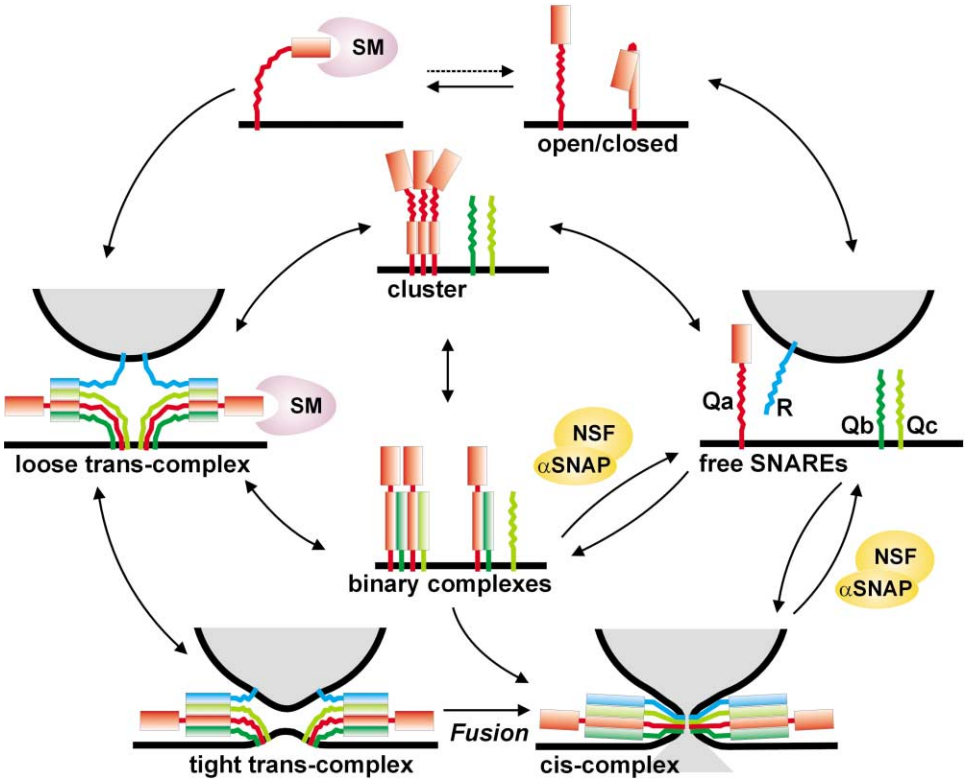

Figure 5. The Conformational Cycle of SNARE Proteins

Free SNAREs contain unstructured SNARE motifs that undergo multiple interactions. Some syntaxins switch between an open and a closed conformation. SM proteins bind either to the closed conformation (Munc18 proteins, not shown) or to the N terminus of syntaxins, either alone or in SNARE complexes. In membranes, SNAREs exist in homo- and heterooligomeric complexes (Laage et al., 2000), segregated in cholesterol-dependent clusters (Lang et al., 2001; Chamberlain and Gould, 2002). SNAREs also form binary and ternary *cis*-complexes (Otto et al., 1997; Ungermann et al., 1998). Formation of *trans*-complexes is thought to be reversible. Before fusion, *trans*-complexes may "breathe", i.e., transit between loose and tight states (Xu et al., 1999). Note that Qb- and Qc-SNAREs may also contain independently folded N-terminal domains.

and accompanied by large conformational changes. Free SNARE motifs are unstructured in solution. When appropriate SNARE motifs are combined, they spontaneously assemble into elongated four-helical bundles in which the four SNARE motifs are in a parallel orientation, with the transmembrane regions emerging from the C-terminal end (Hanson et al., 1997; Lin and Scheller, 1997). Since membrane fusion requires that SNAREs are initially present on both membranes, the structure of the core complex offers a fascinatingly simple mechanism of SNARE function. If assembly proceeds in a zipper-like fashion from the N-terminal end of the SNARE motifs toward the C-terminal membrane anchors, assembly of SNAREs in the opposing membranes pulls the membranes close together (Hanson et al., 1997; Lin and Scheller, 1997). According to this model, formation of such *trans*-complexes would largely overcome the energy barrier for fusion. Indeed, fully assembled core complexes are extraordinarily stable, indicating that SNARE assembly is associated with a major release of energy (Fasshauer et al., 2002). After fusion, the transmembrane regions of the SNAREs are present in the same membrane, resulting in *cis*-complexes (Figure 5) that need to be disassembled for reactivation. This reaction is catalyzed by the ATPase NSF with the soluble NSF-attachment proteins (SNAPs) as cofactors (Söllner et al., 1993a).

The crystal structures of two distantly related core complexes revealed a remarkable structural conservation (Sutton et al., 1998; Antonin et al., 2002). Both complexes form elongated coiled coils of four intertwined α helices corresponding to the four SNARE motifs that occupy specific positions in the helix bundle. The SNARE motifs in each position are distinguished by unique features that allow a classification into four subfamilies. Based on a highly conserved layer of interacting amino acids (three glutamines, one arginine) in the center of the helix bundle, the subfamilies are termed Qa-SNAREs (or syntaxins), Qb-, and Qc-SNAREs (homologs of the N- and C-terminal SNARE motif, respectively, of SNAP-25), and R-SNAREs (VAMPs) (Fasshauer et al., 1998; Bock et al., 2001). Apparently, all functional SNARE complexes contain one copy of the Qa-, Qb-, Qc-, and R-SNARE motifs, respectively (Figure 6). Indeed, sequence comparisons showed that the side chains in the core of the bundle are highly conserved between different complexes, whereas their surfaces are more diverse.

The SNARE model predicts that each of the two fusing membranes needs to contribute at least one SNARE with a transmembrane domain. The distribution of the remaining SNAREs, however, seems to be more variable than originally thought. For instance, *trans*-complexes can be formed during fusion either from two pairs of

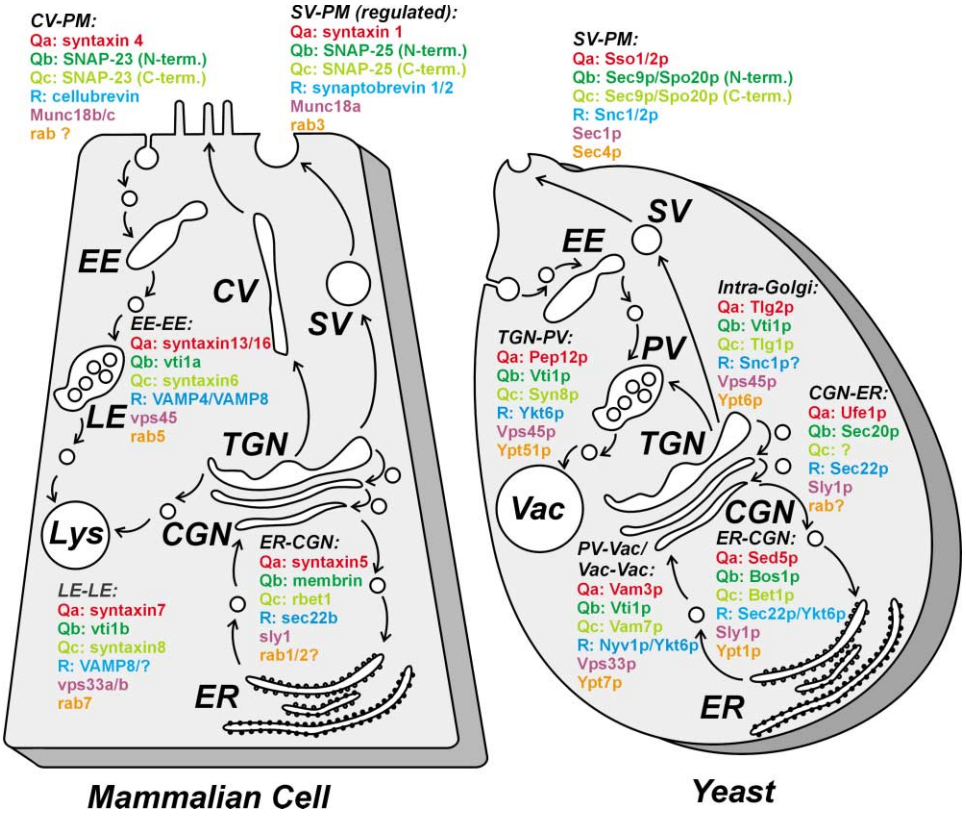

Figure 6. Association of SNARE Complexes, Rab Proteins, and SM Proteins with Trafficking Steps in Mammalian Cells and Yeast

