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Auxiliary subunits of the AMPA receptor: The Shisa family of proteins

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Abstract

AMPA receptors mediate fast synaptic transmission in the CNS and can assemble with several types of auxiliary proteins in a spatio-temporal manner, from newly synthesized AMPA receptor tetramers to mature AMPA receptors in the cell membrane. As such, the interaction of auxiliary subunits with the AMPA receptor plays a major role in the regulation of AMPA receptor biogenesis, trafficking, and biophysical properties. Throughout the years, various 'families' of proteins have been identified and today the approximate full complement of AMPAR auxiliary proteins is known. This review presents the current knowledge on the most prominent AMPA-receptor-interacting auxiliary proteins, highlights recent results regarding the Shisa protein family, and provides a discussion on future research that might contribute to the discovery of novel pharmacological targets of auxiliary subunits.

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Introduction

α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) are ionotropic glutamate receptors expressed at the neuronal postsynaptic membrane and mediate fast synaptic transmission in the CNS [1]. AMPAR subunit composition, phosphorylation state, and changes in the number are well known to directly affect synaptic transmission and plasticity [2]. In addition, AMPARs have been found to associate with a wide range of auxiliary proteins, which play a critical role in regulating intracellular trafficking, expression at the synaptic membrane, and AMPAR functional properties [3]. Differential expression of AMPAR core subunits and their co-assembly with auxiliary proteins dramatically increases receptor diversity and modulatory potential in the CNS [4,5]. The first family of AMPAR auxiliary subunits identified were the transmembrane AMPAR regulatory proteins (TARPs), of which TARP γ2 (stargazin) was found to be absent in the severely ataxic stargazer mouse mutant [6,7]. Since then, the advent of interaction proteomics has enabled the identification of a full complement of AMPAR associated proteins [8,9].

In 2010, interaction proteomics led to the identification of Shisa9/CKAMP44 as a novel AMPAR-interacting protein [10], whose structure and function were distinct from other established auxiliary subunits [11,12]. More specifically, Shisa9 was characterized as a single-pass transmembrane protein that induced fast and strong desensitization of AMPAR currents within the hippocampus. In the past few years, several additional members of the Shisa family have been reported to exert a role as AMPAR modulatory proteins, namely Shisa6/CKAMP52 [13–15], Shisa7/CKAMP59 [13,16], and Shisa8/CKAMP39 [13]. Although the Shisa family members share a high structural similarity, they are very distinct in both their (brain) regional expression profile and their modulation of AMPAR function [13,17]. This review provides a short overview of the known AMPAR auxiliary subunits including their function and effect on AMPARs while highlighting the Shisa protein family.

Auxiliary subunits

AMPARs assemble with a wide range of auxiliary subunits during their life cycle [3,5,18], from the moment of synthesis in the endoplasmic reticulum (ER) to full integration at postsynaptic sites [5]. Multiple studies using electrophysiology, proteomics, imaging, and mutagenesis have elucidated the many important functions of these proteins [5,18]. Apart from AMPAR assembly, trafficking, and expression at the synapse, auxiliary subunits are directly involved in the regulation of the receptor's biophysical properties [3,5].

Stargazin was identified as the first transmembrane protein to interact with the AMPAR [19]. Stargazin (Transmembrane AMPAR Regulatory Protein (TARP) γ2) acts during AMPAR biogenesis between tetramerization and ER exit, is essential for receptor maturation, and affects AMPAR surface expression [20–23]. Other members of the TARP family have subsequently been identified as AMPAR-interacting proteins: Type-I TARPs (subunits γ2- γ4 and γ8) and type-II TARPs (subunits γ5 and γ7) [21,24–26]. In addition to AMPAR maturation and trafficking, TARPs affect the pharmacological and gating properties of the receptor, as observed in both *in vitro* (Table 1) and *ex/in vivo* (Table 2) studies [25,27]. The effects of the TARP proteins on AMPAR conductance properties are specific for each family member and are subunit-dependent [28,29]. Importantly, TARPs have been demonstrated to directly associate with PSD-95 [19,30,31], one of the key organizers of the postsynaptic density (PSD) scaffold. This interaction regulates AMPAR surface trafficking and is essential for both long-term potentiation (LTP) and long-term depression (LTD) in the hippocampus [30,32]. In particular, TARP γ2 phosphorylation facilitates the interaction with PSD-95 and regulates lateral diffusion of AMPARs [32–35]. In addition, TARP γ8 phosphorylation was found to be crucial for CaMKII-dependent LTP [36].

