AMPAR Receptor Trafficking at Excitatory Synapses

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Characterization of Silent Synapses

A resolution came with the discovery of "silent synapses" (Durand et al., 1996; Isaac et al., 1995; Liao et al., 1995), which have only NMDA receptors and rapidly acquire functional AMPA receptors following LTP. The silent synapse hypothesis gained further support from immunocytochemical (Dong et al., 1999; Gomperts et al., 1998) and immunogold electron microscopic (EM) (Kharaiz and Weinberg, 1999; Nusser et al., 1998; Petralia et al., 1999; Racca et al., 2000; Takumi et al., 1999) studies showing that some synapses contain excitatory synapses in the CNS release glutamate, which acts primarily on two types of ionotropic receptors: AMPA receptors and NMDA receptors. AMPA receptors mediate the postsynaptic depolarization that initiates neuronal firing, whereas NMDA receptors initiate synaptic plasticity. Recent studies have emphasized that distinct mechanisms control synaptic expression of these two receptor classes. Whereas NMDA receptor proteins are relatively fixed, AMPA receptors cycle synaptic membranes on and off. A large family of interacting proteins regulates AMPA receptor turnover at synapses and thereby influences synaptic strength. Furthermore, neuronal activity controls synaptic AMPA receptor trafficking, and this dynamic process plays a key role in the synaptic plasticity that is thought to underlie aspects of learning and memory.

**Summary**

Excitatory synapses in the CNS release glutamate, which acts primarily on two types of ionotropic receptors: AMPA receptors and NMDA receptors. AMPA receptors mediate the postsynaptic depolarization that initiates neuronal firing, whereas NMDA receptors initiate synaptic plasticity. Recent studies have emphasized that distinct mechanisms control synaptic expression of these two receptor classes. Whereas NMDA receptor proteins are relatively fixed, AMPA receptors cycle synaptic membranes on and off. A large family of interacting proteins regulates AMPA receptor turnover at synapses and thereby influences synaptic strength. Furthermore, neuronal activity controls synaptic AMPA receptor trafficking, and this dynamic process plays a key role in the synaptic plasticity that is thought to underlie aspects of learning and memory.

**Introduction**

For more than a century it has been suggested that information storage in the brain involves alteration in the strength of synaptic communication between neurons. Such a cellular mechanism requires that synapses show activity-dependent long-lasting changes. Solid experimental evidence for this first came in 1973, when Bliss and Lomo (1973) showed that brief repetitive activation of hippocampal excitatory synapses causes a persistent enhancement in synaptic strength, referred to as long-term potentiation (LTP). Within the next decade it became clear that LTP is initiated by activation of postsynaptic NMDA-type receptors and a rise in postsynaptic calcium.

**Pre- verses Postsynaptic Site of Expression of LTP**

Although researchers unanimously agree upon the site for LTP induction, a protracted debate has ensued whether LTP is expressed pre- or postsynaptically. The most compelling evidence concerning presynaptic expression came from observations that the synaptic failure rate decreases following induction of LTP (Bekkers and Stevens, 1990; Malinow and Tsien, 1990). Based on classical studies at the neuromuscular junction, a decrease in failure rate argues for an increase in the probability of transmitter release. However, other experiments implicated a postsynaptic modulation for LTP expression (Malenka and Nicoll, 1999; Nicoll, 2003).

**Molecular Biology of AMPA Receptors**

AMPA receptors are glutamate-gated channels whose postsynaptic activation provides the primary depolarization in excitatory neurotransmission in brain. These receptors were originally designated as non-NMDA receptors based on pharmacology and kinetics, which distinguishes them from NMDA receptors. However, molecular cloning demonstrates an array of non-NMDA receptors that include both the AMPA-type and the related kainate receptors.
Figure 1. Schematic Diagram of an AMPA Receptor Subunit

All subunits have a similar structure and topology. The N-terminal domain (NTD) is followed by S1, which together with S2 forms the glutamate binding site. Three of the four hydrophobic segments span the membrane, while one dips into the membrane from the cytoplasmic face and contributes to the channel pore. The alternatively spliced flip/flop region and the C-terminal PDZ binding domain are shown.

GluR1, forms a functional homomeric cation channel in oocytes that is activated by glutamate and pharmacologically resembles brain AMPA receptors. The encoded polypeptide has a hydrophobic signal sequence and four hydrophobic regions, which were predicted to span the plasma membrane as α helices. The topology of the receptor was definitively determined by a combination of epitope-tagging (Bennett and Dingledine, 1995) and analysis of glycosylation (Hollmann et al., 1994). The actual topology shows that a large extracellular N-terminal region is followed by a membrane-spanning domain. The second hydrophobic segment—initially thought to traverse the membrane—only dips into the membrane from the cytosolic side. This region forms the pore of the channel. This pore-forming domain is followed by a true transmembrane domain, an extracellular loop, the third transmembrane region, and finally the cytoplasmic tail (Figure 1).

The GluR1 subunit is broadly expressed in most brain regions but is absent from thalamus and mesencephalon (Hollmann and Heinemann, 1994), which are known to express AMPA-sensitive channels. Subsequent homology cloning identified a family of three additional mRNA species (GluR2, GluR3, and GluR4) that are highly related to the original GluR1 subunit (Hollmann and Heinemann, 1994; Wisden and Seeburg, 1993). These subunits represent the complete family of AMPA receptors in brain.

Modulation of AMPA Receptor Properties by Posttranscriptional Modifications

Although heterologously expressed homomeric GluR1 channels pharmacologically resemble those in brain, the channel properties are distinct. AMPA receptors in principal neurons of forebrain form monovalent cation channels that are nonrectifying and strictly impermeable to calcium. However, homomeric channels formed from GluR1, GluR3, or GluR4 are calcium permeable and inwardly rectifying. Homomeric GluR2 channels express poorly on their own but lack calcium permeability and inward rectification. Heteromeric channels formed by co-expression of GluR2 with any of the other subunits express well and form nonrectifying channels that lack calcium permeability and closely resemble channels from principal neurons in brain. The structural determinant for this difference in ion flow maps to a single arginine residue in the pore-forming region in GluR2 that is a glutamine residue in the corresponding position of GluR1, GluR3, and GluR4 (Hume et al., 1991; Verdoorn et al., 1991). Interestingly, the arginine (R) codon (CGG) in GluR2 is actually encoded as a glutamine (Q) codon (CAG) in the genomic DNA sequence. This “Q/R” discrepancy is explained by posttranscriptional mRNA editing, in which the central adenosine base in the GluR2 Q codon is deaminated (Seeburg, 2002). This editing of the “Q/R” site occurs in >99% of GluR2 mRNA transcripts and explains the distinct sequence of GluR2 mRNAs.

Another structural mechanism that determines channel properties is alternative splicing. The second extracellular region of all four AMPA receptor subunits is alternatively spliced as one of two distinct modules (Sommer et al., 1990), designated “flip” and “flop” (Figure 1). This alternative splicing is regulated both developmentally and regionally and influences the pharmacologic and kinetic properties of the channel. AMPA receptors in cultured neurons show an initial fast desensitizing current response to glutamate or AMPA that decays to a small steady-state plateau, whereas the receptors respond to kainate with a smaller nondesensitizing current. Remarkably, a single point mutation (L483Y) in the glutamate binding site blocks AMPA receptor desensitization (Rosenmund et al., 1998).

The flip and flop forms differ in their responses to glutamate and AMPA relative to kainate. That is, the flop versions desensitize much more rapidly than do the flip forms in response to glutamate (Sommer et al., 1990). Interestingly, desensitization is blocked by certain benzothiadiazides, such as cyclothiazide and diazoxide,
which are clinically used as diuretics. Furthermore, the flop channels are less responsive to cyclothiazide, and a single point mutation (S750Q) in the alternatively spliced flip/flop eliminates allosteric modulation by cyclothiazide (Partin et al., 1995). Definitive insight into the molecular mechanisms for AMPA receptor regulation derives from recent structural studies. Although the structure of a full-length channel is not yet available, considerable insight can be derived from comparison of AMPA receptors to distantly related bacterial potassium channels that have been analyzed by X-ray crystallography (Doyle et al., 1998). The tetrameric structure for these K⁺ channels suggests a similar tetrameric stoichiometry for functional AMPA receptors. In the K⁺ channel structure, the three sets of transmembrane domains from each subunit lie in the membrane in a regular pattern, producing 4-fold symmetry. The pore-forming helix lies in the center of this ring to form the ion channel itself. The edited arginine in GluR2 or the corresponding glutamine in the other subunits would be predicted to occur at the apex of this set of pore-forming helices (Tikhonov et al., 2002) and thereby controls ion selectivity as well as rectification.

