HHS Public Access

Author manuscript

*Curr Opin Neurobiol*. Author manuscript; available in PMC 2021 August 01.

Published in final edited form as:

*Curr Opin Neurobiol*. 2020 August ; 63: 95–103. doi:10.1016/j.conb.2020.03.008.

# Assembly of the presynaptic active zone

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## Abstract

In a presynaptic nerve terminal, the active zone is composed of sophisticated protein machinery that enables secretion on a sub-millisecond time scale and precisely targets it towards postsynaptic receptors. The past two decades have provided deep insight into the roles of active zone proteins in exocytosis, but we are only beginning to understand how a neuron assembles active zone protein complexes into effective molecular machines. In this review, we outline the fundamental processes that are necessary for active zone assembly and discuss recent advances in understanding assembly mechanisms that arise from genetic, morphological and biochemical studies. We further outline the challenges ahead for understanding this important problem.

## Introduction

Transmission and processing of information in the brain mostly occurs at synapses, sites of communication between neurons at which a presynaptic nerve terminal contacts a postsynaptic cell. Synaptic transmission is initiated by the fusion of neurotransmitter-containing synaptic vesicles with the plasma membrane. This fusion step is mediated by a set of conserved fusion proteins including SNAREs, their regulators, and $Ca^{2+}$ sensors. While presynaptic fusion rates are low at rest, they dramatically increase upon opening of voltage-gated $Ca^{2+}$ channels in response to action potential firing. Synaptic vesicle exocytosis is executed within less than one millisecond upon presynaptic depolarization, is precisely targeted towards postsynaptic receptors, is remarkably plastic, and is heterogenous across synapse and neuronal types. These important features of synaptic transmission are mediated by sophisticated protein machinery called the active zone (Box 1) [1,2].

In this review, we discuss recent progress and current models of the mechanisms of active zone assembly. The focus of this review is on “primary” active zone proteins, which we narrowly define as the proteins that are preferentially localized to the active zone membrane despite the lack of transmembrane domains, and function together in coupling vesicle fusion to $Ca^{2+}$ influx through $Ca^{2+}$ channels (Box 1, Figure 1). These proteins include RIM, Munc13, Bassoon/Piccolo, Liprin-α, ELKS and RIM-BP. Notably, this definition does not

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Conflict of interest declaration for “Assembly of the presynaptic active zone”, Javier Emperador-Melero and Pascal S. Kaeser Nothing to declare.

include SNARE proteins or $Ca^{2+}$ channels, but instead highlights that one active zone function is to control the relative positioning of these essential proteins [1,2]. We further define active zone assembly as the process that positions and maintains these proteins in a complex that supports exocytotic functions at the presynaptic plasma membrane. Active zone assembly and function are closely linked. Any given component may have assembly roles in recruiting other proteins to the active zone and direct roles in mediating exocytosis of vesicles. The following steps are essential for active zone assembly (Figure 2):

- the generation of active zone proteins, their sorting into axonal transport, and their capture in a nerve terminal
- the assembly of these active zone constituents into protein complexes
- the positioning and anchoring of these complexes at the presynaptic target membrane precisely opposed to postsynaptic receptors

These steps are interconnected and do not necessarily occur in the same order for each protein. We highlight recent progress in dissecting each of these steps, first focusing on the best understood step, assembly of the protein complex, and we explore key challenges that lie ahead.

## The active zone protein complex and its assembly

Superresolution microscopy has revolutionized the characterization of active zone protein complexes and uncovered striking patterning of the active zone. In the vertebrate brain, the large scaffold Bassoon is oriented such that its C-terminus is close to the presynaptic plasma membrane, and its N-terminus reaches tens of nanometers into the presynaptic cytoplasm [3]. Other active zone proteins localize between the Bassoon N- and C-termini [4*], indicating that they are part of the same complex. A similar pattern occurs at the fly neuromuscular junction, where Bruchpilot, a partial homologue of ELKS, is oriented with its N-terminus close to the target membrane and the other presynaptic proteins are organized around it [5,6]. In addition to the patterning perpendicular to the target membrane, the active zone is also patterned laterally.

Active zones of hippocampal excitatory synapses contain several clusters of the scaffold RIM, and these clusters mark individual release sites [7**]. Munc13-1 also forms clusters that correlate with individual secretory sites at hippocampal synapses [8**] and at the fly neuromuscular junction [9,10]. This lateral patterning was also observed in freeze-fracture immuno-electron microscopic experiments, in which Munc13 and $Ca^{2+}$ channel clusters are detected within sub-areas of the active zone [11*]. Hence, morphological studies indicate that the active zone is a highly patterned structure with multiple protein complexes that may be variable in composition within and across synapses.