In recent years, major efforts have been undertaken to identify the full complement of AMPAR-interacting proteins, using immunopurification of brain-derived AMPAR complexes and mass spectrometric analysis [8,9,37]. From these efforts stem the establishment of Cornichon homolog proteins (CNIHs) as an additional auxiliary subunit family [37]. These four-transmembrane domain proteins are expressed widely throughout the brain, and play a role in controlling the export of AMPARs from ER [38–40]. Moreover, CNIHs modulate AMPAR activation, deactivation, desensitization, and synaptic transmission (Tables 1 and 2) [3,37,41–43]. Remarkably, studies have found the association of either TARP γ2 or γ8 with CNIH-2 at hippocampal AMPARs, suggesting an interplay between TARPs and CNIHs [39,41,44].

Using interaction proteomics analysis of the germline-specific gene 1 like (GSG1L), a distant homolog of TARPs was identified as auxiliary subunits of the AMPAR [8,45]. GSG1L directly interacts with AMPARs at synapses and modulates AMPAR trafficking, surface expression, and gating properties (Tables 1 and 2; Figure 1) [8,45–49]. Synapse differentiation induced gene 1 (SynDIG1) was first identified as an AMPAR-interacting transmembrane protein that regulates the development, strength, and number of excitatory synapses [50]. However, since SynDIG1 does not directly affect biophysical properties or AMPAR surface trafficking it was later suggested to serve as a regulatory protein for excitatory synaptogenesis [51]. SynDIG4, also known as proline-rich transmembrane protein 1 (Prrt1), was identified as an AMPAR-associated protein [8,9,45]. SynDIG4 (Prrt1) was shown to be a component of the PSD [52], although the major fraction of Prrt1 colocalizes with GluA1 subunits at extrasynaptic sites in neurons [53]. It was shown to control levels of AMPARs at the cell surface and influence the gating properties of AMPARs (Tables 1 and 2) [54,55]. Ferric-chelate reductase 1 like (FRRS1L), also known as C9orf4 [8], is a single-pass transmembrane protein that effectively associates with the ER-resident CPT1c protein and plays an important role in the regulation of excitatory synaptic strength [56–58]. FRRS1L-CPT1c complexes co-assemble with the AMPAR during early biogenesis in the ER and FRRS1L-CPT1c-containing AMPARs exclusively localize at ER membranes (Figure 1). Brechet et al. have suggested that FRRS1L-CPT1c assemblies act as a 'catalyst' of AMPAR biogenesis and delivery of synaptic AMPARs specifically in the ER and are not present in AMPAR complexes at the synapse [56]. Additional protein quantifications and correlation analyses led to the model that all AMPARs expressed at the cell surface emerge from FSSR1L-containing AMPAR complexes in the ER [56]. In addition, secreted proteins acting at the AMPAR have been found [8,9]. Noelin1 (Olfactomedin-1) was shown to affect the membrane diffusion properties of the receptor while leaving kinetic and conductance properties of the AMPAR unaffected (Tables 1 and 2) [59].

In conclusion, a broad range of proteins can interact with AMPARs at varying stages of its life cycle, from newly synthesized AMPAR tetramers to mature AMPARs in the cell membrane (Figure 1), affecting biogenesis, trafficking, and biophysical properties of the receptor. The modulatory properties of the abovementioned auxiliary proteins on AMPAR surface expression and gating properties are summarized in Table 1 (*in vitro*) and Table 2 (*in vivo*).

