Published in final edited form as:
Neurosci Res. 2003 March ; 45(3): 243–254.

Proteins interactions implicated in AMPA receptor trafficking: a clear destination and an improving route map||

Jeremy M. Henley*
Department of Anatomy, MRC Centre for Synaptic Plasticity, School of Medical Sciences, University of Bristol, Bristol BS8 1TD, UK

Abstract

The mechanisms that regulate α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor (AMPAR), synthesis, transport, targeting and surface expression are of fundamental importance to understand the molecular basis of fast excitatory neurotransmission and synaptic plasticity in the mammalian CNS. An area of intense current interest is how AMPARs are directed to the correct locations in the neuron as and when required. This is a multi-layered problem, which involves complex spatiotemporal coordination of multiple protein interactions. Considerable progress has been achieved in identifying a number of proteins that bind directly to AMPAR subunits and the functional consequences of blocking some of these interactions have been determined. This review highlights recent developments in the field.

Keywords

AMPA receptors; Synaptic plasticity; Synaptic organisation; Protein trafficking; NSF; PDZ proteins; Protein interactions; GFP; Endocytosis; Exocytosis

1. General trafficking processes

General protein transport requires bidirectional vesicle trafficking between cell compartments that are dependent on a range of trafficking molecules (for reviews, see Teasdale and Jackson, 1996; Klumperman, 2000; Mostov et al., 2000). The final destination of proteins is determined by intrinsic targeting motifs encoded in their amino acid sequences and/or by binding other proteins that contain such motifs.

Most vesicle trafficking requires the ordered coating of a donor membrane, budding, fission to form transport vesicles, transport by passive or active vectoral delivery along microtubules (Antonny and Schekman, 2001) and final fusion with the target membrane. The fidelity of these processes is maintained by specific v- and t-SNAREs (Pfeffer, 1996) and by spatial and temporal regulation by vesicle and target membrane lipid interactions via Rab-GTPases, tethering proteins and phosphoinositides (Sato et al., 2001).

As a rule, only fully processed and correctly folded nascent proteins are exported from the ER to the Golgi. Retention in the ER acts as a mechanism for assembling oligomeric transmembrane proteins, probably via determinants within the protein subunits (Romano et al., 1996; Meddows et al., 2001) and once assembled dominant ER retention signals are

||Manuscript accompanied a presentation to the Japan–UK Workshop ‘From Molecules to Memory’, Nara, 29–30 August, 2002.

*Tel.: +44-117-928-8077; fax: +44-117-929-1687. j.m.henley@bris.ac.uk (J.M. Henley)..

Europe PMC Funders Author Manuscripts

sterically masked or overridden (Standley and Baudry, 2000). In addition, association with regulatory subunits in a multimeric receptor complex may also facilitate exit from the ER, for example with cytosolic or transmembrane accessory proteins (Zerangue et al., 1999; Bichet et al., 2000). However, many details of general protein transport remain to be determined. For example, the precise roles of the large family of vesicle coating proteins and small GTPases have not yet been well characterised. It is also unclear how cargo proteins are accumulated in relevant vesicles.

2. Trafficking in neurones

Neurons are highly compartmentalised cells and their proper function relies on the organised localisation of specialised membrane proteins, such as receptors. There are several pathways by which transmembrane proteins can be sorted and trafficked in neurons following exit from the Golgi. For example, AMPARs could be transported inside the neuron by vesicles to the vicinity of their target site and then excoytosed to the membrane (Passafaro et al., 2001) and/or they can be exocytosed directly to the membrane and then move by lateral diffusion and anchored at their target destination (Borgdorff and Choquet, 2002). Another, possibly more specialised alternative, is the transport of mRNAs within the cell and local synthesis of proteins close to their destination (Steward and Schuman, 2001).

Bi-directional vesicle transport has been reported in both axons and dendrites (Foletti et al., 1999). Vesicles are driven by the molecular motor proteins–kinesin and dynein–responsible for movements towards opposite ends of the microtubules. Thus, polarised vesicle trafficking is likely to require specific interactions between domain-specific motor and vesicle cargo. For example, both NMDA receptors (Setou et al., 2000; Washbourne et al., 2002) and AMPARs (Setou et al., 2002) undergo cargo-selective polarised vesicle transport via dendrite specific kinesin motors. Furthermore, targeting will require either address motifs in the cargo proteins or some kind of a recognition domain allowing passing vesicles to be captured at relevant sites.

3. Complexity of the problem

Neurons may possess many thousands of synapses each of which can contain multiple receptors with potentially differing subunit compositions. Furthermore, different synapses contain different receptor complements and in most cases, these can be strongly developmentally and activity regulated. Therefore, even for individual oligomeric receptor types, mechanisms must exist to differentially target receptor proteins of different subunit compositions to specific synapses. The differential regulation of exocytosis, receptor density, spatial segregation, anchoring and endocytosis of receptors at glutamatergic synapses is an area of concerted investigation. A schematic showing questions to be addressed for AMPARs is shown in Fig. 1.

4. AMPAR trafficking is highly regulated

AMPARs are multimeric assemblies of the subunits GluR1–4 (Hollmann et al., 1994). Each subunit comprises ≈ 900 amino acids and has a molecular weight of ≈ 105 kDa. The N-terminus is extracellular, there are three membrane spanning and one re-entrant loop domains and the C-terminus is intracellular (Bennett and Dingledine, 1995; Wo and Oswald, 1995). Some 50–70% of AMPARs are intracellular (Hall et al., 1997; Archibald et al., 1998), with a significant proportion localised within dendrites (Baude et al., 1994; Richmond et al., 1996).

Until a few years ago it was generally accepted that AMPARs within the postsynaptic membrane were relatively static, at least under basal conditions, with a constitutive turnover

of surface expressed receptors in the order of hours to days (Huh and Wenthold, 1997; Mammen et al., 1997; Archibald et al., 1998). However, it has now been shown that GluR2-containing AMPARs undergo rapid *N*-ethylmaleimide-sensitive fusion protein (NSF)-dependent cycles of internalisation and reinsertion in to the postsynaptic membrane with a half-life in the order of a few minutes (Henley et al., 1997; Nishimune et al., 1998; Osten et al., 1998; Song et al., 1998; Luscher et al., 1999; Luthi et al., 1999; Noel et al., 1999; Kim and Lisman, 2001) and these findings have had major implications for understanding the cellular processes underlying synaptic plasticity (for reviews, see (Barinaga, 1999; Morales and Goda, 1999; Malinow et al., 2000). The principle of rapid NSF-dependent recycling has also recently been extended to G-protein coupled receptors via β-arrestins (Miller and Lefkowitz, 2001) and $GABA_A$ receptors via GABARAP (Kittler et al., 2001), suggesting that this may be an important general regulatory synaptic mechanism.

There is an extensive body of literature on the dynamic regulation of AMPARs at synapses. Electrophysiological studies have shown that excitatory synapses can exhibit NMDAR-mediated responses in the absence of functional AMPARs and are therefore postsynaptically ‘silent’ at resting membrane potentials (Isaac et al., 1995; Liao et al., 1995). The ‘unsilencing’ of these synapses via the rapid acquisition of AMPAR responses is likely to be important in NMDAR-dependent synaptic plasticity and neuronal development (Durand et al., 1996). Consistent with this hypothesis, recent immunocytochemical results have confirmed the existence of silent synapses that contain NMDARs but no AMPARs (Liao et al., 1999; Pickard et al., 2001) and have demonstrated that AMPARs can be recruited to the synapse by either spontaneous or stimulated NMDAR activation (Fitzjohn et al., 2001; Liao et al., 2001; Lu et al., 2001; Pickard et al., 2001).

Other recent advances towards better understanding of the molecular mechanisms of synaptic plasticity have come from viral transfection of neurons with GFP-AMPAR subunits. Tetanic synaptic stimulation induced a rapid NMDAR-dependent delivery of GFP-GluR1 into dendritic spines in hippocampal slice preparations (Shi et al., 1999). Native AMPARs in hippocampal neurons have been shown to be assembled from combinations of mainly GluR1/GluR2 or of GluR3/GluR2 (Craig et al., 1993; Wenthold et al., 1996). While these studies have provided valuable information, it is difficult to distinguish between surface expressed and intradendritic localization of the GFP fluorescence. In an elegant series of experiments, Malinow et al. obtained data using GFP-labeled subunits in combination with electrophysiological tagging (Hayashi et al., 2000), where the channel rectification properties of recombinant AMPARs comprising specific subunits are altered by point mutation of s residues within the channel pore, e.g. GluR2(R586Q)-GFP, it has been proposed that there are differential targeting mechanisms for AMPARs comprising either GluR1/GluR2 or GluR2/GluR3 subunit assemblies.

