Molecular determinants of AMPA receptor subunit assembly

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AMPAType (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate) glutamate receptors (AMPARs) mediate postsynaptic depolarization and fast excitatory transmission in the central nervous system. AMPARs are tetrameric ion channels that assemble in the endoplasmic reticulum (ER) in a poorly understood process. The subunit composition determines channel conductance properties and gating kinetics, and also regulates vesicular traffic to and from synaptic sites, and is thus critical for synaptic function and plasticity. The distribution of functionally different AMPARs varies within and between neuronal circuits, and even within individual neurons. In addition, synapses employ channels with specific subunit stoichiometries, depending on the type of input and the frequency of stimulation. Taken together, it appears that assembly is not simply a stochastic process. Recently, progress has been made in understanding the molecular mechanisms underlying subunit assembly and receptor biogenesis in the ER. These processes ultimately determine the size and shape of the postsynaptic response, and are the subject of this review.

Introduction

Ionotropic glutamate receptors (iGluRs) mediate the majority of fast excitatory synaptic transmission in vertebrate central nervous systems (CNSs) [1]. Three subfamilies of glutamate-gated, cation-selective channels mediate rapid signalling: AMPA, NMDA (N-methyl-D-aspartate) and kainate receptors. They play pivotal roles in synaptic formation and maintenance, and in various forms of synaptic plasticity [2,3]. In addition, dysfunction of these receptors results in diverse acute and chronic neurological disorders. iGluRs are widely distributed in the CNS, where they fulfil different functions. AMPA-type glutamate receptors (AMPARs) mediate fast depolarization in glutamatergic transmission, and their exceptionally fast kinetics conveys ‘point-to-point’ signalling [2,4].

In contrast to the skeletal nicotinic acetylcholine receptor, which is built in a predetermined fashion [5], AMPARs are expressed, like many other ion channels, in various subunit combinations; they assemble from four subunits, GluR1–4 (or GluRA–D), into ion channel tetramers [1,6–9]. The selectivity of the assembly process, which determines channel functional properties, is poorly understood. The subunit stoichiometry also regulates vesicular traffic [1,10,11] and synapse-specific targeting of channel tetramers [12–14], and thereby directly impacts synaptic efficacy and plasticity. Moreover, accumulating evidence suggests that the AMPAR composition is not static, but can be altered in response to certain inputs [15–21].

In analogy to K⁺ channels [22], AMPARs are thought to assemble as dimers of dimers [9,23,24]. The N-terminal domain mainly mediates dimer formation; subsequent tetramerisation involves the extracellular S2 loop and the transmembrane segments (including the pore loop; Figure 1a) [23]. Receptor assembly occurs at the endoplasmic reticulum (ER) membrane. Exit from the ER is under stringent quality control, which monitors correct subunit folding and assembly [25]. The efficiency of these processes impacts on ER export kinetics and thereby determines the number of channels available for expression at synapses; this will ultimately tune the responsiveness of a neuron. Interestingly, recent studies indicate that conformational alterations associated with gating motions, such as ligand binding and desensitization (i.e. channel closure in ligand bound state), take place in the ER lumen, where they are sensed by the quality control machinery [26–31]. These findings add iGluRs to a growing list of ER client proteins that require ligands or ‘chemical chaperones’ for efficient folding and export from the ER [32].

In addition to the subunit stoichiometry, AMPAR function is diversified further by RNA processing events, including alternative splicing and RNA editing [1]. Splicing of mutually exclusive exons (termed flip/flop) within the ligand-binding domain (LBD) modulates desensitization kinetics [33]. These two alternative exons are present in all four subunits and are common to vertebrate AMPARs (Figure 2) [34]. Also, RNA editing at two sites modulates AMPAR function — the R/G site within the LBD of GluR2–4 and the Q/R site in the pore loop of GluR2 (Figure 1a; Box 1) [35,36]. These sites are located within subunit interfaces (Figure 1b) and intimately impact receptor assembly [30,37]. Below, we discuss the role of individual domains in the assembly process and review structural parameters of their interaction surfaces. We then describe how these interfaces are modulated by RNA editing and how these modifications evolved in the iGluR lineage (Figure 2; Box 2). Finally, we consider how gating motions, initiated by ligand binding in the ER, contribute to receptor folding and assembly.