Shisa9

The type-I transmembrane protein Shisa9 (CKAMP44) was discovered by interaction proteomics in 2010 and described as the first Shisa family member interacting with AMPARs [10]. Shisa9 specifically associates with AMPARs independent of subunit-type [10]. The 44 kDa protein Shisa9 is widely expressed throughout the brain and is most abundant in the dentate gyrus (DG) of the hippocampus [10]. Recent large-scale single-cell RNA sequencing studies of the hippocampus CA1 revealed the highest expression of Shisa9 in GABAergic interneurons, specifically in CCK^+ and $Reln^+$ subclasses [60]. Shisa9 function in inhibitory cells has not been studied to date and it will be of interest to reveal its role in these neurons. Shisa9 localizes at postsynaptic sites in primary neurons and interacts with multiple scaffold proteins, which are vital for the regulation of synaptic plasticity [61]. For instance, the C-terminal region of the

Table 1

Summary of AMPAR-interacting auxiliary proteins and their modulation of AMPAR surface expression and gating properties *in vitro*.

	AMPAR surface expression	Current amplitude	Deactivation rate	Desensitization rate	Recovery from desensitization rate	Methods used
In vitro						
TARP γ2	↑ [20,28,45,74]	↑ [20,28,75,76]	↓ [8,20,28,37,43,68,75,77]	↓ [8,20,28,43,68,75–77]	↔ [8,44,68,76,77]	[8,28,37]: Oocytes; [20,44,68]: HEK293; [45,74]: HEK; [75]: Oocytes/HEK293; [43,76,77]: HEK293 outside-out patch
TARP γ8	↑ [17]	↑ [17,69]	↓ [29,39,42,44,69,74,77]	↓ [17,29,44,69,77]	↑ [44,69]	[17,29,42,44,69]: HEK293; [39,74]: HEK; [77]: tsA201 outside-out patch
CNIHs	↑ [37,58,74]	↑ [39,74]	↓ [8,37,39,42–44]	↓ [8,37,43,44]	↔ [8,37,42,44]	[8,58]: Oocytes (CNIH-2); [37]: Cultured cells (HeLa)/Oocytes (CNIH-2/3); [39]: HEK (CNIH-2); [42,44,74]: HEK293 (CNIH-2); [43]: HEK 293 outside-out patch (CNIH-3)
GSG1L	↑ [45]	N/A	↓ [8,45,47]	↓ [8,45,47]	↓ [8,46,47]	[8]: Oocytes; [45]: HEK outside-out patch; [46,47]: HEK
SynDIG4 (Prrt1)	N/A	↓ [54]	↓ [54]	↓ [54]	- [54]	[54]: Oocytes, outside-out patch
FRRS1L	- [57,58]	- [57]	N/A	N/A	↓ [56]	[56]: tsA201 outside-out patch; [57]: HEK ; [58]: Oocytes
CPT1c	↔ [58,78]	↑ [78]	N/A	- [78]	- [56]	[56]: tsA201 outside-out patch; [58]: Oocytes; [78]: tsA201 outside-out patch
Noelin1 (Olfacto-medin-1)	N/A	N/A	- [59]	- [59]	- [59]	[59]: HEK293
Shisa9 (CKAMP44)	- [10]	↓ [10,17]	N/A	- [17]	↓ [17]	[10]: Oocytes; [17]: HEK293
Shisa6 (CKAMP52)	↑ [13]	-↓* [13]	↔* [13,14]	↔* [13,14]	↔* [13]	[13]: HEK293/Oocytes; [14]: HEK293
Shisa7 (CKAMP59)	-↓* [13]	-↓* [13]	- [13,16]	↔ [13,16]	↔ [13,16]	[13]: HEK293/Oocytes; [16]: HEK293
Shisa8 (CKAMP39)	-↓* [13]	↓ [13]	-↓* [13]	-↑* [13]	↓ [13]	[13]: HEK293/Oocytes

↑ depicts increase, ↓ decrease, ↔ variable effects/conflicting results, – no effect. *Effect depends on type of GluA subunit. N/A, not available; HEK, human embryonic kidney. The table represents our best approximation of data in the literature.