In organotypic slice cultures, it has been reported that GluR1/GluR2 receptors are added to synapses during plasticity, a process that requires interactions between GluR1 and group I PDZ domain proteins (Hayashi et al., 2000). In this model, CaMKII and LTP drive synaptic expression of GluR1-containing AMPARs. In contrast, GluR2/GluR3 receptors replace existing synaptic receptors in a constitutive manner dependent on interactions by GluR2 with NSF and group II PDZ domain proteins (Shi et al., 2001). Another study using a thrombin cleavage assay in dispersed cell cultures to measure the rate and location of surface expression of AMPAR subunits reported broadly consistent results (Passafaro et al., 2001). They showed that surface insertion of the GluR1 subunit under basal conditions was relatively slow, but was stimulated by NMDAR activation. By contrast, GluR2 exocytosis was rapid and constitutive. GluR1 was inserted initially at extrasynaptic sites and GluR2 was inserted more directly at synapses.

Recently, the small GTPases Ras and Rap have been reported to be involved in the control of AMPAR trafficking and in the postsynaptic signaling underlying synaptic plasticity. Ras mediates activity-evoked increases in GluR1/GluR4 containing AMPAR surface expression at synapses via a pathway that requires p42/44 MAPK activation. In contrast, Rap mediates NMDAR-dependent removal of synaptic GluR2/3-containing AMPARs via a pathway that involves p38 MAPK. The regulation of Ras and Rap, which act as molecular switches, may in turn control AMPAR surface expression at synapses and thereby regulate potentiation and depression of synaptic activity (Zhu et al., 2002).

Overall, these data have been used to propose that the combination of regulated addition and continuous replacement of synaptic receptors may be the mechanism for stabilising long-term changes in synaptic efficacy and could be a general model for how surface AMPAR number is established and maintained. This model relies, to some extent, on the idea that AMPARs comprise mainly GluR1 and GluR4 heteromers *or* GluR2 and GluR3 heteromers. This arrangement would allow independent regulation of the two types of receptor assemblies. However, this is not easily reconciled with the observation that the surface expression of native GluR1 and GluR2 subunits are increased following LTP (Lu et al., 2001). One possible reason for this apparent discrepancy could be the use of different experimental systems. Organotypic cultures are likely to preserve better and possibly different synaptic connections to those formed by dispersed neurons grown in culture.

Several studies have focused on identifying the molecular basis of AMPAR recycling at the postsynaptic membrane. The internalisation of AMPARs is thought to be important in the expression of long-term depression (LTD) triggered by NMDA receptor activation and is mediated by the formation of clathrin-coated pits (Carroll et al., 1999b). Disruption of clathrin-mediated endocytosis, for example by biochemical inhibition of pit formation or overexpression of a dominant negative form of dynamin, effectively blocks the internalisation of AMPAR in a GluR2-dependent manner (Ehlers, 2000; Man et al., 2000a). (For more detailed reviews of AMPAR endo- and exocytosis, see Turrigiano, 2000; Man et al., 2000b; Carroll et al., 2001.)

AMPAR turnover appears to be directly regulated by synaptic activity with removal from the synaptic membrane in a ligand-dependent manner. Increasing synaptic activity using picrotoxin, a GABA receptor antagonist, has been shown to cause a decrease in number of surface expressed AMPAR and size of AMPAR clusters in cultured neurons (Lissin et al., 1999). Conversely, decreasing synaptic activity using tetrodotoxin or AMPAR antagonists causes an increase in synaptic AMPAR (Ehlers, 2000). Exogenous application of glutamate or AMPA causes internalisation and redistribution of AMPARs, but probably only in a subset of synapses as mEPSCs decrease in frequency but not size (Carroll et al., 1999a; Lissin et al., 1999; Beattie et al., 2000). This can occur in both an NMDAR dependent and independent fashion, with differential implications for the fate of internalised receptors. AMPARs internalised in response to NMDAR activation are rapidly recycled and reinserted into the synapic membrane, in contrast, treatment with AMPA causes the internalised receptors to be targeted to protein degradation pathways (Ehlers, 2000; Lin et al., 2000).

5. Intracellular C-terminal domains of AMPARs as sites for protein–protein interaction

Because the C-terminal domains of AMPAR subunits are the predominant cytoplasmic regions, they are likely to be a major factor in the trafficking of the AMPARs. As such, the AMPAR C-termini have been extensively investigated, mainly using yeast two-hybrid and GST pull-down assays, in the search for interacting proteins. A number of protein interactors have been isolated and, in some cases, the roles of individual interactors in receptor

trafficking and surface expression have begun to be elucidated. From these studies, it is becoming increasingly clear that AMPAR complexes get 'handed' from one interacting protein to another in a sequential and probably hierarchical process and these interacting proteins can act as the sorting and delivery mechanisms for the correct delivery to and anchoring at appropriate synapses.

6. PDZ proteins known to interact with AMPARs

AMPAR interactors fall into two main categories; those that bind AMPARs via a PDZ domain and those that do not. The discovery of the role of PDZ domain-mediated protein interactions in the regulation of both AMPARs and NMDARs was a key development. PDZ domains are protein–protein interaction motifs that contain three repeats of ≈ 90 amino acids. PDZ domains are present, either singly or as repeats, in well over 100 otherwise unrelated proteins (Ponting et al., 1997; Songyang et al., 1997). Most PDZ-mediated interactions occur via the recognition of a short motif of three to seven residues located at the extreme C-terminus of the binding protein and provide a mechanism for clustering ion channels and receptors at the plasma membrane and for directing kinases and phosphatases toward their substrates (Garner et al., 2000; Bezprozvanny and Maximov, 2001; Sheng and Sala, 2001).

6.1. Glutamate receptor interacting protein (GRIP) and AMPAR binding protein (ABP)

GRIP is a 130 kDa protein that contains seven PDZ domains, of which domains 4 and 5 mediate binding to the extreme C-terminal ESVKI motif of GluR2 and GluR3 (Dong et al., 1997), but does not bind to GluR1 or GluR4. GRIP has been shown to interact with receptor proteins other than AMPARs (Hirbec et al., 2002). For example, the family of Eph-receptors and their membrane-bound ligands, the ephrins, both bind to GRIP (Hsueh and Sheng, 1998; Torres et al., 1998). Eph-receptors are receptor tyrosine kinases that bind the PDZ domains 6 and 7 of GRIP (as opposed to 4 and 5 for GluR2/3). It is believed that interactions between Eph-receptors and their ligands located on adjacent cells are important for processes involved in neurite extension and axonal guidance (Gale and Yancopoulos, 1997).

ABP is closely related to GRIP with 64–93% homology in their PDZ domains. It has a widespread distribution in the CNS and is enriched in the PSD. ABP exists in two isoforms, one of 130 kDa which also exhibits seven PDZ domains and a shorter 98 kDa isoform which contains only six PDZ domains, of which domains 3, 5 and 6 are capable of binding to the VKI region of the C-Terminal of the GluR2/3 (Srivastava et al., 1998). The second PDZ domain of the 98 kDa isoform of ABP mediates homodimerisation as well as heterodimerisation with GRIP, thus ABP can form multimeric complexes with itself as well as heteromeric complexes with GRIP (Srivastava et al., 1998).

The function of GRIP is not fully resolved, although multiple PDZ domains would suggest it serves a scaffolding/adaptor function comparable to PSD95 (Dong et al., 1997). For example, the PDZ domains other than 4 and 5 could provide a mechanism to link AMPARs to other binding partners of GRIP. Indeed, several GRIP-associated proteins (GRASPs) that bind to distinct PDZ domains within GRIP have been identified (Ye et al., 2000). Of these, GRASP-1 is a neuronal rasGEF associated with GRIP and AMPARs in vivo. Overexpression of GRASP-1 in cultured neurons specifically reduced the synaptic targeting of AMPARs and the subcellular distribution of both AMPARs and GRASP-1 is regulated by NMDAR activation.

A particularly intriguing observation is that GRIP binds kinesins. The kinesin motor proteins drive vesicular transport of synaptic vesicle components to axons and of neurotransmitter receptors to dendrites. Therefore, specific steering proteins must be required to determine

the polarity of transport for axonal or dendritic-bound cargos. It has recently been shown that GRIP directly interacts with and steers kinesin heavy chains to dendrites as a motor for AMPA receptors (Setou et al., 2002).

The subcellular distribution of GRIP was originally described to be synapse specific, but subsequent studies revealed that this protein has a widespread subcellular distribution (Wyszynski et al., 1998). In the cerebellum, GRIP staining is prominent in cell body and proximal dendrites of Purkinje cells. GRIP and GluR2 colocalize in Purkinje cells and is especially prominent in a subset of GluR2-containing cells that also expressed a high level of GluR1. Furthermore, GRIP was seen over the postsynaptic density of asymmetric synapses and at high levels in dendrites of GABA-positive neurons (Burette et al., 1999; Wyszynski et al., 1999).

