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Vertebrate Presynaptic Active Zone Assembly: a Role Accomplished by Diverse Molecular and Cellular Mechanisms

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Abstract Among all the biological systems in vertebrates, the central nervous system (CNS) is the most complex, and its function depends on specialized contacts among neurons called synapses. The assembly and organization of synapses must be exquisitely regulated for a normal brain function and network activity. There has been a tremendous effort in recent decades to understand the molecular and cellular mechanisms participating in the formation of new synapses and their organization, maintenance, and regulation. At the vertebrate presynapses, proteins such as Piccolo, Bassoon, RIM, RIM-BPs, CAST/ELKS, liprin-α, and Munc13 are constant residents and participate in multiple and dynamic interactions with other regulatory proteins, which define network activity and normal brain function. Here, we review the function of these active zone (AZ) proteins and diverse factors involved in AZ assembly and maintenance, with an emphasis on axonal trafficking of precursor vesicles, protein homo- and hetero-oligomeric interactions as a mechanism of AZ trapping and stabilization, and the role of F-actin in presynaptic assembly and its modulation by Wnt signaling.

Keywords Bassoon · CAZ · CAST · ELKS · F-actin · Liprin-α · Munc13 · Piccolo · RIM · RIM-BPs · Wnt

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Introduction

Chemical synapses are specialized junctions that pass signals among neurons or from neuron to non-neuronal cells such as those in muscles or glands. In the CNS, synapses have two structural and functional different regions: (i) the active zone (AZ), a specialized area of the plasma membrane located in the presynaptic neuron, which contains the molecular machinery that regulates neurotransmitter exocytosis from synaptic vesicles; and (ii) the postsynaptic density (PSD), a membrane region of the postsynaptic neuron which has neurotransmitter receptors and signaling apparatus. The strength of synapse communication at the CNS depends on pre- and postsynaptic factors. At the presynapses, synaptic efficacy depends on the frequency and number of vesicles released after stimulation. Synaptic vesicles at the presynaptic terminal are organized, transit between three different pools, and undergo a cycle of exo- and endocytosis. The readily releasable pool (RRP) is located closest to the AZ plasma membrane, followed by the recycling pool and resting pool [1]. AZ proteins maintain the structural and functional integrity of these pools, and also recruit voltage-gated Ca^{2+} channels (VGCC) to the presynaptic membrane to allow fast and synchronous coupling between excitation and release, thereby modulating synaptic strength and presynaptic plasticity.

The conservation of a collection of proteins found in invertebrate and vertebrates AZs suggest that they form a minimal platform necessary for neurotransmitter release. These proteins include Rab3-interacting molecules (RIMs), RIM-binding proteins (RIM-BPs), ELKS, liprin-α, and Munc13. Other vertebrate AZ proteins such as Piccolo and Bassoon are less conserved among species, and they might play a more specialized role in higher organisms. Hence, AZ proteins form a dense accumulation called the cytomatrix at the active zone (CAZ), which is a macromolecular complex that regulates

synaptic vesicle trafficking cycle. The synaptic vesicle cycle involves several steps: SV are recruited to the AZ where they are tethered and docked. Then they are primed so that they can fuse rapidly in response to calcium entry triggered by an action potential [2, 3]. To complete the cycle, synaptic vesicles undergo endocytosis, recycle, and refill with neurotransmitters for a new round of exocytosis.

Synaptogenesis, the formation of synapses between neurons in the nervous system, is a multistep process that requires cell adhesion, transport of synaptic components along neurites, trapping, stabilization of synaptic proteins at sites of newly forming synapses, and maturation. This process and the establishment of protein interactions must be timely and spatially regulated to avoid the development of neurological diseases [4]. There is a multivesicular-mediated mechanism for the transport of AZ and synaptic vesicle proteins from the cell soma into newly forming synapses at distal axonal sites [5–8]. During trafficking, cytoskeletal and motor proteins participate actively [9], and some AZ proteins play a role as adapters between motor proteins and vesicles [10]. Table 1 summarizes the main described functions of these proteins. In the last 20 years, accumulating evidence has shown the involvement of the actin cytoskeleton and regulatory signaling mechanisms participating in the formation and stabilization of new synapses in which the Wnt signaling pathway plays an important role.

Active Zone Proteins: Role in Presynaptic Organization and Function

The overall function of the AZ is to translate an action potential into a chemical signal releasing neurotransmitters into the synaptic cleft. Therefore, AZ proteins have to interact in a coordinated manner for normal synaptic function to be accomplished. During synapse formation, AZ proteins are transported along axons to sites of newly forming presynapses where they interact with other AZ proteins, forming a macromolecular network of interactions. Furthermore, once synapses are established, the AZ undergoes molecular remodeling during the lifespan to support the requirements of synaptic activity and plasticity. Initially, due to their modular structure, AZ proteins were given the role of scaffolding; however, advances in live microscopy, super-resolution microscopy, electron microscopy, and genetic and molecular manipulations combined with studies in invertebrates have deciphered more specific and dynamic functions for some of these proteins in the various steps of neurotransmitter release. Therefore, AZ proteins are more than fixed scaffolds with a role in holding SV; they also participate in SV endocytosis/exocytosis and the maintenance, remodeling, and dynamics of the presynapses, working as a team with specific and shared functions (Fig. 1). The latter is thought to be part of a redundant and protective mechanism. Most AZ proteins have several common

Table 1 Main functions of vertebrate active zone proteins

Protein	MW (kDa)	Function	Reference
Piccolo	550	Scaffolding, CAZ organization	[11, 12]
		Presynapse assembly	[13, 14]
		Modulation of SV reserve pool movement	[12, 18, 20, 21]
		Regulation of F-actin dynamic	[18, 19, 21]
		Regulation synaptic ubiquitination and proteostasis	[42, 44]
		Partipation in the communication between synaptic activity and gene expression	[43]
Bassoon	420	Key scaffolding protein of ribbon synapses	[28, 31, 32, 38]
		Adapter for the transport of PTVs	[10, 109]
		Regulation synaptic ubiquitination and proteostasis	[42, 44]
		Calcium channel clustering	[35, 37]
		Partipation in the communication between synaptic activity and gene expression	[43]
ELKS	120	Scaffolding, CAZ organization	[48–50, 54]
		Negative regulator of inhibitory synapses	[53, 55]
RIM	190	SV priming	[62]
		Calcium channel recruitment to AZ	[66, 74]
		Presynaptic plasticity	[67–69]
RIM-BP	180	Calcium channel recruitment	[77, 78]
Liprin	160	Scaffolding, CAZ organization	[90, 95]
		Presynaptic plasticity	[93–95]
Munc13	200	SV priming and fusion	[103, 104, 106]
		Presynaptic plasticity	[98, 105]

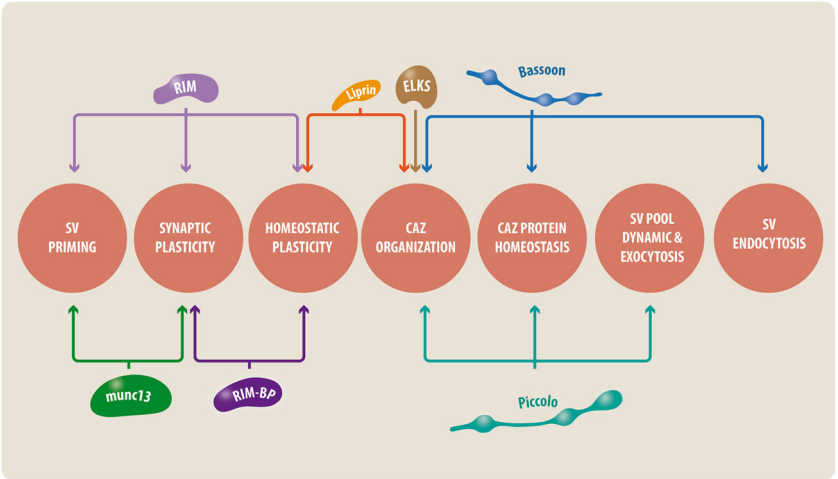

Fig. 1 General functions of main active zone proteins. Vertebrate's central presynapses contain seven functionally relevant proteins: Piccolo, Bassoon, ELKS, RIM, RIM-BP, liprin, and Munc13. The figure summarizes the specific and overlapping functions of these main active zone proteins suggesting that redundant mechanisms protect central synapse structure and function

characteristics: they are multi-domain, interact with other AZ proteins, form temporal complexes with diverse proteins, and continue to be expressed at high levels in the adult brain, emphasizing their permanent role in synapse function. These characteristics situate AZ proteins as key molecular entities to modulate AZ content and efficacy during the lifespan of the synapse.

Vertebrate AZ Proteins

Piccolo

Piccolo was one of the first presynaptic molecules to be described in the vertebrate CNS [11]. There are several characteristics that make Piccolo a candidate for vertebrate CAZ assembly and organization: (a) early appearance, (b) large size, (c) multiple domains (two N-terminal zinc fingers, three coiled-coil, proline-rich region (Q domain), PDZ and two C2 domains), and (d) interaction with proteins of diverse function [11–13]. The multiple interactions described for Piccolo suggest that it is a very versatile molecule. Piccolo interacts directly with the AZ proteins Bassoon, ELKS, liprin-α, and Munc13. Piccolo also binds to GIT1, a GTPase-activating protein of the ADP-ribosylation factor family that participates in functions such as vesicle trafficking, adhesion, and cytoskeletal organization [13, 14]. GIT1 colocalizes with Piccolo at synapses and is part of a multi protein complex, suggesting a role in the organization of the CAZ [14]. Another interaction suggests that Piccolo has a role in SV movement and is with the prenylated Rab acceptor protein 1 (PRA1), which might control SV docking and fusion [12]. Piccolo is also postulated to have a role in synaptic vesicle clathrin-mediated endocytosis because of its interaction with Abp1, an F-actin-binding protein, and the GTPase dynamin [15]. Another possible role for Piccolo is in the scaffolding of voltage-gated calcium through its C2A domain, but its importance has not been further explored [16, 17]. Other recently described interactions of Piccolo, which will be discussed in the "Active zone and Wnt signaling" section, are Daam1 (Disheveled associated activator of morphogenesis 1) [18] and Rho-GEF Trio [19].

A specific shRNAi for Piccolo designed by Leal-Ortiz et al. [20] rendered non-Piccolo immunoreactive bands in western blot analysis of lysates prepared from rat hippocampal neurons [21]. The knock-down (KD) of Piccolo in rat hippocampal neurons did not affect synapse formation since presynaptic and postsynaptic proteins showed normal synaptic targeting [20] suggesting that this protein is not essential for glutamatergic synapse formation, although the loss of Piccolino, the major Piccolo isoform from mouse photoreceptor cells, causes defects in the maturation and ultrastructure of ribbons [22, 23]. When the presynaptic function was evaluated by styryl FM dyes [24] in the Piccolo KD model, no differences were found in the total reserve pool (TRP) of SV but the destaining kinetics of the TRP was more rapid in comparison with the control suggesting changes in the exocytosis of SV. This study showed that Piccolo negatively regulates the exocytosis of SV by modulating the synapsin 1 dynamic at the AZ by a calmodulin kinase II-dependent (CaMKII) mechanism [20] that involves presynaptic F-actin polymerization [21]. Interestingly, no neurotransmission defects were found in a study that used a mouse model with a targeted deletion of exon 14 for the Piccolo gene [25]; however, this Piccolo knock-out (KO) model continued to express presynaptic Piccolo isoforms, which most likely supports Piccolo function in neurotransmitter release [21]. Therefore, Piccolo will be necessary to restrain SV at the reserve pool by acting as the scaffold for several actin-binding proteins, which modulate presynaptic F-actin polymerization. Recently, an invertebrate homolog with conserved Piccolo functions in *Drosophila*, fife, has been described [26]. Fife mutants present a decrease in neurotransmitter release, abnormalities in the presynaptic membranes, and reduced SV clustering [26, 27].