Current mechanistic insight into assembly of active zone complexes strongly rely on gene knockout studies (Table 1). The major message from these studies is that the active zone is a resilient structure. Specifically, ablation of individual active zone proteins, or of active zone protein families, typically results in at most mild assembly deficits, illustrated by impaired localization of select interaction partners. At hippocampal synapses, for example, removing RIMs leads to reduced levels of Munc13-1 [12] and of $Ca_V2$ $Ca^{2+}$ channels [13]. For some

proteins, these functions differ strikingly across synapse types. Three examples are (1) knockout of RIM-BP2 that leads to reduced Munc13-1 levels specifically at mossy fiber synapses [14], (2) a recruitment function of ELKS1 for ubMunc13-2 that is restricted to a small subset of forebrain synapses [15], and roles for RIM in Bassoon clustering in dopamine axons but not at hippocampal synapses [13,16]. Synapse-specific assembly roles may also be revealed in functional phenotypes. Functions of ELKS, for example, differ between excitatory and inhibitory transmission, which may reflect distinct roles in presynaptic assembly [17]. In contrast to these generally mild effects on active zone structure at typical small central synapses, there are morphologically unique synapses with prominent dense projections. There, single proteins often account for the majority of the density, and genetic ablation of the main component leads to a loss of these densities [18-20]. Disrupting these electron densities, however, does not fully abolish exocytosis, suggesting that machinery for secretion remains at least partially intact. The overall resilience of active zone structure indicates that redundant protein interactions within the active zone account for its stability.

An important step forward in understanding redundancy in active zone assembly came from the analysis of compound mutants [21*, 22*,23*]. Simultaneous knockout of RIM and ELKS induced a strong disruption of active zone complexes with loss of Munc13-1, Bassoon, Piccolo, RIM-BP2, and $Ca_V2.1$ $Ca^{2+}$ channels, accompanied by a loss of vesicle docking and tethering [22*]. This study also indicated that the proteins that are concurrently reduced upon RIM and ELKS ablation indeed participate in the same protein complex. In similar studies, ablation of RIM and RIM-BP led to impaired targeting of $Ca^{2+}$ channels at vertebrate synapses and the C.elegans neuromuscular junction [21*,23*], and at mouse hippocampal synapses it was accompanied by loss of Munc13-1, Bassoon, electron dense material at the target membrane and vesicle docking and tethering [21*]. Hence, active zone scaffolding is redundant, and essential scaffolding roles are mediated by the cooperative action of at least RIM, ELKS and RIM-BP.

Liprin-α proteins are important for presynapse assembly in invertebrates [24,25], and localized to active zones at hippocampal synapses [4*]. Interestingly, despite this colocalization with other active zone proteins, the presynaptic levels of Liprin-α were not reduced by the compound RIM/ELKS or RIM/RIM-BP mutants with disrupted active zone structure [4*,21*,22*]. One possibility is that Liprin-α is not part of the same protein complex within an active zone, and hence it does not behave like other active zone proteins in these mutants. Another possibility is that Liprin-α is an early active zone member that recruits other proteins. The latter model is supported by genetic studies in invertebrates. Liprin-α operates upstream of other active zone proteins in synapse formation in C.elegans [26]. At the fly neuromuscular junction, Liprin-α is important for the recruitment of a specific Munc13 isoform [10], and may also control the transport of presynaptic material [25,27]. Vertebrate genetic experiments for Liprin-α, however, are incomplete because they only removed one of the four Liprin-α genes [4*], and future studies should address this limitation.

Assessment of interactions of active zone proteins has long been used to generate models for active zone assembly [1]. As outlined above, however, knockout of single active zone

proteins, or of active zone proteins from the same family, has overall mild effects on active zone structure. Hence, individual protein interactions are unlikely to have a major role in assembly. A new model of active zone assembly that does justice to this point has been proposed. The observation that RIM, RIM-BP and $Ca^{2+}$ channels form droplet-like condensates in vitro led to the model that active zone assembly relies on liquid-liquid phase separation principles [28**]. Here, individual interactions are less important, but instead multiple low affinity interactions drive active zone formation. This mechanism may also be more broadly important for synapse assembly, as vesicle clustering [29] and the assembly of postsynaptic densities [30] may also be mediated by it. The model of phase separation for synapse assembly is attractive because it allows for dynamic regulation of local protein content and enables movement of proteins and vesicles within the assemblies. However, major challenges lie ahead to further develop this model, with the most important question being whether phase condensates form in vivo at synapses.

