Excitatory synaptic transmission in the brain is mediated by ligand-gated ion channels (iGluRs) activated by glutamate. Distinct from other neurotransmitter receptors, the extracellular domains of iGluRs are loosely packed assemblies with two clearly distinct layers, each of which has both local and global 2-fold axes of symmetry. By contrast, the iGluR transmembrane segments have 4-fold symmetry and share a conserved pore loop architecture found in tetrameric voltage-gated ion channels. The striking layered architecture of iGluRs revealed by the 3.6 Å resolution structure of an AMPA receptor homotetramer likely arose from gene fusion events that occurred early in evolution. Although this modular design has greatly facilitated biophysical and structural studies on individual iGluR domains, and suggested conserved mechanisms for iGluR gating, recent work is beginning to reveal unanticipated diversity in the structure, allosteric regulation, and assembly of iGluR subtypes.

Introduction

Glutamate receptor ion channels (iGluRs) are membrane proteins widely expressed in the central nervous system that mediate fast excitatory synaptic transmission in the brain of vertebrates. In the late 1970s, based on their different sensitivity to the ligand N-methyl-D-aspartic acid, vertebrate iGluRs were broadly classified into two subtypes named NMDA and non-NMDA receptors (Watkins and Jane, 2006). In the subsequent 40 years, this classification was extended to include additional subtypes, and a series of 18 human iGluR genes was cloned. Based on their selective ligand-binding properties and DNA sequences, four major classes of iGluRs encoded by 8 gene families have been identified in vertebrates. Each family has distinct developmental and cell-specific expression profiles with unique roles in brain function (Traynelis et al., 2010). The NMDA receptors that play key roles in synaptic plasticity are obligate heteromers formed by coassembly as tetramers of different combinations of the GluN1, GluN2A-GluN2D, GluN3A, and GluN3B subunits, which form Ca\(^{2+}\) permeable ion channels blocked by extracellular Mg\(^{2+}\). The so-called “non-NMDA receptors” are Mg\(^{2+}\) insensitive ion channels encoded by three gene families of which GluA1-GluA4 form AMPA receptors that mediate fast excitatory synaptic transmission at the majority of central synapses. Kainate receptors encoded by two gene families regulate neuronal excitability and are formed by coassembly of GluR5-7 with KA1 or KA2, alternatively named GluK1-GluK5. The remaining two iGluR genes, GluD1 and GluD2, do not form functional channels, despite sharing similar overall architecture to other iGluR subtypes. BLAST searches of the Drosophila genome recently identified a family of 61 iGluR-related genes named iRs, which assemble as heteromeric complexes to form chemosensory receptors (Benton et al., 2009). On the basis of amino acid sequence alignments, these proteins are structurally related to vertebrate iGluRs but have divergent ligand-binding properties (Abuin et al., 2011; Benton et al., 2009). It is likely that structural studies on the ligand-binding domains of these proteins will give unique insights into the molecular mechanisms for chemosensation.

The iGluR family of ligand-gated ion channels has unique structural features distinct from those of other neurotransmitter receptors, raising intriguing questions about their function and assembly that have only recently come into focus. The extracellular domains form 85% of the mass of an iGluR core and can be subdivided into discrete amino terminal and ligand-binding domains; in NMDA receptor subtypes, the cytoplasmic C terminus is extended by an additional 500 residues. Structural studies performed over the past decade focused largely on the extracellular ligand-binding domain expressed in bacteria as a soluble protein genetically isolated from the rest of the receptor. This work has been extensively reviewed and gave unprecedented insight into the molecular mechanisms for ligand recognition and subtype selectivity (Mayer, 2006; Mayer and Armstrong, 2004; Pohlsgaard et al., 2011; Stawski et al., 2010; Traynelis et al., 2010), leading to a consensus view of models for activation and desensitization, with subtle albeit biologically important differences between iGluR subtypes. Recent work shatters this harmony, reinforcing the striking differences between NMDA and non-NMDA receptors that likely have structural origins that are as yet not well understood. This review focuses, for the most part, on more recent structural work on full-length receptors and the amino terminal domain, and captures a snapshot of a very rapidly moving field for which significant advances are anticipated in the near future.

Domain Organization in a Full-Length AMPA Receptor

The first crystal structure for an intact iGluR, an AMPA receptor GluA2 homotetramer purified from the membranes of baculovirus-infected insect cells (Sobolevsky et al., 2009), was a landmark achievement. The structure was solved at a resolution of 3.6 Å using higher-resolution structures of the isolated GluA2 ATD (3HSV 2.3 Å) and LBD (1FTL 1.8 Å) as probes for molecular replacement and as guides for model building; it is doubtful
that the structure could have been solved without this information. The structure was validated by preparing multiple SeMet-labeled constructs with Met mutations used as markers in anomalous difference maps, as well as by preparing Hg derivatives as discussed below. The full-length GluA2 structure revealed numerous unexpected features not anticipated from work on the soluble ligand-binding domains. In the full-length receptor, the extracellular domains are loosely packed assemblies with two clearly distinct layers, each of which is assembled as a dimer of dimers but with different global and local 2-fold axes of symmetry; by contrast, the transmembrane segments share a conserved core architecture found in tetrameric voltage-gated pore loop ion channels with 4-fold symmetry (Figure 1). In each of the four subunits in a tetrameric iGluR assembly, the 280-residue ligand-binding domain (LBD), which forms the filling of the iGluR sandwich structure, is connected by short polypeptide linkers to the 380-residue amino terminal domain (ATD) top layer and a 120-residue ion channel (TM) bottom layer. Unexpectedly, different subunit pairs form dimer assemblies in the ATD and LBD layers; this subunit crossover was entirely unexpected and has many potential consequences for the assembly, activation, and especially the allosteric regulation of iGluRs.

