Metazoan Evolution and Diversity of Glutamate Receptors and Their Auxiliary Subunits

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Highlights

- Ionotropic glutamate receptors (iGluRs) formed by fusion of prokaryotic genes.
- The classification of animal iGluRs is largely incomplete; biased by vertebrate genes.
- More animal iGluRs bind glycine or serine than glutamate.
- AMPA receptor auxiliary subunits greatly diversified in vertebrates
- Animals have four classes of metabotropic glutamate receptors, not three.

Abstract

Glutamate is the major excitatory neurotransmitter in vertebrate and invertebrate nervous systems. Proteins involved in glutamatergic neurotransmission, and chiefly glutamate receptors and their auxiliary subunits, play key roles in nervous system function. Thus, understanding their evolution and uncovering their diversity is essential to comprehend how nervous systems evolved, shaping cognitive function. Comprehensive phylogenetic analysis of these proteins across metazoans have revealed that their evolution is much more complex than what can be anticipated from vertebrate genomes. This is particularly true for ionotropic glutamate receptors (iGluRs), as their current classification into 6 classes (AMPA, Kainate, Delta, NMDA1, NMDA2 and NMDA3) would be largely incomplete. New work proposes a classification of iGluRs into 4 subfamilies that encompass 10 classes. Vertebrate AMPA, Kainate and Delta receptors would belong to one of these subfamilies, named AKDF, the NMDA subunits would constitute another subfamily and non-vertebrate iGluRs would be organised into the previously unreported Epsilon and Lambda subfamilies. Similarly, the animal evolution of metabotropic glutamate receptors has resulted in the formation of four classes of these receptors, instead of the three currently recognised. Here we review our current knowledge on the animal evolution of glutamate receptors and their auxiliary subunits.

1. **Introduction**

Glutamate is the major excitatory neurotransmitter in the mammalian brain (Johnson, 1978; Pascual-Anaya and D'Aniello, 2006). It primarily acts through receptor proteins located at the postsynaptic membrane (Rudy et al., 2015). These receptors are transmembrane proteins that belong to two structurally unrelated families: ionotropic glutamate receptors (iGluRs) and metabotropic glutamate receptors (mGluRs) (Barnes and Henley, 1992; Watkins and Evans, 1981). iGluRs mediate fast synaptic transmission, whereas mGluRs modulate synaptic transmission strength and plasticity (Conn and Pin, 1997; Tikhonov and Magazanik, 2009). iGluRs are ligand-gated ion channels formed by four protein subunits that exhibit different pharmacological and electrophysiological properties (Traynelis et al., 2010). By contrast, mGluRs are G-protein-coupled receptors that activate intracellular signal transduction events.

Pharmacological studies performed during the 1980s and 1990s set the basis for the first classification of iGluRs into three groups or functional types: i) receptors activated by α-amino-3-hydroxy-5-methylisoxazole-propionic acid (AMPA), ii) receptors activated by kainic acid (also referred to as kainate) and iii) receptors activated by n-methyl-D-aspartate (NMDA) (Henley et al., 1989; Stevens, 1986; Watkins et al., 1990). Functionally, AMPA and Kainate receptors are more similar to each other than to NMDA receptors. Both can be activated by AMPA and Kainate, although with different affinities, and share a number of agonists (Swanson and Sakai, 2009). AMPA and Kainate receptors have different electrophysiological properties, as AMPA receptors are rapidly desensitized, whereas Kainate receptors show slower desensitization (Castillo et al., 1997; Erreger et al., 2004; Mosbacher et al., 1994). Studies on NMDA receptors

identified a unique characteristic of this class, as they were found to be activated only if there was a simultaneous presence of ambient glycine, release of glutamate from the presynaptic neuron and a depolarization of the postsynaptic membrane. Because of this they were defined as coincidence detectors (Tang et al., 1999).

When mammalian iGluR subunits were cloned and sequenced it was observed that subunits that form receptors with the same pharmacological properties share higher sequence identity among each other than with subunits from other iGluR classes (Hollmann and Heinemann, 1994). Furthermore, two orphan subunits GluD1 and GluD2, for which no agonist had been described, were cloned and sequenced. As these subunits shared higher sequence identity than with any proteins from the other three classes, they were organized into a new class, referred to as Delta (Lomeli et al., 1993). Thus, first genomic investigations extended the classification of iGluRs to four classes: AMPA, Kainate, NMDA and Delta. Subsequently it was seen that NMDA receptors contained three classes: NMDA1, NMDA2 and NMDA3.

Phylogenetic analysis of vertebrate iGluRs shows that all genes from different species can be classified into one of these classes (Chen et al., 2001; Okamura et al., 2005; Teng et al., 2010). Moreover, these analyses revealed that the subunit-encoding genes within each class are paralogues, i.e., they were formed by duplication events from a single gene (Gogarten and Olendzenski, 1999). Because vertebrate families of iGluR genes expanded as a result of the two rounds of whole-genome duplication (2R) that occurred in the ancestors of present-day vertebrates (Kasahara, 2007; Chen et al., 2001; Holland et al., 1994),

it was assumed that the preduplicative ancestor of vertebrates contained only one gene for each class (AMPA, Kainate, Delta and NMDA), and that non-vertebrates would generally present a similarly reduced repertoire of iGluRs. Systematic phylogenetic studies of iGluR evolution along the animal kingdom has proved this assumption to be wrong.

2. The eukaryotic iGluR gene formed by fusion of multiple prokaryotic genes

The prototypical eukaryotic iGluR subunit has four domains: the N-terminal domain (NTD); the ligand-binding domain (LBD); the transmembrane domain (TMD), which is formed by three transmembrane helices (M1, M3 and M4) and a membrane re-entrant loop (M2, between M1 and M3); and the C-terminal domain (CTD) (Sobolevsky et al., 2009). This structure is common to all known eukaryotic iGluRs, including those from plants and algae (Alberstein et al., 2015; De Bortoli et al., 2016). Interestingly, it has been proposed that this complex architecture emerged by the fusion of multiple prokaryotic genes, each adding new domains (Figure 1). Homologues of iGluR subunits can be traced back to prokaryotic organisms, such as the bacterial GluR0 from *Synechocystis* PCC 6803 (Chen et al., 1999). Nevertheless, prokaryotic iGluR homologues are smaller, as they lack the NTD, the last transmembrane helix (M4) and the CTD (Arinaminpathy et al., 2003; Ger et al., 2010; Tikhonov and Magazanik, 2009).

