Dynamic Regulation of N-Methyl-d-aspartate (NMDA) and α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic Acid (AMPA) Receptors by Posttranslational Modifications*

Published, JBC Papers in Press, October 9, 2015, DOI 10.1074/jbc.R115.652750

Marc P. Lussier†, Antonio Sanz-Clemente‡, and Katherine W. Roche†

From the †Département de Chimie, Université du Québec à Montréal, Montréal, Québec H3C 3P8, Canada, the ‡Department of Pharmacology, Feinberg School of Medicine, Northwestern University, Chicago, Illinois 60611, and the §Receptor Biology Section, NINDS, National Institutes of Health, Bethesda, Maryland 20892

Many molecular mechanisms underlie the changes in synaptic glutamate receptor content that are required by neuronal networks to generate cellular correlates of learning and memory. During the last decade, posttranslational modifications have emerged as critical regulators of synaptic transmission and plasticity. Notably, phosphorylation, ubiquitination, and palmitoylation control the stability, trafficking, and synaptic expression of glutamate receptors in the central nervous system. In the current review, we will summarize some of the progress made by the neuroscience community regarding our understanding of phosphorylation, ubiquitination, and palmitoylation of the NMDA and AMPA subtypes of glutamate receptors.

The brain functions efficiently due to accurate communication between neurons. At excitatory synapses, the amino acid glutamate is released from synaptic vesicles present in presynaptic terminals; glutamate diffuses into the synaptic cleft and binds to the extracellular region of glutamate receptor subunits (GluRs).* Glutamate binding to receptors induces structural modification resulting in ion channels opening in the case of ionotropic glutamate receptors (iGluRs) or activation of intracellular signaling cascades upon activation of metabotropic glutamate receptors (mGluRs). Changes in synaptic strength include both potentiation and depression of excitatory neurotransmission, known as long-term potentiation (LTP) and long-term depression (LTD), mechanisms believed to represent cellular correlates of learning and memory (1–3). Over the last three decades, the development of sophisticated biochemical, cellular, and molecular approaches has allowed for in-depth investigation of the detailed mechanisms regulating the content of GluRs at synapses demonstrating that GluRs are dynamic. As shown in Fig. 1, synaptic glutamate receptor localization is regulated by: 1) lateral diffusion to and from synapses; 2) endocytosis and exocytosis at the plasma membrane; and 3) intracellular routing and sorting through endosomal pathways (4–8).

It is clear from a multitude of studies that a variety of posttranslational modifications (PTMs) control GluR trafficking and synaptic expression. For example, these modifications play essential roles in influencing protein activity, signaling cascades, protein turnover, synaptic localization, and interactions with intracellular proteins or lipids. These PTMs include glycosylation, phosphorylation, and palmitoylation, which constitute the addition of a functional group to a substrate, and ubiquitination and sumoylation, which involve the covalent conjugation of the protein ubiquitin or the small ubiquitin-like modifier (SUMO) protein to a substrate. Although each of these PTMs can modify GluRs, the current review is specifically focused on the phosphorylation, palmitoylation, and ubiquitination of two subtypes of iGluRs: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) and N-methyl-d-aspartate receptors (NMDARs). AMPARs and NMDARs are tetrameric ligand-gated ion channels composed of homologous subunits: AMPARs (GluA1–4) and NMDARs (GluN1; GluN2A-D; GluN3A-B). Each iGluR subunit shares a similar overall topology (Figs. 2 and 3): a long extracellular N-terminal domain (9), a hydrophobic hairpin region forming the pore region that is located between two short intracellular loops (loop1 and loop2), and the first and the second of three membrane-spanning regions. Finally, each subunit has an intracellular C-terminal tail of variable length depending on subtype. The intracellular loops and C-terminal tails have many sites for modifications including palmitoylation, ubiquitination, sumoylation, and phosphorylation, which play critical roles in regulating synaptic expression and intracellular trafficking.

Phosphorylation

Phosphorylation is defined as the reversible addition of a phosphate group (PO₄⁻) to a protein, typically to a Ser, Thr, or Tyr residue, although phosphorylation on His, Arg, or Lys has also been reported (10). The presence of this heavily charged group is important for changing the hydrophobicity and electric charge of a protein region and, therefore, it can result in a change in the protein conformation or interactions with other proteins or cell structures. In the particular case of the GluRs, phosphorylation regulates intracellular trafficking and channel
properties. The best-studied example of the latter is for the AMPAR subunit GluA1 in which the conductance and opening probability are modulated by phosphorylation of Ser-831 (PKC/CaMKII) or Ser-845 (PKA), respectively (11, 12). More recently, the phosphorylation on the NMDAR subunit GluN2B by PKA (Ser-1166) was identified as an important factor affecting Ca^{2+}/H\text{1001}permeation (13).