There are substantial differences in packing of the dimer of dimers assemblies in the ATD and LBD layers, as well as pronounced differences in packing between the ATD and LBD layers for subunits proximal and distal to the global 2-fold axis of symmetry. Thus, although iGluRs can assemble as homotetramers, there are striking symmetry breakdowns at multiple locations in the molecule, which are without precedent in their extent and complexity. This undoubtedly impacts iGluR function, but the consequences have yet to be investigated in depth. In electrophysiological experiments on AMPA and kainate receptors, Cys mutant cross links in the LBD, which link different pairs of subunits within or between dimer assemblies, provided the first evidence for functional effects caused by subunit nonequivalence in homomeric iGluR assemblies (Das et al., 2010; Plested and Mayer, 2009), and further work using this approach should give greater insight into the role of asymmetry in the gating mechanisms of iGluRs.

**Symmetry and Packing in the Extracellular ATD and LBD Layers**

In the amino terminal domain layer of the GluA2 homotetramer there are two identical dimer assemblies, formed by the AB and CD subunit pairs, for which the A and C subunits lie distal to the 2-fold axis of global symmetry (Figure 1). The dimer pairs are related by a central axis of 2-fold symmetry, whereas the subunits within each dimer assembly are related by a local 2-fold axis of symmetry tilted 24° away from the central axis, generating a Y-shaped structure when viewed from the side, and an N-shaped structure when viewed from the top. The protomers in each dimer pair have extensive buried surfaces of around 1400 Å² mediated approximately equally by contacts in the upper and lower lobes of the ATD protomers. By contrast,
the dimer of dimers assembly is formed exclusively by contacts
between the lower lobes of the B and C subunits, which lie prox-
imal to the global axis of 2-fold symmetry. This interface has
a much smaller buried surface, of around 330 Å², and thus the
tetramer assembly is likely to be less stable than the AB and
CD subunit dimers. However, tetramer assemblies essentially
identical to that found in full-length GluA2, have been crystal-
ized for the isolated ATDs of GluA2; for the kainate receptor GluR6
and GluR7 ATDs; and for a heterotetramer assembly of GluR6
and KA2 (Clayton et al., 2009; Jin et al., 2009; Kumar and Mayer,
2010; Kumar et al., 2009, 2011; Rossmann et al., 2011), indi-
cating that, despite the small area mediating the dimer of dimers
assembly, the ATDs are predisposed to assemble in the config-
uration observed in the full-length GluA2 structure. Low-resolu-
tion, single-particle EM images reveal a variety of additional
shapes for purified AMPA receptors, suggesting that other
conformations are likely to occur (Nakagawa et al., 2005), but
additional high-resolution structures are required to give insight
into their molecular organization and functional significance.
For example, a structure that breaks the 3.0-Å barrier would
provide valuable insight into side chain conformations, whereas
breaking the 2.2-Å barrier would pinpoint the position of mecha-
nistically important water molecules. Obviously, the intact AMPA
receptor structure at 3.6-Å resolution lacks such detail.

The ligand-binding domain layer of the full-length GluA2 struc-
ture contains a pair of dimer assemblies generated by the AD
and BC subunits, for which the local 2-fold axes of symmetry for the
dimer assemblies are tilted by 19° with respect to the global axis
of symmetry (Figure 1). The ATD and LBD dimer assemblies are
not aligned with each other, further contributing to symmetry
breakdown within the extracellular domain. The structure of the
pair of LBD dimers is essentially identical to that of water-soluble
LBD constructs engineered from numerous subtypes of iGluRs
(Mayer, 2006; Pehlsgaard et al., 2011; Stawski et al., 2010;
Traynelis et al., 2010), but the full-length GluA2 structure
revealed for the first time how the LBD dimers were packed to
form tetramers. In marked contrast to the extreme separation
of distal and proximal subunits in the ATD dimer of dimers
assembly, in the LBD layer of full-length GluA2, all four subunits
approach the global 2-fold axis of symmetry, and it is possible
with Cys mutants to generate cross-links between protomers
both within and between LBD dimer pairs (Plestied and Mayer,
2009; Sobolevsky et al., 2009). In the GluA2 homotetramer, there
are extensive contacts between the base of the ATD and the top
of the LBD in the peripheral A and C subunits, buried surface
460 Å²; by contrast, the corresponding surfaces for the proximal
B and D subunits are solvent-exposed and connected by an
extended rod-like linker. As a consequence, there is a large,
solvent-filled void in the middle of the GluA2 homotetramer. It
is tempting to speculate that this provides space for the binding
of other proteins, perhaps for the extracellular domains of iGluR
auxiliary subunits or proteins presented by the presynaptic
membrane. Of particular interest from a structural perspective, the
linkers that enjoin the ATD, LBD, and ion channel domains
adopt either of two distinct conformations in different protomers
and mediate transitions between the mismatched 2-fold sym-
betry axes of the ATD and LBD layers, and the 2-fold to 4-fold
symmetry transition between the LBD and ion channel. In the
B and D subunits, which lie proximal to the global 2-fold axis
of symmetry of the ATD layer, the linkers from the ATD to the
LBD adopt extended conformations and mediate subunit cross-
over between dimer pairs in the ATD and LBD layers. The linkers
to the ion channel from the LBD also adopt different conforma-
tions caused by a 9-Å difference in distance from the last β strand
in the lower lobe of the LBD to the pre-M1 helix in the ion channel
layer, which are separated by 22 Å in the AC subunits, but only
13 Å in the BD subunits.