## Anchoring of the active zone at the target membrane

Active zone protein complexes are anchored at the presynaptic plasma membrane opposed to postsynaptic specializations. At excitatory synapses, alignment between active zone complexes and AMPA receptors is executed with remarkable precision, within trans-synaptic nanodomains of ~80 nm in diameter [7**]. Notably, none of the primary active zone proteins has a transmembrane domain, indicating that other molecules must control active zone anchoring and alignment. A recent review [2] speculated on mechanisms for alignment. Here, we discuss plausible models of active zone anchoring. We reason that molecules for the control of anchoring must fulfill three requirements: (1) interaction with the plasma membrane, (2) direct or indirect binding to primary active zone proteins, and (3) localization at the active zone membrane. Three general candidates that may fulfill these criteria are synaptic cell adhesion molecules, ion channels, and membrane lipids.

Synaptic cell adhesion molecules are strong candidates because of their ability to recruit presynaptic material in heterologous synapse formation assays. In these experiments, the accumulation of presynaptic material in nerve terminals formed onto non-neuronal cells expressing a postsynaptic cell adhesion molecule is measured. Amongst presynaptic candidates, neurexins and Leukocyte common Antigen-Related Receptor Protein Tyrosine Phosphatases (LAR-RPTPs) have received significant attention [31]. Neurexins are localized to the active zone, where they form sparse, small clusters [32*]. However, it is unclear how they interact with primary active zone proteins, and deletion of all major neurexins in mice generates a range of heterogenous and synapse-specific phenotypes in synapse formation and function [33], but strong impairments in active zone assembly have not been described. These data make it unlikely that neurexins are the key membrane anchors on their own.

Evidence in support of roles for LAR-RPTPs in anchoring includes the direct binding to Liprin-α [34], their established role in invertebrate presynaptic formation and function [25,35,36], and also potential roles in presynapse formation in cultured mouse neurons [37,38]. In addition to potential anchoring roles, LAR-RPTPs may also conduct functions upstream, as indicated by the observations that fly and worm LAR-RPTPs, and their active zone interaction partner Liprin-α, are involved in axon targeting [35,36,39]. A recent study

found that simultaneous deletion of three LAR genes in mice, PTPRF, PTPRD and PTPRS didn't noticeably impair neurotransmitter release [40], suggesting that these three genes are unlikely to account for all active zone anchoring on their own. Future studies should address nanoscale localization of the endogenous isoforms, and pursue genetic deletion of additional LAR-RPTPs or other potentially redundant anchors, accompanied by characterization of active zone assembly. Additional adhesion molecules such as Syncams, ephrins, cadherins, teneurins and FLRTs have also been implicated in presynaptic development [2,31]. However, specific interactions and roles of these proteins for active zone assembly are not established.

$Ca^{2+}$ channels are plausible candidates for active zone membrane attachment. They could anchor active zone components because they directly bind to them [13], preferentially localize to the presynaptic plasma membrane [2*,11*], and loss- or gain-of-function studies suggest roles in controlling presynaptic structure [41-43]. Anchoring active zones via $Ca^{2+}$ channels would make intuitive sense because it would inherently lead to close proximity of active zone proteins and the channels, an important parameter for synaptic function [2]. At the fly neuromuscular junction, however, the active zone component Brp persisted and remained localized opposed to postsynaptic receptors upon removing α2δ-3, an important $Ca^{2+}$ channel subunit [43]. This argues against a central role of the $Ca^{2+}$ channel protein complex in active zone anchoring at this synapse.

Finally, a complementary hypothesis is that protein interactions with plasma membrane lipids mediate active zone anchoring. The phospholipid phosphatidylinositol-4,5-bisphosphate ($PIP_2$), an important regulator of the synaptic vesicle cycle, binds to RIM, Munc13 and other secretory proteins [44*,45]. In non-neuronal cells, $PIP_2$ is present in small microdomains of ~70 nm [46], similar to the size of presynaptic nanodomains [7**]. However, most interactions of $PIP_2$ with presynaptic proteins necessitate $Ca^{2+}$, indicating that these interactions mediate activity-related processes rather than anchoring. One exception is the $Ca^{2+}$-independent binding of RIM $C_2B$ domains to $PIP_2$ [44*]. While this interaction is important for exocytosis [44*,47], it is not required for RIM-positioning at the active zone as detected at the resolution of STED microscopy [44*]. Hence, $PIP_2$ – RIM interactions are either not necessary for anchoring, or the defects in the distribution of presynaptic RIM proteins are too subtle to be detected.

