Regulation of Presynaptic Release Machinery by Cell Adhesion Molecules

Motokazu Uchigashima, Yasunori Hayashi, and Kensuke Futai

Abstract The synapse is a highly specialized asymmetric structure that transmits and stores information in the brain. The size of pre- and postsynaptic structures and function is well coordinated at the individual synapse level. For example, large postsynaptic dendritic spines have a larger postsynaptic density with higher α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor (AMPAR) number on their surface, while juxtaposing presynaptic terminals have a larger active zone and higher release probability. This indicates that pre- and postsynaptic domains bidirectionally communicate to coordinate assembly of specific molecules on both sides of the synaptic cleft. Cell adhesion molecules (CAMs) that localize at synapses form transsynaptic protein interactions across the synaptic cleft and play important roles in synapse formation and regulation. The extracellular domain of CAMs is essential for specific synapse formation and function. In contrast, the intracellular domain is necessary for binding with synaptic molecules and signal transduction. Therefore, CAMs play an essential role on synapse function and structure. In fact, ample evidence indicates that transsynaptic CAMs instruct and modulate functions at presynaptic sites. This chapter focuses on transsynaptic protein interactions that regulate presynaptic functions emphasizing the role of neuronal CAMs and the intracellular mechanism of their regulation.

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1 Introduction

The synapse is a highly specialized asymmetric structure that transmits and stores information in the brain. The majority of synapses in the central nervous system (CNS) are chemical synapses, which are physically separated into pre- and postsynaptic structures by the synaptic cleft. Although these structures are discrete sites with specific molecular machinery, their functions are well coordinated at the individual synapse level. For example, in excitatory synapses on hippocampal and cortical neurons, large postsynaptic dendritic spines have a larger postsynaptic density with a greater number of α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPARs) on the surface. At the same time, juxtaposing presynaptic terminals have a larger active zone, more docked vesicles, and higher release probability. Intuitively, these observations suggest that larger synapses contribute to high-fidelity synaptic transmission [1–7]. During long-term potentiation (LTP) of synaptic transmission, a persistent expansion of postsynaptic dendritic spines [8, 9] is accompanied by an enlargement of presynaptic structures [10] indicating that pre- and postsynaptic sites coordinate with one another to bring about structural changes. Such coordination of pre- and postsynaptic structure and function ensures more efficient transmission.

Modifications to synaptic elements are not limited in the anterograde direction in which the presynaptic side instructs postsynaptic structure and function. Rather, recent mounting evidence indicates that postsynaptic sites can also retrogradely instruct presynaptic changes. For example, it has been described in cortical and hippocampal circuits that postsynaptic neurons retrogradely regulate presynaptic release probability [11–19]. Hippocampal CA3 neurons project Schaffer collateral fibers and form excitatory synapses with both CA1 pyramidal neurons and inhibitory interneurons. Importantly, the same Schaffer collateral excitatory inputs have different release probabilities depending on the type of postsynaptic neurons they synapse with, indicating that there are target neuron-specific retrograde signals that dictate presynaptic function [12, 14]. Diffusible molecules such as endocannabinoids are considered target cell-specific retrograde messengers (reviews are available from other groups [20, 21]). In addition, recent studies have revealed that CAM-mediated protein complexes also regulate target cell-specific presynaptic function.

During CNS development, CAMs play vital roles in synapse specification and formation by establishing transsynaptic interactions between axonal and dendritic segments [22]. In matured synapses, CAMs are essential for synapse function, plasticity, and maintenance [23–25]. Numerous CAMs, such as cadherin, neuroligin, neurexin, extracellular leucine-rich repeat fibronectin type

III domain-containing protein (Elfn), ephrin, SynCAM, delta glutamate (GluD) receptor, and neuronal pentraxin molecules, generate a vast array of possible combinations between pre- and postsynaptic CAMs [22, 23]. In addition to canonical interactions between pre- and postsynaptic CAMs, non-canonical interactions between presynaptic G-protein-coupled receptors (GPCRs) and postsynaptic CAMs have also been identified [22]. Although some specific transsynaptic interactions of CAMs have been reported to underlie distinct synaptic properties [26–28], elucidating synaptic CAM complexes that dictate synapse identity and function remains a major challenge. Recent multidisciplinary studies integrating electrophysiology, imaging, and mouse genetics have revealed that two CAM-mediated canonical and CAM- and receptor-mediated non-canonical transsynaptic interactions regulate presynaptic functions [22, 23].

Long-term potentiation (LTP) is a phenomenon in which a transient burst of synaptic input causes a long-lasting increase in subsequent synaptic transmission [29]. It is well established that LTP induction requires postsynaptic depolarization combined with the activation of *N*-methyl-*D*-aspartate receptors (NMDARs), and resultant influx of calcium (Ca^{2+}). This triggers a series of biochemical processes including the activation of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII). Expression of LTP is achieved by increasing the number of AMPA receptors (AMPARs) at the synapse through activity-dependent change in AMPAR trafficking and persistent expansion of synaptic structures, which is known as structural LTP (sLTP) [9, 30, 31]. This indicates that structural elements, including CAMs on both sides of the synaptic cleft, coordinate the assembly of synaptic proteins for activity-dependent structural changes.

In this chapter, we first describe CAMs that regulate presynaptic release machinery. Next, we discuss possible mechanisms underlying CAM-mediated regulation of synapse function and structure during plasticity. Finally, we discuss the possibility of an activity-dependent mechanism that sub-synaptically segregates different CAMs.

2 Roles of Transsynaptic Adhesion Molecules in Presynaptic Functions

Presynaptic cell adhesion molecules (CAMs) directly regulate the presynaptic neurotransmitter release via direct interaction with the active zone proteins. Postsynaptic CAMs can retrogradely modulate presynaptic neurotransmitter release through interacting with presynaptic CAMs and GPCRs, which regulate the presynaptic neurotransmitter release via direct or indirect interaction with the transmitter release machinery. This section summarizes the basic properties of several CAMs such as Neurexin, Cadherin, Elfn, and Ephrin, and their functional roles in the neurotransmitter release.