Cysteine mutant cross-linking experiments and crystal struc-
tures of isolated iGluR extracellular domains indicate that key
structural features observed in the GluA2 homotetramer,
including subunit crossover in the ATD and LBD layers, are
conserved in full-length kainate and NMDA receptors (Das
et al., 2010; Furukawa et al., 2005; Karakas et al., 2011; Kumar
and Mayer, 2010; Kumar et al., 2011; Lee and Gouaux, 2011;
Mayer, 2006; Salussolia et al., 2011; Stawski et al., 2010; Trayne-
lis et al., 2010). However, an open question is whether the
packing of subunits in NMDA receptors differs from that in
AMPA and kainate receptors. Although this will only be resolved
by solving the structure of an intact NMDA receptor, there is
accumulating evidence that substantial differences are likely,
and as discussed in more detail later in the review, dimer and
tetramer assemblies for GluN1 and GluN1/GluN2 ATDs have a
strikingly different packing from that in AMPA and kainate
receptors.

Structure of the Ion Channel
The ion channel domain of the GluA2 homotetramer has three
membrane-spanning helices (M1, M3, and M4) plus a pore helix
(M2), and as predicted by amino sequence alignments with other
membrane proteins, it has the expected 4-fold symmetry found
in voltage-gated ion channels, but with inverted topology. The
M3 transmembrane helix from the four subunits forms a bundle
crossing on the 4-fold axis of symmetry, which acts as a barrier
to ion permeation and forms the lining of the pore, while the M1
and M4 helices form the lipid exposed external surface of the
channel, with the M4 helix packing against the M1 helix of an
adjacent subunit. There are large depressions in the lipid-
exposed surface between the M1 and M4 segments of each
subunit, which expose the pore helix to the interior of the lipid
bilayer, suggesting that the interaction of lipids with iGluRs might
be more intense than for membrane proteins with a more
compact membrane-embedded surface. In this context, it is
intriguing to note that for GluR6 mutants with Arg substitutions
in the M3 helix, fatty acids change ion selectivity, suggesting inti-
mate access to the pore, or that these lipids induce changes in
pore conformation (Wilding et al., 2010). Additional novel
features revealed by the GluA2 crystal structure include a pre-
M1 cuff helix, which wraps around the bundle crossing at the
exterior of the pore, nearly parallel to the plane of the membrane,
and different conformations in the three peptide linkers of the
AC and BD subunit pairs, which resolve the 2-fold to 4-fold
symmetry transition between the LBDs and ion channel pore.
These symmetry mismatches were nicely captured in anomal-
difference electron density maps for a complex of GluA2 with
a mercury derivative of the competitive antagonist ZK200775,
for which free Hg present as a contaminant was fortuitously
bound in the LBD, ATD, and ion channel layers (Figure 2). By
contrast to crystal structures for voltage-gated ion channels, in
the GluA2 homotetramer the M2 pore loop and linker to M3, which form part of the turret assembly and binding sites for pore blocking toxins in potassium channels, are disordered and there is no electron density for ions or solvent molecules. This likely results from the different ion coordination properties of nonselective iGluRs compared with potassium channels, as well as the low (3.6 Å) resolution of the GluA2 structure, and possibly the closed pore conformation imposed by the competitive antagonist bound to the LBDs. Thus both higher-resolution structures, as well as a structure of an open state, will be needed to provide insight into the location, chemistry, and number of ion-binding sites in iGluR ion channel domains.

High-Resolution Structures of AMPA and Kainate Receptor ATD Assemblies

Because of the difficulty of expression and crystallization of full-length iGluRs, there continues to be substantial effort devoted toward solving structures for the extracellular domains expressed as soluble proteins genetically excised from the ion channel. By contrast to the bacterial expression systems used to express iGluR LBDs, the ATDs have all been expressed as secreted glycoproteins, using either mammalian or insect cell culture, and their structures solved at resolutions of up to 1.4 Å. Figure 3 shows a library of structures for the isolated ATDs of GluA1, GluA2, GluA3, GluR6, GluR7, and KA2, which reveals a highly conserved homodimer assembly. In the majority of these structures, both the upper and lower lobes of the ATD clam shell contribute almost equally to dimer assembly with a buried surface area of around 1400–1600 Å² (Clayton et al., 2009; Jin et al., 2009; Kumar and Mayer, 2010; Kumar et al., 2009; Rossmann et al., 2011; Yao et al., 2011). By contrast, for the KA2 homodimer assembly the upper lobes have moved apart, and for the GluA3 homodimer the lower lobes have separated (Kumar and Mayer, 2010; Sukumaran et al., 2011); because it is unlikely that homomeric iGluRs are formed in vivo, the functional significance of the different conformations remains to be experimentally tested. For GluA2, GluR6, and GluR7, crystal symmetry operations generate tetramers that closely resemble the structure found in full-length GluA2 (Figure 3B). For GluA1, a novel “head to head” packing of dimer pairs was observed in which contacts are formed between the upper lobe of the ATD assembly. Although this form is not sterically compatible with formation of a full-length tetrameric iGluR assembly, it could plausibly contribute to assembly of AMPA receptor arrays in the postsynaptic membrane (Yao et al., 2011). Currently only one tetrameric structure for a heteromeric complex of a non-NMDA receptor ATD assembly has been reported, that for the GluR6/KA2 combination (Figure 3C); the packing found for the heterotetramer is essentially identical to that of the full-length GluA2 ATD homotetramer, but with the GluR6 subunits forming the dimer of dimers interface and the KA2 subunits at the periphery (Kumar et al., 2011).

