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# Presynaptic terminal differentiation: transport and assembly

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The formation of chemical synapses involves reciprocal induction and independent assembly of pre- and postsynaptic structures. The major events in presynaptic terminal differentiation are the formation of the active zone and the clustering of synaptic vesicles. A number of proteins that are present in the presynaptic active zone have been identified. Recent studies of various mutants have clarified the *in vivo* functions of some of the main players. Time-lapse imaging studies have captured dynamic and transient events in the transport of synaptic components, and therefore provided insight into the early stages of synaptogenesis.

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**Abbreviations**

| | |
|---|---|
| **Bsn** | Bassoon |
| **CAST** | CAZ-associated structural protein |
| **GFP** | green fluorescent protein |
| **KIF1A** | kinesin motor protein |
| **munc** | mammalian homolog of unc |
| **RIM** | Rab3-interacting molecule |
| **Sec5** | secretion-5 |
| **SNARE** | soluble N-ethylmaleimide-sensitive component attachment protein receptor |
| **SV** | synaptic vesicle |
| **SYD** | synapse defective |
| **Syt** | synaptotagmin |
| **UNC** | uncoordinated |
| **VAMP** | synaptobrevin |

## Introduction

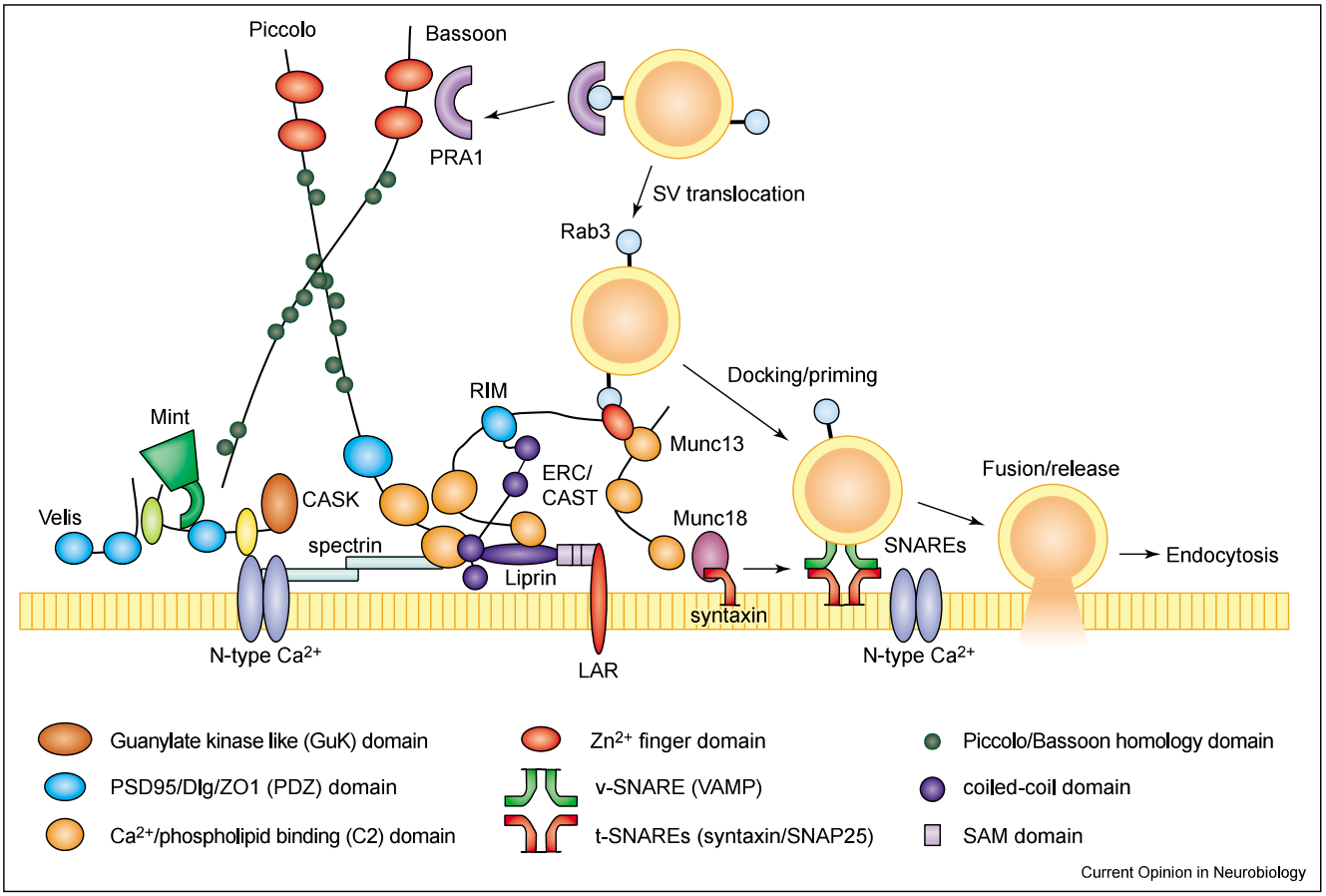
In mature chemical synapses, the ultrastructure of presynaptic terminals consists of densely populated synaptic vesicles forming orderly clusters around the electron-dense structures known as the presynaptic active zones. Active zones are tightly associated with the plasma membrane where they are juxtaposed against the postsynaptic density, and their function is to mediate neurotransmitter release. Recycling of synaptic vesicles takes place at the plasma membrane flanking the active zones.