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Receptor assembly in the ER involves distinct domains

The biogenesis of oligomeric transmembrane proteins occurs at the ER membrane and commences with the co-translational insertion of nascent polypeptides through the Sec61 channel [38]. Subsequent protein folding and assembly is mediated by three physicochemically distinct environments: the cytosol, the lipid bilayer of the rough ER and the ER lumen. Folding of a nascent peptide into its tertiary structure can begin as early as the emergence of the polypeptide from the ribosome, as has been shown for the Kv1.3 K+ channel [39]. Individual domains of eukaryotic proteins appear to acquire their tertiary structure sequentially, which is thought to minimise misfolding of multidomain polypeptides [40].

iGluR subunits can be divided into four distinct domains (Figure 1a,b). The extracellular (and thus the ER-luminal) portion comprises two domains that are homologous to periplasmic binding proteins (PBPs; [41]): the N-terminal LIVBP (leucine/isoleucine/valine-binding protein-like domain) and the adjacent LBD, which is homologous to glutamine-binding protein (Figure 1b). The ion channel domain itself is composed of three transmembrane segments and a re-entrant pore loop, which is distantly related to K+ channel pore loops [42]. The cytoplasmic C-terminal domain interacts with various components of the postsynaptic signalling apparatus, which mediate receptor trafficking and anchorage [1,10,11].

Figure 1. Domain organization of AMPARs and associated interfaces. (a) Schematic of the GluR2 subunit. The N-terminal LIVBP-like domain is in pink. The bipartite LBD (yellow) is composed of the S1 (purple) and the S2 segments (green). The R/G editing site at position 743 is indicated by a red diamond, the flip/flop exon by a blue curve. These two extracellular domains face the ER lumen (depicted by the grey vertical bar) during receptor biogenesis. The ion channel domain within the lipid bilayer (blue) consists of three transmembrane (TM) segments (grey) and the re-entrant pore loop. The Q/R editing site at the pore apex is depicted by a red diamond. (Amino acid positions are numbered according to the mature polypeptide lacking the signal sequence; therefore, in disagreement with [37], the Q/R site is labelled 586.) Two loops (25 and 10 residues in length) and the C terminus face the cytoplasm. (b) Structural depiction of an AMPAR complex. The background colour coding of the individual domains is as in (a). A hypothetical subunit dimer is shown in cyan; the yellow dimer lacks the extracellular portion for clarity. The vertical arrow runs through the twofold symmetric LIVBP and LBD axis, and the fourfold symmetric ion channel axis. Symmetry relations (i.e. twofold and fourfold) are indicated by symbols within the figure. The LIVBP-like domain dimer is represented by the homologous extracellular portion of mGluR1 (PDB code 1EWK) [45]. The LBD dimer is shown in its R/G unedited -Glu-bound form (PDB code 2UXA) [30]. Arg743 sidechains are shown in stick representation and are located at a twofold symmetric subunit interface (red diamond). The vertical grey bar denotes the ER lumen, as in (a). The fourfold symmetric ion channel domain is derived from a homology model [37] based on the atomic coordinates of the prokaryotic KcsA K+ channel. The long α helix corresponds to TM3, the short helix to the ascending part of the pore loop; TM1 and TM4 were not included in the model [37]. Q/R site residues from each subunit are shown in stick form; they are located at a fourfold symmetric subunit interface (red diamond). The C-terminal domain is not included in the model. (c) Back view of an LBD protomer. The S1 (purple) and S2 (green) segments comprise the bipartite LBD [30]. One LBD protomer is shown from the back, with a view onto the elements forming the dimer interface (D, J, b). The Arg743-Asp490 salt bridge, which contributes to S1–S2 interactions, is shown. Cys773 at the C-terminal end is linked to lower lobe Cys718 (indicated in orange); this cysteine bridge is required for folding [30]. The extreme N- and C-terminal ends of the LBD are indicated by black arrows, and the (di-peptide) linker replacing TM1–TM3 [47] is depicted. Tertiary folding of this domain requires translation and ER translocation of the intervening core ion channel (TM1–TM3); it is therefore expected to occur relatively late, probably after the N-termini have engaged in dimer formation.
Box 1. RNA editing.

RNA editing is an enzymatic modification that alters RNA composition by substrate-specific nucleotide insertion, deletion or alteration [84]. Different types of editing have been described in eukaryotes. Editing of mRNA can alter the sequence of the encoded protein, whereas editing within non-coding regions, such as untranslated regions (UTRs) or the introns of pre-mRNA, can affect RNA stability, splicing and RNA interference [36]. In addition, RNA editing is widespread and essential in the processing of tRNA, rRNA and micro RNA (miRNA) [36,84,85].