An important determinant of GRIP binding to GluR2 is the phosphorylation state of the receptor subunit. The serine residue Ser880 in the GluR2 C-terminal sequence (IESVKI) critical for PDZ domain binding is a substrate of PKC and is phosphorylated in vivo. In vitro binding and coimmunoprecipitation studies show that phosphorylation of serine-880 within the GluR2 PDZ ligand significantly decreases GluR2 binding to GRIP1 but not to PICK1 (see below). In cultured hippocampal neurons, Ser880-phosphorylated GluR2 subunits are enriched and colocalized with PICK1 in the dendritic shafts, with very little staining observed at excitatory synapses (Chung et al., 2000b). Similarly, synaptic AMPAR clusters in dendritic spines of Purkinje cells are disrupted by PKC phosphorylation of Ser880 of GluR2 with a corresponding decrease in affinity for GRIP (Hirai, 2001). Significantly induction of cerebellar LTD causes phosphorylation of Ser880 and it has been reported that GluR2 protein released from GRIP is rapidly internalized (Matsuda et al., 2000). A slightly different model has also been proposed in which the maintenance of LTD involves the binding of AMPARs to PDZ proteins inside the neuron to prevent their reinsertion into the membrane, a process that is regulated by PKCα and plays a role during depression (Daw et al., 2000).

6.2. Protein interacting with C-kinase (PICK1)

PICK1 was originally identified by yeast-2-hybrid screening as a protein that interacts with the catalytic subunit of PKCα through its single PDZ domain (Staudinger et al., 1995). It has subsequently been shown to interact with the GluR2 and GluR3 (Dev et al., 1999a; Xia et al., 1999) via the extreme C-terminal PDZ-binding domain. However, the requirements for recognition within this ten amino acid stretch are different for PICK1 and GRIP. A peptide pep2-SVKI with the amino acid sequence NVYGIESVKI blocks the GRIP–GluR2 interaction in a biochemical assay (Li et al., 1999). Two other peptides, ‘GluR2-SVKE’ (NVYGIESVKE), which contains a single amino-acid substitution in the PDZ binding motif and ‘GluR2-EVKI’ (NVYGIEEVKI), in which ser880 is replaced with a glutamate, do not block GRIP binding. Pep2-SVKI and pep2-EVKI blocked the retention of PICK1 by GST-GluR2, while pep2-SVKE had no effect. Thus, pep2-EVKI, in agreement with another recent study (Chung et al., 2000a), selectively blocks the PICK1–GluR2 interaction.

PICK1 has also been shown to be capable of dimerisation through a different site, allowing the possibility for it to aggregate target proteins via its PDZ domains. This has already been demonstrated for AMPARs in heterologous expression systems (Dev et al., 1999b; Xia et al., 1999). PICK1 has been reported to complex with PKCα upon phorbol ester (TPA) induction and the resultant complexes targeted GluR2 in spines. This targeting of PICK1–PKCα complexes to GluR2 in spines caused a decrease in surface GluR2 consistent with a PICK1/PKCα-mediated release of GluR2 from GRIP/ABP binding.

As discussed above, modulation of the surface expression of AMPARs is a key process underlying NMDAR-dependent synaptic plasticity. NMDA application to cultured hippocampal neurons markedly reduces the amount of bound [^{3}H]AMPA to surface expressed receptors of the hippocampal neurons but not to their total membrane fraction (Iwakura et al., 2001). This process was mimicked by phorbol ester and blocked by calphostin C. The NMDA treatment also markedly altered the interaction between the AMPAR subunits and PICK1 with a PKCα-dependent enhancement of the association of GluR2/3 with PICK1. Viral expression of GFP-tagged C-terminal domain of GluR2 (GFP-ct-GluR2) specifically and significantly blocked the NMDA-triggered reduction in [^{3}H]AMPA binding, whereas expression of (GFP-ct-GluR1) had no effect. Co-immunoprecipitation using anti-PICK1 antibodies confirmed that GFP-ct-GluR2 prevented the PICK1 - GluR2/3 interaction consistent with the hypothesis NMDA-induced down-regulation of functional AMPARs involves the interaction between GluR2/3 subunits and PICK1 (Iwakura et al., 2001). Furthermore, it has been shown that PICK1 also binds in neurons and in heterologous cells to PKCα and that the interaction is highly dependent on the activation of the kinase (Perez et al., 2001). The formation of PICK1–PKCα complexes is strongly induced by TPA and PICK1–PKCα complexes are co-targeted with PICK1–GluR2 complexes to dendritic spines, where GluR2 is phosphorylated by PKCα on Ser880. These workers proposed that PICK1 functions as a targeting and transport protein that directs the activated form of PKCα to GluR2 in spines, leading to the activity-dependent release of GluR2 from synaptic anchor proteins and the PICK1-dependent transport of GluR2 from the synaptic membrane.

An alternative scheme is that following LTD internalised receptors bind to GRIP/ABP, which anchors them at the subsynaptic membrane, preventing their rapid re-insertion. However, a proportion of receptors can associate with PICK1 present in spines and in this configuration may be mobile. In this model, the role of PICK1 is likely to be in targeting PKC to GluR2. One form of LTP, namely de-depression (i.e. LTP following prior induction of LTD) AMPARs dissociate from PICK1 or GRIP/ABP and PKC phosphorylates Ser880. This prevents rebinding to GRIP/ABP (Chung et al., 2000a) and allows insertion of receptors (Daw et al., 2000).

6.3. Summary of possible roles for GRIP and PICK1 interactions with GluR2

Based on the findings set out above, it is possible to envisage two separate but not necessarily mutually exclusive models. AMPARs may be secured in intracellular pools via association of the GluR2 subunit with GRIP and/or ABP (Daw et al., 2000; Braithwaite et al., 2002). To release the AMPAR, PICK1 exchanges for GRIP and targets PKC, which then phosphorylates Ser880 of GluR2 thereby preventing the rebinding of GRIP. The Ser880 phosphorylated AMPARs are mobile and available for surface expression. In a variation of this scheme, work from some other laboratories (Chung et al., 2000a; Perez et al., 2001) is more consistent with GRIP immobilising GluR2-containing AMPARs at the synaptic membrane rather than inside the cell. While further work will be required to resolve this apparent discrepancy, it is likely that, depending on the exact experimental protocols used and/or the stimulation history of the neuron and individual synapses, that GRIP is capable of anchoring GluR2-containing AMPARs at either the postsynaptic membrane, at pools inside the dendrite or at both locations. Nonetheless, in both models GRIP is envisaged as an anchoring protein and that phosphorylation of Ser880 of GluR2 destabilises/prevents GRIP, but not PICK1 binding. It is important to note that these interactions between GRIP, PICK1 and GluR2 form one component of a highly complex series of processes that are involved in AMPAR trafficking and surface expression. They will be highly regulated and, in addition, will be influenced by the multiple other interactions taking place in the crowded region of the postsynaptic membrane and elsewhere. For example, the finding that GRIP may steer

AMPAR transport via an interaction with kinesins (Setou et al., 2002) indicates that the roles of individual protein interactions will be dictated in large part by the specific location and micro-environment within the cell at which these interactions take place.

6.4. Synapse associated protein of 97 kDa (SAP97)

SAP97 is a member of the synapse associated protein (SAP) family, whose other members include SAP90 (PSD95), chapsyn110 (PSD93) and SAP102 which have been shown to interact with NMDAR subunits. SAP97 has a presynaptic localisation (Muller et al., 1995) where its function remains unclear. In addition, however, SAP97 can interact selectively with postsynaptically localised GluR1 in co-immunoprecipitation and cross-linking experiments from rat brain (Leonard et al., 1998). Indeed, SAP97 is the only PDZ protein currently known to interact directly with GluR1. SAP97 concentrates at synapses that contain GluR1, but not necessarily GluR2 or GluR3 and is at highest concentration within the postsynaptic density of asymmetric synapses, suggesting that SAP97 may help to anchor GluR1-containing AMPARs at the synapse (Valtschanoff et al., 2000). Another study has indicated that phosphorylation of AMPARs is enhanced by a SAP97–AKAP79 complex that directs PKA to GluR1 via a PDZ domain interaction (Colledge et al., 2000). SAP97 has also been suggested to play a role the provision of new anchoring sites at synapses via a mechanism that involves the binding of autophosphorylated CaMKII to NMDARs. In turn, SAP97 binds to CaMKII and also provides the scaffolding for retaining new GluR1-containing AMPARs (Lisman and Zhabotinsky, 2001). However, a recent report has suggested that interactions involving SAP97 and GluR1 occur early in the secretory pathway, while the receptors are in the endoplasmic reticulum or cis-Golgi (Sans et al., 2001). Few synaptic GluR1-containing receptors were found to associate with SAP97. Furthermore, NMDAR-evoked internalisation of GluR1 did not require SAP97, suggesting that GluR1–SAP97 interactions are involved in AMPAR targeting but not endocytosis and may therefore fulfill a chaperone-like function.