2.1 Neurexin-Mediated Transsynaptic Signaling

Neurexins (Nrxns) were isolated as a family of brain membrane surface proteins that bind α-latrotoxin, a neurotoxin from black widow spider (*Latrodectus mactans*) that functions as a potent trigger for neurotransmitter release [32]. Nrxns are encoded by three genes (Nrxn1–3) and transcribed into longer α (αNrxn1, αNrxn2, αNrxn3), shorter β (βNrxn1, βNrxn2, βNrxn3), and Nrxn1-specific γ (γNrxn1) isoforms each under different promoter [33, 34]. Nrxns are a single transmembrane molecule composed of an extracellular domain carrying an isoform-specific N-terminus and conserved transmembrane and intracellular domains. Extracellularly, Nrxns have various number of LNS (laminin, neurexin, sex-hormone-binding protein) and EGF (epidermal growth factor)-like domains due to extensive alternative splicing, which can generate thousands of Nrxn isoforms [35–39]. Through these domains, Nrxns can bind to specific postsynaptic binding partners [40–42]. Neuroligins (Nlgns) [43, 44], LRRTMs (leucine-rich repeat transmembrane neuronal proteins) [45, 46], $GABA_A$ receptors [47], cerebellins [48, 49], C1q-like (C1ql) proteins [50], SPARCL1 (secreted protein acidic and rich in cysteines 1, also referred to as Hevin) [51], and latrophilins [52] can bind to the sixth LNS domain present in both αNrxns and βNrxns. Interestingly, a variety of molecules critical for synaptogenesis have been reported to bind to specific Nrxn isoforms. For example, neurexophilins [53] and dystroglycan [54] bind to the second LNS domain specific to αNrxns. lgSF21 can promote presynaptic differentiation of inhibitory synapses through the first LNS domain of αNrxn2 [55]. C1ql2/3 can interact with the fifth splicing site of α/βNrxn3, and recruit kainate receptors to synaptic sites [50]. The extracellular domain of αNrxns may further interact with presynaptic α2δ-1 auxiliary subunit of P/Q-type Ca^{2+} channels (Cav2.1) in a cis-configuration, limiting the mobility of a2δ-1 subunits on the cell surface rather than forming a stable complex with α2δ-1 subunits [56]. Intracellularly, Nrxns bind to CASK (mLin-2) and Mint through a PDZ domain-binding motif [57] and also interact with 4.1 protein characterized by FERM (F for 4.1 protein, E for ezrin, R for radixin, and M for moesin) domain proteins [58]. Importantly, CASK, in turn, interacts with Mint, syntenin, and synaptotagmin. Thus, Nrxn is eventually linked to the presynaptic vesicle release machinery.

Nrxns mediate many regulatory functions [24]. One of the most notable functions of Nrxns is the regulation of presynaptic release. Knockout (KO) of all three Nrxn genes causes a decrease in evoked excitatory postsynaptic current (EPSC) amplitude and an increase in paired-pulse ratio at calyx of Held and cerebellar climbing fiber excitatory synapses or cortical somatostatin-positive (Sst+) inhibitory synapses, suggesting a decrease in presynaptic release probability [59, 60]. shRNA-mediated knockdown (KD) of all Nrxn genes in hippocampal primary cultures also lowers synaptic vesicle exocytosis monitored by a genetically encoded exocytosis sensor synapto-pHluorin [61]. Single KO of Nrxn2 gene reduces spontaneous neurotransmitter release at cortical excitatory synapses without changing synapse density [62]. Specific deletion of α isoform of Nrxn3 gene shows a selective

decrease in miniature inhibitory postsynaptic potential (mIPSP) frequency and evoked inhibitory postsynaptic current (IPSC) amplitude and increase in IPSC paired-pulse ratio in cultured mitral/tufted cells of olfactory bulb, indicating a decrease in presynaptic release probability [63]. Ca^{2+} channel dysfunction in the presynaptic active zone is noted as a major mechanism underlying functional impairment of presynaptic release in Nrxn loss-of-function models. KO of all three Nrxn genes disrupts the spatial coupling of Ca^{2+} channels with synaptic vesicles, and removes P/Q-type Ca^{2+} channels from the active zone at calyx synapses [60]. Furthermore, KO of all three Nrxn genes reduces the function of Ca^{2+}-activated BK potassium channels, whose activation depends on their tight association with presynaptic Ca^{2+} channels [60].

αNrxns and βNrxns may distinctly regulate presynaptic release functions through different molecular mechanisms. αNrxn-specific KO impairs Ca^{2+}-dependent neurotransmitter release mediated by P/Q- or N-type Ca^{2+} channels in the brain stem, which can be rescued by αNrxn1 but not βNrxn1 [64, 65]. These findings are supported by a unique cis interaction between αNrxns and the α2δ-1 auxiliary subunit of P/Q-type Ca^{2+} channels [56]. βNrxn-specific KO also impairs action potential-induced Ca^{2+} influx into presynaptic terminals at excitatory synapses in cortical primary cultures and hippocampal acute slices [66]. However, this impairment is caused partly by an increase in the postsynaptic production of endocannabinoids, which retrogradely inhibit neurotransmitter release via the activation of cannabinoid CB1 receptor [66]. On the other hand, the presynaptic phenotype in Nrxn loss-of-function animals depends on the type of synapses deficient in Nrxns. In pan-Nrxn KO mice, presynaptic release probability is not altered at cortical parvalbumin (Pv)+ inhibitory synapses [59]. Nrxn3 KO does not change presynaptic release probability at hippocampal excitatory and inhibitory synapses and olfactory excitatory synapses [63]. These presynaptic phenotypes vary among brain regions, synapse types, and Nrxn loss-of-function model examined, which can be partly due to the diversity in expression patterns of Nrxn genes and complicated developmental compensatory effects of Nrxns or their binding partners.