Overall, Piccolo, in addition to having a clear function in scaffolding and organization of the CAZ, modulates SV

dynamic and homeostasis of CAZ proteins ("Bassoon" section), suggesting a participation in synaptic plasticity (Fig. 2; Table 1).

Bassoon

Bassoon is another large structural protein that interacts with Piccolo and other proteins of the AZ [11, 28]. Bassoon and Piccolo are structurally related proteins sharing ten highly conserved regions [12, 28, 29]. Bassoon contains two N-terminal zinc finger domains, three coiled-coil domains, and a glutamine repeats at its C-terminus [28]. Bassoon differs from Piccolo in that it does not participate directly in F-actin dynamic, but seems to be necessary for the synaptic architecture of other non-CNS synapses because the loss of Bassoon produces structural defects in retinal and cochlea ribbon synapses [30, 31] but not at central synapses. At the retinal photoreceptor synapses, the CAZ contains a specialization called the ribbon complex, which is enriched in the protein Ribeye, and the direct interaction of Bassoon and Ribeye links the ribbon to the AZ compartment, maintaining its integrity [30]. Additionally, Bassoon participates in the early formation of nascent ribbon synaptic sites during retinal ribbon synaptogenesis [32]. Other interactors for Bassoon include the dynein light chains DLC1 and DLC2, which function as a cargo adapter for the Piccolo-Bassoon transport vesicle (PTV), allowing its retrograde trafficking and the synaptic delivery of AZ proteins [10].

The loss of Bassoon at central synapses shows only a reduced number of fusion competent SV and a decrease in the RRP pool size of vesicles at glutamatergic synapses accompanied by an increase in short-term depression and a high number of silent synapses [33, 34]. The latter could be explained by the interaction of Bassoon with RIM-BP, which modulates the recruitment of CaV2.1 (P/Q-type) channels to SV release sites, thereby contributing to the molecular composition of the neurotransmitter release machinery [35]. On the contrary, loss of Bassoon from non-central sensory synapses alters the structure of the presynapses. These synapses contain a structure called ribbon that holds synaptic vesicles close to the AZ and present a tight vesicle-calcium channel coupling. In the absence of Bassoon, ribbons are loss from the synapses of retinal photoreceptors [36] and cochlear inner hair cells [31, 37, 38]. The loss of Bassoon both from the synapses of ribbon-type [37, 38] and Endbuld of Held synapses (a large synapse in the mammalian auditory CNS) [39] synaptic vesicle replenishment and the size of RRP are decreased suggesting an important role for Bassoon in the plasticity of neurotransmitter release.

A recent Bassoon interaction found in a two-hybrid screen is the protein Mover [40], which negatively regulates synapse release probability at the calyx of Held synapses [41]. It is postulated that Bassoon might recruit Mover into this specific type of synapse, which regulates the release probability [41].

Interestingly, two recently described functions shared by Piccolo and Bassoon are the regulation of the homeostasis of synaptic proteins [42] and the communication between

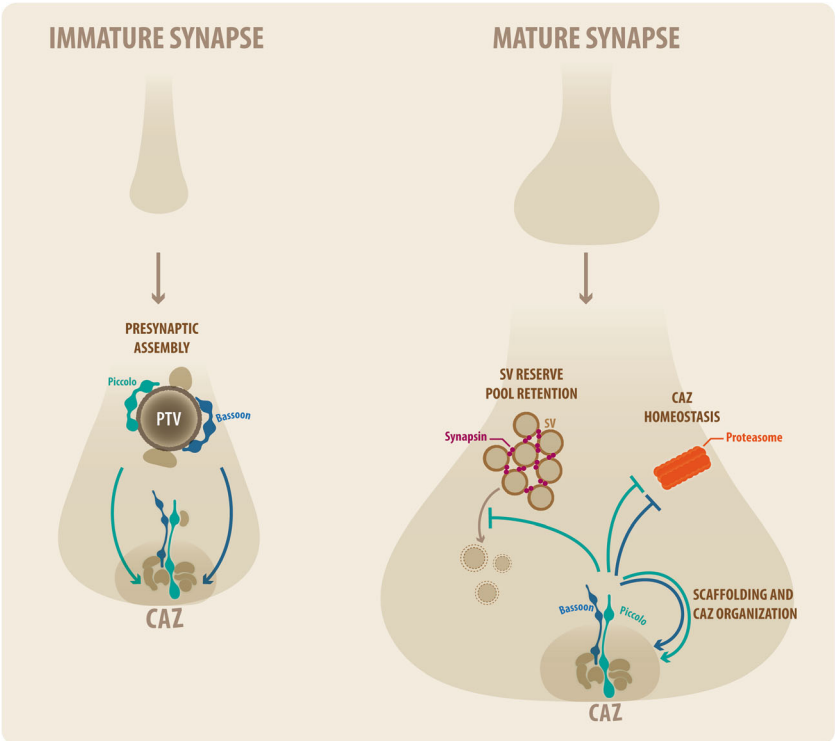

Fig. 2 Roles of Piccolo in immature and mature synapse. Piccolo is the largest protein and one of the most versatile molecules of the active zone. During synaptogenesis, Piccolo's main function is the assembly of new forming presynapses and in mature synapses plays three main functions, some of them share by Bassoon: (i) CAZ organization through its multi-modular structure and interactions; (ii) the dynamic retention of the SV reserve pool acting as an adapter between SV synapsin1, actin-binding proteins, and F-actin; and (iii) stabilization of synaptic proteins by acting together with Bassoon on the ubiquitin-proteosomal system

synaptic activity and gene expression [43]. The regulation of homeostasis is performed in part by their zinc finger domain, which binds to the E3 ubiquitin ligase Siah1, inhibiting its function. Siah1 mediates ubiquitination and proteasome-mediated degradation of specific proteins. In neurons, the absence of Piccolo and Bassoon causes a decrease in the content of synaptic proteins due to an increase in ubiquitination and degradation of several AZ proteins and SV proteins [42]. In the absence of Piccolo and Bassoon, the loss of presynaptic proteins occurs through autophagy, but this intracellular degradative process is blocked in the presence of Bassoon, which interacts and inhibits Atg5, an E3-like ligase essential for autophagy [44]. Therefore, Piccolo and Bassoon stabilize presynaptic proteins and avoid premature synaptic degeneration, suggesting a role in presynaptic efficacy by regulating AZ protein turnover. The other shared function of Bassoon and Piccolo is mediated through their interaction with the C-terminal-binding protein 1/brefeldin A-ADP-ribosylation substrate (CtBP1/BARS) [43], called CtBP1, a transcriptional repressor [45] that is translocated between the presynapses and the nucleus carrying information on synaptic activity to modulate gene expression [30]. Hence, Bassoon and Piccolo anchor CtBP1 to the presynapses, allowing this protein to sense synaptic activity. Similar to Piccolo, Bassoon plays diverse functions at the CAZ that go beyond a simple scaffolding protein (Table 1).

ELKS

ELKSs are proteins with a high content of the amino acids glutamate (E), leucine (L), lysine (K), and serine (S). In vertebrates, two ELKS brain-specific isoforms have been described: ELKS1α [46, 47] and ELKS2α [47, 48]. The multi-coiled-coil nature of ELKS allows it to form oligomeric protein complexes with other CAZ proteins such as Munc-13, RIM1, Piccolo, and Bassoon [48, 49]. Both Piccolo and Bassoon bind directly to a central region of ELKS, and it binds to Munc13-1 indirectly through RIM1α. RIM1α binds through its domain PDZ to the carboxyl terminal of ELKS1α and ELKS2α. Liprin-α binds directly to Piccolo and ELKS. These multiple interactions and the fact that in cultured neurons ELKS might function to recruit or stabilize liprin-α and RIM [48, 50], position these proteins as candidates in the molecular organization of presynaptic AZ [50]. ELKS also interacts with Rab6 in a GTP-dependent manner that suggests a role in trans-Golgi network trafficking [51]. Additionally, the ELKS c-terminal domain also binds to a PDZ domain of syntenin-1, a protein involved in cytoskeletal-membrane organization and trans-membrane protein trafficking [52]. This interaction might also contribute to the molecular organization of the CAZ [52].

Overexpression and in vitro studies with cultured rat neurons suggested that ELKS2α is necessary for neurotransmitter release and that the interaction of ELKS2α with RIMs and Piccolo/Bassoon is required for the function and SV release of the AZ [48, 49]. Deletion of ELKS2α in mice causes an increase in inhibitory synaptic responses and the size of the RRP of SV of inhibitory synapses [53]. Interestingly, there were no changes in the overall structure of these synapses or a functional defect in excitatory synapses. The data suggest that ELKS2α is a negative regulator of inhibitory synapses [53]. Additionally, a scaffold function for ELKS2α has been described in ribbon synapses where ELKS2α KO induces reduced AZ size [54].

To avoid compensation mechanisms between the two brain ELKS isoforms, both genes were removed in hippocampal neurons in culture after synapses were established [55]. The simultaneous loss of ELKS1α and ELKS2α resulted in a 50% decrease in the release of neurotransmitters, accompanied by a 30% decrease in presynaptic Ca^{2+} influx along with a reduction in the probability of release (P) in inhibitory synapses [55]. The findings suggest that a normal influx of calcium into the nerve terminal of hippocampal inhibitory neurons requires direct interaction of ELKS with calcium channels, as was previously reported by Kiyonaka [56]. At excitatory synapses, the removal of ELKS1α and ELKS2α decreases the RRP and neurotransmitter release [57], but P and Ca^{2+} influx are not affected as occurs in inhibitory synapses when both isoforms are removed [55]. As both isoforms are present in both excitatory and inhibitory synapses, the functional differences observed in the depleted neurons might be explained by synapse specificity of other AZ proteins [57]. The removal of both ELKS isoforms did not affect the number of synapses or appearance by electron microscopy, and the deletion did not produce a decrease in levels of presynaptic calcium channels. However, because ELKSs were removed after the synapses were formed in that study, we cannot discard their participation in the formation of new synapses.

In *Drosophila melanogaster*, a crucial role in AZ assembly has been proposed for the ELKS homolog, bruchpilot [58, 59]. The bruchpilot N-terminal is homologous to vertebrate ELKS and has a C-terminal that is different from other AZ proteins. Mutants of bruchpilot lack dense projections (T-bars) and suffer from Ca^{2+} channel-clustering defects. The effect is dramatic because no other isoforms that compensate its function exist in that species. Interestingly, the first study of ELKS in *Caenorhabditis elegans* showed that this protein was a non-essential player in neurotransmitter release [60]. However, a syd-2 gain of function mutant was able to promote synapse formation in the absence of syd-1, which is essential for *C. elegans* synapse formation, but only in the presence of ELKS [61]. The mild effects on synaptic structure observed by the deletion of the two ELKS isoforms in the brain of vertebrates and the studies in *C. elegans* suggest the existence of redundant mechanisms for synapse formation to protect synapse integrity.

RIM

The AZ Rab3-interacting molecule (RIM) [62] is expressed from two RIM vertebrate genes, *Rims1* and *Rims2*, which express five protein isoforms in the brain (RIM1α, RIM1β, RIM2α, RIM2β, and RIM2γ). The RIM molecule has five domains: a N-terminal zinc-finger motif, a central PDZ domain, two C2 domains, and a proline-rich sequence at the carboxy-terminal [63]. RIM1α is located both in the AZ of central synapses and in ribbon synapses and is evolutionary conserved among species. RIM1α, in addition to Rab3, interacts with Munc13-1, liprin-α, and ELKS and forms a protein scaffold at the presynaptic AZ [49]. RIMs also interact with RIM-BPs [64], Ca^{2+} channels [65, 66], and synaptotagmin 1 [65], which suggested a role in the regulation of SV exocytosis.