6.5. Mint1 (LIN-10, X11)

The mint family consists of evolutionarily conserved PDZ-containing adapter proteins from *Candida elegans* to mammalian neurons. Mint1 is a component of macromolecular complexes in the presynaptic and postsynaptic terminals involved in bringing synaptic vesicles to the exocytotic transmitter release site and localising receptors and ion channels in the specific membrane domains (Biederer and Sudhof, 2000; Okamoto et al., 2001).

In *C. elegans*, GLR-1 are AMPA-type glutamate receptors that are expressed postsynaptically at target cells of the sensory neuron ASH and are required for ASH-mediated touch-sensitivity (Hart et al., 1995; Maricq et al., 1995). The localisation of these receptors has been shown to be dependent on the interaction with LIN-10 (Rongo et al., 1998), the *C. elegans* orthologue of mint1/X11 (Borg et al., 1996; Okamoto and Südhof, 1997). Vesicles containing the NMDAR-2B (NR2B subunit) have been shown to be transported along microtubules by KIF17, a neuron-specific molecular motor in neuronal dendrites and that selective transport is accomplished by direct interaction of the KIF17 tail with a complex containing Mint1 and CASK (Setou et al., 2000).

7. Non-PDZ domain-interactions at AMPARs

7.1. N-Ethylmaleimide-sensitive factor (NSF)

The NSF ATPase was originally characterised through its involvement in protein transport activity of the Golgi membranes, then subsequently it was shown to be involved in endoplasmic reticulum (ER) to Golgi transport and endosome fusion (Rothman, 1994). Later, a central role for NSF was identified in docking and/or fusion of synaptic vesicles

(Söllner and Rothman, 1994; Schiavo et al., 1995). In addition to this presynaptic role, NSF was also found to be a constituent of the PSD (Walsh and Kuruc, 1992) and, intriguingly, it was shown to be enriched following transient ischemia (Hu et al., 1998), which enhances synaptic transmission.

GluR2 was first shown to interact with NSF using the yeast-2-hybrid screen (Henley et al., 1997; Nishimune et al., 1998). These findings were confirmed by the demonstration that GluR2 and NSF co-immunoprecipitate from the rat hippocampus along with α- and β-SNAPs in a complex analogous to the t-SNARE syntaxin–NSF–SNAP complex (Osten et al., 1998). The binding site identified as a ten amino acid section of the GluR2 C-terminal domain (Nishimune et al., 1998; Song et al., 1998) and a peptide of this binding site was found to be sufficient for the interaction to occur. The binding site on GluR2 was has no similarity to other NSF binding sites thereby allowing specific functional experiments to be carried out using a synthetic peptide (pep2m) corresponding to this sequence. Partial inhibition of synaptic transmission was observed following perfusion of pep2m to postsynaptic sites indicating that NSF plays a role in a rapid recycling pool of AMPARs, but that there is also a pool of receptors that are not recycled through this mechanism. These experiments also allowed a calculation of a synaptic half-life of <10 min for these rapidly recycled receptors (Nishimune et al., 1998). Infusion of pep2m in to cultured hippocampal neurones demonstrated both a decrease in the frequency but not the amplitude of AMPAR-mediated mEPSCs and chronic viral expression of the pep2m construct caused a dramatic reduction in the AMPAR immunoreactive puncta on the cell surface (Noel et al., 1999). Furthermore, pep2m occludes long term depression (LTD) indicating that LTD expression involves the removal from synapses of a pool of AMPAR controlled by the NSF–GluR2 interactions (Luthi et al., 1999).

7.2. Neuronal activity-regulated pentraxin (Narp)

Narp is a neuronal immediate early gene product which is a member of a secretory family of proteins called the pentraxins. Narp was initially identified using a subtractive cloning strategy in stimulated hippocampus since it remains elevated for nearly 24 h after a single episode of electroconvulsive seizure (ECS) (Tsui et al., 1996). This extracellular protein appears to play a role in the synaptic clustering of AMPAR on aspiny neurones (O'Brien et al., 1999). In cultured neurones from the hippocampus and the spinal cord, it is expressed by a subset of axons and dendrites, being present at excitatory synapses of aspiny, i.e. inhibitory interneurons, but not spiny, i.e. pyramidal cell, neurones. Narp was also found to be on the surface at these synapses and a significant amount of Narp was found in the media around the cells, consistent with its membership of a family of secretory proteins. Transfection studies in spinal neurons demonstrated that synaptic Narp could be derived from both the pre- and postsynaptic neuron which was confirmed by EM studies which revealed Narp in the presynaptic bouton associated with vesicles, within the cleft and in postsynaptic structures. In addition, these transfected spinal neurons showed an increase in the number of excitatory synapses (O'Brien et al., 1999). Repeated ECS on hippocampus caused Narp protein levels to remain elevated, about 6-fold higher than basal levels, at 48 h after the last of a series of five or six ECS given every other day. Therefore, sustained increases in Narp may contribute to changes in excitatory synaptic transmission induced by chronic neuronal stimulation (Reti and Baraban, 2000).

7.3. Stargazin

Stargazin is a mutated protein that is related to the g-1 calcium channel subunit present in the Stargazer mouse (Letts et al., 1998; Hashimoto et al., 1999). Stargazer is an ataxic and epileptic mutant mouse that lacks functional AMPARs on cerebellar granule cells. Stargazin interacts with both AMPAR subunits and synaptic PDZ proteins, such as PSD-95. It has

been reported that the interaction of stargazin with AMPAR subunits is essential for delivering functional receptors to the surface membrane of granule cells, whereas its binding with PSD-95 and related PDZ proteins through a carboxy-terminal PDZ-binding domain is required for targeting the AMPAR to synapses (Chen et al., 2000). Different stargazing isoforms are expressed in other neuronal cell-types, including hippocampal pyramidal cells, leading to the hypothesis that they may perform a similar function in those cell-types. In addition, expression of a mutant stargazin lacking the PDZ-binding domain in hippocampal pyramidal cells disrupts synaptic AMPARs, indicating that stargazin-like mechanisms for targeting AMPARs may be widespread in the central nervous system.

7.4. 4.1N and 4.1G

4.1 Proteins are a family of multifunctional cytoskeletal components (4.1R, 4.1G, 4.1N, 4.1B) derived from four related genes, each of which is expressed in the nervous system. 4.1N is enriched at synapses and colocalises with GluR1 (Walensky et al., 1999). By analogy with the roles of 4.1R in red blood cells, these workers suggested that 4.1N may function to confer stability and plasticity to the neuronal membrane via interactions with multiple binding partners, including the spectrin-actin-based cytoskeleton, integral membrane channels and receptors, and membrane-associated guanylate kinases (Walensky et al., 1999).

Subsequently, a direct interaction between GluR1 and 4.1G and 4.1N proteins was demonstrated (Shen et al., 2000). Both 4.1G and 4.1N bind to a membrane proximal region of the GluR1 C terminus and the site of interaction on 4.1G or 4.1N lies in a defined region in the C-terminal domain. 4.1G and 4.1N may serve to link GluR1 to the actin cytoskeleton as they both contain the binding site for actin complexes. This hypothesis is supported to some extent by the observation that disruption of actin filaments in cultured cortical neurons causes a down-regulation of GluR1 surface expression (Shen et al., 2000).

7.5. Lyn

Lyn is a c-src-like, membrane-associated non-receptor protein tyrosine kinase (PTK) that is highly enriched in a subcellular fraction of nerve growth cones (GCPs). In neurons 1–2% of Lyn associates with AMPAR subunits through its SH2 and SH3 domains (Hayashi et al., 1999). The precise site of interaction on AMPAR subunits was not established but is assumed to be intracellular. PTKs link to the MAPK signalling pathway and Lyn was shown by this study to link AMPAR activation, through this pathway, to activation of expression of brain-derived neurotrophic factor (BDNF) mRNA. BDNF has previously been shown to strengthen the efficiency of synaptic transmission (Thoenen, 1995), so this protein implicates AMPARs in a direct role in synaptic plasticity as well as mediating rapid synaptic transmission.

7.6. Gα

AMPAR, but not NMDAR, signaling in rat cortical neurons has been reported to involve a G-protein coupled to a protein kinase cascade (Wang and Durkin, 1995). Both NMDA and AMPA activated p42 mitogen-activated protein kinase (MAPK) in neurons, but only AMPA-induced MAPK was inhibited by pertussis toxin. Furthermore, AMPA, but not NMDA, caused an association of a G-protein β subunit with a Ras, Raf kinase and MAP/ERK kinase (MEK)-1 complex. This led to the suggestion that AMPAR activation could trigger MAPK activation via a novel mechanism in which G-protein β/γ dimers released from Gα bind to a Ras protein complex causing the activation of Ras, Raf kinase, MEK-1 and finally MAPK. Despite the fact that no sites for G-protein binding have yet been identified on AMPARs, these workers went on to propose that AMPAR activation can generate intracellular signals that are independent of Ca^{2+} and Na^{+} influx through ion

channels. In the absence of intracellular Ca^{2+} and Na^{+}, AMPAR stimulation inhibited pertussis toxin-mediated ADP-ribosylation of Gα (Wang et al., 1997). AMPA also inhibited forskolin-stimulated activity of adenylate cyclase in neurons, suggesting that Gi proteins were activated. In retinal ganglion cells AMPAR activation has also been reported to modulate a G-protein that, in turn, suppresses the inward current through a cGMP-gated channel activated by nitric oxide (NO). The AMPA-induced suppression of the cGMP-gated current was blocked by pertussis toxin suggesting that AMPARs can exhibit a 'metabotropic' activity that antagonizes excitation evoked by NO (Kawai and Sterling, 1999).

