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Cell Biology of Ca^{2+}-Triggered Exocytosis

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Abstract

Ca^{2+} triggers many forms of exocytosis in different types of eukaryotic cells, for example synaptic vesicle exocytosis in neurons, granule exocytosis in mast cells, and hormone exocytosis in endocrine cells. Work over the last two decades has shown that synaptotagmins function as the primary Ca^{2+}-sensors for most of these forms of exocytosis, and that synaptotagmins act via Ca^{2+}-dependent interactions with both the fusing phospholipid membranes and the membrane fusion machinery. However, some forms of Ca^{2+}-induced exocytosis may utilize other, as yet unidentified Ca^{2+}-sensors, for example, slow synaptic exocytosis mediating asynchronous neurotransmitter release. In the following overview, we will discuss the synaptotagmin-based mechanism of Ca^{2+}-triggered exocytosis in neurons and neuroendocrine cells, and its potential extension to other types of Ca^{2+}-stimulated exocytosis for which no synaptotagmin Ca^{2+}-sensor has been identified.

Introduction

Ca^{2+}-induced exocytosis initiates many forms of intercellular communication, as exemplified by synaptic transmission, which begins with Ca^{2+}-triggered synaptic vesicle exocytosis that mediates neurotransmitter release (Fig. 1) [1]. Similarly, neuroendocrine cells secrete hormones by Ca^{2+}-induced exocytosis [2], mast cells release their granule contents upon stimulation by Ca^{2+}-controlled exocytosis [3], and even in T-lymphocytes, Ca^{2+}-triggered exocytosis is functionally essential [4]. The question of how Ca^{2+} triggers exocytosis was first raised by Bernhard Katz's seminal discovery that Ca^{2+} induces synaptic vesicle exocytosis, and thereby initiates synaptic transmission [5]. However, not much progress was made in this question until the discovery of synaptotagmin-1 (Syt1) as a candidate Ca^{2+}-sensor for synaptic exocytosis [6]. Work from many laboratories has provided overwhelming evidence that Syt1 and its homologs function as the primary Ca^{2+}-sensors in most forms of exocytosis, and has elucidated the principal mechanism by which synaptotagmin operates [1]. However, as described below, this work has also raised important new questions about the role of Ca^{2+} in regulating membrane traffic. The present review focuses on the cell biology of Ca^{2+}-triggered exocytosis in neurons and endocrine cells, and tries to relate the emerging synaptotagmin Ca^{2+}-sensor paradigm to these new unanswered questions.

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Synaptic exocytosis

In presynaptic nerve terminals, neurotransmitters are packaged into small synaptic vesicles, and released by Ca^{2+}-triggered exocytosis of synaptic vesicles at the presynaptic active zone (Fig. 1). Three different modes of neurotransmitter release exist (Fig. 2 A):

1. Evoked synchronous release initiates within a millisecond after an action potential induces Ca^{2+}-influx into a presynaptic terminal [8]. Fast synchronous release measured as the postsynaptic response can be fitted by a double exponential function, and thus can be arbitrarily subdivided into a fast and a slow phase [9].
2. Evoked asynchronous release sets in with a delay after an action potential, and is normally negligible [10], either because it is outcompeted by the synchronous release mechanism [11], or because the synchronous release mechanism (i.e., synaptotagmin and complexin, see below) suppresses asynchronous release [12]. However, asynchronous release becomes a dominant form of release in some synapses during high-frequency trains of action potentials, particularly in inhibitory synapses [13-15].
3. Spontaneous 'mini' release, finally, represents the exocytosis of single vesicles [16] that is independent of action potentials, but nevertheless largely Ca^{2+}-dependent [17]. Spontaneous release is induced by resting Ca^{2+}-concentrations, or may be stimulated by stochastic Ca^{2+}-channel openings and/or Ca^{2+}-sparks via Ca^{2+}-influx from internal Ca^{2+}-stores [18].

All three forms of synaptic exocytosis likely perform important physiological functions. Evoked synchronous release represents the primary mode of intercellular communication between neurons, rendering its importance obvious. Bursts of activity are often seen in neurons, suggesting that these trigger asynchronous release which may shape neural function at the circuit level [19]. Spontaneous release may improve the signal to noise ratio at the systems level [20], and/or to contribute to the development and maintenance synapses [21].