Presynaptic functions of Nrxns are also mediated by transsynaptic interactions with postsynaptic binding partners such as Nlgns. Nlgns are encoded by four and five different genes in rodents and humans, respectively. They have one transmembrane region and an extracellular domain that is homologous to acetylcholinesterase but is catalytically inactive. The extracellular domain is crucial for generating an interface for Nrxn binding, which can be regulated by the presence or absence of the insertion at one or two alternative splicing sites for each Nlgn [67]. The corresponding binding interface of Nrxns depends on distinct Nrxn splice variants [24]. Thus, different pairs of Nrxn-Nlgn variants differ in their binding affinities [40, 41]. Intracellularly, Nlgns have a PDZ domain-binding motif that binds to major postsynaptic scaffold proteins, including PSD-95, SAP102, Shank, SSCAM, PICK1 (protein interacting with C-kinase-1), SPAR, and GOPC [68]. Through these interactions, Nrxns and Nlgns bridge the presynaptic release machinery and postsynaptic receptor complex. In an in vitro co-culture assay with neuronal and non-neuronal cells, Nlgns

can induce presynaptic differentiation to recruit presynaptic proteins [69]. Triple KO of Nlgn1–3 reduces the frequencies of both miniature excitatory postsynaptic currents (mEPSCs) and miniature inhibitory postsynaptic currents (mIPSCs). Since synaptic density is normal in triple KO compared to wild-type mice, the decreased frequencies of mEPSCs and mIPSCs reflect reduced presynaptic release probability [70]. In organotypic hippocampal slice cultures, manipulating postsynaptic expression levels of different Nlgns alters presynaptic release probability at specific synapses [27, 71–74]. Simultaneous manipulation of pre- and postsynaptic molecules in organotypic slice cultures is useful to examine functional roles of specific transsynaptic interactions at a given synapse [75, 76]. This approach clarified that Nlgn-mediated alteration of presynaptic release probability is achieved by its Nlgn isoform-specific interactions with presynaptic Nrxns [27, 71, 72]. For example, (i) βNrxn1-AS4 (without the insertion of an exon at the alternative splicing site 4) and Nlgn1+AB (with the insertion of an exon at the alternative splicing sites A and B) pair at hippocampal CA3–CA3 excitatory synapses (Fig. 1a), (ii) αNrxn1-AS4 and Nlgn2+A pair at hippocampal CA1 inhibitory synapses (Fig. 1b), and (iii) αNrxn1+AS4 and Nlgn3Δ (without any exon insertions at any of the alternative splicing sites) pair at hippocampal CA1 inhibitory synapses expressing cholecystokinin, CB1, and vesicular glutamate transporter type 3 (VGluT3) (Fig. 1c) [27, 71, 72]. Such isoform-specific interactions between Nrxns and Nlgn are critical for regulating presynaptic release probability at given synapses. These findings raise the notion that specific Nrxn–Nlgn transsynaptic interactions are responsible for input cell type-dependent molecular mechanisms that control presynaptic release function. Importantly, this view is supported by the distinct expression patterns of Nrxns and Nlgns at individual synapses based on diverse expression patterns of Nrxns in presynaptic neurons across different brain regions and cell types [35, 78, 79] and postsynaptic Nlgn expression that depends on transsynaptic regulation from distinct input cell types [80–85].

Other postsynaptic molecules that bind to Nrxns can be involved in the retrograde modulation of presynaptic release functions via interacting with Nrxns at different synapses. For instance, the deletion of postsynaptic IgSF21, which can bind to αNrxn2, reduces the frequency of mIPSCs and the number of docked synaptic vesicles at hippocampal inhibitory synapses without altering synapse density, suggesting a decrease in presynaptic release probability (Fig. 1d) [55]. Therefore, transsynaptic interactions between αNrxn2 and IgSF21 may contribute to the diversification of presynaptic release function. It has been reported that extracellular C1ql proteins (C1ql2 and C1ql3) and cerebellin1 precursor protein (Cbln1) bridge postsynaptic kainate and delta glutamate (GluD) receptors with presynaptic Nrxns in hippocampal mossy fiber CA3 and cerebellar parallel fiber Purkinje cell synapses, respectively (Fig. 1e, f) [48–50]. Interestingly, reduced release probability has been reported in GluD2 KO mice, which strongly suggests that transsynaptic Nrxn–Cbln1–GluD2 complexes (Fig. 1f) are important for presynaptic structure and/or function [86, 87].