8. Concluding remarks

While significant advances have been made in identifying proteins that interact with the precise roles of each of these interactors and how they relate to each other remains unclear. As illustrated in Fig. 2, more work needs to be carried out to define the developmental, activity-dependent and steady-state processes that regulate changes in AMPAR-mediated neurotransmission. Meeting this challenge represents a significant goal in neuroscience that should lead to the development of intervention and treatment strategies that may provide effective strategies for the treatment of excitoxic and neurodegenerative diseases.

Abbreviations

ABP	AMPA receptor binding protein
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazole propionate
AMPAR	AMPA receptor
CaMK-II	Ca^{2+}calmodulin-dependent protein kinase type II
CNQX	6-cyano-7-nitroquinoxaline-2,3-dione
CNS	central nervous system
EM	electron microscopy
mEPSCs	mini excitatory postsynaptic currents
ER	endoplasmic reticulum
GFP	green fluorescent protein
GluR	glutamate receptor
GluR1–4	AMPAR subunits 1–4
GRIP	glutamate receptor interacting protein
GRASP	GRIP-associated protein
LTD	long-term depression
LTP	long-term potentiation
MAGUK	membrane associated guanylate cyclase
MAP kinase	mitogen-activated protein kinase
mGluRs	metabotropic glutamate receptors
nAChRs	nicotinic acetylcholine receptors
NMDA	*N*-methyl-D-aspartate

NSF	*N*-ethylmalemide-sensitive fusion protein
PDZ	protein binding motif named after first letters of original proteins in which it was identified
PICK1	protein interacting with C kinase
PLC	phospholipase C
PKC	protein kinase C
PSD	postsynaptic density
PSD95	postsynaptic density protein-95 kDa
SAP97	synapse-associated protein-97 kDa
SNAP	soluble NSF attachment protein
SNAP-25	synaptosome-associated protein-25 kDa
t-SNARE	target-associated soluble NSF attachment protein receptor
v-SNARE	vesicle-associated soluble NSF attachment protein receptor.

References

Antonny B, Schekman R. ER export: public transportation by the COPII coach. Curr. Opin. Cell Biol. 2001; 13:438–443. [PubMed: 11454450]

Archibald JM, Perry MJ, Molnar E, Henley JM. Surface expression and metabolic half-life of AMPA receptors in cultured rat cerebellar granule cells. Neuropharmacology. 1998; 37:1345–1353. [PubMed: 9849670]

Barinaga M. New clues to how neurons strengthen their connections. Science. 1999; 284:1755–1757. [PubMed: 10391789]

Baude A, Molnar E, Latawiec D, Mcilhinney RAJ, Somogyi P. Synaptic and nonsynaptic localization of the GluR1 subunit of the AMPA-type excitatory amino acid receptor in the rat cerebellum. J. Neurosci. 1994; 14:2830–2843. [PubMed: 8182442]

Beattie EC, Carroll RC, Yu X, Morishita W, Yasuda H, von Zastrow M, Malenka RC. Regulation of AMPA receptor endocytosis by a signaling mechanism shared with LTD. Nat. Neurosci. 2000; 3:1291–1300. [PubMed: 11100150]

Bennett JA, Dingledine R. Topology profile for a glutamate receptor: three transmembrane domains and a channel-lining reentrant membrane loop. Neuron. 1995; 14:373–384. [PubMed: 7857646]

Bezprozvanny I, Maximov A. PDZ domains: more than just a glue. Proc. Natl. Acad. Sci. USA. 2001; 98:787–789. [PubMed: 11158544]

Bichet D, Cornet V, Geib S, Carlier E, Volsen S, Hoshi T, Mori Y, De Waard M. The I-II loop of the Ca2-channel alpha1 subunit contains an endoplasmic reticulum retention signal antagonized by the beta subunit. Neuron. 2000; 25:177–190. [PubMed: 10707982]

Biederer T, Sudhof TC. Mints as adaptors. Direct binding to neurexins and recruitment of munc18. J. Biol. Chem. 2000; 275:39803–39806. [PubMed: 11036064]

Borg J, Ooi J, Levy E, Margolis B. The phosphotyrosine interaction domains of X11 and FE65 bind distinct sites on the YENPTY motif of amyloid precursor protein. Mol. Cell Biol. 1996; 16:6229–6241. [PubMed: 8887653]

Borgdorff AJ, Choquet D. Regulation of AMPA receptor lateral movements. Nature. 2002; 417:649–653. [PubMed: 12050666]

Braithwaite SP, Xia H, Malenka RC. Differential roles for NSF and GRIP/ABP in AMPA receptor cycling. Proc. Natl. Acad. Sci. USA. 2002; 99:7096–7101. [PubMed: 12011465]

Europe PMC Funders Author Manuscripts

Burette A, Wyszynski M, Valtschanoff JG, Sheng M, Weinberg RJ. Characterization of glutamate receptor interacting protein-immunopositive neurons in cerebellum and cerebral cortex of the albino rat. J. Comp. Neurol. 1999; 411:601–612. [PubMed: 10421871]

Carroll RC, Lissin DV, von Zastrow M, Nicoll RA, Malenka RC. Rapid redistribution of glutamate receptors contributes to long-term depression in hippocampal cultures. Nat. Neurosci. 1999a; 2:454–460. [PubMed: 10321250]

Carroll RC, Beattie EC, Xia H, Luscher C, Altschuler Y, Nicoll RA, Malenka RC, von Zastrow M. Dynamin-dependent endocytosis of ionotropic glutamate receptors. Proc. Natl. Acad. Sci. USA. 1999b; 96:14112–14117. [PubMed: 10570207]

Carroll RC, Beattie EC, von Zastrow M, Malenka RC. Role of AMPA receptor endocytosis in synaptic plasticity. Nat. Rev. Neurosci. 2001; 2:315–324. [PubMed: 11331915]

Chen L, Chetkovich DM, Petralia RS, Sweeney NT, Kawasaki Y, Wenthold RJ, Bredt DS, Nicoll RA. Stargazing regulates synaptic targeting of AMPA receptors by two distinct mechanisms. Nature. 2000; 408:936–943. [PubMed: 11140673]

Chung HJ, Xia J, Scannevin RH, Zhang X, Huganir RL. Phosphorylation of the AMPA receptor subunit GluR2 differentially regulates its interaction with PDZ domain-containing proteins (in process citation). J. Neurosci. 2000a; 20:7258–7267. [PubMed: 11007883]

Chung HJ, Xia J, Scannevin RH, Zhang X, Huganir RL. Phosphorylation of the AMPA receptor subunit GluR2 differentially regulates its interaction with PDZ domain-containing proteins. J. Neurosci. 2000b; 20:7258–7267. [PubMed: 11007883]

Colledge M, Dean RA, Scott GK, Langeberg LK, Huganir RL, Scott JD. Targeting of PKA to glutamate receptors through a MAGUK-AKAP complex (in process citation). Neuron. 2000; 27:107–119. [PubMed: 10939335]

Craig AM, Blackstone CD, Huganir RL, Banker G. The distribution of glutamate receptors in cultured rat hippocampal neurons–postsynaptic clustering of AMPA-selective subunits. Neuron. 1993; 10:1055–1068. [PubMed: 7686378]

Daw MI, Chittajallu R, Bortolotto ZA, Dev KK, Duprat F, Henley JM, Collingridge GL, Isaac JT. PDZ proteins interacting with C-terminal GluR2/3 are involved in a PKC-dependent regulation of AMPA receptors at hippocampal synapses. Neuron. 2000; 28:873–886. [PubMed: 11163273]

Dev KK, Nishimune A, Henley JM, Nakanishi S. The protein kinase Ca binding protein PICK1 interacts with short but not long form alternative splice varients of AMPA receptor subunits. Neuropharmacology. 1999a; 38:635–644. [PubMed: 10340301]

Dev KK, Nishimune A, Henley JM, Nakanishi S. The protein kinase C alpha binding protein PICK1 interacts with short but not long form alternative splice variants of AMPA receptor subunits. Neuropharmacology. 1999b; 38:635–644. [PubMed: 10340301]

Dong H, O'Brien RJ, Fung ET, Lanahan AA, Worley PF, Huganir RL. GRIP: a synaptic PDZ domain-containing protein that interacts with AMPA receptors (see comments). Nature. 1997; 386:279–284. [PubMed: 9069286]

