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Mechanisms of excitatory synapse maturation by trans-synaptic organizing complexes

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Abstract

Synapses are specialized cell-cell adhesion contacts that mediate communication within neural networks. During development, excitatory synapses are generated by step-wise recruitment of pre- and postsynaptic proteins to sites of contact. Several classes of synaptic organizing complexes have been identified that function during the initial stages of synapse formation. However, mechanisms underlying the later stages of synapse development are less well understood. In recent years, molecules have been discovered that appear to play a role in synapse maturation. In this review, we highlight recent findings that have provided key insights for understanding postsynaptic maturation of developing excitatory synapses with a focus on recruitment of AMPA receptors to developing synapses.

Keywords

excitatory synapse maturation; AMPA receptor recruitment; trans-synaptic organizing complexes

Introduction

The developing central nervous system initially generates an abundance of chemical synapses which are later sculpted and refined into the elegant and precise neural networks of the adult brain. The first steps involve several trans-synaptic complexes – collectively referred to as ‘synaptic organizing proteins’ – that initiate and stabilize early synaptic contacts via recruitment of synaptic vesicles to the presynaptic active zone, and N-methyl-D-aspartate (NMDA) receptors to the postsynaptic density [1–4]. PDZ domain proteins such as PSD-95 serve as scaffolds for recruitment of synaptic components required for synapse development [5]. Initial flurries of excitatory synaptogenesis give way to an abundance of silent NMDA receptor-containing synapses subject to activity-dependent strengthening or elimination. Recruitment of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors to the postsynaptic membrane augments glutamatergic transmission and is a critical process in excitatory synapse maturation [6].

Several studies have investigated AMPA receptor trafficking during synaptic plasticity [7–11]; however, it is uncertain if similar or distinct mechanisms underlie initial stages of synapse development. AMPA receptors consist of tetramers of different combinations of one or more subunits termed GluA1–4 with distinct biophysical properties, trafficking, and

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binding partners [8,12]. The C-terminal PDZ-binding motif interacts with cytoplasmic PDZ domain proteins such as PICK1, GRIP/ABP, and SAP-97 [5]. In addition, the PDZ domain protein PSD-95 interacts with AMPA receptors through stargazin [13], the prototypical transmembrane AMPA receptor regulatory protein (TARP). Recent studies have identified a diverse array of additional transmembrane accessory proteins that modulate AMPA receptor function [14]. The relationship between cytoplasmic AMPA receptor-interacting proteins and transmembrane accessory proteins in mature and developing synapse remains unclear.

Neurexin synaptic organizing complexes and AMPA receptor recruitment

Several trans-synaptic signaling systems have been identified that induce pre- or postsynaptic differentiation when presented to axons or dendrites by expression in non-neuronal cells [1–4]. Interestingly, recent results have demonstrated that diverse classes of synaptic organizing molecules form complexes of different members (for a recent and thorough review, see [4]). For example, in addition to interaction with neuroligins, neurexins influence presynaptic differentiation via interaction with the leucine-rich repeat transmembrane proteins (LRRTMs) [15**–17]. Neurexins are also the presynaptic binding partner of the Cbln1-GluD2 complex, a structurally distinct signaling system that directs synapse formation [18]. Thus, a multifaceted system of trans-synaptic mechanisms underlies synapse development.

Different classes of synaptic organizing complexes differentially influence AMPA receptor recruitment at developing synapses (Figure 1; Table 1). Early studies indicated that synaptic organizing complexes promote formation of structural synapses that are capable of releasing neurotransmitter but are postsynaptically silent, and thus, non-functional, due to the lack of AMPA receptors. For instance, clusters of neuroligin-1 and PSD-95 induced by β -neurexin overlap with NMDA but not AMPA receptors [19] even though in theory, AMPA receptors could be recruited to sites of neurexin-neuroligin induced PSD-95 clusters through stargazin [20]. However, GluA1-containing AMPA receptor clustering at neurexin-neuroligin contact sites can be induced upon glutamate application [21], suggesting that neuroligin-neurexin induced synapse maturation is activity regulated. Indeed, increases in synapse numbers in transfected neurons expressing neuroligins require synaptic activity [22]. In addition, overexpression of neuroligin-1 in hippocampal neurons facilitates recruitment of GluA2-containing AMPA receptors (but not GluA1-containing AMPA receptors, consistent with [21]) to PSD-95 clusters induced by neurexin-coated microspheres in an activity-independent manner [23*], suggesting that AMPA receptor recruitment at neurexin-neuroligin contacts is subunit specific.

