Posttranslational Modifications and Receptor-Associated Proteins in AMPA Receptor Trafficking and Synaptic Plasticity

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Abstract

AMPA-type glutamate receptors (AMPA) mediate most fast excitatory synaptic transmission in the mammalian brain. It is widely believed that the long-lasting, activity-dependent changes in synaptic strength, including long-term potentiation and long-term depression, could be the molecular and cellular basis of experience-dependent plasticities, such as learning and memory. Those changes of synaptic strength are directly related to AMPAR trafficking to and away from the synapse. There are many forms of synaptic plasticity in the mammalian brain, while the prototypic form, hippocampal CA1 long-term potentiation, has received the most intense investigation. After synthesis, AMPAR subunits undergo posttranslational modifications such as glycosylation, palmitoylation, phosphorylation and potential ubiquitination. In addition, AMPAR subunits spatiotemporally associate with specific neuronal proteins in the cell. Those posttranslational modifications and receptor-associated proteins play critical roles in AMPAR trafficking and regulation of AMPAR-dependent synaptic plasticity. Here, we summarize recent studies on posttranslational modifications and associated proteins of AMPAR subunits, and their roles in receptor trafficking and synaptic plasticity.

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Introduction

Glutamate and Receptors

The amino acid glutamate is the primary neurotransmitter in excitatory synapses of the vertebrate central nervous system (CNS) and peripheral nervous system (PNS). After release from the presynaptic nerve terminal, glutamate binds to specific receptors on the postsynaptic membrane to conduct excitatory transmission. There are two main types of glutamate receptors (GluRs): ionotropic glutamate receptors (iGluRs), which are ligand-gated ion channels that mediate the fast synaptic transmission, and metabotropic glutamate receptors (mGluRs), which are associated with GTP-binding proteins to exert their physiological effects. Based on their affinity for selective structural analogues of glutamate, iGluRs can be classified into three subtypes: α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type receptor (AMPA), kainate (KA)-type receptor (KAR), and *N*-methyl-D-aspartate (NMDA)-type receptor (NMDAR) (fig. 1A) [1–3].

AMPA Structure

AMPA receptors are heterotetrameric cation channels composed of 4 subunits: GluR1–4 (or GluRA–D), each of which comprises about 900 amino acids with a molecular weight of about 105 kDa, and shares 68–74% amino acid sequence identity. There is also about 70% sequence homology among genes encoding each subunit (fig. 1A) [4]. AMPARs are highly conserved among mammals. For ex-

Fig. 1. AMPAR structure and posttranscriptional modifications. **A** Phylogenetic tree of ionotropic glutamate receptor (iGluR) subunits. AMPAR = α -Amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptor; KAR = kainate receptor; NMDAR = *N*-methyl-D-aspartate receptor; GluR = glutamate receptor subunit; KA = KAR subunit; NR = NMDAR subunit. **B** Schematic of AMPAR subunit at the synaptic membrane. Each subunit consists of an extracellular N-terminus, four hydrophobic regions (TM1–4), two intracellular loops, one extracellular loop and an intracellular C-terminus. TM2 domain is a re-entrant transmembrane hairpin structure that forms the cation pore channel. The extreme N-terminus contains an X-domain and its function remains unknown. The ligand-binding core is composed of two ligand-binding domains: S1 and S2. The intracellular C-terminus is the interaction site for several proteins. **C** Posttranscriptional modifications of AMPAR subunits. All subunits undergo the flip/flop alternative splicing. GluR2 and GluR4 also contain another alternative splicing site in the C-terminus to produce the long and short isoforms, whereas GluR1 only has the long isoform and GluR3 only has the short isoform. In addition, GluR2 contains the Q/R RNA-editing site within the TM2 domain, and GluR2, 3 and 4 have the R/G RNA-editing site right preceding the flip/flop site.

ample, rat, mouse and human GluR1s share 96–97% identical amino acid sequence. Heteromers of GluR1-GluR2 and GluR2-GluR3 predominantly appear in the mature mammalian hippocampus, whereas GluR4-containing forms mainly appear in early postnatal development [5, 6]. Each AMPAR subunit consists of an extracellular N-terminus, three transmembrane-spanning domains (TM1, TM3 and TM4), one re-entrant transmembrane domain (TM2) and an intracellular C-terminus. Therefore, the transmembrane domains form two intracellular loops (L1–2 and L2–3) and one extracellular loop (L3–4). The extracellular N-terminus has two domains: the X-domain and the S1 ligand-binding domain. The X-domain comprises about 400 amino acids and its function is unknown. The S1 ligand-binding domain comprises about 100 amino acids. The other extracellular binding domain S2 is in the L3–4 extracellular loop. S1 and S2 domains form the ligand-binding core. The re-entrant TM2 domain contributes to the cation pore chan-

nel. The intracellular C-terminus is the interaction site for a variety of proteins, which are related to the receptor modification, trafficking and signaling (fig. 1B) [2, 7].

Posttranscriptional Modifications and Receptor Characteristics

In addition to the variation in combinations of subunits, some posttranscriptional modifications also contribute to AMPAR diversity (fig. 1C). All four AMPAR subunits are subject to alternative splicing that can produce two forms: flip and flop. These are encoded by exons 14 and 15 in GluR2, respectively. The splicing position immediately precedes the TM4 domain [8]. The pharmacological properties of the two spliced forms do not show too much difference, but the flip channels are more efficient because they desensitize at a rate that is four times slower than the flop channels. Expression of the two spliced forms shows different cellular distribution and developmental regulation in the brain. For example, in hippo-

campus, only flip forms are found in the CA3 pyramidal cells, whereas there are more flop forms than flip forms in dentate gyrus granule cells. Moreover, flip forms predominantly appear before birth. In contrast, the expression level of flop forms continuously increases after birth up to a level similar to that of the flip forms in the adult [1]. In addition, GluR2 and GluR4 also undergo another alternative splicing in the C-terminal tail to produce long isoforms and short isoforms [9, 10]. Only a small percentage of GluR2 displays a long C-terminus, whereas the long isoform of GluR4 is dominant. The short isoform of GluR4 is mainly found in the cerebellum [9]. GluR1 exists only as the long isoforms and the GluR3 exists only as the short isoforms because of the lack of C-terminal splicing sites.

