Core Proteins of the Secretory Machinery

Thorsten Lang and Reinhard Jahn(✉)

1 SNAREs ... 108
1.1 Structure of the Neuronal SNAREs ... 109
1.2 Assembly and Disassembly of SNAREs: Mechanistic Considerations ... 112
2 Sec1/Munc18 (SM) Proteins ... 115
2.1 SM Protein Interactions with SNAREs ... 116
2.2 Munc18-1–an Oddity among the SM Proteins? ... 116
3 Synaptotagmins ... 117
3.1 Synaptotagmin Family ... 117
3.2 Synaptotagmin 1 as Ca^{2+}-Sensor for Fast Neurotransmitter Release ... 118
3.3 Molecular Mechanism of Synaptotagmin 1 ... 119
4 Rab Proteins ... 119
4.1 Rab3 ... 120
4.2 Rab3 Effectors ... 121
5 Endocytic Proteins ... 121
5.1 Kiss-and-Run Exocytosis/Endocytosis ... 122
5.2 Clathrin-Mediated Endocytosis (CME) ... 123
5.3 Coupling Exocytosis to Endocytosis ... 125
References ... 125

Abstract Members of the Rab, SM- and SNARE-protein families play key roles in all intracellular membrane trafficking steps. While SM- and SNARE-proteins become directly involved in the fusion reaction at a late stage, Rabs and their effectors mediate upstream steps such as vesicle budding, delivery, tethering, and transport. Exocytosis of synaptic vesicles and regulated secretory granules are among the best-studied fusion events and involve the Rab3 isoforms Rab3A-D, the SM protein munc18-1, and the SNAREs syntaxin 1A, SNAP-25, and synaptobrevin 2. According to the current view, syntaxin 1A and SNAP-25 at the presynaptic membrane form a complex with synaptic vesicle-associated synaptobrevin 2. As complex formation proceeds, the opposed membranes are pulled tightly together, enforcing the

Reinhard Jahn
Max Planck Institute for Biophysical Chemistry, Dep. Neurobiology, Am Fassberg 11, 37077 Göttingen, Germany
rjahn@gwdg.de

T.C. Südhof, K. Starke (eds.), *Pharmacology of Neurotransmitter Release. Handbook of Experimental Pharmacology 184*.

© Springer-Verlag Berlin Heidelberg 2008

fusion reaction. Munc18-1 is essential for regulated exocytosis and interacts with syntaxin 1A alone or with SNARE complexes, suggesting a role for munc18-1 in controlling the SNARE-assembly reaction. Compared to other intracellular fusion steps, special adaptations evolved in the synapse to allow for the tight regulation and high membrane turnover rates required for synaptic transmission. Synaptic vesicle fusion is triggered by the intracellular second messenger calcium, with members of the synaptotagmin protein family being prime candidates for linking calcium influx to fusion in the fast phase of exocytosis. To compensate for the massive incorporation of synaptic vesicles into the plasma membrane during exocytosis, special adaptations to endocytic mechanisms have evolved at the synapse to allow for efficient vesicle recycling.

1 SNAREs

SNAREs comprise a superfamily of proteins that function in all membrane fusion steps of the secretory pathway within eukaryotic cells. They are small proteins that vary in structure and size (see Section 1.1), but share an evolutionary conserved stretch of 60–70 amino acids containing eight heptad repeats, which is termed SNARE motif (Brunger 2005). The number of different SNAREs varies between different organisms, ranging from 25 in yeast, 36 in mammals, to over 50 in plants. Each fusion step requires a specific set of four different SNARE motifs that is contributed by three or four different SNAREs, and each of the membranes destined to fuse contains at least one SNARE with a membrane anchor.

Membrane traffic usually consists of a sequence of steps involving the generation of a transport vesicle by budding from a precursor compartment, the transport of the vesicle to its destination, and finally the docking and fusion of the vesicle with the target compartment. SNAREs operate in the very last step of this sequence (Jahn and Scheller 2006). SNAREs on opposed membranes form a complex in "trans" that is mediated by the SNARE motifs and that progressively assembles from the N-terminal tips toward the C-terminal membrane anchors, thus clamping the two membranes together. The energy released during assembly is probably used for overcoming the fusion barrier. During fusion, the complex reorients from "trans" to "cis." Cis-SNARE complexes are unusually stable, and disassembly requires the action of an AAA-ATPase and ATP. Hence, SNAREs undergo a conformational cycle that is crucial for fusion. The cycle is controlled by an array of regulatory factors that are only partially understood (see Section 1.2).

While each fusion step appears to be mediated by a specific set of SNAREs, some SNAREs operate in multiple transport steps where they each interact with different SNARE partners. Conversely, SNAREs of the same subfamily (see Section 1.1) can substitute for each other, at least to a certain degree, in a given transport step. In vitro, there is less specificity in SNARE assembly, suggesting that additional con-

trol mechanisms are involved. According to current concepts, specificity in membrane traffic is achieved by successive layers of regulation that operate upstream of SNARE assembly and that involve members of conserved protein families. Rab-proteins are thought to orchestrate the initial contact between membranes destined to fuse (see Section 4) and to assure that only appropriate organelles are tethered. SM proteins are involved in preparing and proofreading SNAREs for trans-complex formation (see Section 2). The combination of these and possibly additional still unknown mechanisms would guarantee the required specificity in intracellular membrane trafficking.

The SNAREs involved in the fusion of synaptic vesicles and of secretory granules in neuroendocrine cells, referred to as neuronal SNAREs, have been intensely studied and serve as a paradigm for all SNAREs. They include syntaxin 1A and SNAP-25 at the presynaptic membrane and synaptobrevin 2 (also referred to as VAMP 2) at the vesicle membrane. Their importance for synaptic neurotransmission is documented by the fact that the block in neurotransmitter release caused by botulinum and tetanus neurotoxins is due to proteolysis of the neuronal SNAREs (Schiavo et al. 2000). Genetic deletion of these SNAREs confirmed their essential role in the last steps of neurotransmitter release. Intriguingly, analysis of chromaffin cells from KO mice lacking synaptobrevin or SNAP-25 showed that these proteins can be at least partially substituted by SNAP-23 and cellubrevin, respectively (Sorensen et al. 2003; Borisovska et al. 2005), i.e., the corresponding SNAREs involved in constitutive exocytosis.

In the following sections, we limit our discussion to the neuronal SNARE complex that, however, is paradigmatic for most SNARE complexes studied so far.

1.1 Structure of the Neuronal SNAREs

1.1.1 Syntaxin 1A

Syntaxin 1A is a protein composed of 288 amino acids (all numbers refer to rat proteins). Its structure is typical for most SNAREs: the SNARE motif is flanked by an independently folded N-terminal domain and a single transmembrane domain at the C-terminus (Figure 1). The N-terminal domain is composed of an antiparallel three-helix bundle with a small N-terminal extension and is linked to the SNARE motif via a long flexible linker region. Syntaxin is able to interact intramolecularly by folding back its N-terminal domain onto the SNARE motif, resulting in the so-called closed conformation of syntaxin in which the linker is structured and part of the SNARE motif assumes a helical conformation. Syntaxin 1 is highly abundant in neurons and neuroendocrine cells (approximately 1% of total brain protein) but lacking in non-neuronal cells.

