The Glutamate Receptors

The Receptors

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Cover illustration: Fig. 2B, C from Chapter 6, “Structural Correlates of Ionotropic Glutamate Receptor Function,” by Anders S. Kristensen et al.

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Preface

The abundant amino acid glutamate is the principal excitatory neurotransmitter in the mammalian central nervous system. Glutamate exerts its actions on cells via activation of two main classes of receptors. One class, known as the ionotropic glutamate receptors, includes a diverse group of ion channels that, in most cases, are directly gated by glutamate binding. The second class of glutamate receptors, known as metabotropic glutamate receptors, is made up of seven transmembrane-domain proteins that couple to intracellular signaling pathways via heterotrimeric guanosine triphosphate (GTP)-binding proteins. In rodents, at least 22 distinct gene products comprise these two classes of glutamate receptors. In addition to having both ion channels and G protein-coupled receptors, this broad superfamily of receptors encompasses several subunit proteins that do not, in fact, exhibit an affinity for glutamate. These gene products are quite obviously structurally related to other family members and subserve roles in excitatory neurotransmission, and for that reason warrant discussion in a review of the field.

Glutamate receptors are critically important molecules for normal brain function. They transduce the vast majority of excitatory neurotransmission and regulate the strength of both excitatory and inhibitory transmission in the nervous system. Glutamatergic systems are dysfunctional in most neuropathologies, and aberrant receptor function appears to have causative roles in many neurologic diseases. Therefore, it is desirable for all neuroscientists to have a good working knowledge of the general structural and functional properties of these receptors.

The Glutamate Receptors comprises a series of chapters by experts in the study of glutamate receptor function. This book serves as an update to two excellent previous books, *The Ionotropic Glutamate Receptors* and *The Metabotropic Glutamate Receptors*, and is intended to serve as a comprehensive primer on the field of glutamate receptors. In the decade since publication of these earlier volumes, an extraordinary amount of research has produced an abundance of insights into nearly every aspect of glutamate receptor function. This book is intended to cover the significant developments in this fertile period and to give a snapshot of how prominent scientists in the field look to the future of glutamate receptor research.

The amount of material covered is vast, and thus in order to facilitate location of similar aspects of the various receptor subfamilies, we have organized the

book into a series of chapters that follow a similar format. The two main groups of receptors are discussed in separate chapters covering the structure of the receptors, their roles in synaptic plasticity, and the potential therapeutic utility of glutamate receptor ligands. Each subgroup of receptors is discussed in individual chapters covering major areas of emphasis including structure, function, pharmacology, protein–protein interactions, and roles in synaptic transmission and neuromodulation.

The editors hope that this collection will serve as a valuable resource for scientists and students.

Robert W. Gereau, IV, PhD
Geoffrey T. Swanson, PhD

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1

AMPA Receptors

Michael C. Ashby, Michael I. Daw, and John T. R. Isaac

Summary

α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMP ARs) are glutamate-gated ion channels. They are the neurotransmitter receptors that mediate the great majority of fast excitatory synaptic transmission in the mammalian brain and are found throughout the animal kingdom in organisms as diverse as rodents, honeybees, nematode worms, and humans. They are absolutely critical for brain function; for example, infusion of a selective AMPAR antagonist into the rat hippocampus in vivo completely silences excitatory transmission in that region ***(1)***. AMPARs are also required for adaptive changes in the brain, mediating the expression of forms of long-term and short-term synaptic plasticity that are believed to underlie learning and memory, development, and certain neurologic diseases ***(2–5)***. Thus, AMPARs play a central role in brain function, and consequently there is great interest in the development of novel therapies directed at modulating AMPAR function for treatment of neurologic disorders, such as Alzheimer disease and stroke.

Key Words: Glutamate; Ion channel; Excitatory synaptic transmission; Synaptic plasticity; Receptor phosphorylation; Receptor trafficking; Hippocampus.

1. Structure

1.1. Genes

There is remarkable homology among all of the ionotropic glutamate receptor genes that have been identified, suggesting that they may have arisen

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from a common ancestral gene. There are 16 distinct mammalian genes, 4 genes from non-mammalian vertebrates, at least 6 genes from invertebrates, and several genes from plants. A prokaryotic protein called GluR0, which is a glutamate-activated K^+ channel, has substantial homology with the ion channel of glutamate receptors from higher organisms and thus may represent the common ancestor ***(6)***.

In mammals, there are four different α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) subunits: GluR1, GluR2, GluR3, and GluR4 (these subunits are also known as GluR-A, GluR-B, GluR-C. and GluR-D or GLU_{A1}, GLU_{A2}, GLU_{A3}, and GLU_{A4}) ***(7)***. The four different mammalian AMPAR subunits are encoded by separate but related genes that form a single gene family. Although the classification of glutamate receptors was initially based on their pharmacologic properties, the AMPA, kainate, and *N*-methyl-D-aspartate (NMDA) receptor subunits are encoded by similarly distinct families of genes. Although the mammalian AMPAR subunits share ~70% homology, they vary much more from the other ionotropic glutamate receptor subunits (~20%–40% homology). The genes encoding GluR1–4 are named GRIA1–4. They contain multiple intron–exon repeats (17 in mouse GRIA2) and share similar overall structure ***(7)***. The overall size of the genes is likely to be >200 kilobases, whereas the translated protein subunits contain only ~850–900 amino acids ***(8)***.

1.2. Topology and Stoichiometry

There was initially substantial uncertainty about the topology of AMPAR subunits and the other ionotropic glutamate receptors. However, domain mapping of glycosylation and phosphorylation sites and antibody targeting revealed the topology of AMPAR subunits in the membrane ***(9,10)***. This was confirmed by high-resolution structural analyses ***(11)***. All the AMPAR subunit proteins have an extracellular amino (NH_3) terminal and four membrane-associated hydrophobic domains (M1–4). Three of these domains are trans-membrane (M1, 3, and 4), and the other forms a reentrant loop that enters and exits the membrane on the cytoplasmic side without traversing the membrane (M2). This arrangement of M2 means that the C-terminal tail of the protein is intracellular. Transmembrane AMPAR regulatory proteins (TARPs) also are coassembled stoichiometrically with native AMPARs ***(12)*** (Fig. 1).