One of the most common strategies for studying the effects of phosphorylation is the use of mutants in which the phosphorylation is either blocked or mimicked by replacing the phosphorylated residue with a non-polar amino acid (usually alanine) or a negatively charged one (usually aspartate or glutamate), respectively. This approach has proved powerful and provided valuable information, but it is important to recognize the caveats; it may alter some properties of the protein, masking the effect of the phosphorylation. Therefore, it is not uncommon that both phospho-deficient and phospho-mimetic mutations result in a similar phenotype. For this reason, it is preferable to combine a variety of approaches including biochemical characterization of the mutants and the use of complementary techniques (e.g. pharmacological blockade and/or activation of the kinase). Furthermore, a null phenotype with a phospho-mimetic mutation is not uncommon due to a supposed need for the dynamic on and off of true phosphorylation. One could imagine a protein needing phosphorylation for ER egress, but dephosphorylation for stabilization at the synapse, for example, and a surface expression measure could be confounded.

NMDAR Phosphorylation

Phosphorylation is a key regulatory mechanism controlling the trafficking of NMDARs (see Fig. 2 for a list of phospho-sites in the GluN2A/2B C termini). Strikingly, phosphorylation regulates the surface and synaptic expression of NMDARs in a subunit-specific manner, providing a highly plastic and precise mechanism to accurately control different subunits in response to stimuli. For example, GluN2B is internalized in response to synaptic activity resulting in reduced surface expression (Fig. 4). Internalization from the plasma membrane is mediated by clathrin and tightly controlled by the phosphorylation of GluN2B on Tyr-1472 by Fyn/Src kinases. This residue is part of the YEKL endocytic motif that is recognized by the clathrin adaptor AP-2 as a required step to induce GluN2B internalization. GluN2B Tyr-1472 phosphorylation blocks AP-2 binding, thus preventing the endocytosis of the receptor and, therefore, increasing its surface expression (14–16). Fyn/Src can directly bind to the family of membrane-associated guanylate kinase (MAGUK) proteins, including PSD-95 and SAP102. Therefore, GluN2B phosphorylation on Tyr-1472 is promoted by the interaction of the receptor with these scaffolding proteins and, consistently, there is elevated phosphorylation of GluN2B on Tyr-1472 associated with synaptic GluN2B. The phosphorylation of GluN2B Ser-1480 by casein kinase 2 (CK2) inversely controls the phosphorylation of GluN2B Tyr-1472. GluN2B Ser-1480 phosphorylation occurs within the PDZ ligand and...
disrupts the binding of the receptor with MAGUK proteins (17). Therefore, phosphorylation of GluN2B on Ser-1480 disrupts anchoring with the postsynaptic density and allows NMDARs to diffuse laterally to extrasynaptic sites corresponding to dephosphorylation of Tyr-1472 by the action of the phosphatase STEP (18). In addition, the disruption of the PDZ ligand “uncouples” the receptor and Fyn/Src kinases, decreasing phosphorylation of Tyr-1472. Therefore, phosphorylation of GluN2B on Ser-1480 results in a decrease in Tyr-1472 phosphorylation, thus promoting internalization (16). A third phosphorylation site on GluN2B is involved in regulating synaptic expression in an activity-dependent manner (22).

**FIGURE 3. PTMs modify AMPAR intracellular domains.** The AMPAR C termini are substrates for several kinases targeting serine (S), threonine (T), or tyrosine (Y). Also, AMPARs are modified by palmitoylation on cysteines (C) and ubiquitination (UB) on lysines (K). The amino acids (aa) targeted by specific PTMs are depicted.

Phosphorylation can modulate receptor surface expression by regulating endocytosis as described above, but also by affecting protein export from the ER to the plasma membrane. For example, phosphorylation on the obligatory NMDAR subunit GluN1 controls the export of newly assembled receptors to the plasma membrane. Specifically, the PKC- and PKA-dependent phosphorylation of Ser-896 and Ser-897, respectively, on GluN1 promotes the release of the receptor from ER to plasma membrane. Specifically, the PKC- and PKA-dependent phosphorylation of Ser-896 and Ser-897, respectively, on GluN1 promotes the release of the receptor from ER to plasma membrane.