The most prevalent modification in higher eukaryotes is the deamination of adenosine (A) residues to inosine (I) [36]. A-to-I editing was first detected as the unwinding of double-stranded RNA (dsRNA). The enzymes responsible have since been cloned in vertebrates: adenosine deaminases acting on RNA (ADARs) 1–3 [76]. Their significance is clearly demonstrated with mouse mutants: mice lacking ADAR1 are embryonically lethal [86], whereas ADAR2 knockouts are prone to seizures and die within 3 weeks of birth [87]. In addition, loss-of-function mutants of orthologous genes in Caenorhabditis elegans (Adr1 and Adr2) and Drosophila (dAdar) show abnormal behaviour, and gain of Adar function is lethal [36].

Many ADAR substrates are transcripts of genes expressed in the nervous system [88], including vertebrate AMPAR [35]. Editing in exon 11 replaces a glutamine (Q) with an arginine (R) in the pore loop lining the channel (Figure 1a). Q/R editing reduces channel conductance and inward rectification and lowers calcium permeability. This editing normally occurs with almost 100% efficiency and genomically encoding the arginine residue rescues the ADAR2 null phenotype. A developmentally regulated editing site is located in exon 13 of GluR2–4, where a glycine (G) replaces an arginine residue within the LBD, resulting in quicker recovery of AMPARs from desensitization [64]. Both editing sites also alter the biogenesis and trafficking properties of the receptor [30,37]. Editing of AMPARs requires an imperfect inverted repeat sequence in the intron downstream of the editing sites. This editing complementary sequence (ECS) folds back to give dsRNA, which is a substrate requirement for ADARs [36,36]. The importance of editing at these sites is also evident from the conservation of the ECS in vertebrate evolution (Box 2; Figure 2) [89].

Box 2. Evolution of AMPAR RNA processing at subunit interfaces.

RNA processing within the LBD of the functionally dominant GluR2 subunit is highly conserved in vertebrates (Figure 2). AMPAR-like genes in protostomes and primitive chordates are devoid of these processing events. The vertebrate AMPAR gene family is thought to have evolved from a common ancestral subunit in the early chordate lineage (Figure I). The urochordate Ciona intestinalis has a single AMPAR-like subunit with only one exon as the primordial vertebrate flip/flop exon. Hagfish, a primitive jawless vertebrate, has two subunits, both of which have flip and flop exons, as do all subunits of jawed vertebrates. An exon duplication event was proposed to have occurred after the separation of vertebrates from primitive chordates [34]. Functional diversification and alternative splicing of the flip/flop exons probably evolved before the initial expansion of the gene family.

In contrast to hagfish, jawed vertebrates have additional AMPAR-like subunits. The sequence elements required for R/G editing in GluR2–4 show striking conservation, indicating that R/G editing may have appeared during early expansion of the gene family [89]. As shown in Figure 2, the greatest divergence occurs in the terminal pentaloop of the dsRNA substrate. This loop adopts a novel fold [80] and mutations in this sequence affect the efficiency of editing [81]. No vertebrates have so far been discovered with the edited residue genomically encoded.

Constitutive RNA editing occurs at the Q/R site in the GluR2 subunit of sharks and tetrapods (Figure 2) [92]. In some bony fish, this intron sequence has diverged in GluR2 genes (e.g. in Tilapia, an arginine is genomically encoded at the Q/R site). Interestingly, the hagfish GluR2 subunit is intronless in the region encoding the pore loop and has a genomically encoded arginine, indicating that Q/R editing evolved in vertebrates after the separation from jawless fish and that there is strong evolutionary selection for this residue in the GluR2 subunit [92].

within LBD dimers and generates the fourfold symmetric ion channel (Figure 3a) [30]. Below, we consider current insights into the role of individual domains during subunit assembly, starting from the N-terminal domain.

