Trends in Neurosciences

CellPress

Opinion

Splicing, RNA editing, and auxiliary subunits shaped AMPA receptor function through coordinated evolution

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Post-transcriptional regulation of AMPA-type ionotropic glutamate receptors (AMPARs) emerged in early vertebrates alongside a major expansion of AMPAR auxiliary subunits. Recent work shows that two key modifications—alternative splicing of the flip/flop cassette and Q/R site RNA editing—fine-tune channel gating and Ca^{2+} permeability, respectively, through interactions with two distinct auxiliary protein families: the transmembrane AMPA receptor regulatory proteins (TARPs) and the cornichons (CNIHs). I propose that these regulatory layers did not evolve independently but instead coalesced into an integrated system in which splicing, editing, and auxiliary proteins reciprocally shaped each other. This coordinated evolution maximized excitatory signaling diversity during vertebrate brain expansion, helping to explain why perturbations in any layer contribute to neurological disease.

When genomes expanded, vertebrate brains transformed

The mammalian brain's computational capacity arose from several important biological innovations that began nearly 500 million years ago, when vertebrates diverged from invertebrate chordates during the Cambrian period. This evolutionary transition initiated a major expansion in complexity, giving rise to the diverse branches of vertebrates and ultimately to mammals.

A pivotal event in this natural history was the occurrence of two successive whole-genome duplications (the 2R hypothesis) in early vertebrates [1]. These duplications, which occurred after the divergence of urochordates but before the radiation of jawed vertebrates, effectively doubled the genetic repertoire twice [2–4]. As a result, vertebrates inherited a rich array of protein paralogs that could evolve new or specialized functions, particularly in the nervous system, where they contributed to the diversification of receptors, signaling pathways, and transcriptional networks underlying neural complexity [5].

However, gene duplication alone cannot account for the sophistication of the vertebrate brain. Modern studies show that, among other factors, post-transcriptional regulation, especially alternative splicing and RNA editing, further expanded the functional repertoire of neuronal genes without increasing gene number [6,7]. These mechanisms allow a single gene to generate multiple protein isoforms or fine-tune receptor properties, vastly enhancing synaptic signaling diversity and adaptability [8]. Thus, the vertebrate brain's computational power reflects the combined effects of ancient genomic expansion with increased cell number [9] as well as regulatory innovation, transforming a shared genome in vertebrates into the substrate for advanced cognition in mammals with further refinement in humans [9,10].

Highlights

Comparative genomics reveals that alternative splicing, RNA editing, and AMPAR auxiliary subunit expansion emerged together in early vertebrates.

Functional studies reveal that alternative splicing allosterically controls AMPAR nanoscale mobility and auxiliary subunit TARP engagement, uncovering a previously unrecognized regulatory layer.

TARPs and CNIHs expand RNA editing of the channel pore's Q/R site such that Ca^{2+} permeability of AMPARs is a continuum rather than a binary switch, revising a long-standing assumption in the field.

The synchronous emergence of post-transcriptional regulation and auxiliary subunit diversification argues against coincidence and instead suggests a coordinated evolutionary context that created favorable conditions for the elaboration of vertebrate glutamatergic signaling.

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In this article, I illustrate how recent advances in our understanding of **AMPA-type ionotropic glutamate receptors (AMPARs)** (see Glossary) have revealed how the brain's most abundant excitatory neurotransmitter receptor acquired its remarkable diversity of signaling properties. AMPARs are central to fast, millisecond-scale excitatory neurotransmission in the mammalian brain and play essential roles in synaptic plasticity, learning, and memory mechanisms [11]. Their importance is underscored by the association of close to 100 missense mutations in AMPAR subunits with autism spectrum disorders and intellectual disability [12], highlighting their relevance as potential therapeutic targets.

30 years of discovery uncovered the multilayered regulation of AMPA receptors

Our insight into this complexity has emerged from a series of key scientific discoveries spanning the past three decades of neuroscience research. A series of formative studies in the early 1990s demonstrated that AMPA receptors undergo post-transcriptional modification at multiple sites. Specifically, the authors uncovered RNA editing at the Q/R [13,14] and R/G [15] sites and alternative splicing of the flip/flop cassette [16,17], mechanisms that, together, determine both the receptor's responsiveness to L-glutamate and their ability to conduct Ca^{2+} ions, a crucial signaling molecule in synaptic physiology. In receptors lacking Q/R editing, Ca^{2+}-permeable AMPARs undergo voltage-dependent block by intracellular polyamines that enter and occlude the channel pore during depolarization, producing inward rectification and activity-dependent regulation of ion flux (Box 1) [28,29].

Box 1. Post-transcriptional modification of AMPAR subunits

RNA editing of the Q/R and R/G sites occurs cotranscriptionally on **exon–intron double-stranded RNA structures** formed between each site and its downstream **editing complementary sequence**.

Following pioneering work documenting the editing of the Q/R site [14], studies have shown that it requires a precise intronic base-paired stem that presents the target adenosine to an adenosine-to-inosine deaminase [18]. The Q/R site corresponds to a single codon within the pore-forming M2 re-entrant loop of the receptor, where editing converts a genomically encoded glutamine (Q) to an arginine (R). This substitution has profound functional consequences: the positively charged arginine residue dramatically reduces Ca^{2+} permeability, lowers single-channel conductance, and renders AMPARs resistant to voltage-dependent block by endogenous polyamines, thereby linearizing current–voltage relationships [28].