The AMPAR proteins, similar to the other mammalian ionotropic glutamate receptors, have likely evolved through fusion of three gene segments that were once individual bacterial proteins. The amino-terminal domain (NTD) is homologous to the bacterial leucine-isoleucine-valine–binding protein (LIVBP) and forms a large fraction of the total size of the protein (~400 amino acids). Residues within the NTD are important for receptor assembly, may have roles in modulating channel kinetics ***(13,14)***, and potentially play a role in

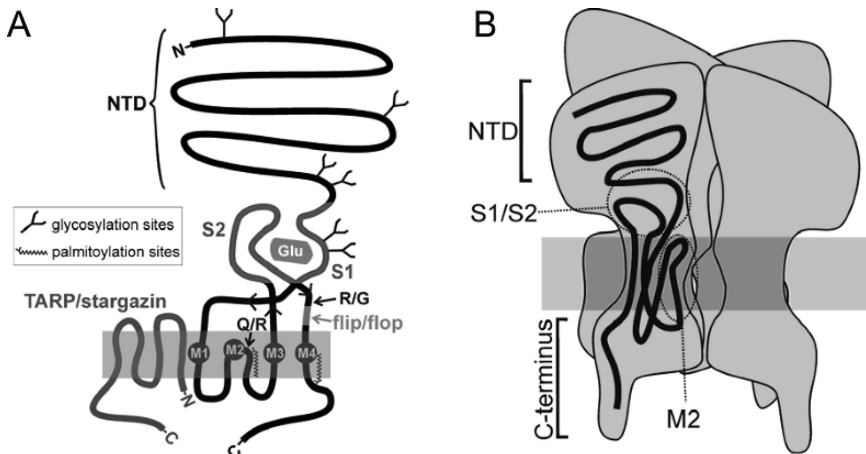

Fig. 1. Topology of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR). **A.** Schematic of an AMPAR subunit in the plasma membrane in association with a transmembrane AMPA receptor regulatory protein (TARP). Glycosylation and palmitoylation sites are indicated. The N-terminal domain (NTD), extracellular ligand-binding domains (S1 and S2), transmembrane domains (M1–4), the flip/flop alternative splicing site, and the RNA editing sites (Q/R and R/G) are also shown. **B.** Three-dimensional representation of the AMPAR complex depicting the arrangement of one subunit within the complex and showing that the M2 region lines the channel.

transsynaptic interactions and the regulation of spine morphology. In this latter respect, overexpression of the GluR2 NTD in isolation can induce changes in neuronal morphology ***(15)***. The ligand-binding domain, which resembles the bacterial lysine-arginine-orthinine binding protein (LAOBP), comprises two separate segments, named S1 and S2. These extracellular polypeptides are interrupted by the ion channel pore, which is structurally similar to bacterial K^+ channels (in particular, the GluR0 protein) ***(11)***. The reentrant M2 transmembrane loop forms the lining of the channel pore, and amino acids in this region determine the selectivity of the ion channel ***(16)*** (Fig. 1). The C-terminal tail is the most variable region between the AMPAR subunits and is the site of subunit-specific protein interactions and phosphorylation sites that modulate AMPAR function ***(17–19)*** (as discussed in more detail later in this chapter). Recently, the structure of the native AMPAR complex has been visualized directly using single-particle electron microscopy (Fig. 2). This reveals an asymmetric organization of the extracellular N-terminal domains of heteromeric receptor complexes, the tight association with TARPs and shows that a conformational change of the extracellular region of the receptor is associated with ligand binding and desensitization ***(20,21)***.

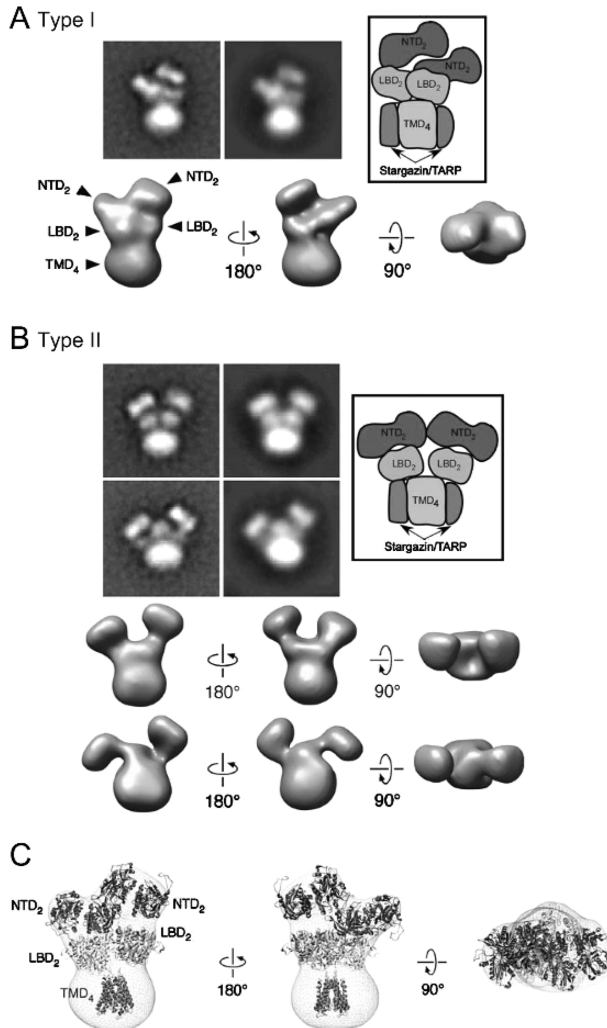

Fig. 2. The structure of the native α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) analyzed using single-particle electron microscopy. **A.** AMPAR purified from brain in type I (nondesensitized) conformation. **Top left:** Two panels showing the averaged image. **Top right:** Schematic of the arrangement of the domains of the native AMPAR–transmembrane AMPA receptor protein (TARP) complex in this type I configuration. **Bottom:** Three-dimensional reconstruction of the AMPAR in the type I conformation. LBD, ligand-binding domain (equivalent to S1 and S2); NTD, N-terminal domain; TMD, transmembrane domain (equivalent to M1–M4). **B.** AMPAR in the two type II (desensitized) conformations (panels as for part A). **C.** Superimposition of related known crystal structures onto the type I AMPAR image. Crystals used are extracellular domain of mGluR1 (NTD_2), ligand-binding domain of

Despite initial controversy, the consensus is that mature functional AMPARs are tetramers ***(22,23)***. Each receptor is formed in the endoplasmic reticulum as a dimer of dimers ***(11)***. That is, the initial stage of formation is the dimerization of two subunits that is dependent on the interactions in the NTD ***(24)***. This is followed by a second dimerization step mediated by associations at the ligand-binding and membrane domains in a process that is also dependent on Q/R editing in M2 ***(25)*** (discussed in detail later). The formation and stabilization of the tetramer is further promoted by NTD interactions.