The N-terminal LIVBP domain

The extracellular portion, which comprises ~80% of a subunit, faces the ER lumen, where it folds and engages in the initial steps of subunit assembly. The N-terminal LIVBP-like domain, which shares ~23% sequence identity with the glutamate-binding domain of the metabotropic receptor mGluR1 (comparing rat GluR2 and rat mGluR1), is believed to determine interaction compatibility by ensuring association of subunit monomers only from a given iGluR subfamily [23]. In the case of AMPARs, dimerisation of heteromers appears to be preferred over homodimerisation [43]. As isolated LIVBP domains form stable dimers in solution [44], this initial interaction is likely to be tight. The crystal structure of the mGluR1 binding core dimer reveals a largely hydrophobic subunit interface, which is formed mainly by two helices (B and C) within the upper lobes of the clamshell-shaped domain [45]. Similarly, upper-lobe segments of the AMPAR N-terminus have been implicated in subtype-specific heteromerisation [24]. Homology modelling indicated that the dimer interface, formed by upper lobe segments of iGluR N-termini, is more hydrophobic in AMPARs, but contains charged residues in kainate receptor N-termini. This difference might underlie the subfamily-specific interactions mediated by this domain [24].

The ~400-residue continuous LIVBP-like segment (Figure 1a) probably adopts its tertiary α,β fold while the remaining polypeptide is still being translated and integrated into the lipid bilayer. Newly synthesized N-termini could therefore associate with other concomitantly translocating nascent chains or assemble with previously synthesized AMPAR monomers. How this first assembly step is achieved is unclear (as it is indeed for many other heteromeric ion channels) and raises the principal question of how subunits preferentially associate with other members of the same subfamily rather than simply homo-oligomerise. As a given mRNA is translated by polysomes, a high concentration of a particular subunit
Figure 2. Evolutionary conservation of regulatory elements required for RNA processing in GluR2. A schematic of the GluR2 protein (from segment 1 [S1] to the C terminus) is aligned with the 3' end of the human GluR2 gene. Colour coding of the protein is as described in Figure 1a. The intron/exon structure is annotated with the editing sites (red diamonds), their corresponding ECS (red), and alternative splicing of flop (o) and flip (i) exons. Below the gene structure, a plot of sequence conservation is shown for the vertebrate GluR2 gene. Sequences were obtained from precomputed alignments of genomes provided by University of California Santa Cruz or Joint Genome Institute, using the VISTA browser [93] and human as the base genome. Alternatively, sequences were found using BLAT ([94]; http://genome.ucsc.edu/) or WU-BLAST 2.0 in Ensembl (http://www.ensembl.org/index.html) of the current genome build using exons 11–16 (amino acids 492–883) of the human GluR2 peptide (P42262) or the gene nucleotide sequence of the inverted repeat. Species marked with an asterisk had sequence for the whole gene region of interest and were used to create a conservation plot; sequences were aligned using mLAGAN ([95]; http://lagan.stanford.edu/lagan_web/index.shtml) and the plot was visualized with SinicView [96]. Multiple sequence
is expected to accumulate at the ER membrane, in a temporally and spatially restricted fashion. In addition, diffusion is limited by the lipid bilayer and probably also by interaction with ER-lumenal chaperone networks, which will restrict ‘collision’ of spatially separated translocation products even further. Together, these conditions are expected to facilitate the formation of homo-oligomers. However, initial dimerisation of homomeric subunits might be of lower affinity than dimer contacts between heteromers and be reversible once a heteromeric partner becomes available (Figure 3a; step Ia). Increasing the ER-dwell time of certain subunits, and thus their availability, could also facilitate heteromerisation. This strategy appears to be employed by the GluR2 subunit, which stably resides in the ER (with a half-life of ~12 h) [46].

The ligand-binding domain

The LBD lies C terminal to the LIVBP, and is split into S1 and S2 segments by the ion channel. Tertiary folding of the LBD thus has to await translocation of the transmembrane segments (Figure 1a,c). Crystal structures of this domain are available for all three iGluR subfamilies [47–51], and details of agonist and antagonist interaction with this domain have been reviewed recently [51,52]. Ligand binding to the crevice between the two lobes of the domain, known as D1 and D2, results in domain closure (Figure 4a). The resulting high-energy transition state is believed to proceed, in parallel, to either channel opening or desensitisation (MSAs) of the editing sites were supplemented with additional sequences (listed below), aligned using ClustalW (http://www.ebi.ac.uk/clustalw/) and visualized using JalView. Additional sequences can be found under the following Genbank accession numbers. Q/R site: AAFC03056666, AAGU01617172, AAGV01116826, AF350048, AF350049, AF350050, AF350051, AF350052; R/G site: AAFC03088020, AAGU01276109, AAGV01116830, AF201347, AF201348; goldfish sequences can be found in [97]. The tetrapod MSA for exon and intron 11 is represented as a consensus sequence. Empty triangles and arrows denote 5’-splice sites and imperfect inverted repeats, respectively. Note that most sequence variation in the inverted repeat sequences and ECS is matched by complementary mutations or gives rise to thermodynamically stable G-U wobble pairs. Also note extensive intronic sequence conservation flanking each of the flip/flop exons.