The enzyme responsible for RNA editing was identified as adenosine deaminase acting on RNA 2 (ADAR2; then called RED1) when it was shown that ADAR2 uniquely and efficiently edits the Q/R site of the GluA2 pre-mRNA [20]. The Q/R site of other AMPAR subunits, namely GluA1, GluA3, and GluA4, remained unedited, and the related isoform ADAR1 showed minimal activity at this position. As a result, edited GluA2(R)-containing receptors support linear synaptic transmission, whereas unedited Q-containing receptors are Ca^{2+} permeable, inwardly rectifying, and can increase vulnerability to excitotoxic signaling [28]. Definitive *in vivo* evidence came from a study in Adar2-null mice, which were shown to lose nearly all Q/R editing, and lethality was rescued only by a 'pre-edited' GluA2(R) allele, establishing ADAR2 as the essential Q/R editor [21].

The R/G site [15] is similarly edited on a short exon–intron hairpin but displays broader enzymatic permissiveness. The site is edited on GluA2–A4 subunits whereas the GluA1 subunit is unaffected. Although **ADAR2** is the predominant editor of the GRIA2 R/G site in neurons, **ADAR1** can also edit this position, particularly in other AMPAR subunits and specific cellular contexts [22,23]. Thus, GRIA2 Q/R editing is essentially an ADAR2-exclusive reaction, whereas R/G editing is ADAR2-dominant but not ADAR2-specific.

The flip/flop cassette is a pair of mutually exclusive exons present in all four AMPA-receptor genes (GRIA1–GRIA4) [17]. These exons encode a short region in the LBD immediately before the TM4 region that imparts distinct desensitization and gating kinetics on AMPARs. The flip variant is enriched in the embryonic brain with increased flop expression in rodent [15–17] and human [24] neurons during postnatal development.

As with all mutually exclusive exons, flip/flop selection is carried out by the canonical spliceosome (U1, U2, U4/U6, U5 snRNPs) using standard splice-site recognition mechanisms [24]. No dedicated enzyme performs this process; instead, exon choice is shaped by *cis*-regulatory elements and *trans*-acting RNA-binding proteins. Although direct biochemical data for GRIA flip/flop regulation are limited, broad neuronal splicing studies show that NOVA, RBFOX, and PTBP proteins regulate many synaptic transcripts through enhancer–silencer interactions [25–27]. These networks are therefore considered likely modulators of flip/flop selection.

Glossary

AMPA-type ionotropic glutamate receptors (AMPARs): AMPARs are tetrameric channels that mediate most fast excitatory neurotransmission in the vertebrate brain. They assemble from GluA1–4 subunits and have modular extracellular, transmembrane, and intracellular domains. Their properties are shaped by RNA editing, alternative splicing, and auxiliary proteins.

Transmembrane AMPA receptor regulatory proteins (TARPs): TARPs are vertebrate-specific AMPAR auxiliary subunits that regulate trafficking, gating, and ion selectivity. By binding AMPAR ligand-binding and transmembrane domains, they slow desensitization, enhance glutamate potency, and modulate Ca^{2+} permeability, coevolving with AMPAR post-transcriptional regulation.

Adenosine-to-inosine (A-to-I) RNA editing: A-to-I RNA editing is a post-transcriptional process in which adenosines are deaminated to inosines, read as guanosine during translation. In AMPAR mRNAs, this modifies key residues in the pore or ligand-binding domains, altering kinetics and ion permeability, and expands protein diversity without gene duplication.

Auxiliary subunits (AMPAR auxiliary subunits): auxiliary subunits are membrane proteins that assemble with AMPAR tetramers to regulate trafficking, surface expression, gating kinetics, and ion permeability. Major vertebrate families include TARPs, CNIHs, Shisa/CKAMPs, SynDIG/Prrts, and GSG1L. Together, they diversify AMPAR signaling by shaping desensitization, deactivation, and Ca^{2+} permeability.

Flip/flop alternative splicing: a mutually exclusive AMPAR splice event in the ligand-binding domain that generates two isoforms differing by few residues but strongly affecting gating. Flip variants slow desensitization and enhance auxiliary subunit modulation, whereas flop variants accelerate desensitization override TARP modulation. This cassette arose early in vertebrate evolution.

R/G site (arginine/glycine site): the R/G site is a conserved AMPAR RNA-editing site in the ligand-binding domain next to the flip/flop cassette. Editing converts arginine to glycine, accelerating recovery from desensitization and shaping synaptic kinetics without altering ion permeation. GluA2–4 are

A second series of transformative discoveries followed in the early 2000s: the identification of a family of auxiliary proteins, the **transmembrane AMPA receptor regulatory proteins (TARPs)**, which regulate AMPAR trafficking to synapses and profoundly modulate receptor gating and ion permeation [30–32]. These findings paved the way for the identification of additional families of AMPAR auxiliary subunits by other laboratories, including the cornichons (CNIHs) [33], Shisa/CKAMP [34], SynDIG [35,36], and GSG1L [19,37,38] proteins, which further diversified AMPAR signaling and synaptic plasticity mechanisms.

