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SYMPOSIUM REVIEW

The dynamic AMPA receptor extracellular region: a platform for synaptic protein interactions

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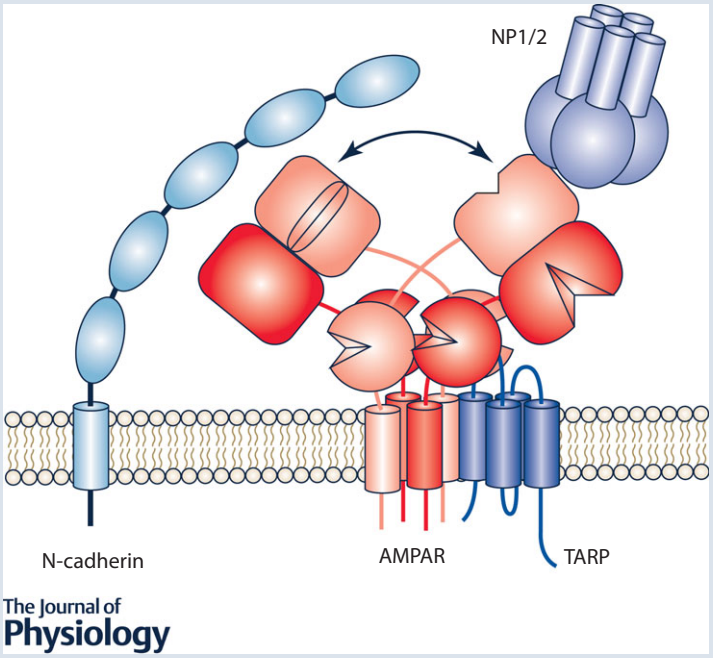
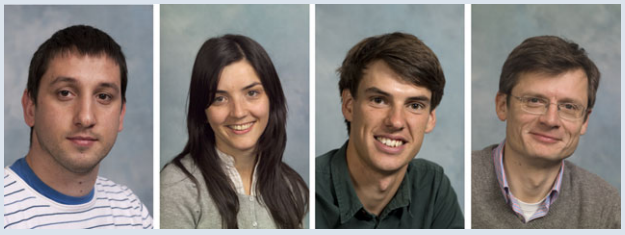


Abstract AMPA receptors (AMPARs) are glutamate-gated cation channels that mediate fast excitatory neurotransmission and synaptic plasticity. Structures of GluA2 homotetramers in distinct functional states, together with simulations, emphasise the loose architecture of the AMPAR extracellular region (ECR). The ECR encompasses ∼80% of the receptor, and consists of the membrane-distal N-terminal domain (NTD) and ligand-binding domain (LBD), which is fused to the ion channel domain. Minimal contacts within and between layers, together with flexible peptide linkers connecting these three domains give rise to an organisation capable of



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dynamic rearrangements. This building plan is uniquely suited to engage interaction partners in the crowded environment of synapses, permitting the formation of new binding sites and the loss of existing ones. ECR motions are thereby expected to impact signalling as well as synaptic anchorage and may thereby influence AMPAR clustering during synaptic plasticity.

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Abstract figure legend The dynamic AMPA receptor extracellular region (ECR). The flexible ECR of AMPARs (red schematic) is expected to permit rearrangements that may impact association with synaptic proteins, three known interaction partners are indicated in blue (N-cadherin, neuronal pentraxin [NP1/2], TARPs). Dynamic reconfigurations could impact AMPAR allosteric signaling as well as diffusion and clustering at synapses.

Abbreviations AMPARs, AMPA receptors; ANM, anisotropic network model; EC, extracellular cadherin (domain); ECR, extracellular region; EM, electron microscopy; iGluRs, ionotropic glutamate receptors; KARs, kainate receptors; LBD, ligand-binding domain; LL, lower lobe (NTD); N-cad, N-cadherin; NMDARs, *N*-methyl-D-aspartate receptors; NPs, neuronal pentraxins; NPR, NP receptor; NTD, N-terminal domain; LTP, long-term potentiation; PSD, postsynaptic density; PVs, parvalbumin-expressing interneurons; TARPs, transmembrane AMPAR regulatory proteins; TMD, transmembrane domain; UL, upper lobe (NTD).