Durand GM, Kovalchuck Y, Konnerth A. Long-term potentiation and functional synapse induction in developing hippocampus. Nature. 1996; 381:71–75. [PubMed: 8609991]

Ehlers MD. Reinsertion or degradation of AMPA receptors determined by activity-dependent endocytic sorting. Neuron. 2000; 28:511–525. [PubMed: 11144360]

Fitzjohn SM, Pickard L, Duckworth JK, Molnar E, Henley JM, Collingridge GL, Noel J. An electrophysiological characterisation of long-term potentiation in cultured dissociated hippocampal neurones. Neuropharmacology. 2001; 41:693–699. [PubMed: 11640923]

Foletti DL, Prekeris R, Scheller RH. Generation and maintenance of neuronal polarity: mechanisms of transport and targeting. Neuron. 1999; 23:641–644. [PubMed: 10482230]

Gale NW, Yancopoulos GD. Ephrins and their receptors: a repulsive topic? Cell Tissue Res. 1997; 290:227–241. [PubMed: 9321684]

Garner CC, Nash J, Huganir RL. PDZ domains in synapse assembly and signalling. Trends Cell Biol. 2000; 10:274–280. [PubMed: 10856930]

Hall RA, Hansen A, Andersen PH, Soderling TR. Surface expression of the AMPA receptor subunits GluR1, GluR2 and GluR4 in stably transfected baby hamster kidney cells. Neuroscience. 1997; 68:625–630.

Hart A, Sims S, Kaplan J. A synaptic code for sensory modalities revealed by analysis of the *C. elegans* GLR-1 glutamate receptor. Nature. 1995; 378:82–85. [PubMed: 7477294]

Hashimoto K, Fukaya M, Qiao X, Sakimura K, Watanabe M, Kano M. Impairment of AMPA receptor function in cerebellar granule cells of ataxic mutant mouse stargazer. J. Neurosci. 1999; 19:6027–6036. [PubMed: 10407040]

Hayashi T, Umemori H, Mishina M, Yamamoto T. The AMPA receptor interacts with and signals through the protein tyrosine kinase Lyn. Nature. 1999; 397:72–76. [PubMed: 9892356]

Hayashi Y, Shi SH, Esteban JA, Piccini A, Poncer JC, Malinow R. Driving AMPA receptors into synapses by LTP and CaMKII: requirement for GluR1 and PDZ domain interaction. Science. 2000; 287:2262–2267. [PubMed: 10731148]

Henley JM, Nishimune A, Nash SR, Nakanishi S. Use of the two-hybrid system to find novel proteins that interact with AMPA receptor subunits. Biochem. Soc. Trans. 1997; 25:838–841. [PubMed: 9388557]

Hirai H. Modification of AMPA receptor clustering regulates cerebellar synaptic plasticity. Neurosci. Res. 2001; 39:261–267. [PubMed: 11248365]

Hirbec H, Perestenko O, Nishimune A, Meyer G, Nakanishi S, Henley JM, Dev KK. The PDZ proteins PICK1, GRIP, and Syntenin bind multiple glutamate receptor subtypes. analysis of PDZ binding motifs. J. Biol. Chem. 2002; 277:15221–15224. [PubMed: 11891216]

Hollmann M, Boulter J, Maron C, Heinemann S. Molecular biology of glutamate receptors. Potentiation of *N*-methyl-D-aspartate receptor splice variants by zinc. Renal Physiol. Biochem. 1994; 17:182–183. [PubMed: 7518953]

Hsueh YP, Sheng M. Eph receptors, ephrins, and PDZs gather in neuronal synapses. Neuron. 1998; 21:1227–1229. [PubMed: 9883715]

Hu B-R, Park M, Martone ME, Fischer WH, Ellisman MH, Zivin JA. Assembly of proteins to postsynaptic densities after transient cerebral ischemia. J. Neurosci. 1998; 18:625–633. [PubMed: 9425004]

Huh KH, Wenthold RJ. Turnover time and regulation of ionotropic glutamate receptors in cultured cerebellar granule cells. Soc. Neurosci. Abstr. 1997; 23:371–373.

Isaac JTR, Nicoll RA, Malenka RC. Evidence for silent synapses: implications for expression of LTP. Neuron. 1995; 15:427–434. [PubMed: 7646894]

Iwakura Y, Nagano T, Kawamura M, Horikawa H, Ibaraki K, Takei N, Nawa H. NMDA-induced AMPA receptor down-regulation involves interaction of carboxyl terminus of GluR2/3 with pick1. Ligand-binding studies using sindvis vectors carrying AMPA receptor decoys. J. Biol. Chem. 2001; 276:40025–40032. [PubMed: 11498531]

Kawai F, Sterling P. AMPA receptor activates a G-protein that suppresses a cGMP-gated current. J. Neurosci. 1999; 19:2954–2959. [PubMed: 10191313]

Kim CH, Lisman JE. A labile component of AMPA receptor-mediated synaptic transmission is dependent on microtubule motors, actin, and *N*-ethylmaleimide-sensitive factor. J. Neurosci. 2001; 21:4188–4194. [PubMed: 11404404]

Kittler JT, Rostaing P, Schiavo G, Fritschy JM, Olsen R, Triller A, Moss SJ. The subcellular distribution of GABARAP and its ability to interact with NSF suggest a role for this protein in the intracellular transport of GABA(A) receptors. Mol. Cell Neurosci. 2001; 18:13–25. [PubMed: 11461150]

Klumperman J. Transport between ER and Golgi. Curr. Opin. Cell Biol. 2000; 12:445–449. [PubMed: 10873822]

Leonard AS, Davare MA, Horne MC, Garner CC, Hell JW. SAP97 is associated with the AMPA receptor GluR1 subunit. J. Biol. Chem. 1998; 273:19518–19524. [PubMed: 9677374]

Letts VA, Felix R, Biddlecome GH, Arikkath J, Mahaffey CL, Valenzuela A, Bartlett FS II, Mori Y, Campbell KP, Frankel WN. The mouse stargazer gene encodes a neuronal Ca2--channel gamma subunit. Nat. Genet. 1998; 19:340–347. [PubMed: 9697694]

Li P, Kerchner GA, Sala C, Wei F, Huettner JE, Sheng M, Zhuo M. AMPA receptor-PDZ interactions in facilitation of spinal sensory synapses. Nat. Neurosci. 1999; 2:972–977. [PubMed: 10526335]

Liao D, Hessler NA, Malinow R. Activation of postsynaptically silent synapses during pairing-induced LTP in CA1 region of hippocampal slice. Nature. 1995; 375:400–404. [PubMed: 7760933]

Liao D, Zhang X, O'Brien R, Ehlers MD, Huganir RL. Regulation of morphological postsynaptic silent synapses in developing hippocampal neurons. Nat. Neurosci. 1999; 2:37–43. [PubMed: 10195178]

Liao D, Scannevin RH, Huganir R. Activation of silent synapses by rapid activity-dependent synaptic recruitment of ampa receptors. J. Neurosci. 2001; 21:6008–6017. [PubMed: 11487624]

Lin JW, Ju W, Foster K, Lee SH, Ahmadian G, Wyszynski M, Wang YT, Sheng M. Distinct molecular mechanisms and divergent endocytotic pathways of AMPA receptor internalization. Nat. Neurosci. 2000; 3:1282–1290. [PubMed: 11100149]

Lisman JE, Zhabotinsky AM. A model of synaptic memory: a CaMKII/PP1 switch that potentiates transmission by organizing an AMPA receptor anchoring assembly. Neuron. 2001; 31:191–201. [PubMed: 11502252]

Lissin DV, Carroll RC, Nicoll RA, Malenka RC, von Zastrow M. Rapid, activation-induced redistribution of ionotropic glutamate receptors in cultured hippocampal neurons. J. Neurosci. 1999; 19:1263–1272. published erratum appears in J. Neurosci. 1999 15, 19(8), 3275. [PubMed: 9952404]

Lu W, Man H, Ju W, Trimble WS, MacDonald JF, Wang YT. Activation of synaptic NMDA receptors induces membrane insertion of new AMPA receptors and LTP in cultured hippocampal neurons. Neuron. 2001; 29:243–254. [PubMed: 11182095]

Luscher C, Xia H, Beattie EC, Carroll RC, von Zastrow M, Malenka RC, Nicoll RA. Role of AMPA receptor cycling in synaptic transmission and plasticity. Neuron. 1999; 24:649–658. [PubMed: 10595516]

Luthi A, Chittajallu R, Duprat F, Palmer MJ, Benke TA, Kidd FL, Henley JM, Isaac JT, Collingridge GL. Hippocampal LTD expression involves a pool of AMPARs regulated by the NSF-GluR2 interaction (see comments). Neuron. 1999; 24:389–399. [PubMed: 10571232]