AMPARs are tetrameric complexes assembled from GluA1–4 subunits and comprise several modular domains (Figure 1A): an extracellular N-terminal domain (NTD), a ligand-binding domain (LBD), a transmembrane domain (TMD) forming the ion channel pore, and an intracellular C-terminal domain (CTD) involved in signaling and trafficking [11]. The flip/flop cassette that undergoes alternative splicing is in the LBD, directly downstream of the R/G editing site

edited at this site, whereas GluA1 remains unedited.

Q/R site (glutamine/arginine site): the Q/R site is a single residue in the narrowest region of the AMPAR pore whose identity is set by RNA editing. The Q form permits Ca^{2+} permeability, whereas the R form strongly limits Ca^{2+} flux and protects against excitotoxicity. Interactions with auxiliary subunits generate a continuum of Ca^{2+} permeability states rather than a strict binary switch.

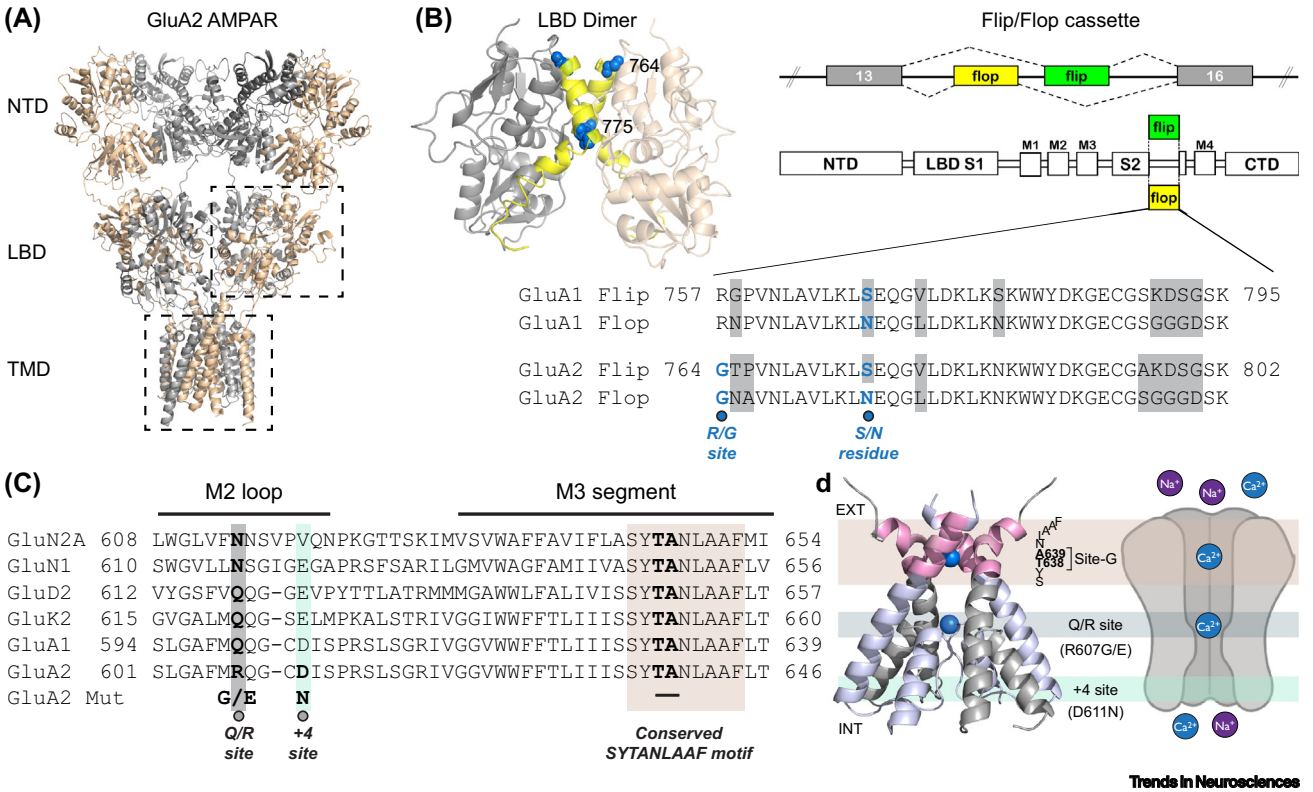

Figure 1. Convergent regulatory elements controlling AMPAR gating and Ca^{2+} permeability. (A) Overall architecture of the AMPAR tetramer (shown for GluA2 in the apo state, PDB: 5L1B), highlighting the extracellular NTD, the bilobed LBD, and the TMD that assembles into the ion channel pore. The intracellular CTD is omitted because its structure remains unresolved. This organization illustrates how ligand-induced conformational changes in the LBD are transmitted to the TMD to control channel gating and ion flow. (B) Structure of the LBD dimer showing the location of the alternatively spliced flip/flop cassette and its exon organization, together with the corresponding amino acid sequences. The flip/flop cassette is generated by mutually exclusive inclusion of two short exons and differs at only a few residues. A single serine/asparagine (S/N) position strongly influences the magnitude and polarity of modulation by TARPs. The cassette lies immediately downstream of the R/G RNA-editing site, where ADAR2-mediated adenosine-to-inosine editing alters an arginine/glycine residue and accelerates recovery from desensitization, defining a regulatory hotspot within the LBD. Adapted from [39] with permission. (C) Sequence alignment of the pore-forming M2 loop and M3 segment across ionotropic glutamate receptor subfamilies. Ca^{2+} permeation is governed primarily by the Q/R site within the narrowest region of the pore and the conserved SYTANLAAF motif in M3, which forms the extracellular vestibule and activation gate. The threonine–alanine pair within this motif defines Site-G, an extracellular Ca^{2+} binding site accessible in the open state. (D) Cryo-EM structure (PDB: 7OCA) and schematic illustrating the spatial relationship between the Q/R site, Site-G, and the nearby + 4 position, which together cooperate to shape Ca^{2+} permeation and ion selectivity. Adapted, with permission, from [40]. AMPAR: AMPA-type ionotropic glutamate receptor; NTD: N-terminal domain; LBD: ligand-binding domain; TMD: transmembrane domain; CTD: C-terminal domain; TARPs: transmembrane AMPA receptor regulatory proteins.