Other neurexin-interacting proteins differentially influence AMPA receptor recruitment at developing synapses. Like neuroligins, all LRRTMs induce presynaptic differentiation in axons when expressed in nonneuronal cells [24]. LRRTM2 was further shown to influence postsynaptic differentiation as follows. Coculture of neurons and nonneuronal cells expressing NMDA receptor subunits and LRRTM2 leads to spontaneous currents [24]. Short-hairpin RNA (shRNA)-mediated knockdown of LRRTM2 in dissociated hippocampal neurons resulted in decreased GluA1 content at synapses and a large (>50%) decrease in evoked synaptic currents [15**]. However, miniature excitatory postsynaptic current (mEPSC) amplitudes were only modestly reduced and mEPSC frequency was unchanged upon LRRTM2 knockdown [15**]. Because the reduction in mEPSC amplitude does not seem sufficient to cause the dramatic decrease in excitatory synaptic transmission, these data suggest that LRRTM2 primarily regulates synapse efficacy and plays a minor role postsynaptic AMPA receptor density regulation. Intriguingly, GluA1 and GluA2, as well as NR1, co-precipitate with LRRTM2 when coexpressed in nonneuronal cells, an interaction requiring the extracellular LRR domain [15**]. Thus, it is tempting to speculate that

LRRTM2 might function similarly to TARPs to modulate AMPA receptor activity such that interaction with neurexin might then selectively influence AMPA receptor function in a subset of synapses. Like TARPs, LRRTM2 interacts with PSD-95 via a C-terminal ECEV motif [15^{**},24]. Deletion of this domain does not impact LRRTM2's synaptic localization [24]; however, the impact on synaptic AMPA receptors was not tested. Analysis of whether LRRTM2 influences channel gating properties of AMPA receptors like TARPs will provide insight to this interesting possibility.

Neurexins have also recently been identified as the presynaptic binding partner of the Cbln1-GluD2 complex [18] responsible for synapse formation between parallel fibers and Purkinje neurons in the cerebellum [18,25]. Loss of Cbln1 or GluD2 in vivo leads to reduced numbers of parallel fiber-Purkinje synapses and increased number of free spines [26,27]. Whether the Cbln1-GluD2-neurexin synaptic organizing complex directly recruits AMPA receptors to developing synapses is unknown; however, an antibody against the putative ligand-binding region of GluD2 destabilizes synaptic AMPA receptors [28], suggesting that GluD2 is involved somehow in AMPA receptor trafficking. Furthermore, expression of GluD2 and GluA1 in nonneuronal cells resulted in glutamate-induced currents and mEPSC-like events that were not observed with GluA1 expression alone [29^{*}]. Still, mEPSC amplitude is unchanged while mEPSC frequency is reduced by 50% in Cbln1 knockout neurons [30]. Together, these data support a model in which the primary role of Cbln1 (perhaps via neurexin) is to promote synapse formation while the secondary role of the GluD2 interaction is to promote synapse maturation. An additional possibility is that the neurexin synaptic organizing complexes function sequentially during synapse development (Figure 2).