**Discussion:** The molecular mechanisms explained above are exclusive for GluN2B-containing NMDARs. GluN2A, the other GluN2 subunit expressed in adult cortex and hippocampus, is subject to differential regulation despite its homology to GluN2B in its C-terminal domain. For example, the PDZ ligand domain of GluN2A is not required for maintaining GluN2A synaptic localization and GluN2A does not interact with CaMKII. Similarly, GluN2A is mainly sorted to degradation after internalization, whereas GluN2B is recycled to the plasma membrane (8). From a functional perspective, it is not surprising that GluN2A is not phosphorylated in its PDZ ligand or affected by CaMKII phosphorylation. However, based on the high degree of sequence identity and the close proximity of any kinases to both GluN2B and GluN2A, the lack of phosphorylation is striking. It certainly serves as a cautionary example as to the pitfalls of relying on sequence motifs to predict regulation by phosphorylation.

There are examples of GluN2A being regulated by phosphorylation, although upstream of the extreme C terminus, which proves to be so critical for GluN2B regulation. For example, the kinase Dyrk1a phosphorylates GluN2A in its C-terminal domain, specifically on Ser-1048, and increases GluN2A surface expression by impairing internalization (23). Unfortunately, the molecular mechanisms regulating this process and the physiological significance of this phosphorylation remain unexplored. However, the fact that significant overexpression of Dyrk1a has been observed in a Down syndrome patient raises the possibility of a role for phosphorylation of GluN2A on Ser-1048 in that disease (23). Similarly, the internalization of GluN3A is also controlled by phosphorylation because a recent study identified the phosphorylation of Tyr-971 on GluN3A, within a novel endocytic domain (Tyr-971,Trp-972, and Leu-973). Strikingly, Tyr-971 phosphorylation promotes the interaction with AP-2 and induces GluN3A internalization, just the opposite effect observed for Tyr-1472 on GluN2B (24).

**Summary:** The regulatory role of phosphorylation in the expression and localization of AMPARs is a complex process involving multiple kinases and PTMs. The interplay between phosphorylation and ubiquitination, as well as the interaction with MAGUK proteins, is crucial for the dynamic regulation of synaptic expression and localization of AMPARs. Further research is needed to elucidate the specific mechanisms underlying these processes and to understand their physiological significance.
GluN2C is analogous to the CaMKII site, Ser-1303, on GluN2B. Although these two analogous residues on different GluN2 subunits are phosphorylated and functionally important, they have divergent consensus sequences resulting in differing kinase specificities.

**AMPAR Phosphorylation**

Since the mid-1990s, the cytosolic C-tails of AMPAR subunits have been shown to be targets of a variety of kinases (12, 27–31), which regulate AMPARs in many important ways including endocytosis, intracellular trafficking, channel conductance, and synaptic plasticity (11, 12, 31–34). As shown in Fig. 3, all four AMPAR subunits (GluA1–4) are demonstrated substrates of at least one of the following kinases: CaMKII, Fyn, JNK, PKA, PKC, and PKG (5, 35). However, most of our knowledge regarding AMPAR phosphorylation is limited to GluA1 and GluA2, which are widely distributed in the brain. Indeed, GluA1/2 heteromers constitute the majority of AMPARs in the hippocampus (36, 37).

GluA1 was the first AMPAR subunit for which the phosphorylation of the C-terminal tail (Fig. 3) was identified at Ser-831 and Ser-845 (12). Subsequent studies showed that CaMKII specifically phosphorylates Ser-831 (31, 32), which leads to an enhanced single channel conductance (11, 32), whereas the phosphorylation of GluA1 Ser-845 by PKA increases the opening probability of homomeric GluA1 (38). Surprisingly, no interacting partners seem to depend on the phosphorylation state of Ser-831, although Ser-831 phosphorylation regulates recycling (39), whereas Ser-845 dephosphorylation correlates with mechanisms associated with LTD (40, 41). Evidence also suggests that the phosphorylation of GluA2 on Tyr-876 and Ser-880 is essential for receptor endocytosis (42–44). Indeed, GRIP1/2 and PICK1 bind to the extreme GluA2 C-terminal region to the PDZ ligand. Phosphorylation provides elegant specificity of binding as phosphorylation of GluA2 on Tyr-876 and Ser-880 disrupts the binding of GRIP1/2, but is still permissive for PICK1 binding to GluA2 to promote internalization and LTD (43, 45).