1.3. Diversity

1.3.1. RNA Splice Variants and Editing

The functional diversity of AMPARs is increased by alternative splicing and editing of subunit RNA. These posttranscriptional modifications generate multiple isoforms of each subunit, producing varied structural and functional properties (Fig. 3). The pre-mRNA transcripts of all of the AMPAR subunits can be alternatively spliced to produce either "flip" or "flop" isoforms ***(26)***. This alternative splicing of adjacent exons results in variation within a 38-amino acid sequence in the extracellular region of the protein, close to the final transmembrane domain (M4). The two isoforms have different expression patterns, channel kinetics, and pharmacologic profiles. Generally, flip variants are expressed early in development, whereas flop isoforms are initially expressed in low abundance and are upregulated in adult animals ***(26,121)***. There are also cell type– and subunit-specific differences in the ratios of flip and flop isoforms, and levels of expression can be modulated by activity and following injury and during disease. However, little is known about regulation of the flip/flop splicing in neurons. Since the flip and flop isoforms can influence receptor formation and stoichiometry ***(27)***, splicing may be important in determining the AMPAR subunit composition. The major functional difference is that desensitization of flip AMPARs in response to glutamate is markedly reduced and slower compared to that of flop-containing receptors, leading to larger steady-state currents ***(28)***. This may be caused by amino acid differences in regions that influence the ligand-binding domain ***(29)***.

Splicing of GluR1, 2, and 4 mRNA at a 5' donor recognition site just after the M4 sequence is responsible for producing variations in the C-terminal tail of these subunits ***(7,8)*** (Fig. 3). GluR2 and GluR4 are expressed as both short- and long-tailed proteins; this is dependent on differential splicing between exons 16

Fig. 2. (Continued) GluR2 (LBD_2), and transmembrane segment of KcsA (TMD_4). Adapted from Nakagawa T, et al. Structure and different conformational states of native AMPA receptor complexes. *Nature* 2005;433(7025):545–549.

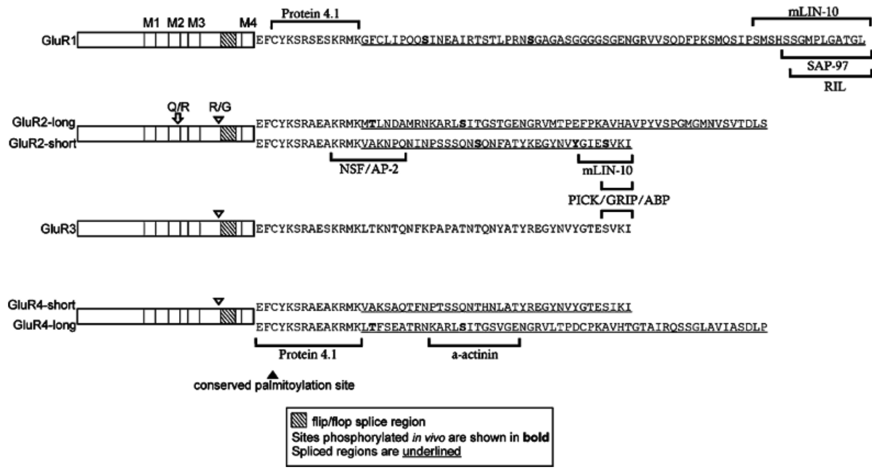

Fig. 3. Sequence alignments of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) subunits showing the membrane-spanning regions and RNA editing sites and highlighting the alternatively spliced regions, phosphorylation sites, and protein–protein interactions in the C-terminus.

and 17 (short-tailed forms are also referred to as GluR2c and GluR4c). In adult brain, >90% of GluR2 subunits are of the short form ***(8)***, whereas the GluR4 subunit is usually, but not exclusively, expressed as the long-tailed form ***(30)***. GluR1 and GluR3 are not alternatively spliced in their C-terminal domains and have long and short tails, respectively. The cytoplasmic C-terminal tails of AMPAR subunits contain a number of residues that are biochemically modified and amino acid sequences that participate in protein–protein interactions. Both of these mechanisms can regulate receptor localization and function ***(17,19)***. Therefore, C-terminal splicing plays an important role in the generation of AMPAR subunits that exhibit distinct regulatory mechanisms. A good example of this is the differential regulation of GluR2 short, the predominant splice variant of this subunit in adult brain, and GluR2 long, which is highly expressed early in development in forebrain and throughout life in olfactory bulb ***(208)***.

AMPAR subunits also undergo RNA editing (Figs. 1 and 3). The most functionally significant editing is that described for the GluR2 subunit. Most mature GluR2 protein contains an arginine residue (R) within the reentrant M2 membrane loop region at position 607 that is genomically encoded to be glutamine (Q) ***(28)***. This change is effected by hydrolytic editing of a single adenosine base in the pre-mRNA to an inosine by the adenosine deaminase enzyme, ADAR2 ***(31)***. The inosine-containing codon is read as an R at residue 607 rather than the genomically encoded Q by the translation machinery. Although this residue is conserved throughout the AMPAR subunit genes,

Q/R editing is restricted to GluR2 because it is dependent on a 10-nucleotide sequence (the editing complementary site [ECS]) specifically found in the intron that precedes the exon encoding the Q/R site of GluR2. In the healthy adult brain, the vast majority of GluR2 subunits are Q/R edited ***(32)***. However, during early development and in certain neurons and glial cells, Q/R editing of GluR2 is not so complete ***(33–35)***.

Q/R editing has several effects on the function of GluR2-containing receptors that will be discussed in the section on ion channel function. The importance of these effects on channel function is shown by the fact that transgenic mutation of the ECS site results in loss of editing of GluR2, and these mice are susceptible to seizures and die by 3 weeks of age ***(36)***. A reduction in Q/R editing efficiency has also been linked to several diseases. Spinal cord motor neurons taken from patients with amyotrophic lateral sclerosis (ALS) exhibit a marked reduction in editing of GluR2 ***(37,38)***, and a reduction in ADAR2 expression and Q/R editing correlates strongly with increased neuronal susceptibility to cerebral ischemia ***(39,40)***. These findings suggest that aberrant regulation of ADAR2 levels or activity may be an important contributor to neuronal dysfunction and excitotoxicity in these disorders. The potential mechanisms underlying regulation of editing and the relative importance of deficient editing compared to reduced GluR2 expression in forming Ca^{2+}-permeable AMPARs remain to be determined.

In GluR2, 3, and 4 pre-mRNAs another adenosine, which is located directly before the flip/flop alternative splice region, can also undergo nuclear editing ***(41)***. The editing causes a change from arginine (R) to glycine (G) and can be mediated by ADAR2 acting at the junction of exon and intron 13 ***(42)***. The R/G editing produces channels that desensitize faster and recover more rapidly from desensitization ***(41,43)***. Although not as complete as Q/R editing, R/G-edited subunits form the majority of AMPARs in adult mouse brain ***(41)***. Changes in the fraction of R/G-edited subunits have been found in hippocampal tissue from epileptic patients ***(44)*** and following ischemia in rats ***(45)***.