In all of the iGluR ATD structures there is a conserved disulfide bond that links α helix B with an extended loop approximately 300 amino acids away. This loop forms a flap capping the surface of the dimer assembly in each subunit, and has been named loop 3, the S-loop, and the hypervariable loop by different groups (Figure 3D). The loop has a different length in each of the major iGluR subtypes and shares very low sequence homology between families. The conformation of the S-loop is variable in the case of the GluA2 ATD for which multiple structures have been solved, whereas for the GluA1 ATD the S-loop quite surprisingly also makes contact between dimer pairs, in the unusual head-to-head assembly not found in other AMPA and kainate receptor ATD crystal structures. Below the S-loop there are extensive intermolecular interactions between the αB and αC helices; at the N terminus of α helix B there is a conserved aromatic residue that slots into a pocket on the dimer partner formed by hydrophobic residues on the αB and αC helices of the dimer partner (Figure 3D). Mutation of the aromatic residue disrupts homodimer and heterodimer assembly in AMPA and kainate receptors (Clayton et al., 2009; Kumar et al., 2011).
In the GluA2 ATD mutation of another upper lobe residue, T78A, also reduces the affinity for dimer assembly (Rossmann et al., 2011). In the lower lobe, intermolecular contacts are made by patches of hydrophobic residues flanked by polar residues that contribute to subtype selective assembly. A quantitative analysis for the effects of mutants on dimer assembly has only been performed for the combination GluR6/KA2, which reveals that intermolecular contacts made by the lower lobe are also major determinants of ATD assembly (Kumar et al., 2011).

All of the non-NMDA receptor ATD structures solved to date are for the apo state; indeed there are no ligands that are known to bind to AMPA and kainate receptor ATDs. For one of the GluA2 ATD crystal structures (PDB 3HSY), Sukumaran et al. (2011) reported that Fo-Fc electron density maps contoured at 3.0 and 2.0 \( \sigma \) reveal “non-protein, non-water molecules in the cleft.” However, rebuilding and refinement of this structure using the deposited structure factors, which reduced \( R_{\text{work}} \) and \( R_{\text{free}} \) by 1.0 and 0.6\%, revealed that all of the features in the electron density maps can be accounted for by water molecules, together with a phosphate ion bound in the cleft and flanked by the side chains of Arg108, Arg135, and Lys273. The crystallization conditions reported in this deposition include 200 mM ammonium phosphate, and in subunit B unambiguous tetrahedral density is present for the ion, the phosphate atom of which remains visible at a contour of >12 \( \sigma \) in omit maps, long after density for the flanking water molecules has disappeared.

Figure 3. A Gallery of iGluR ATD Dimer and Tetramer Structures

(A) An ATD dimer from the full-length GluA2 structure (3KG2) and the isolated ATDs of GluA1 (3SAJ), GluA2 (2WJW, 3H5V, and 3HSY), GluA3 (3O21), GluR6 (3H63), GluR7 (3OLZ), KA2 (3OM0 and 3OM1), and a GluR6/KA2 heterodimer assembly (3QLU). Transparent representations are used for the 2nd and 3rd copies of structures for which more than one dimer assembly was present in the asymmetric unit, and for structures solved by different groups, revealing small changes in subunit orientation, resulting principally from rotations parallel to the two-fold axis of symmetry.

(B) ATD homotetramer assemblies generated by crystallographic symmetry operations for GluA2, GluR6, and GluR7.

(C) The GluR6/KA2 ATD tetramer assembly (3QLV) showing formation of the dimer of dimers interface by the GluR6 subunits.

(D) Stereo view of the GluR6/KA2 ATD heterodimer showing packing between helices B and C in the upper lobes; the S-loop projects into the dimer interface; below this a conserved aromatic residue projects into a hydrophobic slot on the dimer partner.
Thus, although the GluA2 ATD bi-lobed structure can bind organic anions like phosphate, when these are present in crystallization solutions, it remains to be determined whether endogenous ligands can bind instead. Whether the binding of ions or other small molecules produces changes in the conformation of the GluA2 ATD, resulting in allosteric changes in receptor activity via signaling to the LBDs, is also a controversial and significant issue. The association of ATDs in an iGluR tetramer assembly produces steric restraints on the extent and axis of movement that would be possible in response to ligand bindings that are absent in structurally related soluble monomeric proteins like LIVBP. As discussed later, in NMDA receptors this problem appears to have been solved during evolution by a substantial rearrangement of the ATD dimer assembly, which leaves the lower lobes free to move in response to the binding of ligands. By contrast, AMPA and kainate receptors appear to have evolved differently, and because of steric clashes, it is difficult to envision how it is possible for ligands to induce changes in the conformation of ATD dimer assemblies in non-NMDA receptors. However, B-factor analysis reveals that the upper lobes are generally better ordered than the lower lobes, raising the possibility that similar to the natriuretic peptide receptor (NPR) family (He et al., 2001), movement of the lower lobes in an ATD dimer assembly might permit binding of small molecules in the intrasubunit cleft. This mode of binding is distinct from that found in periplasmic proteins like LIVBP, where ligands bind in the cleft located between the upper and lower lobes of single protomers. Modest differences in subunit conformation are observed across the family of AMPA and kainate-receptor ATD structures solved to date, but whether these have functional significance or are the result of differences in crystal packing is unknown. Thus, it remains unclear whether the ATDs of non-NMDA receptors can undergo substantial changes in conformation in response to the binding of allosteric modulators when they are assembled as a tetramer, as has been proposed on the basis of normal mode analysis for the isolated ATDs (Sukumar et al., 2011). Instead, the picture that emerges from comparison of the AMPA and kainate receptor ATD dimer structures is that they have a common low energy conformation that is remarkably conserved between subtypes. There is no single region that confers subtype selective assembly on iGluRs from different gene families; rather, for each family there is an extensive and complimentary dimerization interface involving both lobes in all structures except for the GluA3 and KA2 homodimer assemblies. As discussed below this results in a very stable high-affinity dimer assembly.