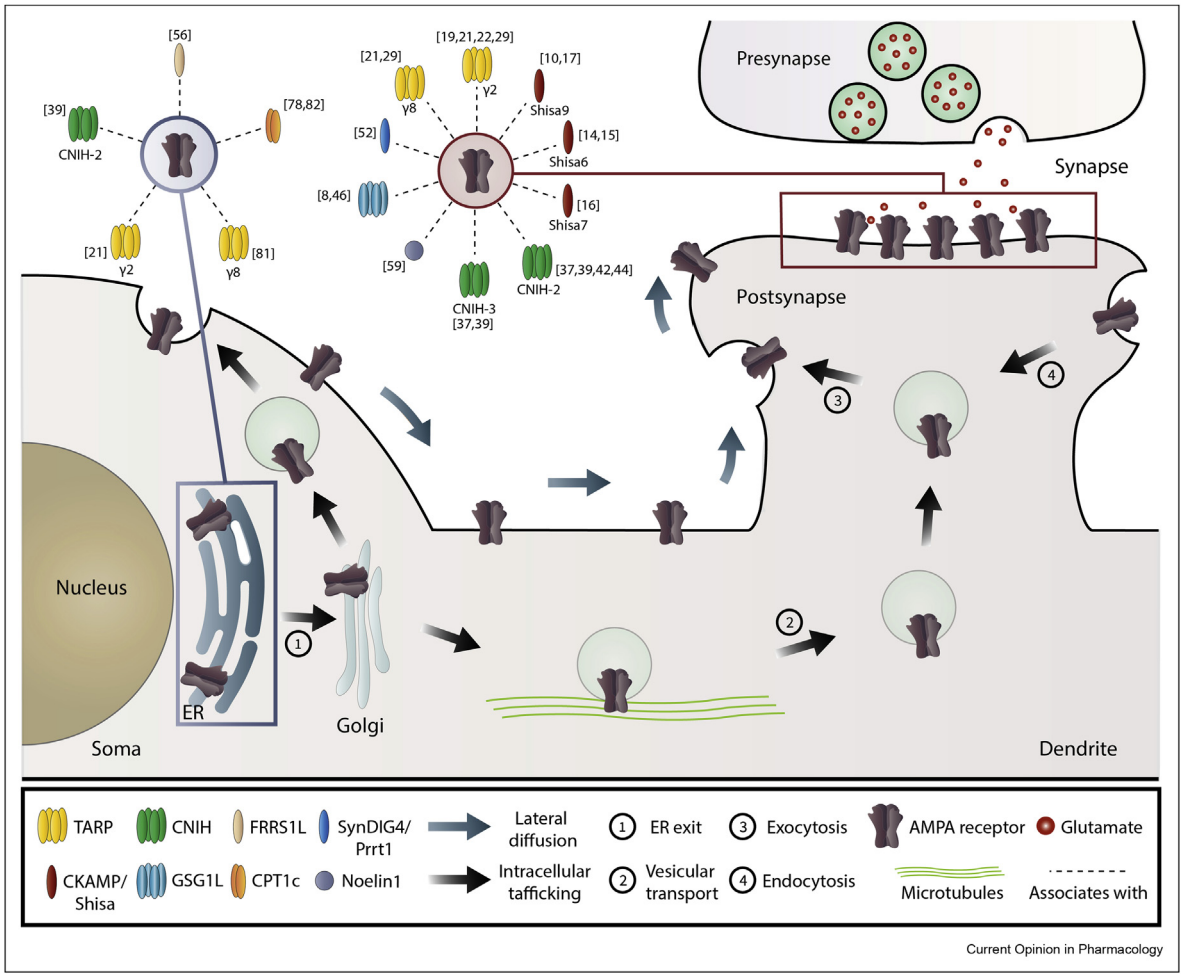
Table 2

Summary of AMPAR-interacting auxiliary proteins and their modulation of AMPAR surface expression and gating properties *ex/in vivo*.