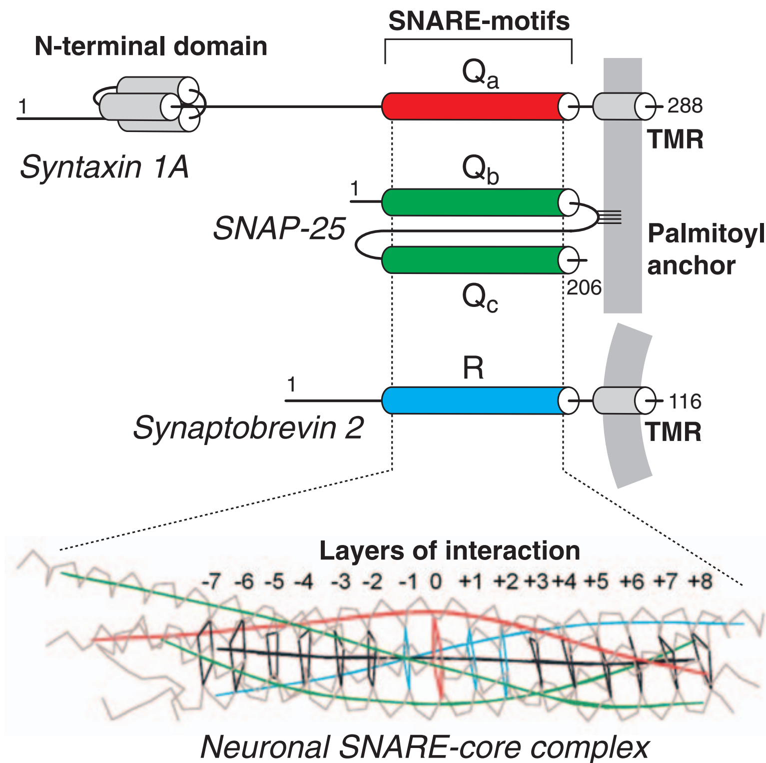

Fig. 1 Structure of the neuronal SNAREs. Upper panel: domain structure of the three neuronal SNARE proteins involved in synaptic vesicle fusion. Syntaxin 1A and SNAP-25 (contains two SNARE motifs) are associated with the presynaptic membrane, whereas synaptobrevin 2 is synaptic vesicle associated. The SNARE motifs form a stable complex (core complex) whose crystal structure has been analyzed (lower panel). In the complex, each of the SNARE motifs adopts an alpha-helical structure, and the four alpha-helices are aligned in parallel forming a twisted bundle (modified from Sutton et al. 1998). Stability of the complex is mediated by layers of interaction (−7 to +8) in which amino acids from each of the four alpha-helices participate (see text).

1.1.2 Synaptobrevin 2/VAMP 2

Synaptobrevin 2 is a small protein composed of 118 amino acids. It contains a SNARE motif with a short N-terminal proline-rich extension but lacks an independently folded N-terminal domain. Like syntaxin 1, the protein possesses a C-terminal transmembrane domain that is connected to the SNARE motif by a short linker (Figure 1). Synaptobrevin is palmitoylated at cysteine residues close to its transmembrane domain. Synaptobrevin 2 is highly expressed in neurons and neuroendocrine cells, but unlike syntaxin 1 it is also present in many non-neuronal tissues albeit at low levels.

1.1.3 SNAP-25

SNAP-25, a protein of 208 amino acids, deviates from the typical SNARE structure in that it has two SNARE motifs, joined by a flexible linker region, but lacks a transmembrane domain (Figure 1). The linker contains a cluster of four palmitoylated cysteine residues by which the protein is anchored at the plasma membrane. SNAP-25 can be phosphorylated at positions Thr138 and Ser187 by cAMP-dependent protein kinase (PKA) and protein kinase C (PKC), respectively. SNAP-25 represents a small subgroup of SNAREs with a similar structure, including SNAP-23, SNAP-29, and SNAP-47. In contrast to the neuron-specific SNAP-25 these SNAREs are ubiquitously expressed.

1.1.4 The Neuronal SNARE Complex

Syntaxin 1A, SNAP-25, and synaptobrevin 2 undergo structural changes when they assemble during membrane fusion. The crystal structure of the core-region of the neuronal SNARE complex (the assembled SNARE motifs) has been analyzed (Figure 1) and turned out to be paradigmatic for all SNARE-core complexes. In the complex, all SNARE motifs adopt an alpha-helical structure and are aligned in parallel, forming a twisted coiled-coil (Sutton et al. 1998). Along the longitudinal axis in the center of the bundle 16 stacked layers of interacting side chains have been identified (Figure 1). Each layer is formed by four amino acids, each contributed by a different SNARE motif. The layers are largely hydrophobic, with the exception of one ionic central layer that contains three glutamines and one arginine, all highly conserved (Fasshauer et al. 1998). The central layer is used as a center of reference for the remaining layers and termed "0"-layer. The layers from the N-terminal region of the SNARE motif to the 0-layer are termed -7 to -1, those upward from the 0-layer $+1$ to $+8$. An attractive working hypothesis is that SNARE motifs resemble a molecular zipper as they assemble from their N-terminal toward their C-terminal regions. According to this model, layers of interaction form sequentially one after another, pulling the opposed membranes stepwise together. During formation of the last layers, membranes would be forced so closely together that they fuse.

1.1.5 Q/R Classification of SNAREs

Initially, SNAREs were classified functionally into t-SNAREs (target-membrane SNAREs, e.g., syntaxin 1A and SNAP-25) or v-SNAREs (vesicle-membrane SNAREs, e.g., synaptobrevin 2). However, this concept cannot be applied to homotypic fusion events and is misleading because SNAREs are grouped together that belong to different subfamilies. A complete and unambiguous grouping is accomplished by the Q-/R-SNARE classification referring to the conserved amino acids present in the "0"-layer. According to the position of the SNARE motif in the structurally conserved SNARE complex, SNARE motifs are classified into Q_a-,

Q_b-, Q_c- and R-SNAREs (Bock and Scheller 2001). Following this classification, syntaxin 1A, SNAP-25. and synaptobrevin 2 represent the Q_a-, Q_b- and Q_c-, and R-SNAREs, respectively (Fasshauer et al. 1998). It turned out later that actually all functional SNARE complexes assigned to trafficking steps in yeast and mammals have a $Q_aQ_bQ_c$R-composition (Hong 2005; Jahn and Scheller 2006).

1.2 Assembly and Disassembly of SNAREs: Mechanistic Considerations

As discussed above, fusion is driven by the assembly of SNAREs mediated by their SNARE motifs. Assembly is associated with a major release of energy, and consequently the SNAREs need to be refueled with energy by the generation of free SNAREs before they are reusable for another round of fusion. Thus, SNAREs undergo cyclic assembly and disassembly, and together the individual reactions involved are referred to as the conformational cycle of SNAREs (Figure 2).

1.2.1 Assembly and Fusion

Free SNAREs are presumably short-lived, as they can form complexes among themselves, including homophilic oligomerization into clusters or with SNARE interacting proteins. It is becoming apparent that initial trans-contact between SNAREs

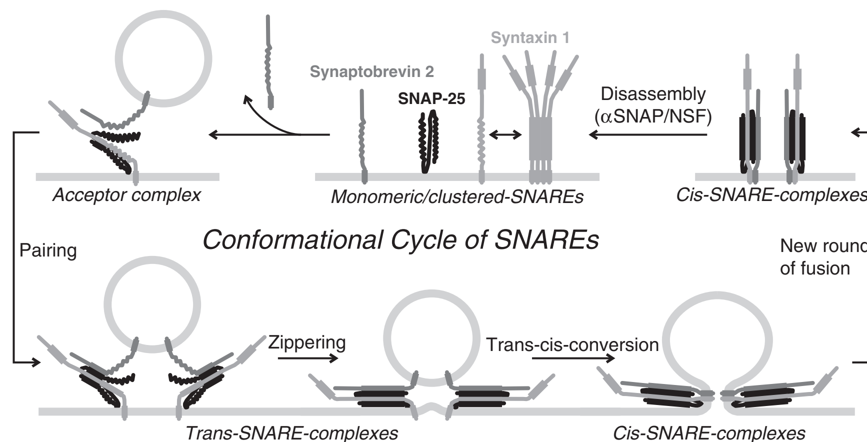

Fig. 2 The conformational cycle of SNAREs. SNAREs cycle between two extreme conformations, the unstructured monomeric SNAREs and the fully assembled cis-SNARE complexes. Initially, SNAREs on the membranes destined to fuse establish trans-SNARE complexes between the opposed membranes. Proceeding SNARE complex assembly forces the membranes tightly together enforcing membrane fusion. The resulting cis-SNARE complexes are disassembled into free SNAREs by the ATPase NSF and its co-factor, a process that consumes ATP and fuels the SNAREs with energy for undergoing a new SNARE cycle (for details see text).