Biochemical purifications have identified three main protein complexes, and each has a key function at the presynaptic active zone [1–4] (Figure 1). The core SNARE (soluble N-ethylmaleimide-sensitive component attachment protein receptor) complex includes syntaxin, synaptobrevin (VAMP), and synaptosomal-associated protein-25 (SNAP25), and is the basic machinery necessary for vesicle docking and fusion with the plasma membrane. A second protein complex that includes mainly Munc18/UNC-18 (mammalian uncoordinated 18/uncoordinated-18), Munc13/UNC-13, and synaptotagmin (syt) interacts with the SNARE complex and regulates the exocytosis of vesicles. The third complex has somewhat variable contents, usually including Piccolo, Bassoon (Bsn), Rab3-interacting molecules (RIMs)/UNC-10, Liprin/synapse defective-2 (SYD-2), CAZ (presynaptic cytomatrix at the active zone)-associated structural protein (CAST/ERC), Velis (vertebrate LIN-7 homolog), and Mints (Munc 18-interacting protein) [5•,6,7]. This complex is proposed to form a cytomatrix structure at the active zone that tethers vesicles and organizes the distribution of endocytosis and exocytosis machineries. UNC-18/Munc18 and UNC-13/Munc13 can also be found associated with RIM/UNC-10, Bassoon, and other matrix complexes [6]. Further interactions among the three basic protein complexes through inter- and intra-molecular binding create a highly organized network that underlies the orderly arrangement of presynaptic terminals (Figure 1). As the functions of the SNARE complex in exocytosis have been reviewed extensively, we review here the current progress on the *in vivo* function of the other active zone proteins and the transport of presynaptic components.

## The functions of cytomatrix proteins at the active zones

Bassoon and Piccolo are two large proteins that share extensive homology (Figure 1, Table 1; [3]). They are present at the active zones of both excitatory and inhibitory synapses, with overlapping expression in most brain regions. The zinc-finger domains of Bassoon and Piccolo can bind to the prenylated Rab3A-associated protein-1 (PRA1) *in vitro* [8]. The central region of Bassoon and Piccolo that contains multiple coiled-coil domains is important for anchoring both proteins to the plasma membrane of the active zone [5•]. Piccolo contains additional motifs that allow it to interact with both

**Figure 1**




Schematic of the proteins that are present in the presynaptic cytomatrix at the active zone. Three major protein complexes (see text) have been identified to structure the cytoskeleton near the active zone and to facilitate docking, priming and fusion of synaptic vesicles (Modified with permission from [57]).

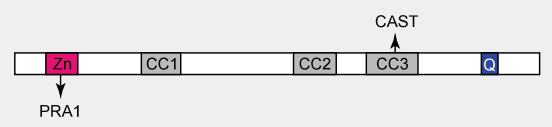
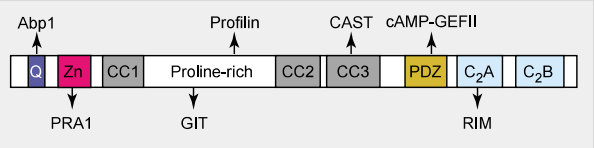
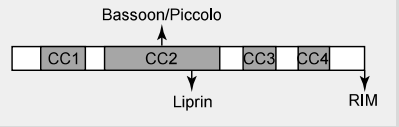
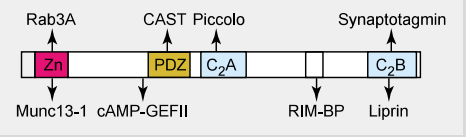
cytoskeletal and signaling molecules that regulate vesicle cycling (Table 1; [8–11]). The large size of Bassoon and Piccolo and their interactions with multiple synaptic proteins support their scaffolding role in the cytomatrix at the presynaptic active zone.

Altrock *et al.* and Dick *et al.* [5•,12•] recently examined the *in vivo* functions of Bassoon using a mouse strain carrying a deletion in the central region of the protein. Although homozygous *Bsn* mutant mice are viable and normal at birth, half of them develop epileptic seizures and die with no obvious brain architecture abnormalities. Different types of synapses display variable defects. The total number and morphology of synapses in the hippocampus and cerebellum are not altered. However, excitatory glutamatergic synapses in the hippocampus are partially inactive because of a failure of vesicle fusion. The epileptic seizures in *Bsn* mutant mice are also suggestive of a decrease in the inhibitory synaptic function, although it is unclear whether or not this is a direct effect of the loss of Bassoon function.

In the retina, Bassoon is required for both the formation and the function of photoreceptor synapses. Photoreceptor synapses have specialized active zones that contain a large-surface electron-dense ribbon band extending from the neurotransmitter release site into the cytoplasm. Bassoon is localized to the base of the ribbons close to the release site [13]. The *Bsn* mutant retina shows a significant reduction in the total number of photoreceptor synapses, and the remaining synapses display structural defects where the ribbons are unable to anchor into the presynaptic active zone region and hence float freely in the cytoplasm. Ectopic ribbon sites and postsynaptic processes are also detected in retinal neurons, which could be a secondary defect triggered by the loss of normal synaptic connectivity. The reduction in the number of synapses and the presence of aberrant active zone structures are compatible with impaired retina function. In *Bsn* mutant mice, the retina still responds to short light flashes as indicated by an electroretinographic recording (ERG). However, the amplitude and oscillation of certain ERG waves are reduced, which