**Thermodynamic Analysis of iGluR Assembly**

Biophysical studies on the isolated LBDs and ATDs have provided valuable insight into the processes driving iGluR assembly. Measurements using multicycle light scattering, dynamic light scattering, and analytical ultracentrifugation revealed that the LBDs of all eukaryotic iGluRs examined to date are monomeric at protein concentrations as high as 250 μM, and thus the Kₜ for dimer and tetramer assembly by the LBDs must be in the high mM range (Furukawa et al., 2005; Sun et al., 2002; Weston et al., 2006b). Viewed in light of the full-length GluA2 structure, the LBDs are almost like a cluster of four buoys tethered by connections to the ATD, but packed more intimately against the membrane-embedded ion channel layer. Thus, in an intact iGluR assembly, intermolecular contacts mediated by the LBD layer of the tetramer assembly must be very weak and would be expected to play only a minor role in the initial stages of receptor assembly. However, intermolecular interactions in the LBD layer do have a profound impact on receptor function. Increasing the strength of subunit interactions via mutations at the LBD dimer interface strongly attenuates desensitization (Chaudhry et al., 2009; Nayeem et al., 2009; Sun et al., 2002; Weston et al., 2006b). On the other hand, electrophysiological experiments on intact GluA2, which measured the rate of formation of disulfide bonds between Cys mutants introduced into the LBD (Plested and Mayer, 2009), and single molecule FRET measurements of the conformational stability of the isolated LBD (Landes et al., 2011), reveal highly dynamic structures undergoing conformational exchange on the millisecond-to-second time scale. Although both approaches highlight the dynamic nature of the LBD assembly, they give no information on the structure of the spectrum of conformational states revealed by kinetic analysis. With current techniques, single-molecule FRET measurements have the additional limitation that they are incapable of measuring conformational changes on the time scale of agonist binding and ion channel activation, which concentration jump experiments with outside-out patch recording, and single channel analysis reveal to occur on the μs time scale.

Biophysical studies on the isolated ATDs of iGluRs have only recently been performed and reveal a spectrum of behavior that significantly affects the processes of iGluR assembly and allosteric modulation. By contrast to extremely weak interactions for the iGluR LBDs, the isolated ATDs show a wide range of affinities for oligomerization, with monomer-dimer Kₜ values ranging from low nM to high mM. Notably, for the subset of AMPA and kainate-receptor iGluRs, which can assemble to form functional homomeric ion channels, the isolated ATDs assemble with very high affinity, whereas for the KA2 subunit, and the NMDA receptor GluN1 and GluN2 subunits, all of which are obligate heteromers, homodimer formation was very weak, Kₜ 0.5 mM for KA2 (Kumar et al., 2011), or not measurable for GluN1 and GluN2 (Karacas et al., 2011). However, high-affinity heterodimers are formed when the ATDs for these subunits are mixed with those from their partners, which coassemble to form native heteromeric assemblies in vivo, i.e., GluR6 for KA2, heterodimer Kₜ 11 nM, and the mix of NR1 and NR2, heterodimer Kₜ 0.7 μM (Karacas et al., 2011; Kumar et al., 2011). These results are consistent with cross-linking experiments in full-length kainate and NMDA receptors, which indicate coassembly of GluR6/KA2 ATDs and NMDA receptor ATDs as heterodimers (Karacas et al., 2011; Kumar et al., 2011; Lee and Gouaux, 2011). For AMPA receptors, a related pattern is observed, with high affinity homodimers formed by the isolated ATDs of GluA1, GluA2, and GluA4, which efficiently form homomeric receptors, whereas for GluA3, homodimer Kₜ 1.2 μM, the heterodimer formed on coassembly with GluA2, its likely partner in vivo, has much higher affinity with a Kₜ of 1 nM (Rossmann et al., 2011).