1.3.2. Heteromeric Subunit Diversity

The great majority of AMPARs in the central nervous system are thought to exist as heteromers ***(46,47)***. AMPAR subunits only assemble with other AMPAR subunits, and this exclusivity of assembly is determined by the specificity of interactions within the NTD ***(13,14)***. The formation of specific AMPAR heteromeric combinations is likely under the control of several factors. In cells in which GluR2 is expressed, the great majority of the AMPARs contain this subunit, and the preferred organization of receptor complexes containing GluR2 is a symmetric heteromer ***(48)***. This is likely linked to Q/R editing in the pore loop of GluR2, which regulates receptor assembly and transit of GluR2-containing dimers out of the endoplasmic reticulum (ER) ***(25,47)***. It was

suggested that a large pool of available, unassembled GluR2 resides in the ER, and this excess of GluR2 ensures that the great majority of AMPARs include GluR2. The result is that there is a predominance of GluR1/2- and GluR2/3-containing receptors in GluR2-expressing cells such as principal neurons ***(46)***. The importance of GluR2 is further highlighted by GluR2-knockout mice, in which a profound disruption in the subunit composition of AMPARs is observed ***(49)***. Therefore, in cells in which Q/R editing of GluR2 is almost complete, the incorporation of GluR2 into functional AMPARs seems simply to depend on the level of GluR2 expression. In this regard, the expression of GluR2 is highly regulated at the transcriptional level ***(50–52)***, and there is evidence that a loss of this regulation contributes to excitotoxicity mediated by pathologic expression of calcium-permeable, GluR2-lacking AMPARs during cerebral ischemia ***(53–55)***.

However, certain cell types exhibit calcium-permeable, GluR2-lacking AMPARs under physiologic conditions, and these cells typically exhibit low levels of GluR2 expression ***(56,57)***. Moreover, there is evidence that cells expressing high levels of GluR2 (e.g., cortical pyramidal neurons) express a minor population of GluR2-lacking, calcium-permeable AMPARs. These receptors can be incorporated at synapses under certain conditions ***(58,59)*** and are involved in the expression of long-term synaptic plasticity ***(60,61)***.

1.4. Posttranslational Modifications

1.4.1. Phosphorylation

Several serine (S), threonine (T), and tyrosine (Y) amino acid residues in the C-terminal tail of AMPARs are targets for phosphorylation (Fig. 3). Details of these sites are listed in the following paragraph, and the implications for AMPAR function are discussed later in the chapter.

GluR1 is phosphorylated in vitro and in vivo at the S831 position by PKC and CaMKII and at S845 by protein kinase A (PKA) ***(62)***. The predominant, short-tailed GluR2 splice variant is phosphorylated at S880 by protein kinase C (PKC) ***(63)***. A third site on the C-terminal tail of GluR2, S863, can also be phosphorylated in vitro by PKC, although its direct effect on receptor function is unclear ***(63)***. Phosphorylation of the predominant GluR4 splice variant (long-tailed) occurs at S842 and can be mediated by PKA, PKC, and CaMKII in vitro ***(64)***.

Several other consensus sequences for phosphorylation exist within the cytoplasmic domains of the various AMPAR subunits that can be phosphorylated in vitro. Some of these sites are conserved, such as the potential PKC target sequence around the T830 residue in GluR3 and long-tailed variants of GluR4 and GluR2 ***(64)***. However, there is inherent danger in extrapolating in vitro information to phosphorylation of AMPARs in the brain. This is exemplified by the early identification of several AMPAR phosphorylation

sites that subsequently were identified on regions of the protein located on the extracellular side of the plasma membrane ***(65)***. Therefore, further work is needed to elucidate the sites of phosphorylation on the AMPAR subunits in the brain and to understand the effects in vivo of such phosphorylation on channel function and trafficking on the characteristics of synaptic receptors. This need is highlighted by the fact that almost nothing is known about phosphorylation of GluR3.

1.4.2. Palmitoylation

Palmitoylation is the reversible addition of the 16-carbon fatty acid palmitate to cysteine amino acids. AMPAR subunits can be palmitoylated at two intracellular cysteine residues, one close to M2 on the intracellular loop and the other in the C-terminal tail proximal to M4 ***(66)*** (Fig. 1). The Golgi-associated palmitoyl transferase GODZ palmitoylates the first of these sites ***(66)***. Palmitoylation promotes association of proteins with specialized membrane domains and thus may be involved in controlling AMPAR association with particular membrane compartments.

1.4.3. Glycosylation

All of the AMPAR subunits have between four and six consensus sites for N-linked glycosylation, at which carbohydrate chains can be added onto extracellular residues of the protein ***(67)*** (Fig. 1). The sites reside in conserved positions of the NTD and the first ligand-binding domain, S1, although GluR2 lacks the sites on the extreme NTD. There is a progressive glycosylation of AMPARs as they pass through the secretory pathway such that mature AMPARs at the plasma membrane exhibit substantial glycosylation, as shown by a decrease in molecular weight of ~4 kDa after in vitro removal of oligosaccharides from native proteins ***(68,69)***. Although the oligosaccharides have been identified ***(70)*** and they are known to be sulfated (in GluR2 at least), it is not known which sites on the AMPAR subunits are glycosylated in the mature protein. Moreover, the role of glycosylation is unclear for AMPARs: Glycosylation is not absolutely required for receptor expression, trafficking, ligand binding, or channel function, but does have an effect on ligand binding and is likely to influence other characteristics ***(68,71,72)***. In this regard, incomplete glycosylation of GluR3 can result in cleavage of the protein by granzyme B that may be involved in generating the autoimmune response underlying Rasmussen syndrome ***(73)***.

2. Function

2.1. In Vitro

AMPARs have a relatively small single-channel conductance and fast kinetics, and they rapidly inactivate and desensitize in the presence of agonists such as glutamate or AMPA ***(74)***. The affinity for the natural agonist,

L-glutamate, is relatively low (compared to NMDA receptors), with a half-maximal effective concentration (EC_{50}) of ~0.5 mM. These biophysical properties result in a fast transient macroscopic current on agonist application; this is observed both for recombinant homomeric AMPARs expressed in heterologous cells such as HEK293 and for native AMPARs in patches excised from neurons (Fig. 4). The kinetics and desensitization of the channel depend on subunit composition, splice variant, and RNA editing. For example, the desensitization properties of AMPARs depend on splicing at the flip/flop site: Flip-variant receptor subunits exhibit slower desensitization than the flop variants and have a nondesensitizing low-conductance state ***(26)***. The AMPAR channel opens to a number of subconductance states between 7 and 50 pS, with those <20 pS predominating ***(74–76)***. In patches excised from neurons, native AMPARs typically exhibit a mean single-channel conductance of ~12 pS ***(77,78)***, which represents a weighted average of all the subconductance states (Fig. 4). Nonstationary fluctuation analysis, which allows single-channel conductance to be estimated from synaptic AMPAR-mediated currents, also indicates that synaptic AMPARs in a variety of neuronal types exhibit a similar mean single-channel conductance of ~12 pS; similar to that observed in excised patches ***(79–81)***.