	AMPAR surface expression	Current amplitude	Deactivation rate	Desensitization rate	Recovery from desensitization rate	Methods used
Ex/In vivo						
TARP γ2	↔ [21,29,42,77,79,80]	↔ [21,29]	- [77]	↓ [80]	↑ [80]	[21]: CN, KO (CB); [29]: CN outside-out patch, KO (CB); [42]: Acute slices (HC); [77]: CN (CB); [79]: IB (CB); [80]: CN (HC)
TARP γ8	↑ [17,29,39,81]	↔ [17,21,29,39,42,81]	↔ [17,39]	↓ [17]	↑ [17,42]	[17]: CN outside-out patch, KO (HC (DG)); [21]: CN, KO (CB); [29]: CN outside-out patch, KO (CB); [39]: IB (HC)/CN, KO (HC (CA1); [42]: CN (CB, HC); [81]: CN (HC)
CNIHs	↑ [39,51,74]	↑ [39]	↔ [39,74]	↔ [39,74]	- [39,42]	[39]: Acute slices (HC)/CN, KO (HC) (CNIH-2); [42]: CN (CB) (CNIH-2); [51]: CN, KD (HC); [74]: CN (CB, HC)
GSG1L	↓ [46]	↓ [46,48]	↔ [46,48]	↔ [46,48]	↑ [46]	[46]: CN, KO (HC (CA1)) [48]: CN, KO (HC (DG))
SynDIG4 (Prrt1)	↑ [55]	↔ [54,55]	- [54]	N/A	N/A	[54]: Acute slices, KO (HC (CA1)); [55]: Acute slices (HC (CA1))
FRRS1L	↔ [56,57]	↑ [56–58]	- [56,57]	N/A	N/A	[56]: CN, KD (HC); [57]: CN, single-cell KO (HC (CA1)); [58]: Acute slices outside-out patch, KO (HC (CA1))
CPT1c	↑ [56,78]	↑ [56,82]	- [56,82]	N/A	N/A	[56]: CN, KD ((HC); [78]: CN (cortex); [82]: CN, KO (HC)
Noelin1 (Olfacto-medin-1)	N/A	- [59]	N/A	N/A	N/A	[59]: CN (HC)
Shisa9 (CKAMP44)	↑ [17,83]	↔* [10,17,83]	↔ [10,17,83]	↔* [10,17,83]	↓ [10,17,83]	[10]: Acute slices outside-out patch, KO (HC (DG))/ nucleated patch, KO, KD (HC); [17]: CN outside-out patch, KO (HC (DG)/nucleated patch (HC (DG)); [83]: Acute slices, nucleated patch, KO (dLGN relay neurons)
Shisa6 (CKAMP52)	↔ [14,15]	- [14,15]	↔* [14,15]	↓ [14]	N/A	[14] IB, KO (HC)/Acute slices, KO (HC (CA1))/ Nucleated patch, KO (HC (CA1)); [15]: IB, KO (CB)/ Acute slices, KO (CB (PCs)
Shisa7 (CKAMP59)	- [16]	- [16]	↓ [16]	N/A	N/A	[16]: IB (HC)/Acute slices, KO (HC (CA1))
Shisa8 (CKAMP39)	N/A	N/A	N/A	N/A	N/A	N/A

↑ depicts increase, ↓ decrease, ↔ variable effects/conflicting results, – no effect. *Effect depends on type of GluA subunit. N/A, not available; CN, cultured neurons; IB, immunoblotting; KO, knockout; KD, knockdown; CB, cerebellum; HC, hippocampus; DG; dentate gyrus; dLGN: dorsal lateral geniculate nucleus; PCs, Purkinje cells. The table represents our best approximation of data in the literature.

Figure 1



Schematic showing a broad range of AMPAR auxiliary proteins at varying stages of their life cycle, highlighting their association with newly synthesized AMPARs in the endoplasmic reticulum (ER) and with AMPARs at the postsynaptic density (PSD). AMPARs and auxiliary subunits are assembled in the ER and Golgi cell soma and subsequently, upon ER (#1, ER exit) and Golgi exit and via microtubular vesicular trafficking (#2, vesicular transport), delivered to the plasma membrane (#3, exocytosis). Via lateral diffusion, surface AMPARs reach the postsynapse. Via endocytosis (#4, endocytosis) AMPARs can be retrieved from the plasma membrane (recycling). Studies have shown that several auxiliary subunits affect AMPAR trafficking (Shisa9 [17]; TARP γ2 [21,22,25,28,29]; γ8 [81]; CNIH-2 [39]; GSG1L [46]; CPT1c [78]; SynDIG4 [55]), with some of the auxiliary proteins acting at specific stages, such as ER exit (TARP γ2 [21]; γ8 [81]) and lateral diffusion (TARP γ2 [30,35]; Noelin1 [59]). Proteins that have been specifically shown to associate with AMPARs within the ER and postsynaptic zone are shown in the figure (supporting literature is given in parentheses). However, the precise auxiliary subunit composition, stoichiometry, as well as the interaction of auxiliary subunits with each other, remains to be determined. Of note, with the exception of SynDIG4 and Noelin1, auxiliary subunits present in the postsynaptic zone are most likely assembled in the ER.