Introduction

Ionotropic glutamate receptors (iGluRs) are cation channels embedded in the postsynaptic density (PSD) opposite presynaptic neurotransmitter release sites. Ion flux through the channel is triggered by the binding of L-glutamate, the major excitatory transmitter in the central nervous system. iGluRs play central roles in synapse development and synaptic plasticity (Traynelis *et al.* 2010); their dysfunction is associated with a variety of neuropathologies (Bowie, 2008). Three iGluR subfamilies, kainate receptors (KARs), *N*-methyl-D-aspartate receptors (NMDARs) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs), contribute different components to the postsynaptic response, whereas the δ receptors (GluDs) mediate synaptogenesis and synapse maintenance primarily in the cerebellum (Yuzaki, 2011). The rapid kinetics of AMPARs permit point-to-point signalling (Geiger *et al.* 1997; Trussell, 1999) and their accumulation at synapses is crucial for synaptic plasticity, which underlies learning (Kessels & Malinow, 2009).

AMPARs predominantly exist as hetero-tetramers, assembled from the GluA1–4 subunits in various stoichiometries (Hollmann & Heinemann, 1994). Assembly is controlled at various levels (Herguedas *et al.* 2013) and ultimately increases the functional repertoire, relative to homomeric receptors. The vast majority of AMPARs incorporate the functionally critical GluA2 subunit, which restricts Ca^{2+} permeability, relieves polyamine block and lowers channel conductance (Isaac *et al.* 2007). Subunit composition and association with various auxiliary subunits (Jackson & Nicoll, 2011) greatly diversifies AMPAR trafficking and signalling at synapses (Traynelis *et al.* 2010).

Like other iGluRs, AMPARs are composed of four domain layers (Fig. 1*A*): a cytoplasmic C-terminus that directs trafficking and synaptic anchorage of the receptor (Shepherd & Huganir, 2007), a transmembrane domain (TMD) forming the channel pore, and an extensive, bipartite extracellular region (ECR), consisting of the ligand-binding domain (LBD) and the membrane-distal N-terminal domain (NTD). The NTD comprises 50% of the receptor and, together with the C-terminus, is the most sequence-diverse region across the four AMPAR paralogues (Fig. 2*A*). The NTD has, so far, been implicated in subunit assembly and clustering at synapses (Sia *et al.* 2007; Kumar *et al.* 2011; Rossmann *et al.* 2011).

AMPARs transit between different functional states: (1) a resting, closed-channel state, (2) active open states, where agonist-binding triggers opening of the channel pore, and (3) desensitised states, where prolonged exposure of agonist induces a closed channel state with agonist still bound. Each subunit can bind glutamate independently and hence a multitude of subconductance states exist (Robert & Howe, 2003), facilitated by the loose architecture of the tetramer. Functional diversity combined with a modular building plan and the need to interact with a variety of auxiliary subunits (Jackson & Nicoll, 2011) renders the AMPA-type iGluR a versatile signalling machine. Here we review recent insights into AMPAR structure and ask how its uniquely flexible architecture will impact receptor operation in the crowded environment of the synaptic cleft.

Structure and organisation of the ECR

Structures of isolated ECR domains have provided insights into iGluR pharmacology and a framework for understanding receptor activation and desensitisation (Sun *et al.*

2002). The architecture of an AMPAR tetramer was first revealed by an antagonist-bound structure of a near-intact GluA2 homomer (Sobolevsky *et al.* 2009), exhibiting a modular architecture with an overall Y-shape, the arms of the Y formed by two NTD dimers (Fig. 1*A*). The structure revealed a symmetry mismatch with twofold symmetry in the LBD and NTD layers and a quasi fourfold symmetric TMD. Furthermore, the NTD and LBD layers engage in domain swapping, with different subunit pairs forming NTD and LBD dimers, a building plan conserved in NMDARs (reviewed in Karakas *et al.* 2015).

A series of recent X-ray crystallography and cryo-electron microscopy (EM) structures of iGluRs provided further insights into their organisation and operation (Chen *et al.* 2014; Durr *et al.* 2014; Karakas & Furukawa, 2014; Lee *et al.* 2014; Meyerson *et al.* 2014; Yelshanskaya *et al.* 2014). AMPAR GluA2 homomers were trapped in different functional states; in addition to the first, antagonist (ZK-200775)-bound, structure (PDB code 3KG2), ligand-free (resting) and agonist-bound (desensitised) states were obtained (reviewed in Karakas *et al.* 2015). These revealed state-dependent reorganisations in the LBD layer at multiple levels: the clamshell cleft, the dimer interface and between LBD dimers, in line with the dynamic nature of this layer discerned from functional experiments (Plested & Mayer, 2009).