The single-channel properties of AMPARs can be regulated dynamically. Phosphorylation of serine 831 on GluR1 causes the homomeric GluR1 AMPARs to open to the higher-conductance states, producing an increase in the weighted mean single-channel conductance ***(82)***. The mean single-channel conductance is also proportional to the concentration of agonist ***(23,83)***: Each subunit is thought to bind ligand independently, and as more agonist molecules are bound to the receptor complex, the predominant subconductance state increases. Recent evidence also demonstrates a strong influence on channel properties of the TARP family of proteins, which includes stargazin ***(84–86)***. Interaction of the AMPAR complex with TARPs slows AMPAR desensitization and deactivation, increases open channel probability, and increases the proportion of channel openings at the higher subconductance levels. In addition, the AMPAR interaction with TARPs dramatically alters the pharmacology of the receptor such that kainate, which is only a partial agonist at recombinant AMPARs lacking TARP, is a full agonist at AMPARs coexpressed with TARPs.

GluR2 is a dominant subunit in determining the biophysical properties of the AMPAR channel ***(74)***. Channels containing GluR2 subunits have a linear current–voltage relationship ***(87)*** and are impermeable to Ca^{2+}, whereas those lacking GluR2 are Ca^{2+} permeable and show inward rectification due to a voltage-dependent block by endogenous polyamines ***(88–90)***. In addition, there is evidence that AMPARs lacking edited GluR2 exhibit considerably higher single-channel conductance than their GluR2-containing counterparts ***(91)***. The

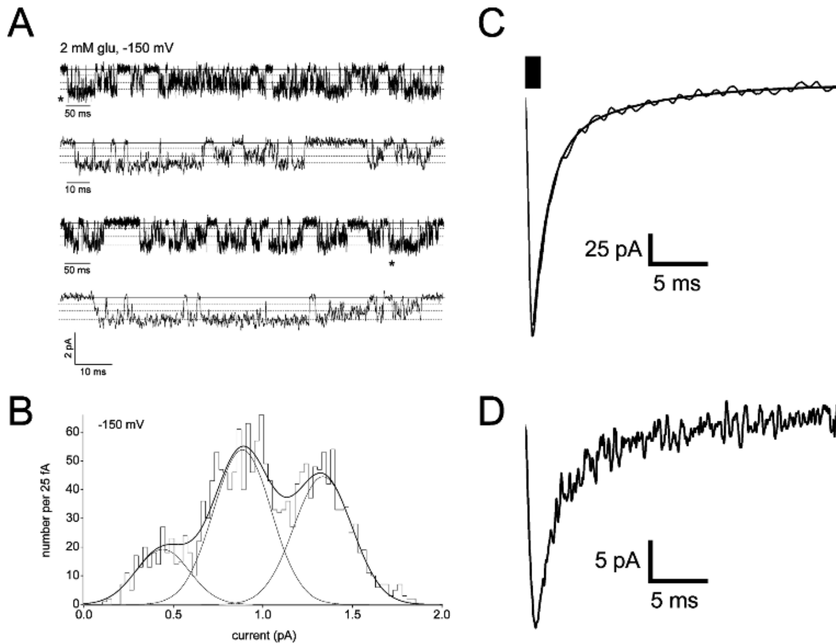

Fig. 4. Electrophysiologic properties of native α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs). **A.** AMPA receptor single-channel records in an outside-out patch excised from a developing cerebellar granule cell. Currents were evoked by glutamate (2 mM) in the presence of cyclothiazide. Dotted lines indicate subconductance levels, the solid line represents zero current, and the second and fourth traces are expanded from the first and third traces, respectively. **B.** Amplitude histogram for experiment shown in panel A, fitted with three Gaussians to show the main subconductance levels (3, 6, and 9 pS) observed in this patch. **C.** Current evoked by a 1-ms application of 1 mM glutamate (gray bar) to an outside-out patch excised from a CA3 pyramidal neuron in the presence of D-AP5 (100 μM), voltage-clamped at –70 mV. The superimposed solid line is a double-exponential fit to the decay (τ_{fast} = 1.3 ms, 82%; τ_{slow} = 7.9 ms, 18%). **D.** AMPAR-mediated miniature excitatory postsynaptic current (EPSC) (averaged) recorded from a stellate cell in neonatal mouse barrel cortex (in the presence of 0 mM Ca^{2+} and 8 mM Sr^{2+}, 100 μM D-AP5, (−70 mV holding potential), showing that the time courses of synaptic AMPAR-mediated responses are very similar to those evoked by rapid glutamate application to AMPARs in an excised patch.
A, B: Reproduced from Smith TC, Howe JR. Concentration-dependent substrate behavior of native AMPA receptors. *Nat Neurosci* 2000;3(10):992–997. C: Lauri, S. and Isaac, J.T.R. (unpublished). D: Reproduced from Bannister NJ, et al. Developmental changes in AMPA and kainate receptor–mediated quantal transmission at thalamocortical synapses in the barrel cortex. *J Neurosci* 2005;25(21):5259–5271.

differences in channel properties of GluR2-containing receptors are due to the presence of arginine at residue 607 as a consequence of the RNA editing of GluR2. This residue is in the reentrant M2 membrane-spanning region that forms the AMPAR ion channel pore (Fig. 1). The additional positive charge introduced into the pore by this editing prevents the passage of divalent Ca^{2+} cations and prevents the polyamine block ***(92)***. The predominance of the edited form of GluR2 in native receptors in the vast majority of postnatal neurons is the reason that the majority of native AMPARs are Ca^{2+} impermeable. However, Ca^{2+}-permeable AMPARs lacking GluR2 are found in certain cell types in the brain, for example, subsets of hippocampal interneurons ***(93)***. The voltage dependence of the polyamine block of the GluR2-lacking AMPAR also produces a novel form of short-term synaptic plasticity mediated by an activity-dependent unblock of the AMPARs ***(94–96)***.

2.2. *In Vivo*

AMPARs mediate the vast majority of fast excitatory synaptic transmission in the mammalian brain. They are expressed in all neuronal types as well as in glia. The major role of AMPARs in mediating excitatory transmission is shown by the profound effect of infusion of an AMPAR antagonist on transmission in the hippocampus in vivo ***(1)***. Activation of native AMPARs by agonist causes a rapid opening of channels permeable to Na^+ and K^+, with a reversal potential around 0 mV in vivo. At synapses this produces a transient inward current rising in a few hundred microseconds and decaying within a few milliseconds. The kinetics of this AMPAR-mediated excitatory postsynaptic current (EPSC) is primarily a function of the kinetic properties of the AMPAR channels combined with the time course of transmitter release ***(74,97,98)*** (Fig. 4). In particular, the time course of decay of the EPSC is primarily mediated by the deactivation properties of the AMPARs, which in turn is influenced by subunit composition, subunit splice variants, and degree of RNA editing. In addition, AMPAR recovery from desensitization also depends on the composition of the receptor complex and can play an important role in limiting the postsynaptic response during high-frequency repetitive activity ***(97)***. The time course of the AMPAR-mediated excitatory postsynaptic potential (EPSP), however, is influenced by a number of additional factors such as the passive electrotonic properties of the particular neuronal type in question, the contributions of subthreshold voltage-gated conductances, and the degree of spontaneous synaptic input to the neuron. Therefore, the time course of the EPSP initiated by the AMPAR-mediated EPSC can vary greatly between cell types.