RNA editing also increases the molecular diversity of AMPAR. In the GluR2, a glutamine codon (CAG) within the TM2 domain is edited to an arginine codon (CIG) (Q/R). Therefore, the GluR2-containing AMPAR, the dominant form in the hippocampus, shows low channel conductance and impermeability to Ca^{2+} , and allows ion influx and efflux, depending on the cell membrane potential. By contrast, the AMPAR lacking GluR2 subunit only allows ion flow into the cell [11, 12]. In addition, in GluR2, GluR3 and GluR4 mRNA, an arginine codon (AGA) preceding the flip/flop site can be altered to a glycine codon (IGA) (R/G), which changes the receptor kinetics properties of desensitization and resensitization [13]. The process of RNA editing is fulfilled by RNA-dependent adenosine deaminase 2 (ADAR2) [14–16].

Composition of the subunits, posttranscriptional modifications, and especially RNA editing, endow AMPARs with substantial diversity and also determine the ion channel characteristics of the receptor. In response to ligand binding, cations Na^+ and Ca^{2+} flow into the cell through AMPARs. Consequently, a fast excitatory postsynaptic response is transmitted. Ca^{2+} is an important second messenger that activates a variety of protein kinases to initiate a series of signaling pathways, which in turn mediate AMPAR modifications and trafficking to promote and maintain synaptic plasticity [7, 17].

AMPA and Synaptic Plasticity

In 1973, Bliss and Lomo [18] demonstrated that repetitive activation of excitatory synapses with high-frequency stimulation (HFS) in the hippocampus, a brain area essential for learning and memory, could cause an enhancement in synaptic strength that could last for hours, days, and even weeks. They defined this long-lasting synaptic strength as long-term potentiation (LTP). In the past two decades, it has been widely accepted that this long-lasting,

activity-dependent change in synaptic strength could be the molecular and cellular basis of experience-dependent plasticities, including learning and memory [19–23], and even drug addiction [24, 25]. Since the discovery of hippocampal CA1 LTP, a variety of other forms of synaptic plasticity have been discovered in the CNS including NMDAR-dependent long-term depression (LTD), NMDAR-independent LTP at moss fiber synapses in the hippocampus, mGluR-dependent LTD in the cerebellum, and endocannabinoid-mediated LTD [25]. The prototypic form of plasticity, NMDAR-dependent LTP, generated at excitatory synapses in hippocampal CA1 pyramidal cells, received the widespread attention [20, 26].

The process of LTP can be divided into two phases: induction or early LTP (E-LTP), initiating synaptic strength, and maintenance or late LTP (L-LTP), retaining the enhancement of synaptic strength for a long time. Interestingly, there is a parallel between LTP and memory, which also has two components: short-term memory and long-term memory [22]. To induce LTP, glutamate is released from the presynaptic membrane and binds to AMPARs and NMDARs. Release of Mg^{2+} blockade activates NMDARs to allow Na^+ and Ca^{2+} to flow into the dendritic spine through NMDARs. Increasing Ca^{2+} in the dendritic spine binds to calmodulin (CaM) to activate the CaM kinase II (CaMKII) [26, 27]. Other kinases involved in LTP induction include cAMP-dependent protein kinase (PKA) [28, 29], protein kinase C (PKC) [30–32], extracellular signal-regulated kinase (ERK) in the mitogen-activated protein kinase (MAPK) cascade [33], phosphatidylinositol 3-kinase (PI₃-kinase) [34, 35], and tyrosine kinase Src [36]. Some phosphatases are also critical for regulation of LTP [37]. The induction of LTP may be involved in multiple intracellular cascades that are redundant and as yet unidentified [38, 39]. Maintenance of LTP requires the synthesis of new proteins. These proteins include AMPAR subunits, some transcriptional factors, and structural proteins to enhance existing synapses by enlarging of dendritic spines and forming new connections [40, 41].

Subunit Rules in Receptor Trafficking

The C-terminal cytoplasmic tail of each AMPAR subunit is unique and could be associated with specific regulatory proteins. These proteins impart distinct spatiotemporal patterns of trafficking and localization to each subunit. Combining the subunits to form heterotetramers introduces more complications. Interestingly, in GluR1 knockout mice, the hippocampal CA1 LTP was absent, and a specific spatial working memory was im-

Fig. 2. AMPAR trafficking from ER to Golgi apparatus and synapse. **A** After assembly, GluR1-GluR2 heteromers are rapidly transferred from ER to the *cis* face of Golgi apparatus through interaction between the GluR1 and PDZ protein synapse-associated protein 97 (SAP97), whereas GluR2-GluR3 heteromers exit the ER much more slowly and require interaction between GluR2 C-terminus and protein interacting with C kinase 1 (PICK1), another PDZ protein. A fraction of GluR2 is retained in the ER with chaperones for an unknown reason. **B** The interaction between the GluR2 C-terminus and PDZ protein glutamate receptor-interacting protein 1/AMPA-binding protein (GRIP1/ABP) is necessary for AMPAR trafficking in dendrites along the microtubular cytoskeleton. Liprin- α serves as an adaptor to link GRIP/ABP with KIF1A, a motor protein. Later, AMPARs are targeted to the dendritic spine along actin filaments through the interaction between the C-terminus of GluR1/GluR4 and protein 4.1N. **C** GluR2-GluR3 receptors continuously and rapidly cycle in and out of synapses to maintain the total number of AMPARs at synapses, whereas GluR1-GluR2 receptors are added into synapses via the extrasynaptic surface during expression of LTP. **D** With induction of LTD, receptors are internalized rapidly.

paired [42–44]. No GluR1-containing AMPARs were delivered to the cell membrane, and no LTP was expressed. These results suggest that the AMPAR subunit GluR1 is critical for LTP expression. Consistently, GluR1 exerts dominant effects over GluR2 in the GluR1-GluR2 heteromeric receptors and controls receptor trafficking and distribution [45–47]. Next, we will focus on the roles of post-translational modifications and AMPAR-associated proteins in receptor trafficking during synaptic plasticity.

