Glutamate is a major excitatory neurotransmitter in the vertebrate brain. Among the ionotropic glutamate receptors, \( \alpha \)-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) glutamate receptors are the major receptors mediating excitatory fast synaptic transmission. AMPA receptors are also responsible for modifying synaptic strength through the regulation of their numbers at synapses. Their high regulatability, therefore, could contribute to the mechanisms of synaptic plasticity. The mechanisms regulating AMPA receptor trafficking have evolved great interest through the decades. Recent studies show that in the brain, AMPA receptors make complexes with transmembrane AMPA regulatory proteins (TARPs), which serve as auxiliary subunits. TARPs are required for AMPA receptor function and trafficking. After the initial discovery of TARPs, several other AMPA receptor auxiliary subunits were identified: CNIH-2, CNIH-3, CKAMP44, SynDIG1, SOL-1, SOL-2 and GSG1L. This review discusses progress in identifying the role of auxiliary subunits in AMPA receptor trafficking.

Keywords: AMPA-type glutamate receptor/auxiliary subunit/synapse/TARP/trafficking.

Abbreviations: AMPA, \( \alpha \)-amino-3-hydroxy-5-methyl-4-isoxazole-propionate; AP-4, adaptor protein 4; CaMKII, calcium/calmodulin-dependent protein kinase II; CKAMP44, cystine-knot AMPA receptor modulating protein 44; C-terminal, carboxy-terminal; CNIH, cornichon homolog; CUB, complement C1r/C1s, Uegf, Bmp1; EPSC, excitatory postsynaptic current; GDPβS, guanosine 5-O-(2-thiodiphosphate); GSG1L, germ-cell-specific gene 1-like; GK, guanylate kinase; HEK-293, human embryonic kidney-293 cells; LTD, long-term depression; LTP, long-term potentiation; N-terminal, amino-terminal; MAGUKs, membrane-associated guanylate kinases; NMDA, \( N \)-methyl-\( \alpha \)-aspartic acid; PKA, protein kinase A; PDZ, PSD-95–discs large–zona occludens 1; PSD, postsynaptic density; SOL-1, -2, suppressor of Lurcher-1, -2; SH3, src homology 3; SynDIG1, synapse differentiation-induced gene 1; v-SNARE, vesicle \( N \)-ethylmaleimide-sensitive factor (NSF) attachment protein receptor.

Excitatory Synapses and \( \alpha \)-Amino-3-hydroxy-5-methyl-4-isoxazole-propionate Receptors

Circuits formed by billions of neurons in the central nervous system underlie a variety of brain functions, including learning and memory. Synapses are neuronal communication sites in these circuits. During synaptic transmission at chemical synapses, neurotransmitters released from presynaptic terminals activate receptors at postsynaptic sites. In a simplified model, excitatory synapses depolarize postsynaptic membranes, causing postsynaptic neurons to fire when the membrane potential is exceeded by a certain amount. By contrast, inhibitory synapses hyperpolarize postsynaptic membranes, causing postsynaptic neurons to cease firing. This kind of neuronal connectivity via dynamically adjusted synaptic transmission maintains neural circuits.