The fast kinetics of the AMPAR-mediated EPSC produces EPSPs with rapid kinetics that provide a precise window for coincidence detection of subthreshold input and that can generate action potentials with a high degree

of precision ***(99–101)***. These features of precise timing also depend on the electrotonic properties of neurons. In electrically compact neurons such as subtypes of cortical GABAergic interneurons or relay cells in brainstem nuclei, AMPAR-mediated EPSPs allow for very high precision timing of input and output ***(102–104)***. Since precise timing and coincidence detection are thought to be critically important features for information processing by cortical networks ***(104,105)***, the rapid kinetics of the AMPAR channel can thus been seen to be vital to the functioning of neural networks.

The AMPAR is also a major target for direct modification during the expression of the predominant forms of long-term synaptic plasticity in the brain, NMDA receptor (NMDAR)-dependent long-term potentiation (LTP) and long-term depression (LTD) ***(4,18,19,106)***. LTP expression involves an increase in AMPAR function that is mediated at least in part by increased phosphorylation of S831 on GluR1 and the rapid incorporation of GluR1-containing receptors at synapses. LTD is expressed by the rapid removal of AMPARs from synapses through a mechanism involving endocytosis and requires dephosphorylation of S845 on GluR1 and GluR2-dependent trafficking. There is a very large body of work investigating such mechanisms, and this is described in appropriate detail in a subsequent chapter. However, of particular relevance here are recent findings that long-term synaptic plasticity can cause a rapid change in the GluR2 subunit composition and as a consequence the biophysical properties of the AMPAR ***(61,107–109)***.

In addition to their well-established postsynaptic function, AMPARs play other roles in the brain. There is accumulating evidence for presynaptic AMPARs regulating transmission ***(110,111)***. The best-characterized presynaptic role for AMPARs is their direct inhibition of γ-aminobutyric acid (GABA) release from basket cell terminals onto cerebellar Purkinje neurons ***(112)***. This is a heterosynaptic presynaptic regulation, with the glutamate being released from climbing fibers onto the same Purkinje neurons, and appears to be mediated by the canonical ionotropic AMPAR mechanism. Recent work also suggests a direct metabotropic AMPAR-mediated presynaptic inhibition at the calyx of Held ***(113)***. It has also been known for several years that certain types of nonneuronal cells in the brain can express functional AMPAR ion channels ***(114–116)***. These AMPARs can be activated by glutamate released from neurons and may be involved in a diverse range of glial processes. These include modulation of astrocytic glutamate transporter function, generation of intracellular calcium transients that influence glial morphology, release of neuroactive substances, regulation of gene expression, and modulation of the extracellular ionic environment ***(114,117,118)***. The finding that certain central nervous system (CNS) precursor cells are known to express AMPARs, which may sense ambient glutamate even before the formation of neuronal synapses,

suggests that there may also be an important role for AMPARs in very early brain development ***(114)***.

3. Expression, Trafficking, and Targeting

AMPARs are widely expressed in the CNS both in neurons and in glia, and are also expressed in many peripheral neurons and in several peripheral nonneuronal cell types ***(114,119)***. In the mammalian CNS there are region-, development-, and cell-specific variations in AMPAR subunit expression that profoundly affect AMPAR function ***(7,119,120)***. In adult brain, there is widespread expression of GluR1 and GluR2 but much more restricted expression of GluR3 and GluR4. In forebrain, including hippocampus and cerebral neocortex, the predominantly expressed subunits are GluR1 and GluR2, with low levels of GluR3 and GluR4 ***(49,57,90,121,122)***. In contrast, the cerebellum, retina, and thalamic reticular nucleus additionally display substantial expression of GluR4 ***(30,123)***. However, there are cell type–specific patterns of expression superimposed on the regional expression profiles. The major neuronal population in the cerebral neocortex and hippocampus—pyramidal cells—primarily express GluR1 and GluR2, resulting in GluR1/2 as the major heteromeric combination in this cell type ***(46,49)***. Although GluR2/3 has been hypothesized as the other major heteromer in cortical pyramidal neurons, expression of GluR3 is low in this cell type (~10% of GluR1 or GluR2 levels) ***(46,49,57,90)***, making it unlikely that GluR2/3 heteromers are expressed in any high level in pyramidal neurons. In the hippocampus, neocortex, retina, and cerebellum, there are populations of GABAergic interneurons that lack GluR2 subunit expression and hence have calcium-permeable AMPARs ***(90,124,125)***. These calcium-permeable AMPARs confer novel properties on synapses in these cell types ***(95,108,126–128)***.

GluR4 expression is low in the great majority of cortical neuronal types. However, GluR4 expression is relatively high in certain cell types in cerebellum such as Bergmann glia and granule cells ***(129,130)***. In addition, Bergmann glia do not express GluR2 and therefore have predominantly calcium-permeable AMPARs; the activity of these calcium-permeable AMPARs has been shown to be important in controlling the structural relationship between the Bergmann glia and cerebellar Purkinje cells ***(131)***. Cerebellar granule cells express high levels of both GluR2 and GluR4, and thus AMPARs in these neurons are very likely GluR2/4 heteromers. This cell type also is a good example of developmental regulation of splicing since there is a progressive switch from flip to flop isoforms of GluR4 mRNA during the first 2 weeks of rat brain development ***(132)***.

AMPAR subunit expression is differentially regulated during development. AMPAR subunit expression is initiated during embryonic development and

rises quickly during early postnatal development to levels of mRNA that are reported to be significantly elevated compared to those in adult brains ***(121)***. There are region- and subunit-specific differences in the developmental changes observed. In particular, GluR4 is expressed early in development in the forebrain, but declines during the first postnatal week; this period is also associated with an increase in GluR2 forebrain expression. There are also cell-specific variations in expression of the flip and flop splice isoforms that are developmentally regulated ***(26,121)***. Expression of AMPAR subunits can also be altered acutely by pathologic events such as cerebral ischemia ***(133,134)*** or drug administration ***(135)*** and by tetanic stimulation of afferents in the hippocampus ***(136)***.