(Figure 1B). In contrast, the Q/R site, which also undergoes RNA editing, is located at the narrowest region of the ion channel pore, making it ideally positioned to regulate ion transport (Figure 1C,D). Almost all native AMPARs in the mammalian brain assemble with two or four TARP auxiliary subunits [19,41,42], with the exception of GSG1L–AMPAR complexes, which have a 2:4 stoichiometry and represent about 5% of all native receptors [37].

Recent work from my laboratory has revealed an interplay between post-transcriptional regulation and auxiliary protein association with AMPARs. Our findings show that flip/flop splicing determines the propensity of AMPARs to engage with TARPs by controlling the nanoscale mobility of AMPARs at rest [42]. Flip variants restrict NTD movement, enabling tighter long-range allosteric coupling to the LBD and slower desensitization via TARPs. Conversely, flop variants enhance NTD mobility, weakening this coupling, overriding TARP modulation, and altering receptor behavior. TARP stoichiometry further diversifies receptor function, generating distinct classes of AMPARs containing either two or four TARP subunits [39,42].

This bottom-up mode of regulation, in which distal extracellular domains and auxiliary subunits influence gating by propagating allosteric effects toward the channel pore, contrasts with the top-down regulation characteristic of N-methyl-D-aspartate receptors (NMDARs), where the NTD exerts dominant control over receptor activity by directly modulating LBD conformation and agonist efficacy [43–45]. In NMDARs, this hierarchical control is largely intrinsic to the receptor subunits themselves and is mediated by stable NTD–LBD interactions rather than by auxiliary proteins. This distinction suggests that the two iGluR families evolved different strategies to coordinate functional activity between their modular domains. Such coordination between allosterically remote domains is now emerging as a general principle in other protein systems [46,47] but remains incompletely understood in ionotropic glutamate receptors (iGluRs, see Outstanding questions). Interestingly, alternative splicing of the flip/flop cassette and TARP subunits engages in a privileged relationship that is not shared with any other auxiliary subunit family including GSG1L [39], a claudinlike protein that shares several structural features with TARPs [48].

In parallel, TARPs, along with cornichon proteins, regulate the Q/R site to fine-tune calcium permeability (Ca^{2+}) across a broad continuum. What was once considered a binary control of Ca^{2+} permeability, set solely by AMPAR **Adenosine-to-Inosine RNA editing (A-to-I RNA editing)** at the Q/R site, is now revealed to be significantly expanded by coassembly with TARP and CNIH proteins [40,49,50]. This tuning arises not only from AMPAR subunit composition but also from auxiliary subunit positioning and their influence on the pore's ion selectivity filter [40]. Interestingly, several missense mutations in the pore region linked to individuals with neurodevelopmental disorders disrupted Ca^{2+} transport, in some cases exceeding the limit achieved with TARP and CNIH modulation [40]. This finding suggests that the occurrence of autism and intellectual disability in some individuals may reflect the dysregulation of Ca^{2+} transport by AMPARs. The actions of TARPs and CNIHs on the Q/R site uncover an expanded role for AMPARs in Ca^{2+} signaling in the mammalian brain, with implications for both physiological function, in terms of Hebbian and homeostatic plasticity, and disease states, as noted for autism and intellectual disability [49] (see Outstanding questions).

Together, these findings position alternative splicing and RNA editing as molecular scalpels that are evolutionarily refined tools unique to the vertebrate brain that sculpt AMPAR diversity and responsiveness (Box 1). By uncovering how these mechanisms influence native receptor function, this work provides new insights into brain signaling and offers promising avenues for therapeutic intervention in neurological disorders.

Vertebrate origins of alternative splicing, RNA editing, and auxiliary protein expansion

Phylogenetic and genomic analyses suggest that alternative splicing and RNA editing of AMPAR genes and the expansion of the different families of auxiliary subunits **(AMPAR auxiliary subunits)** all appeared early in vertebrate evolution (Figure 2) [6,54–56]. None of these features are found in the nervous system of invertebrates, such as flies or worms, or even in our closer invertebrate relatives, such as sea squirts (tunicates). As a result, the emergence of these molecular innovations that shaped AMPAR biology was part of the broader evolutionary push that led to the complexity of the vertebrate brain.