**Table 1**

**The structure and *in vitro* biochemical interactions of the scaffolding active zone proteins.**

| Protein | Motifs | Partners | Structure |
|---|---|---|---|
| Bassoon (mammal) | C2H2 zinc-finger | PRA1 (Prenylated Rab3-interacting protein) [1] |   |
| | Coiled-coil (cc) | CAST [7] | |
| Piccolo (mammal) | Q domain | Abp1 (actin/dynamin-binding protein) [8] |   |
| | C2H2 zinc-finger | PRA1 [1,11] | |
| | Coiled-coil (cc) | CAST [7] | |
| | Other region | GIT (Arf GTPase activating protein) [10] | |
| | Proline rich region | Profilin (actin monomer-binding protein) [1] | |
| | PDZ domain | cAMP-GEFII (guanine nucleotide exchange factor) [9] | |
| | C2 domain | RIM [9] | |
| CAST/ERC (mammal) | Coiled-coil (cc) | Liprin [16], Bassoon/Piccolo [7] |   |
| | PDZ-binding site | RIM [6] | |
| Liprin-α (mammal) | Coiled-coil (cc) | RIM [15], CAST [16], GIT [58] KIF1A (kinesin motor protein) [59] |   |
| SYD-2 (*C elegans*) Dliprin (*Drosophila*) | SAM (sterile alpha motif) domains | LAR (receptor tyrosine phosphatase) [19] | |
| RIM (mammal) | Zinc-fingers | GTP-Rab3 [15], Munc-13 [15] |   |
| | PDZ domain | CAST [6] | |
| UNC-10 (*C. elegans*) | C2 domain | Piccolo [9], Synaptotagmin [15], Liprin [15] | |
| | Other regions | cAMP-GEFII [60], RIM-BP (Rim-binding protein) [15] | |

Listed are reported binding partners for the major presynaptic active zone proteins. The structural motifs for each protein are illustrated in the diagrams, and the arrows point to the proteins that bind the specific domain.

suggests that the synaptic transmission from the ribbon synapse is perturbed.

The alteration of synaptic morphology and function in the *Bsn* mutant demonstrates an *in vivo* role for Bassoon in facilitating vesicle exocytosis. The lack of morphological and functional defects in the majority of central nervous system (CNS) synapses could be because the deletion in the central region does not completely abolish the function of Bassoon, or because the loss of Bassoon function is compensated for by Piccolo. Indeed, the expression of Piccolo increases 1.5 times in the *Bsn* mutant [5•]. Piccolo mutants have not yet been reported. The differential effect of the loss of Bassoon function is consistent with the heterogeneity of synapses. In photoreceptor ribbon synapses, Bassoon apparently plays a prominent part in synapse formation. The heterogeneity might also exist in subpopulations of the same types of synapses, as only a fraction of glutamatergic synapses are inactive in the CNS. It is conceivable that synapses in the cerebellum and hippocampus of *Bsn* mutants develop more flexibility to compensate for the loss of individual proteins and individual synapses in the region.

Bassoon and Piccolo associate with another set of active zone proteins that are known as CAST/ERC, Liprin-α/SYD-2, RIM/UNC-10, Munc13/UNC-13, and Munc18/UNC-18 (Figure 1, Table 1; [7,14••]). RIM/UNC-10 and Munc13/UNC-13 regulate exocytosis through their interactions with the core SNARE complex. CAST/ERC, RIM/UNC-10 and Liprin-α/SYD-2 can bind to each other directly *in vitro* [6,15–17]. Co-expression of CAST and Liprin-α in cultured hippocampal neurons enhances the restriction of Liprin-α at the synaptic sites. The loss of function of the liprin proteins in *Caenorhabditis elegans* and *Drosophila* leads to altered active zone morphology, a

failure of synapse formation, and reduced synaptic transmission [18,19]. How the CAST/Liprin/RIM complex associates with Bassoon and Piccolo is not well understood. It is also not known if these active zone cytomatrix proteins depend on each other for localization *in vivo*. The physical interactions among these active zone proteins might help to anchor the exocytosis event at the active zone cytomatrix regions through CAST and Liprin. Among the identified active zone components, only Bassoon and Piccolo have no obvious homolog in invertebrates. Therefore, the CAST/Liprin/RIM complex can at least function independently of Bassoon and Piccolo.

## UNC-18/Munc18: regulators of vesicle docking and/or priming?

Synaptic vesicles go through the processes of docking (contact with the plasma membrane at active zones) and priming (become competent for vesicle fusion) before releasing neurotransmitters (Figure 1; [2]). The formation of the core SNARE complex alone is sufficient for the fusion and release of neurotransmitter *in vitro*, but not *in vivo*. RIM/UNC-10, Munc13/UNC-13, and Munc18/UNC-18 are proposed to modulate the formation of the SNARE complex *in vivo* [20]. Whereas UNC-13/Munc13 and UNC-10/RIM modulate the priming of synaptic vesicles by promoting SNARE complex formation in *C. elegans*, *Drosophila*, and mouse [15,21–25], the role of Munc18/UNC-18 is more controversial [26]. Weimer *et al.* [27••] recently demonstrated that UNC-18 plays a part in mediating the docking of synaptic vesicles in *C. elegans*, where *unc-18* is a single gene. In *unc-18* mutants, a reduced fraction of synaptic vesicles contact the plasma membrane near the active zone. The reduction in synaptic activity as assessed by physiological recording is more severe than the decrease in the fraction of docked vesicles, which suggests that only some of the docked vesicles are fusion-competent. Syntaxin undergoes conformational change from the 'closed' to 'open' form to allow the formation of the SNARE complex [26]. UNC-18/Munc18 binds specifically to the closed form of syntaxin [26]. There has been speculation that UNC-18/Munc-18 promotes exocytosis through either the trafficking or the activation of syntaxin. Surprisingly, the role of UNC-18 in *C. elegans* does not involve syntaxin directly [27••]. Although the syntaxin level is reduced by 50% in *unc-18* mutants (a phenotype consistent with that of the *Munc18-1* knockout mouse [28]), syntaxin localizes normally in *unc-18* mutants. Increasing the level of either a wild type or a constitutively open form of syntaxin in *unc-18* mutants cannot ameliorate the defects in synaptic activity. The increase in the syntaxin levels in *unc-18* mutants is also unlikely to restore the docking of vesicles at the active zone. Normal vesicle docking has been observed in studies with *Drosophila* syntaxin null mutants [23]. The role of UNC-18 in *C. elegans* synapses resembles the function of Munc18-1 in chromaffin cells, where it is required for docking, but not the release of, dense core vesicles [29]. UNC-18/Munc-18 might mediate docking through its physical interaction with other synaptic proteins, such as Doc and Mint [20]. Whether UNC-18/Munc-18 homologs in other organisms also function independently of or indirectly through syntaxin remains to be examined. Furthermore, the analysis of *C. elegans unc-18* mutants does not rule out the roles of proteins in the family of UNC-18 in priming. For example, in CA1 hippocampal synapses Munc18-1 is required for vesicle fusion, but not docking [28].