The elimination of the major RIM isoform in the mouse brain, RIM1α, did not produce major abnormalities either in the synaptic structure or in the protein composition, but Munc13-1 protein levels were decreased by 60% [67]. However, the RIM1α KO mice showed alterations in synaptic function, such as defects in short-term synaptic plasticity [67], lack of mossy fiber LTP in the hippocampus and the cerebellum [68], and deficits in learning and memory [69]. Additional studies have shown that RIM1α mediates synaptic vesicle docking and priming [3, 67, 70–72] and recruitment of Ca^{2+} channels to the AZ [73–75]. The role in SV docking for RIM1α has been supposed because of its interaction with Rab3 and the priming factor Munc13 [71, 74, 76]. RIMs interact directly with Ca^{2+} channels through its PDZ domain and indirectly through RIM-BPs [74, 77], localizing Ca^{2+} channels to the AZ. Therefore, the interaction of RIM with RIM-BP is necessary for proper localization of Ca^{2+} channels close to the synaptic vesicle release machinery [73, 74, 78]. At the functional level, the depletion of the five isoforms caused, in addition to the defect in priming and neurotransmitter release observed in the single gene deletion, a decrease in Ca^{2+} influx, and diminution of responsiveness and synchronization of release were observed [74]. Elimination of the two RIM-BP isoforms does not affect neurotransmitter release but is necessary for high-fidelity coupling of synaptic transmission [78]. However, simultaneous deletion of RIMs and RIM-BPs affects synapse function by blocking SV priming, delocalizing Ca^{2+} channels, and altering postsynaptic organization, suggesting a redundant function for these two presynaptic proteins [79].

A function worth mentioning, although observed in the *Drosophila* neuromuscular junction, is the role of RIM and RIM-BP in homeostatic plasticity. In homeostatic presynaptic plasticity, different levels of postsynaptic receptor perturbation induce compensatory mechanisms at the presynapses. Both RIM and RIM-BP perform this role by modulating the RRP of synaptic vesicles [80, 81], and RIM-BP in addition regulates the presynaptic Ca^{2+} influx [81].

Hence, the main functions for RIM are SV priming and synaptic plasticity (Table 1).

RIM-BP

Vertebrate RIM-BPs consist of three isoforms containing three SH3-domains, which bind to voltage-gated Ca^{2+} channels and RIM1α, and two-three fibronectin III repeats [64, 82]. RIM-BP1 and RIM-BP2 present different expression pattern in the brain. About the RIM-BP's functions mentioned in previous sections, we can highlight the coupling of voltage-gated Ca^{2+} channels to RIM and Bassoon proteins in order to regulate the strength of synaptic transmission [77]. Hence, brain RIM-BPs, although not essential for synaptic transmission, they are important in the tight coupling between voltage-gated Ca^{2+} channels and the release machinery [78]. Loss of the main hippocampal isoform RIM-BP2 induces an increase in the distance between Bassoon and the voltage-gated Ca^{2+} channel subunit CaV2.1, which explain the decrease in both the vesicular release probability and the defect in short-term plasticity [83].

Liprin-α

The liprin-α family of proteins was identified by their interaction with LAR-RPTPs (LAR family of receptor protein tyrosine phosphatases) [84, 85]. In vertebrates, there are four liprin-α genes, liprin-α1, -α2, -α3, and -α4, all of which are expressed in the brain, but the α1 and α4 isoforms are also expressed in non-neuronal tissues. Liprin-α amino acid organization suggests the presence of coiled-coil at the N-terminal and three C-terminal SAM domains [84]. The liprin-α N-terminal binds to itself, forming homodimers, or binds to the AZ proteins, RIM, and ELKS [86–88]. The C-terminal of liprin-α binds to liprin-β [89], CASK [90], and LAR-type receptor phosphotyrosine phosphatases [84].

In mature hippocampal synapses, liprin-α2 was found to be a very dynamic protein in comparison with Munc13 and Bassoon, which are very stable [91, 92], and through its interactions with RIM1 and CASK, it regulates presynaptic organization and hence SV release in response to network activity [93, 94]. Elimination of liprin-α2 by knockdown in mature hippocampal neurons does not affect the number of active synapses but does alter the efficiency of SV release by regulating RRP size. An ultrastructural analysis shows lengthening of the synapse and a reduced number of docked vesicles. The presence of liprin-α2 at synapses does not depend on depletion of several AZ proteins [95]. Depletion of liprin-α2 decreases the levels of its direct interactors CASK and RIM and other AZ proteins, such as Bassoon, Rab3, Munc18, VAMP2, and synapsin, and vesicular glutamate transporter VGlut and

P/Q voltage-gated Ca^{2+} channel Cav2.1 are partially diminished [95]. Furthermore, in the absence of liprin-α2, the remaining synaptic CASK and RIM become more stable, suggesting a role in the dynamics of these proteins and consequently SV release efficacy.

In *C. elegans* and *Drosophila* loss of liprin-α produced an increase in the size of AZ and affected SV accumulation [61, 96]. Liprin-α participation in presynaptic assembly will be modulated by its interaction with the LAR-type receptor phosphotyrosine phosphatase PTP-3, thereby stabilizing the active zone, Ca^{2+} channels and SV by linking synaptic cell adhesion to core AZ proteins [88].

In summary, vertebrate liprin-α2 association with the CAZ is regulated by synaptic activity and is a key organizer of mature presynapses and modulates the dynamics of RIM and CASK, which regulate synaptic plasticity. In vertebrates, there are no studies of liprin-α2 depletion before synapses are formed, and hence, a role in the assembly of new presynapses cannot be ruled out.

Munc13

The Munc13 family comprehends the three specific brain isoforms, Munc13-1, bMunc13-2, and Munc13-3 [97]. Additionally, there is a ubiquitously expressed Munc13-2 splice variant called ubMunc13-2 [98]. Munc13-1 and ubMunc13-2 have the same domain structure: (a) N-terminal C_2 domain (C_2A) and a Ca^{2+}/CaM-binding site, (b) a central diacylglycerol and phorbol ester–binding C_1 domain and a second C_2 domain (C_2B), and (c) a C-terminus with a Munc13 homology or minimal Munc13 priming domain and a third C_2 domain (C_2C). The isoform Munc13-1 is the most abundant in the brain, and only 10% of cortical and hippocampal synapses contain both Munc13-1 and bMunc13-2 [99]. Both bMunc13-2 and Munc13-3 differ from the other two isoforms at the N-terminus [100–102].

The two main functions described for Munc13 in neurotransmitter exocytosis are SV priming and modulation of presynaptic plasticity. The first function is accomplished by acting on the SNARE/SM protein fusion machinery, resulting in SV competent for exocytosis. Munc13s prime SVs by acting on syntaxin, which is a t-SNARE (acronym for SNAP Soluble NSF Attachment Protein REceptor). A SNARE complex is formed on the vesicle side by SNAP-25, and syntaxin and synaptobrevin proteins are located at the target synaptic membrane. During SV priming, syntaxin-1 shifts from a closed state that binds Munc18-1 toward an open state conformation that is able to form part of the SNARE complex; this last step is accelerated by Munc13s [103]. The isoforms Munc13-1 and ubMunc13-2 bind to the Zn^{2+} finger region of αRIMs via their conserved N-terminal region [97, 104, 105], thereby forming Munc13-RIM1α-Rab3 complexes which are a requisite for Munc13s-mediated SV priming [70]. Although the recruitment of the primary brain Munc13-1 isoform to AZ requires its interaction with RIM1, the anchoring of bMunc13-2 is mediated by ELKS1α in a small subset of synaptic terminals in hippocampal neurons [99]. This synaptic specific anchoring explains the molecular and functional heterogeneity of presynaptic AZs.

As Munc13 KO hippocampal neurons show normal AZ structure [97], a role in AZ assembly is discarded, but the N-terminal region of Munc13-1 may be the hub for the AZ proteins Piccolo, Bassoon, ELKS, and RIM1, which serve as a core for the physical and functional integrity of the protein machinery at the AZ, thereby orchestrating SV priming [106]. However, all AZ proteins seem to have that characteristic according to their multi-interactions.

Mechanisms Modulating Recruitment of AZ Proteins to Sites of Newly Forming Synapses

AZ Proteins, Cell Soma Packing, and Traffic along the Axon.

The generation and maintenance of functional presynaptic sites require time and site-specific delivery of AZ and SV components. In the last two decades, sustained advances have been made in understanding the mechanisms involved in the transport of presynaptic proteins. Those studies have shown that presynaptic proteins are not transported as individual units, but they travel along axons in groups linked to vesicles originating at the Golgi apparatus. In 1998, Nakata et al. [107] used GFP-tagged proteins and laser scan microscopy to show that putative precursors of SV were transported by tubulovesicular organelles, suggesting that SVs are not transported as a mature unit but are synthesized locally by recycling at the nerve terminal [107]. Ahmari et al. strengthened the idea of precursor vesicles as sources of presynaptic specialization [5]. In their study, they used the synaptic vesicle protein VAMP tagged with GFP and time lapse microscopy combined with DIC imaging to study the dynamics of VAMP-GFP in young hippocampal neurons in culture before synapses were formed [5]. They observed mobile packets that stop at sites of axon-dendrite contacts and analysis by electron microscopy showed that the contact areas contained tubulovesicular structures, dense core vesicles and small pleomorphic vesicles with no resemblance to mature synaptic vesicles [5]. Those contact areas apparently became functional presynaptic recycling sites, as evidenced by FM 4-63 uptake, soon after contacts were formed [5, 108]. Other proteins such as SV2, synapsin I, and calcium channel subunit α1 were found on those packets, suggesting that the AZ in bulk might be transported in the VAMP-GFP labeled packets [5] (Fig. 3). Thereafter, a specific AZ precursor vesicle immunoisolated from young rat brains was identified to transport Piccolo and

Bassoon [8]. This vesicle with an 80-nm diameter had a dense core suggesting the transport of secreted synaptogenic factors, and analysis by western blot identified the presence of a plethora of other AZ proteins [8, 48]. However, no synaptic vesicle proteins were detected, suggesting that this factor was a specific AZ protein transport vesicle [8]. This AZ precursor vesicle, named PTV, has an origin at the *trans*-Golgi network (TGN) where Piccolo, Bassoon, and ELKS are recruited [6, 109, 110]. Additional evidence for the hypothesis of a multivesicular mechanism for presynapse formation came from the studies of Tao-Cheng, who used an ultrastructural analysis to show that AZ and SV proteins are transported together in large aggregates, but they are carried in different types of vesicles [7]. Interestingly, not all AZ proteins seem to be associated with the same vesicle (e.g., Munc13-1α is transported in a different TGN-derived vesicle than Piccolo and Bassoon) [6], and RIM1α only seems to associate with PTVs during axonal trafficking [6]. RIM has also been associated with a vesicle that transports neurexin, CASK, and voltage-dependent Ca^{2+} channels [111] (Fig. 3). Hence, as *golgi*-derived PTVs travel along the axon, they are thought to suffer further maturation before reaching nascent presynapses [6] (Fig. 3).