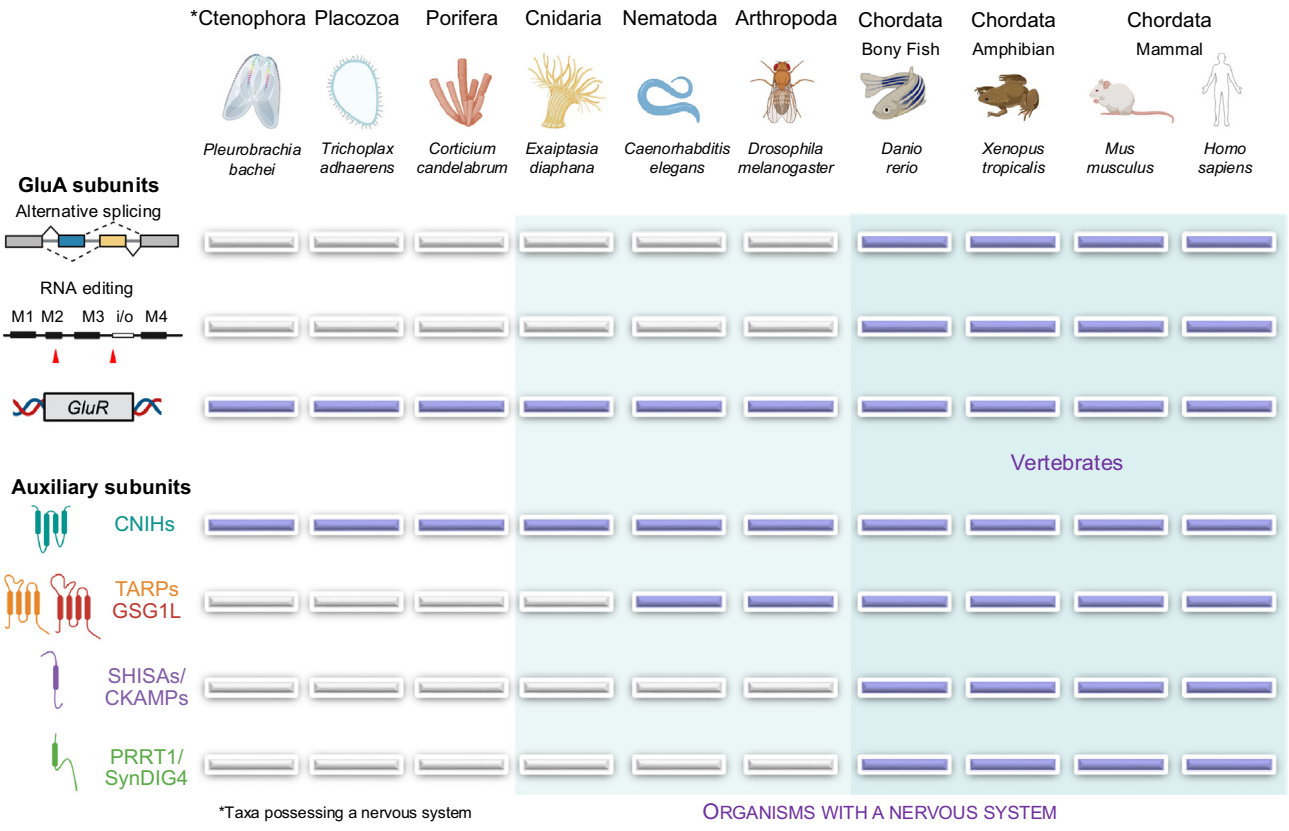

Figure 2. AMPA receptor post-transcriptional processing and auxiliary subunit expansion arose in early vertebrates. Schematic illustrating the evolutionary emergence of AMPAR subunits, post-transcriptional regulatory mechanisms, and auxiliary subunit families across major metazoan phyla. Organisms are arranged (top row) from early branching nonbilaterians to vertebrates, while the left column lists AMPAR features whose presence or absence is indicated across taxa. Ctenophores are positioned as early-branching nonbilaterians for phylogenetic context. Although ctenophores possess neurons and synapselike structures (as denoted by *), genomic analyses indicate that their neural signaling machinery is highly divergent and may represent an independent evolutionary origin rather than a homolog of cnidarian and bilaterian nervous systems [51,52]. Accordingly, their depiction here reflects a homology-based distinction and does not imply the absence of a nervous system. Functional AMPAR-like receptors are present in early diverging animals such as the placozoan *Trichoplax adhaerens*, where they are likely to exhibit Ca^{2+} permeability and display micromolar-range polyamine block, like unedited vertebrate AMPARs [53]. Although these species lack Q/R-site editing, R/G-site editing, and flip/flop alternative splicing, they nevertheless encode a glutamine at the Q/R position (e.g., AKDF1–4) and contain the ancestral SYTANLAAF-like motif, indicating that Ca^{2+} flux and polyamine sensitivity are deeply conserved properties of ionotropic glutamate receptors. Phylogenetic surveys detect cornichon-like (CNIH) sequences in all major metazoan phyla, including early nonbilaterians [54]. However, their functional association with AMPARs appears to become prominent only in vertebrates, coinciding with the emergence of additional auxiliary subunit families such as TARPs, GSG1L, Shisa/CKAMPs, and Protocadherin-related proteins (e.g., SynDIG/PRRTs). The onset of Q/R and R/G RNA editing and flip/flop alternative splicing in early vertebrates parallels the diversification of AMPAR auxiliary subunits, suggesting that these regulatory layers may have coevolved. Together, these innovations dramatically expanded the functional range of vertebrate glutamatergic signaling by enabling multiple tunable modes of gating, trafficking, and Ca^{2+} permeability. Figure created using a combination of original artwork and BioRender.com elements. AMPAR: AMPA-type ionotropic glutamate receptor; CNIH, cornichon-like; TARPs: transmembrane AMPA receptor regulatory proteins.