AMPA Trafficking Regulated by Posttranslational Modifications

Since AMPAR mediates most fast excitatory transmission, its trafficking and cellular distribution must be highly regulated to properly conduct the correct neuronal signals [29, 48]. After synthesis, AMPAR subunits form heteromers in the endoplasmic reticulum (ER) and

the N-terminus of each subunit is critical for the assembly. GluR1-GluR2 heteromers are transferred from the ER to the *cis* face of Golgi apparatus rapidly through interaction between the GluR1 C-terminus and PDZ protein synapse-associated protein 97 (SAP97) [49]; GluR2-GluR3 heteromers exit the ER much more slowly and require interaction between the GluR2 C-terminus and protein interacting with C kinase 1 (PICK1), another PDZ protein (fig. 2A). A portion of GluR2 is retained in the ER as a complex with some chaperones and the function is unknown [50], but the unique GluR2 Q/R RNA editing may be critical [51]. Most of AMPARs are found in the cell body, so they need to undergo a long journey from the *trans* face of the Golgi apparatus to the dendritic spine, where the receptors can exert their functions (fig. 2A). The process is microtubule and actin filament-based and requires some AMPAR subunit interaction proteins as adapters, such as PDZ protein glutamate receptor-interacting protein 1/AMPA-binding

ing protein (GRIP1/ABP) [52, 53], and protein 4.1N [54]. In the last step, AMPARs reach the synapses through two different pathways (fig. 2B). GluR2-GluR3 receptors continuously and rapidly cycle in and out of synapses to maintain the amount of AMPARs at synapses, whereas GluR1-GluR2 receptors and GluR4-containing receptors are added into synapses during synaptic plasticity [21, 45, 46, 55]. In basal conditions, surface insertion of GluR1-GluR2 receptors is slow. While in response to NMDAR activation, receptors are driven into the dendritic spine and redistributed in the dendrites [56]. The delivery routes of the two AMPARs are also different: GluR1-GluR2 receptors are inserted into the extrasynaptic membrane, and later delivered laterally along dendrites to reside stably at the synapse in response to the afferent stimulation, whereas GluR2-GluR3 receptors are inserted more directly into the synaptic membrane [45, 46]. Real-time receptor trafficking also revealed that intracellular AMPARs are delivered into the synaptic membrane via the extrasynaptic and somatic surface, whereas the direct exchange of intracellular receptors with synaptic receptors is slow [57].

It is clear that trafficking of AMPARs to and away from synapses is a mechanism to modulate synaptic strength [21, 24, 27, 29, 58–67]. During LTP expression, more AMPARs are delivered to the postsynaptic membrane (fig. 2C). In contrast, LTD induces receptor internalization (fig. 2D). These findings support the idea that alterations in synaptic strength are directly related to the receptor exocytosis and endocytosis, while AMPAR trafficking is primarily regulated through posttranslational modifications and receptor-associated proteins [63, 68].

Glycosylation and Receptor Stability

Glycosylation can protect proteins from proteolytic degradation. Each AMPAR subunit can be *N*-glycosylated at 4–6 different sites located in the extracellular domains of the protein. Glycosylation increases the receptor subunit molecular weight by about 4 kDa. Ligand binding on crystallized unglycosylated GluR2 S1–S2 domains shows that this protein modification is not necessary for ligand recognition [69]. However, the desensitization-inhibiting lectin concanavalin A (ConA) can potentiate AMPAR subunit except GluR2 currents by direct binding to these carbohydrate side chains [70]. Therefore, *N*-glycosylation mainly facilitates the AMPAR maturation, protects the receptor from proteolytic degradation, and may affect receptor current amplitudes as well. However, the lack of *N*-glycosylation does not significantly affect AMPAR subunit synthesis, assembly, or trafficking [3, 70].

Palmitoylation and Receptor Localization

Palmitoylation of protein is a reversible fatty acylation and regulates protein trafficking and cellular localization. All AMPAR subunits can be palmitoylated at two cysteine sites: one in the transmembrane domain TM2, the other in the intracellular C-terminal region. TM2 palmitoylation results in accumulation of AMPARs in the Golgi apparatus and consequently, a decrease of receptor expression in the cell surface. C-terminal palmitoylation reduces the interaction between receptor and protein 4.1N and mediates agonist-induced AMPAR internalization. Therefore, depalmitoylated receptors are stabilized at the cell surface, whereas palmitoylated receptors are more susceptible to internalization triggered by ligand binding. In addition, activation of GluRs by glutamate stimulation decreases receptor palmitoylation and recruits more AMPARs to the cell surface to mediate synaptic plasticity [71].

Phosphorylation and Receptor Activation

Phosphorylation is the most important molecular mechanism to regulate the ligand-gated ion channels. It can regulate the physiological properties of the channel as well as protein trafficking. With the exception of GluR3, all AMPAR subunits have been reported to be phosphorylated on several amino acid residues by a variety of kinases.

To date, three phosphorylation sites of GluR1 have been reported. All sites are located at the intracellular C-terminus of GluR1. Serine 831 (S831) can be phosphorylated by both PKC [30] and calcium CaMKII [72]; serine 845 (S845) is a protein kinase A (PKA) phosphorylation site [30]. Phosphorylations in S831 and S845 are thought to be important for regulating the GluR1-containing AMPAR trafficking and differentiation of two prototypic synaptic plasticities: hippocampal NMDA-dependent LTP and LTD. According to plasticity history, synapses can be classified into three groups: depressed, naive, and potentiated. LTD induction decreases phosphorylation of S845 in naive synapses and phosphorylation of S831 in potentiated synapses, whereas LTP induction increases phosphorylation of S831 in naive synapses and the phosphorylation of S845 in depressed synapses. In the process, dephosphorylation is introduced by phosphatase PP1/2A [37]. The details that describe how phosphorylation regulates surface expression of AMPAR are not clear, but S845 phosphorylation may facilitate extrasynaptic delivery of GluR1-containing AMPARs and NMDAR activation drives receptors to synapses from extrasynaptic sites by lateral diffusion [45, 73]. It is important to note

that both S831 and S845 are necessary, but not sufficient to deliver AMPARs into synapses. Therefore, other signaling events may be also involved [74, 75]. In addition, transgenic mice with mutations in S831 and S845 still show reduced LTP, although a lack of LTD [76]. Another PKC phosphorylation site in the C-terminus of GluR1, serine 818 (S818), has been reported recently [32]. Phosphorylation of GluR1 S818 is critical in LTP-driven incorporation of AMPARs into the postsynaptic membrane and is suggested to exert its function by facilitating the interaction between GluR1 and a delivery or tethering protein.