In central synapses, synaptic vesicles exist in functionally different pools [22], although this may not be true for neuromuscular synapses [23]. All types of synaptic exocytosis probably utilize the same release-ready pool of vesicles (the so-called readily-releasable pool [RRP]), which accounts only for a small percentage of the total vesicle pool [22]. An operational definition of the RRP is the amount of release triggered by application of hypertonic sucrose, which induces Ca^{2+}-independent exocytosis of all synaptic vesicles in the RRP [24,25]. The morphological correlate of the RRP is unclear; RRP vesicles are distinct from the synaptic vesicles that are morphologically docked at the active zone because the number of docked vesicles does not correlate with the RRP size [26], and a point mutation that activates the SNARE-protein syntaxin-1B simultaneously increases the number of docked vesicles but decreases the RRP size [27].

Endocrine exocytosis

Hormonal exocytosis of endocrine cells operates on large dense-core vesicles (LDCVs) that are probably similar to neuropeptide LDCVs in neurons. LDCV exocytosis has been studied mostly in adrenal chromaffin cells and in pancreatic β-cells, where Ca^{2+}-triggered exocytosis operates in three phases, referred to as the fast, slow and sustained phase [28,29]. The three phases of LDCV exocytosis have been attributed to different vesicle pools, but it is uncertain whether these pools represent physically distinct types of vesicles, or simply different functional states. In addition, spontaneous exocytosis of LDCVs has also been described in chromaffin cells, and also appears to be regulated by Ca^{2+} [30].

Even the fast phase of LDCV exocytosis is much slower than both synchronous and asynchronous synaptic exocytosis (Fig. 1). Nevertheless, LDCV and synaptic exocytosis are very similar, as they appear to use the same Ca^{2+}-triggering mechanisms (see discussion below), and differ primarily in how synaptic vesicles and LDCVs are docked and prepared for fusion (i.e., primed).

Synaptotagmins as Ca^{2+}-sensors for exocytosis

Synaptotagmins are synaptic and secretory vesicle proteins (although some isoforms may be on the plasma membrane) that contain a single N-terminal transmembrane region, and two C-terminal Ca^{2+}-binding C2-domains [6]. 16 mammalian synaptotagmin isoforms were identified, 8 of which bind Ca^{2+} with distinct apparent Ca^{2+}-affinities (Syt1-3, Syt5-7, Syt9, and Syt10 [31-33], Table 1). Synaptotagmins are highly conserved evolutionarily; even all invertebrates express multiple isoforms. However, synaptotagmins are absent from plants and unicellular eukaryotes, suggesting that they emerged coincidentally with animals during evolution.

Synaptic and endocrine exocytosis are mediated by the same fusion machinery composed of SNARE- and SM-proteins as other membrane fusion reactions – in fact, this fusion machinery was discovered at the synapse [34]. SNARE-proteins catalyze fusion by forming a complex that bridges the two fusing membranes, forcing these membranes together, whereas SM-proteins promote fusion by an unknown but essential mechanism. Ca^{2+}-binding to synaptotagmin triggers exocytosis by operating on this fusion machinery with the help of an ancillary protein called complexin. Thus, only six proteins – three SNARE-proteins, one SM-protein (Munc18-1), one synaptotagmin, and complexin – form the core of the Ca^{2+}-triggered exocytosis machinery (Fig. 2B), constituting a molecular clockwork that exhibits an amazing simplicity which we refer to as the synaptotagmin paradigm [34].

Both C2-domains of synaptotagmin bind Ca^{2+}; C2-domains were first shown to represent Ca^{2+}-binding domains in Syt1 [35]. C2-domains are janus-faced domains with 2-3 Ca^{2+}-binding sites on top, and a Ca^{2+}-independent surface on the bottom. Ca^{2+}-binding induces simultaneous synaptotagmin-binding to both the fusing phospholipid membranes, and the assembling SNARE complex (Fig. 3). Complexin activates SNARE-complexes prior to synaptotagmin action, and clamps fusion by preventing complete SNARE-complex assembly until Ca^{2+} binds to synaptotagmin [36,37]. Complexin performs these actions by binding to SNARE complexes, and synaptotagmin dislodges the complexin clamp (Fig. 3 step 5, [38]). Blocking Ca^{2+}-binding to the C2B-domain blocks synchronous exocytosis [39], whereas blocking Ca^{2+}-binding to the C2A-domain decreases exocytosis ~40%, and additionally decreases the apparent Ca^{2+}-cooperativity of exocytosis ~40% [40]. Thus, the two C2-domains of synaptotagmins thus are not equivalent, but they cooperate with each other, with the C2B-domain playing the leading part. Nevertheless, mutations in the C2A-domain alter the overall apparent Ca^{2+}-affinity of synaptotagmin and change the Ca^{2+}-affinity of synaptic exocytosis correspondingly, an observation that provided the formal proof for the Ca^{2+}-sensor of synaptotagmin in exocytosis [41,42]. Biochemically, Ca^{2+}-binding to the C2B-domain is essential both for effective Ca^{2+}-dependent phospholipid binding of synaptotagmin and for displacing the complexin clamp from SNARE complexes. Note that in the latter process, complexin may still remain associated with the SNARE complex, since it likely interacts with the complex via multiple mechanisms.