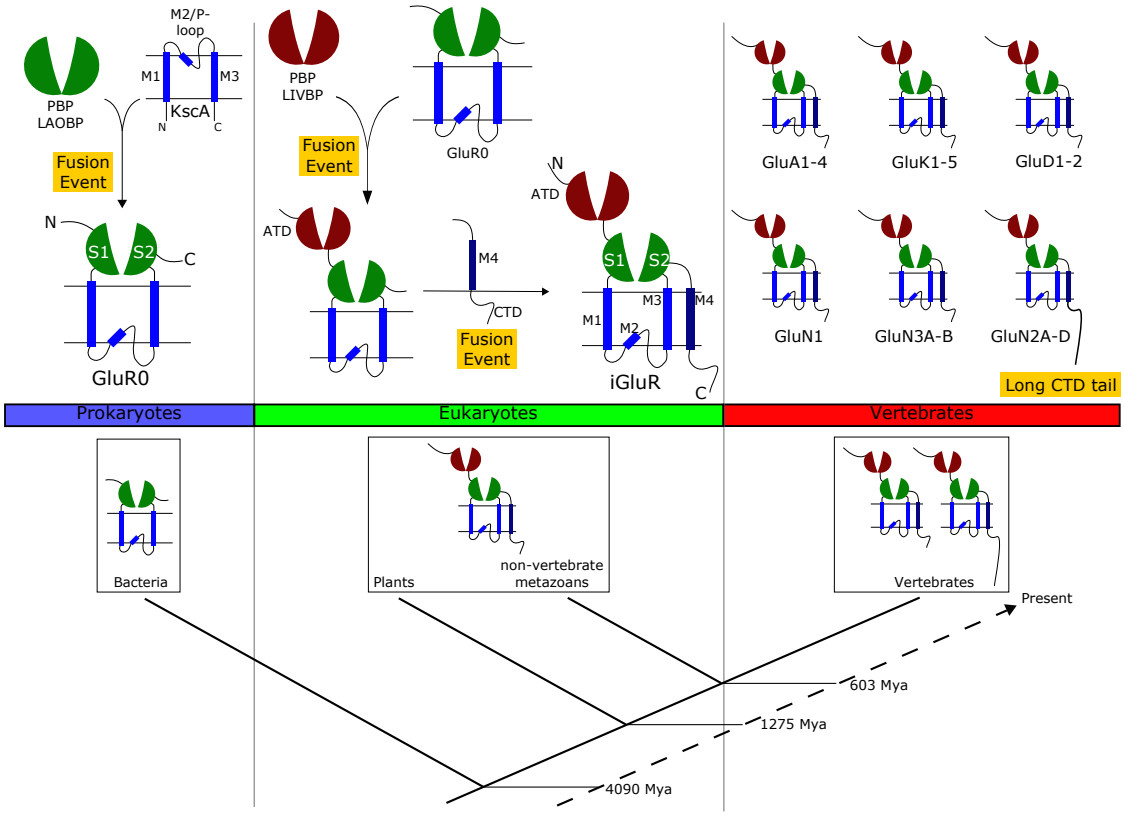


Figure 1. Domain architecture of ionotropic glutamate receptors emerged by the fusion of multiple prokaryotic genes.
Top. Left, representation of the evolutionary events occurred in the prokaryotic ancestor. A LAOBP (lysine-arginine-ornithine-binding periplasmic protein), which forms the LBD, fused with a KcsA channel. Centre, representation of the evolutionary events occurred in the eukaryotic ancestor. A prokaryotic-like iGluR (GluR0) fused with a LIVBP (leucine-isoleucine-valine-binding periplasmatic protein) and with a DNA sequence coding for the M4 and CTD. These two events did not necessarily occur in the order shown. Right, representation of the final domain composition of vertebrate iGluRs. The evolution of a long CTD tail in vertebrate GluN2 subunits is also shown.
Bottom. Evolutionary tree of different organisms with iGluR proteins. The current architecture of iGluRs present in each organism is shown above the organism name. The estimated times of divergence between the different lineages represented are shown at the bottom-right part of the tree.

2.1. Evolution of the ligand-binding and transmembrane-channel-forming domains

The extracellular LBD shows structural homology with prokaryotic lysine-arginine-ornithine-binding periplasmic proteins (LAOBPs) (Forde and Lea, 2007), which are ABC transporters involved in amino acid transport. The LBD structure

consists of two lobes, named S1 and S2. In the folded protein, the interface between these lobes forms the ligand-binding pocket (Armstrong and Gouaux, 2000; Furukawa et al., 2005; Mayer, 2005; Naur et al., 2007; Yao et al., 2008). Yet, in the primary amino acid sequence the two lobes are separated by the M1, M2 and M3 segments. The TMD of bacterial GluR0 and the eukaryotic transmembrane segments M1, M2 and M3 show high structural homology with the potassium channel KcsA found in prokaryotes (Arinaminpathy et al., 2003; Kuner et al., 2003). Because of this it has been proposed that the evolutionary origin of bacterial GluR0s, and by extension their eukaryotic homologues, resulted from the insertion of KcsA between the two lobes of LAOBP (Chiu et al., 1999; Forde and Lea, 2007; Tikhonov and Magazanik, 2009).

Interestingly, GluR0 is a K^+ selective channel, whereas eukaryotic iGluRs are non-selective cationic channels (Burnashev et al., 1995; Jatzke et al., 2002). K^+ selectivity in KcsA and GluR0 is provided by a motif (P loop) located in the M2 helix. This motif, which has the consensus sequence TXVGYG, is absent from eukaryotic iGluRs, with the exception of two iGluRs found in the rotifer (bilateral eukaryote) *Adineta vaga* that also has selective permeability for K^+ ions (Janovjak et al., 2011). How *A. vaga* has retained these ancestral iGluRs in its genome is unknown, nevertheless, this organism can undertake horizontal gene transfer and gene conversion. These processes provide a reasonable explanation for the presence of the ancestral TXVGYG motif in A. vaga (Flot et al., 2013). Thus, although the transmembrane domains M1, M2 and M3 might have an origin in prokaryotic potassium channels (Kuner et al., 2003; Tikhonov and Magazanik, 2009), eukaryotic iGluRs would have lost the TXVGYG motif, presenting Na^+ and/or Ca^{2+} as well as K^+ selectivity. In metazoan iGluRs ion selectivity is

specified by the QRN site, which is also located in the M2 domain (Kuner et al., 2001; Verdoorn et al., 1991; Wollmuth and Sakmann, 1998).

The TMD also encompasses a highly evolutionarily conserved motif within M3. This domain is involved in gating, maintaining a closed channel in the resting state (Kohda et al., 2000; Yuan et al., 2005). M3 also contains a signal for endoplasmic reticulum (ER) export (Horak et al., 2014). This motif, with consensus sequence SYTANLAAF, is present in all iGluRs, including those from bacteria, and also in the bacterial KcsA channel (Chiu et al., 1999; Janovjak et al., 2011; Kuner et al., 2003; Ramos-Vicente et al., 2018).

2.2. Evolution of the N-terminal domain and its relationship with other receptor proteins

The extracellular NTD comprises the first ~400 amino acids of iGluRs (Sobolevsky et al., 2009). It presents binding sites for regulatory small molecules and ions (Masuko et al., 1999; Paoletti et al., 2000; Traynelis et al., 1995). The NTD mediates the tetramerization of the receptor in the ER by interacting with NTDs from other subunits (Hansen et al., 2010) and also the interaction of the tetrameric receptor with other proteins such as N-cadherins, pentraxines or ephrines (Saglietti et al., 2007; Sia et al., 2007; Takasu et al., 2002). The NTD has a ‘venus flytrap’ structure and is homologous to the LBD of prokaryotic leucine-isoleucine-valine-binding periplasmatic proteins (LIVBPs). Interestingly, the extracellular domain of class C G-protein-coupled receptors, which includes mGluRs, is also a homologue of the LBD of prokaryotic LIVBPs (Kumar et al., 2009; O'Hara et al., 1993; Paoletti et al., 2000; Tikhonov and Magazanik, 2009). Because of this structural homology it has been proposed that a LIVBP-encoding sequence inserted at the beginning of the ancestral iGluR0 gene (Figure 1). This

event would have occurred very early in eukaryotic evolution, as all known eukaryotic iGluRs present this NTD. This evolutionary origin would explain the ligand binding properties of the NTD of vertebrate GluN2A/B subunits, as these would conserve the function of its precursor LIVBP (Felder et al., 1999). The protein-protein interactions performed by the NTD have been proposed to be important for the neofunctionalization of GluD2 receptors, which diverged during vertebrate evolution to act as cell adhesion and trans-synaptic signalling molecules rather than as ligand-gated ion channels (Matsuda et al., 2010).

2.3. Origin of the M4 and C-terminal domains, and evolution of iGluR regulation

Eukaryotic iGluRs have a fourth transmembrane segment (M4), which in the primary structure is just after the S2 lobe of the LBD (Sobolevsky et al., 2009). This segment is not present in the prokaryotic GluR0 or KcsA, and it is thus an eukaryotic innovation that appeared early in evolution, as it is present in all known eukaryotic iGluRs (Ger et al., 2010; Salussolia et al., 2013; Tikhonov and Magazanik, 2009). The M4 segment contains a series of highly conserved residues that interact with the M1 and M3 of adjacent subunits, contributing to receptor tetramerization (Salussolia et al., 2011). Importantly, the addition of this transmembrane segment means that eukaryotic iGluR C-termini face the cytoplasmic side of the membrane. This change resulted in the evolution of C-terminal domains that mediate the formation of receptor protein complexes (Bayés et al., 2014; Emes et al., 2008; Frank et al., 2016; Husi et al., 2000; Reig-Viader et al., 2018) and allow for post-translational modifications (Ehlers et al., 1998), both key for the function of iGluRs in animals.

The CTD is also the most variable domain, sharing low sequence identity and having differing lengths between subunits (Ryan et al., 2008). Particularly notable is the increase in CTD length found in vertebrate GluN2 subunits that occurred at the base of this lineage, before the 2R event. After the two genome duplications, each vertebrate GluN2 subunit diverged its CTD to perform different roles in the nervous system (Ryan et al., 2013). Long CTDs are not found in invertebrate GluN2s, which are of similar length to those of other iGluR types (Kenny and Dearden, 2013; Ryan et al., 2008). The sequence elongation of vertebrate GluN2 C-terminal tails created more sites for post-translational modifications and protein-protein interactions, potentially increasing the complexity of the regulation of GluN2 subunit-containing receptors (Kenny and Dearden, 2013; Ryan et al., 2013, 2008).