Glutamate binding regulates AMPA receptor channel gating, and insight into this mechanism derives from crystallographic studies of the glutamate binding domain. As mentioned above, AMPA receptors have a large first extracellular domain, which comprises a 400 amino acid NTD followed by a 200 amino acid module (S1) that shows homology to the glutamate binding protein (QBP) from bacteria (Figure 1). This homology to an amino acid binding protein suggested that S1 might be the site for glutamate binding to AMPA receptors. However, biochemical studies show that glutamate binding requires both the QBP as well as the second extracellular loop, which follows the first pair of transmembrane domains. This second extracellular domain (S2) also shows distant homology to bacterial amino acid binding proteins. Artificially linking the S1 and S2 domains, by removing the intervening transmembrane segments and by adding a short linker, yields a soluble protein that binds to glutamate and related agonists/antagonists with a pharmacology that closely resembles that of the endogenous receptor.

Gouaux and associates determined the structure of this S1/S2 construct by X-ray crystallography (Armstrong et al., 1998). The S1/S2 structure in complex with glutamate or other agonists shows a bidomain kidney-shaped structure. Consistent with its homology to bacterial QBPs, each domain broadly resembles the previously determined structures for these bacterial proteins. Agonists bind in the interdomain crevice of the structure. Interestingly, the domains of the ligand binding core are expanded in the apo state and contract upon ligand binding (Hogner et al., 2002). The extent of domain closure increases in the order of apo > DNQX > kainate > glutamate = AMPA. These structures indicate that agonist-induced domain closure is transduced to the transmembrane domains to gate the channel.

Although functional AMPA receptors are almost certainly tetrameric, the glutamate binding S1/S2 fragment crystallizes as a monomer. This is consistent with previous work suggesting that the NTD and/or the transmembrane domains mediate receptor assembly (Kuusinen et al., 1999). Interestingly, addition of cyclothiazide induces dimerization of the isolated glutamate binding domains (Sun et al., 2002). A similar dimerization is induced by the L483Y mutation that prevents receptor desensitization. Crystallization of these two dimeric forms of the ligand binding domain shows that the cyclothiazide site and the critical Tyr483 occur at the interface of the subunits. These results suggest that stabilization of the intradimer interface reduces receptor desensitization. Indeed, a structure/function analysis based on the dimer interface indicates that mutants that stabilize the dimer reduce desensitization, whereas perturbations that destabilize the interface enhance desensitization (Sun et al., 2002).

It is curious that the ligand binding domains of the AMPA receptors form dimers with a 2-fold axis of symmetry. This is in contrast to the channel-forming region of the receptors that are likely to have a 4-fold rotationally symmetric arrangement in the membrane. Thus, a functional AMPA receptor might properly be regarded as a dimer of dimers. This has important implications from several perspectives. First, the assembly of receptors may occur sequentially: an initial formation of dimers, which subsequently form a tetramer. Furthermore, the extent of ligand binding to each dimeric unit in a receptor may differentially activate channel activity. Future studies of intact AMPA receptors at the atomic level will be necessary to resolve some of these issues.

AMPA Receptor Assembly and Trafficking
As AMPA receptors are the major determinant of postsynaptic excitability, the density of these receptors must be carefully regulated. Indeed, all aspects of receptor synthesis, trafficking, and degradation are tightly controlled in neurons to tune synaptic responsiveness.

Transcriptional Regulation of AMPA Receptors
The first level for controlling receptor density is synthesis, which is primarily regulated by transcription. AMPA receptor subunit mRNAs are found almost exclusively in neurons and certain glia, including Bergmann glia of cerebellum (Galbo et al., 1992) and oligodendrocyte precursor cells throughout brain (Bergles et al., 2000; Patneau et al., 1994). This transcriptional specificity has been studied in the GluR1 and GluR2 genes and maps to the 5′ promoter regions (Borges and Dingledine, 2001; Myers et al., 1998). Best characterized is the GluR2 promoter, which contains several regulatory elements as well as an RE1-silencing transcription factor (REST) site that silences GluR2 expression in nonneuronal cells. Blockade of synaptic activity increases AMPA receptor transcription, a process that is proposed to give homeostasis in synaptic transmission (Turrigiano et al., 1998). How synaptic activity regulates the GluR2 promoter remains uncertain.

Initial Trafficking of AMPA Receptors
Messages for AMPA receptors are translated on the rough endoplasmic reticulum, and transit through the ER represents the first site for receptor assembly and regulation. Within the ER, the N-terminal signal sequence is removed and a high-mannose glycosylation attaches to specific asparagines residues in the first extracellular domain. Dimerization appears to occur in
the ER, and a recent study indicates that the critical Q/R editing site in GluR2 plays a major role in determining ER trafficking of the receptor (Greger et al., 2002). That is, the R residue in GluR2 impedes ER export of the receptor. This is evidenced by a substantial fraction of GluR2 (but not the other receptor subunits) containing glycosylation with high-mannose sugars. Impeding transit of GluR2 receptors through the ER ensures that a large pool of these receptor subunits is available for assembly (Greger et al., 2002). This may explain why all AMPA receptors in principal neurons are heteromeric and contain the GluR2 subunit.

Following their synthesis in the ER, AMPA receptors transit through the Golgi, where the high-mannose sugars are modified to the more complex carbohydrates seen in mature receptors. Receptors are then trafficked either to dendrites or to axons. Some insight into mechanisms that control polarized protein sorting in neurons comes from studies of the GLR-1 subunit in C. elegans. Although the GLR-1-containing sensory neurons in C. elegans lack distinct axons and dendrites, GLR-1 in neurites of these neurons traffics exclusively to postsynaptic sites and is absent from the nearby presynaptic sites. This polarized sorting of GLR-1 is disrupted by mutations in LIN-10, the C. elegans homolog of MINT-1 (Rongo et al., 1998). Proper polarized sorting of GLR-1 also relies on its C-terminal tail. However, the C-terminal tail of GLR-1 does not interact with LIN-10, indicating that additional proteins are involved in this process.

**Dendritic/Postsynaptic Trafficking of AMPA Receptors**

Within the somatodendritic domain, receptors occur either in cytosolic vesicles or on the plasma membrane at both synaptic and extrasynaptic sites. At early time points during neuronal development, AMPA receptors occur diffusely in the dendrite and later become concentrated at postsynaptic sites (Rao et al., 1998). Some work has begun to define the intracellular membrane compartments of dendrites that contain AMPA receptors. Velocity gradient centrifugation of microsomal light membranes from rat brain identifies a fraction containing GluR2/3 but lacking NMDA receptors (Lee et al., 2001). This compartment sediments more slowly than synaptosomes but faster than synaptic vesicles. This sedimentation is consistent with the intermediate size of these GluR2/3 vesicles, which range between 50 and 300 nm in diameter. These intracellular membranes containing GluR2/3 also cofractionate with certain AMPA receptor binding proteins (GRIIP and PICK1, to be discussed below), which may indicate a role for this compartment in receptor trafficking.

The route AMPA receptors take from intracellular vesicles to synapses is also unclear. A critical question is whether the receptors are directly inserted into synaptic sites or if they are first inserted into the extrasynaptic plasma membrane and later diffuse to synapses. At the neuromuscular junction, nicotinic acetylcholine receptors shuttle between the junctional and perijunctional regions in an activity-dependent manner (Akaaboune et al., 1999, 2002). In cultured neurons, single receptor tracking techniques show that AMPA receptors move freely in the extrasynaptic membrane but lose mobility when they enter a synaptic region (Borgdorff and Choquet, 2002; Choquet and Triller, 2003), suggesting that extrasynaptic receptors serve as a reservoir for synaptic receptors. This notion is further supported by the GluR1 knockout mouse, in which the number of synaptic AMPA receptors is normal but the extrasynaptic receptors are greatly diminished (Zamanillo et al., 1999). Finally, based on a thrombin cleavage assay, Passafaro et al. (2001) tracked the membrane insertion of AMPA receptors in cultured neurons and found that expressed GluR1 receptors initially accumulate at extrasynaptic sites, whereas GluR2 receptors are directly inserted into the synapse.

The ultimate density of AMPA receptors that accumulate at synapses determines synaptic strength. Interestingly, the dendritic spine geometry is critical in determining the density of AMPA receptors. Two-photon uncaging of glutamate in cultured hippocampal neurons allowed functional mapping of glutamate receptors (Matsuzaki et al., 2001). This work showed that AMPA receptors are abundant in large mushroom-shaped spines but are faintly present in smaller spines. These smaller spines, which are thought to be precursors of mushroom-shaped spines, contain NMDA receptors and likely reflect “silent synapses.” These studies show that AMPA receptor density is regulated during spine development and that this regulation occurs at the level of single spines (Petralia et al., 1999).