PKC phosphorylates GluR2 at serine 880 (S880) in the C-terminal sequence (IESVKI) for PDZ domain binding to differentially regulate the interaction with PDZ proteins: GRIP1/ABP and PICK1 [77, 78]. Phosphorylation of GluR2 S880 by PKC activation decreases receptor binding to GRIP1, and recruits PICK1 to synapses and facilitates rapid internalization of surface receptors. This indicates GluR2 phosphorylation of S880 is particularly important in regulating the AMPAR internalization during synaptic plasticity. In fact, GluR2 phosphorylation of S880 is a critical event in the induction of cerebellar LTD, not a NMDAR-dependent, but a metabotropic glutamate receptor mGluR1-dependent form of plasticity [79]. Interestingly, another phosphorylation on tyrosine 876 (Y876) of GluR2 introduced by Src family tyrosine kinases has almost the same effects on GluR2 binding to GRIP1/ABP and PICK1, and in turn facilitates the AMPA- or NMDA-induced receptor internalization [36]. In addition, serine 863 of the GluR2 C-terminus is another potential PKC phosphorylation site and its function remains unknown [80].

Like GluR1, GluR4 is a long isoform of AMPAR subunit. The expression of GluR1 and GluR4 in hippocampus is altered during development. GluR4 is critical for the synaptic plasticity during the early postnatal period when GluR1 expression in the hippocampus is low, whereas in the adult hippocampus GluR4 expression level is low and GluR1 is critical for synaptic function [81]. These two long isoform subunits share the conserved PKA phosphorylation site: S845 of GluR1 and S842 of GluR4. Interestingly, phosphorylation of GluR1 S845 is required, but not sufficient for LTP induction, which also needs S818 and S831 phosphorylations, and the PDZ-binding site. In contrast, phosphorylation of GluR4 S842 is both necessary and sufficient to deliver the receptor to the synapse and induce plasticity in early postnatal development [75]. In addition, PKC γ is reported to directly interact with the GluR4 and phosphorylate it at serine 482

(S482) *in vitro*, thereby increasing recombinant GluR4 surface expression to mediate the function of GluR4-containing AMPARs [82]. Threonine 830 (T830) is another potential PKC phosphorylation site of GluR4 and its function remains unknown [83].

PKC Isoforms and AMPA Phosphorylation

Taken together, receptor phosphorylation by kinases, specifically CaMKII and PKC, play critical roles in AMPAR trafficking and plasticity expression. Notably, several members of the PKC family directly phosphorylate AMPAR subunits to mediate plasticity [30, 32, 77, 80, 82, 83]. Recently, a special atypical PKC (aPKC) isoform, PKM ζ has received considerable attention and undergoes intense investigation. Vertebrates express 10 PKC isoforms classified into three groups: conventional PKC (cPKC: α , β I, β II and γ), novel PKC (nPKC: δ , ϵ , η /L and θ), and atypical PKC (aPKC: ζ and ι / λ) (fig. 3A). Each isozyme consists of an N-terminal regulatory region and a conserved C-terminal catalytic domain. The regulatory region contains a pseudosubstrate, an autoinhibitory sequence, which automatically interacts with the catalytic domain and blocks the enzymatic activity. Release of the pseudosubstrate by stimulation of second messengers activates the enzyme [84]. PKM ζ is an isoform of PKC ζ without the regulatory region (fig. 3B). This unique characteristic enables the enzyme to be constitutively activated [85, 86]. PKM ζ is produced from PKM ζ mRNA, transcribed from an internal promoter within the PKC ζ gene (fig. 3C) [86]. Interestingly, phosphorylation introduced by PKM ζ is necessary and sufficient for LTP maintenance [87, 88]. In addition, a PKM ζ inhibitor, myristoylated ζ -pseudosubstrate inhibitory peptide (ZIP), both reverses LTP maintenance *in vivo* and leads to loss of spatial information. These findings suggest that the LTP maintenance mediated by PKM ζ sustains spatial memory [23]. A recent study showed that two sequential steps are involved in LTP maintenance: synthesis and activation of PKM ζ . PI $_3$ -kinase, CaMKII, MAPK, PKA, mTOR (mammalian target of rapamycin) and preexisting PKM ζ regulate *de novo* synthesis of PKM ζ and phosphoinositides-dependent protein kinase-1 (PDK1) activates PKM ζ via phosphorylation of its activation loop [89]. Indeed, PKM ζ causes synaptic potentiation by increasing the number of active AMPARs at the postsynaptic membrane without affecting the unit conductance of receptor channels [90]. This indicates that phosphorylation induced by PKM ζ mediates AMPAR trafficking, instead of altering channel properties.

Fig. 3. The specific structure enables PKM ζ to maintain LTP. **A** PKC family has 10 isoforms classified into three groups: conventional PKC (cPKC: α , β I, β II and γ), novel PKC (nPKC: δ , ϵ , η /L and θ), and atypical PKC (aPKC: ζ and ι / λ). Each isoform consists of an N-terminal regulatory region, which contains cysteine-rich domains (C-1 and C-2) and a pseudosubstrate, and a conserved C-terminal catalytic domain with of serine/threonine kinase activity. **B** The pseudosubstrate in the regulatory region automatically interacts with the catalytic domain and blocks the activity of the enzyme. Release of the pseudosubstrate by stimulation of the second messengers can activate the enzyme. PKM ζ is an isoform of PKC ζ without the regulatory region, so this enzyme is constitutively activated. **C** Afferent stimulation activates the NMDARs and causes Ca^{2+} influx into the dendrite, which in turn activates various Ca^{2+} -dependent kinases to enhance the conductance of receptor channels, to induce LTP expression or early LTP (E-LTP). The influx of Ca^{2+} also triggers an unknown signaling cascade toward the nucleus. As a result, a transcriptional factor binds the internal promoter (P2) of the PKC ζ gene to initiate the PKM ζ mRNA transcription. The PKM ζ mRNA is actively transported to dendrites and produces PKM ζ , which is activated by PDK1 during LTP maintenance or late LTP (L-LTP). Consistent phosphorylation by PKM ζ even without second messengers increases and maintains the amount of active AMPARs at the synapse to sustain LTP.

