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AMPA Receptor Trafficking and Synaptic Plasticity

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■ **Abstract** Activity-dependent changes in synaptic function are believed to underlie the formation of memories. Two prominent examples are long-term potentiation (LTP) and long-term depression (LTD), whose mechanisms have been the subject of considerable scrutiny over the past few decades. Here we review the growing literature that supports a critical role for AMPA receptor trafficking in LTP and LTD, focusing on the roles proposed for specific AMPA receptor subunits and their interacting proteins. While much work remains to understand the molecular basis for synaptic plasticity, recent results on AMPA receptor trafficking provide a clear conceptual framework for future studies.

INTRODUCTION

It is widely believed that a long-lasting change in synaptic function is the cellular basis of learning and memory (Alkon & Nelson 1990, Eccles 1964, Hebb 1949, Kandel 1997). The most thoroughly characterized examples of such synaptic plasticity in the mammalian nervous system are long-term potentiation (LTP) and long-term depression (LTD). A remarkable feature of LTP and LTD is that a short period of synaptic activity (either high- or low-frequency stimulation) can trigger persistent changes of synaptic transmission lasting at least several hours and often longer. This single property initially led investigators to suggest that these forms of plasticity are the cellular correlate of learning (Bliss & Gardner-Medwin 1973, Bliss & Lomo 1973). Work over the past 25 years that has elucidated many properties of LTP and LTD reinforces this view as well as suggests their involvement in various other physiological as well as pathological processes (Martin et al. 2000, Zoghbi et al. 2000).

Much effort in the field has been directed toward understanding the detailed molecular mechanisms that account for the change in synaptic efficacy. For many years, a most basic question remained intractable: Is the change in synaptic strength

during these forms of plasticity primarily due to a pre- or postsynaptic modification? Numerous experiments using a variety of approaches were directed toward answering this question. Surprisingly, they often yielded conflicting conclusions (Kullmann & Siegelbaum 1995). Although many studies suggested primarily postsynaptic modifications (Davies et al. 1989, Kauer et al. 1988, Manabe et al. 1992, Muller et al. 1988), a consistent finding was a change in synaptic failures after LTP (Isaac et al. 1996, Kullmann & Nicoll 1992, Malinow & Tsien 1990, Stevens & Wang 1994). Because synaptic failures were assumed to be due to failure to release transmitter (a presynaptic property), these results were in apparent contradiction. A resolution arrived with the identification of postsynaptically "silent synapses" and the demonstration that they could be converted to active synapses by a postsynaptic modification (Durand et al. 1996, Isaac et al. 1995, Liao et al. 1995). Synapses are postsynaptically silent if they show an NMDA but no AMPA receptor response. Thus, at resting potentials NMDA receptors (NMDARs) are minimally opened, and transmitter release at such a synapse is recorded as a failure. The wholesale appearance of an AMPA response at such synapses during LTP, with no change in the NMDA response, strongly supports a postsynaptic modification consisting of a functional recruitment of AMPA receptors (AMPARs). One potential mechanism envisioned was the rapid delivery of AMPARs from nonsynaptic sites to the synapse, via a mechanism analogous to the exocytosis of presynaptic vesicles during transmitter release.

Two early studies provided support for postsynaptic exocytosis playing a role in synaptic plasticity. One study in hippocampal slices showed that loading postsynaptic cells with toxins that specifically perturb membrane fusion could block LTP (Lledo et al. 1998). Thus, postsynaptic events such as exocytosis were implicated. A separate study in dissociated cultured neurons identified a form of dendritic exocytosis that was mediated by activation of CaMKII (Maletic-Savatic et al. 1998), an enzyme believed to play a critical role in LTP (Lisman et al. 1997). Thus, dendritic exocytosis was further linked to synaptic plasticity. These studies along with the demonstration of the role of silent synapses in LTP provided strong motivation for the development of cellular and molecular techniques that could monitor and perturb trafficking of AMPARs to and away from synapses.

MOLECULAR INTERACTIONS OF AMPA RECEPTORS

AMPA receptors (AMPARs) are hetero-oligomeric proteins made of the subunits GluR1 to GluR4 (also known as GluRA-D) (Hollmann & Heinemann 1994, Wisden & Seeburg 1993). Each receptor complex is thought to contain four subunits (Rosenmund et al. 1998). In the adult hippocampus two species of AMPAR appear to predominate: receptors made of GluR1 and GluR2 or those composed of GluR3 and GluR2 (Wenthold et al. 1996). Immature hippocampus, as well as other mature brain regions, express GluR4, which also complexes with GluR2 to form a receptor (Zhu et al. 2000). Although the extracellular and transmembrane regions of AMPAR subunits are very similar, their intracellular cytoplasmic tails

are distinct (Figure 1). GluR1, GluR4, and an alternative splice form of GluR2 (GluR2L) have longer cytoplasmic tails and are homologous. In contrast, the predominant splice form of GluR2, GluR3, and an alternative splice form of GluR4 that is primarily expressed in cerebellum (GluR4c) have shorter, homologous cytoplasmic tails. Through their C-terminal tails, each subunit interacts with specific cytoplasmic proteins (Figure 1). Most of these AMPAR-interacting proteins thus far identified have single or multiple PDZ domains, which are well-characterized protein-protein interaction motifs that often interact with the extreme C-terminal tails of target proteins (Sheng & Sala 2001). GluR1 forms a group I PDZ ligand, while GluR2, GluR3, and GluR4c form group II PDZ ligands. GluR4 and GluR2L have variant C-terminal tails, and if they interact with classical PDZ-domain proteins is unclear. In a variety of cell types, proteins containing PDZ domains have been implicated in playing important roles in the targeting and clustering of membrane proteins to specific subcellular domains (Sheng & Sala 2001).

GluR1 interacts with the PDZ-domain regions of SAP97 (Leonard et al. 1998) and RIL [reversion-induced LIM gene (Schulz et al. 2001)]. SAP97 is closely related to a family of proteins (SAP90/PSD95, chapsyn110/PSD93, and SAP102) that interact with NMDAR subunits. RIL, on the other hand, may link AMPA receptors to actin. GluR2 and GluR3 interact with glutamate receptor-interacting protein (GRIP) (Dong et al. 1997, 1999) and AMPA receptor binding protein (ABP)/GRIP2 (Dong et al. 1999, Srivastava et al. 1998), proteins with six or seven PDZ domains. GluR2 and GluR3 as well as GluR4c also interact with PICK1 (protein interacting with C-kinase) (Dev et al. 1999, Xia et al. 1999), which contains a single PDZ domain that interacts with both PKCα and GluR2. Other group II PDZ-domain-containing proteins that interact with GluR2, GluR3, and GluR4c have recently been identified and include rDLG6 (Inagaki et al. 1999) and afadin (Rogers et al. 2001). No binding partners have yet been reported for GluR4 and GluR2L.