3. **Evolution and diversity of metazoan iGluRs**

Early genomic and transcriptomic projects in invertebrate model species, particularly in *Caenorhabditis elegans* and *Drosophila melanogaster*, identified clear homologues to mammalian iGluRs (Adams et al., 2000; *C. elegans* Sequencing Consortium, 1998). Many of these iGluRs could be confidently assigned to one of the vertebrate classes (Brockie et al., 2001; Li et al., 2016). Studies in these model animals suggested that invertebrates would present a more limited number of iGluR genes, and that mammalian iGluR classes would be the only ones present in all animals (Brockie et al., 2001a; Greer et al., 2017; Li et al., 2016; Okamura et al., 2005). This idea, that was supported by work on vertebrate iGluRs (Cox et al., 2005; Lin et al., 2006; Ottiger et al., 1995; Schmidt et al., 2009), was initially challenged by studies in other invertebrates, such as *Aplysia californica*, *Lymnaea stagnalis* or *Ciona intestinalis*, as their iGluRs could

not be confidently classified into any of the vertebrate classes (Okamura et al., 2005; Greer et al., 2017).

A comprehensive reconstruction of the animal iGluR phylogeny, which included sequences from many species spanning all major phyla of the metazoan kingdom, showed that invertebrate sequences previously classified as orphan or ancestral (Alberstein et al., 2015; Greer et al., 2017) cluster together, forming new phylogenetic groups (Figure 2a) (Ramos-Vicente et al., 2018). This study resulted in a reorganised classification of metazoan iGluRs, which previously had essentially been based on vertebrate genes. Instead of the six classes previously recognised –AMPA, Kainate, Delta, NMDA1, NMDA2 and NMDA3 – this work established the existence of 12 phylogenetic groups. Thus, the family of iGluRs would be organised into four subfamilies, termed Lambda, NMDA, Epsilon and AKDF (AMPA/Kainate/Delta/Phi). NMDA, Epsilon and AKDF subfamilies contain proteins from basal metazoans (ctenophores, sponges and cnidarians) to invertebrate chordates (the phylum to which vertebrates belong), while the NMDA and AKDF subfamilies contain the known vertebrate proteins. On the other hand, the Lambda subfamily would be restricted to porifera species. Importantly, subsequent studies have corroborated the evolution of metazoan iGluRs into these four subfamilies and have identified most of the classes found within them (Stroebel and Paoletti, 2020).

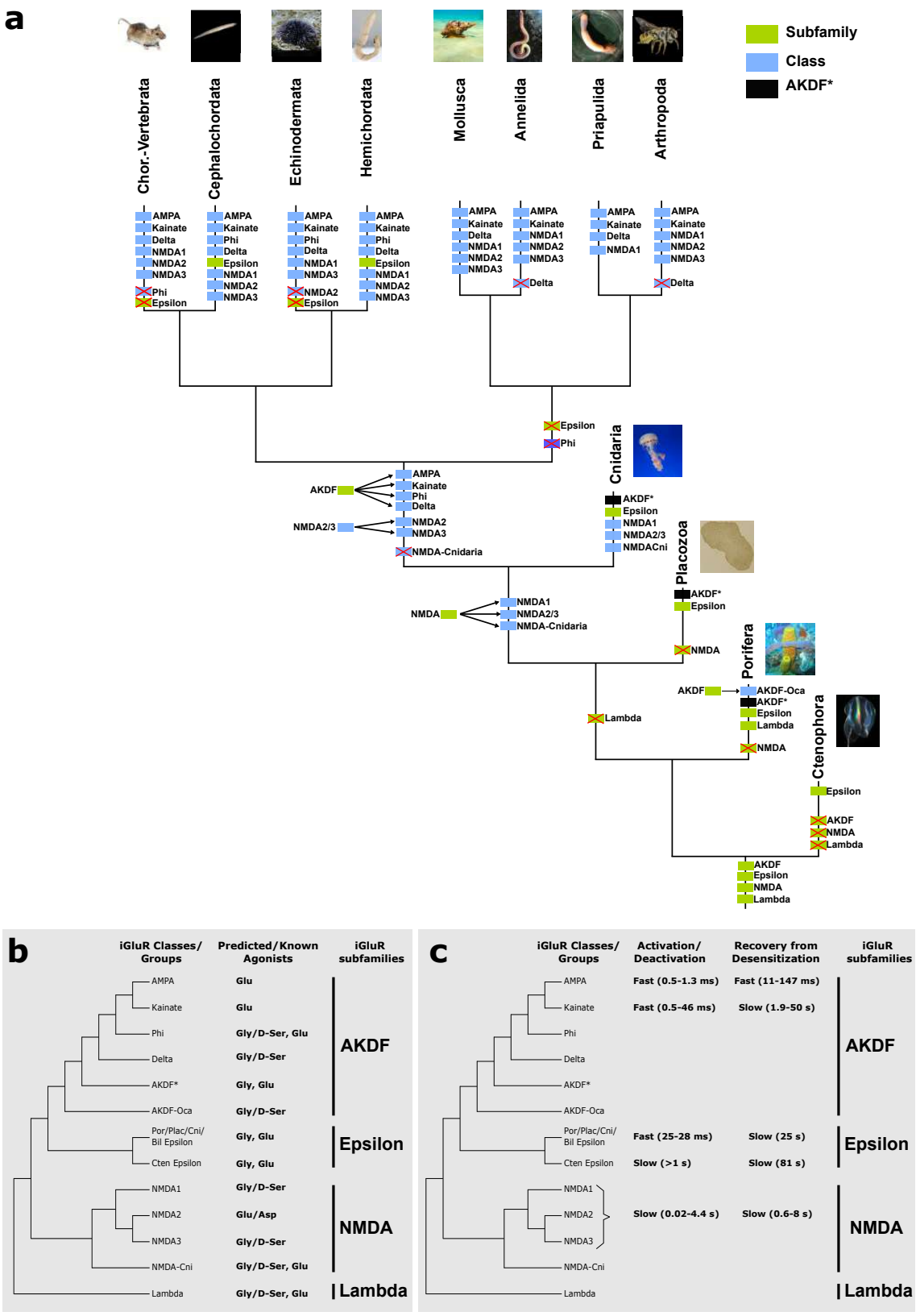


Figure 2. Metazoan evolution of ionotropic glutamate receptors.

a. Summary tree of proposed evolutionary history of iGluRs in the metazoan phylum, and current distribution of iGluR classes in major metazoan lineages. Each branch corresponds with one lineage. Phylogenetic subfamilies are represented by green boxes and classes by light blue boxes. The four subfamilies present in the ancestor of all current metazoan lineages are shown at the base

of the tree. Duplications of subfamilies in ancestors of current lineages are indicated. When a class or subfamily is lost in a lineage or in an ancestor, the corresponding box is crossed out with a red cross.
b. Simplified phylogenetic tree comparing known kinetic characteristics of metazoan iGluR classes and subfamilies. The experimentally estimated times of activation/deactivation and recovery from desensitization are showed at the right of each class. As NMDA receptors are heterotetramers that can contain different stoichiometries of NMDA1, NMDA2 and NMDA3 subunits, the times showed include all studied combinations of these subunits. Class name is provided at the branch tip, and subfamilies are indicated at the right of the tree. Epsilon and Lambda subunits are not currently classified in classes. Por/Plac/Cni/Bil Epsilon represents the Epsilon subfamily subunits from porifera, placozoa, cnidaria and bilateria, Cten Epsilon represents the Epsilon subfamily subunits from ctenophora.
c. Simplified phylogenetic tree comparing known or predicted agonist specificity of metazoan iGluR classes and subfamilies. The specificity for glycine (Gly), glutamate (Glu) or aspartate (Asp) of the subunits belonging to each class or subfamily is showed at the right of their name. Class name is provided at the branch tip, and subfamilies are indicated at the right of the tree. Epsilon and Lambda subunits are not currently classified in classes. Por/Plac/Cni/Bil Epsilon represents the Epsilon subfamily subunits from porifera, placozoa, cnidaria and bilateria, Cten Epsilon represents the Epsilon subfamily subunits from ctenophora. Image credit: Placozoa, author Oliver Voigt, licensed under CC BY-SA 3.0 Germany license; source https://commons.wikimedia.org/wiki/File:Trichoplax_mic.jpg; *P caudatus*, author Shunkina Ksenia, licensed under CC BY 3.0 source https://commons.wikimedia.org/wiki/File:Priapulus_caudatus.jpg; Hemichordata, released under GNU Free Documentation License, source https://commons.wikimedia.org/wiki/File:Eichelwurm.jpg; Cephalochordata, author Hans Hillewaert, licensed under CC BY-SA 4.0 International license, source https://commons.wikimedia.org/wiki/File:Branchiostoma_lanceolatum.jpg.