The evolutionary origin of the 114 base pair exons that encode the flip and flop variants (38 amino acids) of the AMPAR LBD was examined by studying the gene structure of chordate (tunicate, cephalochordate, and vertebrate) and protostome (*Drosophila* and *Caenorhabditis elegans*) AMPAR subunits [57]. Phylogenetic analysis revealed that the mutually exclusive flip and flop exons found in vertebrates evolved from a common sequence. Since primitive chordates (such as tunicates and cephalochordates) possess only a single homologous exon in that position, it was concluded that the duplication event occurred after vertebrates diverged from early chordates [57,58]. Interestingly, gene structure comparisons suggest that a new intron was inserted, separating the original flip/flop exon from the adjacent M4 transmembrane-coding exon, in a chordate ancestor, before the duplication event. Thus, even jawless fishlike lampreys, which are among the most primitive vertebrates, can generate both flip and flop isoforms of their AMPAR subunits, placing the innovation of alternative splicing (**flip/flop alternative splicing**) in AMPARs at the dawn of vertebrate evolution (Figure 3).

RNA editing at the **Q/R site (glutamine/arginine site)** appears to have evolved slightly later than the flip/flop cassette but still within the early vertebrate timeframe. Jawless fish provide an evolutionary snapshot [59–61]. Comparative studies show that jawless vertebrates lack the canonical Q/R editing of GluA2 seen in jawed vertebrates. It was found that the hagfish (*Paramyxine yangi*) *GRIA2* gene is genomically encoded with an arginine (R) at the Q/R site (no glutamine to edit), whereas sharks and bullfrogs carry a genomically encoded glutamine (Q) [59]. Consequently, hagfish GluA2 transcripts are *not* edited, as the gene is intronless around the pore loop and lacks the intronic editing partner sequences required for ADAR-mediated Q to R editing [59,61]. Accordingly, the ability to perform Q/R site editing was most likely acquired after

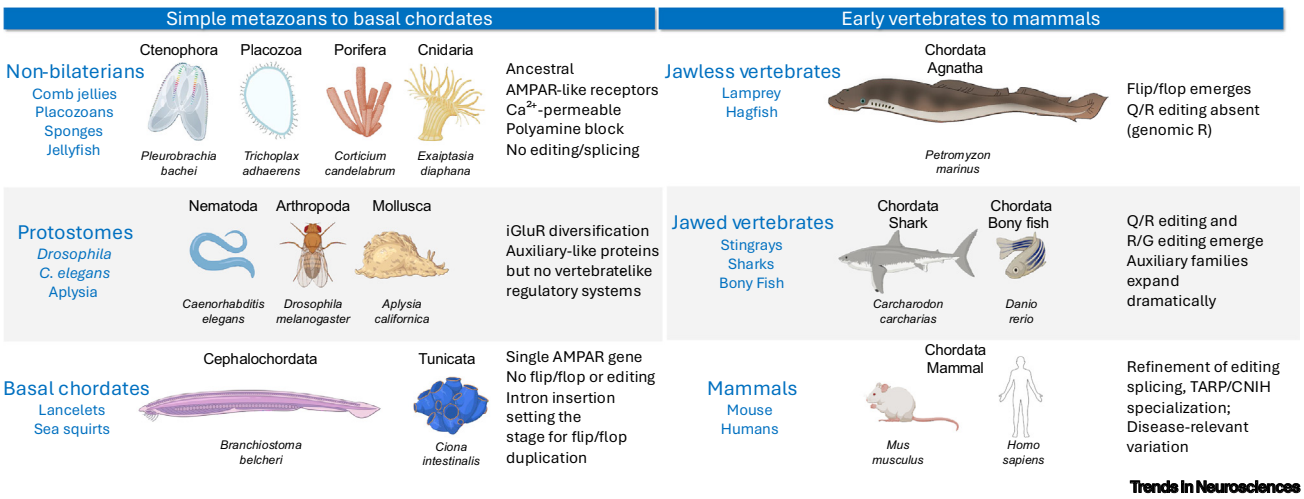

Figure 3. Stepwise emergence of AMPAR regulatory mechanisms during metazoan and vertebrate evolution. Schematic overview summarizing the evolutionary appearance of AMPAR subunits, post-transcriptional regulatory mechanisms, and auxiliary proteins across major metazoan lineages, from early nonbilaterians to mammals. Early-branching nonbilaterians (ctenophores, placozoans, sponges, and cnidarians) possess ancestral AMPAR-like receptors that are Ca^{2+}-permeable and sensitive to polyamine block but lack RNA editing and alternative splicing. In protostomes (nematodes, arthropods, and molluscs), iGluRs diversify substantially, and auxiliarylike proteins are present; however, vertebrate-style regulatory systems, including canonical AMPAR auxiliary subunits and post-transcriptional modulation of pore properties, are absent. Basal chordates (cephalochordates and tunicates) retain a single AMPAR gene without flip/flop splicing or RNA editing, but the appearance of intronic sequences establishes the genomic architecture necessary for subsequent acquisition of these regulatory mechanisms. In jawless vertebrates (agnathans), the flip/flop cassette emerges, whereas the Q/R site remains genomically encoded as arginine, indicating that splicing precedes RNA editing during evolution. In jawed vertebrates, both Q/R and R/G RNA editing arise, coincident with a dramatic expansion of AMPAR auxiliary subunit families, enabling finer control of Ca^{2+} permeability, gating kinetics, and synaptic signaling. In mammals, these mechanisms undergo further refinement through increased splicing complexity, specialization of TARPs and CNIHs, and the emergence of disease-associated genetic variation affecting editing and auxiliary subunit interactions. Figure created using a combination of original artwork and BioRender.com elements. AMPAR: AMPA-type ionotropic glutamate receptors; CNIH, cornichon-like; iGluRs: ionotropic glutamate receptors; TARPs: transmembrane AMPA receptor regulatory proteins.