In summary, no protein interaction or protein family has been found to be essential for membrane anchoring. The most parsimonious working model is that many interactions between active zone proteins and components of the target membrane redundantly contribute to membrane anchoring. In this model, low affinity interactions or liquid-liquid phase separation can also contribute to membrane anchoring [28**].

## Axonal transport and presynaptic capture of active zone proteins

Active zone protein constituents are made and packaged in the soma, sorted into and transported along the axon, and captured at nerve terminals. Despite their central importance, these are the least understood processes in active zone assembly.

Initial work proposed that active zone precursors form in the soma and are transported as discrete units or together with other presynaptic proteins [48,49]. However, the content of

these precursor complexes and how many different precursors exist has remained uncertain. Independently of the complex assembly location, diverse vesicular carriers are likely to regulate the transport of presynaptic components [48,50]. Such carriers may have different cellular origins, and delivery of active zone components may involve interaction with endo-lysosomal pathways [51,52]. The existence of different carriers makes it plausible that different scaffold components are shipped at different developmental time points. Remarkably, the generation of presynaptic material is controlled as early as at the time of nuclear mRNA export [53], which may constitute a very early mechanism of assembly regulation.

A central role for cargo assembly has been shown for the small GTPase arl-8. Loss-of-function mutants in C.elegans lead to premature deposition of presynaptic assemblies in proximal axons [54], and strongly impair axonal transport and presynaptic morphology at the fly neuromuscular junction [51]. Arl-8 associates with the major axonal motor KIF1A to promote anterograde axonal transport and this function is antagonized by the JNK kinase pathway that promotes presynaptic assemblies and suppresses anterograde transport [55]. Interestingly, active zone proteins themselves may promote axonal transport. Liprin-α, for example, has been isolated in a complex with KIF1A [56], and its loss leads to reduced anterograde delivery of presynaptic cargo [27]. A model arises in which regulatory mechanisms exist to promote presynaptic assemblies and simultaneously inhibit their anterograde transport, or vice versa, leading to push-and-pull regulation.

Another important question is how presynaptic material is captured and unlinked from microtubular transport upon arrival in nerve terminals. This could be stochastic, as is possible for dense-core vesicles [57], or local mechanisms in a nerve terminal could actively capture presynaptic cargo. In either case, there must be mechanisms to bias cargo unloading from microtubular transport to nerve terminals as opposed to other axonal compartments. One attractive mechanism of biasing cargo unloading to en passant boutons arises from local control of microtubular dynamics [58**]. This mechanism is rooted in the observation that microtubules are highly dynamic and rich in GTP-tubulin within nerve terminals, and that KIF1A binds less strongly to GTP-tubulin than to GDP-tubulin. Hence, weak motor-microtubule interactions in presynaptic boutons may induce cargo unloading.

Ultimately, better insight into the delivery mechanisms of presynaptic material will be key to understanding active zone assembly, as many questions remain open. How are active zone components shipped to and captured at synapses? Are active zone proteins transported individually or as pre-assembled units? Do all active zone components arrive simultaneously or are they sequentially delivered? If they arrive sequentially, do they have a role in capturing the remaining components? Recent improvements in live imaging and genetic tagging of endogenous proteins may prove instrumental to tackle these questions.

## Outlook

Research has started to shed light on how active zones are assembled, but the understanding is incomplete and lags behind studies of active zone protein function. Throughout this short review, we have discussed plausible working models for the transport, assembly and membrane anchoring of the active zone protein complex, along with pressing questions that

remain open. The key overall goals for future research on active zone assembly beyond these points should include the following:

- *A quantitative assessment of local protein content and protein interactions at an active zone.* Experiments may include determining copy numbers of each protein per active zone, and assessing their assembly in a synaptic context and at atomic resolution. Complementary approaches to determine which components follow phase separation principles within a synapse should also be developed.
- *Assessing the relationship of the active zone to other important presynaptic assemblies.* We have narrowly focused on primary active zone proteins. It will be important to assess how they are connected to other important molecular assemblies, for example the presynaptic cytoskeleton, the endocytotic apparatus, or the machinery that controls local protein homeostasis, and how these various presynaptic machines contribute to assembly and function of one another.
- *Testing how active zones differ across synapses and change in response to activity.* Structural heterogeneity of synapses is essential to control brain function. We are only at the beginning of understanding the diversity of active zone structure and function, and how principles of diversity are employed by synapses during plasticity.