Malinow R, Mainen ZF, Hayashi Y. LTP mechanisms: from silence to four-lane traffic. Curr. Opin. Neurobiol. 2000; 10:352–357. [PubMed: 10851179]

Mammen AL, Huganir RL, O'Brien RJ. Redistribution and stabilization of cell surface glutamate receptors during synapse formation. J. Neurosci. 1997; 17:7351–7358. [PubMed: 9295381]

Man YH, Lin JW, Ju WH, Ahmadian G, Liu L, Becker LE, Sheng M, Wang YT. Regulation of AMPA receptor-mediated synaptic transmission by clathrin-dependent receptor internalization. Neuron. 2000a; 25:649–662. [PubMed: 10774732]

Man HY, Ju W, Ahmadian G, Wang YT. Intracellular trafficking of AMPA receptors in synaptic plasticity. Cell Mol. Life Sci. 2000b; 57:1526–1534. [PubMed: 11092447]

Maricq AV, Peckol E, Driscoll M, Bargmann C. glr-1, a *C.elegans* glutamate receptor that mediates mechanosensory signaling. Nature. 1995; 378:78–81. [PubMed: 7477293]

Matsuda S, Launey T, Mikawa S, Hirai H. Disruption of AMPA receptor GluR2 clusters following long-term depression induction in cerebellar Purkinje neurons. EMBO J. 2000; 19:2765–2774. [PubMed: 10856222]

Meddows E, Le Bourdelles B, Grimwood S, Wafford K, Sandhu S, Whiting P, McIlhinney RA. Identification of molecular determinants that are important in the assembly of *N*-methyl-D-aspartate receptors. J. Biol. Chem. 2001; 276:18795–18803. [PubMed: 11279200]

Miller WE, Lefkowitz RJ. Expanding roles for beta-arrestins as scaffolds and adapters in GPCR signaling and trafficking. Curr. Opin. Cell Biol. 2001; 13:139–145. [PubMed: 11248546]

Morales M, Goda Y. Nomadic AMPA Receptors and LTP. Neuron. 1999; 23:431–434. [PubMed: 10433256]

Mostov KE, Verges M, Altschuler Y. Membrane traffic in polarized epithelial cells. Curr. Opin. Cell Biol. 2000; 12:483–490. [PubMed: 10873817]

Muller BM, Kistner U, Veh RW, Cases-Langhoff C, Becker B, Gundelfinger ED, Garner CC. Molecular characterization and spatial distribution of SAP97, a novel presynaptic protein homologous to SAP90 and the *Drosophila* discs-large tumor suppressor protein. J. Neurosci. 1995; 15:2354–2366. [PubMed: 7891172]

Nishimune A, Isaac JTR, Molnar E, Noel J, Nash SR, Tagaya M, Collingridge GL, Nakanishi S, Henley JM. NSF binding to GluR2 regulates synaptic transmission. Neuron. 1998; 21:87–97. [PubMed: 9697854]

Noel J, Ralph GS, Pickard L, Williams J, Molnar E, Uney JB, Collingridge GL, Henley JM. Surface expression of AMPA receptors in hippocampal neurons is regulated by an NSF-dependent mechanism. Neuron. 1999; 23:365–376. [PubMed: 10399941]

O'Brien R, Xu D, Petralia R, Steward O, Huganir R, Worley P. Synaptic clustering of AMPA receptors by the extracellular immediate-early gene product Narp. Neuron. 1999; 23:309–323. [PubMed: 10399937]

Okamoto M, Südhof T. Mints, Munc18-interacting proteins in synaptic vesicle exocytosis. J. Biol. Chem. 1997; 272:31459–31464. [PubMed: 9395480]

Okamoto M, Nakajima Y, Matsuyama T, Sugita M. Amyloid precursor protein associates independently and collaboratively with PTB and PDZ domains of mint on vesicles and at cell membrane. Neuroscience. 2001; 104:653–665. [PubMed: 11440799]

Osten P, Srivastava S, Inman GJ, Vilim FS, Khatri L, Lee LM, States BA, Einheber S, Milner TA, Hanson PI, Ziff EB. The AMPA receptor GluR2 C terminus can mediate a reversible, ATP-dependent interaction with NSF and α- and β-SNAPs. Neuron. 1998; 21:99–110. [PubMed: 9697855]

Passafaro M, Piech V, Sheng M. Subunit-specific temporal and spatial patterns of AMPA receptor exocytosis in hippocampal neurons. Nat. Neurosci. 2001; 4:917–926. [PubMed: 11528423]

Perez JL, Khatri L, Chang C, Srivastava S, Osten P, Ziff EB. PICK1 targets activated protein kinase Calpha to AMPA receptor clusters in spines of hippocampal neurons and reduces surface levels of the AMPA-type glutamate receptor subunit 2. J. Neurosci. 2001; 21:5417–5428. [PubMed: 11466413]

Pfeffer SR. Transport vesicle docking: SNAREs and associates. Annu. Rev. Cell Dev. Biol. 1996; 12:441–461. [PubMed: 8970734]

Pickard L, Noel J, Duckworth JK, Fitzjohn SM, Henley JM, Collingridge GL, Molnar E. Transient synaptic activation of NMDA receptors leads to the insertion of native AMPA receptors at hippocampal neuronal plasma membranes. Neuropharmacology. 2001; 41:700–713. [PubMed: 11640924]

Ponting CP, Phillips C, Davies KE, Blake DJ. PDZ domains: targeting signalling molecules to sub-membranous sites. Bioessays. 1997; 19:469–479. [PubMed: 9204764]

Reti IM, Baraban JM. Sustained increase in Narp protein expression following repeated electroconvulsive seizure. Neuropsychopharmacology. 2000; 23:439–443. [PubMed: 10989271]

Richmond SA, Irving AJ, Molnar E, McIlhinney RA, Michelangeli F, Henley JM, Collingridge GL. Localization of the glutamate receptor subunit GluR1 on the surface of living and within cultured hippocampal neurons. Neuroscience. 1996; 75:69–82. [PubMed: 8923524]

Romano C, Yang WL, O'Malley KL. Metabotropic glutamate receptor 5 is a disulfide-linked dimer. J. Biol. Chem. 1996; 271:28612–28616. [PubMed: 8910492]

Rongo C, Whitfield CW, Rodal A, Kim SK, Kaplan JM. LIN-10 is a shared component of the polarized protein localisation pathways in neurons and epithelia. Cell. 1998; 94:751–759. [PubMed: 9753322]

Rothman JE. Mechanisms of intracellular protein transport. Nature. 1994; 372:55–63. [PubMed: 7969419]

Sans N, Racca C, Petralia RS, Wang YX, McCallum J, Wenthold RJ. Synapse-associated protein 97 selectively associates with a subset of AMPA receptors early in their biosynthetic pathway. J. Neurosci. 2001; 21:7506–7516. [PubMed: 11567040]

Sato M, Tani E, Fujikawa H, Yamaura I, Arita N, Kaibuchi K. Importance of Rho-kinase-mediated phosphorylation of myosin light chain in vasospasm. Acta Neurochir. Suppl. 2001; 77:49–54. [PubMed: 11563308]

Schiavo G, Gmachl MJS, Stenbeck G, Sollner TH, Rothman JE. A possible docking and fusion particle for synaptic transmission. Nature. 1995; 378:733–736. [PubMed: 7501022]

Setou M, Nakagawa T, Seog DH, Hirokawa N. Kinesin superfamily motor protein KIF17 and mLin-10 in NMDA receptor-containing vesicle transport. Science. 2000; 288:1796–1802. [PubMed: 10846156]

Setou M, Seog D, Tanaka Y, Kanai Y, Takei Y, Kawagishi M, Hirokawa N. Glutamate-receptor-interacting protein GRIP1 directly steers kinesin to dendrites. Nature. 2002; 417:83–87. [PubMed: 11986669]

Shen L, Liang F, Walensky LD, Huganir RL. Regulation of AMPA receptor GluR1 subunit surface expression by a 4.1 N-linked actin cytoskeletal association. J. Neurosci. 2000; 20:7932–7940. [PubMed: 11050113]

Sheng M, Sala C. PDZ domains and the organization of supramolecular complexes. Annu. Rev. Neurosci. 2001; 24:1–29. [PubMed: 11283303]

Shi S, Hayashi Y, Petralia R, Zaman S, Wenthold RKS, Malinow R. Rapid spine delivery and redistribution of AMPA receptors after synaptic NMDA receptor activation. Science. 1999; 284:1811–1816. [PubMed: 10364548]

Shi S, Hayashi Y, Esteban JA, Malinow R. Subunit-specific rules governing ampa receptor trafficking to synapses in hippocampal pyramidal neurons. Cell. 2001; 105:331–343. [PubMed: 11348590]

Söllner T, Rothman JE. Neurotransmission: harnessing fusion machinery at the synapse. Trends Neurosci. 1994; 17:344–348. [PubMed: 7526506]

