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Synaptic localization of neurotransmitter receptors: comparing mechanisms for AMPA and $GABA_A$ receptors

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Abstract

Ionotropic neurotransmitter receptors mediate fast synaptic transmission by localizing at postsynapses. Changes in receptor number at synapses induce synaptic plasticity. Thus, mechanisms for the synaptic localization of receptors in basal transmission and synaptic plasticity have been investigated extensively. Recent findings reveal that synaptic localization of tetrameric AMPA receptors in basal transmission requires the PDZ binding of TARP auxiliary subunits, which modulate receptor properties and pharmacology. On the other hand, pentameric $GABA_A$ receptors require multiple receptor subunits for their synaptic localization in basal transmission. AMPA receptors seem to utilize distinct mechanisms for basal synaptic localization and synaptic insertion during plasticity. Revealing precise mechanisms for receptor synaptic localization may establish new approaches to control synaptic transmission.

Introduction

Synaptic transmission is mediated by neurotransmitters and their receptors. The properties and number of receptors at synapses determine synaptic strength. It is thus of critical interest to reveal the molecular mechanisms determining both receptor properties and receptor number at synapses. In this review, we discuss recent progress toward understanding the synaptic localization of neurotransmitter receptors by comparing findings in AMPA receptors (AMPARs) for excitatory synapses and $GABA_A$ receptors ($GABA_A$Rs) for inhibitory synapses. To reveal mechanisms to stabilize receptors at postsynapses, significant effort has focused on gene knockout and overexpression strategies. However, interpretation of such studies is complicated by the fact that these manipulations may primarily alter receptor protein expression, assembly or trafficking, and secondarily affect the number of receptors at synapses. Thus, a robust alteration in receptor synaptic localization may be observed, but a direct mechanism to stabilize receptors at synapses may not be revealed.

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Therefore, it is important to elucidate how molecules modify the activity and localization of receptors and to identify direct mechanisms to control receptor localization at synapses.

Receptor complexes

Both AMPARs and $GABA_A$Rs are heterooligomeric ion channels comprised of distinct pore-forming subunits. Besides pore-forming subunits, native receptor complexes may contain auxiliary subunits that modulate receptor localization, properties and/or pharmacology.

Native AMPARs assemble with transmembrane AMPAR regulatory proteins (TARPs) auxiliary subunits (Figure 1a). TARPs accelerate AMPAR gating, change affinity and efficacy of pharmacological reagents and regulate the surface expression and synaptic localization of the receptors [1,2]. An additional component of the AMPAR complex, cornichon-like protein (CNIH), was identified by a proteomic approach [3]. In the hippocampus, AMPARs form a tripartite complex with TARPγ-8 and CNIH2, and the expression of CNIH2/3 and the AMPAR subunits GluA1 and GluA2 is significantly reduced in the hippocampus of TARPγ-8 knockout mice [4,5]. CNIH2 slows the decay kinetics of TARPγ-8/AMPARs, but not TARPγ-2/AMPARs [4,6,7]. CNIH2/3 knockout mice show reduced AMPA-evoked currents and accelerated decay kinetics of AMPAR-EPSCs [6], indicating that CNIH modulates the properties of AMPARs in the brain. Interestingly, in *C. elegans*, the cornichon homologue CNI-1 plays a role in AMPAR/GLR-1 trafficking [8]. Thus, CNIHs and TARPs may modulate both the properties and trafficking of AMPARs.

In addition to TARPs and CNIH2/3, recent proteomic studies identified GSG1-l and CKAMP44, both of which modulate the AMPAR function [9–11]. Both CKAMP44 and GSG1-l modulate AMPAR properties in heterologus cells, CKAMP44 knockout mice show changes in AMPAR responses upon repetitive stimulation in the hippocampal dentate gyrus [11], and CKAMP44 and TARPγ-8 co-operatively modulate AMPAR function [12]. It is likely that AMPARs in distinct brain regions form complexes with distinct sets of auxiliary subunits or interactors [13], thus enabling regional diversity of AMPAR function.

Native $GABA_A$Rs are heteropentamers of distinct sets of 19 $GABA_A$R subunits, and this combinatorial assembly provides diversity in $GABA_A$R function (Figure 1b) [14–16]. Concatenation of multiple $GABA_A$R subunits reveals that α1/β2/γ2-containing $GABA_A$Rs are arranged as β2-α1-β2-α1-γ2 [17]. In contrast to AMPARs, no $GABA_A$R auxiliary subunit has been identified. Differences in receptor properties between $GABA_A$Rs expressed in neurons and heterologous cells have been observed [18]. However, given the diversity of $GABA_A$R subunits and the huge number of possible heteropentameric combinations of these subunits, it is difficult to know whether the differences in native and recombinant $GABA_A$Rs are due to differences in subunit combination, or to missing auxiliary subunits of the $GABA_A$R complex. A more thorough analysis of neuronal receptor properties with defined subunit composition will be helpful in identifying missing components of the native $GABA_A$R complex.