It is interesting to note that consensus motifs can be misleading. For example, both PKC and CaMKII recognize very similar sequences (46), but the amino acid sequence surrounding GluA1 Ser-831 does not conform well to the prototypic CaMKII/PKC consensus motif as the residue at position −3 is not basic but hydrophobic (a proline). Thus, it is unclear what other molecular determinants dictate the kinase specificity for Ser-831. In addition to sequence specificity, other factors can modulate receptor phosphorylation such as receptor-binding proteins or other PTMs. For example, SAP97, the only PDZ protein known to bind GluA1, could play a role in regulating PTMs (47). Indeed, a model proposes that SAP97 binds to activated α-CaMKII firmly attached to NMDARs, which provide a solid platform for the synaptic anchoring of newly inserted GluA1-containing AMPARs (48). Thus, SAP97 binding to AMPARs and CaMKII could be a critical mechanism underlying LTP and receptor trafficking (4, 49–55).

Recent studies have revealed the important regulation of GluR trafficking dictated by mechanisms targeting the intracellular loops of GluRs, which include ER retention motifs (56) and residues that are targets for a variety of kinases. Indeed, we found that GluA1 is phosphorylated by CaMKII on Ser-567, a residue in the loop1 region of AMPARs. Surprisingly, phosphorylation on this residue inhibits GluA1-containing AMPAR synaptic insertion under basal condition (57). Instead of promoting AMPAR synaptic expression, the phosphorylation of GluA1 on Ser-567 may represent the first example of an LTD-specific CaMKII substrate that is distinctively different from standard CaMKII substrates such as GluA1 Ser-831 and GluN2B Ser-1303 (58).

It is likely that the AMPAR intracellular loop contains even more regulatory sites, and it seemed unlikely that CaMKII was
the only kinase targeting this region. Indeed, in subsequent studies, we found that CK2 phosphorylates the loop1 region of both GluA1 and GluA2 (59). This study shows that blocking phosphorylation of the major CK2 phosphorylation site on GluA1, Ser-579, impairs AMPAR surface and synaptic expression. Interestingly, CK2 can also phosphorylate, at least in vitro, the GluA1 Ser-567 residue previously identified as a CaMKII phosphorylation site (57). Furthermore, casein kinase 1 is another potential kinase that could regulate AMPARs by phosphorylating the loop1 region of AMPARs (59). Phosphorylation of this region might not only regulate trafficking, but due to the close proximity to the pore region, could potentially have potent effects on channel properties. Thus, the loop1 of AMPARs may represent an overlooked region with great potential for gaining insight into core mechanisms regulating glutamate receptor function.

Through the years, phosphospecific antibodies, phosphopeptide mapping, mass spectrometry analysis, and genetic approaches generated volumes of data substantiating a critical role for AMPAR phosphorylation in regulating synaptic expression and dynamic AMPAR changes during paradigms of synaptic plasticity. However, a recent study by Hosokawa et al. (60) has attempted to tackle the issue of overall stoichiometry of AMPAR phosphorylation using a different biochemical approach, specifically a Phos-tag SDS-PAGE reagent that resolves molecules by molecular weight, as a reflection of their phosphorylated residues. Thus, the distinction between phosphorylated and not phosphorylated species is possible based on their mobility on SDS-PAGE. Using this technique, 4.3% of all GluA1 found in the hippocampus are phosphorylated at Thr-840, whereas phosphorylated GluA1 at Ser-831 and at Ser-845 represent respectively 0.18% and 0.018% of total GluA1 (60). This estimation is in sharp contrast to other studies that have estimated closer to 15% of surface GluA1 is phosphorylated at Ser-845 under steady-state conditions (61). Furthermore, genetic knock-in approaches have found that GluA1-containing mutations at Ser-831 and Ser-845 display impaired synaptic plasticity (62). Therefore, there are conflicting data, but the study by Hosokawa et al. (60) certainly sheds light on the issue of stoichiometry and how it can be more precisely determined. However, detecting low phosphorylation levels at any given time reflects the transient nature of phosphorylation, and thus studying the stoichiometry of PTMs (i.e., phosphorylation or ubiquitination) on substrates is not necessarily a measure of functional relevance because spatial and temporal resolution is missing.

Ubiquitination

In addition to phosphorylation, other PTMs such as palmitoylation and ubiquitination are gaining attention as well. Indeed, the importance of the ubiquitin (UB) system in regulating virtually all aspects of cell function rivals, and may exceed, the role of protein phosphorylation (63). For example, the UB system preserves cell homeostasis by acting as the primary mechanism of protein quality control, membrane protein trafficking, receptor internalization, and degradation (64, 65).