Song I, Kamboj S, Xia J, Dong H, Liao D, Huganir RL. Interaction of the *N*-ethylmaleimide-sensitive factor with AMPA receptors. Neuron. 1998; 21:393–400. [PubMed: 9728920]

Songyang Z, Fanning AS, Fu C, Xu J, Marfatia SM, Chishti AH, Crompton A, Chan AC, Anderson JM, Cantley LC. Recognition of unique carboxyl-terminal motifs by distinct PDZ domains. Science. 1997; 275:73–77. [PubMed: 8974395]

Srivastava S, Osten P, Vilim FS, Khatri L, Inman GJ, States BA, Daly C, DeSouza S, Abagyan R, Valtschanoff JG, Weinberg RJ, Ziff EB. Novel anchorage of GluR2/3 to postsynaptic density by an AMPA receptor binding protein ABP. Neuron. 1998; 21:581–591. [PubMed: 9768844]

Standley S, Baudry M. The role of glycosylation in ionotropic glutamate receptor ligand binding, function, and trafficking. Cell Mol. Life Sci. 2000; 57:1508–1516. [PubMed: 11092445]

Staudinger J, Zhou J, Burgess R, Elledge SJ, Olson EN. PICK1: a perinuclear binding protein and substrate for protein kinase C isolated by the yeast two-hybrid system. J. Cell Biol. 1995; 128:263–271. [PubMed: 7844141]

Steward O, Schuman EM. Protein synthesis at synaptic sites on dendrites. Annu. Rev. Neurosci. 2001; 24:299–325. [PubMed: 11283313]

Teasdale RD, Jackson MR. Signal-mediated sorting of membrane proteins between the endoplasmic reticulum and the Golgi apparatus. Annu. Rev. Cell Dev. Biol. 1996; 12:27–54. [PubMed: 8970721]

Thoenen H. Neurotrophins and neuronal plasticity. Science. 1995; 270:593–598. [PubMed: 7570017]

Torres R, Firestein BL, Dong H, Staudinger J, Olson EN, Huganir RL, Bredt DS, Gale NW, Yancopoulos GD. PDZ proteins bind, cluster, and synaptically colocalize with Eph receptors and their ephrin ligands. Neuron. 1998; 21:1453–1463. [PubMed: 9883737]

Tsui CC, Copeland NG, Gilbert DJ, Jenkins NA, Barnes C, Worley PF. Narp, a novel member of the pentraxin family, promotes neurite outgrowth and is dynamically regulated by neuronal activity. J. Neurosci. 1996; 16:2463–2478. [PubMed: 8786423]

Turrigiano GG. AMPA receptors unbound: membrane cycling and synaptic plasticity. Neuron. 2000; 26:5–8. [PubMed: 10798386]

Valtschanoff JG, Burette A, Davare MA, Leonard AS, Hell JW, Weinberg RJ. SAP97 concentrates at the postsynaptic density in cerebral cortex. Eur. J. Neurosci. 2000; 12:3605–3614. [PubMed: 11029631]

Walensky LD, Blackshaw S, Liao D, Watkins CC, Weier HU, Parra M, Huganir RL, Conboy JG, Mohandas N, Snyder SH. A novel neuron-enriched homolog of the erythrocyte membrane cytoskeletal protein 4.1. J. Neurosci. 1999; 19:6457–6467. [PubMed: 10414974]

Walsh MJ, Kuruc N. The postsynaptic density-constituent and associated proteins characterized by electrophoresis, immunoblotting, and peptide sequencing. J. Neurochem. 1992; 59:667–678. [PubMed: 1629737]

Wang Y, Durkin JP. alpha-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, but not *N*-methyl-D-aspartate, activates mitogen-activated protein kinase through G-protein beta gamma subunits in rat cortical neurons. J. Biol. Chem. 1995; 270:22783–22787. [PubMed: 7559406]

Wang Y, Small DL, Stanimirovic DB, Morley P, Durkin JP. AMPA receptor-mediated regulation of a Gi-protein in cortical neurons. Nature. 1997; 389:502–504. [PubMed: 9333240]

Washbourne P, Bennett JE, McAllister AK. Rapid recruitment of NMDA receptor transport packets to nascent synapses. Nat. Neurosci. 2002; 5:751–759. [PubMed: 12089529]

Wenthold RJ, Petralia RS, Blahos J II, Niedzielski AS. Evidence for multiple AMPA receptor complexes in hippocampal CA1/CA2 neurons. J. Neurosci. 1996; 16:1982–1989. [PubMed: 8604042]

Wo Z, Oswald RE. Unravelling the modular design of glutamate-gated ion channels. Trends Neurosci. 1995; 18:161–168. [PubMed: 7539962]

Wyszynski M, Kim E, Yang F-C, Sheng M. Biochemical and immunocytochemical characterisation of GRIP, a putative AMPA receptor anchoring protein in rat brain. Neuropharmacology. 1998; 37:1335–1344. [PubMed: 9849669]

Wyszynski M, Valtschanoff JG, Naisbitt S, Dunah AW, Kim E, Standaert DG, Weinberg R, Sheng M. Association of AMPA receptors with a subset of glutamate receptor-interacting protein in vivo. J. Neurosci. 1999; 19:6528–6537. [PubMed: 10414981]

Xia J, Zhang X, Staudinger J, Huganir RL. Clustering of AMPA receptors by the synaptic PDZ domain-containing protein PICK1. Neuron. 1999; 22:179–187. [PubMed: 10027300]

Ye B, Liao D, Zhang X, Zhang P, Dong H, Huganir RL. GRASP-1: a neuronal RasGEF associated with the AMPA receptor/GRIP complex. Neuron. 2000; 26:603–617. [PubMed: 10896157]

Zerangue N, Schwappach B, Jan YN, Jan LY. A new ER trafficking signal regulates the subunit stoichiometry of plasma membrane K(ATP) channels. Neuron. 1999; 22:537–548. [PubMed: 10197533]

Zhu JJ, Qin Y, Zhao M, Van Aelst L, Malinow R. Ras and Rap control AMPA receptor trafficking during synaptic plasticity. Cell. 2002; 110:443–455. [PubMed: 12202034]

Europe PMC Funders Author Manuscripts

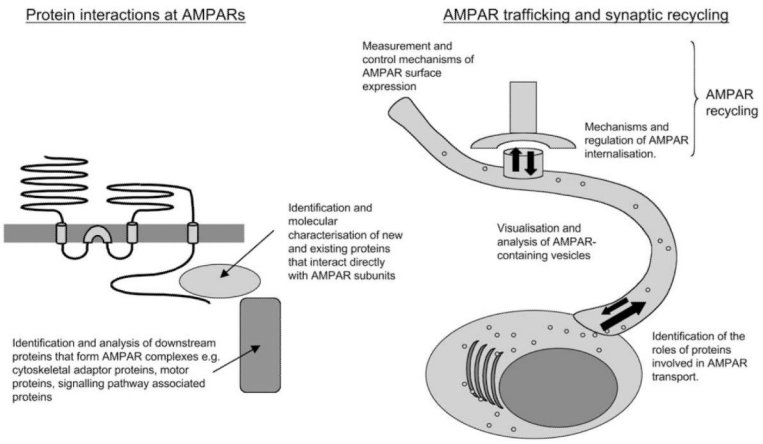

Fig. 1.
Schematic of the outstanding questions in AMPAR trafficking.

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

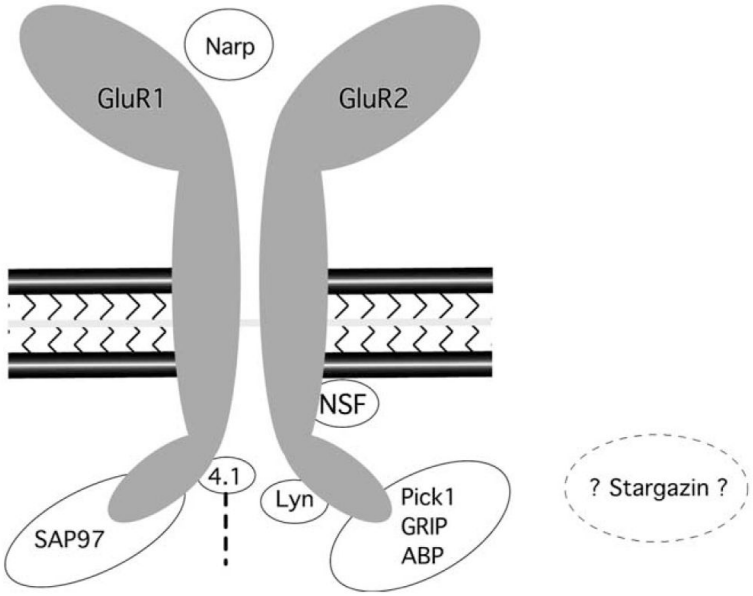

Fig. 2.
Schematic of proteins that bind to GluR1 and GluR2 subunits and their approximate sites of interaction.