Shisa9 protein interacts with PDZ domain proteins, such as PSD-95, thereby providing indirect binding of AMPARs to the PSD [61,62]. Interference with these Shisa9-PDZ interactions profoundly affects synaptic AMPAR function [62].

Shisa9 modulates AMPAR gating properties different from TARP (Tables 1 and 2). Initial studies in both Xenopus oocytes and CA1 pyramidal neurons have shown that overexpression of Shisa9 affects AMPAR channel kinetics, such as deactivation and desensitization rates, whereas *Shisa9* deletion remained without effect in CA1 neurons (Table 2) [10]. In DG granule cells, in which Shisa9 is clearly expressed [17], *Shisa9* deletion resulted in faster deactivation, slower desensitization, and faster recovery from desensitization of AMPAR-mediated currents [17]. Depending on the endogenous expression of Shisa9, its effect on AMPAR surface expression has yielded diverse results (Tables 1 and 2). Although Shisa9 expression in CA1 neurons did not show profound effects in terms of AMPAR surface expression [10,63], overexpression in DG granule cells increased surface expression [17], whereas heterologous expression in HEK cells reduced surface

expression [13]. Co-expression of both Shisa9 and TARP γ8 was proven essential for efficient AMPAR surface expression in the DG (Table 2) [17], with additive effects observed upon a double deletion. Deletion of *TARP γ8* decreased paired-pulse ratio (PPR) at medial and lateral perforant path (MPP, LPP) synapses of DG granule cells, whereas deletion of *Shisa9* increased short-term plasticity [10,17]. On the other hand, deletion of *TARP γ8* fully abolished LTP at MPP synapses of DG granule cells, but deletion of *Shisa9* had no effect [17].

Shisa6

Shisa6 (CKAMP52) was recently identified as an additional constituent of native AMPAR complexes and is closely related to the AMPAR auxiliary protein Shisa9 [8,10,14]. Shisa6 shares the majority of its structural features with Shisa9, as both are type-I single-pass transmembrane proteins that contain an extracellular cysteine-rich domain, a proline-rich intracellular region, and a C-terminal type II PDZ-ligand motif [14,64]. Shisa6 is highly expressed in the cerebellum and all hippocampal subregions [14,65]. Unlike Shisa9, Shisa6 is highly expressed in pyramidal neurons and has moderate expression in two classes of SST^{+} interneurons in the hippocampus CA1 [60]. Shisa6 interacts with AMPAR subunits GluA1, GluA2, and GluA3 in both HEK293 cells and hippocampal tissue, and does so without an evident subunit preference [14]. In addition, Shisa6 interacts with PDZ-domain-containing scaffold proteins, such as PSD-95, through its C-terminal EVTV domain [14]. Shisa6 co-localizes with protein PSD-95 at excitatory postsynaptic sites and is enriched within purified PSD fractions. Overexpression of Shisa6 within hippocampal neurons was shown to constrain lateral surface mobility of AMPARs and confine them to PSD by association with PDZ proteins. Expression of a truncated version of Shisa6, lacking the C-terminal EVTV motif, did not alter AMPAR mobility [14].

Shisa6 modulates AMPAR-mediated currents directly upon co-expression with homomeric GluA1 and heteromeric GluA1/2 receptors within HEK293 cells. The presence of Shisa6 decreased the AMPAR deactivation rate and slowed recovery from desensitization, similar to what was reported for Shisa9 (Table 1) [10]. However, in contrast to the effect of Shisa9, Shisa6 was found to decrease the rate of desensitization and to greatly enhance the steady-state current upon prolonged glutamate application [14]. In CA1 pyramidal cells, deletion of *Shisa6* leads to a decrease in both rise and decay time of AMPAR-mediated miniature excitatory postsynaptic currents (mEPSCs), without affecting the amplitude or frequency [14]. Moreover, the absence of *Shisa6* profoundly impacted short-term plasticity in a frequency-dependent manner [14], resulting in enhanced synaptic depression during high-frequency stimulation (50 Hz). Combined with the enhanced sustained current observed in HEK293 cells, the presence of Shisa6 is suggested to facilitate synaptic transmission by protecting postsynaptic AMPARs against full desensitization.