Some additional proteins interact with the cytoplasmic tails of AMPAR subunits at regions that are not at the exact C terminus. GluR1 interacts with band 4.1N and is linked through it to actin (Shen et al. 2000). The interaction occurs at a region on GluR1 that is homologous with all other subunits, and thus band 4.1N may interact with other AMPAR subunits as well. A surprising finding is that the cytoplasmic tail of GluR2, in addition to interacting with PDZ proteins, also binds to NSF (NEM-sensitive-factor) (Nishimune et al. 1998, Osten et al. 1998, Song et al. 1998), an ATPase known to play an essential role in the membrane fusion processes that underlie intracellular protein trafficking and presynaptic vesicle exocytosis (Rothman 1994). This was particularly surprising given that GluR2 has no sequence homology with the extensively characterized SNARE proteins that previously were thought to be the unique targets for NSF action. Another key component of membrane fusion machinery, α and β SNAPS (soluble NSF attachment proteins) could be co-immunoprecipitated with AMPARs containing GluR2 (Osten et al. 1998), although the molecular regions mediating the presumed interaction between AMPARs and SNAPS remain to be determined.

Because these AMPAR-interacting proteins either contain PDZ domains, are proteins implicated in membrane fusion, or interact with the actin cytoskeleton,

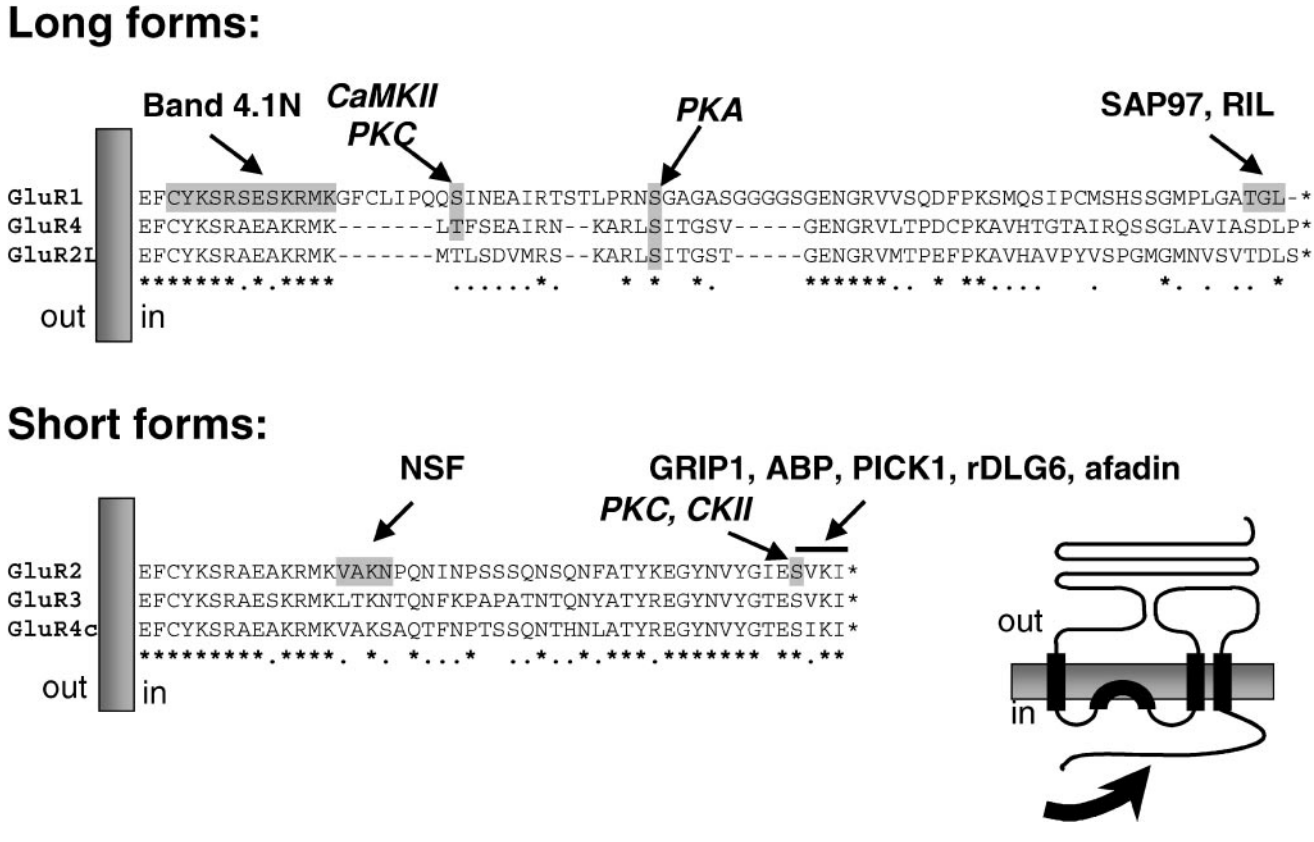

Figure 1 Cytoplasmic carboxyl tail regions of AMPARs, phosphorylation sites, and interacting proteins. Long and short forms of AMPARs are aligned and homologies are indicated (*, identity; ., similarity). Interacting proteins and approximate sites of interaction indicated (. . .). Protein kinases (italics) and sites of phosphorylation indicated (. . .). See text for details.

they have been suggested to play important roles in controlling the trafficking of AMPARs and/or their stabilization at synapses. The proposed specific functions of each of these proteins in controlling AMPAR behavior are discussed in greater detail in the following sections.

AMPA RECEPTOR ENDOCYTOSIS AND LONG-TERM DEPRESSION

Activity-Dependent Loss of Synaptic AMPA Receptors

The first experiments to test directly the idea that activity could influence the number of AMPARs at individual synapses involved chronic (several days) pharmacological manipulations of network activity in dissociated cultured neurons. Increasing network activity by blocking inhibitory synaptic transmission with GABA-A receptor antagonists caused a significant decrease in the proportion of synapses containing epitope-tagged AMPARs with no detectable effect on the level of NMDAR expression at synapses (Lissin et al. 1998). Consistent with this decrease in the content of AMPARs, prolonged increases in the activity of spinal cord and cortical cultures caused a decrease in the amplitude of miniature AMPAR-mediated synaptic currents (O'Brien et al. 1998, Turrigiano et al. 1998). Conversely, applying AMPAR antagonists for hours to days caused an increase in the surface expression of AMPARs at synapses (Liao et al. 1999, O'Brien et al. 1998) and a decrease in the proportion of anatomically defined silent synapses (Liao et al. 1999).