In mammalian cells, only a selection is shown since the composition of many complexes is not known with certainty (for instance, exocytosis also involves syntaxins 2–4) although many additional SNAREs have been assigned individually to distinct trafficking steps (Chen and Scheller, 2001; Hay, 2001). In yeast, defined SNARE complexes can be assigned to most fusion steps (Pelham, 2001; Lewis and Pelham, 2002; Brickner et al., 2001), allowing deduction of some principles. Some SNAREs participate in several fusion events with different partners, such as the R-SNARE Ykt6p and the Qb-SNARE Vti1p. Conversely, a SNARE complex mediating a specific fusion reaction may accept alternative SNAREs. For instance, Ykt6p can substitute for Nyv1p in traffic to the vacuole (Dilcher et al., 2001), and for Sec22p in the fusion of COPII-vesicles with the Golgi (Liu and Barlowe, 2002). Ykt6p is partially soluble and thus may function as a promiscuous wildcard that substitutes for other R-SNAREs in multiple fusion reactions, which might explain why deletions of several yeast R-SNAREs have surprisingly mild phenotypes (Liu and Barlowe, 2002). Note that the trafficking steps from early endosomes and the prevacuolar compartment to the Golgi, and (possible) intra-Golgi trafficking steps are omitted since the composition of the SNARE complexes is not clear, they probably include Sed5p (Qa), Gos1p, Vti1p (Qb), Sft1p, Tlg1p (Qc), Snc1/2p, and Ypt6p (R).

EE, early endosome, CV constitutive vesicle, SV, secretory vesicle, LE, late endosome, Lys, lysosome, TGN, *trans*-Golgi network, CGN, *cis*-Golgi network, ER, endoplasmic reticulum, PV, prevacuolar compartment (corresponding to late endosome), Vac, vacuole (corresponding to lysosome).

membrane-anchored SNAREs on the two membranes or from a single membrane-anchored SNARE on one membrane and a triplet on the other membrane (Cao and Barlowe, 2000; Liu and Barlowe, 2002). These findings disagree with the original v-SNARE/t-SNARE concept (Rothman, 1994) that the localization of SNAREs on transport vesicles and target membranes is predetermined for each fusion step. As a result, the classification of SNAREs into v- and t-SNAREs (which has led to the proposal of complexes that violate the QaQbQcR-rule; McNew et al., 2000) can be misleading. Formation of SNARE complexes is promiscuous in vitro as long as the QaQbQcR-rule is followed (Fasshauer et al., 1999; Yang et al., 1999) although not all of the promiscuous complexes are stable. Similarly, exocytosis in permeabilized PC12 cells can be competed for with cognate and non-cognate SNARE fragments (Chen et al., 1999; Scales et al., 2000), but again some of the non-cognate SNAREs are more efficient than others indicating that promiscuity is not absolute.

Given that there is no evidence that non-cognate complexes assemble in living cells—how is their formation prevented? One explanation may be provided by the differences in N- and C-terminal sequences among SNAREs that surround the SNARE motifs and may dictate differential subcellular localizations and protein interactions. Specificity, however, cannot be due to separate location alone. SNAREs must follow membrane trafficking pathways in order to return to the site of their specific fusion event, resulting in overlapping recycling routes for distinct sets of SNAREs. For instance, secretory vesicles and endosomes contain full sets of SNAREs involved in exocytosis and endosome fusion without apparent crosstalk (Antonin et al., 2000). Thus, there must be mechanisms that distinguish in an upcoming fusion event which of them to use and which to

silence. It is possible that kinetic barriers reduce interactions between non-cognate SNAREs despite the thermodynamic stability of such complexes. Again, the sequences surrounding the SNARE motif may be involved here by binding to reaction-specific partners of SNARE proteins to control their localization and pairing, as for example proposed for the SM proteins (see below).

SNAREs as Fusogens

The hypothesis that SNAREs initially assemble in a *trans*-configuration during fusion to drive membranes into close proximity is supported by a large body of evidence (for recent reviews, see Chen and Scheller, 2001; Rizo and Südhof, 2002). However, there is still no consensus on how precisely SNAREs act. Key experiments have led to opposing views, namely that SNAREs as "minimal fusion machines" do everything, from the initial recognition of membranes to complete fusion (Weber et al., 1998) or that SNAREs act before fusion and are not directly involved in executing fusion (Ungermann et al., 1998). It is possible that both views are partly correct, and that the transition from *trans*- to *cis*-configuration of SNARE complexes catalyses an essential but incomplete step in intracellular fusion. This conclusion is supported by the fact that SNAREs do not appear to mediate the specificity of membrane fusion (although this issue is still controversial, see e.g., McNew et al., 2000), that other conserved proteins, especially SM-proteins, are essential cofactors in fusion, and that the dependence on SNAREs may be, at least in part, bypassed under specialized conditions.

In conclusion, SNAREs remain the best candidates for initiating fusion by inducing transition states that ultimately lead to the opening of a fusion pore. The critical step of bringing membranes close together may, at least in part, be substituted for by other cellular mechanisms that can do the same. This could explain why deficiency of certain SNAREs does not necessarily lead to a complete loss of fusion (see e.g., David et al., 1998; Schoch et al., 2001). However, the SNAREs may provide an additional function which is as important: namely to embed the approximation of membranes into an ordered sequence of reactions. This "embedding" is presumably performed by the parts of SNAREs that are outside of the SNARE motif, and among others require SM proteins.

SM Proteins: Organizers of Fusion?

SM Protein Interactions with SNAREs

SM proteins are hydrophilic proteins of 650–700 residues (Jahn and Südhof, 1999). Wherever examined, deletion of an SM protein stops the respective fusion event, showing that SM proteins are essential for fusion. There are far fewer SM proteins (4 in the yeast and 7 in the human genome) than fusion reactions suggesting that SM proteins are versatile fusion agents that function in multiple reactions. The crystal structures of two only distantly related SM proteins (neuronal Munc18 and yeast Sly1p) revealed a remarkable degree of structural conservation. Apparently, all SM-proteins are arch-shaped molecules consisting of three domains and a major V-shaped cleft in the middle (Misura et al., 2000; Bracher and Weissenhorn, 2002).

SM proteins interact with SNAREs via four distinct types of mechanisms, not all of which involve direct binding. The types of interaction mechanisms correlate with the topographical localization of fusion reactions in the cell, suggesting that different cellular provinces are marked by distinct fusion mechanisms.

In the most widespread type of interaction, the SM protein binds directly to the N-terminal ~20 residues of the corresponding Qa-SNARE. Yeast and vertebrate Sly1 bind to Sed5p/syntaxin 5 and to Ufe1p/syntaxin 18, and yeast and vertebrate Vps45 bind to Tlg2p/syntaxin 16 by this mechanism (Yamaguchi et al., 2002; Dulubova et al., 2002). Binding is sequence-specific and non-promiscuous. The binding site for the N-terminal Qa-SNARE peptide is on the outer surface of the N-terminal domain of the SM protein (Bracher and Weissenhorn, 2002; Dulubova et al., 2003). This type of interaction seems to cover all fusion reactions involving the endoplasmic reticulum and the Golgi complex, making it the dominant mechanism in the synthetic/secretory province of cells. Because the N-terminal binding sequences of syntaxins are distant from the SNARE motifs, the corresponding SM proteins bind equally well to the isolated syntaxins and to assembled SNARE complexes (Peng and Gallwitz, 2002).

The SM proteins Munc18a, 18b, and 18c (which operate in exocytosis) also bind directly to the corresponding Qa-SNAREs (syntaxins 1–4). However, Munc18 binding requires a special "closed" conformation of syntaxins in which the N-terminal domain (that in all Qa-SNAREs characterized so far is composed of a three-helix bundle referred to as H_{abc} domain) contacts part of the SNARE motif (reviewed in Rizo and Südhof, 2002). In stark contrast to the Sly1p/Sed5p-complex, Munc18a binds syntaxin in the central cleft via interactions that involve both the H_{abc} domain and the SNARE motif (Misura et al., 2000). Because the closed conformation of syntaxins is incompatible with SNARE complex formation, Munc18s cannot bind to syntaxins simultaneously with other SNAREs (Pevsner et al., 1994).