Two of these subfamilies, NMDA and AKDF, contain five iGluR classes each, including the six vertebrate classes mentioned above, but also four unreported ones. Thus, the main 12 phylogenetic groups among animal iGluRs would be: the subfamilies (i) Lambda and (ii) Epsilon, for which no classes were defined; the classes from the NMDA subfamily, namely (iii) NMDA1, (iv) NMDA2, (v) NMDA3, (vi) NMDA-Cni and (vii) NMDA2/3; and the classes from the AKDF subfamily, namely (viii) AMPA, (ix) Kainate, (x) Delta, (xi) Phi and (xii) AKDF-Oca. Importantly, classes NMDA2/3 and AKDF-Oca were only identified in one species, and thus further research will be required to validate their existence. Later work on metazoan iGluRs (Stroebel and Paoletti, 2020) has identified even more branches within the AKDF subfamily, suggesting that the number of AKDF classes would be even larger. More metazoan sequences and more extensive analysis of this subfamily will be required to fully understand its evolutionary history (Stroebel and Paoletti, 2020).

Notably, the four subfamilies in which iGluRs are organized are evolutionarily ancient. A recent study has shown that at least three of them, namely Lambda, NMDA and an ancestor of Epsilon-AKDF, appeared before the emergence of metazoans, as some unicellular opistokonts -a clade of eukaryotic organisms that includes fungi, metazoans and closely related unicellular eukaryotes such as choanoflagellates (Torruella et al., 2012)- contain homologous sequences (Tikhonenkov et al., 2020). Nevertheless, other evolutionary scenarios are not discarded in this study, such as unicellular opistokont sequences diverging before the appearance of the NMDA subfamily or these being a sister group of metazoan Epsilon iGluRs (Tikhonenkov et al., 2020). Importantly, during evolution each metazoan lineage experienced independent expansions and losses of different

groups that led to the current set of iGluR subunits found in modern genomes. This resulted in organisms such as cephalochordates or the sponge *Oscarella carmela* having larger sets of iGluRs than vertebrates (Ramos-Vicente et al., 2018). The fact that these organisms have a simpler nervous system (fewer neurons) than vertebrates, or even lack a nervous system at all, suggests that an increase in the set of neurotransmitter receptors is not strictly correlated with an increase in the anatomical complexity of the nervous system.

4. **Evolution of ligand binding selectivity**

The lack of functional data on most invertebrate iGluRs means that their ligand binding selectivity has to be predicted based on their protein sequence and our knowledge from studies with their vertebrate homologs. While this is a fair approach, it is important to keep in mind that work on fly iGluRs has convincingly shown that a very reduced number of amino acid substitutions can dramatically change their ligand binding properties (Li et al., 2016; Mayer 2020). Undoubtedly, our poor understanding of the structure-function relationships in non-vertebrate iGluRs hampers our ability to make highly confident predictions on their ligand selectivity based solely on protein sequence.

Despite their name, iGluRs can be activated by other amino acids and some of their derivatives. Actually, ligand specificity is mainly determined by a fairly small number of residues (Alberstein et al., 2015; Blaise et al., 2004; Pentikäinen et al., 2003). As a general rule, iGluR subunits that present a serine at position 653 (numbering corresponds to rat GluA2) and a non-polar residue at position 655 will bind glycine or D-serine. Instead, when these positions are occupied by a glycine and a threonine, glutamate becomes the preferred ligand (Figures 2b and

3a). The residue at position 704 also plays a role in ligand binding, although it is less evolutionary conserved. In glutamate binding subunits this position is occupied by an aliphatic residue (such as leucine, Figure 3a), or by tryptophan in subunits recognising glycine or D-serine. Importantly, both vertebrate and invertebrate GluN2 subunits present a tyrosine in this position, which together with valine 708 and tyrosine 736 are involved in aspartate binding (Furukawa et al., 2005; Laube et al., 2004).

The combination of residues that allow for aspartate binding in GluN2s has only been found in one iGluR sequence from other phylogenetic groups. This is GluE10 from the ctenophore *Pleurobrachia bachei* (Alberstein et al. 2015, Ramos-Vicente et al., 2018). Nevertheless, this particular subunit has not been investigated experimentally and we do not know if aspartate can act as its agonist. Overall, with the available data, the capacity to recognise aspartate would be essentially exclusive to GluN2 subunits (Figure 2b). Interestingly, NMDA subunits are less selective in the ligands they bind, whereas AMPA and Kainate receptors have glutamate as their only endogenous agonist (Armstrong and Gouaux, 2000; Mayer, 2005; Swanson and Sakai, 2009). For instance, mammalian GluN1 receptors can bind D-alanine, glycine and D-serine, and, with less affinity, L-alanine and L-serine (Furukawa and Gouaux, 2003; McBain et al., 1989; Schell, 2004). GluN2s bind L-glutamate but can also be activated by D-/L-aspartate, homocysteate and cysteinesulfinate (D'Aniello et al., 2003; Do et al., 1988; Furukawa et al., 2005; Watkins and Evans, 1981; Zhang and Nadler, 2009). Finally, GluN3A/B can bind glycine and D-/L-serine (Yao et al., 2008).

a

POSITION					SELECTIVITY	EXAMPLE SUBUNIT
653	655	704	708	736		
G (S/T)	T	L	M	Y	Glutamate	GluA1
G	T	Y	V	Y	Glutamate & Aspartate	GluN2A
S(R)	V/L/A/I/P	W	V	F	Gly/D-Ser	GluN1

b

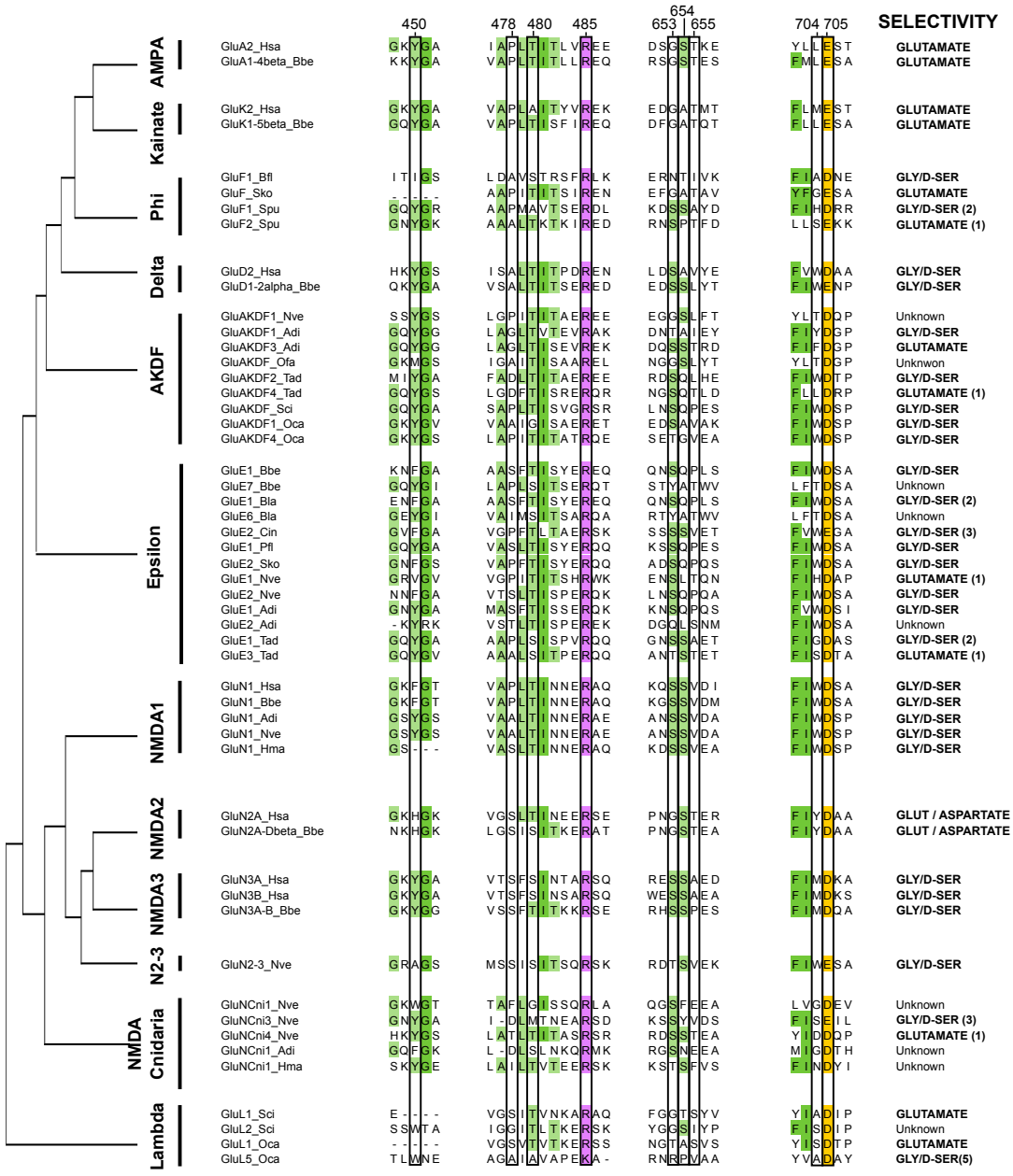


Figure 3. Protein alignment of iGluR residues involved in ligand-binding

a. Primary sequence determinants of ligand selectivity. The main residues involved in ligand selectivity are 653, 655 and 704 (numbering corresponds to rat GluA2). The amino acids at these positions determine ligand preference as shown. Residues involved in D-/L-aspartate binding are in positions 704, 708 and