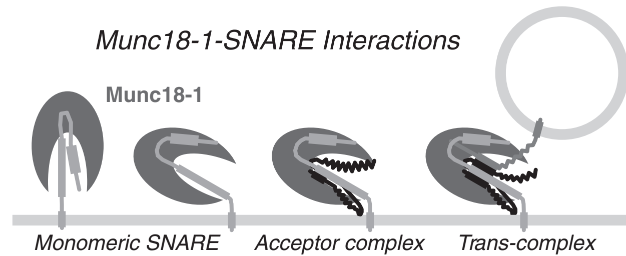

Fig. 3 munc18-1 SNARE-binding modes. The following munc18-1 interactions with monomeric/assembled SNAREs have been proposed. From left, binding of munc18-1 to a closed conformation of syntaxin 1A (Misura et al. 2000), to a half-open conformation of syntaxin or to an acceptor complex formed by syntaxin and SNAP-25 (Zilly et al. 2006), and to an assembled SNARE complex (Dulubova et al. 2007). It is possible that each of the proposed complexes represents an intermediate on a munc18-1 controlled molecular pathway of specific SNARE complex assembly.

(also referred to as "nucleation") is tightly controlled by cellular factors. A key role in preparing SNAREs, and perhaps in controlling nucleation, is assigned to the SM proteins, including its neuronal variant munc18-1, which are discussed more fully below. (see Section 3 and Figure 3).

It is not clear at present whether assembly is regulated after nucleation of trans-complexes or whether nucleation invariantly proceeds toward fusion. In vitro, assembly is completed rapidly after nucleation (Pobbati et al. 2006). However, mutagenesis of side chains in the central layers of the SNARE complex resulted in phenotypes that are best explained by energy minima during SNARE zippering, representing partially zippered and metastable intermediates in the molecular pathway of SNARE assembly. A partially assembled SNARE complex thus may account for the primed state of secretory granules in neuroendocrine cells (Sorensen et al. 2006). A protein thought to act on partially assembled trans-complexes is complexin, a small, soluble protein of 15 kDa (Marz and Hanson 2002). In vitro, complexin binds to SNARE complexes with high affinity, with the central part of complexin forming an α-helix that binds in an antiparallel orientation in the groove between synaptobrevin and syntaxin. While this interaction is thought to stabilize partially assembled SNARE complexes, deletion of complexin in mice lowers the sensitivity of neuronal exocytosis to calcium, resulting in a phenotype resembling that of synaptotagmin knockout mice (see section 3.2). Recently it has been proposed that complexin operates by stabilizing an otherwise metastable trans-SNARE intermediate that requires calcium-dependent displacement of complexin by synaptotagmin to be activated for fusion (Figure 4) (Tang et al. 2006).

The final steps in fusion are only incompletely understood. For instance, it is controversial whether the non-bilayer transition states in fusion are initiated primarily by force, transmitted from the "pull" of the assembling SNARE motifs via the linkers onto the membrane, as suggested by mutagenesis of the linker domain. Alternatively, the function of the SNARE motifs may be confined to close apposition of the membranes, with the final steps being catalyzed by other factors such as a

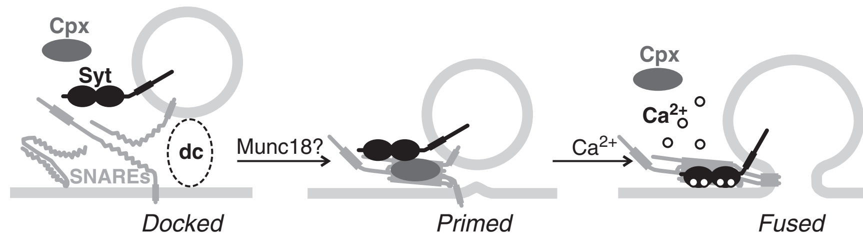

Fig. 4 Stages in synaptic vesicle exocytosis. Putative intermediate steps on the molecular pathway to synaptic vesicle fusion. Vesicle delivery and tethering to the presynaptic membrane most likely involves Rab-proteins and their effectors. So far, the nature of a speculative docking complex (dc) is unclear, but docking appears to be independent from SNARE proteins. In the primed state, SNAREs have assembled into a complex probably stabilized by complexin (Cpx). The fusion reaction is arrested until the intracellular calcium concentration increases. The putative calcium sensor for fast neurotransmitter release, synaptotagmin 1 (Syt), binds to intracellular calcium and in turn triggers fusion by associating with the presynaptic membrane and interacting with the SNARE complex, thereby displacing complexin (Tang et al. 2006).

perturbing “fusogenic” function of the transmembrane domains. For instance, it has been suggested that hetero-dimerization of the transmembrane domains of syntaxin 1A and synaptobrevin 2 facilitate the conversion from a hypothetical hemifusion intermediate state to full fusion (Ungermann and Langosch 2005). Recent evidence supports the involvement of hemifusion intermediates in the SNARE fusion pathway (see, e.g., Yoon et al. 2006). Furthermore, it is still controversial whether the fusion pore, i.e., the first aqueous connection between the vesicles and the extracellular space, is primarily lipidic or whether proteins (e.g., the transmembrane domains of the SNAREs) are part of the transition state.

1.2.2 Other Proteins Involved in the Regulation of SNAREs

In addition to the proteins discussed above, neuronal SNAREs were reported to interact with numerous other proteins in a specific manner, but in most cases both the structural basis and the biological function of these interactions need to be defined. For instance, synaptophysin, a membrane protein of synaptic vesicles, forms a complex with synaptobrevin in which synaptobrevin is not available for interactions with its partner SNAREs syntaxin 1A and SNAP-25, suggesting that this complex represents a reserve pool of recruitable synaptobrevin (Becher et al. 1999) or regulates interactions between the vesicle-associated synaptobrevin and the plasmalemmal SNAREs. Alternatively, it has been suggested that this complex is involved in synaptobrevin sorting to synaptic vesicles.

Munc13 is a 200 kDa protein essential for synaptic vesicle priming. As double knockouts of munc13 and syntaxin in *Caenorhabditis elegans* are rescued by constitutively open syntaxin, it has been suggested that munc13 mediates the transition from closed to open syntaxin (Brunger 2005). Recently, a ternary complex

composed of Rab3, RIM, and munc13 was described, suggesting a function in targeting synaptic vesicles to the priming machinery.

Tomosyn is a soluble protein of 130 kDa with a C-terminal R-SNARE motif that is capable of replacing synaptobrevin in the neuronal SNARE complex. Most available data indicate that tomosyn negatively regulates exocytosis by competing with synaptobrevin in the formation of SNARE complexes (Brunger 2005), thereby leading to the inhibition of synaptic vesicle priming (McEwen et al. 2006).