Ubiquitination is a highly regulated ATP-dependent process that requires the coordinated and sequential action of an E1-activating enzyme, an E2-conjugating enzyme and, finally, an E3 UB ligase. Ultimately, the UB molecule is attached to the substrate via the formation of an isopeptide bond between the C-terminal glycine of UB and an internal lysine within the substrate. The human genome encodes several hundred E3s, and only a few of these have been studied thus far. In mammalian cells, many G protein-coupled receptors and ion channels are ubiquitinated in response to ligand binding (66–74). In addition, the UB-proteasome system influences neuronal activity and glutamatergic neurotransmission. For instance, a study by Ehlers (75) shows that bidirectional homeostatic plasticity triggers activity-dependent ubiquitination and profound modifications of a variety of PSD proteins. This pioneering work, along with studies from other groups, suggests a mechanism for regulating dynamic changes in spine content, morphology, and structure, therefore altering synaptic activity and plasticity (76, 77).

Many studies have identified and characterized the ubiquitination of mammalian iGluRs. For instance, the UB E3 ligases Fbx2 (78) and Mind Bomb-2 (79) ubiquitinate the NMDAR subunits GluN1 and GluN2B in an activity-dependent manner. More recently, GluN2D was shown to be ubiquitinated by Ned4-1 (80). The ubiquitination of AMPARs, on the other hand, was initially demonstrated in Caenorhabditis elegans (81), and it took another decade before studies demonstrated that mammalian AMPAR subunits were actually ubiquitinated by the UB E3 ligases APC-Cdh1, Ned4-1, and RNF167 (68, 69, 72, 73, 82–84). Interestingly, modulation of neuronal activity by repetitive stress induces GluA1 and GluN1 ubiquitination (85). Importantly, two recent proteomic studies performed on rodent brains identified GluN1, GluN2A/2B (Fig. 2), and RNF167 (86, 87). Without a doubt, ubiquitination is important for regulating GluRs, but the mechanisms and implications of AMPAR and NMDAR ubiquitination on health and with respect to synaptic dysfunction remain to be investigated in depth.

Palmitoylation

Another common and important PTM that regulates GluR trafficking is palmitoylation. It is defined by the covalent and reversible union of a palmitic acid molecule (saturated 16-carbon lipid) to a cysteine residue in a given protein. The presence of basic and hydrophobic residues surrounding cysteine appears to create a favorable sequence environment for the reaction (88). This likely explains the propensity of palmitoylated cysteines to be identified near the transmembrane-spanning region for membrane proteins (89–92). The addition of the palmitoyl group increases the hydrophobicity of the protein and, therefore, facilitates the interaction with cellular membranes. Palmitoylation can both stabilize proteins in the plasma membrane and control protein shuttling between intracellular compartments (93). Palmitoylation is mediated by a group of enzymes named palmitoyltransferases (PATs), of which there are 23 in humans with each containing an Asp-His-His-Cys (DHHC) Cys-rich domain that conforms the molecular signature of PATs (94). Conversely, depalmitoylation is mediated by acyl-protein-thioesterases, of which very few have been identified so far.
The function of many neuronal proteins, including NMDARs and AMPARs, is regulated by palmitoylation. The palmitoylation of GluN2A and GluN2B subunits occurs in “two clusters” (Fig. 2). Cluster I, close to the last transmembrane domain of GluN2A and GluN2B, is associated with an increase in the surface expression of the receptor, whereas palmitoylation of Cluster II, located in the middle of the intracellular C-terminus, plays an opposite role to Cluster I and is associated with receptor accumulation in the Golgi apparatus (95, 96). However, the regulation of NMDARs by palmitoylation is complex and participates in interplay with tyrosine phosphorylation for both GluN2A and GluN2B. In addition, PSD-95 and other synaptic proteins important for controlling NMDARs are also palmitoylated, multiplying the complexity of the regulation of NMDARs by this modification (93). Similarly, all four subunits of AMPARs are palmitoylated at two conserved cysteine residues (Fig. 3); one is located within the C-terminal region, and another is located within the second intracellular loop immediately adjacent to the pore region (89, 97). Although in vitro studies show that AMPAR trafficking and membrane expression are regulated by palmitoylation (95, 97), the study by Van Dolah et al. (98) tackled the function of AMPAR palmitoylation in the brain. In this interesting study, intraperitoneal injection of the psychostimulant cocaine (20 mg/kg) in adult male rats up-regulates the palmitoylation of GluA1 and GluA3 AMPAR subunits in the nucleus accumbens. In fact, cocaine causes the redistribution of AMPARs, increasing the intracellular localization, whereas the surface expression was reduced. Although future studies are required to clarify the function of palmitoylation in AMPAR synaptic plasticity, it is clear that AMPAR palmitoylation can be dynamically controlled by extracellular stimuli in various brain regions (89, 97, 98).