736. Nevertheless, in Ctenophores position 653 can be occupied by arginine in glycine binding subunits and serine or threonine in those recognising glutamate. **b.** Representative sequences for AMPA, Kainate, Delta and NMDA classes are shown together with protein sequences from unreported phylogenetic groups found in Ramos-Vicente *et al.*, 2018 and Epsilon iGluRs from ctenophores (Alberstein *et al.*, 2015). Residue numbering (on top) corresponds to mature rat GluA2 sequence. Residues involved in agonist binding are highlighted by a black frame. Residue 450 is involved in Van-der-Waals interactions with the α-carbon of the ligand, residues 485 and 654 engage in electrostatic interactions with the α-carboxyl group, residues 478, 480 and 705 form interactions with the α-amino group and residues 653, 655 and 704 contact the amino acid side chain. iGluR residues are shadowed as follows: yellow for acid residues and pink for basic ones. Higher amino acid conservation is represented by increasing intensity of green background. Known or predicted agonist selectivity is indicated at the right. Overall prediction is based on sequence similarity with vertebrate proteins, but in particular considers the following sequences: (1) similarity with the *M. leidyi* GluE13_Mle sequence (gene reference ML05909A), (2) similarity with *H. sapiens* GluN3A sequence, (3) similarity with *H. sapiens* GluN1 sequence, (4) similarity with AMPA receptor subunits sequence and (5) similarity with *M. leidyi* GluE7_Mle (gene reference ML032222a). For some sequences a reliable prediction cannot be made, these are labelled as 'Unknown'. Figure was modified from Ramos-Vicente *et al.*, 2018.

It has been hypothesized that the ligand promiscuity of NMDA subunits is related to their more ancestral origin; conversely, the specificity of AMPA and Kainate receptors for glutamate has been attributed to their more modern origin, acting as highly specialized receptors (Alberstein et al., 2015; Stroebel and Paoletti, 2020). Nevertheless, iGluR subunits, which based on their primary sequence, are predicted to bind glycine or glutamate are found in all major animal phylogenetic groups of iGluRs, despite of their evolutionary origin (Figure 3b). The fact that AMPA and Kainate receptors have a restricted ligand binding selectivity, in their case for glutamate, would be the exception rather than the rule among iGluR classes. Thus, these findings question the previous notion that glycine binding is more ancestral than glutamate binding. In any case, it is presently not possible to establish which ligand specificity arose first, what seems clear is that subunits recognising glycine or glutamate have coexisted in the same class of receptors

for most of metazoan evolution and that those predicted to bind glycine currently outnumber those that would bind glutamate (Figure 3b). Finally, we cannot rule out that some subunits of the Epsilon, AKDF, NMDA-Cnidaria or Lambda phylogenetic groups have evolved to recognise other ligands, different from those described in vertebrates. This is because the residues they present in the positions involved in ligand binding are very different from those reported in studied iGluR subunits (see sequences classified with 'unknown' selectivity in Figure 3b). An example of an atypical ligand specificity is found in a receptor from *Adineta vaga* (AvGluR1), which can be activated by alanine, methionine or cysteine (Janovjak et al., 2011; Lomash et al., 2013). If these receptors are highly specific for their yet unidentified ligands they could have important biotechnological or biomedical applications.

Vertebrate iGluRs binding glutamate (i.e. AMPA and Kainate) present faster kinetics than glycine or glycine/glutamate binding receptors (Delta or NMDA) (Banke and Traynelis, 2003; Barberis et al., 2008; Cummings et al., 2017; Cummings and Popescu, 2016; Krampfl et al., 2001; Sekiguchi et al., 2002; Swanson and Heinemann, 1998; Vicini et al., 1998; Wyllie et al., 1998). This poses the question if there is an evolutionary history leading to fast, glutamate-binding receptors. Since the kinetics of ionotropic glutamate receptors has only been systematically investigated in mammalian receptors, it is rather speculative to discuss on the evolution of iGluR kinetics. Nevertheless, some pioneer studies have looked into the kinetics of AMPA and Kainate receptors from drosophila (Li et al., 2016; Walker et al., 2016; Wang et al., 2008) as well as the kinetics of Epsilon receptors from ctenophores (Alberstein et al., 2015) and cephalochordates (Ramos-Vicente et al. 2018). With the available data it seems

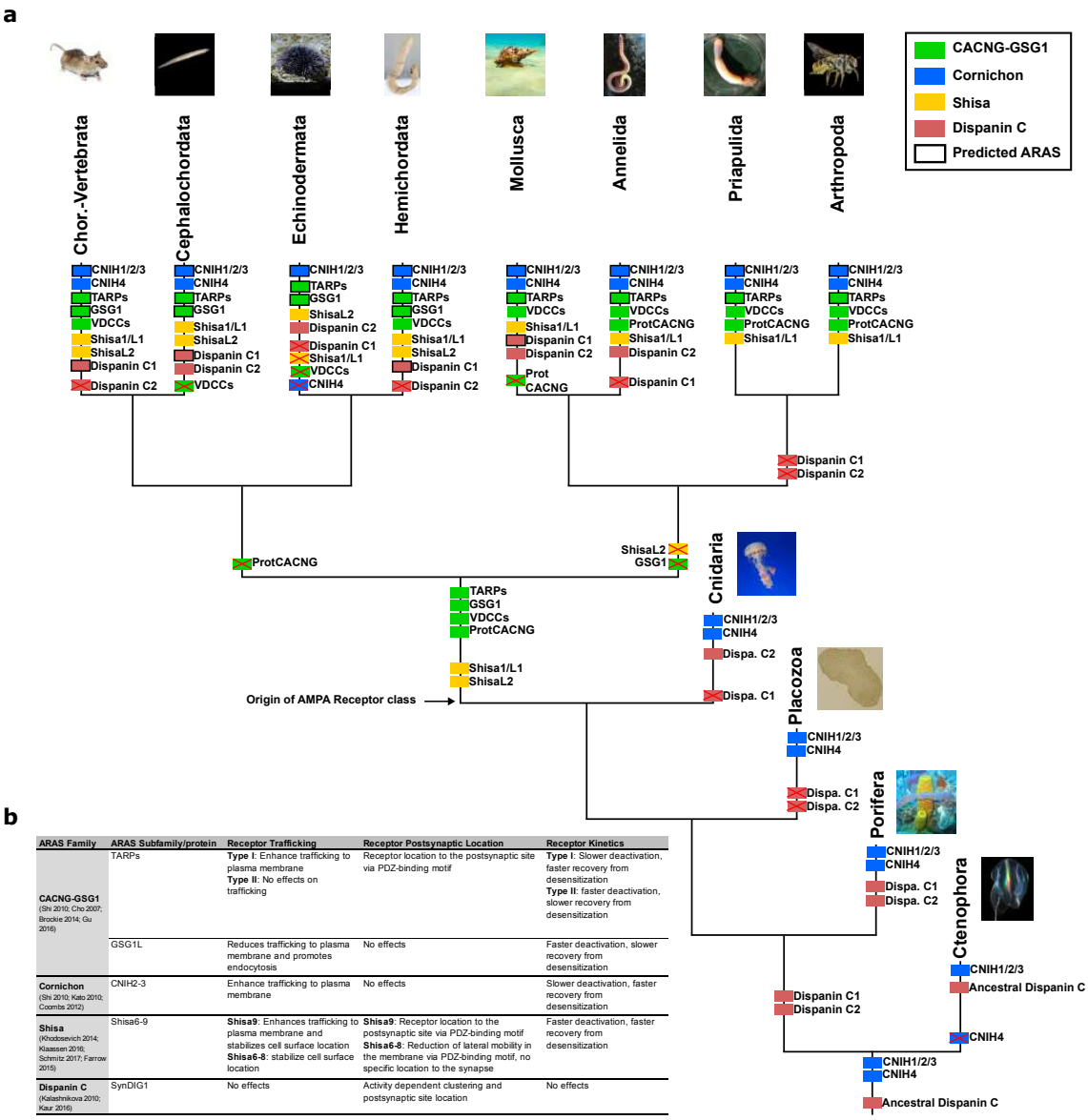
that fast activation/deactivation kinetics would not be exclusive to AMPA and Kainate receptors, as the Epsilon receptor (GluE1), from amphioxus, displays activation/deactivation kinetics in the range of some mammalian Kainate receptors (Figure 2c), although these are not as fast as AMPAR ones. Furthermore, GluE1 was specifically activated by glycine (as predicted by its protein sequence), showing no response to glutamate (Ramos-Vicente et al. 2018). This data would discard an evolutionary trend towards fast, glutamate-binding iGluRs. On the other hand, the fast recovery from desensitization (in the range of milliseconds) characteristic of AMPA receptors, might be exclusive to this class, as all other iGluR types investigated present slow (in the range of seconds) recovery times (Figure 2c). This is the case for mammalian NMDA receptors but also for all Epsilon receptors investigated.