Although these studies demonstrated that prolonged manipulations of activity affected the synaptic distribution of AMPARs, the question remained whether a much more rapid movement of AMPARs might occur. Such rapid movement is mandatory if changes in AMPAR content at synapses contribute to synaptic plasticity such as LTD. One of the first pieces of evidence for a rapid redistribution of AMPARs came from the demonstration that brief applications of glutamate to hippocampal cultures cause a significant loss of AMPARs, detected immunohistochemically, from synaptic sites (Lissin et al. 1999). Consistent with the idea that NMDARs are relatively more stable in the synaptic plasma membrane (Allison et al. 1998), NMDARs appeared to be unaffected. It is now clear that a similar rapid loss of synaptic AMPARs can be triggered by the activation of several different receptors including AMPARs, NMDARs, metabotropic glutamate receptors, and insulin receptors (Beattie et al. 2000, Carroll et al. 1999a, Ehlers 2000, Man et al. 2000, Snyder et al. 2001).

Endocytosis of AMPA Receptors During LTD

Immunocytochemical techniques that distinguish AMPARs in the plasma membrane from AMPARs in intracellular pools have directly demonstrated that the loss of AMPARs due to pharmacological activation of glutamate or insulin receptors is indeed due to internalization of receptors originally on the cell surface. Several

lines of evidence indicate that the endocytosis of AMPARs is analogous to the stimulated endocytosis of G protein–coupled receptors in that AMPAR endocytosis occurs via clathrin-coated pits and requires the activity of dynamin. First, inhibition of clathrin-mediated endocytosis by high concentrations of sucrose, the expression of a dominant negative form of dynamin, or peptide-mediated disruption of the dynamin-amphiphysin complex each blocks the triggered endocytosis of AMPARs (Carroll et al. 1999a, Man et al. 2000, Wang & Linden 2000). Second, following internalization, AMPARs exhibit an increased colocalization and interaction with the clathrin adaptor protein AP2 (Carroll et al. 1999a, Man et al. 2000).

All of the experiments reviewed thus far used pharmacological manipulations to induce AMPAR endocytosis. Thus, the critical question remained whether AMPAR endocytosis actually contributed to LTD. The first experimental support for this idea came from the use of immunocytochemical techniques to examine the distribution of AMPARs following the generation of NMDAR-dependent LTD in hippocampal cultures (Carroll et al. 1999b). LTD caused a decrease in the proportion of synapses containing detectable surface AMPARs while having no effect on the distribution of synaptic NMDARs. Generation of LTD in the hippocampus in vivo subsequently was found to cause a decrease in the number of AMPARs in synaptoneurosomes, providing further evidence for the role of AMPAR endocytosis (Heynen et al. 2000). That the loss of synaptic AMPARs during LTD involves their clathrin-mediated endocytosis is further supported by experiments in which LTD was blocked by loading CA1 pyramidal neurons or cerebellar Purkinje cells with a peptide that disrupts dynamin function (Luscher et al. 1999, Wang & Linden 2000). Importantly, these results were the first demonstration that two forms of LTD that previously were thought to be mechanistically distinct, cerebellar LTD and NMDAR-dependent LTD in the hippocampus, appear to share a common mechanism of expression. Inhibition of endocytosis also blocked the actions of insulin, which can cause a depression of synaptic currents that occludes LTD (Lin et al. 2000, Man et al. 2000).

Intracellular Signaling Pathways That Trigger AMPA Receptor Endocytosis

Given that LTD involves the endocytosis of AMPARs, a critical question is what intracellular signaling pathways trigger this increase in AMPAR trafficking. For NMDAR-dependent LTD a predominant hypothesis is that activation of a calcium-dependent protein phosphatase cascade involving calcineurin and protein phosphatase 1 (PP1) is required for its triggering (Lisman 1989, Mulkey et al. 1994, Mulkey et al. 1993). Thus, a number of laboratories have investigated the role of calcium and protein phosphatase activity and have found that both are involved in the triggering of AMPAR endocytosis. Specifically, the internalization of AMPARs caused by activation of NMDARs was blocked by removing extracellular calcium or application of the membrane permeable calcium chelator BAPTA-AM as well as by specific inhibitors of calcineurin (Beattie et al. 2000, Ehlers 2000). Similarly,

the AMPAR endocytosis triggered by application of AMPA or insulin was blocked by calcineurin inhibitors (Beattie et al. 2000, Lin et al. 2000). The mechanisms by which calcineurin facilitates AMPAR endocytosis are unknown. One attractive hypothesis, based on work on the mechanisms mediating the activity-dependent increase in the endocytosis of presynaptic vesicles, is that calcineurin facilitates endocytosis via its association with dynamin/amphiphysin and the consequent dephosphorylation of components of the endocytic machinery (Beattie et al. 2000, Lai et al. 1999, Slepnev et al. 1998).

The role of PP1 in AMPAR endocytosis is less clear. Pharmacological inhibition of PP1 has been reported to block the endocytosis of AMPARs triggered by NMDA application (Ehlers 2000) but also has been found to have the opposite effect: enhancement of AMPAR endocytosis (Beattie et al. 2000, Lin et al. 2000). Differences in the techniques used to detect internalized AMPARs may have identified different subpopulations of AMPARs in these studies and therefore may have contributed to these differing results. Study of LTD in cerebellar Purkinje cells provides further complexity to the signaling pathways triggering AMPAR endocytosis. Cerebellar LTD requires activation of PKC (Linden 1994), which is required to stimulate the internalization of AMPARs in these cells (Matsuda et al. 2000, Xia et al. 2000). In fact, activation of PKC with a phorbol ester can also drive AMPAR internalization in cultured cortical neurons (Chung et al. 2000). When all of the studies on the signaling pathways involved in AMPAR endocytosis are considered together, it appears that the regulation of AMPAR endocytosis may be cell-type specific. This likely is because the detailed subunit composition of AMPARs differs among cell types and as a consequence so do the protein-protein interactions involving AMPARs that regulate endocytosis.