**Differential Trafficking of GluR Subunits with Long versus Short Cytoplasmic Tails**

An important functional distinction among AMPA receptor subunits is the nature of their cytoplasmic C-terminal tails, which are either long or short. GluR1 always has a long tail and GluR3 a short one. The tails of GluR2 and GluR4 are alternatively spliced such that these subunits can have either long or short tails (Figure 2). Transfection experiments with individual GluR subunits demonstrate that receptors with long versus short tails are differentially delivered to synapses. AMPA receptors containing the GluR1 subunit (long form) require synaptic activity for synaptic delivery, whereas GluR2 (short form) is constitutively inserted into synapses (Passafaro et al., 2001; Piccini and Malinow, 2002; Shi et al., 2001). Furthermore, expression of the C terminus of GluR2 decreases AMPA EPSCs, whereas expression of the GluR1 C terminus has no effect. These dominant-negative experiments suggest that the endogenous receptors behave similarly to the expressed receptors, and, because the endogenous receptors are either GluR1/2 or GluR2/3 heteromers, the results imply that the GluR1 trafficking signal dominates over GluR2. Indeed, when GluR1/2 heteromeric channels are expressed, the activity-dependent trafficking of GluR1 dominates, whereas GluR2/3 heteromeric channels behave like GluR2 homomeric channels and readily move into the synapse.

**AMPA Receptor Interacting Proteins**

Because AMPA receptors themselves lack motor domains, the receptors must associate with protein partners that assist in their trafficking. Indeed, accessory and scaffolding proteins interact with AMPA receptors at numerous subcellular domains. The collection of AMPA receptor partners, which is almost certainly still incomplete, has begun to explain the details of AMPA receptor trafficking.
**PDZ Proteins and Synaptic Scaffolding**

Major insight into mechanisms that regulate trafficking of neuronal receptors, including AMPA receptors, came from the discovery that proteins containing PDZ domains play general roles in scaffolding membrane proteins. PDZ domains are modular protein motifs of approximately 80 amino acids that are found in a variety of dissimilar proteins. These domains often bind to the extreme C-terminal tails of the interacting protein partners; however, PDZ domains can also bind to internal sequences or associate with one another to form protein complexes (Craven and Bredt, 1998; Kornau et al., 1997; Sheng and Sala, 2001). PDZ domain proteins are found throughout the animal kingdom and are even found in bacterial and yeast proteins. Sequencing of the human genome identified >100 PDZ-containing proteins, which makes this one of the most common protein interaction motifs in biology. Additionally, nowhere are PDZ domain proteins found in greater abundance than at neuronal synapses, where they play major roles in organizing receptors and downstream signaling enzymes.

The prototypical PDZ protein is PSD-95/SAP-90, which contains three PDZ domains followed by an SH3 domain and finally a region with homology to guanylate kinase (Cho et al., 1992; Kistner et al., 1993). PSD-95 PDZ domains were first shown to associate with the extreme C-terminal tails of the NR2 subunits of NMDA receptors (Kornau et al., 1997). The C terminus of NR2 receptors terminate with a T/SXV motif that is critical for binding to the type I PDZ domains present in PSD-95. Although PSD-95 does not seem necessary for synaptic clustering of NMDA receptors, PSD-95 links the NMDA receptors to downstream signaling enzymes such as neuronal nitric oxide synthase (Brenman et al., 1996), which is specifically regulated by calcium influx through NMDA receptors.

**Protein Interactions with Long-Form AMPA Receptors**

Although the C termini of AMPA receptor subunits lack the T/SXV sequence and do not bind to PSD-95, the GluR1 subunit binds to the related protein SAP-97 (Leonard et al., 1998). GluR1 terminates with the tetrapeptide, ATGL, which generally conforms to the consensus motif for type I PDZ domain interactions (Figure 2). Indeed, this motif binds specifically to the second PDZ domain from SAP-97 (Cai et al., 2002); surprisingly, the C terminus of GluR1 does not bind to PDZ domains from PSD-95, PSD-93, or SAP-102. Furthermore, the SAP-97/GluR1 binding relies on a tripeptide sequence (SSG) at residues 9–11 near the C terminus of GluR1 (Figure 2).

The role for SAP-97 in regulating AMPA receptors is uncertain. Some work indicates that interactions involving SAP-97 and GluR1 occur early in the secretory pathway—while the receptors are in the endoplasmic reticulum (ER). This conclusion is based on SAP-97’s selective association with receptors having high-mannose glycosylation (Sans et al., 2001). Furthermore, some SAP-97 immunoreactivity occurs in the cytoplasm and decorates the cisternae of the ER, where SAP-97 colocalizes with the GluR1 and GluR2/3 subunits. Both GluR1 and SAP-97 also show some colocalization with BiP, an ER marker within the dendrites of hippocampal neurons. In addition to these cytosolic/ER localizations, SAP-97 also occurs in axon terminals and is highly concentrated at the PSD of asymmetric synapses (Valtschanoff et al., 2000). Importantly, SAP-97 only localizes to the PSD of synapses that contain GluR1.

Transfection of cultured neurons has suggested roles for the GluR1/SAP97 interaction in regulating synaptic AMPA receptors. In dissociated neuronal cultures, transfected SAP-97 concentrates at postsynaptic sites and increases synaptic AMPA receptor density (Rumbaugh et al., 2003). This increase is associated with an enlargement of synaptic spines and an increase in the miniature EPSC frequency. This work is consistent with SAP-97 having both postsynaptic and presynaptic effects on synaptic transmission. However, other experiments involving SAP-97 transfection into hippocampal slice cultures failed to detect a change in the magnitude of AMPA receptor EPSCs (Schnell et al., 2002). Differences between the dissociated and slice culture preparations or alternative splicing of SAP-97 may explain these disparate results. The PDZ binding site at the C terminus of GluR1 also appears necessary for synaptic targeting of these receptors, as transfected GluR1 sub-
units lacking the C-terminal PDZ binding site do not traffic to synapses (Hayashi et al., 2000).

The long forms of AMPA receptor subunits GluR1/4 also participate in non-PDZ domain interactions. The cytoskeletal protein 4.1 binds to the GluR1 C-terminal tail (Figure 2) in a membrane-proximal region that is also conserved with GluR4 (Coleman et al., 2003; Shen et al., 2000). Protein 4.1 associates with GluR1 and GluR4 in vivo and colocalizes with AMPA receptors at excitatory synapses. Mutating the protein 4.1 binding site on GluR4 prevents its surface expression in heterologous cells. Additionally, disruption of actin formation in cultured cortical neurons reduces the surface expression of AMPA receptors, suggesting that protein 4.1 may link AMPA receptors to the neuronal cytoskeleton to mediate surface expression.

Protein Interactions with Short-Form AMPA Receptors

PDZ proteins also interact with the C termini of GluR2 and GluR3 (short-form receptors) (Figure 2). These AMPA receptor subunits terminate with the residues SVKI (Figure 2), which generally conforms to a type II PDZ binding site. Yeast two-hybrid screening with the C-terminal tail of GluR2 yielded two highly related proteins that were named glutamate receptor interacting protein (GRIP) and AMPA receptor binding protein (ABP), which both contain seven consecutive PDZ domains (Dong et al., 1997; Srivastava and Ziff, 1999). Biochemical studies show that PDZ domains 3, 5, and 6 bind to the C termini of GluR2 and GluR3, suggesting that GRIP/ABP could form supramolecular complexes of AMPA receptors. Furthermore, GRIP/ABP bind to themselves and each other through PDZ/PDZ interactions. Mutagenesis reveals a role for GRIP/ABP binding to GluR2 in surface accumulation of AMPA receptors. In transfected hippocampal neurons, a GluR2 mutant lacking the PDZ binding site is transiently transported to the synaptic surface (Osten et al., 2000). However, over time, this mutant does not accumulate at synapses as does wild-type GluR2. Furthermore, mutating a single residue, which blocks binding to GRIP/ABP, reduces the synaptic accumulation of GluR2. These experiments suggest a role for GRIP/ABP in maintaining the synaptic accumulation of the receptors, possibly by limiting receptor endocytosis.

In addition to binding to AMPA receptor subunits, the PDZ domains from GRIP/ABP interact with other synaptic proteins. Yeast two-hybrid screening found that the sixth PDZ domain from GRIP associates with the liprin-α family of proteins that themselves interact with the LAR receptor protein tyrosine phosphatases (Wyszynski et al., 2002). Although liprin-α in C. elegans was isolated in a screen for mutants affecting synaptic targeting of synaptobrevin at nerve terminals, liprin-α in brain is also postsynaptic and coimmunoprecipitates with GRIP/ABP and AMPA receptors. Importantly, dominant-negative constructs that disrupt interaction between GRIP and liprin-α prevent surface expression and dendritic clustering of AMPA receptors. Liprin-α in turn interacts with GIT1, a multidomain protein, that functions as a GTPase-activating activity for the ADP-ribosylation factor family of GTPases that regulate membrane trafficking and actin cytoskeleton (Ko et al., 2003), and expression of dominant-negative constructs that interfere with the GIT1-liprin-α interaction reduce surface clustering of AMPA receptors in neurons.