In vertebrate brains, glutamate is the major neurotransmitter at excitatory synapses. Ionotropic glutamate receptors are pharmacologically classified into three types: \( N \)-methyl-\( \alpha \)-aspartic acid (NMDA), \( \alpha \)-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) and kainate receptors (Fig. 1). NMDA receptors allow \( Ca^{2+} \) and \( Na^{+} \) ions to flow into neurons. The resulting \( Ca^{2+} \) influx activates \( Ca^{2+} \)-dependent enzymes at postsynaptic sites, subsequently triggering signal cascades involved in synaptic plasticity. Synaptic plasticity is defined as a persistent change in synaptic strength. AMPA receptors in the postsynaptic density (PSD), which is an electron-dense area in electron micrographs of the postsynaptic membrane, play a major role in fast synaptic transmission in excitatory synapses (1, 2). AMPA receptors distributed at synapses and extrasynaptic sites, as well as the numbers of synaptic AMPA receptors, play an important role in modulating synaptic strength (3–9). During hippocampal long-term potentiation (LTP), a kind of activity-dependent plasticity and a model for neuronal learning, \( Ca^{2+} \) influx mediated by NMDA receptors eventually causes the insertion of AMPA receptors into the PSD (3–9). On the other hand, during long-term depression (LTD), another type of activity-dependent plasticity, \( Ca^{2+} \) influx mediated by NMDA receptors or metabotropic glutamate receptors results in the removal of AMPA receptors from the PSD (3–9). Therefore, elucidating the mechanisms responsible for regulating AMPA receptor trafficking will aid our understanding of synaptic transmission and ultimately brain function.

AMPA Receptor Trafficking at Synapses

A three-step model describing the trafficking of AMPA receptors at synapses has been proposed (10). In this
model, movement of AMPA receptors takes place by (i) exocytosis of intracellular AMPA receptors to the plasma membrane, (ii) lateral diffusion of surface AMPA receptors and (iii) stabilization of AMPA receptors at the PSD (Fig. 1).

There is much evidence to support the idea that in neurons, AMPA receptors are trafficked to the plasma membrane via the recycling of intracellular vesicles. Inhibition of exocytosis by the light chains of Type B botulinum toxin (Botox), which inactivates the vesicle fusion protein vesicle N-ethylmaleimide-sensitive factor (NSF) attachment protein receptor (v-SNARE), decreases synaptic AMPA receptor activity in hippocampal pyramidal cells (11). On the other hand, inhibition of dynamin-dependent endocytosis by guanosine 5-O-(2-thiodiphosphate) (GDPβS), which inactivates GTPases such as dynamin, induces over 2-fold increase in synaptic AMPA activity (11). These findings indicate that AMPA receptors are continuously transported via endocytosis and exocytosis.

The cycling of AMPA receptors is not specific to the synapse. Clathrin has been shown to mediate the endocytosis of AMPA receptors (12). Exogenous expression of green fluorescent protein-labeled clathrin (clathrin-GFP) in hippocampal neurons revealed that clathrin coats assemble and disassemble repeatedly at certain sites in dendrites and adjacent to the PSD, indicating that active sites of endocytosis lie in dendrites and in perisynaptic areas (13). The insertion of intracellular AMPA receptors into the plasma membrane via exocytosis is not sufficient for enhancing synaptic AMPA receptor activity. Chemically inducing LTP through forskolin-/rolipram-induced protein kinase A (PKA) activation in hippocampal neurons increases surface AMPA receptors expression (14). PKA activation without basal stimulation, however, only induces surface insertion of AMPA receptors (14). These findings indicate that additional steps are required for synaptic trafficking of AMPA receptors.

Surface AMPA receptors laterally diffuse on the plasma membrane and are stabilized at the PSD. Single-particle tracking with video microscopy directly visualized lateral movements of surface AMPA receptors, demonstrating that AMPA receptors undergo alternating cycles of diffusion and spatial stasis (15). These observations support the hypothesis that surface AMPA receptors undergo lateral diffusion and stabilization.

**AMPA Receptor Subunits**

To understand molecular mechanisms underlying the trafficking of AMPA receptors, it is important to elucidate the molecular composition of AMPA receptors. A tetramer consisting of GluA1-4 subunits makes up the channel pore of AMPA receptors (16). GluA1-4 subunits contain a long amino-terminal (N-terminal) extracellular region, a ligand-binding site, three transmembrane domains, a channel pore loop and a carboxyl-terminal (C-terminal) cytoplasmic tail, which interact with NMDA receptors. AMPAR, AMPA receptor; NMDAR, NMDA receptor.