LTP Induction

The profile of induction and maintenance of LTP is becoming clear. Learning or other afferent stimulation activates the NMDARs and causes Ca^{2+} influx into the dendrite, which in turn activates some Ca^{2+} -dependent kinases. Consequently, the conductance of receptor channels is enhanced to induce LTP expression or early LTP (E-LTP). On the other hand, the influx of Ca^{2+} also triggers an unknown signaling cascade toward the nucleus and initiates the internal promoter of the PKC ζ gene to produce PKM ζ mRNA. The PKM ζ mRNA is actively transported to dendrites and translated to produce PKM ζ , which is activated by PDK1 during LTP maintenance or late LTP (L-LTP) (fig. 3C). The consistent second messenger-independent phosphorylation by PKM ζ

increases and maintains the amount of active AMPARs at the synapse [91]. Although both GluR1 and GluR2 C-termini are PKM ζ substrates in vitro [90], it would be more reasonable to propose that PKM ζ phosphorylates some receptor-associated protein to regulate AMPAR trafficking, instead of directly modifying the receptor itself to change channel characteristics. Continuing to investigate possible candidates for PKM ζ substrates is important for uncovering the roles of PKM ζ in the maintenance of synaptic plasticity.

Atypical PKCs and LTP

Coincidentally, both PKC ι / λ and PKM ζ , but not PKC ζ , are expressed in the mammalian brain at a high level, especially in the hippocampus [92]. PKC ι / λ and PKM ζ

have a similar pattern of distribution in the human brain and both are activated after tetanization [85, 86, 93]. In addition, the catalytic domains of PKC ι/λ and PKM ζ are almost identical. The PKM ζ inhibitors chelerythrine and ZIP also theoretically inhibit other aPKCs [23, 87]. Therefore, results concerning PKM ζ cannot exclude PKC ι/λ involvement in synaptic plasticity. Moreover, it is possible that PKC ι/λ plays a similar role as PKM ζ does before the expression of PKM ζ is promoted by afferent stimulation. It is also possible that PKC ι/λ and PKM ζ possess roles at different sites within the cells, because activated PKC ι/λ is bound to the cell membrane, whereas PKM ζ is mainly located in the cytosol, due to its lack of a regulatory region. Application of chelerythrine, an aPKC inhibitor, and a dominant negative inhibitory form, PKM ζ -K281W, also completely eliminated induction of LTP [87]. This would suggest that full-length PKC ι/λ might be involved in LTP induction. If so, phosphorylation by PKC ι/λ may be necessary for LTP induction, whereas later PKM ζ is expressed in dendrites and maintains LTP. In addition, a cytoplasmic and membrane-associated protein named p62 interacts with the pseudosubstrate region in the regulatory N-terminus of aPKC but not classic PKCs [94, 95]. By binding aPKC, p62 serves as a scaffold protein to recruit substrates to the kinase. This suggests to us that p62 might be involved in the regulation of synaptic plasticity through its interaction with PKC ι/λ .

Ubiquitination and Receptor Endocytosis

Ubiquitin has 76 amino acids and belongs to a family of small proteins highly conserved in structure. Ubiquitination, the attachment of a single ubiquitin or a polymeric ubiquitin chain to a protein, can regulate the proteins in many different ways. The prototypical function of ubiquitination is proteolysis through proteasomal pathway. Later, numerous studies revealed that ubiquitination also plays important roles in many other pathways beyond proteolysis [96, 97], such as protein location, activity, etc. The potential functions of the ubiquitin proteasome system (UPS) in synaptic strength have been investigated widely in many species including *Caenorhabditis elegans*, *Aplysia*, *Drosophila*, and mammal [98–104]. Surprisingly, UPS regulates the abundance of both pre-synaptic and postsynaptic proteins [102, 105] to exert multiple roles in synaptic development, presynaptic function and neurotransmitter release, alternation of postsynaptic density (PSD) and plasticity, spine growth and stability [106]. Here, we will focus on the recent progress in understanding ubiquitination as related to AMPAR trafficking and synaptic plasticity.

As mentioned above, trafficking of AMPARs to and away from the postsynaptic membrane is the molecular basis for the change of synaptic strength. The amount of AMPARs at the synapse is regulated through exocytosis and endocytosis mediated by clathrin-coated vesicles [61, 107, 108]. The disruption of components of clathrin-coated vesicles such as dynamin and huntingtin-interacting protein 1 (HIP1) resulted in neurological deficits and a decrease of AMPAR trafficking [107, 109]. Recent studies reveal the UPS plays important roles for AMPAR endocytosis [105, 110], which is critical for LTD [61], induced by treatment of receptor agonists such as NMDA or hormones such as insulin [108]. Ubiquitination also was reported to play important roles in hippocampal LTP, but the detailed mechanism remains unknown [111]. Theoretically, the substrates of ubiquitination could be either the glutamate receptors themselves or receptor-associated proteins [110]. So far, no evidence of direct ubiquitination of AMPAR in mammals has been reported. However, GLR-1, the ortholog of mammalian GluR1 subunit in *C. elegans*, can be ubiquitinated in the intracellular C-terminus. The ubiquitination site (LxEFxYK/RSRxD/EAK) is conserved in all mammalian AMPAR subunits and shares homology with the ubiquitin/endocytic signal of yeast proteins Ste2P and Ste6P [60, 99]. These findings suggest that ubiquitination of GLR-1 may mediate the synaptic strength and GLR-1-containing synapses through regulating the amount of receptor at the synapse in a clathrin adaptin protein (AP180)-dependent manner [99]. Interestingly, ubiquitination of KEL-8, a neuronal protein localized adjacent to GLR-1 on the postsynaptic membrane, is necessary for proteolysis of GLR-1 receptors and may regulate GluRs localization and signaling in postmitotic neurons [112].

In response to the synaptic activity, the PSD proteins, including some postsynaptic scaffolds, become highly ubiquitinated and those alterations, in turn, change downstream effectors like cyclic AMP (cAMP) response element-binding protein (CREB) and ERK-MAPK [105]. In particular, PSD-95, a major PSD scaffolding protein, mediates synaptic plasticity by anchoring NMDARs and AMPARs to the neuronal cytoskeleton or other receptor-associated proteins [113]. In response to NMDAR activation, PSD-95 is ubiquitinated and removed from the synaptic sites by UPS [114, 115]. This results in a decrease of AMPAR expression in the synaptic surface during synaptic plasticity. Interestingly, β -amyloid (A β), a peptide that is derived from amyloid precursor protein (APP) in neurons and widely believed to underlie the pathophysiology of Alzheimer's disease, can drive AMPAR endocy-

tosis and cause loss of dendritic spines and inhibit hippocampal LTP [116, 117].