A domain of GRIP binds to GRIP-associated protein-1 (GRASP-1), a neuronal RasGEF (Ye et al., 2000). GRASP-1 is a modular protein containing a guanine exchange factor site, a Ras binding domain, and a C-terminal PDZ domain. The seventh PDZ domain of GRIP/ABP binds to the GRASP PDZ domain in a PDZ/PDZ domain interaction. Overexpression of GRASP-1 in cultured neurons specifically blocks synaptic targeting of AMPA receptors, suggesting a possible role for GRASP—and thereby neuronal ras signaling—in regulating AMPA receptor distribution.

Although GRIP binds to AMPA receptors at postsynaptic sites of excitatory synapses, highest densities of GRIP/ABP occur in GABAergic neurons and in GABAergic nerve terminals (Wyszynski et al., 1999). GRIP/ABP are also found in neuronal dendrites and cytosol. The synaptic distributions of GRIP and ABP are regulated by their extreme N termini, which are sites for alternative splicing. Importantly, the longer of the two alternatively spliced isoforms of GRIP and ABP contain a cysteine residue that is palmitoylated (Matsuda et al., 1999; Yamazaki et al., 2001), which involves posttranslational addition of a 16-carbon saturated fatty acid (El-Husseini Ael and Breit, 2002). This modification is necessary for trafficking of the long forms of GRIP/ABP to synapses, whereas the unpalmitoylated isoforms reside in the cytosol. In addition to these neuronal localizations, GRIP is widely distributed outside of the brain. Indeed, targeted disruption of GRIP-1 in mice causes embryonic lethality (Bladt et al., 2002). GRIP-1 embryos develop abnormalities of the dermo-epidermal junction, resulting in extensive skin blistering and death at embryonic day 12. Blisters were also found at the lateral ventricle of the brain, suggesting important roles for GRIP at dermo-epidermal junctions as well as at synaptic junctions.

The C termini of GluR2/3 also associate with the PDZ domain of protein kinase Cζ binding protein, PICK1 (Daw et al., 2000; Xia et al., 1999), a postsynaptic scaffold that interacts with several proteins including the Eph family of ephrin receptors (Torres et al., 1998). In heterologous cells, PICK1 induces clustering of AMPA receptors. Overexpression of PICK1 in hippocampal neurons recruits PKCζ to synapses in a manner that is strongly induced by the PKC activator TPA. PICK1 also reduces the plasma membrane levels of GluR2 at synapses consistent with a PKC-facilitated release of GluR2 from its synaptic anchor. As discussed below, differential interactions of GluR2 with PICK1 and GRIP may participate in aspects of activity-dependent synaptic plasticity.

The C-terminal tails of short-form AMPA receptor subunits also associate with non-PDZ proteins that regulate receptor trafficking and plasticity. The C-terminal tails of GluR2/3 associate with N-ethylmaleimide-sensitive fusion protein (NSF), a multimeric ATPase that plays an essential role in membrane fusion (Nishimune et al., 1998; Osten et al., 1998; Song et al., 1998). This NSF interaction occurs in the center of the C-terminal tail of GluR2 upstream from the C-terminal PDZ binding site (Figure 2). As discussed below, this interaction with NSF appears to mediate trafficking or plasma membrane fusion of vesicles containing GluR2.

The mechanism by which NSF regulates AMPA recep-
tor trafficking likely relates to its classical role in controlling membrane fusion. NSF-dependent membrane fusion is central to the SNARE hypothesis, which posits that vSNARE proteins on vesicles mediate membrane fusion by binding specifically to their cognate receptors on the target membrane, the tSNAREs. NSF is recruited to this complex via soluble NSF attachment proteins (SNAPs), which themselves directly bind to the SNARE proteins. The ATPase activity of NSF dissociates the complex, suggesting that NSF functions as a chaperone-like molecule that uses ATP to perturb the structure of associated membrane proteins. In this regard, it is interesting that GluR2 also interacts with the α and β SNAPs (Osten et al., 1998). Furthermore, ATP hydrolysis dissociates this complex (Hanley et al., 2002), analogous to its actions in the regulated assembly of the SNARE membrane fusion apparatus. Interestingly, PICK1 interactions with GluR2 are also disrupted by NSF ATPase activity. Furthermore, α-SNAP dissociates GluR2 from PICK1. In transfected cells, α-SNAP stabilizes GluR2 in the plasma membrane by preventing PICK1-mediated endocytosis. Finally, the NSF binding site on GluR2 also overlaps (Figure 2) with an interaction domain for the clathrin adaptor AP2 (Lee et al., 2002). As discussed below, interactions with this clathrin adaptor protein may participate in aspects of long-term depression.

**Interactions with the Extracellular Domain of AMPA Receptors**

The extracellular domain of AMPA receptors also appears to participate in synaptic trafficking and clustering. The NTD, which precedes the glutamate binding site (Figure 1), interacts with factors that mediate this clustering. The first protein found to interact with the NTD of AMPA receptors is Narp, neuronal activity-regulated pentraxin (O’Brien et al., 1999). Narp was originally identified by a differential screening method to isolate genes whose expression is upregulated by synaptic activity. Narp is similar to a family of secreted multimeric pentraxin proteins. In brain, Narp is expressed in a subset of axons and dendrites and concentrates at excitatory synapses, particularly on the dendritic shafts of aspiny neurons (Mi et al., 2002). Importantly, overexpression of Narp in cultured neurons increases the number of excitatory, but not inhibitory, synapses (O’Brien et al., 1999). Furthermore, Narp recruits AMPA receptors into large aggregates in heterologously transfected cells.

The mechanism by which Narp mediates clustering of AMPA receptors is uncertain; however, mutagenesis indicates that Narp binds to the NTD of AMPA receptors (O’Brien et al., 1999). Furthermore, Narp forms covalent complexes with itself and with its close pentraxin relative NP1 through N-terminal disulfide bonds and coiled-coil domains (Xu et al., 2003). Whereas Narp expression is regulated by activity, NP1 is expressed constitutively in neurons. Together, Narp and NP1 are supradiffusive in their effects on cell surface expression and clustering of AMPA receptors. Because Narp is an immediate-early gene regulated by synaptic activity, this may represent a novel mechanism for activity-dependent clustering of AMPA receptors.

Whereas Narp and NP1 interact with all AMPA receptor subunits, the extracellular domain of GluR2 specifically induces dendritic spines (Passafaro et al., 2003). Overexpression of GluR2 in hippocampal cultures increases spine size and density in excitatory neurons, and, remarkably, induces spine formation in GABA interneurons that otherwise lack spines. This influence of GluR2 is mediated by the extracellular NTD and is not observed with the NTD of GluR1 or GluR3. Furthermore, fusion proteins of the GluR2 NTD perturb spine morphogenesis, suggesting that this pathway may contribute to endogenous spine formation (Passafaro et al., 2003). It will now be important to investigate spine morphology in mutant mice lacking GluR2, which have previously been generated and display specific neuronal phenotypes (Jia et al., 1996).

**Stargazin Regulation of AMPA Receptors**

Stargazin is the first transmembrane protein found to interact with AMPA receptors (Chen et al., 2000). Stargazin is the protein mutated in stargazer mice, which suffer absence epilepsy and cerebellar ataxia (Letts et al., 1998). This cerebellar ataxia is associated with complete absence of functional AMPA receptors in cerebellar granule cells. Genetic mapping of the stargazer locus originally identified the stargazin gene, which encodes a protein containing four transmembrane domains and a cytoplasmic C-terminal tail, which culminates with a PDZ binding site. Stargazin shows some homology to the γ-subunit of voltage-dependent calcium channels (Letts et al., 1998). This homology to calcium channels fits with the epileptic phenotype, as numerous human and mouse models of epilepsy reflect mutations in calcium channels. However, a calcium channel defect does not readily explain the absence of AMPA receptors in cerebellar granule cells.