**Fig. 1** Synaptic transmission and AMPA receptor trafficking. Glutamate released from presynaptic terminal acts on NMDA receptors and AMPA receptors at the synapse. (A) AMPA receptors insertion at synapse. AMPA receptors at intracellular vesicles are trafficked to the cell surface by exocytosis. Surface AMPA receptors laterally diffuse and are stabilized at the PSD. (B) AMPA receptors removal from synapse. AMPA receptors laterally diffuse from the PSD. Surface AMPA receptors are trafficked to the intracellular vesicles by endocytosis. AMPAR, AMPA receptor; NMDAR, NMDA receptor.
Auxiliary subunits GluA1-4 and their protein–protein interactions. As a result, much progress in the understanding of AMPA receptor trafficking has been achieved (3–9, 19, 20). Further studies of auxiliary subunits of AMPA receptor will accelerate progress.

**TARPs as Auxiliary Subunits of AMPA Receptors**

Auxiliary β-subunits of voltage-gated ion channels were first discovered in the 1990s. In 2005, TARPs were identified as the first auxiliary subunits of AMPA-type ionotropic glutamate receptors (21–24).

The essential contribution of TARPs in AMPA receptor function can be seen in stargazer mice, spontaneous mutant mice with a null mutation in the gene encoding prototypical TARP γ-2 (stargazin) (25, 26). Stargazer mice display absence seizures—brief epileptic seizures of sudden onset and termination—and cerebellar ataxia. Synaptic AMPA receptor activity in the cerebellar granule cells of these mice is impaired (27). There are six isoforms of TARPs (γ-2, γ-3, γ-4, γ-5, γ-7 and γ-8), which are classified into two types: Type I comprises the γ-2, γ-3, γ-4 and γ-8 isoforms and Type II comprises the γ-5 and γ-7 isoforms (28, 29). TARP isoforms show diverse expression patterns in the brain, with γ-2 being expressed throughout the brain, especially in the cerebellum (28, 30, 31). The γ-3 isoform is predominantly expressed in the cerebrum and amygdala, and γ-4 is primarily expressed in the basal ganglia (28, 30, 31). The γ-8 isoform, on the other hand, is abundantly expressed in the cerebrum and especially in hippocampus (28, 30, 31). TARPs consist of four transmembrane domains and C-terminal cytoplasmic tails. Type I TARPs contain a typical PDZ-binding motif at the end of their tails, whereas Type II TARPs contain atypical PDZ-binding sites.

The roles of TARPs as auxiliary subunits of AMPA receptors have been well studied. TARPs are non-pore-forming subunits (32) that stably bind to AMPA receptors in the brain (23, 24, 33, 34) to modulate the channel properties of AMPA receptors (21, 29, 35–37). TARPs regulate the trafficking of AMPA receptors and are required for synaptic AMPA receptor activity, as discussed later in this review. From these findings, it is clear that TARPs are bona fide auxiliary subunits of AMPA receptors.

**TARPs Regulate Surface Trafficking of AMPA Receptors**

It has been well confirmed experimentally that TARPs regulate the surface trafficking of AMPA receptors. The cerebellar granule cells of stargazer mice possess only ~10% of the ionic currents mediated by surface AMPA receptors compared with wild-type mice, and hippocampal pyramidal cells of mice deficient in TARP γ-8, a major TARP isoform in hippocampus, also exhibit a comparable reduction in currents (38–40). However, expression of heterologous combinations of TARP isoforms in stargazer mice increases the surface expression of AMPA receptors (21, 22, 29, 35, 41, 42). In other studies, TARP overexpression in neurons increases the surface expression of AMPA receptors both at the soma and dendrites (22, 38, 43). Of the TARP isoforms, Type II TARP γ-5 does not regulate the surface expression of AMPA receptors (29, 32). These results indicate that TARPs, except γ-5, maintain an extrasynaptic pool of surface AMPA receptors.