Further developing the research on active zone assembly will allow for a deeper understanding of synapse function, how it contributes to the computational power of the brain, and how these processes are affected in neurological disease.

## Acknowledgments

Work on active zone assembly and function in the Kaeser laboratory is supported by the National Institutes of Health (R01NS083898, R01NS103484 and R01MH113349 to P.S.K.). We apologize to our colleagues that we could not cite all important active zone work due to space restrictions. We thank Dr. Shan Shan Wang for electron micrographs presented in Figure 1a and all members of the Kaeser laboratory for insightful discussions.

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## Highlights

We are only beginning to understand the mechanisms of active zone assembly
Trafficking, protein complex assembly and membrane anchoring mechanisms are needed
Ablation of individual active zone proteins has mild effects on assembly Assembly mechanisms are highly redundant and no master organizer controls it Liquid-liquid phase separation principles may control active zone assembly

**Box 1.**

## Active zone definitions

The most common active zone definitions have relied on function, morphology or protein biochemistry (also see Figure 1).

**Function.**

The active zone is the specialized area of the presynaptic plasma membrane where synaptic vesicles fuse [59]. Because the presynaptic plasma membrane contains several release sites [2,7**,60], uncertainty in the terminology arises because a presynaptic release site could be considered an independent active zone, or an active zone could contain multiple independent release sites.

**Morphology.**

The active zone has been defined in electron microscopic studies [61,62], where it appears as electron-dense material attached to the presynaptic plasma membrane and opposed to the postsynaptic density. Often, the active zone membrane is associated with electron dense cytoplasmic protrusions, or dense projections. These projections are variable in shape and prominent at some synapses, for example ribbon synapses in the inner ear or retina, or the fly neuromuscular junction [1].

**Biochemistry.**

The active zone is an insoluble protein complex anchored to the presynaptic plasma membrane [1]. Many proteins are present in this complex, including large scaffolding proteins that are preferentially localized to the active zone area (RIM, RIM-BP, ELKS, Bassoon/Piccolo, Liprin-α and Munc13). Additional important proteins that are present but often not restricted to the active zone are SNARE proteins and their regulators, channels and receptors, cell-adhesion proteins, and cytoskeletal proteins.

**Combinations of criteria.**

Recently, superresolution microscopy is used to describe the active zone combining various criteria. For example, nano-scale localization of RIM or Munc13 and of exocytotic events has led to the identification of multiple individual release sites within an active zone [7**,8**]. Similarly, biochemical and morphological parameters have been combined to localize protein complexes that contain multiple active zone members in an ordered fashion [4*,5].

In this review, we use the term active zone to define the presynaptic membrane specialization opposed to the postsynaptic density that contains multiple individual sites for vesicle docking and release. For simplicity, we focus on the primary components of the presynaptic protein complexes that contain RIM, RIM-BP, Munc13, ELKS, Liprin-α, and Piccolo/Bassoon [1]. We further discuss the association of these proteins with important components of the presynaptic plasma membrane, for example $Ca^{2+}$ channels and cell adhesion molecules, but do not include these proteins in the narrow active zone definition that we use here.