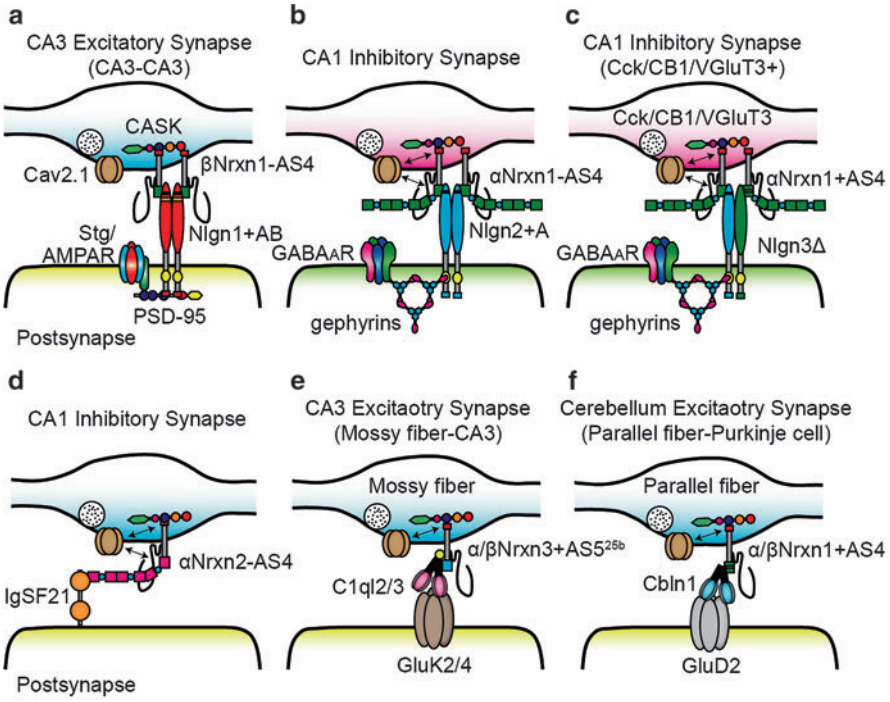

Fig. 1 Schematic diagrams of presynaptic regulation by Nrxn-mediated cis- and transsynaptic protein interactions in the hippocampus. (**a**) Presynaptic βNrxn1-AS4 (βNeurexin1-AS4) and postsynaptic Nlgn1+AB (Neuroligin1+AB) regulate excitatory synaptic transmission in the hippocampal CA3 associational circuit. Nlgn1 forms complexes with AMPARs through PSD-95 and AMPAR auxiliary subunit Stg (stargazin). (**b**) The interaction between presynaptic αNrxn1-AS4 and postsynaptic Nlgn2+A regulates synaptic release at hippocampal CA1 inhibitory synapses. Nlgn2 forms protein complexes with $GABA_A$Rs through gephyrin. αNrxn1 regulates P/Q-type Ca^{2+} channel function through cis interactions. (**c**) Postsynaptic Nlgn3Δ regulates presynaptic release through its interaction with presynaptic αNrxn1+AS4 at CA1 Cck/CB1/VGluT3+ inhibitory synapses. (**d**) Postsynaptic IgSF21 regulates inhibitory synaptic function through its interaction with presynaptic αNrxn2 in the hippocampal CA1 region. (**e**) Extracellular C1ql2/3 bridges postsynaptic kainate 2/4 receptors and presynaptic α/βNrxn3+AS5^{25b} at hippocampal mossy fiber CA3 synapses to regulate excitatory synaptic transmission. (**f**) Extracellular Cbln1 bridges postsynaptic GluD2 receptors and presynaptic α/βNrxn1+AS4 at cerebellar parallel fiber Purkinje cell synapses to regulate excitatory synaptic transmission. (Modified from Uchigashima et al. [77])

2.2 Cadherin-Catenin-Mediated Transsynaptic Signaling

The cadherin superfamily consists of more than 100 members in vertebrates [88]. They are classified into subfamilies that are called classical cadherins and non-classical cadherins. Classical cadherins include N-cadherin and E-cadherin, while non-classical cadherins include desmosomal cadherins, protocadherins, Flamingo/CELSRs (*c*adherin, *E*GF-like, *l*aminin A globular-like [LAG], and *s*even-pass

*r*eceptors), and Fat cadherins [88]. All cadherins mediate Ca^{2+}-dependent homophilic adhesions between cells expressing the same class of cadherin through their extracellular domain-containing repetitive cadherin repeats. Among different cadherins, classical cadherins have been the most extensively studied. Their cytoplasmic domain binds to β-catenin and p120 catenin [89, 90]. β-catenin associates with α-catenin, which is known as an actin-binding protein. These protein–protein interactions likely underlie the mechanism of cadherin-mediated synapse formation and spine stability.

The first evidence of retrograde synaptic control by cadherin was observed in a neuronal co-culture differentiated from mouse embryonic stem (ES) cells lacking neural (N)-cadherin (N-cad, also known as cadherin 2), one of the classical cadherins. In the culture, the absence of postsynaptic N-cad enhanced synaptic depression in response to paired-pulse or high-frequency stimulation suggestive of a reduced readily releasable vesicle pool [91]. Interestingly, the same synaptic phenotypes were observed when the deficiency of N-cad was restricted to postsynaptic neurons in experiments of co-culturing wild-type neurons and ES cell-derived N-cad KO neurons, indicating that postsynaptic N-cad retrogradely controls presynaptic release [91]. Likewise, postsynaptic overexpression of a dominant-negative form of N-cad (DN-N-cad), which lacks extracellular cadherin repeats, reduced the number of presynaptic puncta and changed spine morphology concomitant with the reduction in frequency of mEPSC [92, 93]. Also, postsynaptic DN-N-cad overexpression compromised vesicle endocytotic machinery, which reduced the expression of active zone proteins, the number of total and recycling vesicles, and excitatory presynaptic release probability in primary neurons [94]. These studies demonstrate that N-cad is involved in vesicle recruitment from the readily releasable pool to the active zone and in vesicle recycling pathways [91, 93]. However, curiously, presynaptic expression of DN-N-cad or N-cad shRNAi does not change presynaptic release probability. This indicates homophilic interaction between pre- and postsynaptic N-cad is not required for retrograde regulation of transmitter release and suggests that postsynaptic N-cad interacts with another presynaptic molecule(s) in a non-canonical fashion to influence presynaptic release probability though the putative presynaptic molecule(s) is yet to be identified [94].