**Future Perspectives**

Over the last decade, great advances have been made in identifying the specific regulation of AMPARs and NMDARs by PTMs. Several studies demonstrate crosstalk between two or more PTMs to be important mechanisms of synaptic regulation (99). Functionally, crosstalk may occur within the same protein (cis crosstalk) or between PTMs on two different proteins (trans crosstalk). An example of such crosstalk on glutamate receptors is that, after depalmitoylation of GluA1 on Cys-811, the phosphorylation of GluA1 Ser-818 by PKC enhances binding to 4.1N to drive membrane insertion and the expression of LTP (97). As evidenced by this study, future investigations are required for understanding the synergistic/antagonistic effect of PTMs and the directionality of the crosstalk for identifying new mechanisms implicated in spatial and temporal regulation of AMPARs and NMDARs. Interestingly, using mass spectrometry and various enrichment approaches, a recent study suggests the presence of a global crosstalk directionality in which phosphorylation frequently precedes ubiquitination (100). In conclusion, PTMs represent an important set of mechanisms to regulate protein function and cellular signaling, and the importance and complexity of its code remain a major challenge for our complete understanding of brain function.

**References**

1. Bredt, D. S., and Nicoll, R. A. (2003) AMPA receptor trafficking at excitatory synapses. *Neuron* **40**, 361–379
2. Malenka, R. C., and Bear, M. F. (2004) LTP and LTD: an embarrassment of riches. *Neuron* **44**, 5–21
3. Malinow, R., and Malenka, R. C. (2002) AMPA receptor trafficking and synaptic plasticity. *Annu. Rev. Neurosci.* **25**, 103–126
4. Anggono, V., and Huganir, R. L. (2012) Regulation of AMPA receptor trafficking and synaptic plasticity. *Curr. Opin. Neurobiol.* **22**, 461–469
5. Lu, W., and Roche, K. W. (2012) Posttranslational regulation of AMPA receptor trafficking and function. *Curr. Opin. Neurobiol.* **22**, 470–479
6. van der Sluijs, P., and Hoogenraad, C. C. (2011) New insights in endosomal dynamics and AMPA receptor trafficking. *Semin. Cell. Dev. Biol.* **22**, 499–505
7. Ehlers, M. D. (2013) Dendritic trafficking for neuronal growth and plasticity. *Biochem. Soc. Trans.* **41**, 1365–1382
8. Sanz-Clemente, A., Nicoll, R. A., and Roche, K. W. (2013) Diversity in NMDA receptor composition: many regulators, many consequences. *Neuroscientist* **19**, 62–75
9. Standley, S., and Baudry, M. (2000) The role of glycosylation in ionotropic glutamate receptor ligand binding, function, and trafficking. *Cell. Mol. Life Sci.* **57**, 1508–1516
10. Ciesla, J., Fra&ccaron;zyk, T., and Rode, W. (2011) Phosphorylation of basic amino acid residues in proteins: important but easily missed. *Acta Biochim. Pol.* **58**, 137–148
11. Derkach, V., Barria, A., and Soderling, T. R. (1999) Ca2+/calmodulin kinase II enhances channel conductance of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate type glutamate receptors. *Proc. Natl. Acad. Sci. U.S.A.* **96**, 3269–3274
12. Roche, K. W., O’Brien, R. J., Mammen, A. L., Bernhardt, J., and Huganir, R. L. (1996) Characterization of multiple phosphorylation sites on the AMPA receptor GluR1 subunit. *Neuron* **16**, 1179–1188
13. Murphy, J. A., Stein, I. S., Lau, C. G., Peixoto, R. T., Aman, T. K., Kaneko, N., Aramolaran, K., Saulnier, J. L., Popescu, G. K., Sabatini, B. L., Bell, J. W., and Zukin, R. S. (2014) Phosphorylation of Ser1166 on GluN2B by PKA is critical to synaptic NMDA receptor function and Ca2+ signaling in spines. *J. Neurosci.* **34**, 869–879
14. Lavezzari, G., McCallum, J., Lee, R., and Roche, K. W. (2003) Differential binding of the AP-2 adaptor complex and PSD-95 to the C-terminus of the NMDA receptor subunit NR2B regulates surface expression. *Neuropsychopharmacology* **45**, 729–737
15. Pribylewski, K., Chang, K., Sans, N., Kan, L., Vicini, S., and Wenthold, R. J. (2005) The synaptic localization of NR2B-containing NMDA receptors is controlled by interactions with PDZ proteins and AP-2. *Neuron* **47**, 845–857
16. Sanz-Clemente, A., Matta, J. A., Isaac, J. T., and Roche, K. W. (2010) Casein kinase 2 regulates the NR2B subunit composition of synaptic NMDA receptors. *Neuron* **67**, 984–996
17. Chung, H. J., Huang, Y. H., Lau, L. F., and Huganir, R. L. (2004) Regulation of the NMDA receptor complex and trafficking by activity-dependent phosphorylation of the NR2B subunit PDZ ligand. *J. Neurosci.* **24**, 10248–10259
18. Chen, B. S., Gray, J. A., Sanz-Clemente, A., Wei, Z., Thomas, E. V., Nicoll, R. A., and Roche, K. W. (2012) SAP102 mediates synaptic clearance of NMDA receptors. *Cell Rep.* **2**, 1120–1128
19. O’Leary, H., Liu, W. H., Rorbaugh, J. M., Coultrap, S. J., and Bayer, K. U. (2011) Nucleotides and phosphorylation bidirectionally modulate Ca2+/calmodulin-dependent protein kinase II (CaMKII) binding to the N-methyl-D-aspartate (NMDA) receptor subunit GluN2B. *J. Biol. Chem.* **286**, 31272–31281
20. Sanz-Clemente, A., Gray, J. A., Ogilvie, K. A., Nicoll, R. A., and Roche, K. W. (2013) Activated CaMKII couples GluN2B and casein kinase 2 to control synaptic NMDA receptors. *Cell Rep.* **3**, 607–614
21. Morabito, M. A., Sheng, M., and Tsai, L. H. (2004) Cyclin-dependent kinase 5 phosphorylates the N-terminal domain of the postsynaptic density protein PSD-95 in neurons. *J. Neurosci.* **24**, 865–876
22. Plattner, F., Hernández, A., Kistler, T. M., Pozo, K., Zhong, P., Yuen, E. Y.,
MINIREVIEW: Dynamic Regulation of NMDAR and AMPAR by PTMs