Subunits/domains responsible for synaptic localization

The mechanisms of synaptic localization in basal transmission and synaptic plasticity have been studied extensively. These studies have generally focused on subunits or domains within the receptor complex that mediates synaptic localization, or on receptor interactors that anchor receptors at synapses. A challenge with this approach has been that many identified receptor interactors play no direct role in synaptic localization, but instead play important roles in receptor assembly, cell surface expression, and/or determining overall receptor protein level. Importantly, an alteration of receptor assembly, synaptic localization or subunit expression level can indirectly alter synaptic localization. Thus, experimental approaches are required to distinguish a domain/interactor's role in synaptic localization from its roles in other processes.

Domains responsible for the synaptic localization of the AMPAR complex

Cerebellar GCs of stargazer spontaneous mutant mice, in which TARPγ-2 expression is disrupted, display loss of AMPAR-EPSCs [19]. Overexpression of TARPγ-2 restores AMPAR-EPSCs in GCs from stargazer mice, while overexpression of a TARPγ-2 mutant lacking its PDZ binding domain restores surface activity of AMPARs, but not AMPAR-EPSCs [20]. This strongly supports a specific role of the TARP PDZ binding domain in determining synaptic AMPAR localization in basal transmission in cerebellar GCs. In the hippocampus of both TARPγ-8 knockout mice, as well as TARPγ-8Δ4 knockin mice in which the last 4 amino acids of the PDZ binding domain are deleted, AMPAR-EPSCs are reduced only 30%. This suggests that TARPγ-8 dependent AMPAR-EPSCs are mediated by the TARPγ-8 PDZ binding domain [5,21]. The mechanism for the residual 70% of AMPAR-EPSCs in TARPγ–8 knockout and TARPγ-8Δ4 mice remains unclear. It is possible that other TARP isoforms expressed in the hippocampus (γ-2/3/4/5/7) play redundant roles in facilitating AMPAR-EPSCs, or that AMPARs in the hippocampus, in contrast to those in the cerebellum, may localize at synapses without TARPs. Consistent with a model wherein AMPARs localize to synapses in a TARP dependent manner, a TARPγ-2/3/4 triple knockout is lethal [22].

Subunits responsible for the synaptic localization of $GABA_A$Rs

$GABA_A$Rs are pentamers consisting of three distinct families of pore-forming subunits (α, β, and non-α/β) (Figure 1b). Each subunit has multiple isoforms that play redundant functions. Nonetheless, several knockout mice show a severe reduction in $GABA_A$R-IPSCs. Three mechanisms might underlie a loss or reduction of $GABA_A$R-IPSCs: 1) A loss of synaptic $GABA_A$Rs, due to a loss of $GABA_A$R expression, surface expression or synaptic localization; 2) a loss or reduction in $GABA_A$R conductance due to a change in $GABA_A$R properties or 3) defective GABA release from presynaptic terminals. Therefore, $GABA_A$R knockout or knockin mice showing absent or reduced $GABA_A$R-IPSCs should be examined in detail to distinguish between these possibilities.

A knockout mouse lacking expression of the subunit responsible for synaptic $GABA_A$R localization should show no or reduced $GABA_A$R-IPSCs at synapses where this subunit is a major constituent, and such mutant mice have been described. For example, disruption of