In addition to the regulated endocytosis of AMPARs that plays a critical role in LTD, it is clear that synaptic AMPARs also undergo a constitutive endocytosis that contributes to the basal cycling of AMPARs. Such cycling has been observed using electrophysiological (Kim & Lisman 2001, Luscher et al. 1999, Lüthi et al. 1999, Nishimune et al. 1998, Noel et al. 1999, Shi et al. 2001), biochemical (Ehlers 2000), and immunocytochemical (Lin et al. 2000, Zhou et al. 2001) techniques; the role of specific AMPAR subunits in this cycling has been addressed (see below). Another form of activity-dependent endocytosis that does not require calcium influx or calcineurin can be stimulated by ligand binding to AMPARs, either by competitive antagonists or by AMPA in the absence of receptor activation (Ehlers 2000, Lin et al. 2000). These forms of AMPAR endocytosis are further distinguished from regulated AMPAR endocytosis by differences in the effects of mutations in the carboxyl terminus of GluR2 expressed in HEK293 cells (Lin et al. 2000).

Role of AMPA Receptor Interacting Proteins

NSF-GluR2 INTERACTIONS The first protein that interacts with an AMPAR subunit to receive attention for its possible role in AMPAR trafficking was NSF, which as mentioned above plays a key role in membrane fusion events such as synaptic

vesicle exocytosis (Rothman 1994). Loading CA1 pyramidal cells with peptides that disrupt the interaction of NSF with GluR2 causes a fairly rapid decrease in the size of synaptic currents, which suggests a loss of synaptic AMPARs (Kim & Lisman 2001, Lüscher et al. 1999, Lüthi et al. 1999, Nishimune et al. 1998, Noel et al. 1999, Shi et al. 2001). Depression by such peptides does not occur in animals lacking GluR2, which indicates that the depressive effect is specific for interactions mediated by GluR2 (Shi et al. 2001). More prolonged expression of the peptide caused a decrease in the expression of surface AMPARs identified by immunocytochemistry (Lüscher et al. 1999, Noel et al. 1999), a decrease in the responses of neurons to local application of AMPA (Nishimune et al. 1998), and an ~50% decrease in evoked synaptic transmission (Shi et al. 2001). These results suggest that the synaptic expression of some AMPARs may require an NSF-GluR2 interaction.

This interaction may also be important for controlling the synaptic expression of the population of AMPARs that play a role in synaptic plasticity. Peptide-mediated disruption of the NSF-GluR2 interaction was observed to occlude LTD in hippocampal CA1 pyramidal cells while saturation of LTD occluded any further reduction in synaptic currents due to the peptide (Lüthi et al. 1999, Noel et al. 1999). LTP also was impaired by manipulations that disrupt the function of NSF in postsynaptic cells (Lledo et al. 1998), although these effects were likely not due to a specific disruption of the NSF-GluR2 complex because LTP can still be elicited in knockout mice lacking GluR2 (Jia et al. 1996, Mainen et al. 1998) and because overexpression of the cytoplasmic tail of GluR2 does not block LTP (Shi et al. 2001).

While the results using peptide-mediated disruption of the NSF-GluR2 interaction have supported an important role for this interaction in AMPAR trafficking, the interpretation of these results is dependent on the specificity of the peptide's actions, in particular that the peptides have no effect on the function of NSF in the membrane fusion events required for protein trafficking through the secretory pathway. That the peptide does not have a clear effect in knockout mice lacking GluR2 supports the specificity of its actions (Shi et al. 2001). A complementary approach that obviates this concern is to examine the behavior of mutant forms of GluR2 that do not bind NSF. When such a mutant form of GluR2 is expressed in hippocampal slice cultures, it is not present in the synaptic plasma membrane, whereas wild-type GluR2 can be delivered to synapses without difficulty (Shi et al. 2001). This result is consistent with the experiments using peptides and suggests that the NSF-GluR2 interaction is required either for the delivery of AMPARs to the plasma membrane at synapses or to stabilize AMPARs at the synapses, making them resistant to endocytosis. In contrast, however, a similar construct can be detected at the synaptic surface in hippocampal cultured neurons (Braithwaite & Malenka 2001). In response to AMPA or NMDA application, this construct exhibits enhanced internalization, a result consistent with the suggestion that the NSF-GluR2 interaction plays a role in the stabilization of surface AMPARs. Clearly more work needs to be done on the role of NSF in AMPAR trafficking as well as the role of α- and β-SNAPs that are also closely associated with GluR2 (Osten et al. 1998).

PDZ PROTEINS-GluR2 INTERACTIONS The roles of GRIP, ABP/GRIP2, and PICK1 in AMPAR endocytosis and trafficking have also been addressed using techniques similar to those described above. Loading CA1 pyramidal cells with a peptide that disrupts the GluR2/3-GRIP/ABP interaction caused an increase in synaptic currents in a subset of cells and prevented the generation of LTD (Daw et al. 2000). These results are consistent with the hypothesis that the binding of GRIP/ABP to GluR2/3 stabilizes AMPARs in an intracellular pool and prevents their insertion into the synaptic plasma membrane. Consistent with this idea, following the generation of LTD (which should increase the pool of intracellular AMPARs due to their endocytosis), the peptide increased synaptic strength in a much higher proportion of cells (Daw et al. 2000). Experiments in which a mutant form of GluR2 that does not bind to GRIP/ABP was expressed in hippocampal neurons have also been performed. In one study (Osten et al. 2000), such a mutant GluR2 was targeted appropriately to the synaptic plasma membrane, but its accumulation at synapses was significantly reduced when compared to wild-type GluR2. These findings were consistent with the idea that the association of GluR2 with GRIP and/or ABP is essential for maintaining AMPARs at synapses, perhaps by limiting their endocytosis. Similar results were obtained when a mutant GluR2 that could not bind GRIP/ABP or PICK1 was expressed in hippocampal slice cultures, in that there was no detectable surface expression at synapses of the mutant GluR2, whereas wild-type GluR2 could be readily detected (Shi et al. 2001). Another study, however, found no effect of mutating the GRIP/ABP and PICK1 binding site on GluR2 on its targeting to synapses in hippocampal cultures, although this mutant did exhibit a smaller degree of regulated endocytosis (Braithwaite & Malenka 2001), perhaps because it was not retained in an intracellular pool following its internalization. Taken together, these experiments that attempt to define the role of the interactions of PDZ proteins with GluR2/3 are confusing and do not allow definitive conclusions to be reached. It is conceivable that GRIP/ABP subserves several functions in the delivery, stabilization, and endocytosis of synaptic AMPARs. For example, GRIP/ABP appears to be found both at the membrane and in the cytosol of neurons and thus might stabilize AMPARs in both locations.