In contrast to vertebrate exocytosis, the SM protein mediating yeast exocytosis (Sec1p) does not directly bind to the Qa-SNAREs Sso1p and Sso2p, but only to fully assembled SNARE complexes (Carr et al., 1999). The nature of this interaction, however, is not yet known.

Finally, SM proteins involved in endosomal/vacuolar fusion appear to interact with SNAREs indirectly by forming complexes with SNARE binding proteins. For example, in yeast endosome fusion the SM protein Vps45p does not bind directly to the corresponding Qa-SNARE Pep12p, even though the same SM protein directly binds to the N-terminal peptide of the Qa-SNARE Tlg2p during Golgi fusion and possibly other fusion reactions (Dulubova et al., 2002). Similarly, the SM protein Vps33p functions in vacuole fusion without binding directly to the Qa-SNARE Vam3p (Dulubova et al., 2001; Sato et al., 2001). In these fusion reactions, Vps33p and Vps45p participate in multimeric protein complexes that include the corresponding Rab effectors. Yeast Vps45p forms a complex with Vac1p, which functions as an effector for the Rab Ypt5p. Yeast Vps33p participates in a large complex (the "HOPS" or "VpsC" complex) that binds to the Rab Ypt7p and to assembled SNARE complexes (Figure 7), although the mechanism of binding is not clear (Price et al., 2000a; Seals et al., 2000; Ungermann et al., 2000; Sato et al., 2001).

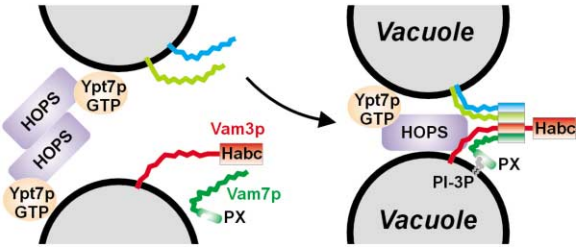

Figure 7. Protein-Protein Interactions Involved in the Homotypic Fusion of Yeast Vacuoles

Tethering and fusion of vacuoles are thought to be controlled by a large protein complex called the HOPS or VpsC complex that is composed of at least six proteins (Vps11p, 16p, 18p, 33p, 39p, and 41p). Left picture: Binding of the HOPS/VpsC complex to GTP-Ypt7p (both of which have to be present on both vacuoles) tethers the vacuoles probably by an interaction between HOPS complexes, although this has not been directly shown (Haas et al., 1994; Seals et al., 2000). Vacuole tethering requires that SNAREs are disassembled (Boeddinghaus et al., 2002), possibly because binding of HOPS complexes to SNARE complexes (see below) competes with their direct interaction. During tethering, the unassembled SNAREs are firmly membrane-attached except for Vam7p, which is recruited to the vacuoles by the binding of its N-terminal PX domain to phosphatidylinositol 3-phosphate (PI-3P) (Cheever et al., 2001). All SNAREs are usually present on both membranes, but are drawn for clarity on only one or the other membrane since it is not required for fusion to have the same SNAREs on both membranes. Right picture: After tethering, the HOPS complex initiates SNARE complex assembly by an unknown mechanism that results in a stable association of the HOPS complex with the assembled core complex; this association is reversed when SNARE complexes are disassembled by NSF and SNAP (Price et al., 2000b; Seals et al., 2000) see, however, (Sato et al., 2001) for an opposing view suggesting that only the isolated Vam3p SNARE binds the HOPS complex). The model does not explain, however, why removal of Ypt7p from vacuoles using an excess of GDI also causes dissociation of Vam7p (Ungermann et al., 2000).

Functions of SM Proteins

At present, most evidence suggests that SM proteins regulate SNARE assembly in a manner that is coupled to membrane attachment. For instance, in vacuole fusion, *trans*-SNARE complexes do not form in the absence of the HOPS complex (Price et al., 2000a; Sato et al., 2001), and in Golgi fusion Tlg2p-containing SNARE complexes do not assemble without Vps45p (Bryant and James, 2001). Furthermore, Sly1p operating in ER to Golgi fusion prevents promiscuous SNARE pairing in vitro (Peng and Gallwitz, 2002) suggesting that the job of SM-proteins may include proofreading of SNAREs. Additional factors, however, are likely to be involved in pairing specificity since a given SM protein can participate in multiple fusion reactions. The only major evidence against a role of SM proteins before SNARE assembly comes from the observation that Sec1p only binds to assembled SNARE complexes (Carr et al., 1999). However, it cannot be excluded that Sec1p is recruited to the fusion site by another mechanism prior to SNARE complex assembly.

Several other functions for SM proteins have been proposed. For example, the decrease in the levels of syntaxin 1 in Munc18a knockouts (Verhage et al., 2000), and of Tlg2p in Vps45p deletion mutants (Bryant and James, 2001) indicated that SM proteins may be syntaxin chaperones. However, not all SM proteins directly bind to syntaxins, and no decrease in Sso1p/2p is observed in Sec1p null mutants in yeast. Alternatively, it was proposed that SM proteins are negative regulators of fusion because Munc18a binding to syntaxin 1 blocks SNARE complex formation in vitro (Pevsner et al., 1994). However, most SM proteins bind to SNARE complexes equally or better than to individual SNAREs, and deletions of SM protein block fusion instead of disinhibiting it. Furthermore, docking of dense core vesicles in chromaffin cells is impaired in Munc18a knockouts, strongly supporting the view that Munc18a operates prior to SNARE complex assembly (Voets et al., 2001). However, it is unlikely that the role of SM-proteins is confined to membrane attachment because the Munc18a knockouts exhibited no change in synaptic vesicle docking and in ER to Golgi traffic Sly1p is essential for fusion but not for tethering (Cao et al., 1998).

How can SM proteins couple membrane attachment mediated by Rab effector complexes to SNARE assembly? As discussed above, the closest links have been identified in endosomal/vacuolar fusion reactions where SM proteins are part of Rab effector complexes that have been shown to regulate SNARE assembly. Possibly SM proteins have a catalytic role in arranging transient protein-protein interactions that are essential for SNARE complex formation during fusion, but at present we do not really understand how SM proteins work at the molecular level.

Ca^{2+}-Regulated Exocytosis: How to Control Fusion Pore Opening

At the neuronal synapse, Ca^{2+} triggers neurotransmitter release by synaptic vesicle exocytosis. Exocytosis occurs only at the active zone of the presynaptic plasma membrane and is the most tightly regulated fusion reaction in biology because precise timing of release is essential for synaptic transmission. Ca^{2+} triggers synaptic vesicle exocytosis with a delay of less than 1 millisecond, possibly less than 100 μsec (Sabatini and Regehr, 1996). The speed of Ca^{2+} action suggests that Ca^{2+} does not induce release by a complex reaction, for example by initiating assembly of new SNARE complexes or causing large conformational changes. Instead, it is likely that the fusion reaction is largely completed before the arrival of trigger Ca^{2+} (reviewed in Südhof, 1995). Maintaining synaptic vesicles at the active zone in a metastable, Ca^{2+}-responsive state requires a specialized protein scaffold that stabilizes the vesicles with at least partially assembled *trans*-SNARE complexes.