Although it is clear that TARPs play an essential role in AMPA receptor trafficking, the mechanisms underlying TARP-mediated AMPA receptor surface expression remains unclear and may differ according to neuronal class or TARP isoform. For example, the cerebellum of stargazer mice, null mutant in TARP γ-2, has large immature pools of AMPA receptors, suggesting that the γ-2 isoform may play a specific role in AMPA receptor maturation in the cerebellum (28). This proposed role of TARP γ-2 is consistent with the finding that γ-2 is involved early on in the AMPA receptor biosynthetic pathway in cerebellar granule cells (41). However, in the hippocampus of mice lacking TARP γ-8, normal EndoH-sensitive GluA2 is observed at the same level as in wild-type mice, indicating that another mechanism also is likely involved (38, 44). These studies suggested that another TARP γ-8 modulate AMPA receptor localization via a receptor stabilization mechanism.

This brief overview of findings suggest that several mechanisms and combinations thereof may underlie TARP-mediated regulation of surface AMPA receptor trafficking.

**TARPs Regulate Somatodendritic Trafficking of AMPA Receptors**

TARPs also regulate the somatodendritic distribution of AMPA receptors. Immunohistochemical staining of hippocampi from TARP γ-8-deficient mice revealed that these mice have fewer dendritic AMPA receptors than wild-type mice (38). On the other hand, overexpression of AMPA receptor subunits resulted in an accumulation of AMPA receptor complexes in the cell body of hippocampal neurons. Overexpression of AMPA receptor subunits along with TARP γ-2 successfully enhances dendritic expression of AMPA receptors (45). Interestingly, in mice deficient in the AP-4 β subunit gene, which encodes a protein involved in vesicular trafficking, AMPA receptors accumulate in the axons of cerebellar Purkinje cells (46). Adaptor protein 4 (AP-4) indirectly interacts with AMPA receptors through the C-terminus of TARPs. Furthermore, a TARP γ-3 mutation, which inhibits the association of TARPs and AP-4, fails to exclude AMPA receptors from the axons of hippocampal neurons (46). These findings suggest that TARPs play a role in the somatodendritic trafficking of AMPA receptors.

**PSD-95 and TARPs Regulate Synaptic Trafficking of AMPA Receptors**

Interestingly, overexpression of PSD-95, a unique scaffold protein located in the PSD, induces synaptic clustering of AMPA receptors but not NMDA receptors
receptors (47, 48). This suggests that overexpression of PSD-95 may open up new slots for AMPA receptors at synapses, thereby enhancing the retention of synaptic AMPA receptors. Furthermore, the diffusion of surface AMPA receptors decreases at PSD-95 clusters (49).

PSD-95 is a membrane-associated guanylate kinase (MAGUK) that contains multiple protein interactive structures: three PDZ domains, a src homology 3 (SH3) domain and a GK domain (Fig. 2). Although PSD-95 cannot directly bind to AMPA receptor subunits, it does bind to TARPs. Type I TARPs can directly bind to the PDZ domains of PSD-95 through its canonical PDZ-binding motif located at the TARP C-terminal end (40, 50). These findings suggest that the PDZ slots of PSD-95 capture and stabilize AMPA receptor complexes at synapses through the C-terminal tail of TARPs.

A model for synaptic trafficking of AMPA receptors that features PSD-95 and TARP has been validated using ΔC mutants, which are TARP mutants that lack a C-terminal tail. Although the overexpression of TARP γ-2 ΔC in hippocampal neurons reduces synaptic AMPA receptor activity in a dominant negative manner (40, 43, 49), it increases the mobility of surface AMPA receptors at synapses (49). In another study, hippocampal pyramidal cells of mutant mice harbouring TARP γ-8 ΔC have reduced synaptic AMPA receptor activity at a level comparable to that of TARP γ-8-deficient mice (44). These results indicate that the C-terminal tails of TARPs are required for stabilizing AMPA receptors at synapses.