Cellular and biochemical studies suggest mechanisms for stargazin regulation of AMPA receptors. Stargazin interacts with all four AMPA receptor subunits, and the PDZ binding site at the C terminus of stargazin associates with the postsynaptic density protein PSD-95 (Chen et al., 2000). Transfection of stargazin into dissociated cerebellar granule cell culture rescues the synaptic AMPA receptor defect. This rescue occurs in the presence of Cd²⁺, which blocks all types of calcium channels, and implies that effects of stargazin on AMPA receptors are independent of calcium channels (Chen et al., 2000). Synaptic targeting of stargazin and associated AMPA receptors requires interaction of the C-terminal PDZ binding site on stargazin with PSD-95. Furthermore, transfection of granule cells with a stargazin isoform lacking the PDZ domain fails to rescue the synaptic AMPA receptors (Chen et al., 2000).

A fascinating aspect of the stargazer phenotype is that extrasynaptic AMPA receptors are also absent in these mice. Although extrasynaptic receptors are not activated by synthetically released glutamate, these receptors can be functionally assessed by bath application of glutamate. Interestingly, transfection of a stargazin mutant lacking the C-terminal PDZ binding site efficiently rescues the extrasynaptic receptors even though it fails to restore the synaptic receptors (Chen et al., 2000). This implies a two-step model for stargazin trafficking of AMPA receptors. That is, for reasons that are as yet unclear, AMPA receptors in cerebellar granule require stargazin to reach the plasma membrane. Stargazin lacking its C-terminal tail escorts AMPA receptors to the cell surface, and, in a second step dependent
upon stargazin interaction with PSD-95, the receptors are delivered to the synapse. This second step of synaptic delivery by stargazin and PSD-95 is regulated by reversible palmitoylation of PSD-95 (Craven et al., 1999; El-Husseini Ael et al., 2002) and may play roles in activity-dependent synaptic plasticity.

The cellular mechanism by which stargazin escorts AMPA receptors to the plasma membrane remains uncertain. This activity can be reproduced in nonneuronal cell and in oocytes. GluR1 was originally cloned by expression in oocytes, so this receptor certainly gets to the plasma membrane when expressed alone (Hollmann and Heinemann, 1994). However, co-injection of oocytes with stargazin massively increases the density of surface AMPA receptors (Chen et al., 2003). This effect is general, as stargazin increases the surface expression of other AMPA receptor subunits. However, the effect is specific, as stargazin does not traffic the kainate receptor GluR6. This specificity fits with the stargazer phenotype, as kainate receptors occur at normal levels on the cerebellar granule cells in stargazer mice (Chen et al., 2003).

Although AMPA receptors are absent from the plasma membrane in cerebellar granule cells in stargazer mice, neurons in forebrain show normal AMPA receptor expression. This suggests either that the cerebellar granule cells are unique in their requirement for stargazin or that related proteins mediate AMPA receptor trafficking in forebrain. Indeed, stargazin is part of a large family of four-pass transmembrane proteins that include γ-subunits of calcium channels and the claudin proteins. Transfection of cerebellar granule cells from stargazer mice with various members of this family identified a subset of four related proteins (stargazin, γ-3, γ-4, and γ-8) that can rescue AMPA receptors in these mutant cells (Tomita et al., 2003). These four proteins are designated the transmembrane AMPA receptor regulatory proteins (TARPs). The TARPs show differential distributions in brain (Tomita et al., 2003). Stargazin is highly expressed in cerebellar granule cells, which explain the defect in stargazer mice. γ-3 occurs in high levels in the cerebral cortex; γ-4 occurs diffusely in brain but is concentrated in corpus striatum and in glia; and γ-8 shows expression specifically in the hippocampus (Figure 3). Mutant mice with targeted disruptions of the TARPs will be necessary to determine roles for these proteins in trafficking of AMPA receptors in forebrain.

Effects of stargazin and PSD-95 on synaptic and extrasynaptic expression of AMPA receptors are also evident in hippocampal cultures. Overexpression of PSD-95 in either dissociated (El-Husseini et al., 2000) or slice cultures (Schnell et al., 2002) causes a large increase in synaptic AMPA receptors. On the other hand, overexpression of stargazin does not alter the synaptic density of AMPA receptors, but remarkably causes a 5- to 10-fold increase in total surface AMPA receptors (Schnell et al., 2002). These results imply that stargazin is the limiting factor for surface expression of AMPA receptors in neurons and that more than 80%–90% of AMPA receptors in hippocampal neurons occur beneath the plasma membrane. This later finding suggests that a large pool of cytosolic AMPA receptor exists for dynamic recruitment by stargazin under physiological conditions.

Finally, overexpression of a stargazin mutant lacking the C-terminal PDZ binding increases surface AMPA receptors but strips away synaptic receptors. These experiments—schematized in Figure 4—suggest that the density of stargazin/PSD-95 at synapse can control the number of synaptic AMPA receptors.

**Presynaptic AMPA Receptors**

Whereas AMPA receptors primarily mediate postsynaptic action, these receptors also occur at certain presynaptic sites. During neuronal development, fine filopodia in the distal axon arbor show high motility and are thought to initiate and form synaptic connections. Glutamate acting on AMPA receptors inhibits the motility of these filopodia (Chang and De Camilli, 2001). Depolarization rapidly recruits axonal AMPA receptors to the surface; furthermore, a fraction of GluR1 and GluR2 copurify with synaptic vesicles, suggesting that vesicular AMPA receptors occur at the nerve terminal and can be recruited to the presynaptic membrane (Schenk et al., 2003).

Presynaptic AMPA receptors can also regulate neurotransmitter release. An elegant physiological study showed that glutamate released from climbing fibers in the cerebellar cortex not only depolarize Purkinje cells but also inhibit GABA release from basket cells impinging on the same Purkinje cells (Satake et al., 2000). These experiments suggest that AMPA receptors occur at the terminals of the basket cells and somehow inhibit transmitter release. It remains uncertain whether AMPA receptor-mediated presynaptic inhibition of release is widespread in brain. The mechanism for trafficking AMPA receptors to presynaptic sites is also unclear; however, it is intriguing that basket cell terminals are unique in having presynaptic PSD-95 (Kistner et al., 1993), which clusters stargazin and AMPA receptors at postsynaptic sites.

**Regulation of AMPA Receptor Function during LTP**

Molecular cloning of AMPA receptors and identification of AMPA receptor interacting proteins have provided insight into the mechanisms that control receptor trafficking during synaptic plasticity. The balance of this review will focus on how AMPA receptor trafficking is controlled during LTP and LTD. We will emphasize how protein interactions with GluR subunits (long versus short; summarized in Figure 5) differentially control receptor trafficking and plasticity.

**Second Messenger Cascades in LTP**

LTP induction requires activation of the NMDA receptor and a rise in dendritic spine calcium, and this calcium rise appears sufficient for LTP (Malenka et al., 1989; Yang et al., 1999). Experiments using pharmacological agents and knockout mice suggest that calcium/calmodulin kinase II (CaMKII) is necessary for LTP (Lisman et al., 2002). In addition, increasing constitutively active CaMKII in neurons (Lledo et al., 1995; Poncer et al., 2002; Shirke and Malinow, 1997) mimics and occludes with LTD (Lledo et al., 1995). These results strongly imply that CaMKII is both necessary and sufficient for LTP (Lisman et al., 2002).

Two general mechanisms have emerged for explaining how CaMKII enhances AMPA receptor function, one involving direct phosphorylation of AMPA receptor...
subunits and the other involving delivery of AMPA receptors to the synapse. CaMKII phosphorylates the C terminus of GluR1, which is also phosphorylated during LTP (Barria et al., 1997b). Closer analysis revealed that CaMKII phosphorylates GluR1 at amino acid S831 (Barria et al., 1997a; Mammen et al., 1997), and this same site is phosphorylated during LTD (Lee et al., 2000). Phosphorylation of S831 increases the single-channel conductance of GluR1 (Derkach et al., 1999), which also increases during LTD (Benke et al., 1998). LTD is diminished in a mouse in which S831 was mutated to prevent phosphorylation (Lee et al., 2003). However, S842, a substrate of protein kinase A (PKA) phosphorylation, was also mutated in this mouse. These findings suggest that CaMKII phosphorylation of synaptic AMPA receptors at S831 can directly contribute to LTD. However, mutating S831 does not impair CaMKII-induced delivery of GluR1 to the synapse in slice culture (Hayashi et al., 2000). Taken together, these results suggest that there are two distinct mechanisms involved in enhancement of AMPA receptor function during LTP: a covalent modification of preexisting synaptic AMPA receptors (at S831) and an activity-dependent delivery of synaptic AMPA receptors.