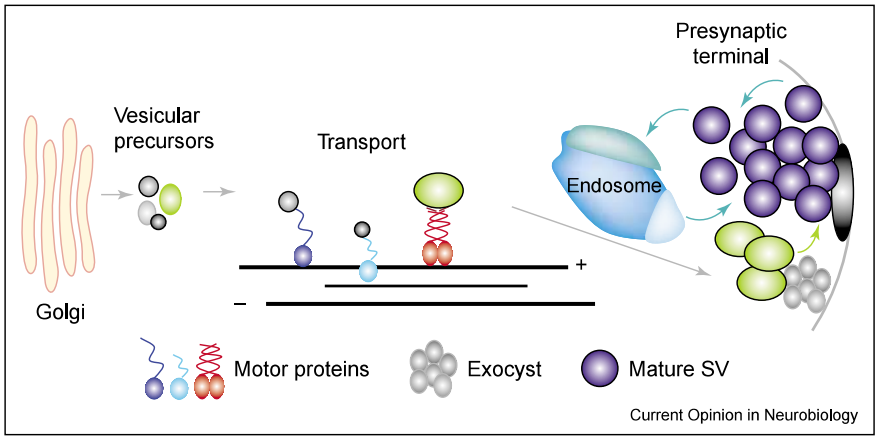
## Quantal transport and assembly of the presynaptic active zone

A fundamental issue relating to synapse formation is how the synaptic components get to the synaptic sites. In an early study in this area [30], experimenters using VAMP-green fluorescent protein (GFP) on cultured hippocampal neurons observed a type of vesicular packet that appeared to contain many cytoplasmic and membrane-associated protein precursors for synaptic vesicles and active zones [30]. Such packets are actively transported in the axons, and are stabilized specifically at sites of synaptic contact. This study has led to the hypothesis that presynaptic components are united early in their biogenesis and sorting pathways, and that membrane remodeling upon synaptic contact subsequently produces the uniform vesicles typical of mature synapses. Recent studies using slightly different methods have revealed additional transporting packets. In particular, the active zone components are transported separately from synaptic vesicles, and are detected earlier than synaptic vesicles at nascent synapses.

Bassoon and Piccolo are among the earliest protein components detected at nascent synapses [31]. Shapira *et al.* [14••] recently showed that many components of the protein complexes forming the matrix (Bassoon, Piccolo, CAST) and functional units (RIM/UNC-10, Munc13/UNC-13 and Munc18/UNC-18) at the active zones could be detected on the same dense core vesicles that are transported along the microtubules to the termini. By fluorescent staining with the antibodies against Piccolo, Bassoon, and RIM, it can be seen that the content, or the level of individual proteins, in a packet appears to vary. Quantification of the protein levels suggests that 2–3 of such active zone packets can deposit sufficient materials to build an active zone at the nascent synaptic termini.

The active zone packets, visualized using Bsn-GFP, appear to be highly motile in young neuronal cells, which coincides with the developmental stage of synaptogenesis [14••]. Initially, the Bsn-GFP packets are present in variable sizes, and are not restricted to nascent active zones. During development, the bigger packets with decreased motility are likely to develop into functional synaptic sites. This suggests that prior to synapse formation, the building materials for presynaptic active zones

**Figure 2**




Transport of presynaptic components. Motor proteins transport different materials or cargoes within the neuron. Some cargoes contain components that are specifically destined for the active zone (black circles and ovals), and some contain synaptic vesicle proteins (gray and green circles). Vesicles from both anterograde transport and local recycling (green and dark blue circles, and endosomes) establish and maintain SV clustering at the synapses. Exocyst (orange and red circles) is required for fusion with plasma membrane of at least one type of anterograde transported vesicle containing SV proteins; and such fusion is necessary for the vesicles to be retained at synapses.

have been pre-assembled and that they can be deposited upon contact with postsynaptic partners to assemble a functional synapse in a timely fashion.

## Synaptic vesicle dynamics: early stage

It is fair to say that the life of a synapse lies in its capacity to accommodate synaptic vesicles and to arrange them in an orderly manner. How are synaptic vesicles generated? Decades of biochemical and cell biological studies have revealed two distinct sources that supply synaptic vesicles (Figure 2; [32]). One is the Golgi apparatus, where vesicles are synthesized *de novo* and transported by anterograde motor proteins to the synaptic terminals. Such vesicles are usually called synaptic vesicle precursors, because they contain proteins that are often not present in the mature synaptic vesicles. The second source is the local plasma membrane, where synapses are derived through endocytosis of the membrane. Studies in mouse, *Drosophila*, and *C. elegans* have provided *in vivo* confirmation of the two pathways. For example, the loss-of-function mutations in kinesin motor protein (KIF1A) and UNC-104 kinesin motor proteins cause retention of synaptic vesicles and precursors in neuronal cell bodies [33,34]. Mutants that are defective in the genes that function in endocytosis, such as dynamin, synaptojanin, and recently endophilin [35•,36•], exhibit drastic reduction and abnormal arrangement of synaptic vesicles at synapses [37–39]. It remains largely unknown, however, how the synaptic vesicle precursors mature and where the very first source of vesicles that enables the endocytosis pathway comes from. Recent progress in combining time-lapse microscopic imaging in normal and mutant neurons has opened up a valuable venue to begin to address these questions.