Although experiments with receptor chimeras indicate that the ATD plays a key role in biosynthesis, by restricting assembly to selected subunit populations (Ayala et al., 2005), the extremely weak interaction of iGluR LBDs raises a conundrum, because it...
would be expected that the ATD provides the major driving force for assembly. However, for AMPA or kainate receptors, deletion of the ATD does not affect the function of homomeric receptors studied in heterologous expression systems (Horning and Mayer, 2004; Pasternack et al., 2002; Plested and Mayer, 2007). For NMDA receptors, which are obligate heteromers, functional channels are also obtained after deletion of the ATD for each of the four GluN2 subunits coexpressed with wild-type GluN1. However, these experiments reveal substantially reduced expression (Rachline et al., 2005), and recent work on assembly of heteromeric kainate receptors, which used the unique ligand-binding properties of the KA2 subunit as a bioassay for coassembly with GluR6, also revealed disrupted assembly when mutations that abolished high-affinity heterodimer assembly were introduced into the KA2 subunit ATD (Kumar et al., 2011). An important control performed for these experiments was the demonstration that these mutations did not interfere with folding and secretion of the KA2 subunit as a bioassay for coassembly with GluR6, also revealed disrupted assembly when mutations that abolished high-affinity heterodimer assembly were introduced into the KA2 subunit ATD (Kumar et al., 2011). An important control performed for these experiments was the demonstration that these mutations did not interfere with folding and secretion of the KA2 subunit ATD. Similar experiments for AMPA receptors reveal that mutations in the GluA2 subunit ATD dimer interface, which would be anticipated to disrupt ATD dimer assembly, also reduce coassembly of heteromeric AMPA receptors, as assayed by changes in receptor function (Rossmann et al., 2011). However, some of these mutations disrupt the expression of correctly folded GluA2 subunit ATDs, and so do not directly test its role in assembly. In addition, the role of the ATD in iGluR assembly is much more complex than a simple recombination based on affinity, because for NMDA receptors there is evidence that the GluN2A subunit ATD contains an ER retention signal that is masked on coassembly with GluN1 (Qiu et al., 2009).

The Emerging Structure of NMDA Receptors

Crystal structures for the isolated ATDs of the NMDA receptor GluN1 and GluN2 subunits are especially interesting because they reveal monomer, dimer, and tetramer assemblies with strikingly different conformations from those for AMPA and kainate receptors, suggesting that the global architecture of subunit packing in NMDA receptor tetramers might differ from that for AMPA and kainate receptors (Figure 4). Although dimer assemblies of the NMDA receptor LBDs closely resemble those for AMPA and kainate receptors (Furukawa et al., 2005), the three NMDA receptor ATD structures solved to date, for rat and Xenopus GluN1 and for rat GluN2B, all exhibit twisted conformations in which the relative orientation of the lower lobe is rotated by approximately 45° compared with the conformation found in AMPA and kainate receptors (Farina et al., 2011; Karakas et al., 2009; Karakas et al., 2011). The rat and Xenopus GluN1 ATD structures are essentially identical (root-mean-square deviation [rmsd] 0.99 Å), with the exception that the rat construct lacks 21 residues encoded by exon 5, which were present in the Xenopus GluN1 splice variant used for crystallization. Like the battery of recently solved AMPA and kainate-receptor structures (Figure 3), the GluN1 ATD was crystallized in the apo state, whereas the GluN2B ATD was crystallized in a complex with Zn²⁺ as well as in the apo state. In addition, an ifenprodil complex was solved for a GluN1/GluN2B ATD heterodimer. It is remarkable that all three GluN2B structures have essentially identical, closed-cleft conformations, with rmsds of 0.52 and 1.0 Å for superposition on the GluN2B apo structure, which in turn is similar to the apo structures of the GluN1 ATDs. There is evidence from functional studies that used either Cys mutant cross-linking

Figure 4. NMDA Receptor ATD Dimer and Tetramer Structures

(A) Stereo view of the rat GluN1 ATD homodimer (3Q41), with the two subunits colored in pale and bright green; the N and C termini marked by blue and red spheres; and the S-loop colored dark blue.

(B) Stereo view of the GluN1/GluN2B heterodimer rotated by approximately 90° from the view in (A), with the Xenopus GluN1 subunit colored pale green, the GluN2B subunit colored gold, and the S-loop, which includes an α-helix colored dark blue; for comparison, the rat GluN1 ATD heterodimer is drawn in transparent shading after superposition using the heterodimer GluN1 subunit coordinates.

(C) Stereo view of the GluN1/GluN2B ATD asymmetric unit contents showing two copies of the ATD heterodimer, with a tetramer formed by crystal contacts mediated by the GluN2B subunits, colored as in (B).
(Stroebel et al., 2011), or chemical modification by MTS reagents with bulky substituents (Gielen et al., 2009), that in their apo state the GluN2A and GluN2B ATDs must be able to adopt an open-cleft conformation; but to date this conformation has not been crystallized, and the extent of the conformational change underlying the open-to-closed transition remains to be established. It is also unknown whether the GluN1 ATD can adopt an open-cleft conformation, or whether like the AMPA and kainate-receptor ATDs, it remains closed in the absence of ligands.

An even more remarkable difference for NMDA and non-NMDA receptor ATDs emerges on inspection of crystal structures for their dimer and tetramer assemblies, but this raises an important caveat. The ATD structures were solved for isolated domains expressed as soluble proteins removed from restraints present in a tetrameric membrane protein assembly. For AMPA and kainate receptors, the similar structure of the isolated ATD and LBD dimer and tetramer assemblies to those found in the full-length GluA2 structure leaves little doubt about their biological significance. However, there are a handful of LBD dimer assemblies in the PDB that likely have no biological significance. Some of these “fakes” are easy to spot, for example dimers in which the subunits are rotated by 180° to generate head to tail assemblies, but for others the changes are more subtle, and can be eliminated only by considering sources of additional information gained, for example, from functional experiments and site-directed mutagenesis. The highly flexible nature of an iGluR tetramer assembly, for which the LBDs undergo large conformational changes during the processes of activation and desensitization, adds to the complexity because multiple conformational states of the ATD and LBD tetramer assemblies must occur. However, at present we have little information on what these should look like. Further difficulties arise if the GluA2 structure cannot be used as a template for NMDA receptors.