In addition, the distal NTD tier showed striking differences between functional states and between iGluR subfamilies (Karakas *et al.* 2015). In both KARs and AMPARs, NTD dimers associate via a small ~330 Å^2 tetramer interface (Fig. 1*A*; green star) (Jin *et al.* 2009; Kumar *et al.* 2011), giving rise to the 'classic' receptor Y-shape (Sobolevsky *et al.* 2009). In KARs this NTD tetramer interface is tighter than in AMPARs: (i) it is evident in solution, in analytical ultracentrifugation experiments, and (ii) it is maintained upon entry into the desensitised state (Kumar *et al.* 2011; Schauder *et al.* 2013; Meyerson *et al.* 2014). By contrast, GluA2 NTD tetramers are labile (Clayton *et al.* 2009) and the tetrameric interface can rupture upon desensitisation (Durr *et al.* 2014; Meyerson *et al.* 2014). Agonist-mediated rearrangements of the NTD layer have been observed in both GluA2 cryo-EM and crystal structures and were seen in earlier studies of native (heteromeric) AMPARs assayed by negative stain EM (Nakagawa *et al.* 2005). Related to this, little (Sobolevsky *et al.* 2009) or no contact (Meyerson *et al.* 2014) between the NTD and LBD layers is apparent in KAR and AMPAR structures. In stark contrast, in NMDARs the NTD layer forms close associations with the LBD layer resulting in a more compact ECR (Karakas *et al.* 2011; Karakas & Furukawa, 2014; Lee *et al.* 2014). This inter-layer interface, in concert with the LBD–NTD linkers, facilitates allosteric communication triggered by NMDAR NTD ligands, which ultimately impact channel open probability (Gielen *et al.* 2009; Yuan *et al.* 2009). A recent GluA2/3 AMPAR heteromer structure, determined by cryo-EM, suggests that AMPARs can also adopt NMDAR-like conformations in their ECR (Herguedas *et al.* 2016).

Impact of the loose ECR organisation

Questions arise when considering the impact of the large, flexible ECR in a synaptic setting. This unique organisation, not evident in other ligand-gated ion

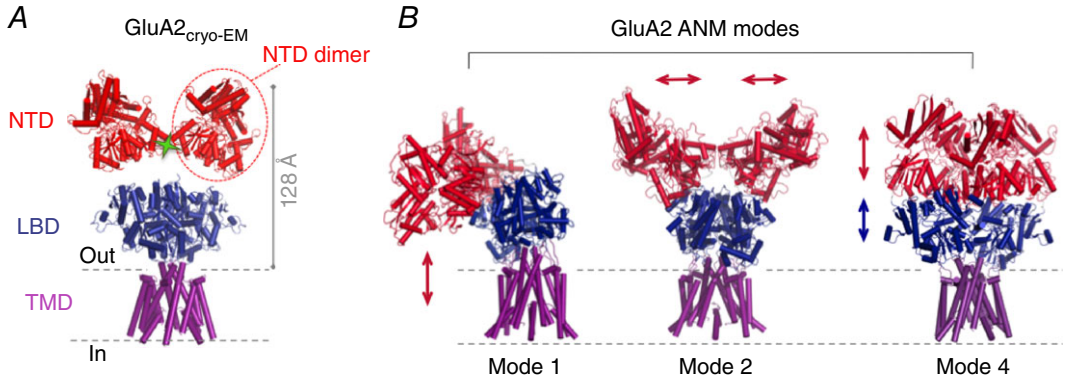

Figure 1. Structure and dynamics of the AMPAR extracellular region (ECR)
A, cryo-EM structure of GluA2 receptor in complex with antagonist ZK200775 (PDB code 4UQJ) with the three receptor layers coloured in red (NTD), blue (LBD) and purple (TMD). An NTD dimer is highlighted (ellipsoid), the NTD tetramer interface is denoted with a green star. *B*, ANM modes (low frequency) of the GluA2 crystal structure in complex with ZK200775 (PDB code 3KG2) showing the different conformation states of the ECR in the various modes (Dutta *et al.* 2015). In mode 1 the complete NTD layer bends down towards the membrane. In mode 2 the two NTD dimers separate and approach the upper leaflet of the membrane. In mode 4, the NTD and LBD layers adopt a compact conformation resembling the crystal structures of NMDARs.

channel families (Unwin, 2005; Kawate *et al.* 2009; Miller & Aricescu, 2014), is expected to permit substantial structural dynamics, which may enable transient interactions in the crowded environment of synapses. Associations with synaptic components could trigger reconfigurations of the AMPAR tetramer (Abstract figure) and ultimately impact receptor signalling, trafficking, diffusion and anchorage. In addition to the drastic rearrangements of the NTD layer upon desensitisation, flexibility within the ECR is emphasised by GluA2 crystal structures in complex with a snail toxin (Chen *et al.* 2014). The toxin wedges between the NTD and LBD, pushing the NTD layer up by ∼10 Å relative to the original antagonist-bound GluA2 (PDB 3KG2), a displacement readily accommodated by the peptide linkers connecting the NTD and LBD. Clearly a deeper understanding of NTD–LBD linker flexibility and conformational dynamics is of interest.