Although GRIP/ABP and PICK1 bind to the same sites on GluR2 and GluR3, their interactions, at least with GluR2, can be regulated independently. Phosphorylation of serine 880 in the PDZ-binding domain of GluR2 greatly decreases the affinity of GluR2 for GRIP/ABP but not for PICK1 (Chung et al. 2000, Matsuda et al. 1999). This phosphorylation is enhanced by activation of PKC leading to a decreased association of GRIP with GluR2 in vitro, in HEK293 cells, and in Purkinje cells (Chung et al. 2000, Matsuda et al. 1999). In cultured neurons, activation of PKC with phorbol esters also causes a redistribution of PICK1 and PKCα to synaptic sites (Chung et al. 2000, Perez et al. 2001) and a decrease in the level of GluR2 in the synaptic plasma membrane (Perez et al. 2001), presumably due to an increase in its internalization (Chung et al. 2000, Matsuda et al. 2000). Thus the phosphorylation state of serine 880 on GluR2, via its differential effects on the binding of GluR2 to GRIP/ABP and PICK1, may be important for

influencing the subcellular localization of AMPARs and may play a role in some forms of LTD.

Work on the role of GluR2/3-PDZ protein interactions in hippocampal and cerebellar LTD suggests that the functions of GRIP/ABP and PICK1 may differ among cell types. As mentioned above, a peptide that inhibits GRIP/ABP and PICK1 interaction with GluR2 impairs LTD in CA1 pyramidal cells and causes an increase in synaptic strength in most cells following the prior induction of LTD (Daw et al. 2000). In contrast, a peptide designed to inhibit specifically only the PICK1-GluR2/3 interaction has no effect on either LTD or basal synaptic strength. The investigators also found that the increase in synaptic currents due to the peptide was prevented by PKC inhibitors, which suggests that PKC activity is required for the recycling of internalized AMPARs back to the plasma membrane (Daw et al. 2000). Taken together these results are consistent with the hypothesis that disrupting the binding of GRIP/ABP to GluR2 impairs the retention of AMPARs in an intracellular pool and allows them to return to the synaptic plasma membrane, thereby preventing the maintenance of LTD. Furthermore, the return of AMPARs to the plasma membrane requires PKC activity.

In contrast to NMDAR-dependent LTD in the hippocampus, cerebellar LTD studied in cultured Purkinje cells was impaired by several different manipulations aimed at specifically disrupting the PICK1-GluR2/3 interaction: loading cells with peptides designed to disrupt this interaction or antibodies directed against the PDZ domain of PICK1 as well as expression of mutant PICK1-GST fusion proteins (Xia et al. 2000). Furthermore, application of a phorbol ester, which elicits LTD in cultured Purkinje cells, causes phosphorylation of serine 880 on GluR2 and internalization of GluR2-containing surface AMPARs (Matsuda et al. 2000). Although these results do not rule out a role of GRIP/APB in cerebellar LTD, they are most consistent with a role for PICK1 binding to GluR2/3 in priming AMPARs for endocytosis or stabilizing endocytosed receptors in intracellular pools. Consistent with the first of these alternatives, overexpression of PICK1 in cultured hippocampal neurons was found to reduce the level of surface expression of GluR2 (Perez et al. 2001). Indeed, in contrast to the conclusions of Daw et al. (2000), a similar role for the PICK1-GluR2/3 interaction in hippocampal LTD has recently been suggested based on the findings that induction of LTD in hippocampal slices increases phosphorylation of serine 880 in GluR2 and a peptide that specifically inhibits the PICK1-GluR2/3 interaction enhances basal synaptic strength and impairs LTD in CA1 pyramidal cells (Kim et al. 2001).

Clearly, we currently do not have a thorough understanding of the functions of the cytosolic proteins that interact with AMPAR subunits; however, it is equally clear that these interactions do play important roles in controlling AMPAR content at synapses and thereby synaptic strength. Some of the confusion on their role in LTD may be because there are cell-type specific differences in the role these proteins play. In addition, each protein may play multiple roles in the delivery, stabilization, and/or removal of synaptic AMPARs, and thus, the consequences of perturbing the interactions of an individual protein with AMPARs may vary

depending on the specific method used to impair protein function and the assays used to monitor the consequences of this manipulation. For example, optical and electrophysiological methods may have different sensitivities for the detection of synaptic AMPARs. It is also possible that AMPAR trafficking in different experimental preparations (e.g., dissociated cultured neurons versus brain slices) may differ. Perhaps regulatory mechanisms are differentially expressed as a consequence of different levels of signals (synaptic or growth factors, etc.) impinging on neurons in these different preparations.

AMPA RECEPTOR DELIVERY TO SYNAPSES AND LTP

Subcellular Steady-State Distribution of AMPA Receptors

A number of studies over the past few years have tested the notion that silent synapses lack AMPARs and that AMPARs are rapidly delivered to synapses during LTP. An important requirement for this model is that there be a pool of nonsynaptic AMPARs near synapses available for delivery. Several studies have used microscopic techniques to examine distribution of glutamate receptors at and near synapses in rat brains (Baude et al. 1995, Kharazia et al. 1996, Martin et al. 1993, Molnar et al. 1993, Nusser et al. 1998, Petralia et al. 1999, Petralia & Wenthold 1992, Takumi et al. 1999). While the concentration of AMPARs is normally higher at synapses, these studies generally find ample amounts of nonsynaptic AMPARs on both surfaces and intracellular regions of dendrites. Indeed, given the much larger space occupied by nonsynaptic regions, nonsynaptic AMPARs appear to outnumber synaptic AMPARs by quite a large margin (Shi et al. 1999). The distance between these nonsynaptic receptors and synaptic regions is a few microns, a distance that could be traversed in seconds by membrane-trafficking processes. Importantly, recent studies using postembedding immunogold techniques (Nusser et al. 1998, Petralia et al. 1999, Takumi et al. 1999) found that a sizable fraction of synapses in CA1 hippocampus lacks or has very few AMPARs, while most synapses have NMDARs. The fraction of synapses lacking AMPARs is greater earlier in development, consistent with the electrophysiological observations that silent synapses are more prevalent at these ages (Durand et al. 1996, Isaac et al. 1997, Liao & Malinow 1996, Rumpel et al. 1998, Wu et al. 1996). While some studies in dissociated cultured neurons support these views (Gomperts et al. 2000, Liao et al. 1999), others do not (Renger et al. 2001), possibly due to different culture conditions.