the evolutionary split between jawless and jawed vertebrates. To date, invertebrate studies have failed to establish Q/R editing in iGluRs. For example, an analysis of the sea squirt (ascidian chordate, *Ciona intestinalis*), considered the closest living relative of vertebrates [62], found that its single AMPA-like receptor gene has only a Q codon that is therefore not subject to RNA editing [63]. Knockdown of the single AMPA-like receptor disrupted early development in *Ciona intestinalis*, suggesting that its calcium permeability may be critical for these events [64,65] (see Outstanding questions).

The **R/G editing site** is also confined to vertebrates (Figures 2 and 3). First discovered in rodent GluA2–4 AMPAR subunits [15], subsequent work has found that it is present in teleost fish AMPARs while absent from invertebrates [66,67]. Cloning studies in fish identified three AMPAR subunits, fGluR2α and fGluR2β, whose deduced amino acid sequence most closely resembles the mammalian GluA2 subunit [67], and the fGluR3α, which displays the highest sequence identity to the GluA3 subunit [66]. Genomic sequence analysis of GluR2α and fGluR2β transcripts revealed that they are subject to RNA editing at the R/G site but not the Q/R site, as noted above, though transcripts from both subunits had alternate choices of the flip and flop [67]. Similarly, variants of fGluR3α were created by alternative splicing and RNA editing at the R/G site [66], demonstrating that early vertebrates acquired both the flip/flop splicing cassette and the enzymatic RNA editing of AMPARs, mechanisms that were likely absent in even the closest nonvertebrate ancestors.

Finally, the expansion of AMPAR auxiliary subunit families occurred in the same broad window of evolutionary time as RNA editing. Genomic analyses of the major known families of AMPAR auxiliaries, which include TARPs, CNIHs, and CKAMPs/Shisa proteins, show little to no expansion in invertebrates but underwent a burst of diversification in vertebrates [54,56] (Figures 2 and 3). Nevertheless, studies on invertebrates have shown that AMPAR-like proteins require coexpression with a TARP auxiliary subunit to exhibit functionality such as reported in the sea squirt, *Ciona intestinalis* [63], and invertebrates, such as the worm, *Caenorhabditis elegans*, and the fruit fly, *Drosophila melanogaster* [68–70]. During vertebrate evolution, AMPAR auxiliary subunit families expanded by independent but convergent processes of neo- and subfunctionalization, which resulted in the plethora of families present in the vertebrate genome [19]. Although genes encoding the pore-forming subunits of AMPARs had already appeared and even diversified in ancestral bilaterian species [53], the expansion in auxiliary subunits occurred much later during early vertebrate evolution [54,56] (Figures 2 and 3).

Coincidence or coevolution?

Given that post-transcriptional regulatory mechanisms and auxiliary subunit families emerged within a similar evolutionary window, it is tempting to ask whether these features were independently acquired but subsequently coselected because of their complementary functional effects, or whether their temporal convergence simply reflects parallel evolutionary trajectories shaped by broader constraints on receptor function.

As noted above, early vertebrates underwent whole-genome duplications and a general increase in regulatory complexity, giving rise to many new genes and RNA-processing innovations, such as alternative splicing, that have been augmented in mammals [71]. It could therefore be argued that the flip/flop splicing cassette evolved simply as one of many new alternative exons that emerged in expanding genomes, with ADAR-mediated RNA editing being independently adopted by multiple gene families around the same time. Similarly, the diversification of auxiliary subunits may have reflected a broader genome-wide expansion of membrane protein families, rather than a process specific to AMPARs. From this perspective, AMPAR splicing, editing,

and coassembly with auxiliary subunits may each have been independently acquired and subsequently selected to fine-tune excitatory signaling in the context of increasingly complex vertebrate neural circuits, thereby establishing a permissive molecular framework rather than causally driving nervous system complexity; the overlap in their timing would thus be correlative rather than deterministic.

Several lines of evidence support a more tightly coupled, causally linked evolution of these features in AMPARs. For instance, the flip/flop cassette differs by only a few amino acids [17], yet a single residue (S/N residue, Figure 1B) among them precisely determines AMPAR sensitivity to TARP modulation [39,42]. It is as if the emergence of the flip/flop cassette had been sculpted to toggle the TARP allosteric effect, making it a highly specific outcome that seems unlikely to have arisen by chance. Although flop isoforms intrinsically alter AMPAR gating kinetics, their selective value is most apparent in the context of native receptors, which almost invariably coassemble with TARPs. In this setting, a flop isoform that attenuates or suppresses auxiliary subunit modulation would be unlikely to confer a meaningful advantage prior to the emergence of TARPs. Thus, the presence of the flop cassette and its conserved sequence suggests that once TARPs appeared, strong selective pressure acted on AMPAR genes to evolve mechanisms for modulating or constraining TARP interactions as needed. This interpretation aligns well with the broad range of kinetic properties observed in native AMPARs [72], which closely parallels those seen in recombinant receptors when TARP type, stoichiometry (2:4 or 4:4), and the contribution of flip or flop isoforms are varied [42,73]. Together, these observations point to reciprocal selection: changes in AMPAR structure were favored in response to alterations in auxiliary proteins and vice versa. In other words, the diversification of AMPAR genes and the expansion of the TARP family exerted mutual selective pressures, each evolving in response to the other over time.