ECR dynamics are also emphasised in simulations of GluA2 (PDB 3KG2) using the anisotropic network model (ANM), which have revealed low-energy modes of motion that the receptor may sample (Dutta *et al.* 2015; Krieger *et al.* 2015). This model only considers Cα atoms, i.e. is 'coarse-grained' and thus enables calculation of global motions accessible to a given structure under equilibrium conditions. The first modes describe the energetically most favourable conformational changes (Bahar *et al.* 2010), thus offering a view onto the ensemble of sub-states encoded by the structure (examples of which are shown in Fig. 1*B*).

Here we summarise three ANM modes of GluA2 (PDB code 3KG2), which are illustrated in Fig. 1*B*. In mode 1, the entire AMPAR ECR bends down towards the upper leaflet of the lipid bilayer, where it may interact with AMPAR auxiliary subunits such as the TARPs (see below; Fig. 2). Similarly, mode 2 shows a downward bending and separation of the two NTD dimers, somewhat resembling desensitised conformations captured by crystallography and EM (Nakagawa *et al.* 2005; Durr *et al.* 2014; Meyerson *et al.* 2014). In mode 2 the two NTD dimers also approximate and could be crosslinked via an engineered disulfide bridge across the NTD tetramer interface (at GluA2 K262C), providing an experimental validation of the simulation data (Dutta *et al.* 2015). Interestingly, in mode 4 vertical stretching and compression of the ECR is seen. The compression results in close approximation between the NTD and LBD reminiscent of the NMDAR conformation (Karakas & Furukawa, 2014; Lee *et al.* 2014) (Fig. 1*B*). This motion suggests that an interface between the NTD and LBD layers can also form in AMPARs and permit allosteric regulation in this iGluR subtype, which appears unlikely in the loose, Y-shape arrangement (Fig. 1*A*). Indeed, a recent cryo-EM structure of an AMPAR GluA2/3 heteromer (captured in an apo-state) reveals interfaces between NTD and LBD layers, analogous to GluA2 mode 4 (Herguedas *et al.* 2016). As a consequence of these dynamics, platforms for protein interactions in the ECR are likely to be reconfigured, altering the binding of known associates (described below) and generating alternative binding sites. These reconfigurations may stabilise conformations and thereby impact signal transmission.

AMPAR-interacting proteins at synapses: TARPs and beyond

The NTD provides a substantial, sequence-diverse docking platform (Fig. 2*A*) stretching approximately midway into the synaptic cleft where it approaches the presynaptic machinery. The cleft of a hippocampal synapse has an estimated width of ∼24 nm, as estimated by cryo-EM (Zuber *et al.* 2005). The ECR of antagonist-bound GluA2 can reach a vertical height of up to 13 nm (in a recent cryo-EM structure; PDB 4UQJ; Meyerson *et al.* 2014).

AMPAR NTDs preferentially exists as heterodimers, forming tight (low nanomolar) assemblies (Rossmann *et al.* 2011) and generating a large surface area of ∼32,000 Å^2. Since sequence identity between the four AMPAR NTDs is only ∼56% (the LBD and TMD are ∼90% identical), different AMPAR heteromers could *selectively* engage synaptic components via the NTD, potentially resulting in subtype-specific diffusion and anchorage (discussed further below; Fig. 3).

AMPARs interact with a large number of accessory proteins (>30; Schwenk *et al.* 2012; Shanks *et al.* 2012), a unique feature among iGluRs (i.e. for KARs only Neto1 and Neto2 have been described to date; Zhang *et al.* 2009). Some act as auxiliary subunits that alter receptor signalling and trafficking (Jackson & Nicoll, 2011) while others, mostly cytosolic factors, route and scaffold the receptor at synapses (including the membrane-associated guanylate kinases (MAGUKs); Shepherd & Huganir, 2007). The function of the majority of AMPAR-interacting proteins is currently elusive.

AMPAR auxiliary subunits have a profound impact on multiple aspects of AMPAR signalling, including gating kinetics, ion permeation, voltage dependence (i.e. polyamine block) and receptor pharmacology (reviewed in Jackson & Nicoll, 2011). Native AMPARs most likely exist in complex with a variety of these subunits, which differ between different neuronal cell types and perhaps even between individual synapses. All auxiliary subunits characterised to date are transmembrane proteins and include: the tetraspanning TARPs (transmembrane AMPAR regulatory proteins) (Chen *et al.* 2000) and GSG1L (germ cell-specific gene 1-like) protein (Schwenk *et al.* 2012; Shanks *et al.* 2012), the three-transmembrane CNIHs (cornichon-2 and -3; Schwenk *et al.* 2009) and CKAMP (cysteine-knot AMPAR-modulating protein)-44,

-52, -39 and -59 (also known as Shisa-9, -6, -8, -7), single membrane-spanning factors (Schwenk *et al.* 2009; Farrow *et al.* 2015). Their interaction regions with the receptor are largely unknown.