the β3 or γ2 subunit reduces $GABA_AR$ activity significantly [23–26]. Knock out of the β3 subunit in mice reduces GABA-evoked whole-cell currents in both acutely dissociated and cultured neurons [24,26]. Most β3 knockout mice die neonatally, supporting a critical role for β3 in animal survival [24]. Primary cortical cultured neurons from β3 knockout mice display $GABA_AR$-miniature IPSCs (mIPSCs) with accelerated decay kinetics but normal amplitude [24]. This result indicates either that β3 does not mediate synaptic localization, or that its function in synaptic localization is redundant with β1 or β2 subunits. Mice lacking expression of all three β subunits might be a useful tool to reveal the roles of β subunits in the brain. Neurons with disrupted γ2 expression show an 80% reduction in $GABA_AR$-mIPSC frequency [25]. Importantly, in primary cortical cultured neurons from γ2 knockout mice, α1 subunit does not localize at GAD-positive synapses, indicating that γ2 modulates the synaptic localization of α1 subunit. The synaptic localization of $GABA_ARs$ is restored by overexpression of the γ2, but not α2, subunit in γ2 knockout neurons [27]. In addition, the γ2 TM4 domain plays a critical role in synaptic localization [27], although the precise contribution of the γ2 TM4 domain remains unclear. As mentioned above, besides the γ2 and β3 knockouts, other knockout mice, including α subunit knockouts, show reduced $GABA_AR$-IPSCs. As strategies are developed to address complexities arising from $GABA_AR$ heterogeneity and subunit redundancy in vivo, systematic analyses of the precise contributions of each subunit to receptor expression, assembly, surface expression and synaptic localization may become possible.

Synaptic anchors and synaptic localization during plasticity

Proteins enriched at excitatory and inhibitory synapses may anchor AMPARs and $GABA_ARs$, respectively.

Excitatory synapses

Many AMPAR/TARP interactors have been proposed to modulate AMPAR localization, and this diversity may reflect a high degree of complexity in the protein networks comprising the postsynaptic density (PSD) [28–31]. Due to space limitations, in this review we focus on PSD-95-like MAGUKs that interacts with the PDZ domain of TARPs responsible for synaptic localization of AMPARs.

A proteomic analysis identified the protein PSD-95 as a major PSD component [32]. PSD-95, along with other members of the PSD-95-like MAGUK protein family, associate with the TARP PDZ binding domain [20,33], and PSD-95 knockout mice show a reduction in AMPAR-EPSCs in basal transmission in the hippocampus [34,35]. These results suggest that PSD-95-like MAGUKs anchor AMPARs at synapses through TARPs. Interestingly, the interaction of PSD-95 with the TARP PDZ binding domain is regulated by TARP phosphorylation (Figure 2). TARPγ-2 is highly phosphorylated at synapses, and nine serine residues are phosphorylated in neurons [36]. TARP knockin mice, in which all nine serine residues are replaced with either alanine (non-phospho mimic) or aspartic acid (phospho-mimic), were generated. Whereas phospho-mimic TARPγ-2 knockin mice show increased AMPAR-EPSCs at the cerebellar MF-GC synapses, non-phospho-mimic TARPγ-2 knockin mice show a reduction in AMPAR-EPSCs [37]. Phosphorylating TARPs or neutralizing negative chargd lipids dissociates TARPs from lipids, enhances TARP binding to PSD-95 in

vitro, and increases AMPAR-EPSCs in neurons [37]. These results show the importance of TARP phosphorylation in basal transmission.

One challenge in studying mechanisms of AMPAR synaptic localization is that the number of AMPARs at synapses is dynamically regulated during synaptic plasticity [38]. Recent findings suggest that AMPARs may localize to synapses under basal conditions and during synaptic plasticity by distinct mechanisms (Figure 2). Whereas AMPAR-EPSCs in basal transmission are reduced in a PSD-95 knockout mouse, long-term potentiation (LTP) is enhanced [34,35,39]. Complementing these findings, TARPγ-8 knockout mice display a severe reduction in LTP, whereas normal LTP is observed in TARPγ-8Δ4 mice lacking the PDZ binding domain [5,21]. These results indicate a critical role for the TARP-PSD-95 interaction in basal transmission, but not in LTP. LTP requires insertion of AMPARs into synapses. In the simplest case, disrupting the mechanism for AMPAR insertion during LTP would block LTP without affecting basal transmission. GluA1 knockout mice and GluA1 double phospho-knockin mice show normal basal transmission but disrupted LTP [40,41], suggesting that the GluA1 subunit somehow facilitates AMPAR synaptic insertion during LTP. Recently, however, a reserve pool of glutamate receptors for LTP was shown to be independent from AMPARs. In neurons with disrupted all AMPAR expression, overexpressed KARs are inserted into synapses upon LTP induction [42]. The mechanism for how this reserve pool of receptors is stabilized, then translocated to the synapse during LTP, remains unclear.

Inhibitory synapses

Among molecules enriched at inhibitory postsynapses, gephyrin and neuroligin-2 have received particular attention, and their molecular links with $GABA_A$Rs have been extensively studied.