Following endocytosis, AMPARs in the early endosome are sorted via two different pathways. They are either recycled back to the cell surface or degraded through the lysosome, depending on the activations of NMDARs and AMPARs. With NMDAR activity stimulated by glutamate, AMPARs are internalized in a Ca^{2+} -dependent manner, dephosphorylated by protein phosphatases, rephosphorylated by PKA, and at last rapidly reinserted in the synaptic membrane via recycling endosomes. On the other hand, without NMDAR activity, AMPARs are internalized in a Ca^{2+} -independent manner, and then targeted to the lysosomes for degradation via late endosomes [59]. Consistently, in response to AMPA stimulation, internalized AMPARs enter the recycling system and reappear on the surface quickly, whereas in response to insulin treatment, internalized AMPARs are diverted into a distinct compartment, a non-recycling pathway [60]. Those reinserted AMPARs are a very important source of receptors for LTP expression [118]. The recycling endosomes and vesicles are also merged into spines to promote and maintain the growth of spines induced by synaptic plasticity [48, 119]. Interestingly, chemically induced LTP (chemLTP) can drive strong exocytosis of AMPARs, but with a small loss of NMDARs on the spine surface. In addition, spines grow larger before collecting AMPARs on their surface [120]. So it is possible that recruitment of AMPARs to the spine surface requires accumulation of certain spine components, specifically, tethering proteins at the spine site.

AMPA-Interacting Proteins in Receptor Trafficking

Unquestionably, the localization and trafficking of AMPARs are both an extremely complicated and highly regulated process. Many proteins interact with different AMPAR subunits to regulate receptor trafficking [7, 121, 122]. We will discuss some of the more critical proteins.

GluR1-Interacting Proteins

Synapse-associated protein 97 (SAP97) is the first protein reported to directly interact with GluR1 subunit [123]. This PDZ-containing protein belongs to a protein family named synapse-associated protein (SAP) and other family members include some NMDAR subunit-interacting proteins such as SAP90 (PSD95), chapsyn110 (PSD93) and SAP102 [121]. SAP97 has three PDZ domains and interacts with the very C-terminal of GluR1

with its second PDZ domain [123, 124]. SAP highly accumulates at GluR1-containing synapses, so SAP may serve as an anchoring molecule to help GluR1 reside stably in the synaptic surface [125]. The interaction between SAP97 and GluR1 first occurs in the receptor secretory pathway and is essential for the transport of the receptor from the endoplasmic reticulum to the *cis* face of Golgi apparatus (fig. 2A) [49]. The interaction of SAP97 with A kinase anchoring protein 79 (AKAP79) can target PKA to GluR1 for S845 phosphorylation and may be involved in the LTD induction [126, 127]. Phosphorylation by CaMKII regulates SAP97 targeting and interaction with NMDAR subunit NR2A [128, 129]. Interestingly, PDZ domain-containing proteins including SAP 97 are widely believed to play critical roles in AMPAR trafficking and LTP expression [46, 74]. However, transgenic mice lacking the C-terminal PDZ-binding site of GluR1 show normal GluR1 synaptic localization and unimpaired hippocampal CA1 LTP [130].

Proteins 4.1G/N are the other GluR1-interacting proteins [54]. They belong to a family of multifunctional cytoskeletal components (4.1B/G/N/R) originally isolated from erythrocytes and are essential for assembly and maintenance of the actin cytoskeleton. 4.1N is enriched in synapses, whereas 4.1G is expressed in all cells [131]. Both 4.1G and 4.1N bind to the intracellular membrane proximal region of GluR1. Particularly, 4.1N interacts with GluR1 *in vivo* and colocalizes with AMPARs at excitatory synapses. Destruction of actin filaments in cultured cortical neurons significantly decreases GluR1 surface expression. Taken together, 4.1G/N may serve as adapters to link GluR1 to the actin cytoskeleton [54]. The actin filaments, but not microtubules, are abundant in the dendritic spine [132]. These observations suggest that GluR1-containing AMPARs are delivered to synapses along actin filaments (fig. 2B). Interestingly, GluR4 also has a 4.1 binding site and the 4.1 proteins may affect GluR4 trafficking and localization in a manner similar to that found for GluR1 [133].

GluR2-Interacting Proteins

GRIP/ABP binds to the C-terminus of GluR2/3 via PDZ domains. GRIP contains seven PDZ domains with a molecular weight of 130 kDa. Its fourth and fifth PDZ domains bind the very C-terminal motif of GluR2/3 (ESVKI) [134]. ABP is a close relative of GRIP and has two splice variants: the short 98-kDa isoform named ABP-S has six PDZ domains and the third, fifth and sixth mediate binding to GluR2/3; the long 130-kDa isoform named ABP-L or GRIP2 has seven PDZ domains [135].

Both GRIP and ABP are ubiquitously expressed in the CNS and enriched in the PSD, but GRIP can be detected before the emergence of AMPARs during embryonic development, whereas the expression pattern of ABP resembles AMPARs [136]. The function of GRIP has not been fully elucidated, but its multiple PDZ domains may endow the protein to function as an adapter. In fact, several proteins can bind to its PDZ domains other than the fourth and the fifth [137]. Some other proteins may regulate AMPARs signaling and trafficking via GRIP. For example, GRIP-associated protein-1 (GRASP-1) is a rasGEF in neurons and associated with GRIP and AMPARs in vivo. Overexpression of GRASP-1 in cultured neurons causes a down-regulation of synaptic targeting of AMPARs [137]. Through GRIP, AMPARs are linked to activity-dependent Ras signaling and trafficking events and the caspase pathway [138]. In another case, both of the Eph receptors, a family of receptor tyrosine kinases, and their ephrin ligands, bind to the sixth and seventh PDZ domains of GRIP [139] and the interaction that may be involved in neurite extension and axonal guidance [140], and recruitment of intracellular GRIPs to membrane lipid rafts [141]. The interactions of GRIP with kinesin heavy chain [53] and microtubule-associated protein (MAP)-1B light chain (LC) [142] may link AMPAR to microtubular motor protein trafficking. Liprin- α interacts with GRIP and is necessary for AMPAR targeting [143]. Liprin- α can also associate with KIF1A, another member of the kinesin superfamily of molecular motors [144]. In addition, interaction between liprin- α and GIT1 is also necessary for AMPAR targeting [145]. Taken together, AMPAR trafficking along the microtubular cytoskeleton in the dendrite is mediated by interaction between GRIP and several microtubular motor proteins (fig. 2A).