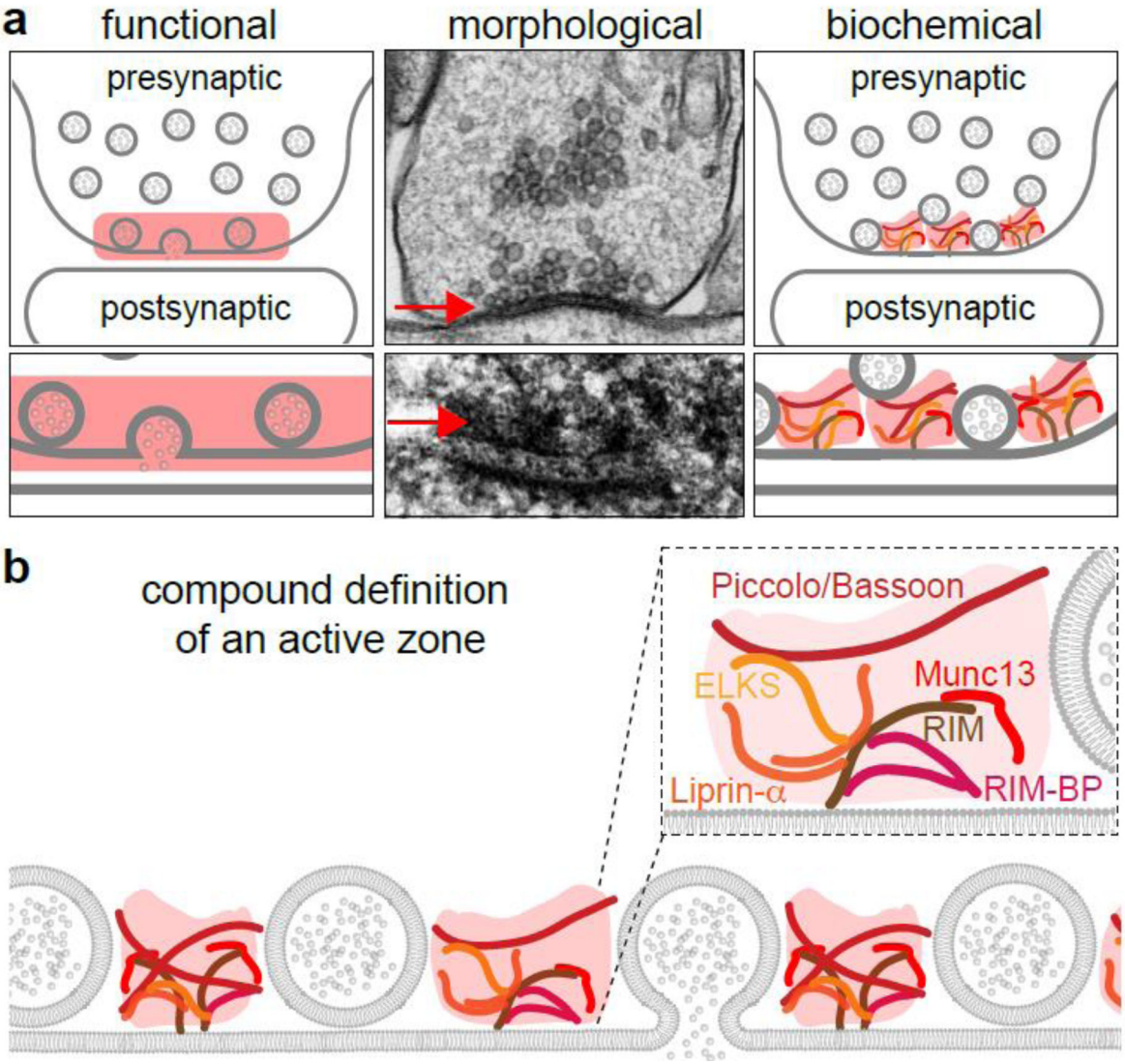


**Figure 1: Active zone definitions and protein content.**

**a**. In the past, multiple active zone definitions (Box 1) have been used. These are summarized in panel a) with overviews (top) and zoom-ins (bottom). A functional definition (left) is depicted as the area of the membrane where vesicles fuse. Synapse electron micrographs (middle, example images from cultured hippocampal neurons) have been used to describe the active zone morphologically (red arrows), where it appears as electron dense material upon phospho-tungstic acid staining (bottom). Biochemical purifications have isolated a detergent insoluble presynaptic protein complex, which has led to a biochemical definition of the active zone (right). **b**. For this review, we use a combination of criteria to define the active zone. In this compound definition, an active zone contains multiple individual sites for exocytosis, and protein complexes formed by RIM, RIM-BP, ELKS, Liprin-α, Munc-13, and Piccolo/Bassoon. These proteins assemble into molecular machines that mediate and regulate synaptic vesicle exocytosis.