The story of synaptic trafficking of AMPA receptors during plasticity is more intriguing. PSD-95 overexpression in neurons induces synaptic AMPA receptor potentiation (47, 48), suggesting that PSD-95 may play a role in AMPA receptor stabilization during plasticity. However, mutant TARP γ-8 ΔC mice have normal LTP induction in the hippocampus, even though interaction between TARP and PSD-95 is absent (44). This clearly means that AMPA receptor trafficking during activity-dependent plasticity is mediated by a quite different mechanism than that employed in the association of PSD-95 and TARPs during the basal state. Further studies will be required to understand all the mechanisms underling synaptic trafficking of AMPA receptors during plasticity, including the possible role of other auxiliary subunits.

**Synaptic AMPA Receptors Are Regulated by TARP Phosphorylation**

The phosphorylation of the C-terminal tails of TARPs plays a crucial role in controlling synaptic strength through AMPA receptors (51). TARP γ-2 possesses nine phosphorylation sites (51). Compared with extrasynaptic regions, TARP γ-2 at synapses is highly phosphorylated (51). Synaptic AMPA receptor activity in cerebellar granule cells is enhanced in TARP γ-2 phospho-mimic-mutant mice but reduced in non-phospho-mimic-mutant mice (52). These results indicate that phosphorylation of TARPs regulates synaptic AMPA receptors.

TARP phosphorylation has been proposed to regulate activity-dependent synaptic trafficking of AMPA receptors. Some phosphorylation sites in TARPs depend on calcium-/calmodulin-dependent protein kinase II (CaMKII), a kinase involved in hippocampal LTP (51). The overexpression of TARP γ-2 non-phospho-mimic mutant in neurons prevents hippocampal LTP induction and cerebellar LTD induction, whereas overexpression of wild-type TARP γ-2 does not (51, 53). CaMKII induces diffusional trapping of surface AMPA receptors at synapses in a TARP phosphorylation-dependent manner (54). These results suggest that phosphorylation of TARPs plays a role in activity-dependent synaptic trafficking of AMPA receptors.

Models have been proposed for the role of lipid bilayers in a molecular mechanism underlying TARP phosphorylation-dependent trafficking (52). We determined that TARP phosphorylation-dependent trafficking is regulated by the interaction between TARPs and lipid bilayers (52). The C-terminal tail of TARP γ-2 binds to lipid bilayers in an electrostatic manner, and

---

**Fig. 2 Schematic structures of AMPA receptor subunits, auxiliary subunits and synaptic scaffolds.** GluA1-4 are AMPA receptor subunits. TARPs are auxiliary subunits of AMPA receptors that were the first of its kind to be identified. GSG1L, CNIIH-2, CNIIH-3, SynDIG1, CKAMP44, SOL-1 and SOL-2 have also been proposed to be auxiliary subunits of AMPA receptors. PSD-95 is a unique scaffold protein found at PSDs. N, N-terminus; C, C-terminus; PSD, postsynaptic density; PDZ, postsynaptic density-95 disc large–zona occludens 1 domain.
this interaction inhibits the binding of TARP to PSD-95 (52). TARP γ-2 phosphorylation regulates its interaction with lipids through its negatively charged phosphates and subsequently regulates binding activity of TARP to PSD-95 through lipid interactions. These findings show that TARP phosphorylation regulates synaptic trafficking of AMPA receptors through lipid bilayers in an electrostatic manner. The role of TARP phosphorylation in activity-dependent plasticity suggests that another mechanism may also be involved, because the TARP C-terminus plays a limited role in hippocampal LTP (44).