Three synaptotagmins (Syt1, Syt2, and Syt9) function as Ca^{2+}-sensors for synaptic exocytosis, but exhibit distinct expression patterns and properties [43]. Syt2 triggers release much faster than Syt1 and operates in synapses relying on fast signaling, such as the calyx of Held synapse

or the neuromuscular junction [8-10,44]. Syt9 triggers release with a significantly slower timecourse than either Syt1 or Syt2, and is primarily expressed in the reward pathway [43].

In endocrine LDCV exocytosis, the major Ca^{2+}-sensors are Syt1 and Syt7, another Ca^{2+}-binding synaptotagmin [45-50]. Single deletions of Syt1 or Syt7 in chromaffin cells impair preferentially the fast or slow phase of LDCV exocytosis, respectively, suggesting that the two synaptotagmins mediate different phases, whereas double deletions of Syt1 and Syt7 block both phases [48]. Point mutations in Syt1 that change its Ca^{2+}-affinity change the apparent Ca^{2+}-affinity of exocytosis in chromaffin cells correspondingly, similar to the synapse [46]. Whereas at least Syt2 can substitute for Syt1 in chromaffin exocytosis [51], Syt7 cannot substitute for Syt1 in synaptic exocytosis [43], and Syt7 deletions have no significant effect on synaptic exocytosis [52]. The reason for this selectivity remains unclear, as Syt7 is very similar to Syt1, and only differs from Syt1 in lacking an N-terminal intravesicular N-glycosylation site and in exhibiting a higher apparent Ca^{2+}-affinity [32]. In chromaffin cells, Syt1 may also be involved in docking of LDCVs since LDCV docking was impaired in cells from Syt1 KO mice [53]. However, the mechanism of this change remains unclear because synapses lacking Syt1 exhibit no major docking phenotype, and a similar docking phenotype was observed in chromaffin cells but not synapses in other mutant mice [27].

Synaptotagmins have also been implicated in non-neuronal and non-endocrine forms of Ca^{2+}-induced exocytosis. In mast cells, Syt2 functions as the major Ca^{2+}-sensor for exocytosis [54]. In fibroblasts, Syt7 may mediate Ca^{2+}-triggered lysosome exocytosis [55], although this has been disputed [56]. It is notable that no definitive functions were described for 4 of the 8 Ca^{2+}-binding synaptotagmin isoforms (i.e., Syt3, Syt5, Syt6, and Syt10). These synaptotagmins form a highly homologous subgroup that exhibit Ca^{2+}-dependent phospholipid- and SNARE-binding similar to Syt1, Syt2, Syt7, and Syt9, and likely also function in exocytosis.

Dual-Ca^{2+}-sensor model for synaptic exocytosis

The most precise definition of synaptic transmission was achieved at the giant calyx of Held synapse in the brainstem that allows simultaneous patching of pre- and postsynaptic cells [57,58]. Measurements in the calyx synapse provided estimates of the Ca^{2+}-affinity (10-100 μM) and Ca^{2+}-cooperativity (~5 Ca^{2+}-ions) of neurotransmitter release [44,57,58]. As shown in mutant mice, this release is mediated by Syt2 as Ca^{2+}-sensor [9,44].

Calyx synapses normally exhibit little asynchronous release, and produce almost only synchronous release even at stimulation frequencies of 100 Hz, making it impossible to analyze asynchronous release biophysically. In Syt2-deficient synapses, however, asynchronous release could be analyzed in isolation, uncontaminated by a more dominant synchronous release component [44]. Such analyses uncovered an asynchronous component of exocytosis in calyx synapses that displayed an apparent Ca^{2+}-cooperativity of exocytosis of only ~2 Ca^{2+}-ions, whereas synchronous release operated with an apparent Ca^{2+}-cooperativity of ~5 Ca^{2+}-ions, although both exhibited similar Ca^{2+}-affinities (~40 μM). Because of the different Ca^{2+}-cooperativities but similar Ca^{2+}-affinities of synchronous and asynchronous exocytosis, low Ca^{2+}-concentrations preferentially but incompletely activate asynchronous release in wild-type synapses, whereas higher Ca^{2+}-concentrations, such as those achieved following an action potential, preferentially and completely activate synchronous release [44].