The traffic of presynaptic precursor vesicles involves motor proteins that transport cargo bidirectionally along actin and microtubule cytoskeletal tracks. Actin employs myosin, and microtubules use kinesin and dynein as motors. The specificity of the transport is provided by a molecular adapter that is part of the vesicle. In the case of PTVs, Bassoon interacts with DLC1 and DLC2 and functions as a cargo adapter for retrograde trafficking of this vesicle [10]. Although PTVs move both anterograde and retrograde, their net movement is anterograde; however, the retrograde movement regulated by Bassoon is necessary for synaptic delivery of AZ proteins [10]. Furthermore, syntabulin, a kinesin-1 family member 5B motor adaptor protein [112], mediates the transport of AZ components through an unknown vesicle adapter during synapse formation and during synaptic plasticity [113].

Thus, the assembly of the presynapse seems to occur by the simultaneous deposition of SV proteins and AZ proteins, which are carried in different types of vesicles (Fig. 3). Precursor vesicles for synaptic vesicles (STVs) and AZ proteins will then be transported in axonal aggregates [7, 114], which co-pause at common axonal sites, probably responding to unidentified local signals specific for each type of vesicle [115]. Therefore, the sites in the axon where these vesicles deliver their content seem to be predefined and independent of the existence of a neuronal contact [114]. The signals determining the stop and clustering of AZ proteins at newly forming synapses remain to be identified.

Homo and Hetero-Oligomerization as a Mechanism of AZ Trapping/Assembly

After biogenesis and axonal trafficking, presynaptic proteins have to be trapped and then maintained at the AZ of mature synapses. The conformation state of the protein might be relevant for its correct trapping and posterior proteome assembly.

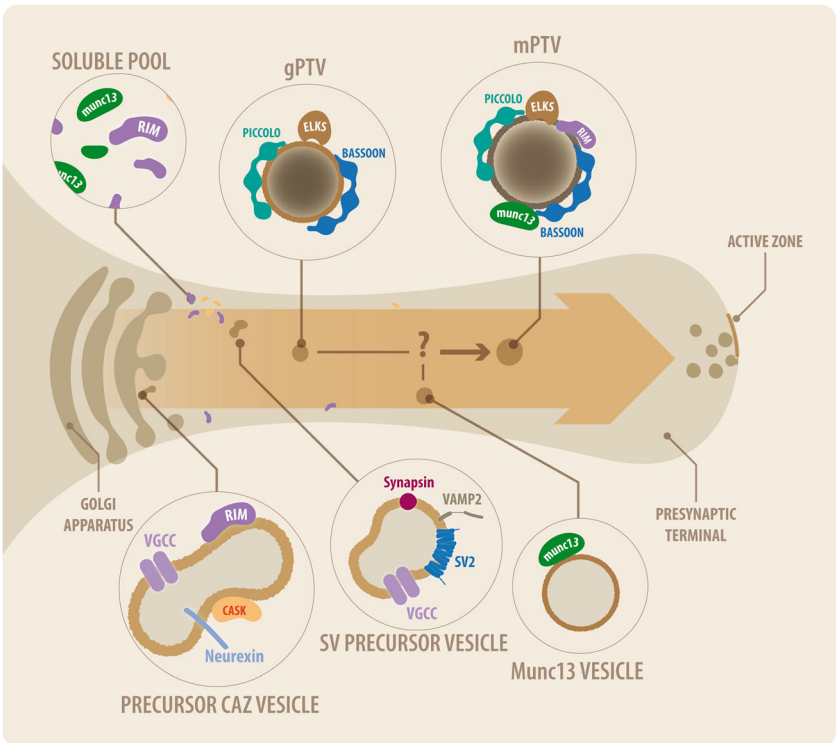

Fig. 3 Schematic representation of the precursor vesicles model of active zone formation. The figure shows the mechanism of axonal transport of several presynaptic proteins during synapse formation. Piccolo, Bassoon, and ELKS exit the trans-Golgi network associated to Golgi-PTV (gPTV), with Munc13 using a different Golgi-derived vesicle. RIM and Munc13 also associate to a soluble pool. During its traffic along the axon, RIM and Munc13 are loaded by an unknown mechanism into gPTV, which turns into mature PTV (mPTV). Synaptic vesicle proteins and other presynaptic protein use different Golgi-derived precursor vesicles with pleomorphic shapes

Several years ago, we proposed a theoretical model of AZ protein trapping [116] that postulates that particular presynaptic proteins undergo a prion-like concentration-dependent conversion, adopting a conformation that will stimulate their own aggregation and aggregation of other proteins. Generally, domains that aggregate in prion proteins are rich in the amino acids glutamine (Q) and asparagine (N). Two vertebrate presynaptic proteins with this characteristic are Piccolo and Bassoon, which have Q-rich areas. Interestingly, Bassoon and Piccolo are known to be homo- and heterodimerizing binding partners [28, 110, 117] and form large aggregates when overexpressed in neurons and heterologous cells [15, 110, 118], and in the case of Bassoon, segments of the protein that do not contain Q domain are unable to interact with the AZ. These presynaptic proteins, with prion-like domains, seem to be under the control of a specific enzyme that modulates their state of aggregation. That is the case with the *Drosophila* AZ protein, bruchpilot, the homolog of ELKS in vertebrates that contains regions rich in Q or Q/N and is essential for the structural integrity of the *Drosophila* AZ. Bruchpilot travels along the axon and is associated with a protein complex that contains the motor adaptor protein Aplip1 [119]. The presence of Aplip1 allows proper transport in axons and avoids premature aggregation of the bruchpilot molecular complex [119], an event regulated by the Serine Arginine Protein Kinase (SRPK)79D. SRPK79D was identified in *Drosophila* by two parallel studies [120, 121] and was found to colocalize with the T-bar-associated protein bruchpilot in both axons as synapses. A mutated SRPK79D causes nerve bruchpilot aggregates in motoneurons [120]. Curiously, although ELKS does not have Q domains, it has a tendency to aggregate if expressed in heterologous cells. Therefore, the presence of coiled-coil domains in its structure, the region homologous to the N-terminus of bruchpilot, might also be the key in its self-clustering and binding to other AZ proteins [48, 49].

A similar oligomerization mechanism has been observed in *C. elegans* because a mutation in a small protein called Arl-8 produces abnormal clustering of Rab-3, UNC-10, and SYD-2 (the last two are homologous to the mammalian RIM and liprin, respectively) close to the cell body, suggesting a premature and abnormal delivery of AZ proteins. Therefore, Arl-8 would be necessary to avoid ectopic aggregation of presynaptic proteins in this nematode [122]. The abnormal accumulation in these mutants was partially suppressed by mutation in a JNK MAP kinase pathway. Hence, in *C. elegans* AZ proteins, aggregation is regulated by the interplay between Arl-8 and the JNK MAP kinase pathway.

Therefore, a conserved and specialized mechanism exists in vertebrate and invertebrate neurons that regulates the temporo-spatial aggregation of particular presynaptic proteins to avoid premature AZ protein interactions, trapping, and assembly, and the regulating molecules need to be identified.

AZ Proteins and Its Relationship with the F-actin Dynamic

Actin is globular and is the most abundant protein in the majority of eukaryotic cells, playing several roles in cells, such as cellular movement, scaffolding, and intracellular trafficking. Actin has the capacity to polymerize and form filamentous-actin (F-actin) by a highly dynamic process that is under the control of diverse known and unknown clues that determine the function of actin in a specific time and space manner. During CNS development, neurons migrate and develop axons and dendrites to build an intricate network of communication. Axon outgrowth occurs until a contact with the appropriate postsynaptic partner triggers formation of a synapse. During all these processes, the actin cytoskeleton and mainly F-actin participates actively. It is well known that actin is present in young presynaptic terminals and is involved in the assembly and development of presynaptic specializations, participating as a primordial scaffold [123–126]. Accordingly, AZ assembly and structure in young neurons but not in old neurons is F-actin dependent, as disrupting agents such as latrunculin block presynapse formation [125]. However, in mature presynapses, F-actin plays primarily a structural role holding SV and preventing its premature non-regulated fusion [124, 127], thereby modulating synaptic transmission and efficacy [128]. Interestingly, AZ and SV precursor vesicles that deposit at newly forming presynapses utilize different and not well-understood mechanisms of F-actin dynamics, indicating the existence of two structurally separate F-actin pools at this location early during synaptogenesis [129].

There is not much knowledge about which AZ proteins participate in presynaptic F-actin assembly. In *C. elegans*, the AZ protein NAB-1/neurabin mislocalizes if F-actin is disassembled by latrunculin [130]. NAB-1/neurabin is an actin-binding protein that recruits AZ proteins SYD-1 and SYD-2 (liprin-α) (core proteins in *C. elegans* AZ assembly) acting as a bridge between F-actin and AZ proteins during synapse development [130]. In the case of vertebrates, Piccolo is the only AZ protein known to be required for the activity-dependent assembly of presynaptic F-actin through its interaction with actin-binding proteins. These proteins are Profilin2 [29], Epac2 [131], Abp1 [15], GIT1 [14], and Daam1 [18]. The role of these interactions has been described in mature synapses where they regulate the delivery and recycling of SVs at the presynaptic terminal [18]. In this respect, it is postulated that Piccolo serves as a platform coordinating the activity of Profilin2, GIT1, and Daam1 with the spatial assembly of F-actin, which is necessary for the recruitment of CaMKII and the regulation of the kinetics of Synapsin1a during activity-dependent exocytosis [18, 20, 132]. In other words, the interaction of Piccolo with these proteins will hold SV at the reserve pool by modulating synapsin I through F-actin assembly [20, 132].

Active Zone and Wnt Signaling

Wnt signaling plays diverse functions in the development of the mature nervous system. During brain development, Wnt proteins play critical roles in cell differentiation, migration, neurite polarization, and synapse assembly and plasticity [133–135]. In the adult nervous system, Wnt signaling is required for synapse maintenance, synaptic activity, and plasticity [136–139]. There are 19 Wnt ligands [140] that activate three alternative signaling pathways: (1) the canonical Wnt/β-catenin pathway, (2) the Wnt-Planar Cell Polarity (Wnt/PCP) pathway, and (3) the Wnt/calcium pathway [134, 141, 142]. All three pathways are activated by the binding of Wnt ligand to a Frizzled (Fz) receptor, which activates intracellular dishevelled (Dvl) protein. In the canonical pathway, the Wnt ligand signals through β-catenin, which enters the nucleus to activate Wnt target genes. In the Wnt/PCP pathway, the Wnt ligand binds to its receptor Fz, thereby activating Dvl, which signals through two independent and parallel pathways activating the small GTPases Rho and Rac. The activation of Rho GTPase occurs through Daam1 leading to the activation of the Rho-associated kinase Rock and consequently cytoskeletal organization [143]. The other pathway signaling through Rac, which in turn activates c-Jun N-terminal kinase (JNK) targeting gene transcription that culminates with the reorganization of the cytoskeleton [144, 145]. In the Wnt/Ca^{2+} pathway, the ligand binds to Fz receptors, activating classical G protein pathways and phospholipase C (PLC), which acts on phosphatidylinositol 4,5-biphospahte (PIP2) and produces diacylglycerol (DAG) and inositol triphosphate (IP_3). This process generates an increase in intracellular Ca^{2+} that activates Ca^{2+}-dependent proteins and the transcription factor nuclear factor associated with T cells (NFAT) to promote the transcription of target genes [139, 146].