A number of other kinases have been more tentatively linked to LTD. PKA is implicated in various aspects of NMDA receptor-dependent LTD, especially in a protein synthesis-dependent late phase (Abel et al., 1997; Impey et al., 1996). However, other studies have addressed the role for PKA in the early phase (first hour) of NMDA receptor-dependent LTD. Pharmacological blockade of PKA (Blitzer et al., 1995; Otmakhova et al., 2000) reduces LTD, suggesting a “gating” role for PKA in LTD (Blitzer et al., 1995). PKA phosphorylation of S845 in GluR1 increases the peak open probability (Banke et al., 2000). LTD decreases phosphorylation of this site (see below), whereas subsequent LTD increases S845 phosphorylation (Lee et al., 2000). In addition, phosphorylation of S845 accompanies the surface reinsertion of GluR1 (Ehlers, 2000). Pharmacological manipulations together with site-directed mutagenesis of S845 of GluR1 showed that phosphorylation of this site is necessary but not sufficient for the delivery of GluR1 to synapses during LTD (Malinow, 2003). The critical role of PKA phosphorylation of S845 could explain the impairment of LTD in the knockin mice in which both S831 and S845 were mutated (Lee et al., 2003).

Because CaMKII levels are low during the first postnatal week (Kelly and Vernon, 1985), Yasuda et al. (2003) recently addressed the signaling cascades involved in LTD during development. They found that LTD depends upon CaMKII at ages greater than postnatal day 20 (P20) but is independent of CaMKII at P7–P8. In contrast, inhibition of PKA blocked LTD at P7–P8 but had no effect at >P27. Protein kinase C (PKC) (Ling et al., 2002), MAP kinase (English and Sweatt, 1997; Zhu et al., 2002), and PI3 kinase (Man et al., 2003) have also been implicated in LTD. Whether these other kinases play permissive roles or are downstream of CaMKII is not entirely clear, although recent work on MAP kinase (Zhu et al., 2002) argues for a downstream role.

**SAP-97/GluR1 Interaction in LTD**

The PDZ ligand on GluR1 appears critical for activity-dependent trafficking of AMPA receptors, since mutating this site prevents incorporation of GluR1 into both spines visualized with microscopy (Piccini and Malinow, 2002) and synapses measured electrophysiologically (Hayashi et al., 2000). In addition, surface expression

**Figure 3. Differential Distribution of Star- gazin-like TARP**s in Brain

In situ hybridization shows the localization of TARP mRNAs in sagittal sections of rat brain. Stargazin (STG, green) is concentrated in cerebellum and cerebral cortex, γ-3 (blue) is expressed in cerebral cortex, γ-4 (purple) is expressed diffusely, and γ-8 (orange) is concentrated in hippocampus. A merged image of these distributions is below.
Figure 4. Regulation of AMPA Receptors by Stargazin and PSD-95
Schematic model demonstrates how stargazin (STG) and PSD-95 control AMPA receptor density at synaptic and extrasynaptic sites. (A) AMPA receptors occur both at the cell surface in association with stargazin and in cytoplasmic vesicles lacking stargazin. The C-terminal tail of stargazin associates with PSD-95, and this anchors associated AMPA receptors to synapses. (B) Overexpression of PSD-95 GFP recruits extrasynaptic AMPA receptors to the synapse. (C) Overexpression of stargazin GFP shuttles cytoplasmic AMPA receptors to the plasma membrane but does not increase the number of synaptic AMPA receptors, which requires additional PSD-95. (D) Overexpression of stargazin lacking the C-terminal PDZ binding site (STG/H9004C) similarly brings cytoplasmic AMPA receptors to the cell surface but also decreases synaptic AMPA number due to lack of STG/H9004C binding to PSD-95.

and inducible exocytosis of GluR1 is impaired when the C terminus is mutated (Passafaro et al., 2001). It is postulated that LTP activates CaMKII, which then phosphorylates an unknown protein that in turn interacts with the PDZ motif of GluR1 (Hayashi et al., 2000; Shi et al., 2001). While an obvious candidate for this interacting protein is SAP97, the synaptic clustering of GluR1-containing receptors in dissociated culture appears to be normal in mice expressing a truncated form of SAP97 (Klocker et al., 2002). Since SAP-97 is the only protein known to bind to the PDZ domain of GluR1, it will be of interest to examine further the possible role of SAP97 in LTP and determine if other scaffolding proteins can bind to the C terminus of GluR1.

Figure 5. Trafficking of Synaptic AMPA Receptors in LTP and LTD
Glutamate receptors that contain a subunit with a long cytoplasmic tail require activity for delivery to synapse. A number of proteins including CaMKII, PKA, SAP-97, and TARPs are thought to participate in this delivery. Some evidence suggests that the receptors may be initially inserted extrasynaptically by exocytosis and then shuttled laterally to the synapse. Dephosphorylation of residues in the long-tailed receptors may participate in LTD. Receptors that contain only short tails constitutively cycle into and out of the synapse. Proteins including calcineurin, PP1, NSF/AP2, PICK1, and ABP/GRIP regulate various steps in constitutive receptor cycling and/or LTD.
**Endogenous AMPA Receptors and LTP**

Much of the work on AMPA receptor trafficking has involved the use of recombinant AMPA receptors. Although this approach permits study of isoform specificity in trafficking, the overexpression of homomeric receptors involved in these experiments is a limitation. Perhaps the most convincing study of endogenous receptor trafficking is that of Liu and Cull-Candy (2000). These authors found that tetanic stimulation of cerebellar parallel fiber synapses onto stellate cells rapidly changes the property of the synaptic AMPA receptors. Normally, these receptors lack GluR2 and show pronounced inward rectification. However, within 15–30 min of tetanic stimulation, the synaptic response loses its rectification due to the replacement of GluR2-lacking AMPA receptors with ones containing GluR2. This switch in AMPA receptors is triggered by calcium entry through the GluR2-lacking receptors. In other experiments, to address whether endogenous AMPA receptors are driven to the synapse during LTP (Shi et al., 2001) and by experience (Takahashi et al., 2003), the cytoplasmic tail of GluR1, which should disrupt the protein–protein interactions with the C terminus of GluR1, has been used. Expression of this tail prevents LTP and the experience-dependent delivery of AMPA receptors. The large enhancement in endogenous AMPA receptor EPSCs that results from expression of PSD-95 (Schnell et al., 2002) shares many features with LTP (Stein et al., 2003). In addition, this enhancement occludes LTP and greatly increases LTD (Stein et al., 2003), indicating a shared mechanism.

A number of studies have used LTP models in dissociated cell culture to examine the activity-dependent trafficking of endogenous AMPA receptors. In most cases these studies have relied on the analysis of miniature EPSCs and pharmacological treatments, but a thorough characterization of this “LTP” has not been performed. Nevertheless, brief pharmacological activation of NMDA receptors in culture can rapidly cause insertion of endogenous AMPA receptors. In other experiments, to test whether endogenous AMPA receptors are driven to the synapse during LTP (Shi et al., 2001) and by experience (Takahashi et al., 2003), the cytoplasmic tail of GluR1, which should disrupt the protein–protein interactions with the C terminus of GluR1, has been used. Expression of this tail prevents LTP and the experience-dependent delivery of AMPA receptors. The large enhancement in endogenous AMPA receptor EPSCs that results from expression of PSD-95 (Schnell et al., 2002) shares many features with LTP (Stein et al., 2003). In addition, this enhancement occludes LTP and greatly increases LTD (Stein et al., 2003), indicating a shared mechanism.

In summary, the GluR1 subunit of the AMPA receptor apparently plays a role in LTP. Consistent with this, mature GluR1 knockout mice lack LTP in the CA1 region of hippocampus (Zamanillo et al., 1999), and the defect is rescued by genetically expressing GluR1 (Mack et al., 2001). However, that these same mature mice show some LTP in the dentate gyrus (Zamanillo et al., 1999) and also show LTP in the CA1 region in young mice (Mack et al., 2001) indicates that GluR1-independent mechanisms for LTP exist. It is possible that GluR4, which shares similarities in its C-terminal tail, can substitute for GluR1 in the young animals (Zhu et al., 2000). In addition, the presence of hippocampal LTP in the GluR2/3 knockout mice (Meng et al., 2003) also supports the central role for GluR1 (the remaining subunit) in LTP.

**Does LTP Play a Role in Synapse Development?**

Processes underlying LTP have been postulated to also underlie aspects of synapse development. This predicts that synapses would initially have only NMDA receptors and as a consequence of NMDA receptor activation would acquire AMPA receptors. Indeed, in slices (Durand et al., 1996; Hsia et al., 1998; Isaac et al., 1997; Rumpel et al., 1998), high-density cultures (Liao et al., 1999), and intact optic tectum (Wu et al., 1996), newly formed synapses generate primarily NMDA receptor-mediated responses. Furthermore, anatomical studies demonstrate that newly formed synapses are relatively deficient in AMPA receptors (Petralia et al., 1999). In the barrel cortex, the ability to generate LTP can occur at thalamocortical synapses only during a narrow window that corresponds to the critical period, suggesting that LTP participates in the synaptic rearrangement that occurs during this period (Cair and Malenka, 1995).