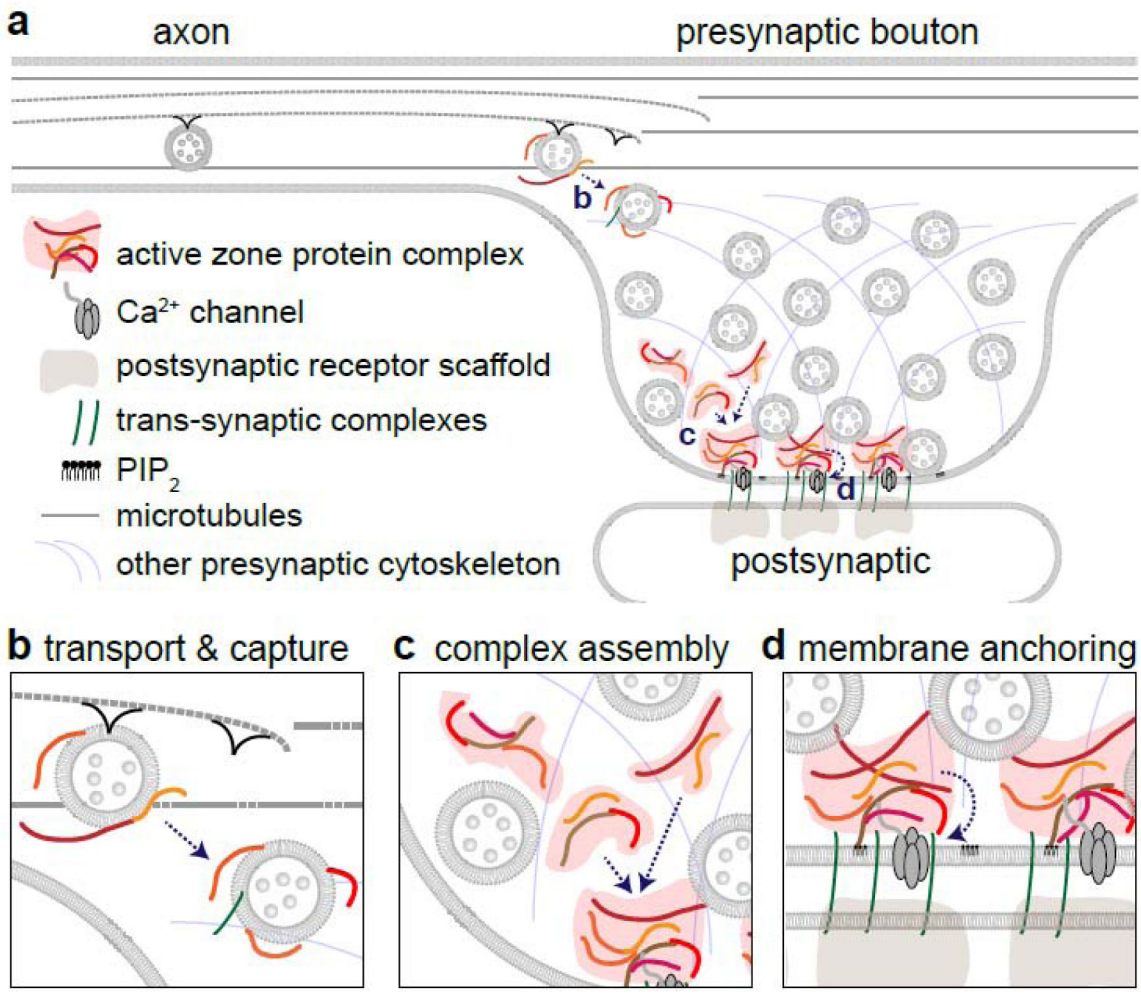


**Figure 2: Main processes in active zone assembly.**

**a - d**. Overview (a) and zoom-ins (b-d) of the main processes that are necessary for active zone assembly. These processes include the transport of active zone material and its capture in presynaptic nerve terminals (b), the assembly of the material into an active zone protein complex (c), and the anchoring of this complex at the target membrane (d). These steps may occur in a variable order for different active zone proteins or subcomplexes, and future research should continue to dissect each of these processes.

**Table 1.**

Key assembly phenotypes in active zone protein knockouts.