Wnt signaling has shown to play a role not only in axon guidance and remodeling but also participates in presynaptic assembly [136] (Table 2). In the cerebellum, granular cells secrete Wnt7a, which induces mossy fiber axonal spreading and branching accompanied by an increase in the clustering of synapsin I [147, 148]. Similar results were observed in rat hippocampal neurons where Wnt7a stimulates the clustering of synaptophysin and induces recycling and exocytosis of SV [149]. The ligand Wnt7b induces clustering of VAMP2 both in mossy fibers and hippocampal neurons as early as 15 min of treatment [150]. The AZ protein Bassoon clustering is increased in 10 DIV hippocampal cultures treated with Wnt7b [150], and Dvl was found to be necessary for the clustering of this AZ protein [150]. Additionally, Wnt3a/Fz1 in hippocampal neurons in culture stimulates the clustering of Bassoon and increases the number of functional presynaptic sites [151]. Interestingly, the Wnt-mediated effect of presynaptic clustering of both synaptic proteins and AZ proteins are observed at 15–30 min, suggesting that immediate local changes induce clustering [149–151].

As mentioned above, there would be two distinct pools of F-actin at new forming presynapses, one that participates in the recruitment of AZ proteins and another that participates in the recruitment of SV proteins during synapse formation [129]. As clustering of AZ proteins and SV proteins is induced both by Wnt3a and Wnt7a and requires Dvl, the Wnt pathway diverges after Dvl, acting specifically on those F-actin pools. Piccolo might act as a link between Wnt signaling and the cytoskeleton because it interacts with AZ proteins, diverse actin-binding proteins (see "Piccolo" section) and with two proteins of the Wnt pathway: (1) Daam1, which has been postulated to modulate actin dynamics through Wnt/PCP signaling [18, 143, 152, 153], and (2) Rho-GEF Trio, which also interacts with Bassoon [19] and is an activator of the Rho family of GTPases [154] and F-actin dynamics. Interestingly, Piccolo interacts with Daam1 only when it is in its open activated conformation [18]. The latter suggests that presynaptic assembly in young neurons and/or synaptic efficacy in mature neurons mediated by Piccolo might be regulated by the Wnt/PCP signaling (Fig. 4).

Nervous system integrity requires that synapse assembly be tightly coordinated with synapse maturation and maintenance. Fulfillment of this requisite depends on precise control of both protein and organelle synthesis and degradation. Some of the postulated mechanisms that account for control of neuronal protein and organelle half-life include involvement of the ubiquitin/proteosomal system [155–157] and autophagy [158, 159]. Another likely requirement includes tight regulation of local calcium levels in order to support neuronal life and synaptic stability [160, 161]. A defect in either of these systems leads to neuronal degeneration. Although it is not the focus of the present work to review the mechanisms that regulate the intracellular processes involved in synapse maintenance the evidence suggests a role for the Wnt pathway. In

Table 2 Wnt ligands inducing synaptic protein clustering

Wnt ligand	Neuron type studied	Synaptic protein clustering	Reference
Wnt7a	Cerebellum granular cells	Synapsin I	[147, 148]
Wnt7a	Hippocampal neurons	Synaptophysin	[149]
Wnt7b	Mossy fiber/hippocampal	VAMP2	[150]
Wnt7b	Hippocampal neurons	Bassoon	[150]
Wnt3a	Hippocampal neurons	Bassoon	[151]

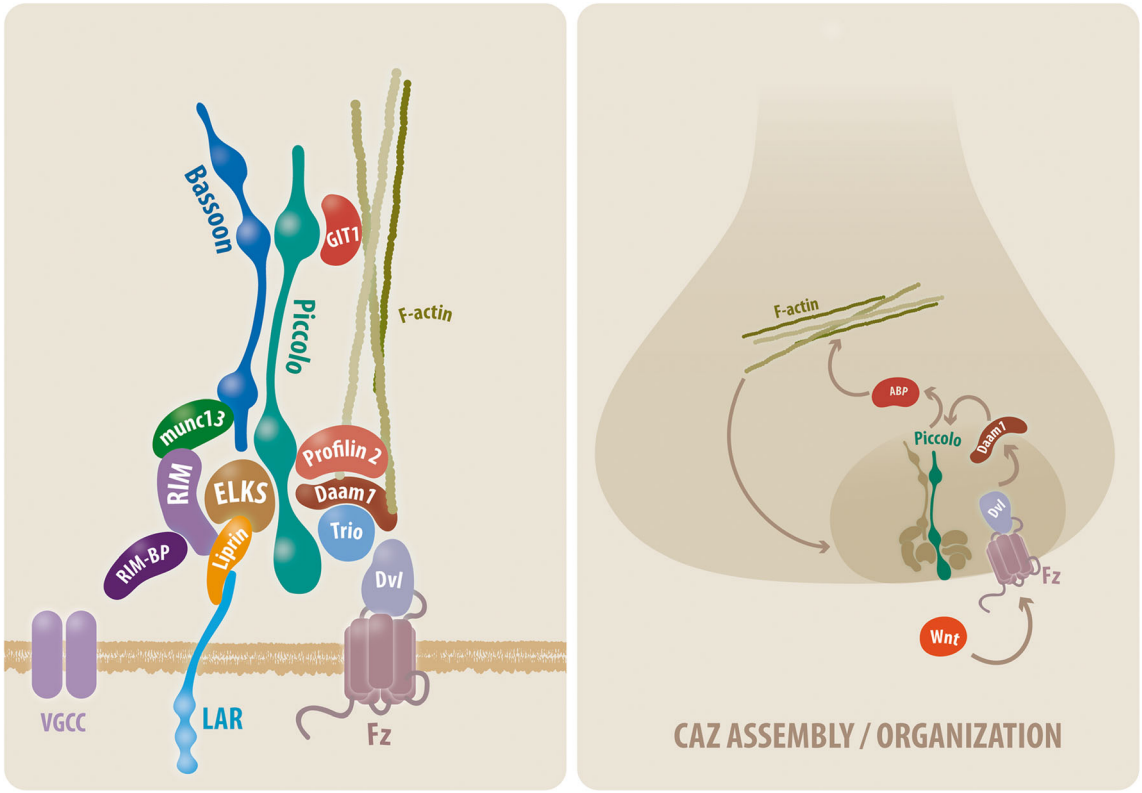

Fig. 4 Piccolo could be an important link between Wnt signaling and presynaptic assembly. In the *left panel* is represented Piccolo as the hub between active zone proteins, F-actin dynamic, and Wnt signaling molecules. The *panel on the right* represents a simplified model of how the Wnt signaling pathway would mediate the assembly of the active zone through Piccolo. Piccolo through its interaction with actin-binding proteins (ABP), and molecules of the Wnt signaling would allow communication of the Wnt signaling with the organization of the active zone

fact, Wnt signaling, in addition to playing a role in the development of the central nervous system, is one of the mechanisms postulated to regulate synaptic stability. It has been shown that neuronal activity promotes the stability of synapses by modulating the levels of endogenous-secreted Wnts [162–165]. Incubation of neurons with Dickkopf, a secreted Wnt pathway antagonist, delocalizes pre- and postsynaptic components in mature and stable hippocampal synapses inducing disassembly of synapses in mature neurons [166]. Also, it was recently reported that Wnt5a is necessary for maintaining dendritic arbor and spines in the adult hippocampus [167]. Future studies are needed to decipher the downstream signals of the Wnt receptors that would control the assembly and maintenance of the synapse.

Concluding Remarks

In vertebrate CNS synapses, there is no protein to which a strict role in AZ assembly can be attributed, mainly because studies of loss of function do not show an evident abnormal presynaptic structure. However, this lack of evidence does not mean that an alteration in one of the AZ proteins could produce an abnormal synaptic function over time. Three presynaptic mechanisms that will contribute to stability of vertebrate central synapses are the following: (1) AZ proteins with overlapping functions, (2) additional isoforms, and (3) the existence of proteins with related structural domains.

In the vertebrate brain, the AZ proteins Piccolo, Bassoon, RIM, RIM-BP, Munc13, liprin-α, and ELKS form an interaction network that gives the structural framework to the CAZ and allows regulated communication between them to respond efficiently to synaptic demands. These proteins also interact temporarily with other molecules that regulate F-actin dynamics and consequently synaptic plasticity according to the prevailing physiological situation. This group of proteins participates directly in the recruitment of Ca^{2+}-channels in which RIM and Munc13 have a more specialized role in docking and priming of SV, and Bassoon with Piccolo will be key in the stability of several presynaptic proteins. Furthermore, among AZ proteins, Piccolo seems to be one of the most versatile component of the CAZ due to its participation in multiple functions at the presynapses. Interestingly, a newly emerging function of some AZ proteins is to communicate with the transcription apparatus in the nucleus to inform the actual synaptic activity.

Therefore, although substantial information has been accumulating about the AZ interactome and functions of its

constituents, there are many questions that remain to be answered, such as the cell signaling pathways regulating the dynamics of these interactions during synapse formation and plasticity, and how a defect in any of these interactions is translated to cognitive impairment in childhood and adulthood.

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Compliance with Ethical Standards

Competing Interests The authors declare that they have no conflicts of interest.