Just recently, an essential role for Shisa6 in the facilitation of cerebellar procedural memory formation has been described [15]. In the study of Peter et al., it was shown that Shisa6 is prominently expressed by cerebellar Purkinje cells (PCs). Shisa6 proteins colocalize and associate with GluA2-containing AMPARs in cerebellar PCs and modulate AMPAR gating properties, such as AMPAR deactivation [15]. The absence of *Shisa6* resulted in a reduction of mEPSC amplitude and rise time and an increased deactivation of AMPAR-mediated currents in PCs, as well as impaired facilitation of functional parallel fiber (PF) to PC excitation. Whereas AMPAR expression, measured in the synaptic membrane fraction, does not seem to be affected by deletion of *Shisa6* in the hippocampus [14], cerebellar levels of AMPAR subunits are significantly reduced upon *Shisa6* deletion [15], in agreement with the effects observed on mEPSC amplitude in both regions. Furthermore, Shisa6 is required for PF-PC synapse LTP, whereas deletion of the AMPAR-interacting protein does not affect LTD of those synapses [15].

Shisa7

Shisa7 (CKAMP59), recently identified as an AMPAR-interacting protein [13,16], shares all major structural characteristics present in Shisa6 and Shisa9, including the N-terminal Cysteine-rich region and the C-terminal PDZ type II binding motif [16]. Shisa7 is highly expressed in the hippocampus, striatum, amygdala, cortex, and olfactory bulb [13,16]. *In situ* hybridization analyses from the Allen Brain Atlas shows high expression of *Shisa7* in both CA regions and the DG of the hippocampus, similar to Shisa6 [65]. Shisa7 is expressed in pyramidal neurons in the hippocampus CA1, with low interneuron expression [60]. Shisa7 specifically associates with the AMPAR in native hippocampal protein complexes and directly binds to homomeric GluA1, GluA2, and GluA3 receptors upon HEK293 co-expression, with no evident preference for a specific subunit [10,13,14,16]. PSD-95 is the most prominent PDZ domain-containing protein that interacts with Shisa7 in the hippocampus, with a crucial role for the C-terminal EVTV motif in this interaction [16]. In comparison to Shisa6, Shisa7 AMPAR-mediated AMPAR mobility has not been investigated.

In 2015, Farrow et al. reported that co-expression of Shisa7 in HEK293 cells does not affect most AMPAR-mediated current kinetics, except for the current amplitude mediated by GluA2-containing AMPARs [13]. More recently, it was established that Shisa7 can alter several AMPAR desensitization kinetics

(desensitization time, steady-state current, and recovery from desensitization), whereas channel kinetics such as AMPAR deactivation and rise time are indeed not affected [16]. Moreover, AMPAR expression levels in the synaptic membrane fraction in *Shisa7* KO mice do not differ from expression levels in Shisa7 WT mice, resembling findings of Shisa6 studies (Table 2) [14,16]. In contrast to both Shisa9 and Shisa6, Shisa7 does not affect short-term plasticity in the hippocampus [10,14,16]. These findings suggest that also Shisa7 modulates AMPAR gating properties in a unique way, compared to other members of the Shisa protein family (Tables 1 and 2). Furthermore, Shisa7 seems to have a crucial role in both the initiation as well as the maintenance of hippocampal LTP [16]. *Shisa7* KO mice showed a slower initiation of LTP as well as a decrease in the maintenance phase, compared to WT animals.

Interestingly, in 2019, Shisa7 was identified as a $GABA_AR$-interacting protein [66]. Han et al. showed that Shisa7 localized at GABAergic inhibitory synapse and that it is a critical protein for inhibitory transmission. Using cocultures of hippocampal neurons with HEK293 cells, co-expression of Shisa7 and $GABA_ARs$ resulted in a reduction of both the decay time constant of spontaneous inhibitory postsynaptic currents (sIPSCs) as well as the time constant of deactivation, indicating modulation of the kinetics of GABAergic transmission and $GABA_ARs$ by Shisa7. Moreover, deletion of *Shisa7* increased the decay time constant of mIPSCs in CA1 pyramidal cells and decreased the expression of $GABA_AR$ subunits at the inhibitory PSD [66]. In opposition to previous studies [13,16], Han et al. have reported that Shisa7 does not colocalize with glutamatergic synaptic markers in primary cultures [66].