Postsynaptic AMPAR subunits, GluAs, are considered as non-canonical mediators for N-cad. N-cad forms a protein complex with GluAs in vivo [95], and the extracellular N-terminal domain of GluA2 interacts directly with N-cad in cis and in trans [96]. Importantly, the extracellular N-terminal domain of GluA1, another AMPAR subunit, failed to interact with N-cad in primary excitatory neurons, highlighting the N-cad–GluA2 interaction as a unique transsynaptic mechanism at the synapse. This heterophilic interaction could be an important mechanism for AMPAR trafficking, retrograde regulation of synaptic transmission, and coordination between pre- and postsynaptic structure and functions. Consistently, Vitureira et al. reported that acute postsynaptic GluA2 KD reduced presynaptic release probability and occluded the effects of postsynaptic overexpression of DN-N-cad in primary excitatory neurons, indicating that GluA2 forms a complex with N-cad in cis and

regulates presynaptic release through a retrograde mechanism [94]. On the other hand, acute KD of postsynaptic GluA1, 2, or 3 reduced the size of the readily releasable pool without changing presynaptic release probability through a signaling pathway that does not involve N-cad in primary hippocampal neurons. Therefore, N-cad may contribute to synaptic structure and function through both hemophilic and heterophilic (with GluAs) interactions (Fig. 2) [97].

The intracellular-binding partners of presynaptic N-cad mediate the effect on presynaptic release machinery. Neuron-specific β-catenin KO reduced the number of releasable vesicles and exacerbated synaptic depression during high-frequency stimulation, although interpretation of this result is complicated by the fact that both pre- and postsynaptic β-catenin were knocked out in this study [98]. Consistently, postsynaptic overexpression of β-catenin resulted in an increase in mEPSC frequency, suggesting retrograde regulation by postsynaptic β-catenin/cadherin interactions, although there is an alternative possibility that β-catenin overexpression increased the number of functional synapses [99].

In contrast, cis interactions between presynaptic N-cad and catenin regulate presynaptic release probability through establishing protein complex with the β-catenin-interacting protein p140Cap (p130Cas-associated protein, also known as SRC

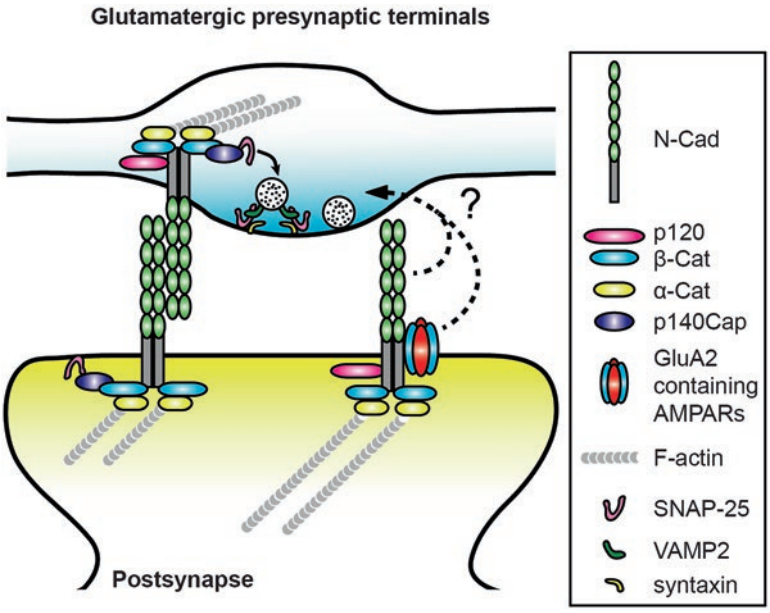

Fig. 2 Schematic diagram of N-cadherin-mediated cis- and transsynaptic regulation of neurotransmitter release at excitatory synapses. Postsynaptic N-cad, β-catenin, and GluA2-containing AMPARs retrogradely regulate excitatory presynaptic release in primary excitatory neurons. In contrast, cis interactions between presynaptic cadherin and β-catenin regulate presynaptic release probability through their interactions with p140Cap

kinase signaling inhibitor 1 [Srcin1] or SNAP-25-interacting protein [SNIP]) in cortical excitatory circuits (Fig. 2) [100]. p140Cap is expressed on both the pre- and postsynaptic sides. On the presynaptic side, p140Cap regulates transmitter release and spine structure through interacting with SNAP-25 while on the postsynaptic side, p140Cap modulates excitatory postsynaptic transmission through interacting with PSD-95 [101, 102]. Li et al. presented that presynaptic but not postsynaptic N-cadherin and β-catenin regulate presynaptic release probability through their interactions with presynaptic p140Cap, suggesting that the N-cad/β-catenin/p140Cap/SNARE protein complex is important for synaptic release.

In summary, both pre- and postsynaptic N-cad are capable of regulating presynaptic release. Further investigation is essential to evaluate how the postsynaptic N-cad/AMPAR complex regulates presynaptic release machinery. It is certainly interesting that postsynaptic N-cad regulates release retrogradely; however, findings are limited to culture systems. A more direct approach, such as acute KD or KO of N-cad in postsynaptic neurons in vivo, is essential to elucidate retrograde N-cad function in native brain circuits.

2.3 Transsynaptic Regulation of Presynaptic Release by mGluR and CAM Interactions

Type III metabotropic glutamate receptors (mGluR6/7/8) are predominantly localized at the presynaptic termini, and modulate neurotransmitter release by activating inhibitory G-proteins ($G_{i/o}$) [103]. Recent evidence has provided that postsynaptic CAMs regulate presynaptic release probability by the activation of type III mGluRs (Fig. 3).