Figure 3. Model depicting the vectorial ‘zipping-up’ of interfaces during subunit assembly. (a) Steps encompassing dimer (I) and tetramer (II) formation are shown (see text). Individual steps that could occur during dimerisation (I) are labelled a–d. Initially, LIVBP domains will interact (step Ib), which might be reversible (step a), depending on the affinity of the interaction partners. In step lc, the LBD dimer interface forms; stabilization of this interface may promote alignment and/or packing of ion channel helices to form tetramerisation-competent dimers (step Id). This step is facilitated by Arg743 and L483Y. Alignment of dimers during tetramerisation (II) will involve the packing of transmembrane helices. Re-entrant pore loops might flip into the lipid bilayer (schematised in b) to form the pore. Tetramer formation is likely to involve new interfaces between subunits within the extracellular portion of the receptor; these interfaces and their overall symmetry are poorly understood at present (see [54,55]). ER-export competence appears to require relaxation into a desensitized-like state (fib) [30]; see also [31]). This step is facilitated by N754D, a GluR2 mutation that destabilizes the LBD interface and accelerates desensitization. Acquisition of this conformation may itself be an intermediate step, that is, it could be specifically recognized by a trans-acting factor, such as Stg, that is required for forward traffic (IH Greger et al. unpublished observations). Colour coding is as in Figure 1b. (b) The potential flipping of pore loops into the lipid bilayer may occur at the level of a dimer or during tetramerisation. As pore loops do not traverse the bilayer and therefore do not represent bona fide transmembrane segments, they might equilibrate into lipid via a Sec61-independent mechanism. This process could be affected by the editing state of the Q/R site and by the strength of LBD dimer contacts. Colour coding of the ion channel domain is as in Figure 1a.
In all LBD structures, twofold symmetrical dimers were observed, with the two protomers arranged in a back-to-back fashion (Figure 4a). The dimer interface, which buries around 1150 Å² of solvent-accessible surface area per protomer in GluR2, is formed by the upper lobes, and involves helices D and J and the first interdomain β strand (Figure 1c); interface contacts are mediated by polar and hydrophobic interactions[47].

In contrast to the LIVBP, isolated LBDs do not exist as dimers in solution (at concentrations below 1 mM)[54], revealing a much looser interaction in this part of the receptor. The LBD constitutes the ‘muscle’ of the receptor and conveys gating motions to the channel in response to ligand binding/unbinding, underlining the necessity of conformational flexibility in this region. Tightening of the interface by mutation or by the allosteric modulator cyclothiazide dramatically attenuates desensitization, suggesting that desensitization involves interface opening[52,54,55]. Specifically, mutation of Leu483 to tyrosine (in GluR2) creates favourable cation–π interaction across the interface, which stabilizes dimers dramatically [54] (Figure 4a). Using a GluR2 mutant that constrains the receptor in a desensitized-like state (S729C), Armstrong et al. recently examined the extent of LBD rearrangement upon desensitization [56]. When compared with an ‘undesensitized’ (L483Y) structure, they observed complete rupture of the D1 interface and formation of a new interface involving the D2 segments. As the ion channel is attached to D2, the increased proximity of the lower lobes suggested a mechanism for channel closure upon desensitization [56].

Low-resolution single-particle electron microscopy images of native AMPARs demonstrated that exposure to glutamate resulted in a large proportion of receptors with widely separated N-termini, compared to a preparation that also included the desensitization blocker cyclothiazide. These data reflect the profound conformational change initiated by LBD dimers upon desensitization [57]. Recent experiments with kainate receptors reveal that tightening of D1 interface contacts via cysteine linkages similarly attenuates desensitization for this iGluR subfamily, implying that a related mechanism underlies desensitization [31,58]. However, the molecular determinants of this process differ between AMPA and kainate receptors [59,60]. It is worth noting that AMPARs lacking the LIVBP domain produce functional homomeric receptors when expressed in heterologous cells [61,62]. GluR0, a prokaryotic K+-selective iGluR, lacks the LIVBP domain. The GluR0 LBD exists as a dimer in solution, with a $K_d$ of ~800 nM [63]; the tighter contacts might compensate for the lack of the N-terminal domain. In contrast to GluR2, the dimer interface is closed towards its N-terminal end, where additional interdomain hydrogen bonds are formed (in the vicinity of the R/G editing site). GluR0 desensitization kinetics are about three orders of magnitude slower than AMPAR kinetics [7]. However,
GluR0 is likely to exist as a homotetramer and thus might not require the ‘selector’ function exhibited by AMPAR LIVBPs; neither does this channel require the fast kinetics that are essential for synaptic AMPARs. The GluR0 LBD appears to combine both the assembly and gating functions.