Based on the biophysical definition of asynchronous release, a dual-Ca^{2+}-sensor model was proposed which at present provides the most precise description of evoked synaptic vesicle exocytosis for synapses [44]. The model assumes that the synchronous Ca^{2+}-sensor synaptotagmin competes with an unknown asynchronous Ca^{2+}-sensor, with the asynchronous Ca^{2+}-sensor binding Ca^{2+} more slowly but at lower concentrations than the synchronous

Ca^{2+}-sensor. It should be noted that although the dual-Ca^{2+}-sensor model is the best available, it does not take into account the intrinsic heterogeneity of synapse vesicles. Vesicles likely differ in their proximity to Ca^{2+}-channels, which is a major determinant for the probability and speed of exocytosis [59]. Moreover, the number of SNARE complexes on a primed vesicle probably contributes to the Ca^{2+}-sensitivity of this vesicle, but differs between vesicles [27]. Finally, synaptic vesicles exhibit size heterogeneity [60], resulting in variations in the postsynaptic signal. In addition, the dual-Ca^{2+}-sensor model also does not include the possibility that at least at some synapses, synaptotagmin may inhibit the asynchronous Ca^{2+}-sensor [12,17]. Thus, despite the fact that the dual-Ca^{2+}-sensor model is currently the best available, it is far from perfect.

Synaptotagmin as a Ca^{2+}-sensor for spontaneous release

At a synapse, lowering the extracellular Ca^{2+}-concentration partially blocks spontaneous mini release; incubating synapses with membrane-permeable Ca^{2+}-buffers, however, or infusing Ca^{2+}-buffers into the calyx presynaptic terminal, blocks almost all spontaneous release [9, 17]. These results suggested that the majority of spontaneous release is Ca^{2+}-dependent, but raised the question what Ca^{2+}-sensor mediates this effect. Interestingly, knockin mutations in Syt1 that change its apparent Ca^{2+}-affinity caused corresponding effects on the frequency of spontaneous release. Specifically, when the apparent Ca^{2+}-affinity of Syt1 is decreased ~2-fold by the R233Q mutation, the frequency of spontaneous release is decreased ~2-fold, whereas an increase in Ca^{2+}-dependent SNARE-complex binding by the D232N mutation of Syt1 caused a correspondingly large increase in spontaneous release frequency [17]. These effects suggested that most spontaneous release is induced by Ca^{2+}-binding to Syt1, with the Ca^{2+} derived from resting Ca^{2+}-levels, Ca^{2+}-influx via stochastically opening Ca^{2+}-channels, or Ca^{2+}-sparks. However, a recent study showed that deletion of another Ca^{2+}-binding protein, Doc2, causes a partial decrease in mini frequency, indicating that this protein may contribute to the Ca^{2+}-regulation of spontaneous release [61].

Strikingly, although point mutations in Syt1 modulate spontaneous release, deletion of Syt1 or Syt2 increase spontaneous release dramatically, despite blocking evoked synchronous release [9,12,44]. The ‘new’ spontaneous release in Syt1-deficient synapses is still Ca^{2+}-dependent, but activated at lower extracellular Ca^{2+}-concentrations with a lower apparent Ca^{2+}-cooperativity than wild-type spontaneous release [17]. Thus, the increased spontaneous release in Syt1 KO synapses (or, for that matter, also in Syt2 KO synapses) exhibits the properties of asynchronous release as determined in the calyx synapse [44]. These results indicate Syt1 and Syt2 might generally inhibit asynchronous release. An alternative explanation is that spontaneous release represents a completely separate cell-biological pathway, as suggested by reports that evoked and spontaneous synaptic release are using different synaptic vesicle pool [62,63]. If so, the Syt1 and Syt2 deletions may not actually disinhibit the asynchronous Ca^{2+}-sensor, but instead activate this separate pathway.

Other potential Ca^{2+}-sensors for exocytosis

Which Ca^{2+}-sensor mediates asynchronous release and other Ca^{2+}-dependent types of exocytosis in which Syt1, Syt2, Syt7 and Syt9 don’t act as Ca^{2+}-sensors? Naturally, prime candidates are the other four Ca^{2+}-binding synaptotagmins that have no known function (Syt3, Syt5, Syt6, and Syt10). However, at least for asynchronous release, this candidacy is doubtful since these synaptotagmins bind Ca^{2+} via a mechanism akin to that of Syt1, with a likely Ca^{2+}-binding stoichiometry of ~5, whereas asynchronous release exhibits a much lower apparent Ca^{2+}-stoichiometry.