With these caveats in mind, the NMDA receptor ATD structures are fascinating in their own right. In the three crystallographically independent GluN1 ATD dimers, one from Xenopus, and two from rat, intermolecular contacts are mediated exclusively via the upper lobes of the ATD clamp shell, with a buried surface of 1140 Å² per subunit. Because of the 45° twist of the lower lobes in each protomer, away from the axis of dimer symmetry, the lower lobe plays no role in dimer assembly. Different from the ATD dimer assemblies for AMPA and kainate receptors, where α-helix B from one subunit faces α-helix C of its dimer partner, in the GluN1 homodimer the subunits are displaced laterally, with a 90° rotation of one subunit compared with the assembly of AMPA and kainate-receptor ATDs. As a consequence, in the GluN1 homodimer, α-helix C is in contact with α-helix C in its dimer partner, whereas α-helix B is located at the lateral edge of the dimer assembly. Superposition of the GluN1/GluN2B heterodimer on the GluN1 homodimer, using coordinates for one of the GluN1 protomers, reveals a strikingly different dimer assembly, in which the GluN2B subunit is rotated by 180° compared with the GluN1 homodimer (Figure 4). Despite this, the buried surface in the GluN1/GluN2B heterodimer, 1100–1200 Å² per subunit, is similar in size to that in the GluN1 homodimer, although the contacts are mediated by different sets of interactions between α helices B and C. In addition, for the GluN1/GluN2B heterodimer, a loop connecting β strand 6 to α-helix G in the lower lobe of the GluN2B subunit projects into the dimer interface and makes contact with the upper lobe of the GluN1 subunit. Overall, a superposition using coordinates for the upper lobes of the ATDs reveals that the GluN1/GluN2B heterodimer assembly more closely resembles that found in AMPA and kainate receptors than the GluN1 homodimer.

The GluN1/GluN2B ATD crystal structure contains 2 dimer pairs in the asymmetric unit, and these form a trimer with the “dimer of dimers” interface mediated by the GluN2 subunits (Figure 4), consistent with Cys mutant cross-linking experiments on the LBD and the predicted tetrameric organization of an NMDA receptor (Sobolevsky et al., 2009). However the dimer of dimers interface is completely different from that found in AMPA and kainate receptors, is formed by the upper not lower lobes of the ATD protomers, and involves contacts mediated by the S-loop. Without additional information, it is impossible to tell whether the GluN1/GluN2B NCS tetramer corresponds to a native NMDA receptor ATD conformation, or whether it occurs only in the crystal lattice. Perhaps wisely, Karakas et al. (2011) chose not to discuss the GluN1/GluN2B tetramer assembly and focused on the dimer assembly and its role in allosteric modulation.

Gel filtration and sedimentation analysis reveals that the isolated ATDs for GluN1 and GluN2 are monomeric at protein concentrations above 1 mg/ml but form high-affinity heterodimers when mixed (Karakas et al., 2011). Thus the functional significance of GluN1 ATD homodimer crystal structures could be questionable. It is striking, however, that the rat and Xenopus structures are nearly identical; that they were solved in different space groups (rat P3,2, Xenopus P2,2,2,1); and that in the Xenopus structure there are two identical dimers generated by crystallographic and noncrystallographic symmetry; thus, despite low affinity for self-association, the GluN1 ATDs appear to have a low energy state favoring dimer assembly in solution, as also supported by single-particle EM analysis (Farina et al., 2011). On the basis of these observations, it has been proposed that the formation of NR1 homodimers occurs as an assembly immediately before exchange with GluN2 subunits (Farina et al., 2011); similar proposals have been made for biosynthesis of heteromeric AMPA receptors, during which GluA2 is likely to form high-affinity homodimer assemblies (Rossmann et al., 2011). Dimer dissociation and subunit exchange would likely require mechanisms that stabilize the fold of individual iGluR subunits before assembly as a tetramer. Possibly, the exchange could occur before the ion channel segments are inserted into the lipid bilayer, or perhaps there are novel membrane proteins that act as chaperones and stabilize lipid embedded iGluR monomers and dimers. Very little is known about these early stages of channel assembly.

**Structural Insights into Allosteric Modulation by Drugs and Endogenous Ions**

The NMDA-receptor ATDs contain binding sites for drugs with therapeutic potential in neurological and psychiatric disease. Based on homology with bacterial periplasmic binding proteins like LIVBP, models have been developed in which ligands bind within the clamshell for individual ATD protomers, such that the resulting domain closure “pulls” on the LBD layer, reducing the stability of the agonist-bound active dimer conformation (Gielen...
Ifenprodil binds within the interface between the GluN1 and GluN2B subunits, and not deep within the cleft as previously assumed. Zn\(^{2+}\) binds at the lateral edge of the interdomain cleft in the GluN2B ATD structure, and not deep within the cleft as is often depicted in cartoons. The Zn\(^{2+}\) ion is bound by residues in the upper (His 127) and lower (Glu 284) lobes, and thus likely stabilizes the closed-cleft conformation. The NR2B ATD also binds a cluster of Na\(^+\) and Cl\(^{-}\) ions, which are located deeper the interlobe cleft in the Zn\(^{2+}\) complex and apo state structures. The function of these Na\(^+\) and Cl\(^{-}\) ion-binding sites is unknown (Karakas et al., 2009).

It is interesting that there are binding sites for unidentified cations in the ATDs of GluR6 and GluR7 (Kumar and Mayer, 2010; Kumar et al., 2009); the GluN1 subunit ATD also has a binding site for monovalent cations, which like that in kainate-receptor LBDs can bind either Na\(^+\), K\(^+\), or Rb\(^+\). The functional effects of ion binding at these sites in iGluR ATDs, which are present in loops projecting from the core of the protein, are also unknown.