The LBD is attached to the ion channel and orchestrates gating (Figure 1b). Assembly of the ion channel domain is influenced by LBD contacts and by RNA editing; these steps are discussed in the section below.

**RNA editing, interface contacts and GluR2 assembly properties**

As mentioned above, the LBD interface in AMPARs is modified by flip/flop splicing and R/G editing; both events modulate gating kinetics [33,35,64,65] and receptor assembly [30,37]. Editing at the R/G site of GluR2–4 results in a dramatic change in the sidechain chemistry at the top of the interface, where adenosine deaminase acting on RNA (ADAR) action replaces Arg743 with glycine (Figures 2, and 4a). This modification, which endows the receptor with functional diversity, is unique to vertebrate AMPARs and is accomplished by a highly conserved intron sequence immediately downstream of the editing site (Box 1; Figure 2). Most other vertebrate and invertebrate iGluR subtypes constitutively express arginine at this position; an exception is squid, which harbours a genomically encoded glycine in the two non-NMDA-like subunits cloned so far [66]. How does the R/G modification affect interface contacts? The X-ray structure of the unedited LBD (in a flip background) revealed an unexpected arrangement of the Arg743 rotamers across the dimer interface. The sidechains were observed in an extended conformation, projecting towards one another to within 3.4 Å (rather than pointing upward toward the solvent; Figure 4a) [30]. The distance between their Cα atoms across the interface is ~1 Å less than that seen in the edited glycine structure (solved in the flop background [47]). Together, these structural data indicated an unconventional Arg–Arg interaction facilitated by surrounding negative charges, such as the highly conserved Asp490 sidechain (Figure 1c) [30]. Whether this interaction actually stabilizes dimers is currently unclear. Functional data do not fully resolve this issue, as the kinetics of entry into desensitization depend on a given subunit and on the flop/flop splice form. They are indeed slower for flop Arg743 isoforms (as would be expected for an interface stabilizer) but are faster for flip arginine isoforms [65]. In kainate receptors, the equivalent arginine (Arg775) forms a salt bridge with an adjacent aspartate (Asp776) of the opposite subunit [49,50]. This electrostatic glue does not appear to stabilize the interface, as judged by the high $K_d$ value obtained from analytical ultracentrifugation runs (~6 mM) [58] and by the fast desensitization kinetics characteristic of kainate receptors [58,59].

Interestingly, the GluR2 Arg743 form has a greater tendency to (homo)tetramerise; it also folds more efficiently and buds from the ER at higher rates than the Gly743 form [30]. These effects were observed in four GluR2 backgrounds (i.e. flip/flop and Q/R), both in neurons and in heterologous cells. Previous experiments had shown a role for RNA editing in AMPAR assembly, whereby editing at the Q/R site attenuated tetramerisation of fully edited GluR2 flop homomers in neurons and restricted subsequent ER export [37]. The Q/R site lies at a four-fold symmetric interface, at the apex of the pore loop (Figure 1b); it was suggested that juxtaposing four arginine-containing pore loops during the second assembly step, the dimerisation of dimers, was rate limiting (Figures 3b and 4b). Testing a panel of amino acids at this position revealed that most efficient tetramerisation was achieved with glutamine and tryptophan at the pore apex (hydrophobic residues were not tested) [37]. The fact that assembly proceeded more efficiently with glutamine than with asparagine indicated that, apart from charge, a specific sidechain length is critical. Four glutamine sidechains, one from each pore loop, could engage in favourable hydrogen bonding (Figure 4c) [67], whereas tryptophan residues might engage in stabilizing stacking interactions during pore formation [37].