Synaptic active zones contain RIM, a multidomain protein that functions as an effector for Rab3 that is the most abundant Rab protein on synaptic vesicles. Different from other fusion reactions, binding of Rab3 to RIM does not appear to attach synaptic vesicles to the active zone because deletion of RIM or Rab3 does not significantly alter vesicle attachment (reviewed in Dobrunz and Garner, 2002). Deletion of RIM or Rab3, however, has dramatic effects on Ca^{2+}-triggered exocytosis, suggesting that binding of Rab3 to RIM functions in regulating and maintaining the activation of synaptic vesicles on the active zone. In addition to binding Rab3, RIM also binds Munc13-1, RIM-BPs, ERCs, and α-liprins, all of which are multidomain proteins of the active zone (Wang et al., 2000; Betz et al., 2001; Wang

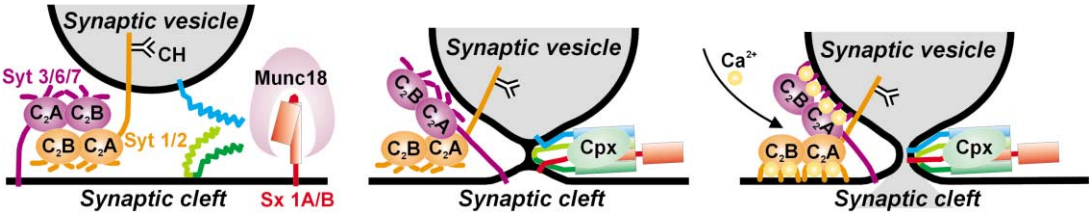

Figure 8. Concerted Action of Synaptotagmins on Synaptic Vesicles and on the Presynaptic Plasma Membrane in Synaptic Vesicle Exocytosis
See text for details. Please note that the hemifusion intermediate is hypothetical, and that any metastable fusion intermediate will satisfy the model. Syt, synaptotagmin, Cpx, complexin, Sx1A/B, syntaxin 1A and B.

et al., 2001b; Schoch et al., 2002; Wang et al., 2002; Ohtsuka et al., 2002). As a result, active zones contain large, biochemically insoluble protein complexes that are nucleated by the Rab3-effector protein RIM as a central component and are involved in synaptic vesicle fusion mediated by SNAREs and Munc18a.

Ca^{2+} triggers release at micromolar concentrations with at least two time components: a synchronous, rapid component (~0.1–5 milliseconds) that requires higher Ca^{2+} concentrations and an asynchronous, slower component (~5–500 milliseconds) that is activated at lower Ca^{2+} concentrations (Barrett and Stevens, 1972; Goda and Stevens, 1994; Atluri and Regehr, 1998). The fast component dominates at low, and the slow component at high stimulation frequencies (Hagler and Goda, 2001). Ca^{2+} triggers release by binding to Ca^{2+} sensors that are localized at the site of exocytosis and that bind multiple Ca^{2+} ions. In forebrain synapses, the Ca^{2+} sensor for the fast component is most likely the synaptic vesicle protein synaptotagmin 1 (Perin et al., 1990; Brose et al., 1992). Synaptotagmin 1 binds multiple Ca^{2+} ions via two C_2-domains, called the C_2A- and C_2B-domains (Ubach et al., 1998; Fernandez et al., 2001). Both C_2-domains form phospholipid complexes that bind Ca^{2+} with an apparent affinity of 3–30 μM free Ca^{2+} similar to the apparent affinity of fast release (Schneggenburger and Neher, 2000; Bollmann et al., 2000). Direct evidence that Ca^{2+} binding to synaptotagmin 1 triggers fast release was obtained with knockin mice (Fernandez-Chacon et al., 2001, 2002). These mice revealed that a mutation in the C_2A-domain, which decreased the Ca^{2+} affinity of the double C2A/B-domain fragment approximately 2-fold, also decreased the apparent Ca^{2+} affinity of exocytosis approximately 2-fold. In contrast, other C_2-domain mutations that do not affect the apparent Ca^{2+} affinity of the double C_2-domain fragment of synaptotagmin 1 had no effect. It has been argued that this conclusion does not hold true for *Drosophila* (Robinson et al., 2002), but in the *Drosophila* experiments the apparent Ca^{2+} affinity of mutant synaptotagmin 1 was measured with a method that does not adequately monitor Ca^{2+} binding to both C_2-domains (Fernandez et al., 2001), and the apparent Ca^{2+} affinity of release was not determined. However, the relative importance of Ca^{2+} binding to the C_2A- and C_2B-domains of synaptotagmin 1 remains unclear (see Robinson et al., 2002; Mackler et al., 2002; Yoshihara and Littleton, 2002; Shin et al., 2003), largely because Ca^{2+} binding affinities of the double C_2-domain fragments of synaptotagmin 1 mutants and of the corresponding release are often not determined.

Although Ca^{2+} binding to synaptotagmin 1 is instrumental in triggering fast release, it is not the only Ca^{2+} sensor in release, probably not even in fast release. It is likely that synaptotagmin 1 functions to accelerate an underlying slower Ca^{2+}-dependent process which may be coincidental with the asynchronous release component. What other Ca^{2+} sensors might mediate the slow, asynchronous component of release? Synaptotagmin 1 is part of a large gene family (Südhof, 2002), and other synaptotagmins are prime candidates for the slow component of release because they are also localized to synapses but exhibit higher apparent Ca^{2+} affinities than synaptotagmin 1 (Sugita et al., 2002; Shin et al., 2002; Fukuda et al., 2002). However, the precise functions and localizations of these "other" synaptotagmins continue to be discussed, and thus it is not yet clear whether they are involved in Ca^{2+}-mediated fusion or in other intracellular processes.

How does synaptotagmin 1 trigger fast release? Since synaptotagmin 1 binds Ca^{2+} best in a complex with phospholipids, it seems likely that it directly influences the lipidic transition states during fusion. Based on studies with liposomes, this by itself may be sufficient to trigger fusion pore opening (Cevc and Richardsen, 1999). In addition to phospholipids, synaptotagmin 1 binds to SNARE proteins, to itself, and to many other proteins in vitro (reviewed in Südhof, 2002; Chapman, 2002). A model for synaptotagmin 1 action is suggested by studies on small soluble proteins called complexins that selectively bind to assembled SNARE complexes containing syntaxin 1 (McMahon et al., 1995; Pabst et al., 2000). Knockout of both complexins impairs, but does not abolish fast Ca^{2+}-triggered exocytosis (Reim et al., 2001), suggesting that complexins are ancillary factors that promote the function of synaptotagmin 1. Complexins bind to fully assembled SNARE complexes close to the transmembrane regions and stabilize the complexes (Pabst et al., 2000; Chen et al., 2002). Together these results indicate that synaptotagmin 1 acts on a state of synaptic vesicles in which SNARE complexes have fully assembled, suggesting that SNARE complex assembly creates a metastable fusion intermediate, perhaps a stalk-like state (see model in Figure 8). Ca^{2+}-triggered insertion of the synaptotagmin C_2-domains is sufficient to destabilize the intermediate and to trigger fusion pore opening. This model postulates a primary role for Ca^{2+}-dependent phospholipid binding, as supported by recent results demonstrating that Ca^{2+}-triggered SNARE interactions by synaptotagmin 1 are not essential for fast Ca^{2+}-triggered release, although such

interactions may be important in other Ca^{2+}-regulated stages of exocytosis (Shin et al., 2003).

Concluding Remarks

In spite of enormous progress in studying membrane fusion, we are still only at the beginning of a molecular understanding. Major questions remain. For example, what is the structure and composition of non-bilayer transition states during fusion, how are these transition states related to the conformational changes of viral fusion proteins, and by what mechanisms do multicomponent fusion machines operate? Only an initial identification of crucial protein-protein interactions has been achieved, and their relevance for fusion is not always clear. We do not really understand the biophysical basis of fusion, we do not know what determines fusion specificity, and we have limited insight into the critical events that induce lipid mixing in SNARE mediated fusion.

Due to the molecular complexity and dynamics of fusion reactions, novel concepts are needed that go beyond the linear reaction sequences which dominate current models (such as the sequence tethering, docking, priming, triggering, and fusion). Complex systems with multiple components that are connected by protein networks exhibit non-intuitive kinetic properties. So far, theories describing such systems (de Jong, 2002; Arkin, 2001) have not been applied to membrane fusion, in part due to the lack of reliable kinetic data describing individual sub-reactions. Another interesting question relates to the flow of energy and the involvement of conformational changes. Protein-protein interactions are not without costs: the higher the affinity, the more energy needs to be spent to dissociate such interactions. Current techniques are heavily biased toward identifying high-affinity interactions, and important low-affinity interactions remain to be discovered. Conversely, current techniques are prone to in vitro artifacts that are based on sticky proteins whose binding is detected by sensitive techniques. Of the reactions discussed above, only three require energy input for recharging (GDP-GTP exchange of Rabs, disassembly of SNAREs, and removal of Ca^{2+} after an action potential to dissociate synaptotagmin from phospholipids). The corresponding energy-consuming steps (SNARE assembly, GTP hydrolysis, and Ca^{2+} influx) appear to be largely responsible for ensuring that the reaction proceeds in one direction. Obviously, energy is needed for other steps related to fusion, e.g., movements involving molecular motors and the reversible phosphorylation of proteins and lipids, with additional energy-dependent reactions waiting to be defined.