How early do functional vesicles appear? An excellent imaging study using VAMP-GFP and FM4-64 (N-[3-triethylammoniumpropyl]-4-[6-(4-[diethylamino] phenyl) hexatrienyl] pyridinium dibromide) [40•] has observed active vesicular traffic at the growth cone, a stage preceding any synaptic contacts. In the cultured rat visual cortical neurons, VAMP-GFP containing vesicles are present in motile growth cone filopodia. Such vesicles contain a number of synaptic proteins that are found in mature synaptic vesicles. They are able to fuse with the plasma membrane and recycle, raising the possibility that the rare fusion events of such vesicles to plasma membrane might be sufficient to trigger the endocytosis that is necessary for replenishing the synaptic vesicle pool at mature synapses. Furthermore, these vesicles move bi-directionally at a speed that is slower than that of KIF1A, which suggests that the synaptic vesicle (SV) transport might use multiple motors or different motors at different stages (Figure 1).

Further insight into synaptic vesicle biogenesis has come from an elegant genetic analysis on the exocyst complex in the *Drosophila* nervous system. The exocyst is a multi-subunit protein complex that is necessary for direct vesicle fusion to specific membrane sites [41,42]. Components of the exocyst complex are present in growth cones [43–45]. Murthy *et al.* [46••] generated null mutant flies that lack the exocyst complex because of a deletion in the Sec5 (secretion 5) gene. In these flies, neurons arrest at the neurite outgrowth stage, and neuromuscular junctions fail to grow once maternal Sec5 has run down, which suggests an essential role for exocyst in the addition of new material to the membrane. Using a genetic method that allows them to determine the efficacy of

addition of newly synthesized proteins in sec5 null neurons, the authors show that vesicles containing newly synthesized Syt-GFP are present along the length of the axon, but are rarely seen in the synaptic boutons. Yet, as revealed by immunocytochemical and electrophysiological assays, synaptic vesicles are concentrated at the synapses and vesicle exocytosis is normal in Sec5 mutant synaptic boutons. Thus, this study suggests two distinct vesicle fusion mechanisms (Figure 2). Abolishing exocyst function causes a failure of the newly synthesized Syt-GFP labeled vesicles to be retained in the synapses because they cannot fuse with the membrane. It remains to be analyzed how the morphology and the chemical content of the Sec5-dependent Syt-GFP vesicles differ from those of the Sec5-independent synaptic vesicles at the synaptic terminal.

## A new role for β-catenin: localizing synaptic vesicles

At mature synapses, synaptic vesicles are divided into three functional pools by their mobility and their electrophysiological capacity: the readily releasable pool (RRP), the resting pool, and the reserve pool [47]. Morphologically, the RRP corresponds to the vesicles docked at the membrane, and the resting and reserve pools to the undocked vesicles. Actin is the major cytoskeletal element involved in tethering the undocked vesicles [48], and the actin binding phosphoprotein synapsin has an essential role in maintaining the reserve pool [49–51]. Using conditional mouse mutants, a recent study has revealed a surprising role of β-catenin in synaptic vesicle localization [52••]. β-catenin is involved in two classical signaling pathways: the cadherin mediated cell adhesion at membrane junctions in which it interacts with α-catenin and bridges cadherins to actin, and the Wnt signaling pathway in which it acts along with T cell factor/lymphoid enhancer binding factor (TCF/LEF) to control gene transcription. The involvement of β-catenin at the postsynaptic development and, in particular, in dendritic spine dynamics has been intensely examined in hippocampal neurons, where it functions mainly through its regulation of cadherin-mediated cell adhesion [53]. By ablating the function of β-catenin selectively in hippocampal pyramidal neurons, Bamji *et al.* [52••] found a 40% reduction in the number of undocked pools of synaptic vesicles at the synapse, accompanied with an increase in the total number of synapses. Time-lapse imaging in the cultured neurons lacking β-catenin reveals that synaptic vesicle proteins are diffusely localized, but that the localization and the number of other presynaptic active zone components including Bassoon and N-cadherin are not affected. These observations suggest that the increase in synapse number is likely to be a compensatory effect to counteract the reduction in synaptic vesicle number. Furthermore, the fluorescent puncta from synaptophysin-GFP exhibit rapid and random movements that are different from the previously reported transport packets. These results support the conclusion that β-catenin has a specific role in localizing synaptic vesicles during presynaptic assembly. Interestingly, this function of β-catenin does not depend on the domains of β-catenin that are known to interact with α-catenin or TCF/LEF; rather it requires its PDZ (PSD-95/Discs-large/ZO1) binding domain [52••]. The known PDZ domain-binding partners of β-catenin include Veli and SCAM/MAGI-1 (synaptic scaffolding molecule/membrane-associated guanylate kinase with inverted domain organization) [54,55]. Disrupting the Veli PDZ domain has only slight effects on SV localization, which suggests that β-catenin has other PDZ domain binding partners [52••].

## Conclusions

With the increasing effort put into generating knockout mutant mice, the *in vivo* functions of biochemically identified molecules at synapses are being gradually defined and clarified. These studies, together with the information from model invertebrate organisms, have helped us to learn the themes and variations that underlie synapse formation. Combining live imaging analysis with genetic mutant studies provides an invaluable and more accurate assessment of the dynamic cellular process in both wild type and mutant cells. Cells constantly produce synaptic components, which are constantly transported along the axons and are ready to assemble synapses at targeted sites. A plethora of cell surface molecules in target recognition have been identified in recent years [56]. An exciting frontier is how the target recognition event leads to local trapping of the mobile transport materials and how the synaptic precursors mature during the synaptic assembly.