Tan, C., Hawasli, A. H., Cooke, S. F., Nishi, A., Guo, A., Wiederhold, T., Yan, Z., and Bibb, J. A. (2014) Memory enhancement by targeting Cdk5 regulation of NR2B. *Neuron* **81**, 1070–1083

Grau, C., Arató, K., Fernández-Fernández, I. M., Valderrama, A., Sindreu, C., Fillat, C., Ferrer, I., de la Luna, S., and Altafar, X. (2014) DYRK1A-mediated phosphorylation of GluN2A at Ser1048 regulates the surface expression and channel activity of GluN1/GluN2A receptors. *Front. Cell. Neurosci.* **8**, 331

Chowdhury, D., Marco, S., Brooks, I. M., Zandueta, A., Rao, Y., Haucke, V., Wesseling, J. F., Tavalin, S. J., and Pérez-Otaño, I. (2013) Tyrosine phosphorylation regulates the endocytosis and surface expression of GluN3A-containing NMDA receptors. *J. Neurosci.* **33**, 4151–4164

Scott, D. B., Blanpied, T. A., Swanson, G. T., Zhang, C., and Ehlers, M. D. (2001) An NMDA receptor ER retention signal regulated by phosphorylation and alternative splicing. *J. Neurosci.* **21**, 3063–3072

Chen, B. S., and Roche, K. W. (2009) Growth factor-dependent trafficking of cerebellar NMDA receptors via protein kinase B/Akt phosphorylation of NR2C. *Neuron* **62**, 471–478

Blackstone, C., Murphy, T. H., Moss, J. S., Baraban, J. M., and Huganir, R. L. (1994) Cyclic AMP and synaptic activity-dependent phosphorylation of AMPA-prefering glutamate receptors. *J. Neurosci.* **14**, 5785–5793

McClade-McCulloh, E., Yamamoto, H., Tan, S. E., Brickey, D. A., and Esteban, J. A. (2005) NMDA receptor-dependent activation of the small GTPase Rab5 drives the removal of synaptic AMPA receptors during hippocampal LTD. *Neuron* **45**, 81–94

Hayashi, T., and Huganir, R. L. (2004) Tyrosine phosphorylation and regulation of the AMPA receptor by SRC family tyrosine kinases. *J. Neurosci.* **24**, 6152–6160

Chung, H. J., Xia, J., Scannevin, R. H., Zhang, X., and Huganir, R. L. (2000) Phosphorylation of the AMPA receptor subunit GluR2 differentially regulates its interaction with PDZ domain-containing proteins. *J. Neurosci.* **20**, 7258–7267