**Role of Domain Closure in Activation by Partial Agonists**

The first structural studies on iGluR LBDs, for the GluA2 subunit, revealed different extents of domain closure, the extent of which was remarkably correlated with the efficacy for activation by “partial agonists,” ligands that produce less than the maximal response at saturating concentrations (Armstrong and Gouaux, 2000; Jin et al., 2003). There is little doubt that these studies captured low-energy conformations of partial agonist complexes, because subsequent free-energy calculations performed using molecular dynamics simulations reveal a similar conformational spectrum with well-defined minima for individual partial agonists (Lau and Roux, 2007; Lau and Roux, 2011). The MD results also illustrate the obvious—that crystal structures capture just one of an ensemble of low-energy populations, and that transitions between some of these states will be frequent and require little additional energy beyond that provided by thermal fluctuation. As would be expected, NMR experiments on the LBD of GluA2 also revealed that, in the absence of a crystal lattice, GluA2 complexes with partial agonists sample a range of conformational states (Fenwick and Oswald, 2008; Mal'tsev et al., 2008). The idea that the extent of LBD domain closure alone was not the sole determinant of iGluR activity first came from the results of functional studies that used mutations in the GluA2 LBD. AMPA, which is a full agonist for wild-type GluA2, produced only 38% of the response to glutamate for the L650T mutant; however, crystal structures for the mutant LBD AMPA complex revealed two conformations, with full and partial domain closure (Armstrong et al., 2003). Because the GluA2 L650T mutant can close to the same extent as wild-type, this raised the idea that the stability of the fully closed LBD conformation, which presumably gates ion channel activity, was also an important determinant of agonist efficacy. Recent studies on an extended suite of AMPA receptor partial agonists have revealed that even for wild-type GluA2, complexes with a single ligand can be crystallized in different conformations and provide another example of a fully closed conformation of the GluA2 LBD in complex with a partial agonist (Poon et al., 2011).
Electrophysiological experiments have also revealed that disruption of interlobe contacts, which were identified from analysis of GluA2 LBD crystal structures, reduces the stability of the LBD agonist complex (Robert et al., 2005; Weston et al., 2006a; Zhang et al., 2008), whereas introducing additional contacts has the opposite effect (Weston et al., 2006a). These results are consistent with the idea that the binding of agonists is a multi-step process in which ligand-induced changes in the extent of domain closure, in turn permit the formation of interlobe contacts that stabilize the closed cleft conformation and affect agonist efficacy. Although our ability to understand the energetics of this process remains limited, an attractive idea is that to activate ion channel gating, both partial and full agonists must produce the same extent of domain closure. This process is less favorable for partial agonists because, most of the time, these ligands populate low-energy states with intermediate extents of domain closure, resulting in nonproductive binding with respect to activation of ion channel gating.

**Future Directions**
The availability of a full-length iGluR structure, combined with high-resolution structures for the isolated ATD and LBDs for multiple iGluR subtypes, has given unprecedented insight into the molecular function of this important family of neurotransmitter receptors. More than 150 high-resolution structures for ligand-binding domain complexes for multiple iGluR subtypes with agonists, partial agonists, competitive antagonists, and allosteric modulators of desensitization reveal with unrivaled clarity the mechanisms underlying the subtype selective pharmacology that formed the historical basis for classification of iGluR subtypes (Mayer, 2006; Pehlsgaard et al., 2011; Stawski et al., 2010; Traynelis et al., 2010). Work is rapidly progressing on the amino terminal domains, but here key questions remain to be addressed.

Almost 10 years ago a model was put forward for the mechanism of desensitization involving “dissociation” of the upper lobes of the LBD dimer assembly (Sun et al., 2002). At the level of the LBD, this model remains essentially intact and in parts is fully supported by the full-length GluA2 structure. However, beyond the level of the ligand-binding domains, our level of understanding rapidly decreases. Mapping the model of desensitization onto the full-length GluA2 structure requires rearrangements in ATD dimers and also in the ATD dimer of dimers assembly (Sobolevsky et al., 2009), such that inter-subunit cross-links introduced in the ATD should inhibit the process of desensitization. In fact, the opposite result was obtained for rapidly desensitizing kainate receptors (Das et al., 2010). For the ion channel pore, the GluA2 structure indicates that there should be large conformational changes during channel opening, but at present these cannot be predicted with any degree of accuracy, highlighting the need for structures in both the open and desensitized states. Although experiments on the mechanism of activation have resulted in a working model that can explain in broad principle the action of partial agonists, the precise molecular details remain vague and will likely be solved only when MD calculations of long duration can be performed on full-length iGluRs—a daunting computational challenge.

Experimental work on full-length iGluRs, and especially for heteromeric assemblies, also remains stunningly difficult because of the requirements for large-scale membrane protein expression in eukaryotic cells, combined with the inherent conformational instability of iGluRs as a result of their layered assembly in addition to their highly asymmetric shape, which limits the opportunities for packing required to obtain well-ordered crystals that diffract to high resolution. Significant structural work remains to be done because without structures for the apo state of NMDA receptor ATD assemblies, it is impossible to build realistic models for the conformational changes underlying allosteric modulation by drugs like ifenprodil. Structural information is also important to understand the molecular basis for recently reported intrinsic subtype selective differences in NMDA receptor basal activity (Gielen et al., 2009; Yuan et al., 2009). Finally, a major challenge will be to understand the molecular basis for co-assembly and modulation of iGluRs by a growing family of auxiliary subunits (Jackson and Nicoll, 2011; Straub et al., 2011).

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