## Acknowledgements

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## References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Dresbach T, Qualmann B, Kessels MM, Garner CC, Gundelfinger ED: **The presynaptic cytomatrix of brain synapses.** *Cell Mol Life Sci* 2001, **58**:94-116.

2. Jahn R, Lang T, Sudhof TC: **Membrane fusion.** *Cell* 2003, **112**:519-533.

3. Garner CC, Kindler S, Gundelfinger ED: **Molecular determinants of presynaptic active zones.** *Curr Opin Neurobiol* 2000, **10**:321-327.

4. Brose N, Rosenmund C, Rettig J: **Regulation of transmitter release by Unc-13 and its homologues.** *Curr Opin Neurobiol* 2000, **10**:303-311.

5. • Altrock WD, tom Dieck S, Sokolov M, Meyer AC, Sigler A, Brakebusch C, Fassler R, Richter K, Boeckers TM, Potschka H *et al.*: **Functional inactivation of a fraction of excitatory**

**synapses in mice deficient for the active zone protein bassoon.** *Neuron* 2003, **37**:787-800.
The authors examined the *in vivo* functions of Bassoon by generating a knockout mouse strain that lacked the central region of the protein. They showed that this deletion leads to abnormal localization of the protein, and an increase in expression of its homolog protein, Piccolo. They found that Bassoon is not essential for synapse formation in the majority of CNS synapses. Only a small fraction of excitatory synapses are defective in neurotransmitter release.

6. Ohtsuka T, Takao-Rikitsu E, Inoue E, Inoue M, Takeuchi M, Matsubara K, Deguchi-Tawarada M, Satoh K, Morimoto K, Nakanishi H *et al.*: **Cast: a novel protein of the cytomatrix at the active zone of synapses that forms a ternary complex with RIM1 and munc13-1.** *J Cell Biol* 2002, **158**:577-590.

7. Takao-Rikitsu E, Mochida S, Inoue E, Deguchi-Tawarada M, Inoue M, Ohtsuka T, Takai Y: **Physical and functional interaction of the active zone proteins, CAST, RIM1, and Bassoon, in neurotransmitter release.** *J Cell Biol* 2004, **164**:301-311.

8. Fenster SD, Kessels MM, Qualmann B, Chung WJ, Nash J, Gundelfinger ED, Garner CC: **Interactions between Piccolo and the actin/dynamin-binding protein Abp1 link vesicle endocytosis to presynaptic active zones.** *J Biol Chem* 2003, **278**:20268-20277.

9. Fujimoto K, Shibasaki T, Yokoi N, Kashima Y, Matsumoto M, Sasaki T, Tajima N, Iwanaga T, Seino S: **Piccolo, a $Ca^{2+}$ sensor in pancreatic beta-cells. Involvement of cAMP-GEFII.RIM2.Piccolo complex in cAMP-dependent exocytosis.** *J Biol Chem* 2002, **277**:50497-50502.

10. Kim S, Ko J, Shin H, Lee JR, Lim C, Han JH, Altrock WD, Garner CC, Gundelfinger ED, Premont RT *et al.*: **The GIT family of proteins forms multimers and associates with the presynaptic cytomatrix protein Piccolo.** *J Biol Chem* 2003, **278**:6291-6300.

11. Wang X, Kibschull M, Laue MM, Lichte B, Petrasch-Parwez E, Kilimann MW: **Aczonin, a 550-kD putative scaffolding protein of presynaptic active zones, shares homology regions with RIM and Bassoon and binds profilin.** *J Cell Biol* 1999, **147**:151-162.

12. • Dick O, tom Dieck S, Altrock WD, Ammermuller J, Weiler R, Garner CC, Gundelfinger ED, Brandstatter JH: **The presynaptic active zone protein bassoon is essential for photoreceptor ribbon synapse formation in the retina.** *Neuron* 2003, **37**:775-786.
The authors used the same Bassoon mutant mice as Altrock *et al*. [5•] to examine its role in the retina. In contrast to the mild defects in the hippocampus, they found that Bassoon is required for both the formation and the function of photoreceptor ribbon synapses.

13. Dick O, Hack I, Altrock WD, Garner CC, Gundelfinger ED, Brandstatter JH: **Localization of the presynaptic cytomatrix protein Piccolo at ribbon and conventional synapses in the rat retina: comparison with Bassoon.** *J Comp Neurol* 2001, **439**:224-234.

14. •• Shapira M, Zhai RG, Dresbach T, Bresler T, Torres VI, Gundelfinger ED, Ziv NE, Garner CC: **Unitary assembly of presynaptic active zones from Piccolo-Bassoon transport vesicles.** *Neuron* 2003, **38**:237-252.
In this study, the authors confirmed that the active zone transport vesicles contain most of the active zone components by western blotting or immunogold antibody staining. By quantifying the protein level of Bassoon, Piccolo and RIM at the active zone, they concluded that most active zones result from the fusion of 2–3 active zone transport packets. Using the Bassoon-GFP marker, they found that in the early stage of synapse development the active zone transport packets are not restricted to synaptic sites and are highly motile in early stages of synaptogenesis, but are stabilized at the synaptic sites when synapses form.

15. Schoch S, Castillo PE, Jo T, Mukherjee K, Geppert M, Wang Y, Schmitz F, Malenka RC, Sudhof TC: **RIM1alpha forms a protein scaffold for regulating neurotransmitter release at the active zone.** *Nature* 2002, **415**:321-326.

16. Ko J, Na M, Kim S, Lee JR, Kim E: **Interaction of the ERC family of RIM-binding proteins with the liprin-alpha family of multidomain proteins.** *J Biol Chem* 2003, **278**:42377-42385.