Synaptic organizing activity of GluA subunits

Narp (neuronal activity-regulated pentraxin receptor), a member of the neuronal pentraxin (NP) family, mediates AMPA receptor clustering at synaptic contacts onto interneurons [31,32]. Narp (also known as NP2) and NP1 are secreted molecules while NPR (neuronal pentraxin receptor) is an integral membrane protein. All three members are capable of forming hetero-oligomers whereby Narp and NP1 become membrane bound through association with NPR [33]. Interaction of the extracellular N-terminal domain (NTD) of the AMPA receptor GluA4 subunit is required for presynaptically-derived NPR/NP1-mediated GluA4 synaptic recruitment [34]. Synaptic recruitment of GluA4 required coexpression with neuroligin-1 in nonneuronal cells [34], suggesting the tantalizing possibility of a trans-synaptic complex consisting of NP1-NPR-GluA4 to promote synapse maturation via interaction with neuroligin-1. Consistent with this possibility, NP1/2 knockout neurons have reduced AMPA receptor mediated synaptic transmission reflected in an increased number of silent synapses during eye-specific refinement in the retinogeniculate system [35^{**}]. NP1/2 are required for segregation of eye-specific retinal ganglion cell projections to the dorsal lateral geniculate nucleus [36], a process that involves activity-dependent synapse formation and elimination. NPR has also been implicated in AMPA receptor recruitment during homeostatic scaling [37], suggesting dual roles for NPs in synapse maturation and plasticity.

AMPA receptor recruitment activity of other synaptic organizing complexes

The ephrins and Eph receptors are axon guidance and signaling molecules that are increasingly appreciated for their roles in synapse formation and maturation. For example, postsynaptic EphB2 influences AMPA receptor localization through PDZ binding domain interactions [38]. Interestingly, recent experiments implicate a role for postsynaptic ephrins and presynaptic Eph receptors in synapse maturation. EphrinB2 activation by soluble EphB4 selectively stabilizes surface AMPA receptors in dissociated neuronal cultures [39].

mEPSCs recorded from ephrinB2 knockout neurons were decreased in amplitude compared to controls [39**], suggesting that AMPA receptor content was reduced in the absence of ephrinB2. A phosphorylation-dependent interaction between ephrinB2 and GRIP was required for this effect [39]. Similarly, amplitude, but not frequency, of mEPSCs was reduced in ephrinB3 mutants and ephrinB3 mutant mice exhibit reduced AMPA receptor-mediated synaptic transmission [40], suggesting that synapse strength is affected by ephrinB3 loss of function. Together, these findings suggest that ephrinB2 interacts with GRIP to recruit and maintain AMPA receptors at synapses, while ephrinB3 is capable of strengthening excitatory synaptic transmission.

Another axon guidance family of molecules, leukocyte common antigen-related (LAR), appears to be co-opted at the synapse for specialization and development. Presynaptic LAR family protein tyrosine phosphatase receptors (PTRPs) signal through postsynaptic netrin-G ligand NGL-3 to induce excitatory synapse development [41]. While it appears that the LRRs of NGL-3 predominantly induces presynaptic differentiation, it has also been shown to selectively cluster postsynaptic excitatory components, including GluA2 AMPA receptor subunits [42]. In the same study, the investigators found that frequency, but not amplitude, of mEPSCs was reduced with NGL-3 knockdown [42], arguing that this molecule may largely work to establish and maintain nascent excitatory synapses, but whether it functions directly in synaptic strengthening or maturation requires closer investigation. A third LRR domain-containing protein family that may also play a role in synaptic maturation is the synaptic adhesion-like molecules, or SALMs. Coimmunoprecipitation experiments showed that SALM2 interacts with both NMDA and AMPA receptors and regulates the maturation of excitatory synapses [43]. Recent studies showed that SALM5 knockdown reduces both amplitude and frequency of mEPSCs and mIPSCs [44*], suggesting SALM5 promotes both excitatory and inhibitory synaptic differentiation.

Finally, a recent study identified SynDIG1 (synapse differentiation induced gene 1), a conserved type II transmembrane protein, as a novel regulator of excitatory synapse maturation [45**]. SynDIG1 coimmunoprecipitates with AMPA receptor subunits in heterologous cells and brain extracts and knock-down of SynDIG1 in dissociated rat hippocampal neurons reduces AMPA receptor content at developing synapses by ~50% as determined by immunocytochemistry and electrophysiology [45**]. However, SynDIG1 did not influence NMDA receptor containing synapses, suggesting that SynDIG1 is a selective regulator of excitatory synapse maturation. Whether SynDIG1 functions via a trans-synaptic complex to regulate synaptic maturation remains to be determine.