Elfns are primarily expressed at postsynaptic sites and act as target neuron-specific retrograde mediators that regulate presynaptic release machinery. Two Elfn proteins, Elfn1 and Elfn2, consist of a single transmembrane domain and extracellular leucine-rich repeat domain that bind with membrane proteins. Elfn1 is specifically expressed in somatostatin-positive (Sst+) stratum oriens/lacunosum-moleculare (O-LM) inhibitory interneurons but not in parvalbumin-positive (Pv+) interneurons in the hippocampus. Importantly, KD or KO of Elfn1 in either O-LM or cortical Sst+ interneurons robustly reduced synaptic facilitation suggestive of increased presynaptic release by postsynaptic Elfn1 dysfunction. This indicates that Elfn1 negatively regulates presynaptic glutamate release onto Sst+ inhibitory interneurons (Fig. 3a) [104–106]. In addition, overexpression of Elfn1 in Pv+ interneurons, which do not normally express Elfn1, was sufficient to reduce presynaptic release probability, suggesting that Elfn1 is a necessary postsynaptic CAM that can influence target cell-specific release modulation. Importantly, in vitro binding assays elucidated that Elfns directly bind to type III metabotropic glutamate receptors (mGluR) including mGluR6 and mGluR7 [106–109].

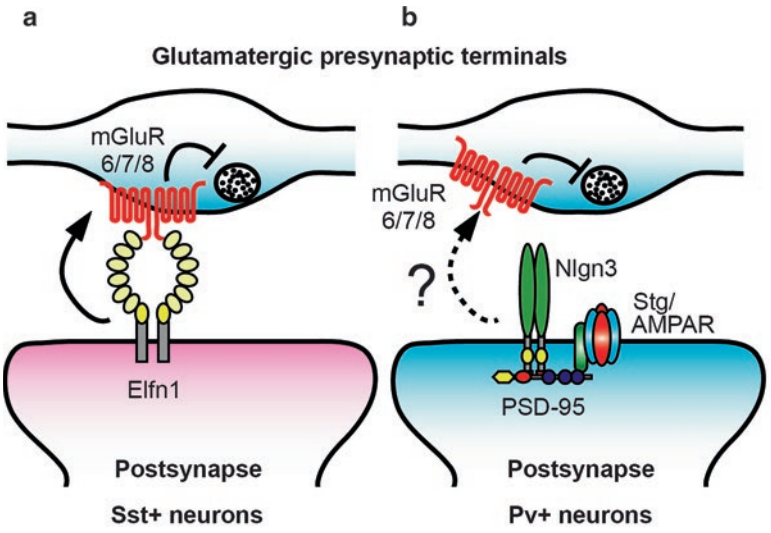

Fig. 3 Schematic diagrams of target neuron-specific regulation of neurotransmitter release at excitatory synapses through mGluRs and CAM interactions. Postsynaptic Nlgn3 (neuroligin 3) (**a**) and Elfn1 (**b**) expressed in Pv+ and Sst+ interneurons, respectively, retrogradely regulate synaptic release of excitatory neurotransmitters through presynaptically expressed type III mGluRs. It is not known whether Nlgn3 physically interacts with presynaptic mGluRs

Interestingly, the formation of the transsynaptic Elfn1-mGluR7 complex activates mGluR7 in a glutamate-independent fashion, which represents a novel GPCR signaling cascade in the brain [105].

In contrast to Elfn1, Elfn2 is highly expressed in excitatory neurons. KO of Elfn2 caused reduced expression of mGluRs in total brain lysate and increased basal excitatory synaptic transmission [107]. The increased basal excitatory synaptic transmission in Elfn2 KO mice might reflect a disruption of mGluR-mediated suppression of synaptic release. It is particularly interesting to test whether other leucine-rich repeat (LRR) family proteins, such as LRRTMs, affect target cell-specific presynaptic modulation.

Nlgn3 is another CAM that regulates neurotransmitter release via interacting with presynaptic type III mGluRs (Fig. 3b). Polepalli et al. demonstrated that Pv+ interneuron-specific Nlgn3 KO impairs type III mGluR-mediated suppressions of EPSC amplitudes and presynaptic release probability at Pv+ interneuron–pyramidal cell excitatory synapses, leading to the alteration of hippocampal network activity that underlies learning and memory [110]. Although a direct interaction between Nlgn3 and type III mGluRs has not been reported yet, this finding supports that type III mGluR-mediated presynaptic functions can be controlled by multiplexed transsynaptic signaling that involves distinct postsynaptic CAMs such as Elfns and Nlgn3.

2.4 Ephrin Receptor–Ephrin Ligand Mediated Transsynaptic Signaling

Ephrin ligand family interacts with its receptor family, Eph. Both ephrin ligands and Eph receptors are divided into two subclasses, A and B. EphrinA ligands are tethered to the membrane through GPI-linkage anchors and specifically bind to EphA receptors, while ephrinB ligands associate with the plasma membrane through a transmembrane domain and preferentially bind to EphB receptors. The intracellular carboxy-terminal tail of Eph receptors contains a tyrosine kinase domain, SAM protein interaction domain, and a consensus motif for binding to PDZ domain-containing proteins. Interestingly, several Eph receptors bind synaptic PDZ domain proteins such as the glutamate receptor-interacting protein 1 (GRIP1), protein interacting with C-kinase-1 (PICK1), syndecan-binding protein syntenin, and Ras-binding protein AF-6 [111, 112]. EphrinB ligands also have PDZ domain-binding motifs in the carboxy-terminal region, which can mediate interactions with syntenin, PICK1, GRIP1, and GRIP2 [112–114]. Thus, Eph receptors and ephrinB ligands are linked to the synaptic scaffold through PDZ-mediated protein interactions. Both EphA and EphB receptors have been detected mainly in postsynaptic sites [111, 115, 116], but some Eph receptors are also expressed in presynaptic terminals [117]. In contrast, the synaptic localization of ephrin ligands differs between subtypes. In the adult hippocampus, ephrinB2 is expressed mainly in CA1 pyramidal cells and is more abundant at postsynaptic sites [118–120] whereas ephrinB3 is expressed in dentate gyrus granule cells and targets presynaptically to the mossy fiber axons and termini [118, 120, 121].