The cellular processes that control AMPAR gene expression are not fully understood. GluR2 is the best-studied subunit because its expression is critical in determining the biophysical properties of AMPARs. Transcription of GluR2 is under control of several independent initiation sites in the promoter that are not individually essential ***(52)***. Expression is strongly biased toward neurons due to silencer elements in the GluR2 promoter that are under control of REST (a multi–zinc finger repressor) ***(137)***. Furthermore, REST expression triggered by ischemic insult has been implicated in the downregulation of GluR2 expression, which then leads to an increase in calcium-permeable AMPARs that mediate the excitotoxicity in the hippocampus ***(54)***. Recent data also suggest that GluR2 mRNA translation can be suppressed by untranslated leader sequences that may or may not be present in the mRNA molecule, depending on which site was used for initiation of transcription ***(50)***. In relation to synaptic plasticity, there has been great interest in the idea that expression of AMPARs can be rapidly and locally regulated in dendrites. This concept arose from the observation of polyribosomes in the vicinity of synapses ***(138)***, and it has been shown that new AMPAR protein, GluR1 and GluR2 subunits in particular, can be synthesized in neuronal dendrites ***(58)***.

AMPAR localization at the subcellular level has been intensely studied using immunocytochemistry, biochemistry, and immunogold electron microscopy ***(139–141)***. A substantial proportion of the AMPARs are on the surface of the cell at any one time. Since the major role of AMPARs is to mediate synaptic transmission, it is not surprising to find that the neuronal surface receptors are not homogeneously distributed but have a tendency to cluster at postsynaptic sites (Fig. 5) ***(141–144)***. This clustering is likely to be ultimately mediated by protein–protein interactions that act to link AMPARs to scaffolds in the postsynaptic density (PSD). Attempts have been made to measure the extent of the clustering by using functional and morphologic methods ***(140,145–150)***. The results indicate that the number of AMPARs correlates strongly with the size of the postsynaptic density, whereas the number of NMDARs is independent of synapse size ***(147,150–153)***. These studies suggest that the

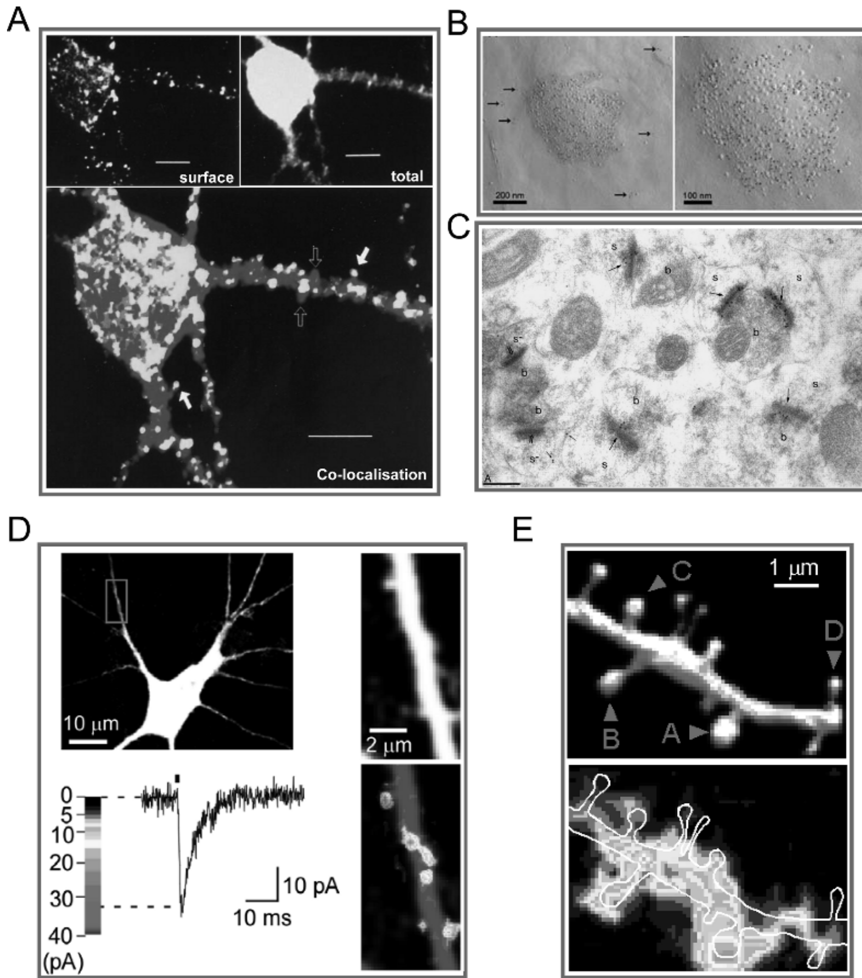

Fig. 5. Native α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) distribution in neurons. **A.** Immunocytochemical localization of surface (**upper left**) and total (**upper right**) GluR1 in a cultured hippocampal neuron using a subunit-specific antibody directed to the extracellular N-terminal (the lower panel shows the superimposed image). This demonstrates that some spines lack surface GluR1 but contain intracellular GluR1. **B.** Electron micrographs of sodium dodecyl sulfate–digested freeze-fracture replicas from neonatal rat cerebellum labeled with a pan-AMPAR antibody (GluR1–4) and a secondary conjugated to 5-nm gold particles. **Left:** The extent of the postsynaptic density (PSD) is highlighted in gray, and extrasynaptic AMPARs are indicated by arrows. **Right:** Higher-power image of the PSD showing extensive labeling. **C.** Immunogold electron micrograph of juvenile hippocampal CA1 stratum radiatum labeled with GluR2/3 antibody (b, presynaptic bouton; s, spine), showing high-density AMPAR labeling of PSD (*large closed arrows*)

density of AMPARs within the PSD can be >1000 μm^{-2}, whereas the density of extrasynaptic receptors is likely tens to hundreds of times lower (Fig. 5).

The density of AMPARs at synapses can vary greatly among cell types and developmental stages. The best-characterized example of this is for the hippocampus, where synapses onto CA1 pyramidal cells show highly variable numbers of AMPARs and the average number of receptors increases during development ***(140,146,150,151,154)***. In an extreme example of this variation, it is clear that there is a fraction of synapses lacking AMPARs (but containing NMDARs), which are termed "silent" synapses. Silent synapses are particularly evident early in development and can be unsilenced acutely during LTP ***(154)***. In addition, there are differences in AMPAR distribution along dendrites. In particular, this has been described for CA1 pyramidal cells, in which a distance-dependent scaling of synaptic strength and AMPAR number at synapses along the apical dendrites is observed ***(155)***. This is mediated by increasing numbers of GluR1-containing AMPARs at progressively more distal synapses ***(156)*** and is believed to normalize synaptic strength by compensating for increased dendritic filtering of synapses more distal to the cell body.