1.2.3 Disassembly of SNARE Complexes

After membrane fusion, all neuronal SNAREs reside in the plasma membrane. Their assembled SNARE motifs are aligned in parallel and the TMRs of syntaxin 1A and synaptobrevin 2 are close to each other in the same membrane. These cis-SNARE complexes are of remarkable stability and do not disassemble spontaneously into free SNAREs. Reactivation of the SNAREs is mediated by the ATPase NSF (N-ethylmaleimide-sensitive factor). NSF is required for all intracellular trafficking steps, and its function is to disassemble cis-SNARE complexes into free SNAREs. NSF is a member of the AAA-protein family (ATPases associated with other activities) that generally appear to be involved in disentangling protein complexes and protein aggregates (Hanson and Whiteheart 2005). Unlike many other AAA-ATPases, NSF cannot act alone–it needs co-factors termed SNAPs (soluble NSF attachment protein), represented by three isoforms termed α-, β-, and γ-SNAP. SNAPs bind to the cis-SNARE complex first and in turn recruit NSF, followed by stimulation of its ATPase activity. The hydrolysis of ATP induces major conformational changes resulting in the disassembly of the entire complex into its free constituents. In this uncomplexed state, the neuronal SNAREs are probably most susceptible to cleavage by clostridial neurotoxins.

2 Sec1/Munc18 (SM) Proteins

SM proteins were initially discovered during genetic screens in yeast and *C. elegans* for mutants showing defects in membrane traffic and secretion (Toonen and Verhage 2003). They comprise a small family of cytosolic proteins of 650–700 amino acids with seven members in mammals and four members in yeast. Although not yet documented unequivocally in each case, it appears that each trafficking step catalyzed by SNAREs is dependent on one of the SM proteins. Due to the small number of SM proteins, it is evident that some of them operate in more than one fusion reaction. In contrast to SNAREs, no functional redundancy has been observed so far, but SM proteins from distant species are capable of replacing each other provided they participate in the same trafficking step. Wherever investigated, genetic deletion of an SM protein leads to a block of the corresponding fusion reaction, indicating that their role in membrane fusion is essential. SM proteins most likely exert their

function upon regulating SNARE assembly, although also a role in docking has been suggested (Voets et al. 2001). Although significant progress has been made in recent years, it has turned out to be surprisingly tricky to unravel how they work, with major questions still being open.

2.1 SM Protein Interactions with SNAREs

All SM proteins interact with SNAREs, either directly or indirectly in complex with other proteins. Furthermore, strong genetic interactions have been documented between SM proteins and SNAREs. In some cases deletion of an SM protein is associated with a reduced expression level of its respective SNARE binding partner (Gallwitz and Jahn 2003).

The crystal structure of mammalian and squid munc18 and of yeast Sly1p shows a remarkable degree of structural conservation. SM proteins are composed of three domains that form an arch-shaped molecule with a central cleft (Misura et al. 2000; Bracher and Weissenhorn, 2002). Surprisingly, however, a confusing variety of binding modes between SM proteins and SNAREs has been observed (see, e.g., Figure 3). Both munc18 and Sly1p directly bind to the corresponding Qa-SNAREs syntaxin 1 and Sed5p (syntaxin 5), respectively, but in a completely different manner. In the crystal structure of the munc18-1-syntaxin 1A complex, syntaxin is arrested in the closed conformation, being inserted in the central cleft of munc18-1. In stark contrast, Sed5p binds only with a short N-terminal peptide that precedes its helical N-terminal domain, and the binding site on Sly1p is represented by a small groove on the surface of the SM protein, with no involvement of the central cleft. The latter binding mode has also been described for yeast and mammalian SM proteins involved in trafficking steps of the ER, the Golgi, the trans-Golgi network, and early endosomes (Toonen and Verhage 2003; Rizo and Südhof 2002), and it thus appears that the binding mode between munc18 and syntaxin 1 is unique among the family.

2.2 Munc18-1–an Oddity among the SM Proteins?

Munc18-1 (and its ortholog unc-18 in *C. elegans*) are essential for exocytosis. Knockout of munc18-1 in mice results in a nervous system that initially develops normally but in which synapses are totally silent – one of the most dramatic phenotypes of synaptic proteins that highlights the essential role and the lack of redundancy of the protein. Despite this dramatic phenotype, it has been remarkably difficult to reconcile the physiological findings obtained from synapses containing deleted, overexpressed, or otherwise manipulated munc18 with its biochemical properties studied in vitro. As discussed above, munc18-1 binds to syntaxin 1A with high affinity in a manner that clamps the N-terminal domain of syntaxin onto its

SNARE motif, effectively preventing syntaxin from binding to its partner SNAREs SNAP-25 and synaptobrevin 2. However, an inactivation of syntaxins' SNARE function is exactly the opposite of what one would expect from an essential protein, and (except for a debated study in *Drosophila*) even massive overexpression of munc18 does not appear to affect exocytosis (Gallwitz and Jahn 2003). Thus, it has been debated whether the "closed" conformation of syntaxin 1 in the munc18 represents a nonphysiological extreme situation that does not occur in intact cells, particularly since no other SM protein interferes with the formation of SNARE complexes (see below). Hence, the search has been on for munc18 SNARE complexes with different properties that may be closer to the physiological situation. Recently, evidence was provided showing that in native membranes munc18-1 stabilizes a half-closed conformation of syntaxin that still is capable of engaging in SNARE assembly (Zilly et al. 2006). Furthermore, it has been shown that munc18-1 activates SNARE-mediated membrane fusion in a reconstituted liposome system (Shen et al. 2007), and can bind directly to the assembled SNARE complex (Dulubova et al. 2007). Thus it is becoming apparent that munc18, in addition to its ability to form a complex with closed syntaxin 1, is capable of interacting with the neuronal SNAREs in other binding modes that are not inhibitory but rather may promote assembly of SNARE complexes (Figure 3). Such binding modes are more compatible with all other SM proteins that bind to partially or even fully assembled SNARE complexes. Although much more work needs to be done, a picture is emerging according to which SM proteins may assist in the formation of SNARE acceptor complexes needed for trans-SNARE interaction, and in doing so they may also be involved in proofreading of SNAREs, thus differentiating between cognate and noncognate SNAREs (Peng and Gallwitz 2002).

With the focus clearly being on the SNAREs, it needs to be borne in mind that SM proteins interact with a diverse array of additional proteins, in some cases even forming stable complexes (e.g., the SM protein Vps33p is part of the HOPS/VpsC complex needed for vacuole fusion in yeast). For instance, munc18 binds to the cytoplasmic protein Mint and it has been suggested that munc18-1 binding to Mint could regulate exocytosis by syntaxin-independent interactions (Schütz et al. 2005; Ciufo et al. 2005).

3 Synaptotagmins

3.1 Synaptotagmin Family

Synaptotagmins comprise a small family of single-membrane spanning proteins that are expressed in neurons and neuroendocrine cells. So far 16 members have been identified in vertebrates (Craxton 2004). They contain an N-terminal transmembrane domain followed by a variable linker region and two C2 domains which are connected by a short linker (Südhof 2002). Some synaptotagmins have additional short

N-terminal domains that in some cases are glycosylated (Syt 1 and 2) or carry a disulfide bond (Syt 3, 5, 6, and 10). The two C2 domains (C2 stands for second constant sequence, as defined when the first C2 domains were identified in protein kinase C isoforms) are termed C2A and C2B and generally bind three and two calcium ions, respectively, although some synaptotagmins do not bind calcium. Synaptotagmins are found both on synaptic and secretory vesicles (Syt 1, 2, and 9) and on the plasma membrane (e.g., Syt 3 and 7). More recently, additional proteins have been discovered that are similar to synaptotagmins in that they possess C2 domains and membrane anchors. Best characterized are the ferlins which comprise a membrane protein family with four to seven C2 domains and a single TMR at their C-terminus (see, e.g., Washington and Ward 2006). As for the synaptotagmins, recent evidence suggests that ferlins also play a role in Ca^{2+}-dependent exocytosis such as that involved in membrane repair in muscle cells (requiring dysferlin) and in vesicle release in the hair cells of the inner ear (otoferlin).