Acknowledgments

The authors thank Drs. Bill Wickner (Dartmouth), Hugh Pelham (Cambridge, United Kingdom), and Winfried Weissenhorn (Grenoble, France) for useful hints and making available unpublished data; and Drs. W. Almers, (Portland), L. Chernomordik (Bethesda), E. Neher, D. Gallwitz, G. Fischer von Mollard, C. Schütte (all Göttingen, Germany), J. Rizo (Dallas), and one of the reviewers for invaluable advice and for a critical reading of the manuscript. We also thank H. Sebesse (Göttingen, Germany) for the preparation of Figures 1 and 2. R.J. is supported by the Gottfried Wilhelm Leibniz-Program of the Deutsche Forschungsgemeinschaft.

References

Antonin, W., Holroyd, C., Fasshauer, D., Pabst, S., von Mollard, G.F., and Jahn, R. (2000). A SNARE complex mediating fusion of late endosomes defines conserved properties of SNARE structure and function. EMBO J. *19*, 6453–6464.

Antonin, W., Fasshauer, D., Becker, S., Jahn, R., and Schneider, T.R. (2002). Crystal structure of the endosomal SNARE complex reveals common structural principles of all SNAREs. Nat. Struct. Biol. *9*, 107–111.

Araki, S., Kikuchi, A., Hata, Y., Isomura, M., and Takai, Y. (1990). Regulation of reversible binding of smg p25A, a ras p21-like GTP-binding protein, to synaptic plasma membranes and vesicles by its specific regulatory protein, GDP dissociation inhibitor. J. Biol. Chem. *265*, 13007–13015.

Archer, D.A., Graham, M.E., and Burgoyne, R.D. (2002). Complexin regulates the closure of the fusion pore during regulated vesicle exocytosis. J. Biol. Chem. *277*, 18249–18252.

Arkin, A.P. (2001). Synthetic cell biology. Curr. Opin. Biotechnol. *12*, 638–644.

Atluri, P.P., and Regehr, W.G. (1998). Delayed release of neurotransmitter from cerebellar granule cells. J. Neurosci. *18*, 8214–8227.

Barrett, E.F., and Stevens, C.F. (1972). The kinetics of transmitter release at the frog neuromuscular junction. J. Physiol. *227*, 691–708.

Betz, A., Thakur, P., Junge, H.J., Ashery, U., Rhee, J.S., Scheuss, V., Rosenmund, C., Rettig, J., and Brose, N. (2001). Functional interaction of the active zone proteins Munc13–1 and RIM1 in synaptic vesicle priming. Neuron *30*, 183–196.

Bock, J.B., Matern, H.T., Peden, A.A., and Scheller, R.H. (2001). A genomic perspective on membrane compartment organization. Nature *409*, 839–841.

Boeddinghaus, C., Merz, A.J., Laage, R., and Ungermann, C. (2002). A cycle of Vam7p release from and PtdIns 3-P-dependent rebinding to the yeast vacuole is required for homotypic vacuole fusion. J. Cell Biol. *157*, 79–89.

Bollmann, J.H., Sakmann, B., Gerard, J., and Borst, G. (2000). Calcium sensitivity of glutamate release in a calyx-type terminal. Science *289*, 953–957.

Bracher, A., and Weissenhorn, W. (2002). Structural basis for the Golgi membrane recruitment of Sly1p by Sed5p. EMBO J. *21*, 6114–6124.

Breckenridge, L.J., and Almers, W. (1987). Currents through the fusion pore that forms during exocytosis of a secretory vesicle. Nature *328*, 814–817.

Brickner, J.H., Blanchette, J.M., Sipos, G., and Fuller, R.S. (2001). The Tlg SNARE complex is required for TGN homotypic fusion. J. Cell Biol. *155*, 969–978.

Brose, N., Petrenko, A.G., Südhof, T.C., and Jahn, R. (1992). Synaptotagmin: a Ca^{2+} sensor on the synaptic vesicle surface. Science *256*, 1021–1025.

Bryant, N.J., and James, D.E. (2001). Vps45p stabilizes the syntaxin homologue Tlg2p and positively regulates SNARE complex formation. EMBO J. *20*, 3380–3388.

Bullough, P.A., Hughson, F.M., Skehel, J.J., and Wiley, D.C. (1994). Structure of influenza haemagglutinin at the pH of membrane fusion. Nature *371*, 37–43.

Cao, X., and Barlowe, C. (2000). Asymmetric requirements for a Rab GTPase and SNARE proteins in fusion of COPII vesicles with acceptor membranes. J. Cell Biol. *149*, 55–66.

Cao, X., Ballew, N., and Barlowe, C. (1998). Initial docking of ER-derived vesicles requires Uso1p and Ypt1p but is independent of SNARE proteins. EMBO J. *17*, 2156–2165.

Carr, C.M., Grote, E., Munson, M., Hughson, F.M., and Novick, P.J. (1999). Sec1p binds to SNARE complexes and concentrates at sites of secretion. J. Cell Biol. *146*, 333–344.

Cevc, G., and Richardsen, H. (1999). Lipid vesicles and membrane fusion. Adv. Drug Deliv. Rev. *38*, 207–232.

Chamberlain, L.H., and Gould, G.W. (2002). The v- and t-SNARE proteins that mediate Glut4 vesicle fusion are localised in detergent-

insoluble lipid rafts present on distinct intracellular membranes. J. Biol. Chem. *277*, 49750–49754.

Chanturiya, A., Chernomordik, L.V., and Zimmerberg, J. (1997). Flickering fusion pores comparable with initial exocytotic pores occur in protein-free phospholipid bilayers. Proc. Natl. Acad. Sci. USA *94*, 14423–14428.

Chapman, E.R. (2002). Synaptotagmin: a Ca^{2+} sensor that triggers exocytosis? Nat. Rev. Mol. Cell Biol. *3*, 498–508.

Cheever, M.L., Sato, T.K., de Beer, T., Kutateladze, T.G., Emr, S.D., and Overduin, M. (2001). Phox domain interaction with $PtdIns_3P$ targets the Vam7 t-SNARE to vacuole membranes. Nat. Cell Biol. *3*, 613–618.

Chen, Y.A., and Scheller, R.H. (2001). SNARE-mediated membrane fusion. Nat. Rev. Mol. Cell Biol. *2*, 98–106.

Chen, Y.A., Scales, S.J., Patel, S.M., Doung, Y.C., and Scheller, R.H. (1999). SNARE complex formation is triggered by Ca^{2+} and drives membrane fusion. Cell *97*, 165–174.

Chen, X., Tomchick, D.R., Kovrigin, E., Arac, D., Machius, M., Südhof, T.C., and Rizo, J. (2002). Three-dimensional structure of the complexin/SNARE complex. Neuron *33*, 397–409.

Chernomordik, L.V., Melikyan, G.B., and Chizmadzhev, Y.A. (1987). Biomembrane fusion: a new concept derived from model studies using two interacting planar lipid bilayers. Biochim. Biophys. Acta *906*, 309–352.

Chernomordik, L.V., Leikina, E., Kozlov, M.M., Frolov, V.A., and Zimmerberg, J. (1999). Structural intermediates in influenza haemagglutinin-mediated fusion. Mol. Membr. Biol. *16*, 33–42.

David, D., Sundarababu, S., and Gerst, J.E. (1998). Involvement of long chain fatty acid elongation in the trafficking of secretory vesicles in yeast. J. Cell Biol. *143*, 1167–1182.

de Jong, H. (2002). Modeling and simulation of genetic regulatory systems: a literature review. J. Comput. Biol. *9*, 67–103.

Dilcher, M., Köhler, B., and Fischer von Mollard, G. (2001). Genetic interactions with the yeast Q-SNARE VTI1 reveal novel functions for the R-SNARE YKT6. J. Biol. Chem. *276*, 34537–34544.

Dobrunz, L.E., and Garner, C.C. (2002). Priming plasticity. Nature *415*, 277–278.