Protein interacting with C kinase (PICK1) has a BAR (Bin/amphiphysin/Rvs) domain and a PDZ domain, through which the dimer of PICK1 could bind to the C-terminus of GluR2 [146] and the catalytic domain of PKC α [147]. So, PICK1 may target the AMPARs to an active PKC α [148]. S880 phosphorylation of GluR2 by PKC upon phorbol ester (TPA) induction releases GluR2-containing AMPARs from GRIP1 binding, and increases receptor binding to PICK1. As a result, more PICK1 traffic to synapses and GluR2-containing AMPARs are internalized rapidly to result in cerebellar and hippocampal LTD [78, 79]. T876 phosphorylation has the similar effects on GluR2 binding to ABP/GRIP and PICK1 [36]. An interesting model may explain the functions of PICK1 and GRIP/ABP in GluR2-containing AMPAR internal-

ization. PICK1 BAR domain interacts with its PDZ domain and also interacts with GRIP/ABP linker II region. Binding to GluR2 or PKC α interrupts the intramolecular interaction and facilitates the intermolecular interaction of PICK1 BAR domain with GRIP/ABP linker II region. Then PKC α phosphorylates S880 of GluR2 and the phosphorylated GluR2 is released from GRIP/ABP to bind to PICK1. Finally, the PICK1 BAR domain directs receptor internalization [149]. PICK1 may also be involved in the formation of extrasynaptic membrane pools of GluR2-containing AMPAR which later could be targeted to the synaptic membrane via lateral trafficking [6]. The interaction between PICK1 and GluR2 is also necessary for receptor exit from the ER (fig. 2B) [51, 149]. Considering PICK1 interacts with more than a dozen proteins besides GluR2, PKC α , and GRIP/ABP [150], the AMPAR trafficking regulated by GRIP is expected to be more complicated. A recent study revealed that the neuronal endosomal protein NEEP21 may be involved in the GluR2-containing AMPAR sorting and reinsertion into the cell surface by binding with GRIP and GluR2 subunit [151].

The interaction between ATPase *N*-ethylmaleimide-sensitive fusion protein (NSF) and AMPAR subunit GluR2 was first detected by using the yeast two-hybrid screen [152, 153]. A region in the GluR2 C-terminus (KRMKVAKNPQ) is responsible for NSF binding with asparagine 851 playing an essential role [153]. NSF is a well-known multihomomeric ATPase that plays a central role in docking and fusion of synaptic vesicles [154]. Perfusion of a synthetic peptide pep2m mimicking the NSF-binding site on GluR2 to postsynaptic sites partially blocked synaptic transmission [155]. Therefore, NSF may play a role in the regulated delivery of GluR2-containing AMPARs from the postsynaptic surface to the lysosome during LTD [47]. Overexpression of NSF in cultured hippocampal neurons caused a down-regulation in surface expression of AMPARs, but the total AMPAR expression was unaffected [156]. Interestingly, both PICK1 and NSF are necessary for calcium-permeable AMPAR plasticity (CARP), and dynamically exchange AMPARs lacking GluR2 with GluR2-containing AMPARs in the synapse during CARP to mediate the calcium permeability of AMPAR [6].

AP2 belongs to the family of assembly particles and other members include AP1 and AP3. Particularly, AP2 complex, a well-characterized clathrin adapter involved in endocytosis from the cell surface, consists of four subunits: α , β , σ and μ adaptins. Clathrin adapters are essential for endocytosis by linking membrane proteins to clathrin and facilitating assembly of clathrin coats. There-

fore, association of clathrin adapters to the cytoplasmic domains of receptors is a key to promote receptor endocytosis [157]. Activity-dependent AMPAR internalization occurs via dynamin-dependent clathrin-coated vesicles [61, 107, 108]. Interestingly, AP2 associates with GluR2 in almost the same region as the NSF-binding site. In fact, application of pep2m blocks both AP2 and NSF binding to GluR2 [155]. Although sharing the same binding site on GluR2, AP2 and NSF play distinct roles in the maintenance and removal of AMPARs at the synapse.

Other AMPAR-Interacting Proteins

Transmembrane AMPAR regulatory protein (TARP) family has four members: γ -2 (or stargazin), γ -3, γ -4 and γ -8. Stargazin, the prototypical TARP, is the first identified transmembrane interactor of AMPARs [5, 158]. Stargazin is mutated in the stargazer mice that show a phenotype of the absence epilepsy and cerebellar ataxia, which results from the lack of functional AMPAR channels in cerebellar granule cells [158–160]. These four proteins may function as the auxiliary components of neuronal AMPARs [5, 161] and are spatiotemporally expressed during differentiation and development in the CNS. γ -4 is dominant during early development; stargazin is the only expressed TARP in cerebellar granule cells, but lacking from the hippocampal CA1 region; γ -8 is highly expressed all over the hippocampus [162]. Different distribution of TARPs would implicate differential and developmental control of activity-dependent trafficking affairs. Stargazin is a four-transmembrane protein and targets AMPARs to the granule cell surface [162]. The intracellular C-terminus of stargazin binds to some PDZ proteins including PSD-95 to mediate synaptic trafficking and clustering of AMPARs [158]. Stargazin also can be phosphorylated in an activity-dependent pattern to regulate hippocampal synaptic strength [163, 164]. In addition, stargazin controls AMPAR channel gating by slowing glutamate-induced AMPAR deactivation and desensitization, and enhancing the channel conductance [163, 165]. Both extracellular and intracellular regions of stargazin interact with AMPARs [162] and the interaction may be direct because immunoprecipitation of stargazin also pulled down other AMPAR-interacting proteins [5], although which domain of GluRs is responsible for stargazin binding remains unknown. A recent study revealed that a point mutation in the glutamate-binding region of GluR1 destroys stargazin's effects on receptor trafficking and channel gating. This finding suggests that the glutamate-binding domain of AMPARs could be the extracellular binding site for TARPs [166].

Soluble NSF attachment protein (SNAP) has three forms: α , β and γ , and is critical in membrane fusion. Particularly, α - and β -SNAPs interact with the C-terminus of the GluR2 subunit [167]. As the name indicated, SNAPs can bind NSF [168], but they associate with AMPARs independently with an increase of the amount of AMPARs binding SNAP in the presence of NSF because AMPARs can recruit more SNAPs indirectly via NSF [167]. SNAP is another AMPAR interactor involved in vesicle budding and fusion beside NSF and may play a role in regulating synaptic expression of AMPARs through a direct association with receptors [169, 170]. In addition, SNAPs and NSF can disassemble the GluR2-PICK1 complex to mediate AMPAR surface expression [171].