As mentioned, the energy barrier of arginine pore formation is lowered by alterations of LBD dimer contacts, as Q/R-edited GluR2 containing Arg743 has a greater tendency to tetramerise than its Gly743 counterpart [30]. In addition, the interface stabilizer L483Y promotes tetramerisation of fully edited GluR2, perhaps by aligning subunits within a dimer (Figure 3a; step Id). Whether Arg743 contacts indeed stabilize intersubunit contacts is at present unclear. Arg743 could also facilitate LBD folding by linking the S1 and S2 segments; Arg743 forms a salt bridge with Asp490, which itself projects from a loop within S1 (Figure 1c). Together, these data revealed that RNA editing alters GluR2 assembly properties: unedited GluR2 is competent to form homomers, whereas editing restricts self-assembly, implying that fully edited GluR2 preferentially heterotetramerises (Figure 5).

**ER exit of tetramers and functional quality control**

L483Y GluR2 mutants tetramerise, but exit from the ER inefficiently [30]. Stabilizing the LBD interface thus overcomes a rate-limiting step in the assembly pathway, but this step needs to be reversible, that is, once assembled the channel might have to collapse into a low-energy, desensitized-like state (Figure 3a; step Ib). This conformational transition appears to render the channel ER-export competent [30]; it could result in chaperone dissociation or facilitate association with a transport factor. A potential candidate for the latter scenario is the AMPAR- auxiliary subunit stargazin (Stg; Figure 3a) [68]. Stg and its relatives, the transmembrane AMPA receptor regulatory proteins (TARPs), are required for early secretory trafficking [69]; they interact with the LBD, slow gating kinetics and appear to recognize specific conformational states of the LBD [68,70]. N754D, a GluR2 mutant that accelerates desensitization, promoted budding from the ER in neurons [30], but not in Hek293 cells (authors’ unpublished observations). This might reflect that Stg, which is not expressed in Hek293 cells, mediated efficient trafficking of N754D GluR2 in neurons. In addition, we noticed that GluR2 harbouring the lurcher mutation A622T, which reduces desensitization of GluR1 [71], produced a retention phenotype (authors’ unpublished observations). Recent experiments revealed that kainate receptors, when locked in the
open state, are also retained in the ER [31]; as kainate receptors do not interact with Stg, other components of the ER quality control machinery will be involved in sensing ER-export-competent, quaternary conformations of iGluRs.

These data, together with the finding that ligand binding appears to be required for ER transit [29], indicate that conformational changes underlying gating motions (i.e. resting-open-desensitized) are sensed during iGluR biogenesis in the ER. l-Glu, which is highly concentrated in the cytosol [29], could reach luminal AMPARs through GluR2 (i.e. the R/G site, although unedited GluR2 traffics more efficiently than partially edited biogenesis in the ER). L-Glu, which is highly concentrated (i.e. resting-open-desensitized) are sensed during iGluR that conformational changes underlying gating motions, which lie subunit interfaces and alter gating kinetics, are ideally suited to influence AMPAR biogenesis. In fact, the finding that flip splice forms traffic more efficiently in Hek293 cells [74] might partly explain why flip channels display functional dominance over flop forms in this system [75].

**Outlook**

RNA processing within the LBD is developmentally regulated [35,64]. As these changes also alter assembly properties [30], they could encourage formation of GluR2-containing tetramers during development (Figure 5). ADARs, the enzymes responsible for adenosine-to-inosine editing (Box 1; [76]), are under sophisticated negative feedback control [36,77,78] and are regulated by diverse external cues [79–81]. If alterations in neuronal network activity were to alter ADAR levels, regulation of AMPAR assembly could be envisaged. As editing is manifested at the level of subunit mRNA, the resulting change in post-synaptic AMPAR responsiveness would most likely follow a slow time course and might play a role during long-term adjustments, such as those occurring during homeostatic plasticity [3].

AMPAR assembly does not seem to operate stochastically. Different tetramer combinations can be targeted to selected dendritic subdomains within a given neuron [12–14]. In addition, synapses can ‘employ’ functionally different tetramers in response to altered input patterns [15–21,82]; this regulation might involve local synapse-specific remodelling of AMPARs [83]. Taken together, these findings demonstrate that neurons are able to tailor AMPAR complexes depending on their specific needs, which will increase their computational capacity substantially. Whether these different tetramers are assembled in response to altered activity patterns or, alternatively, whether ‘pre-assembled’ complexes are specifically trafficked remains an open question.

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