Synaptotagmin 1 is the founding member of the synaptotagmin family, and it is also the most intensely studied isoform. Together with Syt2, Syt1 functions as synaptic Ca^{2+}-sensor that couples Ca^{2+}-influx with fast transmitter release (Chapman 2002). Intriguingly, some of the plasma membrane associated synaptotagmins have a 10-fold higher binding affinity for Ca^{2+}. Together with the finding that Syt7 can function as Ca^{2+}-sensor for exocytosis in chromaffin cells but not in neurons, it is conceivable that low-calcium-affinity synaptotagmins (Syt1 and 2) trigger fast neurotransmitter release in neurons (see Section 3.2), whereas plasma-membrane-associated Syt7 may function as calcium sensor for exocytosis in neuroendocrine cells. In addition, having synaptotagmins on both the plasma and the vesicle membrane may result in a system of complementary Ca^{2+}-sensors regarding the sensitivity to intracellular calcium signals.

3.2 Synaptotagmin 1 as Ca^{2+}*-Sensor for Fast Neurotransmitter Release*

Genetic deletion of Syt1 in *Drosophila* and mice leads to the loss of the fast, Ca^{2+}-dependent phase of transmitter release that follows the arrival of an action potential in the presynaptic nerve terminal (Chapman 2002). Disruption of Ca^{2+}-binding to either of the C2 domains severely inhibits the function of synaptotagmin in mediating fast synchronous transmitter release, with the disruption of the C2B-domain being more severe than that of the C2A domain. Furthermore, when mutant synaptotagmins exhibiting either reduced or increased Ca^{2+}-affinity are expressed in mice lacking synaptotagmin I a close correlation was observed between the Ca^{2+}-affinity and the Ca^{2+} -dependence of neurotransmitter release, all confirming that Syt1 is an essential link between Ca^{2+}-influx and the synaptic fusion machinery. Interestingly, lack of synaptotagmin does not abolish exocytosis, as a "normal" SNARE-dependent exocytotic response is attainable when exocytosis is triggered by α-latrotoxin (the active ingredient of black widow spider venom).

3.3 Molecular Mechanism of Synaptotagmin 1

C2 domains are represented by stable, mostly β-stranded folds, with the Ca^{2+}-binding site at one end of the elongated domain. The Ca^{2+}-binding site only incompletely coordinates the Ca^{2+}-ions. Consequently, the affinity of the free C2 domains for Ca^{2+}-is rather low but dramatically increases in the presence of acidic phospholipids. Thus, the C2 domains mediate Ca^{2+}-binding to membranes that in the case of Syt1 is further enhanced in the presence of phosphatidylinositol (4, 5) bisphosphate (PIP_2). Furthermore, synaptotagmin binds to both isolated syntaxin 1 and SNAP-25 as well as to partially (containing SNAP-25 and syntaxin) and fully assembled SNARE complexes in vitro. For SNARE binding, both Ca^{2+}-dependent and Ca^{2+}-independent interactions have been described. Mutagenesis studies involving partial or full disruption of Ca^{2+}-dependent binding to phospholipids and of binding to SNAREs have suggested that each of these interactions is required for the function of Syt1.

As in the case of munc18-1, it has been tricky to delineate the molecular mechanism of Syt1 action, and many details are still unclear (Rizo et al. 2006). The problems in understanding the mechanism of synaptotagmin, despite major efforts, highlight the fact that we do not yet have a good understanding of the status of the fusion machinery including SNAREs and associated proteins in the docked and primed state that synaptotagmin is acting upon. Ca^{2+}-dependent binding of the C2-domains to membranes may clamp the membranes tightly together (as recently suggested). Furthermore, membrane binding may destabilize bilayers at the site of fusion, facilitating the formation of transition states (such as hemifusion) or destabilizing the transition states themselves. Furthermore, as discussed above, Syt1 is capable of displacing complexin from the surface of the SNARE complex in a Ca^{2+}-dependent manner. It was proposed that these proteins act in sequence, with complexin stabilizing a labile SNARE trans-intermediate that then is driven toward fusion by Syt1 upon displacement of complexin.

4 Rab Proteins

Rabs are small (20–29 kDa) ubiquitously expressed proteins. They represent monomeric GTPases which belong to the Ras GTPase superfamily. So far 11 members are known in yeast and more than 60 in mammalian cells (Schultz et al. 2000), whereas the numbers reflect the complexity of membrane trafficking pathways in these different organisms. Rabs cycle between the cytosol and the membrane of the trafficking organelle. This cycle is controlled by conformational changes that are regulated by guanine-nucleotides, thus providing a molecular switch, with the membrane-bound GTP-form being "on" and the GDP-form being "off." GTP-Rabs bind to proteins termed effectors that only recognize Rabs in their GTP-bound state. A large diversity of Rab effectors is known, many being specific for a single or for small subsets of Rabs.

Rabs function as master regulators of membrane docking and fusion. Fusion can only occur if membrane contact is established, and numerous lines of evidence suggest that contact is orchestrated by Rabs. In fact, selective activation of Rabs at a given site ensures directionality and specificity of membrane docking and fusion (Grosshans et al. 2006), similar to the GTPases Sar1 and Arfs that are involved in coat recruitment during vesicle budding. In some cases, distinct Rab domains are present on the same organelle where they are involved in different transport steps.

As Rabs share a common structure and are highly homologous, the structural diversity of their effectors probably reflects the versatile functions of these GTPases as molecular switches. This diversity is highlighted by the functional diversity of Rab effectors. For instance Rab27a regulates transport of melanosomes to the cell periphery by binding to its effector melanophilin. Melanophilin associates with the actin motor myosin-Va. Rab-mediated tethering of membranes in preparation for fusion involves multimeric complexes. One of the best-studied examples is the exocyst, an octameric protein complex that tethers secretory vesicles to the plasma membrane in yeast. For homotypic membrane fusion of early endosomes (or of yeast vacuoles), Rab effectors interact with SNAREs such as the Rab5 effector EEA1 that binds to syntaxin-13.

4.1 Rab3

Despite major efforts, the precise role of Rab proteins in synaptic exocytosis is still not clear. One of the most abundant synaptic Rab proteins is Rab3, which is selectively localized to synaptic vesicles and that is represented by four homologous isoforms (Rab3A, Rab3B, Rab3C, and Rab3D). Of these, Rab3A is the most abundant and best studied (Südhof 2004). Rab3A undergoes a synaptic vesicle association and dissociation cycle coupled to calcium-stimulated exocytosis and recovery after stimulation.

Like all Rabs, the GTP-bound form of Rab3A is anchored in the synaptic vesicle membrane via a covalently bound geranylgeranyl moiety. When exocytosis is triggered, Rab3A-bound GTP is hydrolyzed to GDP, and the resulting Rab3A-GDP forms a complex with GDI (guanine dissociation inhibitor) in which the geranylgeranyl anchors are enveloped, leading to the dissociation of the GDI-Rab3A-GDP complex from the synaptic vesicle membrane. Rab3A is then recruited again to the synaptic vesicle membrane by a poorly understood mechanism involving binding of a specific GEF (guanine nucleotide exchange factor) to Rab3A and GDP exchange by GTP.

The tight coupling of exocytosis to the Rab3A association and dissociation cycle suggested a key role for Rab3A and its effectors in mediating directionality of synaptic vesicle traffic. However, mice lacking Rab3A are viable and have an only moderate synaptic dysfunction. In neurons derived from the hippocampal CA1 region, an alteration of the short-term plasticity was observed. In contrast, in neurons derived from the hippocampal CA3 region short-term plasticity was unaltered, but

Rab3A was essential for mossy-fiber long-term potentiation. However, a complete genetic analysis in mice showed that Rab3 is essential for survival in mice and that the Rab3 isoforms are functionally redundant (Schlüter et al. 2004).