References

1. Alabi AA, Tsien RW (2012) Synaptic vesicle pools and dynamics. Cold Spring Harb Perspect Biol 4:a013680. doi:10.1101/cshperspect.a013680
2. Garner CC, Kindler S, Gundelfinger ED (2000) Molecular determinants of presynaptic active zones. Curr Opin Neurobiol 10:321–327
3. Schoch S, Gundelfinger ED (2006) Molecular organization of the presynaptic active zone. Cell Tissue Res 326:379–391. doi:10.1007/s00441-006-0244-y
4. Torres VI, Vallejo D, Inestrosa NC (2017) Emerging synaptic molecules as candidates in the etiology of neurological disorders. Neural Plast 2017:1–25. doi:10.1155/2017/8081758
5. Ahmari SE, Buchanan J, Smith SJ (2000) Assembly of presynaptic active zones from cytoplasmic transport packets. Nat Neurosci 3:445–451. doi:10.1038/74814
6. Maas C, Torres VI, Altrock WD et al (2012) Formation of Golgi-derived active zone precursor vesicles. J Neurosci 32:11095–11108. doi:10.1523/JNEUROSCI.0195-12.2012
7. Tao-Cheng J-H (2007) Ultrastructural localization of active zone and synaptic vesicle proteins in a preassembled multi-vesicle transport aggregate. Neuroscience 150:575–584. doi:10.1016/j.neuroscience.2007.09.031
8. Zhai RG, Vardinon-Friedman H, Cases-Langhoff C et al (2001) Assembling the presynaptic active zone: a characterization of an active one precursor vesicle. Neuron 29:131–143
9. van den Berg R, Hoogenraad CC (2012) Molecular motors in cargo trafficking and synapse assembly. Advances in experimental medicine and biology, In, pp. 173–196
10. Fejtova A, Davydova D, Bischof F et al (2009) Dynein light chain regulates axonal trafficking and synaptic levels of bassoon. J Cell Biol 185:341–355. doi:10.1083/jcb.200807155
11. Cases-Langhoff C, Voss B, Garner AM et al (1996) Piccolo, a novel 420 kDa protein associated with the presynaptic cytomatrix. Eur J Cell Biol 69:214–223
12. Fenster SD, Chung WJ, Zhai R et al (2000) Piccolo, a presynaptic zinc finger protein structurally related to bassoon. Neuron 25:203–214
13. Gundelfinger ED, Reissner C, Garner CC (2016) Role of bassoon and piccolo in assembly and molecular organization of the active zone. Front Synaptic Neurosci 7:19. doi:10.3389/fnsyn.2015.00019
14. Kim S, Ko J, Shin H et al (2003) The GIT family of proteins forms multimers and associates with the presynaptic cytomatrix protein piccolo. J Biol Chem 278:6291–6300. doi:10.1074/jbc.M212287200
15. Fenster SD, Kessels MM, Qualmann B et al (2003) Interactions between piccolo and the actin/dynamin-binding protein Abp1 link vesicle endocytosis to presynaptic active zones. J Biol Chem 278:20268–20277. doi:10.1074/jbc.M210792200
16. Gerber SH, Garcia J, Rizo J, Südhof TC (2001) An unusual C(2)-domain in the active-zone protein piccolo: implications for Ca(2+) regulation of neurotransmitter release. EMBO J 20:1605–1619. doi:10.1093/emboj/20.7.1605
17. Müller CS, Haupt A, Bildl W et al (2010) Quantitative proteomics of the Cav2 channel nano-environments in the mammalian brain. Proc Natl Acad Sci U S A 107:14950–14957. doi:10.1073/pnas.1005940107
18. Wagh D, Terry-Lorenzo R, Waites CL et al (2015) Piccolo directs activity dependent F-actin assembly from presynaptic active zones via Daam1. PLoS One 10:e0120093. doi:10.1371/journal.pone.0120093
19. Terry-Lorenzo RT, Torres VI, Wagh D et al (2016) Trio, a rho family GEF, interacts with the presynaptic active zone proteins piccolo and bassoon. PLoS One 11:e0167535. doi:10.1371/journal.pone.0167535
20. Leal-Ortiz S, Waites CL, Terry-Lorenzo R et al (2008) Piccolo modulation of synapsin1a dynamics regulates synaptic vesicle exocytosis. J Cell Biol 181:831–846. doi:10.1083/jcb.200711167
21. Waites CL, Leal-Ortiz SA, Andlauer TFM et al (2011) Piccolo regulates the dynamic assembly of presynaptic F-actin. J Neurosci Off J Soc Neurosci 31:14250–14263. doi:10.1523/JNEUROSCI.1835-11.2011
22. Regus-Leidig H, Ott C, Löhner M et al (2013) Identification and immunocytochemical characterization of piccolino, a novel piccolo splice variant selectively expressed at sensory ribbon synapses of the eye and ear. PLoS One 8:e70373. doi:10.1371/journal.pone.0070373
23. Regus-Leidig H, Fuchs M, Löhner M et al (2014) In vivo knockdown of piccolino disrupts presynaptic ribbon morphology in mouse photoreceptor synapses. Front Cell Neurosci 8:259. doi:10.3389/fncel.2014.00259
24. Cochilla AJ, Angleson JK, Betz WJ (1999) Monitoring secretory membrane with fm1-43 fluorescence. Annu Rev Neurosci 22:1–10. doi:10.1146/annurev.neuro.22.1.1
25. Mukherjee K, Yang X, Gerber SH et al (2010) Piccolo and bassoon maintain synaptic vesicle clustering without directly participating in vesicle exocytosis. Proc Natl Acad Sci 107:6504–6509. doi:10.1073/pnas.1002307107
26. Bruckner JJ, Gratz SJ, Slind JK et al (2012) Fife, a Drosophila Piccolo-RIM homolog, promotes active zone organization and neurotransmitter release. J Neurosci 32:17048–17058. doi:10.1523/JNEUROSCI.3267-12.2012
27. Bruckner JJ, Zhan H, Gratz SJ et al (2017) Fife organizes synaptic vesicles and calcium channels for high-probability neurotransmitter release. J Cell Biol 216:231–246. doi:10.1083/jcb.201601098
28. Dieck S, Sanmartí-Vila L, Langnaese K et al (1998) Bassoon, a novel zinc-finger CAG/glutamine-repeat protein selectively localized at the active zone of presynaptic nerve terminals. J Cell Biol 142:499–509
29. Wang X, Kibschull M, Laue MM et al (1999) Aczonin, a 550-kD putative scaffolding protein of presynaptic active zones, shares homology regions with rim and bassoon and binds profilin. J Cell Biol 147:151–162
30. Dieck S, Altrock WD, Kessels MM et al (2005) Molecular dissection of the photoreceptor ribbon synapse. J Cell Biol 168:825–836. doi:10.1083/jcb.200408157

31. Khimich D, Nouvian R, Pujol R et al (2005) Hair cell synaptic ribbons are essential for synchronous auditory signalling. Nature 434:889–894. doi:10.1038/nature03418
32. Regus-Leidig H, Dieck S, Brandstätter JH (2010) Absence of functional active zone protein bassoon affects assembly and transport of ribbon precursors during early steps of photoreceptor synaptogenesis. Eur J Cell Biol 89:468–475. doi:10.1016/j.ejcb.2009.12.006
33. Altrock WD, Dieck S, Sokolov M et al (2003) Functional inactivation of a fraction of excitatory synapses in mice deficient for the active zone protein bassoon. Neuron 37:787–800
34. Hallermann S, Fejtova A, Schmidt H et al (2010) Bassoon speeds vesicle reloading at a central excitatory synapse. Neuron 68:710–723. doi:10.1016/j.neuron.2010.10.026
35. Davydova D, Marini C, King C et al (2014) Bassoon specifically controls presynaptic P/Q-type Ca2+ channels via RIM-binding protein. Neuron 82:181–194. doi:10.1016/j.neuron.2014.02.012
36. Dick O, Dieck S, Altrock WD et al (2003) The presynaptic active zone protein bassoon is essential for photoreceptor ribbon synapse formation in the retina. Neuron 37:775–786
37. Frank T, Rutherford MA, Strenzke N et al (2010) Bassoon and the synaptic ribbon organize Ca^2+ channels and vesicles to add release sites and promote refilling. Neuron 68:724–738. doi:10.1016/j.neuron.2010.10.027
38. Jing Z, Rutherford MA, Takago H et al (2013) Disruption of the presynaptic cytomatrix protein bassoon degrades ribbon anchorage, multiquantal release, and sound encoding at the hair cell afferent synapse. J Neurosci Off J Soc Neurosci 33:4456–4467. doi:10.1523/JNEUROSCI.3491-12.2013
39. Mendoza Schulz A, Jing Z, Sánchez Caro JM et al (2014) Bassoon-disruption slows vesicle replenishment and induces homeostatic plasticity at a CNS synapse. EMBO J 33:512–527. doi:10.1002/embj.201385887
40. Ahmed S, Wittenmayer N, Kremer T et al (2013) Mover is a homomeric phospho-protein present on synaptic vesicles. PLoS One 8:e63474. doi:10.1371/journal.pone.0063474
41. Körber C, Horstmann H, Venkataramani V et al (2015) Modulation of presynaptic release probability by the vertebrate-specific protein mover. Neuron 87:521–533. doi:10.1016/j.neuron.2015.07.001
42. Waites CL, Leal-Ortiz SA, Okerlund N et al (2013) Bassoon and piccolo maintain synapse integrity by regulating protein ubiquitination and degradation. EMBO J 32:954–969. doi:10.1038/emboj.2013.27
43. Ivanova D, Dirks A, Montenegro-Venegas C et al (2015) Synaptic activity controls localization and function of CtBP1 via binding to bassoon and piccolo. The EMBO journal 34:1056–1077. doi:10.15252/embj.201488796
44. Okerlund ND, Schneider K, Leal-Ortiz S et al (2017) Bassoon controls presynaptic autophagy through Atg5. Neuron 93:897–913.e7. doi:10.1016/j.neuron.2017.01.026
45. Chinnadurai G (2009) The transcriptional corepressor CtBP: a foe of multiple tumor suppressors. Cancer Res 69:731–734. doi:10.1158/0008-5472.CAN-08-3349
46. Deguchi-Tawarada M, Inoue E, Takao-Rikitsu E et al (2004) CAST2: identification and characterization of a protein structurally related to the presynaptic cytomatrix protein CAST. Genes Cells Devoted Mol Cell Mech 9:15–23
47. Wang Y, Liu X, Biederer T, Südhof TC (2002) A family of RIM-binding proteins regulated by alternative splicing: implications for the genesis of synaptic active zones. Proc Natl Acad Sci U S A 99:14464–14469. doi:10.1073/pnas.182532999
48. Ohtsuka T, Takao-Rikitsu E, Inoue E et al (2002) 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 158:577–590. doi:10.1083/jcb.200202083
49. Takao-Rikitsu E, Mochida S, Inoue E et al (2004) Physical and functional interaction of the active zone proteins, CAST, RIM1, and bassoon, in neurotransmitter release. J Cell Biol 164:301–311. doi:10.1083/jcb.200307101
50. Ko J, Na M, Kim S et al (2003) Interaction of the ERC family of RIM-binding proteins with the liprin-alpha family of multidomain proteins. J Biol Chem 278:42377–42385. doi:10.1074/jbc.M307561200
51. Monier S, Jollivet F, Janoueix-Lerosey I, et al. (2002) Characterization of novel Rab6-interacting proteins involved in endosome-to-TGN transport. Traffic (Copenhagen, Denmark) 3:289–297.
52. Ko J, Yoon C, Piccoli G et al (2006) Organization of the presynaptic active zone by ERC2/CAST1-dependent clustering of the tandem PDZ protein syntenin-1. J Neurosci Off J Soc Neurosci 26:963–970. doi:10.1523/JNEUROSCI.4475-05.2006
53. Kaeser PS, Deng L, Chávez AE et al (2009) ELKS2alpha/CAST deletion selectively increases neurotransmitter release at inhibitory synapses. Neuron 64:227–239. doi:10.1016/j.neuron.2009.09.019
54. Dieck S, Specht D, Strenzke N et al (2012) Deletion of the presynaptic scaffold CAST reduces active zone size in rod photoreceptors and impairs visual processing. J Neurosci 32:12192–12203. doi:10.1523/JNEUROSCI.0752-12.2012
55. Liu C, Bickford LS, Held RG et al (2014) The active zone protein family ELKS supports Ca2+ influx at nerve terminals of inhibitory hippocampal neurons. J Neurosci Off J Soc Neurosci 34:12289–12303. doi:10.1523/JNEUROSCI.0999-14.2014
56. Kiyonaka S, Nakajima H, Takada Y et al (2012) Physical and functional interaction of the active zone protein CAST/ERC2 and the -subunit of the voltage-dependent Ca2+ channel. J Biochem 152:149–159. doi:10.1093/jb/mvs054
57. Held RG, Liu C, Kaeser PS (2016) ELKS controls the pool of readily releasable vesicles at excitatory synapses through its N-terminal coiled-coil domains. elife. doi:10.7554/eLife.14862
58. Wagh DA, Rasse TM, Asan E et al (2006) Bruchpilot, a protein with homology to ELKS/CAST, is required for structural integrity and function of synaptic active zones in Drosophila. Neuron 49:833–844. doi:10.1016/j.neuron.2006.02.008
59. Kittel RJ, Wichmann C, Rasse TM et al (2006) Bruchpilot promotes active zone assembly, Ca2+ channel clustering, and vesicle release. Science 312:1051–1054. doi:10.1126/science.1126308
60. Deken SL, Vincent R, Hadwiger G et al (2005) Redundant localization mechanisms of RIM and ELKS in *Caenorhabditis elegans*. J Neurosci 25:5975–5983. doi:10.1523/JNEUROSCI.0804-05.2005
61. Dai Y, Taru H, Deken SL et al (2006) SYD-2 Liprin-α organizes presynaptic active zone formation through ELKS. Nat Neurosci 9:1479–1487. doi:10.1038/nn1808
62. Wang Y, Okamoto M, Schmitz F et al (1997) Rim is a putative Rab3 effector in regulating synaptic-vesicle fusion. Nature 388:593–598. doi:10.1038/41580
63. Wang Y, Südhof TC (2003) Genomic definition of RIM proteins: evolutionary amplification of a family of synaptic regulatory proteins. Genomics 81:126–137
64. Mittelstaedt T, Schoch S (2007) Structure and evolution of RIM-BP genes: identification of a novel family member. Gene 403:70–79. doi:10.1016/j.gene.2007.08.004
65. Coppola T, Magnin-Luthi S, Perret-Menoud V et al (2001) Direct interaction of the Rab3 effector RIM with Ca2+channels, SNAP-25, and synaptotagmin. J Biol Chem 276:32756–32762. doi:10.1074/jbc.M100929200
66. Gandini MA, Felix R (2012) Functional interactions between voltage-gated Ca2+ channels and Rab3-interacting molecules (RIMs): new insights into stimulus–secretion coupling. Biochim Biophys Acta Biomembr 1818:551–558. doi:10.1016/j.bbamem.2011.12.011