Dulubova, I., Yamaguchi, T., Gao, Y., Min, S.W., Huryeva, I., Südhof, T.C., and Rizo, J. (2002). How Tlg2p/syntaxin 16 'snares' Vps45. EMBO J. *21*, 3620–3631.

Dulubova, I., Yamaguchi, T., Arac, D., Li, H., Huryeva, I., Min, S.W., Rizo, J., and Südhof, T.C. (2003). Convergence and divergence in the mechanism of SNARE binding by Sec1/Munc18-like proteins. Proc. Natl. Acad. Sci. USA *100*, 32–37.

Dulubova, I., Yamaguchi, T., Wang, Y., Südhof, T.C., and Rizo, J. (2001). Vam3p structure reveals conserved and divergent properties of syntaxins. Nat. Struct. Biol. *8*, 258–264.

Durrer, P., Galli, C., Hoenke, S., Corti, C., Glück, R., Vorherr, T., and Brunner, J. (1996). H^+-induced membrane insertion of influenza virus hemagglutinin involves the HA2 amino-terminal fusion peptide but not the coiled-coil region. J. Biol. Chem. *271*, 13417–13421.

Eckert, D.M., and Kim, P.S. (2001). Mechanisms of viral membrane fusion and its inhibition. Annu. Rev. Biochem. *70*, 777–810.

Epand, R.M., and Epand, R.F. (2000). Modulation of membrane curvature by peptides. Biopolymers *55*, 358–363.

Fasshauer, D., Sutton, R.B., Brünger, A.T., and Jahn, R. (1998). Conserved structural features of the synaptic fusion complex: SNARE proteins reclassified as Q- and R-SNAREs. Proc. Natl. Acad. Sci. USA *95*, 15781–15786.

Fasshauer, D., Antonin, W., Margittai, M., Pabst, S., and Jahn, R. (1999). Mixed and non-cognate SNARE complexes–characterization of assembly and biophysical properties. J. Biol. Chem. *274*, 15440–15446.

Fasshauer, D., Antonin, W., Subramaniam, V., and Jahn, R. (2002). SNARE assembly and disassembly exhibit a pronounced hysteresis. Nat. Struct. Biol. *9*, 144–151.

Fernandez, J.M., Neher, E., and Gomperts, B.D. (1984). Capacitance measurements reveal stepwise fusion events in degranulating mast cells. Nature *312*, 453–455.

Fernandez-Chacon, R., Königstorfer, A., Gerber, S.H., Garcia, J., Matos, M.F., Stevens, C.F., Brose, N., Rizo, J., Rosenmund, C., and Südhof, T.C. (2001). Synaptotagmin I functions as a calcium regulator of release probability. Nature *410*, 41–49.

Fernandez, I., Arac, D., Ubach, J., Gerber, S.H., Shin, O., Gao, Y., Anderson, R.G., Südhof, T.C., and Rizo, J. (2001). Three-dimensional structure of the synaptotagmin 1 C2B-domain: synaptotagmin 1 as a phospholipid binding machine. Neuron *32*, 1057–1069.

Fernandez-Chacon, R., Shin, O., Königstorfer, A., Matos, M.F., Meyer, A.C., Garcia, J., Gerber, S.H., Rizo, J., Südhof, T.C., and Rosenmund, C. (2002). Structure/function analysis of Ca^{2+} binding to the C2A domain of synaptotagmin I. J. Neurosci. *22*, 8438–8446.

Fischer von Mollard, G., Südhof, T.C., and Jahn, R. (1991). A small GTP-binding protein (rab3A) dissociates from synaptic vesicles during exocytosis. Nature *349*, 79–81.

Fisher, R.J., Pevsner, J., and Burgoyne, R.D. (2001). Control of fusion pore dynamics during exocytosis by Munc18. Science *291*, 875–878.

Fukuda, M., Kowalchyck, J.A., Zhang, X., Martin, T.F., and Mikoshiba, K. (2002). Synaptotagmin IX regulates Ca^{2+}-dependent secretion in PC12 cells. J. Biol. Chem. *277*, 4601–4604.

Garoff, H., and Cheng, R.H. (2001). The missing link between envelope formation and fusion in alphaviruses. Trends Microbiol. *9*, 408–410.

Goda, Y., and Stevens, C.F. (1994). Two components of transmitter release at a central synapse. Proc. Natl. Acad. Sci. USA *91*, 12942–12946.

Graham, M.E., and Burgoyne, R.D. (2000). Comparison of cysteine string protein (Csp) and mutant α-SNAP overexpression reveals a role for csp in late steps of membrane fusion in dense-core granule exocytosis in adrenal chromaffin cells. J. Neurosci. *20*, 1281–1289.

Haas, A., Conradt, B., and Wickner, W. (1994). G-protein ligands inhibit in vitro reactions of vacuole inheritance. J. Cell Biol. *126*, 87–97.

Hagler, D.J., and Goda, Y. (2001). Properties of synchronous and asynchronous release during pulse train depression in cultured hippocampal neurons. J. Neurophysiol. *85*, 2324–2334.

Hanson, P.I., Roth, R., Morisaki, H., Jahn, R., and Heuser, J.E. (1997). Structure and conformational changes in NSF and its membrane receptor complexes visualized by quick-freeze/deep-etch electron microscopy. Cell *90*, 523–535.

Hay, J.C. (2001). SNARE complex structure and function. Exp. Cell Res. *271*, 10–21.

Heinz, F.X., and Allison, S.L. (2001). The machinery for flavivirus fusion with host cell membranes. Curr. Opin. Microbiol. *4*, 450–455.

Hermann, G.J., Thatcher, J.W., Mills, J.P., Hales, K.G., Fuller, M.T., Nunnari, J., and Shaw, J.M. (1998). Mitochondrial fusion in yeast requires the transmembrane GTPase Fzo1p. J. Cell Biol. *143*, 359–373.

Hsu, S.C., Hazuka, C.D., Foletti, D.L., and Scheller, R.H. (1999). Targeting vesicles to specific sites on the plasma membrane: the role of the sec6/8 complex. Trends Cell Biol. *9*, 150–153.

Hung, C.H., Srivastava, M., and Pollard, H.B. (1996). Membrane fusion protein synexin (annexin VII) as a Ca^{2+}/GTP sensor in exocytotic secretion. Proc. Natl. Acad. Sci. USA *93*, 10797–10802.

Jahn, R., and Südhof, T.C. (1999). Membrane fusion and exocytosis. Annu. Rev. Biochem. *68*, 863–911.

Jahn, R., and Grubmüller, H. (2002). Membrane fusion. Curr. Opin. Cell Biol. *14*, 488–495.

Kemble, G.W., Danieli, T., and White, J.M. (1994). Lipid-anchored influenza hemagglutinin promotes hemifusion, not complete fusion. Cell *76*, 383–391.

Kozlov, M.M., and Markin, V.S. (1983). Possible mechanism of membrane fusion. Biofizika *28*, 255–261.

Kozlov, M.M., and Chernomordik, L.V. (1998). A mechanism of protein-mediated fusion-coupling between refolding of the influenza hemagglutinin and lipid rearrangements. Biophys. J. *75*, 1384–1396.

Kozlovsky, Y., and Kozlov, M.M. (2002). Stalk model of membrane fusion: solution of energy crisis. Biophys. J. *82*, 882–895.

Kuhn, R.J., Zhang, W., Rossmann, M.G., Pletnev, S.V., Corver, J., Lenches, E., Jones, C.T., Mukhopadhyay, S., Chipman, P.R., Strauss, E.G., et al. (2002). Structure of dengue virus: implications for flavivirus organization, maturation, and fusion. Cell *108*, 717–725.

Kuzmin, P.I., Zimmerberg, J., Chizmadzhev, Y.A., and Cohen, F.S. (2001). A quantitative model for membrane fusion based on low-energy intermediates. Proc. Natl. Acad. Sci. USA *98*, 7235–7240.

Laage, R., Rohde, J., Brosig, B., and Langosch, D. (2000). A conserved membrane-spanning amino acid motif drives homomeric and supports heteromeric assembly of presynaptic SNARE proteins. J. Biol. Chem. *275*, 17481–17487.