Presently, it cannot be excluded that despite the apparently highly specific function of certain Rabs in intracellular trafficking pathways there is redundancy with respect to vesicle docking in the synapse. The surprising diversity of Rabs on highly purified synaptic vesicles (more than 30 different Rabs) supports the view that multiple Rabs are required for synaptic vesicle recycling, which may have overlapping functions.

4.2 Rab3 Effectors

Rabphilin and RIM1α/2α represent two different classes of effectors that bind to Rab3-GTP (Südhof 2004). They have structural similarities, as both contain two C_2 domains, an N-terminally located zinc-finger domain that mediates binding to GTP-Rab3, and sites for phosphorylation by PKA that are located in the center of the proteins.

Rabphilin 3A is a soluble protein that is recruited to the membrane of synaptic vesicles by Rab3A and C in a GTP-dependent manner, closely coupling it to the Rab3 cycle. Like synaptotagmin, it has two functional C2 domains in the C-terminal region, but unlike synaptotagmins, it does not contain a transmembrane domain.). Rabphilin is phosphorylated by various kinases in a stimulation-dependent manner. Rabphilin knockout mice are viable and do not show a major synaptic phenotype, thus providing no clue for the function of this protein.

In contrast, genetic deletion of RIM1α in mice revealed that the protein is required for long-term potentiation both in the hippocampus and in the cerebellum. Biochemical experiments revealed that RIM1α is part of a presynaptic protein scaffold containing "active zone proteins" that is required for normal release of neurotransmitters. No change in the number of docked vesicles was observed, however, suggesting that other proteins are needed for the docking of synaptic vesicles at active zones.

5 Endocytic Proteins

Exocytosis of synaptic vesicles or of regulated secretory granules results in incorporation of membrane into the plasma membrane. For maintaining the cell surface area constant, homeostatic mechanisms are required that assure a rapid and efficient re-internalization of the incorporated vesicle membranes. Different types of vesicle recycling pathways are discussed for synapses (Figure 5), including fast retrieval of the vesicle at the site of exocytosis, called "kiss-and-run," a slower clathrin-dependent pathway, and other clathrin-independent retrieval pathways (Royle and

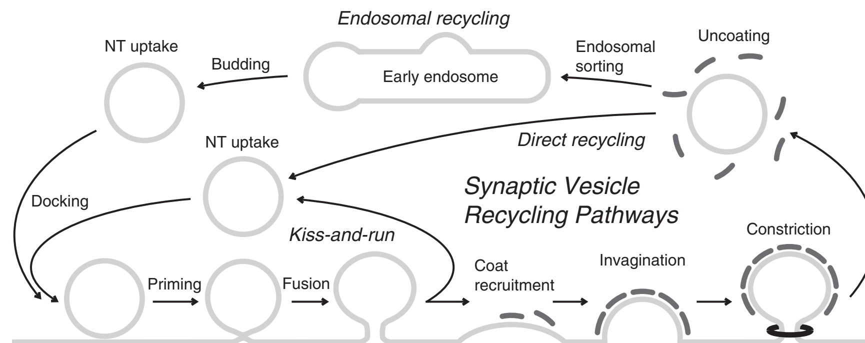

Fig. 5 Synaptic vesicle recycling in the synapse. For synaptic vesicle recycling, several endocytic mechanisms appear to co-exist in synaptic nerve terminals. In the case of fast "kiss-and-run" exocytosis/endocytosis, the fused vesicle does not collapse into the membrane but is retrieved directly by a fast process. The molecular machinery underlying this pathway is unknown. Vesicles that have fully collapsed into the membrane are recycled by clathrin-mediated endocytosis. Clathrin, along with other proteins, is involved in membrane invagination (see figure and text) and leads finally to the formation of a constricted pit. The GTPase dynamin (black ring) mediates membrane scission of the constricted pit. After removal of the clathrin coat, two pathways are possible (direct recycling and recycling via the early endosome). In all cases, before fusion the recycled vesicles have to be loaded with neurotransmitters (NT).

Lagnado 2003). Which of the pathways dominates seems to depend on the type of neuron and the duration and intensity of the exocytotic stimulus. Apparently, the fast "kiss-and-run" pathway is preferred at low stimulation frequency for retrieving vesicles of the readily releasable pool. At higher stimulation frequencies the slow clathrin-dependent, endosomal recycling pathway is activated (Rizzoli and Betz 2005). Whereas no synapse is functional without an intact clathrin-dependent recycling pathway, similarly firm evidence for the need of the "kiss-and-run" pathway is not available. In fact, most of the evidence for "kiss-and-run" is either at the detection limit of the respective techniques, or of indirect nature. Furthermore, no protein machinery that is specific for this pathway has been identified. For these reasons, doubts persist whether such a pathway does exist at all in synapses. Another debated issue relates to the function of endosomal intermediates. Although synapses possess functional early endosomes it is unclear whether endocytosed vesicles must pass through an endosomal intermediate during each recycling or whether endosomes can be bypassed, with synaptic vesicles reforming directly after clathrin uncoating (Südhof 2004).

5.1 Kiss-and-Run Exocytosis/Endocytosis

According to the classical view, synaptic vesicles completely flatten during exocytosis, which is followed by retrieval of the membrane components by clathrin-dependent endocytosis. Evidence for direct retrieval (kiss-and-run) was provided

more than 30 years ago by Bruno Ceccarelli. At the neuromuscular endplate, Ceccarelli and co-workers were unable to observe reduction of vesicle numbers and the appearance of coated vesicles during low-stimulation frequencies, although synaptic vesicles acquired an extracellular fluid-phase marker in a stimulus-dependent fashion (Ceccarelli et al. 1973). When exocytotic events were captured by electron microscopy using rapid shock-freezing of stimulated synapses, exocytotic events were visible, with vesicles opening to the extracellular space by means of a pore. It was proposed that synaptic vesicles do not necessarily flatten into the membrane but instead can undergo rapid and transient fusion with the presynaptic membrane, a mechanism that was termed "kiss-and-run" (Fesce et al. 1994). This mechanism would allow for neurotransmitter release without the loss of synaptic vesicle identity, and therefore without any need of membrane recycling. Indeed, evidence for "reversible" fusion has since been provided from secretory cells with much larger vesicles. For instance, capacitance measurements on a variety of secretory cells showed that exocytosis, observed as a stepwise increase in capacitance, is frequently followed by a decrease of similar size (Fernandez et al. 1984; Breckenridge and Almers 1987). The molecular mechanisms that would mediate the scission of the fused, but incompletely flattened synaptic vesicles are still unclear. In chromaffin cells, in which the regulated organelles are large secretory granules, available data indicate that dynamin-1 may be responsible for the direct rapid retrieval pathway while dynamin 2 is involved in slow clathrin-mediated endocytosis (Artalejo et al. 2002). However, it is unclear how these findings are exactly related to the many types of nonclassical recycling pathways in nerve terminals that may differ with respect to retention of shape, protein, or lipid of the fused vesicle before its reinternalization. However, as a generally accepted terminology for the different fast nonclassical modes is not available, they are usually termed "kiss-and-run" exocytosis/endocytosis.