Shisa8

The least studied AMPAR-interacting member of the Shisa protein family is Shisa8 (CKAMP39). The highest expression of Shisa8 was observed in the cerebellum and olfactory bulb, with almost no expression in other brain regions [13]. In the study of Farrow et al., co-expression of Shisa8 and AMPAR subunits GluA1 and GuA2 in Xenopus oocytes showed that it co-immunoprecipitated with both subunits, and modulated AMPAR gating properties [13]. In HEK293 cells, Shisa8 significantly affected AMPAR-mediated current kinetics, such as current amplitude, as well as AMPAR desensitization and recovery from desensitization. However, some differences were observed for deactivation- and desensitization kinetics between GluA1-mediated currents and GluA2-mediated currents, when modulated by Shisa8. The effects of Shisa8 on AMPAR gating properties in neurons remains unknown since this effect has not been studied to date. With lacking data on the endogenous neuronal function, Shisa8 can currently not be regarded as a true AMPAR auxiliary protein.

Perspective: pharmacological targeting of interfaces between AMPARs and auxiliary proteins

Auxiliary proteins interacting with the AMPAR play a critical role in the CNS by modulating receptor synthesis, trafficking, surface expression, and gating properties. With current knowledge, a broad range of AMPAR auxiliary proteins seems to modulate the receptor's function at different cellular locations, from the newly synthesized tetramers at the early stage of biogenesis to mature AMPARs in the cell membrane. Throughout the years, various 'families' of proteins have been identified and today the approximate full complement of AMPAR auxiliary proteins is known. With this parts list identified, the journey into the discovery of specific functions of individual proteins or combinations thereof has only begun. After the discovery of the Shisa protein family, the precise examination of their role in allosteric modulation of AMPAR function and membrane diffusion has significantly contributed to the detailed understanding of mechanisms acting on AMPAR-mediated fast synaptic transmission and plasticity. This holds true for many of the identified AMPAR-associated proteins.

Interestingly, the expression levels of various auxiliary subunits have been identified by determining molecular profiles of AMPAR auxiliary subunits across distinct brain regions and recently even down to single cells. Distinct cellular expression of auxiliary proteins may lead to functionally unique combinatorial actions on the AMPAR [17]. With respect to this, the composition and stoichiometry of auxiliary subunits also vary greatly within AMPAR complexes [4,67]. The stoichiometry of TARPs is best understood since recent cryo-electron microscopy (cryo-EM) studies have provided structural information on the AMPAR-TARP complex [68–70]. In addition, structural analyses have revealed the molecular mechanism underlying the modulation of AMPAR function by Stargazin/TARP $\gamma 2$ [71,72]. However, structural analyses of the AMPAR interaction with other auxiliary subunits, such as Shisa proteins, or even with multiple different auxiliary proteins, are absent.

Importantly, both functional and structural information of the combinatorial action of auxiliary proteins on the AMPAR is opening new avenues for the rational design of compounds acting at the molecular interfaces of the auxiliary proteins and AMPAR [73]. This may provide unprecedented possibilities for modulating AMPAR receptor function. In conclusion, examining the subcellular points of interaction between auxiliary subunits and AMPARs, the cell-type-specific auxiliary subunit expression, the precise auxiliary subunit composition, and stoichiometry, as well as the interaction of Shisa proteins with each other and/or with other auxiliary subunits together at the AMPAR, might all contribute to potential new pharmacological interventions.

Author contributions

August B Smit: Conceptualization, Writing and editing, Supervision. **Mazyar Abdollahi Nejat:** Writing – original draft, Writing. **Sabine Spijker:** Visualization, editing. **Remco Klaassen**: Investigation, editing.

Conflict of interest statement

Nothing declared.

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* of special interest
** of outstanding interest

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