Gephyrin has been widely used as a marker for inhibitory synapses (Figure 1b). Gephyrin was originally co-purified with glycine receptors from an amino-strychnine affinity-column [43], and was subsequently found to localize to both glycinergic and GABAergic inhibitory synapses [44]. $GABA_A$Rs and gephyrin may mutually stabilize each other at synapses. Spinal cord and primary hippocampal cultured neurons from gephyrin knockout mice show a reduction in synaptic localization of some, but not all, $GABA_A$R subunits [45], supporting a model in which $GABA_A$Rs localize to synapses by both gephyrin dependent and independent mechanisms. On the other hand, disruption of the $GABA_A$R γ2 or α subunits redistributes gephyrin from synapses [25,46–48], and interaction of gephyrin with α and β subunits has been shown [49–51]. These results support a model in which $GABA_A$Rs first stabilize gephyrin at GABAergic synapses, followed by gephyrin-dependent recruitment of additional synaptic $GABA_A$Rs [52]. Importantly, a molecule initiating the synaptic localization of $GABA_A$Rs and gephyrin remains unidentified.

The neuroligin-neurexin complex plays a critical role in synaptic transmission. Neuroligin-2 (NL2) is enriched at inhibitory synapses, whereas NL1 is enriched at excitatory synapses [53,54] (Figure 1). Postsynaptic overexpression of NL2 promotes the formation of both excitatory and inhibitory synapses in primary cultured neurons, whereas overexpression of NL1 or NL3 preferentially promotes excitatory synapse formation, suggesting that NL2

predominantly acts at inhibitory postsynapses [54,55]. Two models have been proposed by which NL2-neurexin may regulate $GABA_A$Rs. First, the cytoplasmic domain of NLs may interact with gephyrin and collybistin to induce synaptic localization of $GABA_A$Rs [56,57]. Supporting this model, knocking out any one of these three components in mice reduces $GABA_A$R-mIPSCs [45,55,56,58], although in each case some $GABA_A$Rs remain at synapses, possibly due to redundancy by other isoforms, for example, NL4 [59]. On the other hand, postsynaptic neurexin interacts directly with $GABA_A$Rs to modulate their function, but not their synaptic localization, by a NL-independent mechanism [60]. Stoichiometric information about the native $GABA_A$R/neurexin complex in the brain may reveal key details about this mechanism. Similar to gephyrin and NL2, the dystrophin-glycoprotein complex (DGC) is also enriched in GABAergic synapses [61]. $GABA_A$Rs are redistributed in dystrophin mutant mice [62]. However, in primary hippocampal neurons, disrupting the expression of dystroglycan, a critical DGC component, does not alter the distribution of $GABA_A$Rs [63]. Thus, there appears to be heterogeneity in the requirement for DGC in $GABA_A$R clustering at synapses.

Recently, the ADAMTS like protein Madd-4 was identified as a modulator of $GABA_A$R synaptic localization by *C. elegans* genetic screening [64](Figure 1b). In Madd-4 mutants, both L-AchRs and $GABA_A$Rs redistribute to extrasynaptic sites. MADD-4 has long and short splicing isoforms, which result from alternative promoters. Selective deletion of the short isoform causes $GABA_A$Rs to redistribute to cholinergic synapses, whereas overexpression of the long isoform in GABAergic neurons recruites L-AChR to GABAergic synapses. These results suggest that MADD-4 is a critical synaptic organizer of both GABAergic and cholinergic synapses in *C. elegans*. It will be interesting to see whether the mammalian homologue of MADD-4, Punctin, regulates synaptic localization of $GABA_A$Rs.

Conclusions

Following neurotransmitter release, synaptic strength is determined by the properties and number of neurotransmitter receptors at postsynapses. Recent findings have shed light on mechanisms for the synaptic localization of neurotransmitter receptors. Here we compare mechanisms for the synaptic localization of tetrameric AMPARs and pentameric $GABA_A$Rs by focusing on the constituents of the respective receptor complexes in vivo, and the domains and interactors responsible for their synaptic localization. Although many interactors have been proposed as described above, due to the limited space in this review we focused on the role of PSD-95 like MAGUKs in the synaptic localization of the AMPAR/TARP complex, and the role of gephyrin, NL2 and MADD-4/Punctin in the synaptic localization of $GABA_A$Rs. Figure 1 highlights interesting similarities and differences in our current understanding of AMPAR and $GABA_A$R synaptic localization. Neurexin-neuroligin complexes may play roles in regulating both AMPARs and $GABA_A$Rs through intermediate interactors. On the other hand, AMPARs have auxiliary subunits, but $GABA_A$R auxiliary subunits have not yet been found. Determining whether $GABA_A$Rs utilize auxiliary subunits will be a key step toward elucidating their mechanism of synaptic localization.