Two major classes of cytosolic Ca^{2+}-binding proteins are known, EF-hand proteins and C2-domain containing proteins. Calmodulin, the most important EF-hand Ca^{2+}-binding protein,

appears to enhance neurotransmitter release in both excitatory and inhibitory synapses without directly participating in asynchronous exocytosis [64]. Numerous other EF-hand Ca^{2+}-binding proteins are also expressed in brain, but most function as Ca^{2+}-buffers or as Ca^{2+}-regulated enzymes. Many C2-domain proteins are expressed in brain, most of which contain a single C2-domain and are involved in signal transduction. A smaller subset of C2-domain proteins contains multiple C2-domains, such as synaptotagmins (Fig. 4). Although the function of most of these multiple C2-domain proteins is unknown, the synaptotagmin paradigm suggests that at least some of them are involved in membrane traffic, rendering them candidates for exocytotic Ca^{2+}-sensors.

1. *Ferlins* contain at least six C2-domains and a C-terminal transmembrane region; the two C-terminal C2-domains include canonical Ca^{2+}-binding sequences. Ferlins are required for sperm exocytosis in C. elegans [65]. Of the 5 mammalian ferlins, dysferlin is involved in Ca^{2+}-dependent exocytosis of repair vesicles in muscle [66], and otoferlin is required for Ca^{2+}-dependent exocytosis in hair cells [67], although the mechanisms involved have not been defined.

2. *MCTPs* (multiple C2-domain transmembrane proteins) are evolutionarily conserved proteins with 3 C2-domains containing canonical Ca^{2+}-binding sequences, and a C-terminal transmembrane region. The functions of MCTPs are unknown [68].

3. *E-Syts* (extended synaptotagmins) are also evolutionarily conserved transmembrane proteins. E-Syts contain an N-terminal transmembrane region like synaptotagmins, followed by a single SMP-domain and either five C2-domains (E-Syt1) or three C2-domains (E-Syt2 and E-Syt3). Only the N-terminal C2-domain contains the requisite Ca^{2+}-binding sequence, and exhibits Ca^{2+}-dependent phospholipid binding [69]. No functional data on E-Syts are available.

4. *Synaptotagmin-like proteins (SLPs)* represent a large and heterogeneous class of proteins with two C-terminal C2-domains but without transmembrane regions. An evolutionarily conserved subset of SLPs contains N-terminal Rab-binding sequences and zinc-finger domains (including rabphilin); another subset of SLPs that is not evolutionarily conserved lacks the N-terminal Zinc-finger domain (Doc2s, slp2c, slp3b). In vertebrate synapses, rabphilin is important for re-priming of synaptic vesicles [70]. Doc2 proteins appear to bind SNARE proteins and phospholipids tighter than Syt1, and deletion of Doc2α and Doc2β partly reduces spontaneous release [61]. In chromaffin cells, Doc2β plays a role in Ca^{2+}-dependent priming and exocytosis [71], but the overall function of Doc2 proteins and other SLPs remains unclear.

5. *Copines* are soluble double C2-domain proteins of unknown function that bind to phospholipids [72]. Copines are evolutionarily conserved, and contain two N-terminal C2-domains with canonical Ca^{2+}-binding sequences and a unique C-terminal domain.

Conclusions

A universal mechanism by which Ca^{2+}-binding to synaptotagmins triggers exocytosis has emerged over the last decade. This mechanism mediates most Ca^{2+}-triggered exocytosis using a pas-de-deux of synaptotagmins and complexin acting on SNARE complexes and phospholipid membranes. However, new intriguing questions have emerged. Are forms of exocytosis for which no synaptotagmin Ca^{2+}-sensor has been identified, such as asynchronous release, mediated by an atypical synaptotagmin, or by novel Ca^{2+}-sensor, for example one of the other multiple C2-domain proteins (Fig. 4)? How do the Ca^{2+}-sensors for synchronous and asynchronous release intersect in synaptic exocytosis – do they compete, or do synaptotagmins and complexin inhibit the alternative pathway? What is the role of ferlins in exocytosis in

Ca^{2+}-triggered exocytosis? Answering these questions will significantly advance the field beyond the synaptotagmin paradigm.