67. Schoch S, Castillo PE, Jo T et al (2002) RIM1alpha forms a protein scaffold for regulating neurotransmitter release at the active zone. Nature 415:321–326. doi:10.1038/415321a
68. Castillo PE, Schoch S, Schmitz F et al (2002) RIM1α is required for presynaptic long-term potentiation. Nature 415:327–330. doi:10.1038/415327a
69. Powell CM, Schoch S, Monteggia L et al (2004) The presynaptic active zone protein RIM1alpha is critical for normal learning and memory. Neuron 42:143–153
70. Deng L, Kaeser PS, Xu W, Südhof TC (2011) RIM proteins activate vesicle priming by reversing autoinhibitory homodimerization of Munc13. Neuron 69:317–331. doi:10.1016/j.neuron.2011.01.005
71. Gracheva EO, Hadwiger G, Nonet ML, Richmond JE (2008) Direct interactions between *C. elegans* RAB-3 and rim provide a mechanism to target vesicles to the presynaptic density. Neurosci Lett 444:137–142. doi:10.1016/j.neulet.2008.08.026
72. Koushika SP, Richmond JE, Hadwiger G et al (2001) A post-docking role for active zone protein rim. Nat Neurosci 4:997–1005. doi:10.1038/nn732
73. Han Y, Babai N, Kaeser P et al (2015) RIM1 and RIM2 redundantly determine Ca2+ channel density and readily releasable pool size at a large hindbrain synapse. J Neurophysiol 113:255–263. doi:10.1152/jn.00488.2014
74. Kaeser PS, Deng L, Wang Y et al (2011) RIM proteins tether Ca2+ channels to presynaptic active zones via a direct PDZ-domain interaction. Cell 144:282–295. doi:10.1016/j.cell.2010.12.029
75. Graf ER, Valakh V, Wright CM et al (2012) RIM promotes calcium channel accumulation at active zones of the Drosophila neuromuscular junction. J Neurosci Off J Soc Neurosci 32:16586–16596. doi:10.1523/JNEUROSCI.0965-12.2012
76. Lu J, Machius M, Dulubova I et al (2006) Structural basis for a Munc13–1 homodimer to Munc13–1/RIM heterodimer switch. PLoS Biol 4:e192. doi:10.1371/journal.pbio.0040192
77. Hibino H, Pironkova R, Onwumere O et al (2002) RIM binding proteins (RBPs) couple Rab3-interacting molecules (RIMs) to voltage-gated Ca(2+) channels. Neuron 34:411–423
78. Acuna C, Liu X, Gonzalez A, Südhof TC (2015) RIM-BPs mediate tight coupling of action potentials to Ca2+−triggered neurotransmitter release. Neuron 87:1234–1247. doi:10.1016/j.neuron.2015.08.027
79. Acuna C, Liu X, Südhof TC (2016) How to make an active zone: unexpected universal functional redundancy between RIMs and RIM-BPs. Neuron 91:792–807. doi:10.1016/j.neuron.2016.07.042
80. Muller M, Liu KSY, Sigrist SJ, Davis GW (2012) RIM controls homeostatic plasticity through modulation of the readily-releasable vesicle pool. J Neurosci 32:16574–16585. doi:10.1523/JNEUROSCI.0981-12.2012
81. Müller M, Genç Ö, Davis GW (2015) RIM-binding protein links synaptic homeostasis to the stabilization and replenishment of high release probability vesicles. Neuron 85:1056–1069. doi:10.1016/j.neuron.2015.01.024
82. Wang Y, Sugita S, Sudhof TC (2000) The RIM/NIM family of neuronal C2 domain proteins. Interactions with Rab3 and a new class of Src homology 3 domain proteins. J Biol Chem 275:20033–20044. doi:10.1074/jbc.M909008199
83. Grauel MK, Maglione M, Reddy-Alla S et al (2016) RIM-binding protein 2 regulates release probability by fine-tuning calcium channel localization at murine hippocampal synapses. Proc Natl Acad Sci U S A 113:11615–11620. doi:10.1073/pnas.1605256113
84. Serra-Pagès C, Kedersha NL, Fazikas L et al (1995) The LAR transmembrane protein tyrosine phosphatase and a coiled-coil LAR-interacting protein co-localize at focal adhesions. EMBO J 14:2827–2838
85. Serra-Pagès C, Medley QG, Tang M et al (1998) Liprins, a family of LAR transmembrane protein-tyrosine phosphatase-interacting proteins. J Biol Chem 273:15611–15620
86. Taru H, Jin Y (2011) The liprin homology domain is essential for the homomeric interaction of SYD-2/liprin- protein in presynaptic assembly. J Neurosci 31:16261–16268. doi:10.1523/JNEUROSCI.0002-11.2011
87. Ko J, Na M, Kim S et al (2003) Interaction of the ERC family of RIM-binding proteins with the liprin—family of multidomain proteins. J Biol Chem 278:42377–42385. doi:10.1074/jbc.M307561200
88. Dai Y, Taru H, Deken SL et al (2006) SYD-2 liprin-alpha organizes presynaptic active zone formation through ELKS. Nat Neurosci 9:1479–1487. doi:10.1038/nn1808
89. Astigarraga S, Hofmeyer K, Farajian R, Treisman JE (2010) Three Drosophila liprins interact to control synapse formation. J Neurosci Off J Soc Neurosci 30:15358–15368. doi:10.1523/JNEUROSCI.1862-10.2010
90. Olsen O, Moore KA, Fukata M et al (2005) Neurotransmitter release regulated by a MALS–liprin-α presynaptic complex. J Cell Biol 170:1127–1134. doi:10.1083/jcb.200503011
91. Kalla S, Stern M, Basu J et al (2006) Molecular dynamics of a presynaptic active zone protein studied in Munc13-1-enhanced yellow fluorescent protein knock-in mutant mice. J Neurosci 26:13054–13066. doi:10.1523/JNEUROSCI.4330-06.2006
92. Tsuriel S, Fisher A, Wittenmayer N et al (2009) Exchange and redistribution dynamics of the cytoskeleton of the active zone molecule bassoon. J Neurosci 29:351–358. doi:10.1523/JNEUROSCI.4777-08.2009
93. Spangler SA, Jaarsma D, De Graaff E et al (2011) Differential expression of liprin-α family proteins in the brain suggests functional diversification. J Comp Neurol 519:3040–3060. doi:10.1002/cne.22665
94. Wei Z, Zheng S, Spangler SA et al (2011) Liprin-mediated large signaling complex organization revealed by the liprin-α/CASK and liprin-α/liprin-β complex structures. Mol Cell 43:586–598. doi:10.1016/j.molcel.2011.07.021
95. Spangler SA, Schmitz SK, Kevenaar JT et al (2013) Liprin-α2 promotes the presynaptic recruitment and turnover of RIM1/CASK to facilitate synaptic transmission. J Cell Biol 201:915–928. doi:10.1083/jcb.201301011
96. Zhen M, Jin Y (1999) The liprin protein SYD-2 regulates the differentiation of presynaptic termini in *C. elegans*. Nature 401:371–375. doi:10.1038/43886
97. Augustin I, Rosenmund C, Südhof TC, Brose N (1999) Munc13-1 is essential for fusion competence of glutamatergic synaptic vesicles. Nature 400:457–461. doi:10.1038/22768
98. Junge HJ, Rhee J-S, Jahn O et al (2004) Calmodulin and Munc13 form a Ca2+ sensor/effector complex that controls short-term synaptic plasticity. Cell 118:389–401. doi:10.1016/j.cell.2004.06.029
99. Kawabe H, Mitkovski M, Kaeser PS et al (2017) ELKS1 localizes the synaptic vesicle priming protein bMunc13-2 to a specific subset of active zones. J Cell Biol 216:1143–1161. doi:10.1083/jcb.201606086
100. Brose N, Hofmann K, Hata Y, Südhof TC (1995) Mammalian homologues of *Caenorhabditis elegans* unc-13 gene define novel family of C2-domain proteins. J Biol Chem 270:25273–25280
101. Koch H, Hofmann K, Brose N (2000) Definition of Munc13-homology-domains and characterization of a novel ubiquitously expressed Munc13 isoform. Biochem J 349:247–253
102. Basu J, Shen N, Dulubova I et al (2005) A minimal domain responsible for Munc13 activity. Nat Struct Mol Biol 12:1017–1018. doi:10.1038/nsmb1001
103. Ma C, Li W, Xu Y, Rizo J (2011) Munc13 mediates the transition from the closed syntaxin–Munc18 complex to the SNARE complex. Nat Struct Mol Biol 18:542–549. doi:10.1038/nsmb.2047