Lang, T., Bruns, D., Wenzel, D., Riedel, D., Holroyd, P., Thiele, C., and Jahn, R. (2001). SNAREs are concentrated in cholesterol-dependent clusters that define docking and fusion sites for exocytosis. EMBO J. *20*, 2202–2213.

Lentz, B.R., Malinin, V., Haque, M.E., and Evans, K. (2000). Protein machines and lipid assemblies: current views of cell membrane fusion. Curr. Opin. Struct. Biol. *10*, 607–615.

Lescar, J., Roussel, A., Wien, M.W., Navaza, J., Fuller, S.D., Wengler, G., Wengler, G., and Rey, F.A. (2001). The Fusion glycoprotein shell of Semliki Forest virus: an icosahedral assembly primed for fusogenic activation at endosomal pH. Cell *105*, 137–148.

Lewis, M.J., and Pelham, H.R. (2002). A new yeast endosomal SNARE related to mammalian syntaxin 8. Traffic *3*, 922–929.

Lin, R.C., and Scheller, R.H. (1997). Structural organization of the synaptic exocytosis core complex. Neuron *19*, 1087–1094.

Lindau, M., and Almers, W. (1995). Structure and function of fusion pores in exocytosis and ectoplasmic membrane fusion. Curr. Opin. Cell Biol. *7*, 509–517.

Liu, Y., and Barlowe, C. (2002). Analysis of Sec22p in endoplasmic reticulum/Golgi transport reveals cellular redundancy in SNARE protein function. Mol. Biol. Cell *13*, 3314–3324.

Liu, J., Wang, S., Hoxie, J.A., LaBranche, C.C., and Lu, M. (2002). Mutations that destabilize the gp41 core are determinants for stabilizing the simian immunodeficiency virus-CPmac envelope glycoprotein complex. J. Biol. Chem. *277*, 12891–12900.

Luo, Z.L., Matthews, A.M., and Weiss, S.R. (1999). Amino acid substitutions within the leucine zipper domain of the murine coronavirus spike protein cause defects in oligomerization and the ability to induce cell-to-cell fusion. J. Virol. *73*, 8152–8159.

Mackler, J.M., Drummond, J.A., Loewen, C.A., Robinson, I.M., and Reist, N.E. (2002). The C2B Ca^{2+}-binding motif of synaptotagmin is required for synaptic transmission in vivo. Nature *418*, 340–344.

Markin, V.S., and Albanesi, J.P. (2002). Membrane fusion: stalk model revisited. Biophys. J. *82*, 693–712.

Martin, I., and Ruysschaert, J.M. (2000). Common properties of fusion peptides from diverse systems. Biosci. Rep. *20*, 483–500.

McMahon, H.T., Missler, M., Li, C., and Südhof, T.C. (1995). Complexins: cytosolic proteins that regulate SNAP receptor function. Cell *83*, 111–119.

McNew, J.A., Parlati, F., Fukuda, R., Johnston, R.J., Paz, K., Paumet, F., Söllner, T.H., and Rothman, J.E. (2000). Compartmental specificity of cellular membrane fusion encoded in SNARE proteins. Nature *407*, 153–159.

Melikyan, G.B., White, J.M., and Cohen, F.S. (1995). GPI-anchored influenza hemagglutinin induces hemifusion to both red blood cell and planar bilayer membranes. J. Cell Biol. *131*, 679–691.

Misra, S., Miller, G.J., and Hurley, J.H. (2001). Recognizing phosphatidylinositol 3-phosphate. Cell *107*, 559–562.

Misura, K.M., Scheller, R.H., and Weis, W.I. (2000). Three-dimensional structure of the neuronal-Sec1-syntaxin 1a complex. Nature *404*, 355–362.

Noguchi, H., and Takasu, M. (2001). Fusion pathways of vesicles: a Brownian dynamics simulation. J. Chem. Phys. *115*, 9547–9551.

Novick, P., and Guo, W. (2002). Ras family therapy: Rab, Rho and Ral talk to the exocyst. Trends Cell Biol. *12*, 247–249.

Odorizzi, G., Babst, M., and Emr, S.D. (2000). Phosphoinositide signaling and the regulation of membrane trafficking in yeast. Trends Biochem. Sci. *25*, 229–235.

Ohtsuka, T., Takao-Rikitsu, E., Inoue, E., Inoue, M., Takeuchi, M., Matsubara, K., Deguchi-Tawarada, M., Satoh, K., Morimoto, K., Nakanishi, H., and Takai, Y. (2002). Cast, a novel protein of the cytomatrix at the active zone of synapses that forms a ternary complex with RIM1. J. Cell Biol. *158*, 577–590.

Otter-Nilsson, M., Hendriks, R., Pecheur-Huet, E.L., Hoekstra, D., and Nilsson, T. (1999). Cytosolic ATPases, p97 and NSF, are sufficient to mediate rapid membrane fusion. EMBO J. *18*, 2074–2083.

Otto, H., Hanson, P.I., and Jahn, R. (1997). Assembly and disassembly of a ternary complex of synaptobrevin, syntaxin, and SNAP-25 in the membrane of synaptic vesicles. Proc. Natl. Acad. Sci. USA *94*, 6197–6201.

Pabst, S., Hazzard, J.W., Antonin, W., Südhof, T.C., Jahn, R., Rizo, J., and Fasshauer, D. (2000). Selective interaction of complexin with the neuronal SNARE complex. Determination of the binding regions. J. Biol. Chem. *275*, 19808–19818.

Pelham, H.R. (2001). SNAREs and the specificity of membrane fusion. Trends Cell Biol. *11*, 99–101.

Peng, R., and Gallwitz, D. (2002). Sly1 protein bound to Golgi syntaxin Sed5p allows assembly and contributes to specificity of SNARE fusion complexes. J. Cell Biol. *157*, 645–655.

Pereira-Leal, J.B., and Seabra, M.C. (2001). Evolution of the Rab family of small GTP-binding proteins. J. Mol. Biol. *313*, 889–901.

Perin, M.S., Fried, V.A., Mignery, G.A., Jahn, R., and Südhof, T.C. (1990). Phospholipid binding by a synaptic vesicle protein homologous to the regulatory region of protein kinase C. Nature *345*, 260–263.

Pevsner, J., Hsu, S.C., and Scheller, R.H. (1994). n-Sec1: a neural-specific syntaxin-binding protein. Proc. Natl. Acad. Sci. USA *91*, 1445–1449.

Pfeffer, S. (2003). Membrane domains in the secretory and endocytic pathways. Cell *112*, this issue, 507–517.

Price, A., Seals, D., Wickner, W., and Ungermann, C. (2000a). The docking stage of yeast vacuole fusion requires the transfer of proteins from a *cis*-SNARE complex to a Rab/Ypt protein. J. Cell Biol. *148*, 1231–1238.

Price, A., Wickner, W., and Ungermann, C. (2000b). Proteins needed for vesicle budding from the Golgi complex are also required for the docking step of homotypic vacuole fusion. J. Cell Biol. *148*, 1223–1229.

Reim, K., Mansour, M., Varoqueaux, F., McMahon, H.T., Südhof, T.C., Brose, N., and Rosenmund, C. (2001). Complexins regulate a late step in Ca^{2+}-dependent neurotransmitter release. Cell *104*, 71–81.

Rizo, J., and Südhof, T.C. (2002). Snares and Munc18 in synaptic vesicle fusion. Nat. Rev. Neurosci. *3*, 641–653.

Robinson, I.M., Ranjan, R., and Schwarz, T.L. (2002). Synaptotagmins I and IV promote transmitter release independently of Ca^{2+} binding in the C_2A domain. Nature *418*, 336–340.

Root, M.J., Kay, M.S., and Kim, P.S. (2001). Protein design of an HIV-1 entry inhibitor. Science *291*, 884–888.

Rothman, J.E. (1994). Mechanisms of intracellular protein transport. Nature *372*, 55–63.

Sabatini, B.L., and Regehr, W.G. (1996). Timing of neurotransmission at fast synapses in the mammalian brain. Nature *384*, 170–172.

Sato, T.K., Overduin, M., and Emr, S.D. (2001). Location, location, location: membrane targeting directed by PX domains. Science *294*, 1881–1885.

Scales, S.J., Chen, Y.A., Yoo, B.Y., Patel, S.M., Doung, Y.C., and Scheller, R.H. (2000). SNAREs contribute to the specificity of membrane fusion. Neuron *26*, 457–464.