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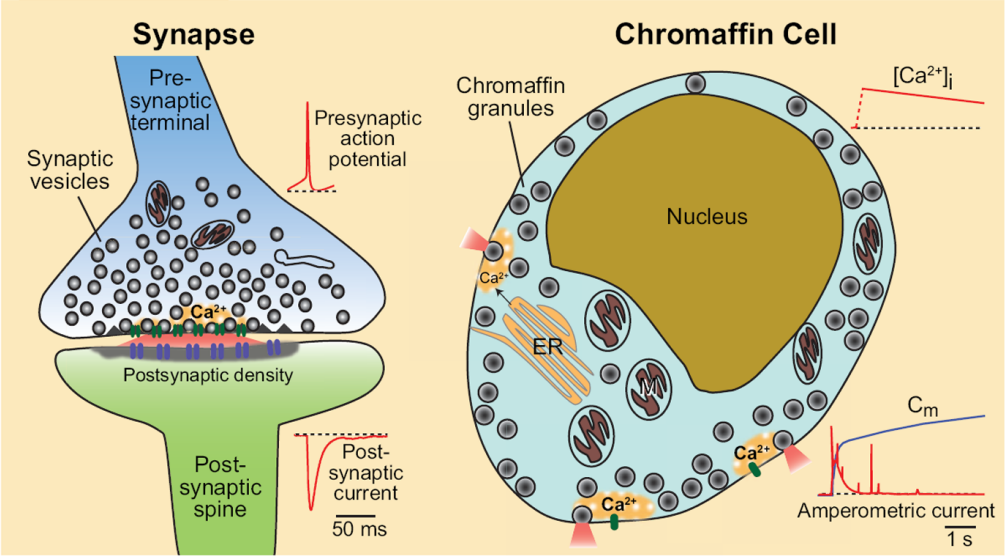

Figure 1. Synaptic and endocrine Ca^{2+}-triggered exocytosis

At a synapse (**left**), neurotransmitters are packaged into small synaptic vesicles, which are docked at the active zone adjacent to voltage-dependent Ca^{2+}-channels. A presynatpic action potential (*insert*) gates Ca^{2+}-influx into the terminal, thereby triggering vesicle exocytosis. The released transmitters produce a postsynaptic current (*insert*) which can be recorded by whole-cell patch clamping. In endocrine cells (**right**), hormones are packaged into LDCVs, which are generally not docked. Upon sustained increases in cytosolic Ca^{2+}, as obtained during stimulation or Ca^{2+}-uncaging (*insert*), exocytosis is triggered with a significantly slower time course than at a synapse, as measured by amperometry or capacitance (Cm; *insert*). Note that Ca^{2+}-channel and release sites are not tightly coupled in endocrine cells. ER, endoplasmic reticulum; M, mitochondrion. Traces are shown purely for demonstration purposes.

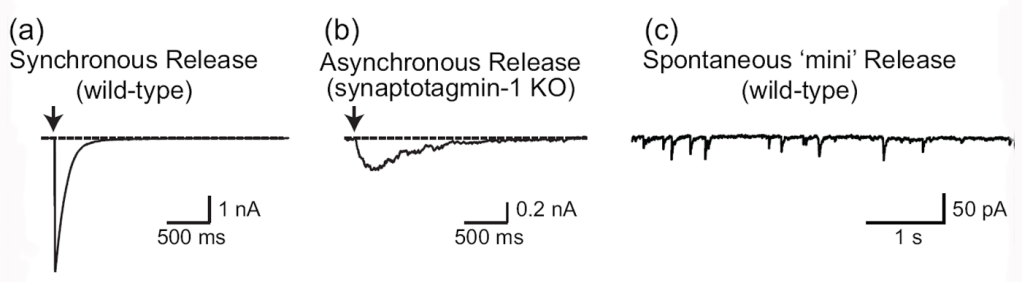

Figure 2. Synaptic vesicle exocytosis detected by whole-cell patch clamp recordings

Images depict representative traces of postsynaptic currents illustrating the three different forms of synaptic exocytosis: evoked synchronous release from wild-type synapses (a), evoked asynchronous release from Syt1-deficient synapses (b) and spontaneous mini release (c). Note that asynchronous release also can be recorded in some wild-type neurons upon high-frequency stimulation.