Based on secondary structure prediction and on homology to mouse claudin-15, a TARP-related protein (Suzuki *et al.* 2014), the extracellular portion of TARPs is minimal (Fig. 2*B*) and they are therefore expected to engage the TMD and the membrane-proximal LBD. Recent studies started to identify the TARP-binding sites on GluA2 and AMPAR binding sites on TARPs and CNIH3 (Cais *et al.* 2014; Shanks *et al.* 2014). Unexpectedly, an interaction between TARPs (γ-2 and γ-8) and the NTD emerged from peptide array mapping and the binding of TARP γ-2 to GluA2 was reduced upon deletion of the NTD in immunoprecipitations, further supporting an interaction between TARPs and the membrane-distal NTD (Cais *et al.* 2014). NTD bending towards the lipid bilayer, as suggested by ANM simulations (modes 1 and 2; Fig. 1*B*) (Krieger *et al.* 2015), and by early single particle EM (Nakagawa *et al.* 2005), may facilitate the NTD–TARP interaction, most likely enabled by the NTD–LBD linkers (Fig. 3; right panel). Deletion of linker residues indeed altered the TARP modulation of AMPAR gating kinetics (Cais *et al.* 2014).

In addition to gating, the NTD–TARP contact could impact AMPAR sequestration at synapses. AMPARs are highly dynamic and constantly exchange between synaptic and extrasynaptic sites (Choquet & Triller, 2013). Long-term potentiation (LTP)-type stimuli that induce plasticity recruit additional AMPARs into synapses and result in their immobilisation (Newpher & Ehlers, 2008; Opazo & Choquet, 2011). Within the postsynaptic density (PSD) AMPARs are concentrated in ~70 nm-wide nanodomains of approximately 30 receptors/domain (Nair *et al.* 2013) (an AMPAR ECR has a sphere of ~12 nm diameter, which would be consistent with this estimate). Anchorage to the PSD can be mediated (i) via the receptor C-termini harbouring PDZ motifs (Barry & Ziff, 2002), and (ii) via TARPs, which directly bind to PSD-95 (Chen *et al.* 2000; Schnell *et al.* 2002), a prominent postsynaptic scaffolding factor (Bats *et al.* 2007). The latter appears to be crucial, as interfering with the TARP/PSD-95 linkage increases AMPAR mobility whereas deletion of the C-terminal PDZ motif (in GluA1 and GluA2) has little effect (Bats *et al.* 2007; Hoze *et al.* 2012; Kerr & Blanpied, 2012) and deletion of the entire C-terminal tail still permits LTP (Granger *et al.* 2013).

Receptor activation has been suggested to result in 'shedding of the TARP' and hence AMPAR release from