5. **iGluR auxiliary subunits expanded in vertebrates**

iGluRs interact with other synaptic proteins that regulate their function and trafficking (Bissen et al., 2019). Glutamate receptor auxiliary subunits are among the best characterized of these partners (Jackson and Nicoll, 2011). In mammals, AMPA receptors have been shown to interact with a great variety of transmembrane proteins, which act as auxiliary subunits (Bissen et al., 2019; Greger et al., 2017). Known AMPA receptor auxiliary subunits (ARASs) belong to unrelated protein families that also contain members with functions unrelated to AMPA receptors (Ramos-Vicente and Bayés, 2020). These are: (i) transmembrane AMPA receptor regulatory proteins (TARPs), members of the CACNG family (Chu et al., 2001; Kato et al., 2007; Tomita et al., 2003); (ii) CNIH2 and CNIH3 from the Cornichon family (Castro et al., 2007; Sauvageau et al., 2014; Schwenk et al., 2009); (iii) Shisa6 to Shisa9, from the Shisa family

(Bourdon et al., 2002; Hedge and Mason, 2008; von Engelhardt, 2019); (iv) GSG1L from the Claudin superfamily (Chen and Gouaux, 2019; Choi et al., 2008; Shanks et al., 2012); and (v) SynDIG1, a protein from the Dispanin C family (Kalashnikova et al., 2010).

Protein homologues of vertebrate TARPs have been reported acting as ARASs in invertebrate model organisms, including the fruit fly *D. melanogaster*, *C. elegans* and the honeybee *Apis mellifera* (Walker et al., 2006; Wang et al., 2008). A homologue of Cornichon proteins, a family with two ARASs in vertebrates, has been reported functioning as an AMPA receptor auxiliary protein in *C. elegans*, but not in other invertebrate models, including the fruit fly (Bökel et al., 2006; Brockie et al., 2013). The animal evolutionary history of the four protein families containing mammalian ARASs has recently been reported (Figure 4a) (Ramos-Vicente and Bayés, 2020). The phylogenies of these families have revealed that the diverse set of ARASs found in vertebrate synapses arouse from neo- and subfunctionalization events in four independent protein families; these processes occurred after the 2R, early in vertebrate evolution (He and Zhang, 2005; Ramos-Vicente and Bayés, 2020). These phylogenetic studies showed that Cornichons are the most ancient ARASs, while TARPs would have appeared together with AMPA receptors in the bilaterian ancestor. Thus, in invertebrate bilaterians, Cornichons and TARPs would act as auxiliary subunits, as has been experimentally proven in the nematode *C. elegans* (Cornichons and TARPs) and the arthropod *D. melanogaster* (TARPs) (Brockie et al., 2013; Walker et al., 2006; Wang et al., 2008).



ARAS Family	ARAS Subfamily/protein	Receptor Trafficking	Receptor Postsynaptic Location	Receptor Kinetics
CACNG-GSG1 (Shi 2010; Cho 2007; Brockie 2014; Gu 2016)	TARPs	**Type I**: Enhance trafficking to plasma membrane **Type II**: No effects on trafficking	Receptor location to the postsynaptic site via PDZ-binding motif	**Type I**: Slower deactivation, faster recovery from desensitization **Type II**: faster deactivation, slower recovery from desensitization
	GSG1L	Reduces trafficking to plasma membrane and promotes endocytosis	No effects	Faster deactivation, slower recovery from desensitization
Cornichon (Shi 2010; Kato 2010; Coombs 2012)	CNIH2-3	Enhance trafficking to plasma membrane	No effects	Slower deactivation, faster recovery from desensitization
Shisa (Khodosevich 2014; Klaassen 2016; Schmitz 2017; Farrow 2015)	Shisa6-9	**Shisa9**: Enhances trafficking to plasma membrane and stabilizes cell surface location **Shisa6-8**: stabilize cell surface location	**Shisa9**: Receptor location to the postsynaptic site via PDZ-binding motif **Shisa6-8**: Reduction of lateral mobility in the membrane via PDZ-binding motif, no specific location to the synapse	Faster deactivation, faster recovery from desensitization
Dispanin C (Kalashnikova 2010; Kaur 2016)	SynDIG1	No effects	Activity dependent clustering and postsynaptic site location	No effects

Figure 4. Metazoan evolution of auxiliary subunits of AMPA class ionotropic glutamate receptors

a. Summary tree of proposed evolutionary history of ARAS containing families in the metazoan phylum, and current distribution of their subfamilies in major metazoan lineages. Each branch corresponds with one lineage. ARASs containing families are represented by different colour boxes. CACNG, Cornichon, Shisa and Dispanin C families correspond to green, blue, yellow and red boxes, respectively. Subfamilies that based on the phylogeny are predicted to act as ARASs are framed in black. First appearance of ARASs containing families in ancestors of current lineages are indicated. The origin of AMPA class in the ancestor of bilaterians is indicated by a black arrow. When a class or subfamily is lost in a lineage or in an ancestor, the corresponding box is crossed out with a red cross. Image credit: Placozoa, author Oliver Voigt, licensed under CC BY-SA 3.0 Germany license; source https://commons.wikimedia.org/wiki/File:Trichoplax_mic.jpg; *P caudatus*, author Shunkina Ksenia, licensed under CC BY 3.0 source https://commons.wikimedia.org/wiki/File:Priapulus_caudatus.jpg; Hemichordata,

released under GNU Free Documentation License, source https://commons.wikimedia.org/wiki/File:Eichelwurm.jpg; Cephalochordata, author Hans Hillewaert, licensed CC BY-SA 4.0 International license, source https://commons.wikimedia.org/wiki/File:Branchiostoma_lanceolatum.jpg. **b.** Table summarising the main functional effects that ARAS have on glutamate receptors. These can be divided in three groups: Effects on receptor trafficking, Receptor Postsynaptic location and receptor kinetics. Relevant references for each ARAS family are also indicated.

In vertebrates, a subfunctionalization event in the Cornichon CNIH1/2/3 subfamily allowed for the specialization of CNIH2 and CNIH3 into ARASs, while CNIH1 took over what was the most likely role of the ancestral gene, which is to traffic TGFα to the secretory pathway (Castro et al., 2007; Ramos-Vicente and Bayés, 2020). The increase in Cornichon ARASs in vertebrates has not substantially contributed to the overall load of vertebrate ARASs, since invertebrates have one and vertebrates two (Ramos-Vicente and Bayés, 2020). Similarly, the family of Dispanin C would include only one ARAS in both invertebrates and vertebrates. Although the function of two out of its three members is unknown. By contrast, the expansion of TARPs and the neofunctionalization of four Shisa proteins into ARASs would have significantly increased the number of ARASs in vertebrates (Ramos-Vicente and Bayés, 2020). The recruitment of all these diverse proteins as ARASs allowed a further increase in the variability of glutamatergic postsynaptic responses (Bissen et al., 2019; Khodosevich et al., 2014). The increased modulation in the responses to neurotransmitter release added a new layer of complexity to glutamatergic transmission, potentially enabling the development of more complex neuronal circuitries and elaborated behaviours (Grant, 2016; Kenny and Dearden, 2013; Ramos-Vicente and Bayés, 2020; Thomas and Hayashi, 2013).

Most ARASs not only regulate AMPA receptor trafficking and location, but also modulate their electrophysiological properties (Buonaroti et al., 2019; Cho et al., 2007; Kott et al., 2009). This modulation depends on the auxiliary subunit composition of the AMPA receptor complex, adding more variability and regulatory options to the synaptic transmission mediated by them (Kato et al., 2010; Khodosevich et al., 2014). The main AMPAR functional domains modulated by ARASs can be grouped into: i) intracellular receptor trafficking, ii) receptor localization and stabilization at the postsynapse and iii) channel kinetics (Figure 4b). Importantly, all these functions can be modulated in opposed directions and, as a general rule, each vertebrate ARAS will modulate it only in one direction. Even members of the same phylogenetic group can have opposed effects on AMPAR function. For example, Type I TARPs enhance AMPAR traffic to the plasma membrane, while GSG1L reduces it. ARASs from the same subfamily can also have totally opposed effects, for example in channel kinetics. Type I TARPS will make AMPAR have slower deactivation rates, while Type II TARPs enhance this feature (Figure 4b). Since the only invertebrate ARAS investigated are TARPSs and Cornichons we ignore if invertebrate homologs to the other ARAS will also modulate AMPAR function or if, contrarily, this function has appeared after the neo/subfunctionalization of these protein families in vertebrates. Nevertheless, our hypothesis is that, as for invertebrate TARPs and Cornichons, invertebrate Shisas or Dispanin C will modulate to some degree AMPA or other related receptors. In any case, the fine-tuning of AMPAR modulation by ARAS has most likely occurred during early vertebrate evolution, as indicated by the fact that vertebrate paralogues that have only one invertebrate homologue modulate in opposed directions AMPAR function.