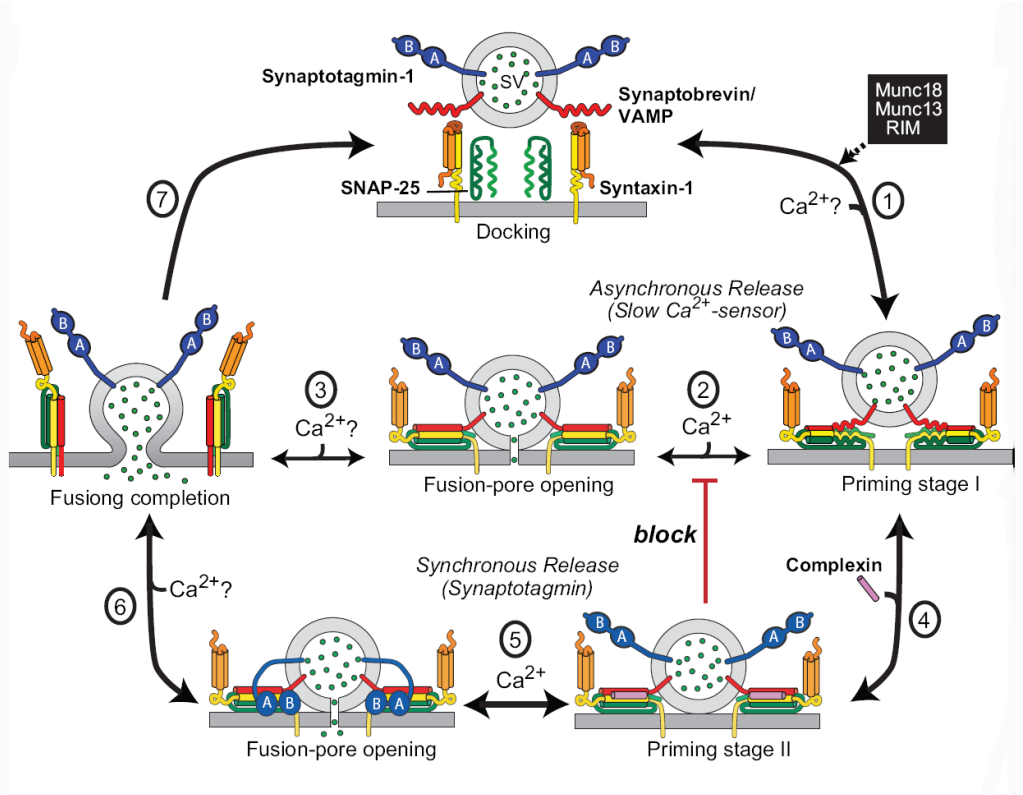

Figure 3. Model of the molecular steps mediated synaptic vesicle exocytosis (modified from [38])

Synaptic vesicles are docked at the active zone of a presynaptic terminal with unassembled SNARE complexes (top)m and are then primed for release by partial SNARE-complex assembly that is catalyzed by Munc18, Munc13, and RIM (step 1). At least in inhibitory synapses, this priming process might be further modulated by ELKS2. The primed vesicles form the substrate for two main pathways of Ca^{2+}-triggered neurotransmitter release: asynchronous release (steps 2 and 3), in which full assembly of SNARE complexes leads to fusion-pore opening followed by complete fusion (step 3); and synchronous release (steps 4,5 and 6), in which "superpriming" by binding of complexins to assembled SNARE complexes (step 4) activates and freezes SNARE complexes in a metastable state (referred to as priming stage II). This stage is then substrate for fast Ca^{2+}-triggering of release when Ca^{2+}-binding to Syt1 induces its binding to phospholipids and to SNARE complexes, with the latter reaction displacing complexin and resulting in fusion-pore opening (step 5) and full fusion (step 6). Both the synchronous and the asynchronous release pathway can mediate spontaneous 'mini' release, depending on the local Ca^{2+}-microdomain. Synaptotagmin and complexin clamp **(block, in red)** the unidentified slow Ca^{2+}-sensor which mediates the asynchronous release; this clamping is relieved when Ca^{2+} binds to Syt1, allowing competition between Syt1 and the asynchronous Ca^{2+}-sensor during high-frequency stimulation [12,44].