Outlook and open questions

While significant progress in our understanding of postsynaptic maturation has been made, several open questions exist. The spine, for example, is the predominant site of excitatory synapses and a hallmark of synapse maturation. Spine size correlates with the abundance of AMPA receptors, but what is the relationship between AMPA receptor recruitment and spine morphogenesis? The extracellular NTD of the GluA2 subunit is important for the formation, growth and/or maintenance of dendritic spines [46] and involves interaction with N-cadherin [47]. EphB receptors influence motility of dendritic filopodia and spine morphogenesis and are required for synaptogenesis only when filopodia are most abundant and motile [48]. In addition, EphA5-ephrinA5 interaction induces expression of PSD-95-NMDA receptor complexes and morphological spine maturation [49]. Activation of neuregulin-ErbB4 signaling stabilizes synaptic AMPA receptors [50] via extracellular interactions whereas its tyrosine kinase activity is important for dendritic morphological changes [51]. Thus, it is likely that additional molecular mechanisms underlie the coordination of synapse maturation and spine morphogenesis. Additional avenues of

research include understanding the relationship between AMPA receptor recruitment during early stages of synapse development and later stages of synapse plasticity. In addition, what is the relationship between the various synaptic organizing proteins, transmembrane AMPA receptor accessory proteins, cytoplasmic AMPA receptor interacting proteins, and AMPA receptor recruitment? Future work is needed to sort these diverse molecular mechanisms into a unifying model of synapse maturation.

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Figure 1. AMPA receptor recruitment by select synaptic organizing complexes

Shown are interactions of trans-synaptic adhesion and other complexes with implications in AMPA receptor recruitment at excitatory synapses. A presynaptic SALM or SynDIG1 ligand has yet to be identified. Neurexin-Neuroigin-1 interaction requires synaptic activity (indicated by the long arrow) to affect GluA1-containing AMPA receptors through CaMKII, whereas other postsynaptic AMPA receptor mediators interact directly with GluA subunits or scaffolding proteins. Because of limited space, some complexes are shown in close proximity to or interacting with a single AMPA receptor-scaffold complex. This is not meant to imply a tertiary interaction.

Figure 2. Hypothetical time course of neurexin-induced synapse maturation

Presynaptic Neurexin interacts with Cbln1-GluD2 complex to induce synapse formation. Subsequent interaction of Neurexin with postsynaptic Neuroligin-1 might then lead to NMDA receptor recruitment at a nascent synapse while subsequent interaction of Neurexin with LRRTM2 might also lead to AMPA receptor recruitment. With time and activity, these mechanisms and others combine to form a stable, mature and active synapse replete with AMPA receptor trafficking and stabilizing machinery. Any given synapse might have one or any combination of the mechanisms shown, and the complement of trans-synaptic adhesion and AMPA receptor regulating machinery is likely to affect its potentiation capacity and strength of transmission at all points in the synapse's lifetime.

Table 1

Modulation of AMPA receptors by select synaptic organizers

Molecule	Interaction with AMPA-R	Action on AMPA-R	Physiological effect at synapses	Effect on synaptic transmission	Reference
Neurotrophin-1		Recruits GluA2			[23*]
LRRTM2	Yes	Influences surface GluA1, GluA2 levels	Small decrease in mEPSC amplitude	Decreased evoked currents upon knockdown	[15**]
Cbln1-GluD2		GluD2 promotes GluA1 ion channel function	mEPSC frequency decrease in Cbln1 KO		[29*] [30]
NP1/2, NPR	Yes	Clusters GluA4 (requires neuroligin-1)	Increased silent synapses in NP1/2 KO	Reduced excitatory transmission in NP1/2 KO	[34] [35**]
ephrinB2/3	Yes via phosphodependent interaction with GRIP (ephrinB2)	Stabilizes surface AMPA-R	Decreased mEPSC amplitude (ephrinB2 or B3 KO)	Reduced excitatory transmission in ephrinB3 KO	[39**] [40]
NGL3		Clusters GluA2	Decreased mEPSC frequency upon knockdown		[42]
SALM5			Decreased mEPSC amplitude and frequency upon knockdown		[44*]
SynDIG1	Yes	Clusters GluA2	Decreased mEPSC frequency and amplitude		[45**]