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

18. Zhen M, Jin Y: **The liprin protein SYD-2 regulates the differentiation of presynaptic termini in *C. elegans*.** *Nature* 1999, **401**:371-375.

19. Kaufmann N, DeProto J, Ranjan R, Wan H, Van Vactor D: ***Drosophila* liprin-alpha and the receptor phosphatase Dlar control synapse morphogenesis.** *Neuron* 2002, **34**:27-38.

20. Toonen RF, Verhage M: **Vesicle trafficking: pleasure and pain from SM genes.** *Trends Cell Biol* 2003, **13**:177-186.

21. Koushika SP, Richmond JE, Hadwiger G, Weimer RM, Jorgensen EM, Nonet ML: **A post-docking role for active zone protein RIM.** *Nat Neurosci* 2001, **4**:997-1005.

22. Castillo PE, Schoch S, Schmitz F, Sudhof TC, Malenka RC: **RIM1alpha is required for presynaptic long-term potentiation.** *Nature* 2002, **415**:327-330.

23. Broadie K, Prokop A, Bellen HJ, O'Kane CJ, Schulze KL, Sweeney ST: **Syntaxin and synaptobrevin function downstream of vesicle docking in *Drosophila*.** *Neuron* 1995, **15**:663-673.

24. Augustin I, Rosenmund C, Sudhof TC, Brose N: **Munc13-1 is essential for fusion competence of glutamatergic synaptic vesicles.** *Nature* 1999, **400**:457-461.

25. Aravamudan B, Fergestad T, Davis WS, Rodesch CK, Broadie K: ***Drosophila* UNC-13 is essential for synaptic transmission.** *Nat Neurosci* 1999, **2**:965-971.

26. Rizo J, Sudhof TC: **Snares and Munc18 in synaptic vesicle fusion.** *Nat Rev Neurosci* 2002, **3**:641-653.

27. •• Weimer RM, Richmond JE, Davis WS, Hadwiger G, Nonet ML, Jorgensen EM: **Defects in synaptic vesicle docking in unc-18 mutants.** *Nat Neurosci* 2003, **6**:1023-1030.
In this study, the authors examined the synaptic defects of *C. elegans unc-18* mutants by ultrastructural and electrophysiological analysis. They demonstrated that there is a vesicle docking defect in *unc-18* mutants. They tested if UNC-18 functions through syntaxin by overexpressing wild type and 'open' form syntaxin in *unc-18* mutants. Neither overexpression nor open form syntaxin can rescue the synaptic defects, which suggests that UNC-18 might function through other proteins, or indirectly through syntaxin.

28. Verhage M, Maia AS, Plomp JJ, Brussaard AB, Heeroma JH, Vermeer H, Toonen RF, Hammer RE, van den Berg TK, Missler M *et al.*: **Synaptic assembly of the brain in the absence of neurotransmitter secretion.** *Science* 2000, **287**:864-869.

29. Voets T, Toonen RF, Brian EC, de Wit H, Moser T, Rettig J, Sudhof TC, Neher E, Verhage M: **Munc18-1 promotes large dense-core vesicle docking.** *Neuron* 2001, **31**:581-591.

30. Ahmari SE, Buchanan J, Smith SJ: **Assembly of presynaptic active zones from cytoplasmic transport packets.** *Nat Neurosci* 2000, **3**:445-451.

31. Zhai RG, Vardinon-Friedman H, Cases-Langhoff C, Becker B, Gundelfinger ED, Ziv NE, Garner CC: **Assembling the presynaptic active zone: a characterization of an active one precursor vesicle.** *Neuron* 2001, **29**:131-143.

32. Hannah MJ, Schmidt AA, Huttner WB: **Synaptic vesicle biogenesis.** *Annu Rev Cell Dev Biol* 1999, **15**:733-798.

33. Yonekawa Y, Harada A, Okada Y, Funakoshi T, Kanai Y, Takei Y, Terada S, Noda T, Hirokawa N: **Defect in synaptic vesicle precursor transport and neuronal cell death in KIF1A motor protein-deficient mice.** *J Cell Biol* 1998, **141**:431-441.

34. Hall DH, Hedgecock EM: **Kinesin-related gene unc-104 is required for axonal transport of synaptic vesicles in *C. elegans*.** *Cell* 1991, **65**:837-847.

35. • Schuske KR, Richmond JE, Matthies DS, Davis WS, Runz S, Rube DA, van der Bliek AM, Jorgensen EM: **Endophilin is required for synaptic vesicle endocytosis by localizing synaptojanin.** *Neuron* 2003, **40**:749-762.
Please see annotation to Fabian-Fine *et al*. [36•].

36. • Fabian-Fine R, Verstreken P, Hiesinger PR, Horne JA, Kostyleva R, Zhou Y, Bellen HJ, Meinertzhagen IA:

**Endophilin promotes a late step in endocytosis at glial invaginations in *Drosophila* photoreceptor terminals.** *J Neurosci* 2003, **23**:10732-10744.

This study and that of Schuske *et al*. [35•] demonstrate an *in vivo* requirement of endophilin in SV endocytosis, and provide further clarification of the mechanisms of endophilin function.

37. De Camilli P, Takei K: **Molecular mechanisms in synaptic vesicle endocytosis and recycling.** *Neuron* 1996, **16**:481-486.

38. Hinshaw JE: **Dynamin and its role in membrane fission.** *Annu Rev Cell Dev Biol* 2000, **16**:483-519.

39. Harris TW, Schuske K, Jorgensen EM: **Studies of synaptic vesicle endocytosis in the nematode *C. elegans*.** *Traffic* 2001, **2**:597-605.

40. • Sabo SL, McAllister AK: **Mobility and cycling of synaptic protein-containing vesicles in axonal growth cone filopodia.** *Nat Neurosci* 2003, **6**:1264-1269.