Optical Detection of Recombinant AMPA Receptor Trafficking During LTP

To monitor AMPAR trafficking in living tissue, one study generated and acutely expressed GFP-tagged GluR1 receptors in organotypic hippocampal slices (Shi et al. 1999). While slices of tissue provide a more challenging experimental preparation to examine receptor trafficking, this tissue was used, rather than dissociated

neurons, since there had been little success in generating LTP using standard electrophysiological protocols in dissociated neurons. These recombinant GluR1-GFP receptors are functional, and their cellular distribution can be monitored with two-photon microscopy. Upon expression, these receptors distribute diffusely throughout the dendritic tree. Interestingly, they remain in the dendritic shaft regions, with little encroachment into dendritic spines, which are the sites of excitatory contacts. This restriction from synapses is in contrast with what is found in dissociated cultured neurons in which expression of recombinant GluR1 concentrates at synapses (Lissin et al. 1998, Shi et al. 1999). In slices, little movement of GluR1-GFP was detected in the absence of stimulation. However, high-frequency synaptic activation generated LTP-induced movement of GFP-tagged receptors to the surface of dendritic shafts as well as to dendritic spines. These movements of GFP-tagged receptors were detected over the course of about 15–30 min and were prevented by blockade of NMDARs. The tagged receptors remained in at least some spines for at least 50 min. This study concluded that GuR1-containing receptors are maintained in reserve at the dendritic shaft and can be delivered to synapses during LTP.

A number of studies have made findings that strengthen these conclusions. Adult knockout mice lacking GluR1 cannot generate LTP, indicating that this subunit plays a critical role (Zamanillo et al. 1999). In a follow-up study, GluR1-GFP was genetically inserted into these GluR1 knockout mice and GFP fluorescence was detected in dendritic spines (Mack et al. 2001). This distribution differs from what is observed when GluR1-GFP is acutely expressed in hippocampal slices before LTP but resembles the distribution after LTP. These observations are consistent with the view that an LTP-like process drives the genetically expressed GluR1-GFP into synapses when the animals are alive. This study also found that LTP was rescued by expression of only ~10% of the normal amount of GluR1. This further supports the view that normally there is an overabundance of GluR1 available for generating LTP.

Electrophysiological Tagging to Monitor Synaptic Delivery of Recombinant AMPA Receptors

While optical studies provide important information regarding receptor distribution, the location of a receptor (even with electron microscopic resolution) cannot unambiguously reveal its contribution to synaptic transmission. One approach to address this issue used electrophysiologically tagged recombinant AMPARs. Such receptors differed in their rectification from endogenous receptors. Rectification is an intrinsic biophysical property of a receptor that can be detected as the ratio of the response observed at −60 mV to that at +40 mV. Most endogenous AMPARs contain the GluR2 subunit and can pass current equally well in both inward and outward directions. In contrast, AMPARs lacking GluR2 (or containing GluR2 that is genetically modified) exhibit profound inward rectification such that they can pass minimal current in the outward direction when the cell is depolarized to +40 mV. Thus, incorporation of recombinant AMPARs into synapses and their contribution

to real synaptic transmission can be monitored functionally. With this assay for AMPAR delivery, it has been possible to show that LTP and overexpression of active CaMKII induce delivery of GluR1-containing receptors into synapses (Hayashi et al. 2000). An interaction between GluR1 and a PDZ-domain protein is necessary for LTP or CaMKII to drive synaptic delivery of GluR1, as point mutations in the PDZ-binding region of GluR1 prevent its synaptic delivery. Neither the identity of the GluR1-interacting PDZ-domain protein(s) responsible for LTP, nor the subcellular site where this (these) critical interaction(s) occurs is known.

One important finding observed in mice lacking GluR1 is that LTP is neither absent in all brain regions [e.g., LTP in dentate gyrus is present (Zamanillo et al. 1999)] nor in all ages [e.g., LTP in CA1 is present in juvenile animals (Mack et al. 2001)]. This suggests that AMPAR subunits other than GluR1 may play critical roles in activity-dependent synaptic plasticity. Indeed, the CA1 hippocampal region in immature animals, as well as the dentate gyrus in older animals, contain GluR4, a subunit with considerable homology to GluR1. Studies using electrophysiological assays to monitor the synaptic delivery of recombinant GluR4 indicate that this subunit mediates activity-dependent AMPAR delivery in immature hippocampus (Zhu et al. 2000). Interestingly, this delivery of recombinant GluR4 to synapses required NMDAR activity (i.e., delivery was blocked by APV) but not CaMKII activity.

As expression of GluR4 in hippocampus decreases to nearly undetectable levels by postnatal day 10, the LTP observed in CA1 hippocampus of juvenile (~postnatal day 28) animals that lack GluR1 may be mediated by other AMPAR subunits. It is possible that this role is played by GluR2L, the alternative splice form of GluR2 with a cytoplasmic tail that resembles GluR1 and GluR4 (Hollmann & Heinemann 1994, Wisden & Seeburg 1993) (Figure 1). Indeed, recent results indicate activity-driven synaptic delivery of recombinant GluR2L (J. Zhu, J. Esteban, R. Malinow, unpublished observations). Results from experiments that used peptides loaded directly into postsynaptic cells suggest that interactions between GluR2 and PDZ-domain proteins may also be necessary to execute some other forms of synaptic potentiation in hippocampus (Daw et al. 2000) as well as in spinal cord (Li et al. 1999).

Synaptic Delivery of Endogenous Receptors

While the studies described above monitored synaptic delivery of recombinant AMPARs, other studies have tested if such a process occurs for endogenous receptors. In one study (Heynen et al. 2000) the amount of GluR1 and GluR2 in synaptoneurosomes (a fraction of brain extracts enriched for synaptic membranes) was increased following generation of LTP in the hippocampus in vivo. This increase was blocked by APV, and no change was detected in the level of NR1, the major NMDAR subunit. This study is consistent with a previous in vivo study that found increased AMPAR binding in hippocampal regions following LTP induction (Maren et al. 1993). At the mossy fiber synapse onto granule cells in cerebellum, tetanic stimulation induces replacement of calcium-permeable AMPARs with calcium-impermeable AMPARs (Liu & Cull-Candy 2000). This protocol does not