Fig. 5. (Contiuned) and lower-density labeling of extrasynaptic membrane (*small closed arrows*). Spines lacking AMPARs are also evident (*open arrows*, s⁻). Scale bar = 200 nm. **D.** Functional mapping of surface AMPAR distribution in cultured hippocampal neurons using two-photon glutamate uncaging. **Top left:** Fluorescence image of a neuron with the region of interest highlighted (*box*). **Top right:** Higher-magnification image of the region of interest. **Bottom left:** AMPAR-mediated current (2pEPSC) evoked by two-photon glutamate uncaging on a spine; false color scale of the current amplitude is indicated. **Bottom right:** False color image map of 2pEPSC amplitude superimposed on the region of interest in experiments in which glutamate is uncaged at numerous locations on the dendrite. This demonstrates that "hot spots" of response to glutamate exist on dendrites. **E. Top:** Three-dimensional reconstruction of the fluorescence image of a region of dendrite from a cultured hippocampal neuron. **Bottom:** Superimposition of a false color image map of 2pEPSC amplitude showing that hot spots correlate with large spines.
A: Reproduced from Richmond SA, et al. Localization of the glutamate receptor subunit GluR1 on the surface of living and within cultured hippocampal neurons. *Neuroscience* 1996;75(1):69–82 (see original publication for color images). B: Modified from Tanaka J, et al. Number and density of AMPA receptors in single synapses in immature cerebellum. *J Neurosci* 2005;25(4):799–807. C: Reproduced from Nusser Z, et al. Cell type and pathway dependence of synaptic AMPA receptor number and variability in the hippocampus. *Neuron* 1998;21(3):545–559. D, E: Reproduced from Matsuzaki M, et al. Dendritic spine geometry is critical for AMPA receptor expression in hippocampal CA1 pyramidal neurons. *Nat Neurosci* 2001;4(11):1086–1092. (see original publication for color images).

The AMPARs present intracellularly are found at different stages of the canonical secretory pathway. As described previously, the assembly of AMPARs in the ER is influenced by subunit-specific interactions and editing of the Q/R site in GluR2 ***(14,25,47)***. The regulated ER exit of GluR2 results in a large GluR2 pool in the ER relative to the other subunits. ER export of glutamate receptors is influenced by the unfolded protein response (UPR) ***(157,158)***, and a subpopulation of AMPARs is known to associate with the ER chaperones BiP and calnexin, which may influence receptor folding or maturation ***(159,160)***. It was recently shown that the UPR is induced in the absence of the AMPAR-binding protein stargazin, suggesting that stargazin acts to promote transit of mature receptors through the ER ***(157)***. Stargazin may also influence subsequent transit of AMPARs through the Golgi apparatus via an interaction with the Golgi-enriched protein nPIST ***(161)***.

Post-Golgi vesicular trafficking of AMPARs to the cell surface has been the subject of intense investigation ***(18,19,162)***. AMPARs are continually delivered to the surface of neurons ***(163,164)*** on relatively rapid time scales. Furthermore, synaptic AMPAR responses are rapidly decreased on infusion of antibodies that block the function of *N*-ethylmaleimide–sensitive fusion protein (NSF) ***(165)*** or toxins that cleave proteins mediating vesicular fusion ***(166)***. Such toxins also block the induction of forms of LTP that are dependent on AMPAR delivery to synapses ***(167)***. The vesicles on which these toxins act have not been identified, but recent evidence suggests that AMPAR exocytosis promoted during LTP ***(168)*** delivers receptors that have come through recycling endosomes ***(169,170)***. This suggests that there is a pool of AMPARs that recycle rapidly between the plasma membrane and internal vesicles, and that exocytosis and endocytosis have major roles in transport of AMPARs to and from synapses.

A recent study using a photoactivatable irreversible antagonist (ANQX) failed to detect such rapid recycling of native AMPARs ***(171)***, but instead supported a role for an alternative mode of AMPAR trafficking, lateral diffusion in the plasma membrane. Rapid lateral movement of AMPARs in the membrane of neurons was directly visualized for the first time recently ***(172)***, demonstrating that a significant proportion of surface AMPARs move around the plasma membrane at relatively rapid rates and can exchange between extrasynaptic and synaptic sites ***(173)***. The rate of AMPAR diffusion in the membrane can be influenced by activity, proximity to postsynaptic sites, changes in intracellular calcium concentration, and dendritic spine morphology ***(172,174,175)***. These findings, along with the studies indicating that AMPARs move laterally away from synapses prior to removal from the plasma membrane ***(164,176,177)***, suggest an important role for diffusion within the plasma membrane as a regulated trafficking mechanism for AMPARs. Overall, this leaves a complex picture of AMPAR trafficking in which the dynamic interplay of vesicular trafficking, lateral diffusion, and protein–protein interactions

determines the distribution of AMPARs on the surface of neurons. In future work it will be important to elucidate the relative roles of these mechanisms and how they interact with one another.

4. Interaction Partners

A number of proteins have been identified that bind directly to AMPARs. In the following we summarize interactors or classes of interacting proteins for which some function is well established. In addition to these are other, less-well-studied interactions, such as GluR2–Lyn kinase ***(178)*** and the GluR1 interaction with $G\alpha_i$ ***(179)***, the significance of which is unclear.

4.1. N-Ethylamide-Sensitive Fusion Protein (NSF) and Adaptor Protein 2 (AP2)

NSF is a protein that is known to be involved in membrane fusion ***(180)***; therefore it was of great interest when NSF was found to interact directly with the C-terminal of GluR2 ***(165,181,182)*** (Fig. 3). This interaction is at a membrane proximal site, and α- and β-SNAPs can also coassemble with the NSF-GluR2 complex ***(182)***. In whole-cell recordings from CA1 pyramidal cells, acute disruption of the NSF–GluR2 interaction using specific peptides, or blockade of NSF ATPase activity with a function-blocking antibody present in the whole-cell pipette causes a rapid depression in EPSC amplitude ***(165, 181)***. This reduction in synaptic AMPAR function is due to a loss of surface receptors ***(166,183,184)***. The effects of disrupting this interaction appear to be activity dependent ***(166,185)***. Taken together, these data suggest that the NSF–GluR2 interaction is important for maintaining AMPARs at synapses during synaptic transmission. The role of the NSF–GluR2 in the maintenance of basal transmission appears to be related to the mechanisms underlying synaptic plasticity because the decrease in EPSC amplitude caused by disrupting the NSF–GluR2 interaction is reversibly occluded by NMDAR-dependent LTD ***(166,184)***. These findings can be explained if the reduction in surface AMPARs caused by blocking the GluR2–NSF interaction results in the complete removal of a population of synaptic AMPARs available for internalization during LTD.

There is also evidence that AP2, a protein critical for clathrin-dependent endocytosis that acts as an adaptor for cargo to be internalized ***(186)***, associates with GluR2 in the same region as NSF ***(187)***. Although AP2 coimmunoprecipitates with GluR2-containing AMPARs, a direct interaction has not been demonstrated; therefore it is unclear whether AMPARs are directly recruited by this interaction for clathrin-dependent endocytosis. However, there is good evidence that clathrin-mediated endocytosis is required for the internalization of AMPARs during NMDAR-dependent LTD ***(106)***, and the GluR2–AP2 association is required for LTD ***(187)***. Therefore, a simple