However, an essential role for NMDA receptor activity or any synaptic activity in synapse formation has been challenged. Chronic removal of NMDA receptor function either by genetic deletion (Kutsuwada et al., 1996; Nakazawa et al., 2002) or by pharmacological blockade (Gomperts et al., 2000; Liao et al., 1999; Luthi et al., 2001; Rao and Craig, 1997) does not prevent appearance of synaptic AMPA receptors. In addition, blockade of all known glutamate receptors and addition of tetrodotoxin to block action potentials do not alter the initial formation of excitatory synapses. In autaptic culture preparations, both AMPA and NMDA currents appear as soon as synaptic transmission is detectable (Gomperts et al., 2000). Transient blockade of NMDA receptors during synapse formation in slice culture delays the acquisition of synaptic AMPA receptors, but chronic blockade of NMDA receptors has no effect (Zhu and Malinow, 2002). These results suggest that, although NMDA-dependent mechanisms contribute to the recruitment of synaptic AMPA receptors, NMDA-independent mechanisms can fully substitute.

**Regulation of AMPA Receptor Function during LTD**

NMDA receptor-dependent LTD, discovered after LTP (Dudek and Bear, 1992), is induced by low-frequency synaptic stimulation (~1 Hz) for a prolonged period (~15 min). That activation of the NMDA receptor in a hippocampal neuron can cause either LTP or LTD might seem paradoxical. It appears that LTD requires a high level of intracellular calcium that acts on a low-affinity calcium-dependent kinase, i.e., CaMKII. One the other hand, LTD involves a high-affinity calcium-dependent phosphatase cascade involving calcineurin and protein phosphatase 1 (Lisman, 1989; Mulkey et al., 1993, 1994). Studies linking these latter processes to the downregulation of synaptic AMPA receptor function have provided important insight into the cellular mechanisms underlying LTD.

**Activity Removes Synaptic AMPA Receptors**

A variety of manipulations can downregulate synaptic AMPA receptors. Initial experiments using chronic treatments showed that blocking synaptic inhibition with picROTOXIN for a week in hippocampal cultures markedly decreases the number of synaptic AMPA receptors and the size of the AMPA receptor EPSC but causes little change in synaptic NMDA receptors or the NMDA receptor EPSC (Lissin et al., 1998). Similar manipulations in spinal (O’Brien et al., 1998) or cortical (Turrigiano et al., 1998) cultures also decrease the amplitude of miniature.
AMPA receptor EPSCs. Unlike the results in the hippocampus, studies in cortical neurons found that the change in the AMPA receptor EPSC is accompanied by a similar change in the NMDA receptor EPSC (Watt et al., 2000). These studies show that neurons modulate their synaptic strength by changing the number of synaptic AMPA receptors in an activity-dependent manner. However, given the chronic nature of the treatments, it is unclear whether these results have direct bearing on the specific mechanisms underlying LTD.

These initial studies were followed up by studies in which more rapid changes in activity were induced. Using field stimulation in neuronal cultures, Carroll et al. (1999b) developed a protocol that induces a long-lasting depression that closely resembles NMDA receptor-dependent LTD in slices. With this preparation, LTD was accompanied by a selective loss of GluR1-labeled synaptic puncta within 15 min. Other experiments show that agonist binding to the AMPA receptor induces a rapid decrease in labeling of synaptic AMPA receptors (Beattie et al., 2000; Carroll et al., 1999a; Lin et al., 2000; Lissin et al., 1999; Zhou et al., 2001).

There is some disagreement on the mechanism by which the selective activation of AMPA receptors causes their internalization. Initial studies suggested that the internalization might be the direct consequence of agonist occupancy of the receptor (Lissin et al., 1999). In support of this mechanism, chelating intracellular calcium with BAPTA-AM has no effect on the internalization (Ehlers, 2000). However, voltage-dependent calcium channel blockers suppress AMPA receptor endocytosis (Beattie et al., 2000; Lin et al., 2000; Zhou et al., 2001), suggesting that calcium does play a role and that more than one mechanism may be involved. Disagreement also exists concerning the fate of the AMPA receptors internalized by AMPA application. Lin et al. (2000) concluded that the receptors quickly reappear on the cell surface, whereas Ehlers (2000) found that these AMPA receptors are targeted to late endosomes and lysosomes. Finally, actin depolymerization was found to be involved in AMPA receptor internalization in one study (Zhou et al., 2001) but not in another (Beattie et al., 2000).

Activation of NMDA receptors (Beattie et al., 2000; Carroll et al., 1999a; Ehlers, 2000; Zhou et al., 2001), mGluR receptors (Snyder et al., 2001; Xiao et al., 2001), and insulin receptors (Lin et al., 2000; Man et al., 2000) can also cause a loss of synaptic AMPA receptors. NMDA-induced AMPA receptor endocytosis (Beattie et al., 2000; Ehlers, 2000; Zhou et al., 2001) resembles LTD in its requirement for calcium influx and activation of the calcium-dependent protein phosphatase calcineurin (Beattie et al., 2000; Ehlers, 2000). Interestingly, calcineurin regulates endocytosis of synaptic vesicles in nerve terminals. The NMDA-induced endocytosis of AMPA receptors is followed by rapid membrane reinsertion of the receptors (Ehlers, 2000).

What Is the Mechanism for the Activity-Dependent Loss of Surface AMPA Receptors?

One of the initial steps for AMPA receptor endocytosis may involve ubiquitination. Recent work on GRIP-1, an AMPA receptor subtype in C. elegans, indicates that the level of receptor ubiquitination controls the abundance of synaptic receptors (Burbea et al., 2002). Furthermore, the loss of synaptic receptor by ubiquitination was prevented by a genetic mutation that blocked endocytosis, suggesting that ubiquitination of the receptor precedes endocytosis. A major mechanism for removal of surface receptors is via clathrin-dependent endocytosis. A GFP-tagged clathrin construct helped identify specific dendritic membrane domains adjacent to the postsynaptic density that are dedicated to endocytosis (Bianpiheid al., 2002). Thus, synaptic receptors destined for endocytosis must first migrate to juxtasynaptic sites. Activated AMPA receptors colocalize with AP2, a marker of endocytic-coated pits, and AMPA receptor endocytosis is blocked by expression of a dominant-negative form of dynamin, which also prevents formation of clathrin-coated pits (Carroll et al., 1999a).

The studies discussed above suggest that hippocampal LTD, which is accompanied by a decrease in the number of synaptic AMPA receptor puncta (Carroll et al., 1999b), may involve clathrin-dependent endocytosis of AMPA receptors. Blockade of endocytosis with either GDPβS or a peptide that prevents the interaction between amphiphysin and dynamin causes a run up in basal synaptic transmission, presumably due to blockade of constitutive cycling of AMPA receptors, and inhibition of LTD (Luscher et al., 1999). Insulin has been reported to cause internalization of AMPA receptors in the hippocampus, and this depression occludes LTD (Man et al., 2000). In addition, inhibiting postsynaptic clathrin-mediated endocytosis blocks both LTD and the insulin-induced depression. Similar results were found for cerebellar LTD (Wang and Linden, 2000), indicating a shared mechanism between these two forms of LTD.

Short- versus Long-Tailed GluR Subunits and LTD

As discussed above, GluR1 (a long-tailed subunit) is critical for LTP; however, GluR1 has also been implicated in LTD. During LTD, the PKA site on GluR1, S845, is dephosphorylated, whereas LTD induction in previously potentiated synapses leads to dephosphorylation of the CaMKII site, S831 (Lee et al., 2000). In mice in which these two sites have been mutared, LTD is absent and AMPA receptor internalization induced by NMDA application is also lacking (Lee et al., 2003). The mechanism by which the phosphorylation state of GluR1 affects AMPA receptor internalization is unknown.

Most studies of LTD have focused on the role of the short-tailed AMPA receptor subunits GluR2/3, which, as covered earlier, have a type II PDZ ligand motif shown to interact with GRIP1/ABP (Dong et al., 1997, 1999; Srivastava et al., 1998) and PICK1 (Dev et al., 1999; Xia et al., 1999). In addition, the cytoplasmic tail of GluR2 binds to NSF (Nishimune et al., 1996; Osten et al., 1998; Song et al., 1998). As discussed below, these interactions appear critical for LTD.