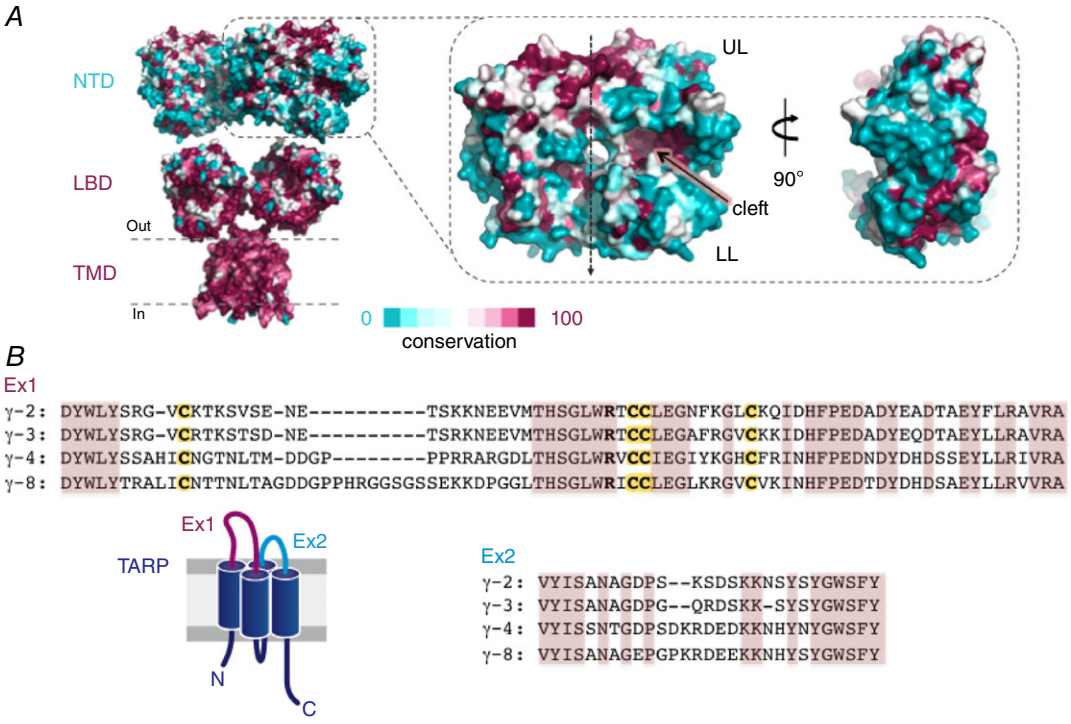

Figure 2. Sequence variations in AMPAR NTDs and the TARP ECR
A, surface representation of GluA2 homotetramer (PDB 4UQJ) coloured by sequence conservation among sub-units (left). While the TMD and LBD are highly sequence conserved (purple), the NTD contains great variability (cyan). This NTD sequence variability is shown in expanded format on the right. *B*, schematic and sequence alignment among the two TARP extracellular loops (Ex1, Ex2), highlighting their variability between family members.

the PSD-95–TARP anchorage (Tomita *et al.* 2004), in turn enabling rapid diffusion away from synaptic sites (Constals *et al.* 2015) and endocytosis. The NTD–TARP interaction would be of consequence as NTD sequence diversity together with variation in the TARP binding region between the TARP members (Fig. 2*A* and *B*) may selectively stabilise some AMPAR heteromers over others and thereby differentially affect their expression at synapses. Selective sequestration of AMPARs could also be mediated by other components associating with the ECR, as discussed below.

Other synaptic components interacting with the AMPAR ECR: N-cadherin and pentraxins

AMPAR ECR reconfigurations accompanying TARP binding may alter the binding of other NTD-targeting factors including N-cadherin (N-cad) and neuronal pentraxins (NPs) (Fig. 3; Abstract figure). N-cad, a homophilic, Ca^{2+}-dependent cell-adhesion molecule is associated with AMPARs through intracellular interactions, mediated by the N-cad-associated protein δ-catenin (NPRAP; neural plakophilin-related armadillo repeat protein) (Silverman *et al.* 2007; Heisler *et al.* 2014). NPRAP interacts with GRIP1/2 (glutamate receptor interacting protein), multi-PDZ scaffolding factors that in turn bind to AMPAR subunits GluA2 and GluA3 (Barry & Ziff, 2002). However, N-cad has also been shown to bind the GluA2 NTD directly (Saglietti *et al.* 2007) although the details of this interaction are elusive.

The N-cad extracellular region is composed of five extracellular cadherin (EC) domains, each forming a seven-stranded β-barrel (Harrison *et al.* 2011). When stabilised in an extended (crescent-shaped) conformation by Ca^{2+} ions, binding to the inter-EC linkers, N-cad would arch above the AMPAR (Fig. 3). Removal of Ca^{2+} shifts N-cad into a 'floppy' conformation that disfavours trans-synaptic, homophilic interactions but promotes associations with AMPARs (Nuriya & Huganir, 2006; Tai *et al.* 2008). The five ECs are not identical in sequence, but whether there is any specificity for binding the NTD is not known. Also, whether the AMPAR–N-cad interaction occurs in *cis* (Nuriya & Huganir, 2006; Morita *et al.* 2009) or in *trans* (Saglietti *et al.* 2007) needs further clarification. The flexible AMPAR ECR could 'scan' the membrane-proximal ECs of Ca^{2+}-bound, extended N-cad and may access all five ECRs of the Ca^{2+}-free, floppy N-cad conformer (Fig. 3). Initially postulated to bind the