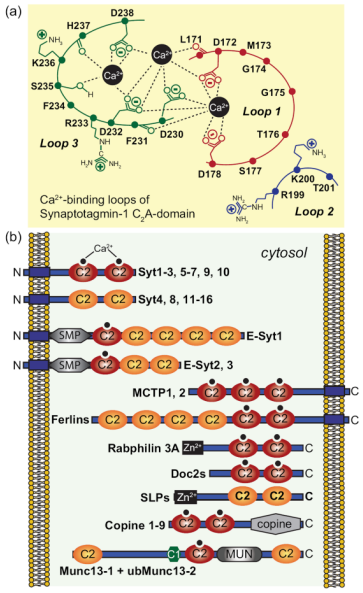

Figure 4. Structures of multiple C2-domain proteins

A. Schematic diagram of the top Ca^{2+}-binding loops of the Syt1 C2A-domain (modified from [41]). The C2A-domain has three top loops, of which loops 1 and 3 form three Ca^{2+}-binding sites. Five aspartate residues, one serine residue and two backbone carbonyl groups coordinate the three bound Ca^{2+} ions. Residues are shown in single-letter aminio acid code and are identified by residue numbers corresponding to mouse Syt1.

B. Domain structures of proteins with multiple C2-domains that contain or lack a transmembrane domain. The mammalian genome encodes four classes of multiple C2-domain proteins containing transmembrane regions: synaptotagmins (Syt1-16; note that 'Syt17' does not contain a transmembrane region, but is membrane-anchored via palmitoylated cysteine residues), extended synaptotagmins (E-Syt1-3), multiple C_2-domain and transmembrane proteins (MCTP1-2) and ferlins (including otoferlin, dysferlin, myoferlin and 2 other uncharacterized ferlin like proteins). Examples of soluble C2-domain containing proteins are also included. C2-domains with canonical Ca^{2+}-binding consensus sequences are labeled as Ca^{2+}-binding C2-domains, although Ca^{2+}-binding has not yet been tested to all of these domains. C2-domains were assigned based on the conserved domain database of the NCBI; some proteins, especially ferlins, may have additional unpredicted C_2-domains that do not precisely fit the consensus sequence, as well as alternative transcripts with fewer C_2-domains.

Table 1

Properties of synaptotagmins

Protein	Class	Localization	Ca^{2+}-binding	Special properties	Function	References
Syt 1	A	Synaptic vesicles LDCVs	Yes	N-terminal *N*-glycosylation Phospholipids and SNARE binding	Ca^{2+}-sensor for fast exocytosis	6,10,12,31–33,39,43,74
Syt2	A	Synaptic vesicles LDCVs?	Yes	N-terminal *N*-glycosylation Phospholipid and SNARE binding	Ca^{2+}-sensor for fast exocytosis	8,9,31–33,43,44,54,74
Syt3	C	Plasma membrane	Yes	Disulfide bonds at N-terminus Phospholipid binding	Ca^{2+}-sensor for exocytosis?	31–33,74
Syt4	D	Postsynaptic?	No*	Aspartate to Serine substitution in C2A domain loops	unknown*; not essential for survival	73,74
Syt5	C	Plasma membrane	Yes	Disulfide bonds at N-terminus Phospholipid bindings	Ca^{2+}-sensor for exocytosis?	31–33,74
Syt6	C	Plasma membrane	Yes	Disulfide bonds at N-terminus Phospholipid binding	Ca^{2+}-sensor for exocytosis?	31,74
Syt7	B	LDCVs Synapses?	Yes	Multiple splicing isoforms Phospholipid and SNARE binding	Ca^{2+}-sensor for LDCV exocytosis	47–50,74
Syt8	F	primarily GIT tract	No	Only Syt isoform that is expressed at highest levels outside of brain	Not known	31,74
Syt9	A	Synaptic vesicles	Yes	Phospholipid and SNARE binding	Ca^{2+}-sensor for fast exocytosis	43,74
Syt10	C	Plasma membrane	Yes	Disulfide bonds at N-terminus Phospholipid binding	Ca^{2+}-sensor for exocytosis?	31,74
Syt11	D	Unknown	No	Similar to Syt4, but more abundant	unknown	74
Syt12	F	Synaptic vesicles	No	Phosphorelated by PKA	May regulate miniature release	31,74,75
Syt13	F	Not known	No		Not known	31,74
Syt14	E	Not known	No	Syt14-16 form a group of related and evolutionarily conserved isoforms	Not known	76
Syt15	E	Not known	No	Syt14-16 form a group of related and evolutionarily conserved isoforms	Not known	76
Syt16	E	Not known	No	Syt14-16 form a group of related and evolutionarily conserved isoforms	Not known	76

Synaptotagmins are defined by the presence of an N-terminal transmembrane region, and two C-terminal C2-domains. Each synaptotagmin protein is encoded by a different gene; some are differentially spliced (e.g., Syt7). Classes of synaptotagmins are proposed defined by sequence homologies, conservation, and functions

*Syt4 binds to Ca^{2+} in *Drosophila* and has been implicated in postsynaptic exocytosis.