Newly Discovered Auxiliary Subunits of AMPA Receptors

The discovery of TARPs as auxiliary subunits of AMPA receptors prompted the search for new auxiliary subunits. In addition to TARPs, cornichon homolog 2 (CNH2), CNH3, cystine-knot AMPA receptor modulating protein 44 (CKAMP44), synapse differentiation-induced gene 1 (SynDIG1), suppressor of Lurcher-1 (SOL-1), SOL-2 and germ-cell-specific gene 1-like (GSG1L) have been proposed as auxiliary subunits of AMPA receptors (55–60). Compared with TARPs, little is known about what roles these newly found auxiliary subunits play in AMPA receptor trafficking.

CNH2 and CNH3 were identified by proteomic analysis of purified AMPA receptor complexes from rat brain (55). CNHs consist of a short cytoplasmic N-terminus, three transmembrane domains and a short extracellular C-terminus (Fig. 2). Expression of CNHs in a heterologous system increases surface expression of AMPA receptors (55). On the other hand, TARPs-deficient mice exhibit reduced surface expression of CNH2 in hippocampus (61). This result means that surface trafficking of CNHs depends on TARPs.

CKAMP44 was identified through a proteomic approach using purified AMPA receptor complexes from mouse brains (57). CKAMP44 is a Type II transmembrane protein ending in a class II PDZ-binding motif (Fig. 2). CKAMP52 was also identified from purified AMPA receptor complexes (54). The role CKAMP44 plays in AMPA receptor trafficking remains unknown. CKAMP44 overexpression in neurons failed to affect the surface expression and synaptic clustering of AMPA receptors (57).

SynDIG1 was originally identified in microarray analyses as a gene associated with neuronal differentiation (62). SynDIG1 is a Type II transmembrane protein containing one transmembrane domain (Fig. 2). SynDIG1 has been proposed to regulate synaptic trafficking of AMPA receptors. SynDIG1 overexpression in hippocampal neurons increases mEPSC amplitude and frequency, suggesting enhanced synaptic clustering of AMPA receptors (56). SynDIG1 downregulation by shRNA reduces synaptic AMPA receptor activity (63).

SOL-1 was identified as a suppressor gene for Lurcher, a gain-of-function mutant of GLR-1, which is an AMPA receptor homolog of C. elegans (64). SOL-1 and SOL-2 are Type I transmembrane proteins containing extracellular complement C1r/C1s, Uegf, Bmp1 (CUB) domains (Fig. 2). Worms (C. elegans) deficient in SOL-1 and SOL-2 exhibit normal surface expression and synaptic clustering of GLR-1 (60, 64). These findings indicate that SOL-1 and SOL-2 are unnecessary for AMPA receptor trafficking.

GSG1L was identified through proteomic analysis of purified AMPA receptor complexes from rat brains (34, 59). GSG1L is similar to TARPs in structure (Fig. 2). GSG1L expression in a heterologous system of human embryonic kidney-293 (HEK-293) cells enhanced surface expression of AMPA receptors, as did TARPs, suggesting that GSG1L contributes to the surface trafficking of AMPA receptors (59).

Conclusion

Glutamate is a major neurotransmitter in the vertebrate brain. Among glutamate receptors, synaptic AMPA receptors mediate major fast transmission in the brain. In order to understand brain function, the molecular mechanisms underlying AMPA receptor trafficking need to be elucidated. Recent studies show that TARPs regulate AMPA receptor trafficking as auxiliary subunits, and they do so by modulating surface expression AMPA receptors and synaptic stabilization. Subsequently, several novel auxiliary subunits of AMPA receptors have been identified, but very little has been revealed about their roles in AMPA receptor trafficking. The presence of numerous auxiliary subunits enables diverse regulation of AMPA receptors. Further studies investigating these auxiliary subunits will extend our understanding of AMPA receptor trafficking and their role in brain function, including learning and memory.

Funding

This work was supported by The Naito Foundation and National Center for Geriatrics and Gerontology.

Conflict of interest

None declared.

References

controls hippocampal AMPA receptor number, distribution and synaptic plasticity. *Nat. Neurosci.* 8, 1525–1533.