Brown, T. C., Tran, I. C., Backos, D. S., and Esteban, J. A. (2005) NMDA receptor-dependent activation of the small GTPase Rab5 drives the removal of AMPA receptors during hippocampal LTD. *Neuron* **45**, 81–94

Banke, T. G., Bowie, D., Lee, H., Huganir, R. L., Schousboe, A., and Traynelis, S. F. (2012) PKC phosphorylates GluA1-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor GluR1 subunit. *J. Biol. Chem.* **273**, 19518–19524

Lisman, J. E., and Zhabotinsky, A. M. (2001) A model of synaptic memory: a CaMKII/PP1 switch that potentiates transmission by organizing an AMPA receptor anchoring assembly. *Neuron* **31**, 191–201

Malenka, R. C., Kauer, J. A., Perkel, D. J., Mauk, M. D., Kelly, P. T., Nicoll, R. A., and Waxham, M. N. (1989) An essential role for postsynaptic calmodulin and protein kinase activity in long-term potentiation. *Nature* **340**, 554–557

Malinow, R., Schulman, H., and Tsien, R. W. (1989) Inhibition of postsynaptic PKC or CaMKII blocks induction but not expression of LTP. *Science* **245**, 862–866

Wyllie, D. J., and Nicoll, R. A. (1994) A role for protein kinases and phosphatases in the Ca2+-induced enhancement of hippocampal AMPA receptor-mediated synaptic responses. *Neuron* **13**, 635–643

Lisman, J., Yasuda, R., and Raghavachari, S. (2012) Mechanisms of CaMKII action in long-term potentiation. *Nat. Rev. Neurosci.* **13**, 169–182

Kessels, H. W., and Malenka, R. C. (2012) NMDA receptor-dependent long-term potentiation and long-term depression (LTP/LTD). *Cold Spring Harb. Perspect. Biol.* **4**, a005710

Nicoll, R. A., and Roche, K. W. (2013) Long-term potentiation: peeling the onion. *Neuropharmacology* **74**, 18–22

Nasu-Nishimura, Y., Jaffe, H., Isaac, J. T., and Roche, K. W. (2010) Differential regulation of kainate receptor trafficking by phosphorylation of distinct sites on GluR6. *J. Biol. Chem.* **285**, 2847–2856

Lu, W., Isozaki, K., Roche, K. W., and Nicoll, R. A. (2010) Synaptic targeting of AMPA receptors is regulated by a CaMKII site in the first intracellular loop of GluA1. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 22266–22271

Coultrap, S. J., Freund, R. K., O’Leary, H., Sanderson, J. L., Roche, K. W., Dell’Acqua, M. L., and Bayer, K. U. (2014) Autonomous CaMKII mediates both LTP and LTD using a mechanism for differential substrate site selection. *Cell Rep.* **6**, 431–437

Luscher, C., and Malenka, R. C. (2012) NMDA receptor-dependent long-term potentiation and long-term depression (LTP/LTD). *Cold Spring Harb. Perspect. Biol.* **4**, a005710

Dell’Acqua, M. L., and Bayer, K. U. (2014) Autonomous CaMKII mediates both LTP and LTD using a mechanism for differential substrate site selection. *Cell Rep.* **6**, 431–437

Luscher, C., and Malenka, R. C. (2012) NMDA receptor-dependent long-term potentiation and long-term depression (LTP/LTD). *Cold Spring Harb. Perspect. Biol.* **4**, a005710

Dell’Acqua, M. L., and Bayer, K. U. (2014) Autonomous CaMKII mediates both LTP and LTD using a mechanism for differential substrate site selection. *Cell Rep.* **6**, 431–437

Luscher, C., and Malenka, R. C. (2012) NMDA receptor-dependent long-term potentiation and long-term depression (LTP/LTD). *Cold Spring Harb. Perspect. Biol.* **4**, a005710

Dell’Acqua, M. L., and Bayer, K. U. (2014) Autonomous CaMKII mediates both LTP and LTD using a mechanism for differential substrate site selection. *Cell Rep.* **6**, 431–437

Luscher, C., and Malenka, R. C. (2012) NMDA receptor-dependent long-term potentiation and long-term depression (LTP/LTD). *Cold Spring Harb. Perspect. Biol.* **4**, a005710

Dell’Acqua, M. L., and Bayer, K. U. (2014) Autonomous CaMKII mediates both LTP and LTD using a mechanism for differential substrate site selection. *Cell Rep.* **6**, 431–437

Luscher, C., and Malenka, R. C. (2012) NMDA receptor-dependent long-term potentiation and long-term depression (LTP/LTD). *Cold Spring Harb. Perspect. Biol.* **4**, a005710