| Protein | Model & references | Protein deleted | Secretion | Changes in presynaptic ultrastructure | Changes in active zone protein levels and/or localization |
|---|---|---|---|---|---|
| **RIM (and related proteins)** | C. elegans NMJ [47,63] | unc-10 | ↓↓ | increased total vesicle numbers, reduced numbers of docked vesicles (CF and HPF) | n.a. |
| | C. elegans NMJ [64*] | Clarinet | ↓ | ~25% decreased synapse area and numbers of synaptic vesicles, and 25-50% increased number of docked vesicles (HPF) | ~20% reduction in Syd2-GFP (IF) |
| | Drospohila NMJ [65*] | Fife | ↓↓ | ~20% reduction in T-bar width and ~50% reduction in docked synaptic vesicles (CF and HPF) | Impaired organization of Bruchpilot (IFS) and ~20% reduction in $Cac^{GFP}$ intensity (IF) |
| | Mouse hippocampal neurons 12,13] | RIM1α,β, RIM2α,β,γ | ↓↓↓ | ~50% reduction of docked vesicles without defects on bouton size or vesicle numbers (CF) | ~50% reductions in $Ca_V2.1$ and Munc13-1 (IF) |
| **Liprin- α** | C. elegans NMJ [24] | Syd2 | n.a. | ~100% increase in active zone length (CF) | n.a. |
| | Drosophila NMJ [25] | dLiprin-α | ↓↓ | ~100% increase in active zone area (CF) | n.a. |
| | Mouse hippocampal neurons [4*] | Liprin-α3 | ↓ | ~25% reduction in docked vesicles (CF and HPF) | ~20% decrease in Munc13-1 (IFS) |
| **ELKS** | Drosophila NMJ [5,18] | Bruchpilot | ↓↓ docked vesicles (CF and HPF) | Loss of T-bars, ~25% decrease in | 25-50% reduction in $Cac^{GFP}$ accumulation (IF and IFS) |
| | Mouse hioppocampal neurons [17,66] | ELKS 1α, ELKS2α | ↓↓ | No changes observed (CF) | Impaired $Ca^{2+}$ entry at synapses, but $Ca_V2$ localization unimpaired (IF) |
| | Mouse hippocampus and cortex [15] | ELKS 1α, ELKS2α | n.a. | n.a. | Impaired recruitment of bMunc13-2 to a subset of synapses (IF) |
| **RIM-BP** | Drosophila NMJ [16] | dRBP | ↓↓↓ | Partial loss of T-bar (CF, HPF) | ~25% reduction in accumulation of $Cac^{GFP}$ (IF or IFS) |
| | Mouse hippocampal synapses [14] | RIM-BP2 | ↓↓ (mossy fiber), → other synapses | ~25% docking deficit selectively at mossy fiber synapses (HPF) | ~50% loss of Munc13-1 clusters selectively at mossy fiber synapses (IFS) |
| **Munc13** | C. elegans NMJ [67] | Unc13 | ↓↓↓ | ~100% increase in docking and vesicle numbers (CF) | n.a. |
| | Drosophila NMJ | Dunc13 | ↓↓↓ [68] | ~100% increase in docking, ~25% increase in vesicle numbers (CF) | n.a. |
| | Mouse hippocampal slice cultures [69] | Munc13-1, Munc13-2 | n.a. | Loss of tightly docked vesicles (HPF) | n.a. |
| **Piccolo/ Bassoon** | Mouse cortical neurons [70] | Piccolo and Bassoon * | → | ~25% decrease in docking and vesicle numbers (CF) | n.a. |
| | Mouse hippocampal neurons [71] | Bassoon | ↓ | n.a. | ~25% decrease in $Ca_V2.1$ and RIM-BP2 (IF) |

| Protein | Model & references | Protein deleted | Secretion | Changes in presynaptic ultrastructure | Changes in active zone protein levels and/or localization |
|---|---|---|---|---|---|
| **RIM/ ELKS** | Mouse hippocampal neurons | RIM1α,β, RIM2α,β,γ ELKS1α, ELKS2α [4*,22*] | ↓↓ | Loss of docked and tethered vesicles (CF and HPF) loss of ~50% of RIM-BP2 and $Ca_V2.1$ (IF, WB, IFS) | Loss of most Munc13-1, Bassoon, and Piccolo, |
| **RIM/RIM-BP** | C elegans NMJ and sensory neurons [23*] | unc-10, Rimb-1 | n.a. | n.a. | >50% decrease in GFP-unc-2 ($Ca_V2$ homologue) puncta, and loss of GFP-unc-2 clustering (IF) |
| | Mouse hippocampal neurons [21*] | RIM1α,β, RIM2α,β,γ RIM-BP1, RIM-BP2 | ↓↓↓ | Loss of docked and tethered vesicles, impaired dense projections (CF) | Loss of most Munc13-1 and of >50% of Bassoon (WB) |

This table summarizes effects of selected genetic ablation experiments with a focus on mutations that detected changes in active zone structure. Effects on neurotransmitter release, presynaptic ultrastructure and active zone composition are indicated. For release, phenotypes are described as downward arrows (from↓ to ↓↓↓ depending on the strength of the impairment), or as horizontal arrows (→) when no phenotype was detected.

NMJ = neuromuscular junction, IF = immunofluorescence followed by diffraction limited light microscopy, IFS = Immunofluorescence followed by superresolution microscopy, WB = western blot, CF = electron microscopy upon chemical fixation, HPF = electron microscopy upon high pressure freezing, n.a. = no data available in the cited papers,

* Bassoon levels were lowered by knockdown of RNA in this study.