6. Conserved and unique iGluR functions in non-vertebrate animals

Glutamate is the major excitatory amino acid in the adult CNS of vertebrates, and glutamate receptors are expressed in almost all neuronal types mediating fast excitatory transmission (Hawrylycz et al., 2012). iGluRs are also key to synaptic plasticity and thus have a pivotal role in learning and memory (Barnes and Slevin, 2003; Kessels and Malinow, 2009; Malenka and Bear, 2004). Furthermore, plasticity processes are also important for the development of glutamatergic synapses (Kerchner and Nicoll, 2008), and so iGluRs also have an important role in CNS development (Chen and Lipton, 2006; Pachernegg et al., 2013; Passafaro et al., 2001). As in vertebrates, glutamatergic neurotransmission is pivotal in the invertebrate CNS (Pascual-Anaya and D'Aniello, 2006). Glutamatergic synapses participate in the generation of different behaviours (Brockie et al., 2001a, 2001b; Zheng et al., 1999). iGluRs also participate in synaptic plasticity in invertebrates, as has been shown for model organisms of memory formation such as the gastropods *A. californica* and *L. stagnalis* (Ha et al., 2006; Roberts and Glanzman, 2003). Thus, these molecular mechanisms of memory formation would have appeared at least in the ancestor of all bilaterians, being conserved in different lineages of protostome and deuterostome clades (Greer et al., 2017). Invertebrate iGluRs are also implicated in the development of glutamatergic neuronal circuits (Brockie and Maricq, 2003; Hirai et al., 2017). Apart from their role in the CNS, iGluRs have been found to modulate and control the activity of the neuromuscular junction (NMJ) in invertebrates, although the primary transmission between the presynaptic motor neuron and the muscle cell is mediated by acetylcholine as in vertebrates (Colombo and Francolini, 2019; Devlin, 2001; Fox and Lloyd, 1999; Thapliyal and Babu, 2018). iGluRs from the Kainate class are present at the NMJ of arthropods, and this class show a specific

expansion in this lineage (Li et al., 2016; Ramos-Vicente et al., 2018). These iGluRs are located at the postsynaptic muscle cell membrane, and they not only modulate synaptic activity, but also play the same role as acetylcholine receptors in the vertebrate NMJ, mediating the activation of the muscle cell (Menon et al., 2013; Rivlin et al., 2004; Wu and Cooper, 2012). A predominant function of iGluRs in the NMJ is also found in the basal metazoan phylum ctenophora. This suggests that early in metazoan evolution neuromuscular transmission was controlled by glutamate, and in the ancestor of cnidarians and bilaterians this function switched to acetylcholine, leaving glutamatergic transmission with a secondary regulatory role in other phyla (Colombo and Francolini, 2019; Moroz et al., 2014). Then, arthropods independently recovered glutamate as the primary transmitter at the NMJ (Devlin, 2001; Rivlin et al., 2004; Thapliyal and Babu, 2018; Wu and Cooper, 2012). In invertebrate organisms without a CNS, such as cnidarians, iGluRs also play a role in neurons and synaptic transmission generating rhythmic patterns and controlling, for instance, feeding behaviours (Kass-Simon et al., 2003; Pierobon, 2012; Pierobon et al., 2004).

Metazoans without nervous systems, including sponges and placozoans, also possess their own set of iGluRs, and in some species the number of genes encoding iGluR subunits is comparable to that of vertebrates (Ramos-Vicente et al., 2018). These receptors belong to the same subfamilies as the rest of metazoan iGluRs (Ramos-Vicente et al., 2018; Tikhonenkov et al., 2020). This evidence highlights the importance of glutamatergic signalling through iGluRs performing non-synaptic functions (Rodriguez et al., 2013). In other eukaryotes that lack nervous systems, iGluRs carry out different functions. In plants, iGluRs are involved in diverse functions: root development (Ni et al., 2016),

carbon/nitrogen metabolic balance (Kang and Turano, 2003), response to damage and innate immune response (Kwaaitaal et al., 2011; Mousavi et al., 2013), abiotic and biotic stress tolerance (Kang et al., 2004, 2006; Kim et al., 2001), cold and touch sensing (Meyerhoff et al., 2005), stomata closing and reproduction (Cho et al., 2009; Michard et al., 2011). Interestingly, chemotaxis is a non-synaptic function of iGluRs shared between plants and metazoans, suggesting that this might be the most ancestral function of these receptors, at least in eukaryotes (Harlow et al., 2015; Liu et al., 2009; Ortiz-Ramírez et al., 2017).

7. **Evolution of metabotropic glutamate receptors unravelled a Class IV**

Metabotropic glutamate receptors modulate glutamatergic transmission strength (Conn and Pin, 1997). These receptors are G-protein-coupled receptors, and thus act through second messengers to modulate many synaptic molecular events, such as ion channel conductivity (Niswender and Conn, 2010). In vertebrates, these receptors are divided into three groups, which vary in their pharmacology and downstream effects: class I, class II and class III (Conn and Pin, 1997; Wright and Schoepp, 1996). In contrast to iGluRs, mGluRs have glutamate as their sole endogenous ligand, with the exception of mGluR3, which also binds N-acetyl aspartylglutamate (NAAG) (Koehl et al., 2019; Mitri et al., 2004; Neale and Olszewski, 2019). Phylogenetic analyses indicate that the three groups were present at least in the ancestor of all vertebrates, and the diversity of receptors within each group was generated by the 2R (Haug et al., 2013). Nevertheless, the metazoan phylogeny of mGluRs showed that in bilateral species there are four mGluR classes that appeared together by gene duplication in the ancestor of all bilaterians (Figure 5) (Ramos-Vicente et al., 2018).