104. Betz A, Thakur P, Junge HJ et al (2001) Functional interaction of the active zone proteins Munc13-1 and RIM1 in synaptic vesicle priming. Neuron 30:183–196
105. Dulubova I, Lou X, Lu J et al (2005) A Munc13/RIM/Rab3 tripartite complex: from priming to plasticity? EMBO J 24:2839–2850. doi:10.1038/sj.emboj.7600753
106. Wang X, Hu B, Zieba A et al (2009) A protein interaction node at the neurotransmitter release site: domains of aczonin/piccolo, bassoon, CAST, and rim converge on the N-terminal domain of Munc13-1. J Neurosci Off J Soc Neurosci 29:12584–12596. doi:10.1523/JNEUROSCI.1255-09.2009
107. Nakata T, Terada S, Hirokawa N (1998) Visualization of the dynamics of synaptic vesicle and plasma membrane proteins in living axons. J Cell Biol 140:659–674
108. Friedman HV, Bresler T, Garner CC, Ziv NE (2000) Assembly of new individual excitatory synapses: time course and temporal order of synaptic molecule recruitment. Neuron 27:57–69
109. Shapira M, Zhai RG, Dresbach T et al (2003) Unitary assembly of presynaptic active zones from piccolo-bassoon transport vesicles. Neuron 38:237–252
110. Dresbach T, Torres V, Wittenmayer N et al (2006) Assembly of active zone precursor vesicles: obligatory trafficking of presynaptic cytomatrix proteins bassoon and piccolo via a trans-Golgi compartment. J Biol Chem 281:6038–6047
111. Fairless R, Masius H, Rohlmann A et al (2008) Polarized targeting of neurexins to synapses is regulated by their C-terminal sequences. J Neurosci 28:12969–12981. doi:10.1523/JNEUROSCI.5294-07.2008
112. Su Q, Cai Q, Gerwin C et al (2004) Syntabulin is a microtubule-associated protein implicated in syntaxin transport in neurons. Nat Cell Biol 6:941–953. doi:10.1038/ncb1169
113. Cai Q, Pan P-Y, Sheng Z-H (2007) Syntabulin-kinesin-1 family member 5B-mediated axonal transport contributes to activity-dependent presynaptic assembly. J Neurosci 27:7284–7296. doi:10.1523/JNEUROSCI.0731-07.2007
114. Sabo SL, Gomes RA, McAllister AK (2006) Formation of presynaptic terminals at predefined sites along axons. J Neurosci 26:10813–10825. doi:10.1523/JNEUROSCI.2052-06.2006
115. Bury LA, Sabo SL (2011) Coordinated trafficking of synaptic vesicle and active zone proteins prior to synapse formation. Neural Dev 6:24. doi:10.1186/1749-8104-6-24
116. Fernandez F, Torres V, Zamorano P (2010) An evolutionarily conserved mechanism for presynaptic trapping. Cell Mol Life Sci 67:1751–1754
117. Dresbach T, Hempelmann A, Spilker C et al (2003) Functional regions of the presynaptic cytomatrix protein bassoon: significance for synaptic targeting and cytomatrix anchoring. Mol Cell Neurosci 23:279–291
118. Jose M, Nair DK, Altrock WD et al (2008) Investigating interactions mediated by the presynaptic protein bassoon in living cells by Foerster's resonance energy transfer and fluorescence lifetime imaging microscopy. Biophys J 94:1483–1496. doi:10.1529/biophysj.107.111674
119. Siebert M, Böhme MA, Driller JH et al (2015) A high affinity RIM-binding protein/Aplip1 interaction prevents the formation of ectopic axonal active zones. elife. doi:10.7554/eLife.06935
120. Nieratschker V, Schubert A, Jauch M et al (2009) Bruchpilot in ribbon-like axonal agglomerates, behavioral defects, and early death in SRPK79D kinase mutants of Drosophila. PLoS Genet 5:e1000700. doi:10.1371/journal.pgen.1000700
121. Johnson EL, Fetter RD, Davis GW (2009) Negative regulation of active zone assembly by a newly identified SR protein kinase. PLoS Biol 7:e1000193. doi:10.1371/journal.pbio.1000193
122. Klassen MP, Wu YE, Maeder CI et al (2010) An Arf-like small G protein, ARL-8, promotes the axonal transport of presynaptic cargoes by suppressing vesicle aggregation. Neuron 66:710–723. doi:10.1016/j.neuron.2010.04.033
123. Dai Z, Peng HB (1996) Dynamics of synaptic vesicles in cultured spinal cord neurons in relationship to synaptogenesis. Mol Cell Neurosci 7:443–452. doi:10.1006/mcne.1996.0032
124. Bernstein BW, DeWit M, Bamburg JR (1998) Actin disassembles reversibly during electrically induced recycling of synaptic vesicles in cultured neurons. Brain Res Mol Brain Res 53:236–251
125. Zhang W, Benson DL (2001) Stages of synapse development defined by dependence on F-actin. J Neurosci Off J Soc Neurosci 21:5169–5181
126. Nelson JC, Stavoe AKH, Colón-Ramos DA (2013) The actin cytoskeleton in presynaptic assembly. Cell Adhes Migr 7:379–387. doi:10.4161/cam.24803
127. Wang XH, Zheng JQ, Poo MM (1996) Effects of cytochalasin treatment on short-term synaptic plasticity at developing neuromuscular junctions in frogs. J Physiol:187–195
128. Kim CH, Lisman JE (1999) A role of actin filament in synaptic transmission and long-term potentiation. J Neurosci Off J Soc Neurosci 19:4314–4324
129. Bleckert A, Photowala H, Alford S (2012) Dual pools of actin at presynaptic terminals. J Neurophysiol 107:3479–3492. doi:10.1152/jn.00789.2011
130. Chia PH, Patel MR, Shen K (2012) NAB-1 instructs synapse assembly by linking adhesion molecules and F-actin to active zone proteins. Nat Neurosci 15:234–242. doi:10.1038/nn.2991
131. Fujimoto K, Shibasaki T, Yokoi N et al (2002) Piccolo, a Ca2+ sensor in pancreatic beta-cells. Involvement of cAMP-GEFII.Rim2. Piccolo complex in cAMP-dependent exocytosis. J Biol Chem 277:50497–50502. doi:10.1074/jbc.M210146200
132. Waites CL, Garner CC (2011) Presynaptic function in health and disease. Trends Neurosci 34:326–337. doi:10.1016/j.tins.2011.03.004
133. Dickins EM, Salinas PC (2013) Wnts in action: from synapse formation to synaptic maintenance. Front Cell Neurosci 7:162. doi:10.3389/fncel.2013.00162
134. Inestrosa NC, Varela-Nallar L (2014) Wnt signaling in the nervous system and in Alzheimer's disease. J Mol Cell Biol 6:64–74. doi:10.1093/jmcb/mjt051
135. Varela-Nallar L, Alfaro IE, Serrano FG et al (2010) Wingless-type family member 5A (Wnt-5a) stimulates synaptic differentiation and function of glutamatergic synapses. Proc Natl Acad Sci 107:21164–21169. doi:10.1073/pnas.1010011107
136. Inestrosa NC, Arenas E (2010) Emerging roles of Wnts in the adult nervous system. Nat Rev Neurosci 11:77–86. doi:10.1038/nrn2755
137. Budnik V, Salinas PC (2011) Wnt signaling during synaptic development and plasticity. Curr Opin Neurobiol 21:151–159. doi:10.1016/j.conb.2010.12.002
138. Cerpa W, Gambrill A, Inestrosa NC, Barria A (2011) Regulation of NMDA-receptor synaptic transmission by Wnt signaling. J Neurosci Off J Soc Neurosci 31:9466–9471. doi:10.1523/JNEUROSCI.6311-10.2011
139. Oliva CA, Vargas JY, Inestrosa NC (2013) Wnts in adult brain: from synaptic plasticity to cognitive deficiencies. Front Cell Neurosci 7:224. doi:10.3389/fncel.2013.00224
140. Toledo EM, Colombres M, Inestrosa NC (2008) Wnt signaling in neuroprotection and stem cell differentiation. Prog Neurobiol 86:281–296. doi:10.1016/j.pneurobio.2008.08.001
141. Logan CY, Nusse R (2004) The Wnt signaling pathway in development and disease. Annu Rev Cell Dev Biol 20:781–810. doi:10.1146/annurev.cellbio.20.010403.113126
142. Gordon MD, Nusse R (2006) Wnt signaling: multiple pathways, multiple receptors, and multiple transcription factors. J Biol Chem 281:22429–22433. doi:10.1074/jbc.R600015200

143. Habas R, Kato Y, He X (2001) Wnt/Frizzled activation of rho regulates vertebrate gastrulation and requires a novel formin homology protein Daam1. Cell 107:843–854
144. Rosso SB, Sussman D, Wynshaw-Boris A, Salinas PC (2005) Wnt signaling through Dishevelled, Rac and JNK regulates dendritic development. Nat Neurosci 8:34–42. doi:10.1038/nn1374
145. Rosso SB, Inestrosa NC (2013) WNT signaling in neuronal maturation and synaptogenesis. Front Cell Neurosci 7:103. doi:10.3389/fncel.2013.00103
146. Inestrosa NC, Montecinos-Oliva C, Fuenzalida M (2012) Wnt signaling: role in Alzheimer disease and schizophrenia. J NeuroImmune Pharmacol 7:788–807. doi:10.1007/s11481-012-9417-5
147. Lucas FR, Salinas PC (1997) WNT-7a induces axonal remodeling and increases synapsin I levels in cerebellar neurons. Dev Biol 192:31–44. doi:10.1006/dbio.1997.8734
148. Hall AC, Lucas FR, Salinas PC (2000) Axonal remodeling and synaptic differentiation in the cerebellum is regulated by WNT-7a signaling. Cell 100:525–535
149. Cerpa W, Godoy JA, Alfaro I et al (2007) Wnt-7a modulates the synaptic vesicle cycle and synaptic transmission in hippocampal neurons. J Biol Chem 283:5918–5927. doi:10.1074/jbc.M705943200
150. Ahmad-Annuar A, Ciani L, Simeonidis I et al (2006) Signaling across the synapse: a role for Wnt and dishevelled in presynaptic assembly and neurotransmitter release. J Cell Biol 174:127–139. doi:10.1083/jcb.200511054
151. Varela-Nallar L, Grabowski CP, Alfaro IE et al (2009) Role of the Wnt receptor Frizzled-1 in presynaptic differentiation and function. Neural Dev 4:41. doi:10.1186/1749-8104-4-41
152. Liu W, Sato A, Khadka D et al (2008) Mechanism of activation of the formin protein Daam1. Proc Natl Acad Sci U S A 105:210–215. doi:10.1073/pnas.0707277105
153. Young KG, Copeland JW (2010) Formins in cell signaling. Biochimica et Biophysica Acta (BBA) - Molecular Cell Research 1803:183–190. doi:10.1016/j.bbamcr.2008.09.017
154. Bateman J, Van Vactor D (2001) The trio family of guanine-nucleotide-exchange factors: regulators of axon guidance. J Cell Sci 114:1973–1980
155. Mabb AM, Ehlers MD (2010) Ubiquitination in postsynaptic function and plasticity. Annu Rev Cell Dev Biol 26:179–210. doi:10.1146/annurev-cellbio-100109-104129
156. Fernández-Chacón R, Wölfel M, Nishimune H et al (2004) The synaptic vesicle protein CSP alpha prevents presynaptic degeneration. Neuron 42:237–251
157. Wheeler TC, Chin L-S, Li Y et al (2002) Regulation of synaptophysin degradation by mammalian homologues of seven in absentia. J Biol Chem 277:10273–10282. doi:10.1074/jbc.M107857200
158. Iwata A, Christianson JC, Bucci M et al (2005) Increased susceptibility of cytoplasmic over nuclear polyglutamine aggregates to autophagic degradation. Proc Natl Acad Sci U S A 102:13135–13140. doi:10.1073/pnas.0505801102
159. Pandey UB, Nie Z, Batlevi Y et al (2007) HDAC6 rescues neurodegeneration and provides an essential link between autophagy and the UPS. Nature 447:859–863. doi:10.1038/nature05853
160. Kumar A, Bodhinathan K, Foster TC (2009) Susceptibility to calcium dysregulation during brain aging. Front Aging Neurosci 1:2. doi:10.3389/neuro.24.002.2009
161. Bezprozvanny I (2009) Calcium signaling and neurodegenerative diseases. Trends Mol Med 15:89–100. doi:10.1016/j.molmed.2009.01.001
162. Yu X, Malenka RC (2003) β-catenin is critical for dendritic morphogenesis. Nat Neurosci 6:1169–1177. doi:10.1038/nn1132
163. Chen J, Park CS, Tang S-J (2006) Activity-dependent synaptic Wnt release regulates hippocampal long term potentiation. J Biol Chem 281:11910–11916. doi:10.1074/jbc.M511920200
164. Wayman GA, Impey S, Marks D et al (2006) Activity-dependent dendritic arborization mediated by CaM-kinase I activation and enhanced CREB-dependent transcription of Wnt-2. Neuron 50:897–909. doi:10.1016/j.neuron.2006.05.008
165. Gogolla N, Galimberti I, Deguchi Y, Caroni P (2009) Wnt signaling mediates experience-related regulation of synapse numbers and mossy fiber connectivities in the adult hippocampus. Neuron 62:510–525. doi:10.1016/j.neuron.2009.04.022
166. Purro SA, Dickins EM, Salinas PC (2012) The secreted Wnt antagonist Dickkopf-1 is required for amyloid β-mediated synaptic loss. J Neurosc Off J Soc Neurosci 32:3492–3498. doi:10.1523/JNEUROSCI.4562-11.2012
167. Chen C-M, Orefice LL, Chiu S-L et al (2017) Wnt5a is essential for hippocampal dendritic maintenance and spatial learning and memory in adult mice. Proc Natl Acad Sci U S A 114:E619–E628. doi:10.1073/pnas.1615792114