5.2 Clathrin-Mediated Endocytosis (CME)

As has been shown by genetic approaches and by in vitro reconstitution, the molecular machinery for clathrin-mediated endocytosis (CME) in nerve terminals is generally similar to that involved in CME of non-neuronal cells. However, at the synapse internalization and recycling of vesicle membranes occurs in seconds, much faster than, e.g., ligand-induced, receptor-mediated endocytosis that occurs at a slower time scale. In fact the synapse possesses several adaptations of the CME pathway that may be responsible for these differences. First, endocytic proteins are highly enriched in synapses, including clathrin, AP2, epsin, eps15 (epidermal growth factor pathway subunit 15), amphiphysin, and synaptojanin. In addition, several of the major CME proteins express neuron-specific isoforms, such as dynamin, AP180, syndapin 1, clathrin light chain, and intersectin. Second, within the nerve terminal the endocytic machinery is localized close to sites of vesicle exocytosis (Roos and Kelly 1999). Hence, there is no need for long-distance diffusion from exocytotic

to endocytic sites when the clathrin machinery is activated. Third, recent evidence suggests that the components of synaptic vesicles may remain clustered prior to internalization instead of dispersing into the membrane (see, e.g., Willig et al. 2006), thereby avoiding the need for elaborate sorting to reconcentrate vesicle components in the plasma membrane.

CME involves sequential and morphologically distinguishable steps, including coat recruitment and assembly on the membrane, invagination, formation of a constricted pit, fission, and uncoating. Coat recruitment and invagination are initiated by endocytic adaptors like AP-2 and stonin that function in the selection of cargo molecules and the initiation of the assembly of the clathrin coat and other factors which are required for the shaping of the vesicle. The endocytic adaptors bind to a type of lipid enriched in the cytoplasmic leaflet of the presynaptic membrane (phosphatidylinositol (4,5)-bisphosphate), to clathrin and other accessory proteins, and to cargo-sorting signals of, e.g., the synaptic vesicle transmembrane protein synaptotagmin (Maldonado-Báez and Wendland 2006; Di Paolo and De Camilli 2006). Although it appears that adaptor-lipid binding initiates the process, stable recruitment of adaptors to the plasma membrane requires cooperation between theses three types of adaptor-interactions (coincidence detection). Adaptor binding to clathrin and the neural specific AP180 leads to the formation of lattice-like structures with basic units of a trimer of clathrin (named triskelion). These assemble into larger baskets, and the interaction of epsin and AP-180 with the polymerizing clathrin lattice is supposed to promote membrane curvature, finally leading to the invagination of the membrane. Such invaginations are morphologically visible in electron micrographs as clathrin-coated pits. As has been revealed more recently by using fluorescent proteins for the study of endocytosis, clathrin-coated pits are not static but dynamic structures exchanging components with the soluble pool of endocytic proteins (Edeling et al. 2006).

The next step is the formation of a constricted pit, followed by fission of the membrane. Membrane fission is mediated by the mechanochemical GTPase dynamin that appears to wrap around the neck of the clathrin-coated pit (Takei and Haucke 2001). Recruitment of dynamin to its site of action is facilitated by amphiphysin, a protein that not only binds to dynamin but also to AP2 and clathrin. Furthermore, dynamin binds to phosphatidylinositol (4,5)-bisphosphate by its pleckstrin homology domain. The binding of GTP to dynamin in complex with amphiphysin has been suggested to redistribute dynamin close to the neck region, resulting in the formation of a constricted pit. GTP-hydrolysis leads to a conformational change of dynamin accompanied by a constriction of the dynamin ring around the neck. This mechanism probably generates the membrane fission event, resulting in a clathrin-coated vesicle ready for transport into the cytosol. Dynamin may thus be regarded as the counterpart of the SNAREs, with the SNAREs mediating fusion and dynamin mediating fission.

Uncoating requires an interaction with the uncoating ATPase Hsc70. Apparently, however, hydrolysis of phosphatidylinositol (4,5)-bisphosphate is required, which is carried out by the protein synaptojanin. Synaptojanin has two phosphatase domains, and in its absence clathrin-coated vesicles accumulate. Furthermore, the

coated vesicle-associated protein auxilin is needed for uncoating. This protein recruits Hsc70 and stimulates its ATPase activity.

It is still unclear whether uncoated recycling vesicles must pass through an endosomal intermediate before synaptic vesicles are re-formed. Synaptic vesicles contain high concentrations of the protein machinery required for endosome fusion (Takamori et al. 2006), and synaptic organelles that have just undergone endocytosis are capable of homotypic fusion. However, it is possible that the endosomal intermediate serves as a backup rather than a mandatory intermediate, with vesicles being directly regenerated after uncoating without the involvement of an additional fusion and budding step.

5.3 Coupling Exocytosis to Endocytosis

In the "kiss-and-run" mode exocytosis and endocytosis are directly coupled to each other, while in the case of classical complete vesicle fusion, exocytosis and slow clathrin-mediated endocytosis are timely and spatially separated. However, it appears that also in the latter case exocytosis and endocytosis occur coordinated, as both are stimulated by an increase of the cytoplasmic calcium concentration. It has been shown that after calcium entry the enzyme phospho-inositol-5 kinase Iγ, which is enriched in the synapse, catalyzes the synthesis of phosphatidylinositol (4,5)-bisphosphate and that this mechanism is important for synaptic vesicle trafficking (Di Paolo et al. 2004). As many proteins involved in clathrin-mediated endocytosis are recruited to the plasma membrane by binding to phosphatidylinositol (4,5)-bisphosphate (e.g., amphiphysin, dynamin, epsin, AP-180, and AP-2) it is attractive to speculate that elevated levels of calcium mediate the recruitment of endocytic proteins to the plasma membrane by this mechanism. The increased level of phosphatidylinositol (4,5)-bisphosphate could be in part degraded by synaptojanin that thereby initiates the disassembly of the clathrin coat. Hence, calcium-induced transient increases in the level of phosphatidylinositol (4,5)-bisphosphate appear to play a central role for coupling exocytosis to clathrin-mediated endocytosis. In addition, it has been demonstrated that calcium also leads to the dephosphorylation of endocytic proteins as amphiphysin, dynamin, and synaptojanin, which in vitro is important for efficient coat assembly (Cousin and Robinson 2001).

References

Artalejo CR, Elhamdani A, Palfrey HC (2002) Sustained stimulation shifts the mechanism of endocytosis from dynamin-1-dependent rapid endocytosis to clathrin- and dynamin-2-mediated slow endocytosis in chromaffin cells. Proc Natl Acad Sci USA 99:6358–63

Becher A, Drenckhahn A, Pahner I, Margittai M, Jahn R, Ahnert-Hilger G (1999) The synaptophysin-synaptobrevin complex: a hallmark of synaptic vesicle maturation. J Neurosci 19:1922–31