It has been reported that transsynaptic retrograde signaling from postsynaptic EphB receptors to presynaptic ephrinB ligands contributes to the induction of an NMDAR-independent LTP between hippocampal mossy fibers and CA3 pyramidal neurons. Interfering with EphB/ephrinB transsynaptic signaling by the application of soluble EphB2 receptor or ephrinB1 ligand peptides occluded or blocked mossy fiber LTP, while expression of a dominant-negative form of ephrinB3 ligand reduced LTP [121, 122]. Interestingly, ephrinB3 KO mice exhibited normal mossy fiber LTP [121]. This lack of effect may be due to developmentally compensating effects by ephrinBs.

3 Roles of Transsynaptic Interactions in Synaptic Plasticity

A number of studies have shown that LTP is accompanied by synaptic translocation of major players necessary for LTP expression including AMPARs, α-actinin, drebrin, cofilin, CaMKIIα/β, β-catenin, and actin [9, 30, 99, 123, 124]. Furthermore, LTP induction causes the expansion of presynaptic boutons [10] and enlargement of active zones [125, 126]. These observations suggest both pre- and postsynaptic components increase alongside LTP. Therefore, it is likely that CAMs are

translocated to the synapse as part of a process of rebuilding larger postsynaptic structures. Indeed, transsynaptic Nrxn–Nlgn interactions mediate LTP expression in hippocampal CA1 synapses. The extracellular domain of Nlgn1 forms cis- and transsynaptic interactions with postsynaptic NMDARs and presynaptic Nrxns, respectively [127]. Acute KD of Nlgn1 completely blocked LTP in hippocampal dentate gyrus synapses [128], presumably due to reduced NMDAR function. Importantly, Wu et al. replaced endogenous Nlgn1 with a mutated Nlgn1 that cannot interact with βNrxn1 but continues to interact with NMDARs in hippocampal CA1 pyramidal neurons. This Nlgn1 mutant failed to induce LTP, indicating that transsynaptic Nlgn1–βNrxn1 binding is important for LTP [129]. Importantly, sLTP is abolished by application of the extracellular domain of βNrxn1 that blocks βNrxn-mediated transsynaptic interactions [130]. This also supports the significance of Nlgn–βNrxn interaction in LTP. It is widely accepted that the expression of LTP is largely postsynaptic and increases the number of AMPARs in the spines without changing presynaptic release probability [29]. Why is presynaptic βNrxn1 necessary for LTP? Does βNrxn1 simply anchor proper synaptic localization of Nlgn1 or reassemble presynaptic protein complex through cis interaction (see Sect. 2.2)?

The recent development of super-resolution microscopy has revealed the presence of transsynaptic nanocolumns or nanomodules, which represent the alignment of presynaptic transmitter release machinery and postsynaptic receptors within the synaptic contact [23, 131, 132]. Many of the excitatory synapses in hippocampal dissociated culture each contain one nanocolumn with some containing more than one [131]. Because synaptic AMPARs are not saturated with glutamate at the synaptic cleft [133, 134], it is possible that the formation of such nanocolumns enhances synaptic transmission efficacy. Indeed, glycine-induced chemical LTP increases the number of nanocolumns, which allows for the accumulation of more proteins under the alignment [131, 132, 135].

An obvious question is what adjusts pre- and postsynaptic alignment and how neuronal activation can modulate this process. Postsynaptic Nlgn1 and LRRTM both bind presynaptic Nrxn and colocalize with AMPAR nanodomains to potentially mediate the alignment [136, 137]. Nlgn1 can be phosphorylated by CaMKII at its intracellular carboxyl tail. This phosphorylation is necessary for activity-driven surface expression of Nlgn1 [138]. Alternatively, CaMKII has been recently found to form self-condensate in a manner triggered by Ca^{2+}/calmodulin stimulation via liquid–liquid phase separation [139, 140]. Liquid–liquid phase separation is a phenomenon where biological macromolecules such as proteins and nucleic acids, often through multimeric interactions, undergo spontaneous condensation that can generate >100-fold greater concentrations of macromolecules. Indeed, multiple pre- and postsynaptic proteins can undergo this phenomenon [140–143]. Interestingly, CaMKII segregates AMPARs together with Nlgn from NMDARs through a Ca^{2+}/calmodulin-triggered mechanism [139] (Fig. 4a). In this way, liquid–liquid phase separation of CaMKII can generate receptor nanodomains at the synapse where specific CAMs can co-segregate together under the regulation of neuronal activity (Fig. 4b). Such mechanisms might regulate the activity-dependent alignment of components of transsynaptic nanocolumns.

4 Future Directions

Much work has elucidated the functions of CAMs but many unresolved questions remain. First, the crosstalk between different CAM-mediated transsynaptic interactions remains unknown. A single CAM can interact with different binding partners at the synaptic cleft. For instance, postsynaptic Nlgn3 can potentially regulate presynaptic functions via interacting with presynaptic Nrxns, protein tyrosine phosphatase δ, or mGluRs [72, 110, 144]. However, it remains elusive whether these three distinct pathways synergistically contribute to presynaptic functions or compete against each other. Moreover, intracellular singling pathways can be also shared by different CAM-mediated transsynaptic interactions. Further studies are necessary for a better understanding of the crosstalk of CAM-mediated signalings that underlie presynaptic functions.

Second, a number of studies have identified non-canonical transsynaptic interactions between receptors and CAMs such as GluA2-N-cad (Sect. 2.1), Elfn1-mGluR6/7 (Sect. 2.3), and Nlgn3-mGluR (Sect. 2.3) as regulators of synapse function. Additional structural, physiological, and imaging studies are essential to reveal the roles of transsynaptic receptor and CAM complexes on presynaptic function and structure.