GluR2-NSF Interaction and LTD

The role for the NSF/GluR2 interaction has been probed by applying intracellularly a short peptide (pep2m/G10) that mimics the NSF interaction site on GluR2 (Nishimune et al., 1998). Acute application of the peptide via the patch pipette causes a rapid and selective decrease in the AMPA EPSC that stabilizes at approximately a 20%-40% depression (Kim and Lisman, 2001; Luscher et al., 1999; Luthi et al., 1999; Nishimune et al., 1998; Noel et al., 1999; Shi et al., 2001), suggesting the existence of two pools of receptor, one labile and one fixed. This depression does not occur in the GluR2 knockout mouse.
(Shi et al., 2001), emphasizing the selective effect of this peptide. The peptide-induced depression reflects loss of synaptic AMPA receptors, since immunocytochemical studies show a marked decrease in surface AMPA receptors (Luscher et al., 1999; Noel et al., 1999). The effects of mutating the NSF binding site in GluR2 have given seemingly conflicting results. In slice culture, the mutated GluR2 is not delivered to the synapse as assayed electrophysiologically (Shi et al., 2001). However, a similar receptor construct is delivered normally to the cell surface in dissociated cultures as assayed immunocytochemically (Braithwaite et al., 2002; Lee et al., 2002), but the regulated internalization of these mutant receptors was impaired. This observation may help explain why the peptide-induced depression of AMPA-EPSCs is dependent on synaptic stimulation (Luscher et al., 1999). The NSF-dependent delivery mechanism also appears to mediate LTD because the peptide-induced depression occludes LTD (Luscher et al., 1999; Luthi et al., 1999).

Recent results (Lee et al., 2002) suggest that the action of the NSF peptide is complex. The clathrin adaptor protein AP2 interacts with a site that overlaps with the NSF binding site, and the pep2m/G10 peptide disrupts both NSF and AP2 binding (Figure 2). Remarkably, a more specific peptide that selectively disrupts the NSF interaction still depresses synaptic transmission, but this depression does not occlude LTD. In addition, a peptide that selectively disrupts the AP2 interaction causes no change in synaptic responses but completely prevents LTD. These results suggest that the NSF/GluR2 interaction maintains synaptic strength by promoting the cycling of GluR2-containing AMPA receptors, whereas AP2 removes synaptic AMPA receptors during LTD.

**PDZ Domain Interactions with GluR2/3 in LTD**

The type II PDZ ligand in GluR2 also regulates synaptic plasticity. Importantly, a critical serine (S880) within the PDZ binding site is phosphorylated by PKC, and this phosphorylation prevents the association of GluR2 with GRIP and ABP (Chung et al., 2000; Matsuda et al., 1999, 2000) but promotes binding to PICK1 (Chung et al., 2000; Matsuda et al., 1999; Perez et al., 2001). Phosphorylation of S880 disperses GluR2 clusters and decreases surface GluR2-containing receptors in both cerebellar Purkinje neurons (Matsuda et al., 2000) and hippocampal neurons (Chung et al., 2000; Perez et al., 2001).

The role of this phosphorylation in LTD has been most thoroughly studied in the cerebellum. Transfecting a GluR2 construct with a point mutation that prevents phosphorylation of S880 into Purkinje cells fails to rescue LTD in the GluR2 knockout mouse (Chung et al., 2003). These data suggest that AMPA receptors are stabilized at the synapse by the binding of GluR2-containing receptors to GRIP/ABP and that disrupting this binding results in loss of synaptic receptors (Matsuda et al., 2000). However, peptides that specifically prevent binding of PICK1 to GluR2 also block LTD (Xia et al., 2000). Thus, PICK1 may either prime the receptors for internalization or, alternatively, PICK1 may bind to internalized AMPA receptors and prevent their reinsertion (Xia et al., 2000).

The role of PDZ protein interactions with GluR2 in NMDA receptor-dependent LTD is less clear. Although hippocampal LTD is accompanied by a phosphorylation of S880, it is not mediated by PKC (Kim et al., 2001), and hippocampal LTD does not have the appropriate pharmacology to implicate a direct role for PKC. Nevertheless, a small peptide that prevents binding of GluR2 to both GRIP/ABP and PICK1, and another peptide that blocks GluR2 binding to PICK1 but not to GRIP/ABP, enhances baseline synaptic responses and inhibits hippocampal LTD (Kim et al., 2001). However, results from another laboratory (Daw et al., 2000; Hirbec et al., 2003) appear to be somewhat at odds with this scenario. Infusing the peptide that disrupts GluR2 binding to GRIP/ABP and PICK1 enhances baseline responses, albeit in a subset of inputs, and blocks LTD. Interestingly, these effects of the peptide required PKC activity. However, a peptide that specifically blocks the interaction with PICK1 had no effect, either on baseline responses or on LTD. Thus, these authors postulate that the GRIP/ABP interaction helps stabilize an intracellular pool of AMPA receptors and that this is regulated by PKC phosphorylation of S880 in GluR2.

**Comparison of Cerebellar and NMDA Receptor-Dependent LTD**

Since hippocampal LTD and cerebellar LTD both involve activity-dependent loss of synaptic AMPA receptors, it has been assumed that these two forms of LTD share basic mechanisms. However, it is clear that important differences exist. First, hippocampal LTD is normal both in the GluR2 (Jia et al., 1996; Meng et al., 2003) and the GluR2/3 (Meng et al., 2003) double knockout mouse, whereas cerebellar LTD is absent in the GluR2 knockout mouse (Chung et al., 2003). These results not only distinguish between these two forms of LTD, but also raise questions about the postulated role of the GluR2 subunit in hippocampal LTD. Second, activation of PKC with phorbol esters can, by itself, induce cerebellar LTD (Crepel and Krupa, 1988; Linden and Connor, 1991) through phosphorylation of S880 (Chung et al., 2003). By contrast, phorbol esters cause a modest increase in AMPA-mediated mEPSCs in the hippocampus (Carroll et al., 1998; Wang et al., 1994). Third, inhibitors of PKC block cerebellar LTD (Linden and Connor, 1991) but have no effect on hippocampal LTD (Kim et al., 2001; Oliet et al., 1997). A possible explanation for these differences is that the AMPA receptors in cerebellar Purkinje cells lack the GluR1 subunit, whereas GluR1 is abundant in hippocampal neurons. Perhaps GluR1 negates the PKC regulation of the GluR2 C-terminal trafficking signals.

**Future Directions**

It has been thirty years since the discovery of LTP, yet the field has only recently focused on regulation of AMPA receptors. Remarkable progress has been made in uncovering molecular mechanisms that control synaptic AMPA receptors; nevertheless, many important issues remain unresolved. An emerging theme is that AMPA receptors containing particular subunit combinations follow distinct trafficking rules. Receptors containing the long-tail forms (GluR1 and 4) apparently require synaptic activity and protein phosphorylation to access the synapse, whereas receptors containing only short tails (GluR2 and 3) cycle constitutively into and out of the synapse. How LTP is maintained when receptors cycle into and out of the synapse remains uncertain.
Many of the subunit rules for AMPA receptor trafficking derive from experiments using protein overexpression, interfering peptides, or dominant-negative constructs. Studies using mutant mice have begun to test the robustness of these models. The lack of LTP in the CA1 region of hippocampus in the GluR1 knockout mouse supports the critical role of GluR1 in this plasticity. However, that LTP is normal in the young GluR1 knockout mouse and is present in the dentate gyrus of the adult knockout raises concerns about the generality of the model. That hippocampal LTD is normal in mice lacking GluR2 and GluR3 questions the work suggesting a critical role of GluR2 in hippocampal LTD. It will be of interest to know whether the GluR1 knockout mouse expresses LTD in hippocampus.

Given these caveats concerning subunit specificity in LTP and LTD, it seems valuable to consider how genetic mutations of AMPA receptor subunits influence basal synaptic transmission. In the GluR1 knockout, the number of synaptic AMPA receptors is unaltered despite a vast reduction in extrasynaptic AMPA receptors (Zamanillo et al., 1999). What is the role of extrasynaptic AMPA receptors? Do they provide a reserve pool for synapses? Is shuttling between synaptic and extrasynaptic sites regulated? Basal synaptic transmission is also normal in the GluR1 phosphorylation-deficient mutant (Lee et al., 2003). Although GluR2/GluR3 double knockout mice have decreased hippocampal field EPSPs, neurons from these mice show normal frequency and amplitude of miniature EPSCs, indicating a normal number of synaptic AMPA receptors (Meng et al., 2003). Why is basal synaptic transmission maintained despite substantial defects in synaptic plasticity in these multiple strains of GluR mutant mice?

These studies of transgenic mice with GluR subunits mutations suggest that regulation of synaptic AMPA receptor number can be largely independent of subunit composition, but instead may use more general signals. Stargazin and related TARPs bind to all AMPA receptor subunits, and NARP binds to the N-terminal domain of all AMPA receptor isoforms. Future studies are needed to determine how these or other generalized types of AMPA receptor interactions regulate basal transmission as well as synaptic plasticity.

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