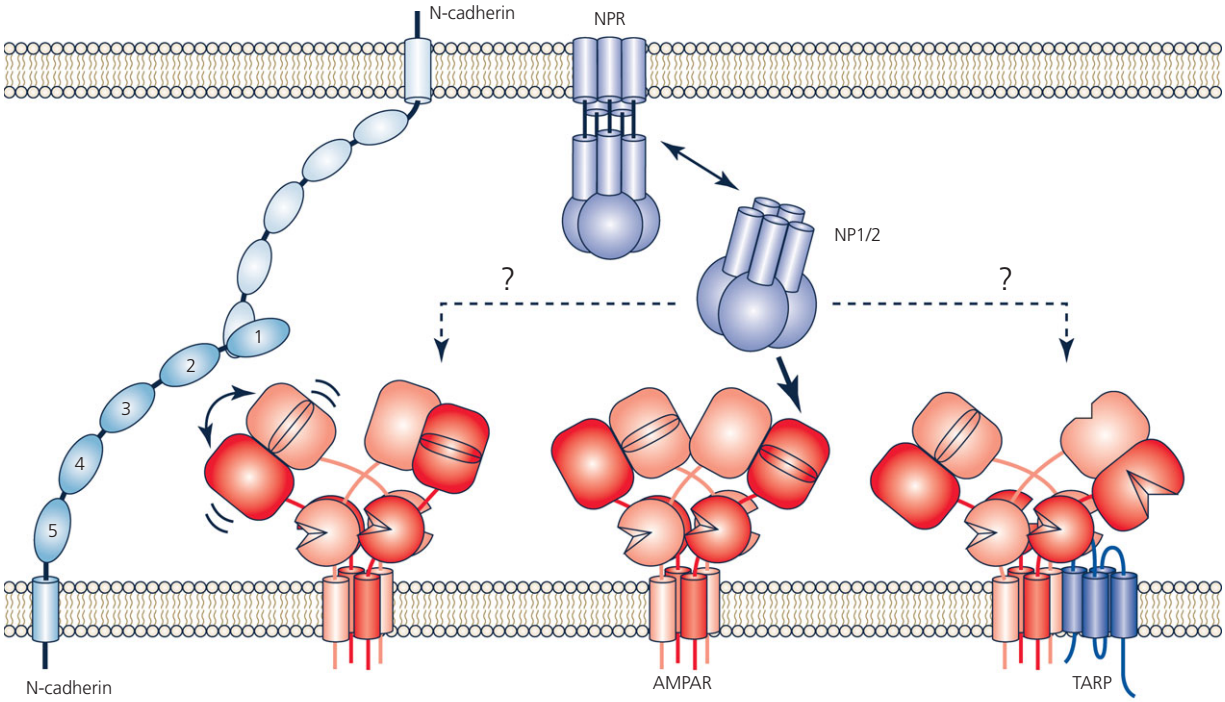

Figure 3. Possible interactions of the AMPAR ECR at synapses
The motions available to the AMPAR (red) allow interactions with a variety of synaptic partners from TARPs at membrane height (right) to possible trans-synaptic interactions with pentraxins (centre). Reconfiguration of the AMPAR-NTD would allow scanning for interaction partners at different levels, for example different EC domains of N-cadherin (left, numbered 1–5). Different configurations are likely to produce mutually exclusive interactions, conferring partner-specific consequences on AMPAR signalling and synaptic clustering in LTP.

NTD front face (Saglietti *et al.* 2007), a secondary N-cad binding site at the GluA2 NTD–LBD linker has recently been described. This requires a specific *N*-glycosylation signature on the linker and aids surface AMPAR trafficking and stabilisation (Takeuchi *et al.* 2015).

The proposed interaction between N-cad and GluA2 would stabilise GluA2-containing AMPARs at synapses and control the balance between synaptic anchorage and AMPAR endocytosis. This makes it a candidate for LTD (long-term depression) induction, as shown for mGluR-mediated LTD (Morita *et al.* 2009; Zhou *et al.* 2011; Mills *et al.* 2014). Modulation of N-cad expression levels or altering AMPAR binding due to the extracellular calcium concentration could directly influence AMPAR synaptic content and turnover. Concordantly the regulatory role of N-cad has been linked to multiple forms of plasticity (LTP: Tang *et al.* 1998; Bozdagi *et al.* 2000; Mills *et al.* 2014; structural: Mendez *et al.* 2010; LTD: Zhou *et al.* 2011; short-term plasticity (STP): Jungling *et al.* 2006). How AMPAR ECR dynamics impact N-cad binding, which EC domain(s) associate(s) with the NTD, and how this interaction is influenced by TARPs (Fig. 3; Abstract figure) and other NTD interactors remain open questions.