Third, the roles of transsynaptic interactions during plasticity are still largely unknown. Although ample studies have elucidated synaptic protein dynamics in dendritic spines during LTP, our knowledge is limited to cis interactions in the postsynaptic density. Much less is known about synaptic dynamics that regulate presynaptic molecular architecture. While it is generally accepted that LTP is expressed postsynaptically during the first hour following stimulation, structural studies have consistently provided evidence for the precise matching of the size and function of the presynaptic active zone and postsynaptic density [4, 7]. Therefore, presynaptic sites should match up with postsynaptic spines at some point. In the future, deciphering the constructive process of synapse modification after LTP induction, from changes in synaptic CAMs to rearrangements of presynaptic structures and vesicular release machinery, will be crucial in elucidating pre- and postsynaptic roles in LTP.

Fourth, recent research has demonstrated that Nlgns expressed in astrocytes regulate synapse development [145]. This indicates that presynaptic Nrxns can form transsynaptic complexes with astrocytic Nlgns as well. It is particularly interesting to highlight the differing roles of postsynaptic and astrocytic Nlgns in presynaptic release and structure.

Fifth, CAM-mediated regulation in modulatory systems is poorly understood. While numerous studies indicate that CAMs regulate fast neurotransmitter release including that of glutamate and GABA, fewer studies have tested CAM functions in central neuromodulatory systems, such as those mediated by dopaminergic and serotonergic signals, which are propagated mainly via volume transmission. Because the expression of CAMs is also detected at dopaminergic synapses [82], pre- and postsynaptic CAMs at these synapses might regulate presynaptic release like that at fast asymmetric synapses. Highlighting the roles of CAMs in modulatory systems will be an intriguing field of investigation.

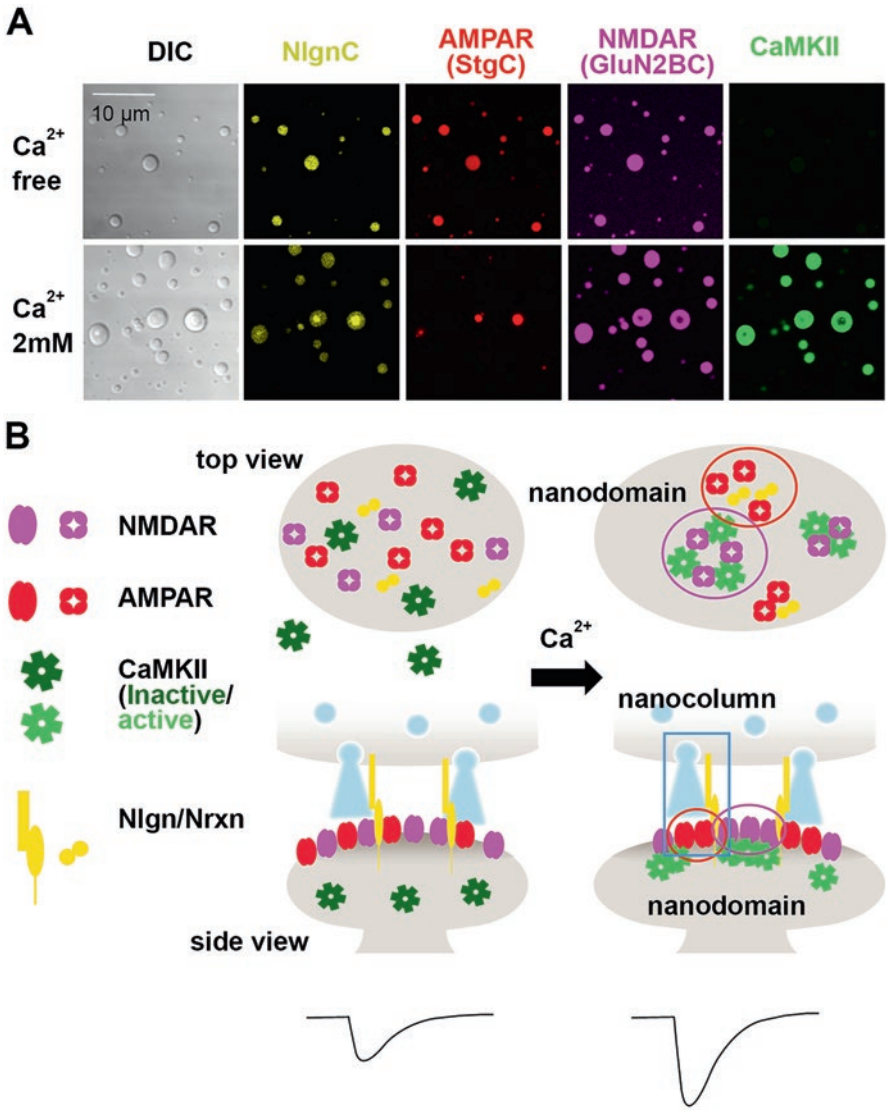

Fig. 4 Formation of synaptic nanocolumns by liquid–liquid phase separation. (**a**) Liquid–liquid phase separation of protein solution containing Nlgn (neuroligin) carboxyl tail (yellow), Stg (stargazin) carboxyl tail (red), NMDAR subunit GluN2B carboxyl tail (magenta), PSD-95 (unstained), calmodulin (unstained), and CaMKII (green). In the absence of Nlgn, AMPARs and NMDARs form homogeneous condensation. CaMKII remains in the diluted phase. Upon stimulation by Ca^{2+}, Nlgn and AMPARs form phase-in-phase surrounded by NMDARs and CaMKII. (**b**) Functional implications of liquid–liquid phase separation. Under resting conditions, AMPARs and NMDARs are mixed. The number of AMPARs beneath the transmitter release site is limited. Upon activation of CaMKII, AMPARs undergo liquid–liquid phase separation with PSD proteins and form nanodomains of AMPARs and NMDARs. Nlgn is condensed together with an AMPAR nanodomain, thereby bringing AMPARs beneath the transmitter release site and forming a synaptic nanodomain. This leads to more efficient synaptic transmission. (Modified from Hosokawa et al. [139])

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