In this study, the authors used VAMP-GFP and FM4-64 to detect SV-like vesicles at the growth cones. They found that such vesicles were present in most of the filopodia and that they were able to fuse with the plasma membrane and recycle, providing a glimpse of SV dynamics at early stages.

41. Lipschutz JH, Mostov KE: **Exocytosis: the many masters of the exocyst.** *Curr Biol* 2002, **12**:R212-R214.

42. Hsu SC, Hazuka CD, Foletti DL, Scheller RH: **Targeting vesicles to specific sites on the plasma membrane: the role of the sec6/8 complex.** *Trends Cell Biol* 1999, **9**:150-153.

43. Brymora A, Valova VA, Larsen MR, Roufogalis BD, Robinson PJ: **The brain exocyst complex interacts with RalA in a GTP-dependent manner: identification of a novel mammalian Sec3 gene and a second Sec15 gene.** *J Biol Chem* 2001, **276**:29792-29797.

44. Vega IE, Hsu SC: **The exocyst complex associates with microtubules to mediate vesicle targeting and neurite outgrowth.** *J Neurosci* 2001, **21**:3839-3848.

45. Hsu SC, Ting AE, Hazuka CD, Davanger S, Kenny JW, Kee Y, Scheller RH: **The mammalian brain rsec6/8 complex.** *Neuron* 1996, **17**:1209-1219.

46. •• Murthy M, Garza D, Scheller RH, Schwarz TL: **Mutations in the exocyst component Sec5 disrupt neuronal membrane traffic, but neurotransmitter release persists.** *Neuron* 2003, **37**:433-447.

In this study, the authors generated mutant flies lacking functional exocysts. Neurons were arrested at outgrowth, and neuromuscular junctions failed to expand. According to electrophysiological and immunocytochemical analysis, synaptic vesicle release at the existing boutons was normal. However, using an elegant genetic trick, they showed that newly synthesized SV-like vesicles were transported normally, but were unable to be retained at synapses, presumably because of a failure to fuse with the plasma membrane. This study reveals a source of SV whose retention/maturation depends on exocyst-mediated membrane fusion.

47. Sudhof TC: **The synaptic vesicle cycle revisited.** *Neuron* 2000, **28**:317-320.

48. Doussau F, Augustine GJ: **The actin cytoskeleton and neurotransmitter release: an overview.** *Biochimie* 2000, **82**:353-363.

49. Rosahl TW, Spillane D, Missler M, Herz J, Selig DK, Wolff JR, Hammer RE, Malenka RC, Sudhof TC: **Essential functions of synapsins I and II in synaptic vesicle regulation.** *Nature* 1995, **375**:488-493.

50. Li L, Chin LS, Shupliakov O, Brodin L, Sihra TS, Hvalby O, Jensen V, Zheng D, McNamara JO, Greengard P *et al.*: **Impairment of synaptic vesicle clustering and of synaptic transmission, and increased seizure propensity, in synapsin I-deficient mice.** *Proc Natl Acad Sci USA* 1995, **92**:9235-9239.

51. Takei Y, Harada A, Takeda S, Kobayashi K, Terada S, Noda T, Takahashi T, Hirokawa N: **Synapsin I deficiency results in the structural change in the presynaptic terminals in the murine nervous system.** *J Cell Biol* 1995, **131**:1789-1800.

52. •• Bamji SX, Shimazu K, Kimes N, Huelsken J, Birchmeier W, Lu B, Reichardt LF: **Role of beta-catenin in synaptic vesicle localization and presynaptic assembly.** *Neuron* 2003, **40**:719-731.

In this study, the authors generated a conditional mutant mouse that lacks β-catenin function specifically in hippocampal pyramidal neurons. They found an overall reduction of SVs from the reserve pool. Using live imaging analysis, they demonstrated a previously unknown function of β-catenin in localizing SVs through its PDZ-binding domain. This further implicates other unknown PDZ-containing proteins in SV clustering.

53. Togashi H, Abe K, Mizoguchi A, Takaoka K, Chisaka O, Takeichi M: **Cadherin regulates dendritic spine morphogenesis.** *Neuron* 2002, **35**:77-89.

54. Perego C, Vanoni C, Massari S, Longhi R, Pietrini G: **Mammalian LIN-7 PDZ proteins associate with beta-catenin at the cell-cell junctions of epithelia and neurons.** *Embo J* 2000, **19**:3978-3989.

55. Dobrosotskaya IY, James GL: **MAGI-1 interacts with beta-catenin and is associated with cell-cell adhesion structures.** *Biochem Biophys Res Commun* 2000, **270**:903-909.

56. Yamagata M, Sanes JR, Weiner JA: **Synaptic adhesion molecules.** *Curr Opin Cell Biol* 2003, **15**:621-632.

57. Garner C: **Microposter.** *Trends Neurosci* 2002, **25**:251.

58. Shin H, Wyszynski M, Huh KH, Valtschanoff JG, Lee JR, Ko J, Streuli M, Weinberg RJ, Sheng M, Kim E: **Association of the kinesin motor KIF1A with the multimodular protein liprin-alpha.** *J Biol Chem* 2003, **278**:11393-11401.

59. Ozaki N, Shibasaki T, Kashima Y, Miki T, Takahashi K, Ueno H, Sunaga Y, Yano H, Matsuura Y, Iwanaga T *et al.*: **cAMP-GEFII is a direct target of cAMP in regulated exocytosis.** *Nat Cell Biol* 2000, **2**:805-811.

60. Ko J, Kim S, Valtschanoff JG, Shin H, Lee JR, Sheng M, Premont RT, Weinberg RJ, Kim E: **Interaction between liprin-alpha and GIT1 is required for AMPA receptor targeting.** *J Neurosci* 2003, **23**:1667-1677.