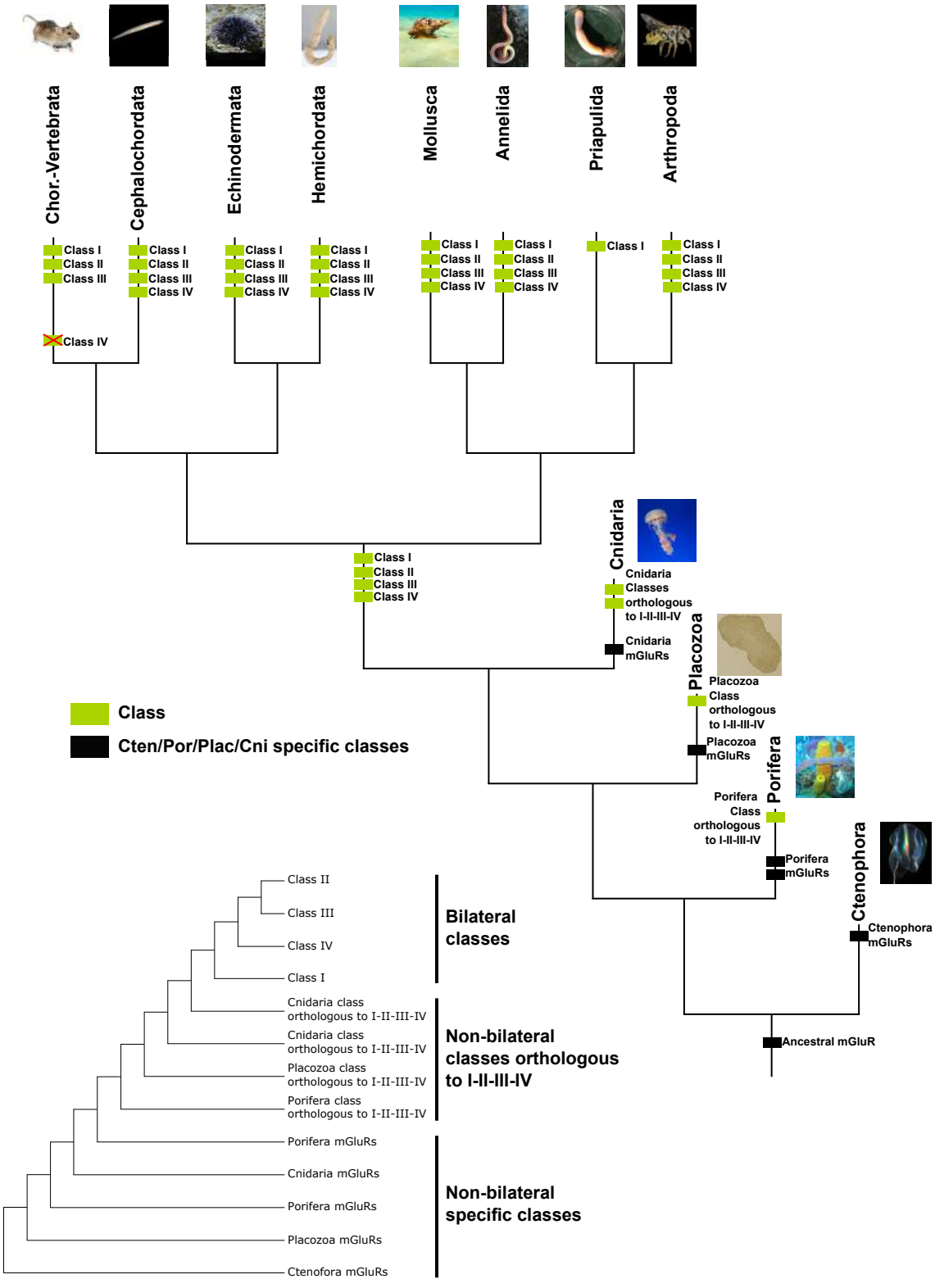


Figure 5. Metazoan evolution of metabotropic glutamate receptors
Summary tree of proposed evolutionary history of mGluRs in the metazoan phylum, and current distribution of mGluR classes in major metazoan lineages. Each branch corresponds with one lineage. Phylogenetic classes are represented by light blue boxes. The ancestral mGluR present in the ancestor of all metazoan lineages is shown at the base of the tree. The four classes present in the ancestor of bilateral metazoans are shown at the rooting branch of these lineages, after the split of cnidaria. When a class is lost in a lineage or in an

ancestor, the corresponding box is crossed out with a red cross. A simplified phylogenetic tree of metazoan mGluR classes is also provided. Class name is provided at the branch tip. Classes are grouped depending on which lineages they belong to and orthology bilateral classes, non-bilateral classes orthologous to I-II-III-IV and non-bilateral specific classes) and is indicated at the right of the tree. The exact topology of non-bilateral specific classes is still not well resolved (Ramos-Vicente et al., 2018). Image credit: Placozoa, author Oliver Voigt, licensed under CC BY-SA 3.0 Germany license; source https://commons.wikimedia.org/wiki/File:Trichoplax_mic.jpg; *P caudatus*, author Shunkina Ksenia, licensed under CC BY 3.0 source https://commons.wikimedia.org/wiki/File:Priapulus_caudatus.jpg; Hemichordata, released under GNU Free Documentation License, source https://commons.wikimedia.org/wiki/File:Eichelwurm.jpg; Cephalochordata, author Hans Hillewaert, licensed under CC BY-SA 4.0 International license, source https://commons.wikimedia.org/wiki/File:Branchiostoma_lanceolatum.jpg.

These comprise the three well-studied vertebrate classes (I to III) and a bilaterian class (IV) that includes known insect mGluRs that do not belong to classes I to III (Mitri et al., 2004). Class IV mGluRs have not been found in vertebrate species, indicating that this class has been lost in this lineage. Interestingly, in *D. melanogaster* class IV receptors have been reported to be involved in taste sensing and bind the plant toxin L-canavanine. The switch in class IV mGluR ligand specificity is due to a substitution in two of the seven residues binding glutamate in proteins from classes I to III (Mitri et al., 2009). Insect class IV receptors act through $G_{i/o}$ proteins, like class II and III mGluRs (Devambez et al., 2013), and their shared interaction with this family of G-protein alpha subunits is in accordance with their higher degree of sequence homology and their phylogenetic origin (Conn and Pin, 1997; Ramos-Vicente et al., 2018).

mGluRs from non-bilateral phyla, that is from cnidarians, placozoans, porifera and ctenophora, diverged before the appearance of bilaterian classes.

Sequences from these species form classes orthologous to I-IV. These classes are formed exclusively by sequences from species of the same lineage, and thus probably originated by specific expansions (Ramos-Vicente et al., 2018). Altogether, the evolution of mGluR classes in metazoans differs considerably from that of iGluRs: while the four iGluR subfamilies were present before the emergence of metazoans, mGluR classes found in vertebrates appeared later in evolution, in the ancestor of bilaterians (Ramos-Vicente et al., 2018; Tikhonenkov et al., 2020). Interestingly, as in the case of iGluRs, organisms with simple nervous systems or even those without one, such as the cnidarians *Hydra magnipapillata* and *Nematostella vectensis*, the sponges *Sycon cilliatum* and *Leucosolenia complicata*, and the placozoan *Trichoplax adhaerens,* have more mGluRs than vertebrates (Ramos-Vicente et al., 2018). This would argue in favour of the notion that the increase in neurotransmitter receptor number is not correlated with more complex nervous systems (Liebeskind et al., 2015; Moroz and Kohn, 2015; Sakarya et al., 2007). In bilaterians, mGluRs experienced few duplications or losses, keeping their number low and highly conserved among phyla with diverse nervous system complexities (Ramos-Vicente et al., 2018). Only vertebrates show an increase in mGluR number, mainly due to the retention of the four class III proteins that arose from the 2R event (Bayés et al., 2017; Ramos-Vicente et al., 2018). These receptors mainly modulate synaptic release of glutamate from the presynaptic terminal, regulating synaptic transmission strength (Higgs et al., 2002).

8. **Concluding remarks and future perspectives**

The vertebrate expansion of synaptic protein families has been proposed as a general mechanism contributing to the increase in nervous system complexity

and behavioural repertoires observed along animal evolution (Bayés et al., 2017; Emes et al., 2008; Emes and Grant, 2012; Grant, 2016; Nithianantharajah et al., 2013). This increase in the synaptic proteome brought associated an expanded ability to regulate neurotransmission (Grant, 2016; Kenny and Dearden, 2013; Thomas and Hayashi, 2013). For instance, the recruitment of diverse proteins to function as ARASs occurred in vertebrates probably added further variability to glutamatergic responses (Ramos-Vicente and Bayés, 2020). Furthermore, the addition of post-translational modifications and RNA editing of iGluR subunits represented a new layer of regulation leading to higher synapse and signalling complexity in vertebrates (Kung et al., 2001; Thomas and Hayashi, 2013). The expansion in synapse proteome complexity and the differential expression of paralogues gave rise to an increase in synaptic molecular types, which have been shown to have differential spatial distribution across the brain and lifespan (Cizeron et al., 2020; Zhu et al., 2018).

Nevertheless, recent data indicates that the number of genes encoding ligand-gated ion channels is similar between organisms with different nervous system complexities (Alberstein et al., 2015; Liebeskind et al., 2015; Moroz and Kohn, 2015; Ramos-Vicente et al., 2018). Furthermore, research in the cephalochordate *Branchiostoma lanceolatum* (amphioxus) indicates that the majority of these genes are expressed in the nervous system, thus likely having a synaptic role also in non-vertebrate species (Ramos-Vicente et al., 2018). iGluRs have also been found in metazoans that lack a nervous system, such as sponges and the placozoan *Trichoplax adhaerens*, and in some species in similar numbers as in vertebrates (Alberstein et al., 2015; Ramos-Vicente et al., 2018; Riesgo et al., 2014; Srivastava et al., 2008). Thus, evolution of nervous system complexity as

measured by brain size does not simply correlate with an increase in the number of genes coding for glutamate neurotransmitter receptors (Liebeskind et al., 2015; Moroz et al., 2014; Moroz and Kohn, 2015; Ramos-Vicente et al., 2018). Although this might suggest that iGluRs represent an exception to the general model of synaptic proteome evolution, caution is required because the contribution made by glutamate receptors to synapse diversity in different species remains to be examined.

It is now clear that glutamate receptors arose before nervous systems, and that they also play a role in non-neuronal cell types in metazoans. This is consistent with the notion that their role in neuronal transmission, synaptic plasticity and learning is a specialized adaptation of their broader biological function. Future investigations on the evolution of other synaptic protein families could shed more light on the evolution of this structure. Focus might be put on proteins with a role in synaptic transmission regulation, in order to give strength or rule out the hypothesis that more elaborated nervous systems could correlate with an increased ability to fine tune synaptic function (Kenny and Dearden, 2013; Kung et al., 2001; Ramos-Vicente and Bayés, 2020; Thomas and Hayashi, 2013).

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