Similarly, at the Q/R editing site, the principal advantage of switching to an edited GluA2 subunit is protection against excessive Ca^{2+} influx. Mice engineered to express increasing levels of unedited GluA2 in native AMPA receptors show correspondingly greater Ca^{2+} permeability, with the most severe phenotype leading to early-onset epileptic seizures and death by 3 weeks of age [74,75]. Thus, in early vertebrates, safeguarding neurons from such detrimental Ca^{2+} overload became critical, particularly as excitatory glutamatergic signaling expanded and auxiliary subunits such as TARPs and CNIHs extended AMPAR gating capabilities. The evolutionary record indicates that Q/R editing emerged at a pivotal moment, not in simple chordates, where excitatory bursts were likely weaker and shorter, but in vertebrates, where prolonged, TARP-enhanced synaptic responses could drive lethal Ca^{2+} accumulation. Our recent finding that TARP and CNIH auxiliary subunits fine-tune Ca^{2+} permeability [40] underscores that the edited (R) versus unedited (Q) distinction is not a simple on/off switch, but part of a finely calibrated regulatory system. From a coevolutionary perspective, once mechanisms such as alternative splicing, RNA editing, and auxiliary subunit diversification arose, they rapidly became integrated. The phylogenetic concordance is striking: no vertebrate species exhibits an expanded repertoire of TARP genes without flip/flop splicing, nor do we find flip/flop cassettes in the absence of auxiliary subunit proliferation. The synchronous emergence of post-transcriptional regulation of AMPARs and the diversification of auxiliary subunits thus argues strongly against coincidence, reflecting instead coordinated evolutionary pressures that shaped vertebrate excitatory signaling.

A more refined interpretation is that these evolutionary innovations arose independently, yet once they coexisted within the same organism, they quickly developed synergistic interactions rather than remaining isolated. This interdependence likely imposed reciprocal selective pressures, locking them into a shared coevolutionary trajectory. Consequently, it seems improbable that the expansion of auxiliary subunits and the emergence of alternative splicing and RNA editing in

AMPARs occurred entirely independently. Our recent findings, alongside the foundational research from the past 3 decades, illustrate how multiple molecular layers including gene family expansion, mRNA processing, and protein–protein interactions can evolve in concert. Together, these processes have driven the leap in functional complexity necessary for the sophisticated synaptic signaling that characterizes the workings of the vertebrate brain as well as providing a valuable framework to understand where neurological disease may arise.

Concluding remarks

The computational sophistication of the vertebrate brain emerged from the coordinated evolution of post-transcriptional regulatory mechanisms rather than from genome duplication alone. Alternative splicing, RNA editing, and the diversification of AMPA receptor auxiliary subunits together form an integrated program that expanded synaptic signaling capacity by refining receptor function and assembly. These mechanisms likely coevolved in a tightly coupled manner, repurposing molecular machinery already present in invertebrates to support the increasing demands of vertebrate excitatory transmission.

Importantly, these same regulatory layers remain dynamically deployed during brain development, with developmental- and cell-type-specific control of R/G editing [15], flip/flop splicing [16], and auxiliary subunit expression [31,37] further diversifying AMPAR function across circuits and maturation stages. Tracing this evolutionary logic from early vertebrates to mammalian AMPA receptors highlights how relatively modest molecular innovations, when integrated across regulatory layers, can generate profound functional complexity. Beyond illuminating the origins of neural computation, this framework also provides a basis for understanding how disruptions to these processes may contribute to neurological disease.

Outstanding questions

What physiological functions might Ca^{2+} entry through ancestral AMPAR-like channels support in placozoans (e.g., *Trichoplax adhaerens*), where they mediate nonsynaptic signaling, or in molluscs (e.g., *Aplysia californica*), where they function at chemical synapses?

Is polyamine block an ancestral mechanism for limiting Ca^{2+} permeability in invertebrate AMPAR-like channels that is superseded later in evolution by Q/R editing?

Does R/G-site editing also influence the allosteric effects of TARPs, CNIHs, or other auxiliary subunits? If so, might multiple post-transcriptional mechanisms have coevolved with auxiliary-protein diversification to shape vertebrate glutamatergic signaling?

How do post-transcriptional AMPAR modifications and auxiliary subunit expression vary across brain regions, cell types, and development? Are they disrupted in central nervous system disorders? Do these regulatory programs diverge across vertebrate species?

How are the long-range allosteric pathways linking flip/flop splicing, R/G and Q/R editing, and auxiliary subunit positioning structurally integrated within the AMPAR tetramer? Can future studies map how these post-transcriptional layers converge on shared allosteric routes?

What are the physiological roles of the newly described continuum of Ca^{2+}-permeable GluA2-containing AMPARs? Do distinct synapse types use specific points along this continuum to tune Hebbian and/or homeostatic plasticity?

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Declaration of interests

The author declares no competing interests.

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