Borisovska M, Zhao Y, Tsytsyura Y, Glyvuk N, Takamori S, Matti U, Rettig J, Südhof T, Bruns D (2005) v-SNAREs control exocytosis of vesicles from priming to fusion. EMBO J. 24:2114–26
Bracher A, Weissenhorn W (2002) Structural basis for the Golgi membrane recruitment of Sly1p by Sed5p. EMBO J 21:6114–24
Breckenridge LJ, Almers W (1987) Final steps in exocytosis observed in a cell with giant secretory granules. Proc Natl Acad Sci USA 84:1945–49
Brunger AT (2005) Structure and function of SNARE and SNARE-interacting proteins. Q Rev Biophys 38:1–47
Ceccarelli B, Hurlbut WP, Mauro A (1973) Turnover of transmitter and synaptic vesicles at the frog neuromuscular junction. J Cell Biol 57:499–524
Ciufo LF, Barclay JW, Burgoyne RD, Morgan A (2005) Munc18-1 regulates early and late stages of exocytosis via syntaxin-independent protein interactions. Mol Biol Cell. 16:470–82
Chapman ER (2002) Synaptotagmin: a Ca(2+) sensor that triggers exocytosis? Nat Rev Mol Cell Biol 3:498–508
Cousin MA, Robinson PJ (2001) The dephosphins: dephosphorylation by calcineurin triggers synaptic vesicle endocytosis. Trends Neurosci 24:659–65
Craxton M (2004) Synaptotagmin gene content of the sequenced genomes. BMC Genomics 5:43
Di Paolo G, De Camilli P (2006) Phosphoinositides in cell regulation and membrane dynamics. Nature 443:651–7
Di Paolo G, Moskowitz HS, Gipson K, Wenk MR, Voronov S, Obayashi M, Flavell R, Fitzsimonds RM, Ryan TA, De Camilli P (2004) Impaired PtdIns(4,5)P2 synthesis in nerve terminals produces defects in synaptic vesicle trafficking. Nature 431:415–22
Dulubova I, Khvotchev M, Liu S, Huryeva I, Südhof TC, Rizo J (2007) Munc18-1 binds directly to the neuronal SNARE complex. Proc Natl Acad Sci USA. 2007 Feb 14; 104, 2697–702
Edeling MA, Smith C, Owen D (2006) Life of a clathrin coat: insights from clathrin and AP structures. Nat Rev Mol Cell Biol 7:32–44
Fasshauer D, Sutton RB, Brunger AT, Jahn R. (1998) Conserved structural features of the synaptic fusion complex: SNARE proteins reclassified as Q- and R-SNAREs. Proc Natl Acad Sci USA. 95:15781–6
Fernandez JM, Neher E, Gomperts BD (1984) Capacitance measurements reveal stepwise fusion events in degranulating mast cells. Nature 312:453–5
Fesce R, Grohovaz F, Valtorta F, Meldolesi J (1994) Neurotransmitter release: fusion or 'kiss-and-run'? Trends Cell Biol 4:1–4
Gallwitz D, Jahn R. (2003) The riddle of the Sec1/Munc-18 proteins – new twists added to their interactions with SNAREs. Trends Biochem Sci 28:113–16
Grosshans BL, Ortiz D, Novick P (2006) Rabs and their effectors: achieving specificity in membrane traffic. Proc Natl Acad Sci U S A 103:11821–7
Hanson PI, Whiteheart SW (2005) AAA+ proteins: have engine, will work. Nat Rev Mol Cell Biol 6:519–29
Hong W (2005) SNAREs and traffic. Biochim Biophys Acta 1744:493–517
Jahn R, Scheller RH. (2006) SNAREs–engines for membrane fusion. Nat Rev Mol Cell Biol 7:631–43
Maldonado-Baez L, Wendland B (2006) Endocytic adaptors: recruiters, coordinators and regulators. Trends Cell Biol 16:505–13
Marz KE, Hanson PI (2002) Sealed with a twist: complexin and the synaptic SNARE complex. Trends Neurosci 25:381–3
McEwen JM, Madison JM, Dybbs M, Kaplan JM (2006) Antagonistic regulation of synaptic vesicle priming by Tomosyn and UNC-13. Neuron 51:303–15
Misura KM, Scheller RH, Weis WI (2000) Three-dimensional structure of the neuronal-Sec1-syntaxin 1a complex. Nature 404:355–62
Peng R, Gallwitz D (2002) Sly1 protein bound to Golgi syntaxin Sed5p allows assembly and contributes to specificity of SNARE fusion complexes. J Cell Biol 157:645–55
Pobbati AV, Stein A, Fasshauer D (2006) N- to C-terminal SNARE complex assembly promotes rapid membrane fusion. Science 313:673–6

Rizo J, Chen X, Arac D (2006) Unraveling the mechanisms of synaptotagmin and SNARE function in neurotransmitter release. Trends Cell Biol 16:339–50
Rizo J, Südhof TC (2002) Snares and Munc18 in synaptic vesicle fusion. Nat Rev Neurosci 3:641–53
Rizzoli SO, Betz WJ (2005) Synaptic vesicle pools. Nat Rev Neurosci 6:57–69
Roos J, Kelly RB (1999) The endocytic machinery in nerve terminals surrounds sites of exocytosis. Curr Biol 9:1411–14
Royle SJ, Lagnado L (2003) Endocytosis at the synaptic terminal. J Physiol 553:345–55
Schiavo G, Matteoli M, Montecucco C (2000) Neurotoxins affecting neuroexocytosis. Physiol Rev 80:717–66
Schlüter OM, Schmitz F, Jahn R, Rosenmund C, Sudhof TC (2004) A complete genetic analysis of neuronal Rab3 function. J Neurosci 24:6629–37
Schultz J, Doerks T, Ponting CP, Copley RR, Bork P (2000) More than 1,000 putative new human signalling proteins revealed by EST data mining. Nat Genet 25:201–04
Schütz D, Zilly F, Lang T, Jahn R, Bruns D (2005) A dual function for Munc-18 in exocytosis of PC12 cells. Eur J Neurosci 21:2419–32
Shen J, Tareste DC, Paumet F, Rothman JE, Melia TJ (2007) Selective activation of cognate SNAREpins by Sec1/Munc18 proteins. Cell 128:183–95
Sorensen JB, Wiederhold K, Müller EM, Milosevic I, Nagy G, de Groot BL, Grubmüller H, Fasshauer D (2006) Sequential N- to C-terminal SNARE complex assembly drives priming and fusion of secretory vesicles. EMBO J 25:955–66
Sorensen JB, Nagy G, Varoqueaux F, Nehring RB, Brose N, Wilson MC, Neher E. (2003) Differential control of the releasable vesicle pools by SNAP-25 splice variants and SNAP-23. Cell 114:75–86
Südhof TC (2004) The synaptic vesicle cycle. Annu Rev Neurosci 27:509–47
Südhof TC (2002) Synaptotagmins: why so many? J Biol Chem 277:7629–32
Sutton RB, Fasshauer D, Jahn R, Brunger AT (1998) Crystal structure of a SNARE complex involved in synaptic exocytosis at 2.4 A resolution. Nature 395:347–53
Takamori S, Holt M, Stenius K, Lemke EA, Gronborg M, Riedel D, Urlaub H, Schenck S, Brugger B, Ringler P, Muller SA, Rammner B, Grater F, Hub JS, De Groot BL, Mieskes G, Moriyama Y, Klingauf J, Grubmüller H, Heuser J, Wieland F, Jahn R (2006) Molecular anatomy of a trafficking organelle. Cell 127:831–46
Takei K, Haucke V (2001) Clathrin-mediated endocytosis: membrane factors pull the trigger. Trends Cell Biol 11:385–91
Tang J, Maximov A, Shin OH, Dai H, Rizo J, Südhof TC (2006) A complexin/synaptotagmin 1 switch controls fast synaptic vesicle exocytosis. Cell 126:1175–87
Toonen RF, Verhage M (2003) Vesicle trafficking: pleasure and pain from SM genes. Trends Cell Biol. 13:177–86
Ungermann C, Langosch D (2005) Functions of SNAREs in intracellular membrane fusion and lipid bilayer mixing. J Cell Sci 118:3819–28
Voets T, Toonen RF, Brian EC, de Wit H, Moser T, Rettig J, Südhof TC, Neher E, Verhage M (2001) Munc18-1 promotes large dense-core vesicle docking. Neuron 31:581–91
Washington NL, Ward S (2006) FER-1 regulates Ca2+ – mediated membrane fusion during C. elegans spermatogenesis. J Cell Sci 119:2552–62
Willig KI, Rizzoli SO, Westphal V, Jahn R, Hell SW (2006) STED microscopy reveals that synaptotagmin remains clustered after synaptic vesicle exocytosis. Nature 440:935–9
Yoon TY, Okumus B, Zhang F, Shin YK, Ha T (2006) Multiple intermediates in SNARE-induced membrane fusion. Proc Natl Acad Sci USA 103:19731–6
Zilly FE, Sorensen JB, Jahn R, Lang T (2006) Munc18-bound syntaxin readily forms SNARE complexes with synaptobrevin in native plasma membranes. PLoS Biol 4:e330