As further insight is gained into the manner by which receptors localize to synapses, general principles governing the synaptic localization of all receptors may emerge. On the other hand, it may become apparent that each receptor type localizes to synapses via a distinct mechanism. Because distinct mechanisms corresponding to distinct receptor types could serve as targets for drug discovery, a complete understanding of these mechanisms may allow us to develop therapeutic strategies to manipulate synaptic activity rapidly in patients with neuropsychiatric disease.

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Highlights

- Molecular constituents of ionotropic neurotransmitter receptors in the brain
- Receptor subunits responsible for synaptic localization of AMPAR and $GABA_AR$
- Anchoring proteins for AMPARs and $GABA_ARs$ at each synapse

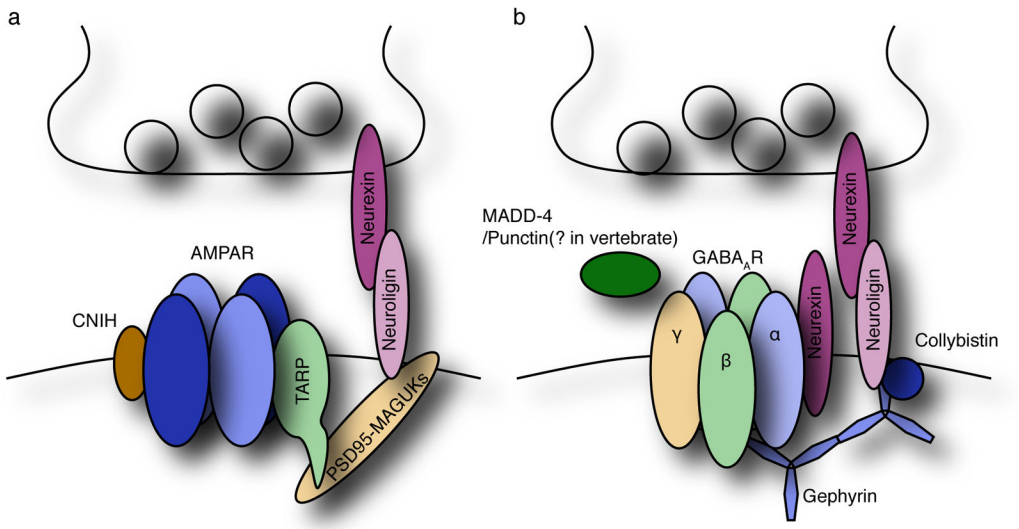

Figure 1. Organization of AMPA receptor synapses and GABA$_A$ receptor synapses

(a) AMPA receptors form a complex with TARPs and PSD-95 like MAGUKs, which also bind to neuroligins. (b) Synaptic localization of GABA$_A$Rs is modulated by neuroligin-2, neurexin, collybistin and gephyrin.

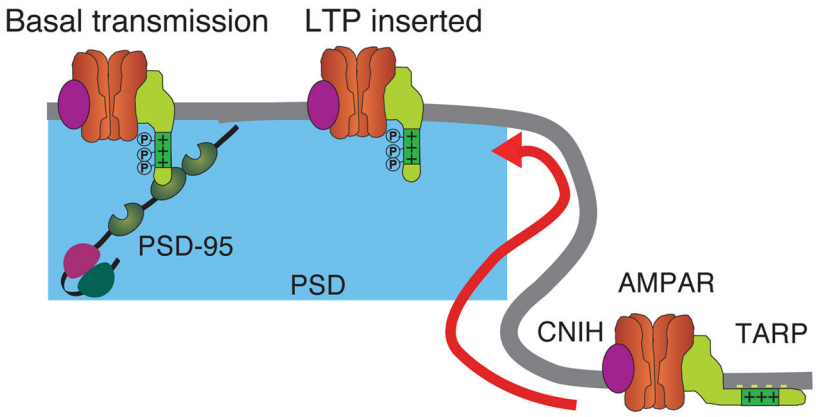

Figure 2. Synaptic localization of AMPA receptor complex in basal transmission and plasticity

In the hippocampus, TARP forms a tripartite complex with TARPs and CNIH2/3. In basal transmission, TARP-dependent synaptic localization of AMPA receptors is mediated by interaction of the TARPγ-8 PDZ binding with PSD-95 like MAGUKs. During plasticity, AMPARs inserted into synapses independent of the TARP PDZ binding and PSD-95 MAGUKs.