enhance transmission but is likely to represent a protective mechanism. One study in cultured hippocampal slices detected an increase of AMPARs in synaptic membranes following pharmacological induction of LTP (Broutman & Baudry 2001). This was blocked by brefeldin A, implicating a process involving protein trafficking between ER/Golgi and surface membrane. A number of in vitro studies on dissociated cultured neurons have also examined delivery of endogenous AMPARs to synapses. In one study, an LTP of miniature excitatory postsynaptic currents could be triggered following brief pharmacological stimulation of NMDA receptors (Lu et al. 2001). This LTP was accompanied by a rapid insertion of endogenous AMPARs that could be blocked by interfering with SNARE-dependent exocytosis. Similarly, Liao et al. show in dissociated cultured neurons chronically kept in APV that washing out APV allows spontaneous activity to stimulate NMDARs, and this leads to the rapid delivery of native AMPARs into synapses previously without AMPARs (e.g., silent synapses) (Liao et al. 2001). Such a stimulus can also recruit CaMKII to synapses (Liao et al. 2001, Shen & Meyer 1999), where it may bind to NMDA receptors (Bayer et al. 2001, Leonard et al. 1999, Strack & Colbran 1998, Strack et al. 2000). The relation between CaMKII translocation to synapses and AMPAR delivery to synapses has not been established, although an intriguing model proposes a necessary link between these processes (Lisman & Zhabotinsky 2001). An actin-dependent process underlying the delivery of AMPARs during LTP is supported by experiments showing that agents that perturb actin function block LTP (Kim & Lisman 1999, Krucker et al. 2000).

Role of AMPA Receptor Phosphorylation in Synaptic Delivery

There has been considerable evidence indicating that protein kinases play critical roles in the generation of LTP (Bliss & Collingridge 1993, Madison et al. 1991, Malenka & Nicoll 1999). Some kinases [e.g., CaMKII; (Lisman et al. 1997)] are thought to mediate directly the signals leading to LTP, while others [e.g., PKA; (Blitzer et al. 1995)] may "gate" (i.e., modulate) its generation. The targets of these kinases responsible for mediating or gating LTP have been the source of considerable investigation. During LTP the CaMKII-phosphorylation site on GluR1, Ser831, is phosphorylated (Barria et al. 1997a, Barria et al. 1997b, Mammen et al. 1997). Such phosphorylation can increase conductance through GluR1 receptors (Derkach et al. 1999), and AMPARS show increased conductance during LTP (Benke et al. 1998). Thus, it was of considerable interest to determine if phosphorylation of Ser831 is required for synaptic delivery of GluR1-containing receptors. However, mutations on GluR1-Ser831 that prevent its phosphorylation by CaMKII do not prevent delivery of the receptor to synapses by active CaMKII (Hayashi et al. 2000) or by LTP (S-H. Shi & R. Malinow, unpublished observations). Thus, CaMKII must be acting on a different target to effect synaptic delivery of GluR1. Recent studies indicate that CaMKII can control a synaptic rasGAP (Chen et al. 1998, Kim et al. 1998) and thereby increase ras activity. Ras activity appears to be necessary to generate LTP and is the downstream effector of CaMKII that

drives synaptic delivery of AMPARs (J. Zhu, L. Van Aelst, & R. Malinow unpublished observations). This conforms with results indicating a critical role for MAP kinase, a downstream effector for ras, in LTP (English & Sweatt 1996, 1997).

Interestingly, mutations at Ser845, the PKA phosphorylation site of GluR1 (Roche et al. 1996), do prevent delivery of GluR1 to synapses by active CaMKII or LTP (Shi & Malinow 2001). Phosphorylation at this site of GluR1 also accompanies surface reinsertion of receptors (Ehlers 2000) and LTP induction after prior LTD (Lee et al. 2000). Phosphorylation at this site by exogenous application of drugs that raise cAMP does not induce delivery of recombinant GluR1 (Shi & Malinow 2001). Thus, PKA phosphorylation of GluR1 is necessary, but not sufficient, for its synaptic delivery, i.e., phosphorylation of Ser845 acts as a gate. Of note, the PKA-scaffolding molecule, AKAP (a kinase anchoring protein), binds to SAP97 and thereby effectively brings PKA to GluR1 (Colledge et al. 2000). Thus, it is possible that the PDZ mutation on GluR1 blocks its synaptic delivery, at least in part, because it prevents PKA phosphorylation at Ser845. Of note, SAP97 associates with GluR1 primarily in intracellular sites (Sans et al. 2001), consistent with its playing a role in making GluR1 competent for synaptic delivery (Figure 2).

Recent studies indicate that activity-driven phosphorylation of GluR4 by PKA is necessary and sufficient for delivery of these recombinant AMPARs to synapses during early development (Esteban & Malinow 2001). Such phosphorylation relieves a retention interaction that, in the absence of synaptic activity, maintains GluR4-containing receptors away from the synapse. Thus, a mechanism (PKA phosphorylation of AMPARs) that mediates plasticity early in development (with GluR4) becomes a gate for plasticity (with GluR1) later in development. Increasing requirements over development may be one way that plasticity becomes more specific and also recalcitrant with age.

GENERAL TRAFFICKING MECHANISMS

A key question has been if plasticity acts by directly modulating a process that is responsible for turning over receptors at synapses (e.g., increasing rate of delivery or decreasing rate of removal) or if there are distinct processes responsible for plasticity and receptor turnover. One recent study (Shi et al. 2001) examined this question and argues for distinct AMPARs responsible for LTP and receptor turnover (Figure 2). AMPARs composed of GluR1 and GluR2 (or any receptor with a long cytoplasmic tail along with GluR2) participate in regulated delivery. In the absence of electrical activity, these receptors are restricted from accessing synapses. LTP (for GluR1-containing receptors) or spontaneous activity (for GluR4-containing receptors) drives these receptors (along with associated scaffolding) into synapses. The long cytoplasmic tails, and not the short cytoplasmic tails, of GluR1/GluR2 heteromers are critical for this activity-dependent synaptic delivery. Receptors composed of GluR2 and GluR3 continuously replace

synaptic GluR2/GluR3 receptors in a manner that maintains transmission constant. How can this model explain long-term changes in synaptic receptor number following plasticity that enhances transmission? At some point after their synaptic delivery, receptors containing GluR1 or GluR4 become replaceable by GluR2/GluR3 receptors. The scaffolding associated with GluR1 or GluR4 [called "slot" complexes (Shi et al. 2001)] must somehow control this. One study provides evidence for replacement of synaptic GluR4-containing receptors by GluR2/GluR3 receptors (Zhu et al. 2000). This occurs over the course of days after the activity-driven delivery of GluR4-containing receptors.