Lyn is a Src-family non-receptor protein tyrosine kinase and is highly expressed in CNS. About 1–2% of Lyn in the cerebellum associates with GluR2-containing or GluR3-containing AMPARs via its Src homology region consisting of SH2 and SH3 domains [172]. Lyn is activated by AMPAR in a Ca^{2+} - and Na^{+} -independent manner and promotes the MAPK-signaling cascade to initiate expression of brain-derived neurotrophic factor (BDNF), which is involved in the regulation of synaptic plasticity besides in neuronal survival and differentiation [173–176]. Therefore, in addition to synaptic transmission via cation influx, AMPARs may be directly involved in mediating intracellular signal from the cell surface to the nucleus through the Lyn-MAPK cascade and mediate synaptic plasticity by regulating BDNF expression [172].

LIN-10 is a membrane-associated protein that mediates localization of GLR-1 in *C. elegans* [177]. Its human ortholog, mLin-10, directly associates with GluR1 and GluR2 subunits through a PDZ domain-dependent mechanism and mediates the receptor trafficking [178]. X11L, a homolog of mLin-10, can bind the p65 subunit of NF- κ B to regulate A β production in neurons and may indicate a novel means to control the progression of Alzheimer's disease [179]. In addition, GluR1 subunit was detected in association with the guanine-nucleotide-binding protein ($\text{G}\alpha_{\text{il}}$) and stimulation with AMPA can regulate $\text{G}\alpha_{\text{il}}$ function in a Ca^{2+} - and Na^{+} -independent manner [180]. This suggests that AMPAR could exert a metabotropic function besides the ion channel.

Future Directions

Cumulative studies reveal that AMPAR-interacting molecules play critical roles in AMPAR-related synaptic plasticity. Some interacting molecules regulate posttrans-

lational modification of the receptors, while others mediate receptor trafficking, cellular localization and signal transduction cascades. Undoubtedly, AMPAR posttranslational modifications and AMPAR-associated proteins are the key to mediate receptor trafficking, which is the underlying molecular mechanism for synaptic plasticity. Despite more than two decades of intense research [58], the complete mechanism of AMPAR trafficking is not clear. For proteins whose binding sites on AMPAR subunits are determined such as 4.1N, SAP97, GRIP/ABP, PICK1, AP2, NSF and TARPs, their functions in AMPAR trafficking are basically understood (fig. 4). For those proteins whose binding sites on the AMPAR subunit remain unknown, continued efforts to characterize the binding sites will shed light on their functions in the regulation of synaptic strength [122]. Unquestionably, more AMPAR-interacting proteins will be discovered and will

Fig. 4. Interactions between AMPARs and associated proteins. 4.1G/N and SAP97 interact with the GluR1 subunit, whereas AP2, NSF, GRIP/ABP and PICK1 interact with the GluR2 subunit. Both extracellular and intracellular regions of TARPs interact with AMPARs, but the intracellular binding site is unknown. Other AMPAR-associated proteins include Lyn, LIN-10 and G α , but the binding sites are unclear.

allow us to develop a more complete picture of AMPAR interactions. Those AMPAR-associated proteins could be newly discovered or some existing proteins with novel functions in plasticity. As discussed previously, p62, the aPKC adapter, is a possible AMPAR-associated protein. p62 is a cytoplasmic and membrane-associated protein with a molecular weight of about 62 kDa. It is also named zeta protein kinase C interacting protein (ZIP, different from ZIP inhibitor of PKM ζ) and was initially identified as a phosphotyrosine-independent ligand of the Src homology 2 (SH2) domain of p56^{lck} [94]. The p62 protein possesses 6 domains, which endow it with the ability to associate with many other proteins (fig. 5): a PB1 domain for aPKC binding, a ZZ-type Zinc finger domain for binding aPKC substrates [95, 181–183], a tumor necrosis factor (TNF) receptor-associated factor 6 (TRAF6)-binding domain, two PEST domains for proteolytic recognition, and a ubiquitin-associated (UBA) domain. p62 is concentrated in hippocampus [95]. Based on its specific structure, p62 serves as a scaffold to recruit substrates of aPKC through its PB1 domain and ZZ-type finger domain [184]. In addition, p62 also functions as an adapter protein in the process of polyubiquitination of many proteins, especially membrane-associated receptors, and specifically binds proteins with K63-polyubiquitin attached through its C-terminal UBA domain, which initiates ubiquitination-mediated receptor endocytosis [185]. Furthermore, p62 may function as an intracellular shuttling factor [186]. Interestingly, the p62 knockout mice exhibit impaired spatial learning and memory, a hippocampal-dependent process [M.W. Wooten, unpubl. data]. Defective spatial learning and memory often correlates with impaired hippocampal LTP. These results suggest that p62 may play a yet to be defined role in regulating synaptic transmission.

Theoretically, PKC ϵ/λ may play an important role for early expression of LTP (E-LTP), whereas PKM ζ is essential for LTP maintenance [23, 87]. If so, p62 could target

Fig. 5. p62 primary structure. p62 possesses 6 domains: a PB1 domain consisting of a SH2-binding site and an aPKC interaction domain (AID), a ZZ-type finger domain binds aPKC substrates, a tumor necrosis factor (TNF) receptor-associated factor 6 (TRAF6)-binding domain, two PEST domains for proteolytic recognition, and a ubiquitin-associated (UBA) domain.

potential substrates to PKC ϵ /λ to mediate E-LTP through aPKC-dependent phosphorylation. In addition, p62 could also serve as an adapter to facilitate the ubiquitination at the PSD to mediate endocytosis of the receptor itself or of some receptor-associated proteins [110]. Investigating the possible roles of p62 in synaptic plasticity may help to uncover additional information on the molecular and cellular mechanisms of synaptic plasticity.

Neurotrophins have been shown to modulate numerous aspects of synaptic transmission and neural plasticity [187]. Recently it has been shown that BDNF can regulate the expression and synaptic delivery of AMPAR subunits [176]. In this scenario, BDNF activates both PKC and CaMKII to phosphorylate S831 and possibly S818 in

the C-terminal tail of GluR1, leading to synaptic incorporation of GluR1. Also it is possible that BDNF may induce the phosphorylation of a regulatory protein that facilitates insertion or delivery of the GluR1 subunit into the synapse. Possible regulation of LTP by BDNF/PKC and p62 as it relates to learning and memory is currently underway in our laboratory.

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