Schneggenburger, R., and Neher, E. (2000). Intracellular calcium dependence of transmitter release rates at a fast central synapse. Nature *406*, 889–893.

Schoch, S., Deak, F., Konigstorfer, A., Mozhayeva, M., Sara, Y.,

Südhof, T.C., and Kavalali, E.T. (2001). SNARE function analyzed in synaptobrevin/VAMP knockout mice. Science *294*, 1117–1122.

Schoch, S., Castillo, P.E., Jo, T., Mukherjee, K., Geppert, M., Wang, Y., Schmitz, F., Malenka, R.C., and Südhof, T.C. (2002). RIM1α forms a protein scaffold for regulating neurotransmitter release at the active zone. Nature *415*, 321–326.

Seals, D.F., Eitzen, G., Margolis, N., Wickner, W.T., and Price, A. (2000). A Ypt/Rab effector complex containing the Sec1 homolog Vps33p is required for homotypic vacuole fusion. Proc. Natl. Acad. Sci. USA *97*, 9402–9407.

Sesaki, H., and Jensen, R.E. (2001). UGO1 encodes an outer membrane protein required for mitochondrial fusion. J. Cell Biol. *152*, 1123–1134.

Shin, O.H., Rhee, J.S., Tang, J., Sugita, S., Rosenmund, C., and Südhof, T.C. (2003). Binding to the Ca^{2+} binding site of the synaptotagmin 1 C2B domain triggers fast exocytosis without stimulating SNARE interactions. Neuron *37*, 99–108.

Shin, O.H., Rizo, J., and Südhof, T.C. (2002). Synaptotagmin function in dense core vesicle exocytosis studied in cracked PC12 cells. Nat. Neurosci. *5*, 649–656.

Siegel, D.P. (1993). Energetics of intermediates in membrane fusion: comparison of stalk and inverted micellar intermediate mechanisms. Biophys. J. *65*, 2124–2140.

Skehel, J.J., and Wiley, D.C. (2000). Receptor binding and membrane fusion in virus entry: the influenza hemagglutinin. Annu. Rev. Biochem. *69*, 531–569.

Söllner, T., Bennet, M.K., Whiteheart, S.W., Scheller, R.H., and Rothman, J.E. (1993a). A protein assembly-disassembly pathway in vitro that may correspond to sequential steps of synaptic vesicle docking, activation, and fusion. Cell *75*, 409–418.

Söllner, T., Whiteheart, S.W., Brunner, M., Erdjument-Bromage, H., Geromanos, S., Tempst, P., and Rothman, J.E. (1993b). SNAP receptors implicated in vesicle targeting and fusion. Nature *362*, 318–324.

Stegmann, T., Doms, R.W., and Helenius, A. (1989). Protein-mediated membrane fusion. Annu. Rev. Biophys. Biophys. Chem. *18*, 187–211.

Sugita, S., Shin, O.H., Han, W., Lao, Y., and Südhof, T.C. (2002). Synaptotagmins form a hierarchy of exocytotic Ca^{2+} sensors with distinct Ca^{2+} affinities. EMBO J. *21*, 270–280.

Sutton, R.B., Fasshauer, D., Jahn, R., and Brünger, A.T. (1998). Crystal structure of a SNARE complex involved in synaptic exocytosis at 2.4 Å resolution. Nature *395*, 347–353.

Südhof, T.C. (1995). The synaptic vesicle cycle: a cascade of protein-protein interactions. Nature *375*, 645–653.

Südhof, T.C. (2002). Synaptotagmins: why so many? J. Biol. Chem. *277*, 7629–7632.

Tamm, L.K., and Han, X. (2000). Viral fusion peptides: a tool set to disrupt and connect biological membranes. Biosci. Rep. *20*, 501–518.

Titorenko, V.I., and Rachubinski, R.A. (2000). Peroxisomal membrane fusion requires two AAA family ATPases, Pex1p and Pex6p. J. Cell Biol. *150*, 881–886.

Ubach, J., Zhang, X., Shao, X., Südhof, T.C., and Rizo, J. (1998). Ca^{2+} binding to synaptotagmin: how many Ca^{2+} ions bind to the tip of a C_2-domain? EMBO J. *17*, 3921–3930.

Ungermann, C., Sato, K., and Wickner, W. (1998). Defining the functions of *trans*-SNARE pairs. Nature *396*, 543–548.

Ungermann, C., Price, A., and Wickner, W. (2000). A new role for a SNARE protein as a regulator of the Ypt7/Rab-dependent stage of docking. Proc. Natl. Acad. Sci. USA *97*, 8889–8891.

Verhage, M., Maia, A.S., Plomp, J.J., Brussaard, A.B., Heeroma, J.H., Vermeer, H., Toonen, R.F., Hammer, R.E., van den Berg, T.K., Missler, M., et al. (2000). Synaptic assembly of the brain in the absence of neurotransmitter secretion. Science *287*, 864–869.

Voets, T., Toonen, R.F., Brian, E.C., de Wit, H., Moser, T., Rettig, J., Südhof, T.C., Neher, E., and Verhage, M. (2001). Munc18-1 promotes large dense-core vesicle docking. Neuron *31*, 581–591.

Wang, Y., Sugita, S., and Südhof, T.C. (2000). The RIM/NIM family of neuronal C2 domain proteins. Interactions with Rab3 and a new class of Src homology 3 domain proteins. J. Biol. Chem. *275*, 20033–20044.

Wang, C.T., Grishanin, R., Earles, C.A., Chang, P.Y., Martin, T.F., Chapman, E.R., and Jackson, M.B. (2001a). Synaptotagmin modulation of fusion pore kinetics in regulated exocytosis of dense-core vesicles. Science *294*, 1111–1115.

Wang, X., Hu, B., Zimmermann, B., and Kilimann, M.W. (2001b). Rim1 and rabphilin-3 bind Rab3-GTP by composite determinants partially related through N-terminal β-helix motifs. J. Biol. Chem. *276*, 32480–32488.

Wang, Y., Liu, X., Biederer, T., and Südhof, T.C. (2002). A family of RIM-binding proteins regulated by alternative splicing: implications for the genesis of synaptic active zones. Proc. Natl. Acad. Sci. USA *99*, 14464–14469.

Weber, T., Paesold, G., Galli, C., Mischler, R., Semenza, G., and Brunner, J. (1994). Evidence for H^+-induced insertion of influenza hemagglutinin HA2 N-terminal segment into viral membrane. J. Biol. Chem. *269*, 18353–18358.

Weber, T., Zemelman, B.V., McNew, J.A., Westermann, B., Gmachl, M., Parlati, F., Söllner, T.H., and Rothman, J.E. (1998). SNAREpins: minimal machinery for membrane fusion. Cell *92*, 759–772.

Weissenhorn, W., Dessen, A., Harrison, S.C., Skehel, J.J., and Wiley, D.C. (1997). Atomic structure of the ectodomain from HIV-1 gp41. Nature *387*, 426–430.

Whyte, J.R.C., and Munro, S. (2002). Vesicle tethering complexes in membrane traffic. J. Cell Sci. *115*, 2627–2637.

Wu, S.K., Zeng, K., Wilson, I.A., and Balch, W.E. (1996). Structural insights into the function of the Rab GDI superfamily. Trends Biochem. Sci. *21*, 472–476.

Xu, T., Rammner, B., Margittai, M., Artalejo, A.R., Neher, E., and Jahn, R. (1999). Inhibition of SNARE complex assembly differentially affects kinetic components of exocytosis. Cell *99*, 713–722.

Yamaguchi, T., Dulubova, I., Min, S.W., Chen, X., Rizo, J., and Südhof, T.C. (2002). Sly1 binds to Golgi and ER syntaxins via a conserved N-terminal peptide motif. Dev. Cell *2*, 295–305.

Yang, B., Gonzalez, L., Prekeris, R., Steegmaier, M., Advani, R.J., and Scheller, R.H. (1999). SNARE interactions are not selective-implications for membrane fusion specificity. J. Biol. Chem. *274*, 5649–5653.

Yoshihara, M., and Littleton, J.T. (2002). Synaptotagmin I functions as a calcium sensor to synchronize neurotransmitter release. Neuron *36*, 897–908.