In addition to C-terminal scaffolding factors, NPs have been shown to induce postsynaptic AMPAR clustering via the NTD (O'Brien *et al.* 1999) and their impact must be considered in the light of AMPAR ECR reconfigurations at synapses (Fig. 3). NPs, a family of Ca^{2+}-dependent lectins, include the secreted NP1 and NP2, as well as the membrane-bound NP receptor (NPR). They harbour a C-terminal pentraxin domain, targeting the NTD and mediating AMPAR clustering, and an N-terminal coiled coil domain, allowing NP multimerisation, which underlies their clustering ability (O'Brien *et al.* 2002; Xu *et al.* 2003). NP1 and NP2 are secreted from glutamatergic terminals, directing AMPAR aggregation at postsynaptic sites somewhat analogous to cerebellins, secreted C1q-type proteins, which mediate GluD2 clustering at parallel fibre–Purkinje cell synapses in the cerebellum (Yuzaki, 2011). NPR is an integral membrane protein, which may facilitate a trans-synaptic interaction via secreted NPs and AMPARs (Sia *et al.* 2007; Pelkey *et al.* 2015). Whereas NP1 is constitutively expressed (Xu *et al.* 2003), NP2 is induced by neuronal activity (O'Brien *et al.* 1999), fine-tuning the synaptic response by regulating AMPAR density at synapses (Chang *et al.* 2010).

NP–NTD interactions have so far been characterised for GluA4 only (Sia *et al.* 2007), with GluA4-containing AMPARs requiring NP2 and NPR to be stabilised at postsynaptic shaft synapses in parvalbumin-expressing interneurons (PVs) (Chang *et al.* 2010; Pelkey *et al.* 2015). Despite this, there is evidence of NP interactions with all AMPAR subunits (O'Brien *et al.* 1999; Xu *et al.* 2003; Sia *et al.* 2007). The molecular details of NP interaction with AMPAR NTDs are currently unclear. This is of interest given the sequence diversity between AMPAR NTDs (Fig. 2*A*), hence the scope for stabilising *specific* AMPAR combinations and potentially permitting the fine control required for synaptic regulation. Significant questions also remain about the synapse specificity of this interaction. At present, pentraxins appear to play a predominant role at interneuronal synapses, being highly enriched at synapses formed between pyramidal cells and parvalbumin-positive interneurons (PVs) (Xu *et al.* 2003; Chang *et al.* 2010). NP2/NPR double knockout mice exhibit PV cell dysfunction affecting the entire hippocampal circuitry, without an apparent effect on Schaffer collateral–CA1 spine synapses (Pelkey *et al.* 2015). Whether AMPAR stability at shaft synapses also requires the TARP/PSD-95 scaffold is not known and raises questions about the combinatorial interaction possibilities of the AMPAR NTD.

Another group of secreted lectins, the galectins, have the potential to modulate AMPAR and KAR gating kinetics, in a subunit-selective manner. The eel galectin Cgn1 substantially slowed the desensitisation kinetics of recombinant, homomeric GluA4 and GluK2 KAR; similar, albeit more subtle effects were seen with mammalian galectins (Copits *et al.* 2014). The more substantial galectin modulation observed with KARs *versus* AMPARs may be explained by the fact that KARs are *N*-glycosylated more extensively and glycans are also present on the LBD; in AMPARs, *N*-glycans are restricted to the NTD and the NTD–LBD linker. The effect on desensitisation was dependent on the location and type of the glycan and was also observed in the presence of auxiliary subunits (TARP γ-2 in the case of GluA4 and Neto-1 for GluK2) (Copits *et al.* 2014). Lastly, AMPAR proteomics screens have unearthed other secreted factors including Noelins and brorins (Schwenk *et al.* 2012), which will interact with the LBD, the NTD or with both ECR domains simultaneously. Their direct impact on the receptor and their influence on the action of other ECR-targeting interactors is currently unknown.

Conclusion

The ECR, particularly in non-NMDARs, is unique in both its vertical extent (~13 nm in PDB 4UQJ) and its flexible organisation, an architecture dramatically deviating from other ligand-gated ion channels such as Cys-loop (Unwin, 2005; Miller & Aricescu, 2014) and P2X receptors (Kawate *et al.* 2009). In addition to a highly dynamic LBD layer, the interface between the NTD dimers ruptures specifically in AMPARs (Nakagawa *et al.* 2005; Durr *et al.* 2014; Meyerson *et al.* 2014) permitting substantial reconfiguration of the ECR. Here we postulate that this organisation will have consequences at synapses where AMPARs are surrounded by a large number of pre- and postsynaptic interaction

partners. Together with the sequence diversity of the NTD this setting would permit a multitude of potentially selective associations with consequences on signalling through the ion channel TMD and perhaps on retrograde ('outward') trans-synaptic signalling. Since AMPAR location at synapses is tightly controlled (Opazo & Choquet, 2011), the ECR may turn out to be a key player in receptor positioning, clustering and stabilisation in synaptic plasticity.

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Additional information

Competing interests

None declared.

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