A recent independent study in cultured dissociated neurons used expression of recombinant AMPAR subunits that contained a thrombin cleavable epitope tag in their extracellular domains to monitor surface delivery of receptors. This clever study supports this view that plasticity and constitutive receptor turnover are controlled by different AMPAR species (Passafaro et al. 2001). Furthermore, this study provided important spatial and temporal information regarding synaptic delivery of these different AMPAR complexes. Receptors containing GluR2/GluR3 are continuously delivered to the spine surface with a time constant of $\sim$15 min and unaffected by agents that perturb plasticity. On the other hand, the rate of surface delivery of GluR1-containing receptors is greatly enhanced by stimulation (in this dissociated cultured system, by either glycine, NMDA, or insulin). Their surface appearance takes place in dendritic (extrasynaptic) regions, with subsequent movement into spine regions.

The movement of AMPARs from cytosol to dendritic surface and subsequently to synaptic regions, described above for receptors containing GluR1, had previously been proposed for GluR4-containing receptors by a study examining the protein stargazin (STG) (Chen et al. 2000). Animals without the transmembrane protein STG lack AMPAR responses in granule cells of the cerebellum (Hashimoto et al. 1999). AMPAR responses to both synaptic and exogenously applied transmitter are absent, although intracellular AMPARs levels are normal. Acute expression of STG rescues responses to synaptic and exogenous transmitter application. Interestingly, expression of STG with a mutation at its cytoplamic tail rescues responses to exogenous application, but not synaptic responses. This suggests that interactions controlled by STG cytoplasmic tail are important for movement of receptors from extrasynaptic (surface) regions to synaptic regions. The relation between STG and regulated or continuous receptor delivery described above has not been established.

In the context of the model that proposes two AMPAR species (Figure 2), one participating in activity-dependent delivery, the other in continuous replacement, it is not clear what receptor types are removed by LTD. Both GluR1/GluR2 and GluR2/GluR3 contain GluR2 carboxyl tails that appear to be critical for LTD (see above). The occlusion of continuous receptor cycling following LTD (Lüthi et al. 1999) suggests that LTD acts by removal of GluR2/GluR3 receptors. However, Shi (2000) finds that overexpression of GluR3 carboxyl tails depresses transmission (presumably by interfering with continuous GluR2/GluR3

delivery) but does not prevent LTD. Overexpression of GluR1 carboxyl tails neither depresses transmission nor blocks LTD. This seems to argue that LTD removes GluR1/GluR2 heteromers by interactions requiring GluR2. Agents that perturb GluR2 interactions may independently block continuous receptor cycling (of GluR2/GluR3) and activity-dependent removal of GluR1/GluR2, receptors that normally do not participate in cycling.

OPEN QUESTIONS

Lynch & Baudry (1984) proposed almost two decades ago that LTP is due to an increase in the number of synaptic glutamate receptors. However, the idea did not gain universal favor and a vigorous exchange over the ensuing decades debated the pre- and postsynaptic contributions to the expression of LTP. Thus, the general acceptance of postsynaptic silent synapses and AMPAR trafficking as playing important roles in synaptic plasticity represent significant advances in the field. They provide a clear conceptual framework that should continue to facilitate studies aimed at determining which molecules play critical roles in LTP and LTD and exactly what role they play. Historically, many molecules have been suggested to be necessary for LTP since their genetic removal or pharmacological inhibition impairs LTP (Sanes & Lichtman 1999). However, to state that a molecule or a process is necessary for LTP (or LTD) is no longer particularly meaningful without specifying exactly what role it plays as a direct mediator of these synaptic phenomena or as a modulator of the molecular events known to be necessary for their triggering or expression.

From our vantage point, a number of significant questions remain open. 1. What are all of the critical targets downstream of NMDAR activation that are responsible for LTP and LTD? 2. How do these downstream targets communicate with the machinery that controls AMPAR trafficking? 3. Does AMPAR trafficking play the same important role in NMDAR-independent forms of LTP and LTD, of which there are several? 4. Which proteins, known to associate with AMPARs, control regulated and continuous trafficking? 5. What other proteins interact with and control AMPAR trafficking? 6. How is a transient delivery/removal of AMPARs transformed to a persistent increase/decrease in the number of synaptic receptors? 7. How is this persistence maintained in the face of protein turnover? 8. What are the differences in AMPAR trafficking between different cell types? Different brain regions? 9. What different circuit functions do different trafficking properties subserve? 10. How do environmental and behavioral stimuli influence AMPAR trafficking? 11. How does AMPAR trafficking impact behavior? 12. Do dysfunctions in AMPAR trafficking contribute to neurological or neuropsychiatric disorders? Given the large number of creative and hardworking scientists actively working on many of these questions, it is our hope and belief that many of these will be answered before we contribute another review on AMPAR trafficking and synaptic plasticity.

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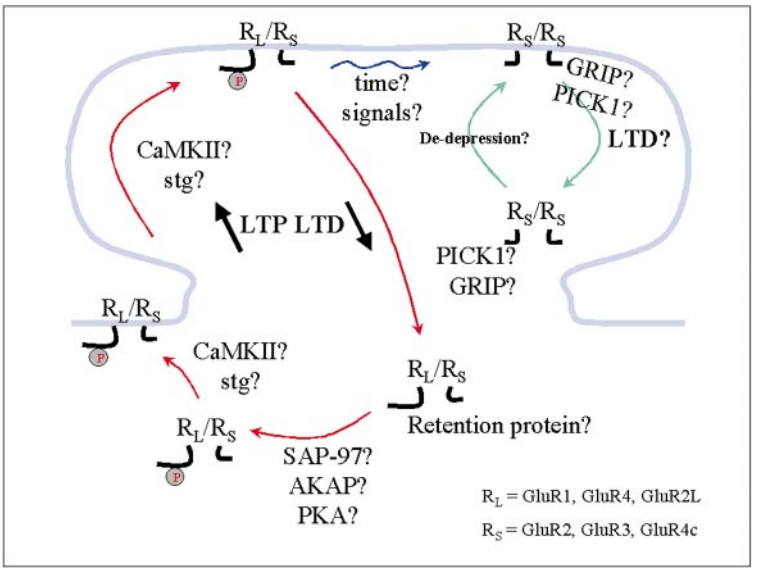

Figure 2 Different steps in trafficking of AMPARs. Note participation in regulated (*red*) or continuous (*green*) trafficking depends on AMPAR composition. Potential molecules or processes controlling specific steps are shown. Phosophorylation at Ser845 by PKA is indicated. See text for details.