SYMPOSIUM REVIEW

Building of AMPA-type glutamate receptors in the endoplasmic reticulum and its implication for excitatory neurotransmission

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Abstract AMPA-type glutamate receptors (AMPARs), the key elements of fast excitatory neurotransmission in the brain, are receptor ion channels whose core is assembled from pore-forming and three distinct types of auxiliary subunits. While it is well established that this assembly occurs in the endoplasmic reticulum (ER), it has remained largely enigmatic how this receptor-building happens. Here we review recent findings on the biogenesis of AMPARs in native neurons as a multistep production line that is defined and operated by distinct ER-resident helper proteins.

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and we discuss how impairment of these operators by mutations or targeted gene-inactivation leads to severe phenotypes in both humans and rodents. We suggest that the recent data on AMPAR biogenesis provide new insights into a process that is key to the formation and operation of excitatory synapses and their activity-dependent dynamics, as well as for the operation of the mammalian brain under normal and pathological conditions.

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Abstract figure legend Assembly of AMPARs occurs in the endoplasmic reticulum (ER). Recent work has unravelled this biogenesis as a multistep assembly line that is defined and operated by a distinct set of helper proteins and that controls the number and composition of AMPARs in excitatory synapses.

Introduction

AMPA-type glutamate receptors (AMPArs) are key to virtually any aspect of excitatory neurotransmission in the mammalian brain: they drive synapse formation, mediate the excitatory current required for the synaptic point-to-point response and endow synapses with activity-dependent dynamics that are fundamental for memory formation and learning (Silver et al. 1992; Bredt & Nicoll, 2003; Cull-Candy et al. 2006; Isaac et al. 2007; Collingridge et al. 2010). Most famous in this respect is the phenomenon of ‘long-term potentiation’ (LTP), an enhancement of synaptic signal transduction that results from activity-triggered recruitment of additional receptor channels into the postsynaptic membrane (Bliss & Collingridge, 1993; Shi et al. 1999; Kennedy et al. 2010; Granger et al. 2013; Huganir & Nicoll, 2013; Penn et al. 2017; Choquet, 2018).

The decisive, but easily forgotten, prerequisite for all AMPAR-mediated functions located at (distinct sites of) the plasma membrane is the building of the receptor channels in the intracellular membrane compartment(s) of the endoplasmic reticulum (ER; Fig. 1). There, functional AMPARs must be properly assembled from two types of subunits: the pore-forming GluA1–4 proteins and the members of three families of auxiliary proteins, the transmembrane AMPA-receptor regulating proteins (TARPs), the cornichon homologues (CNIHs) and the germ cell-specific gene 1-like (GSG1l) protein (Fig. 2 and Table 1; Chen et al. 2000; Tomita et al. 2005; Schwenk et al. 2012, 2014; Zhao et al. 2019). These auxiliary proteins, which all exhibit a four transmembrane (TM) domain architecture, bind to the GluA tetramers at two distinct pairs of binding sites (with full occupancy of these sites being the preferred appearance of AMPARs in the rodent brain) and together with the tetrameric pores build the core of the receptor channels (Schwenk et al. 2012; Twomey et al. 2017a; Herguedas et al. 2019; Nakagawa, 2019; Zhao et al. 2019). Functionally, the auxiliary subunits profoundly impact the aforementioned channel properties of the AMPAR assemblies, in particular ion permeation and gating kinetics, and they distinctly control the various processes behind the trafficking of the receptor complexes, as well as their stability/dwell-time at the plasma membrane and their subcellular localization (Table 1; Tomita et al. 2005; Milstein et al. 2007; Coombs et al. 2012; Shanks et al. 2012; Boudkakazi et al. 2014; Bowie, 2018; Choquet, 2018; for overview see recent reviews: Huganir & Nicoll, 2013; Greger et al. 2017; Choquet, 2018; Chen & Gouaux, 2019; von Engelhardt, 2019; Kamalova & Nakagawa, 2021).
Hetero-oligomeric AMPAR cores assembled from four GluAs and up to four auxiliary subunits, as observed in the rodent brain, have been successfully reconstituted in virtually any type of heterologous expression system without requirement of additional partner proteins. In this respect, it came as a surprise that comprehensive proteomic analyses of native AMPARs recently identified a set of proteins that assemble with the core subunits in the ER (Brechet et al. 2017), but are not part of the AMPARs at the cell surface where an additional set of proteins, mostly transmembrane or secreted proteins, interact with the core subunits (detailed in Table 1, ‘GluA-interactome’). Interestingly, these selective ER interactors, which all (previously) lacked annotations of primary functions or links to AMPARs in public databases, were found to be fundamental for the assembly of AMPAR complexes in native neurons and their deletion profoundly hampered excitatory synaptic transmission and its dynamics (Brechet et al. 2017). In addition, several recent genetic studies using whole-exome sequencing on patients suffering from severe intellectual disability unravelled protein FRRS1l, a key player among the ER-selective interactors of the receptor core, as the disease-causing gene, thus highlighting the importance of AMPAR biogenesis and the underlying molecular machinery in the ER (Madeo et al. 2016; Shaheen et al. 2016; Brechet et al. 2017). In light of these recent findings, we will review our current understanding of the building of AMPAR assemblies in the ER (Fig. 1) and discusses its significance for excitatory synaptic transmission in the mammalian brain and its implications for the formation of ion channel proteins and protein complexes in general.

**Biogenesis of native AMPARs**

The first definitive view on the appearance of GluA proteins in the ER of native tissue was provided by native gel-separations of ER-derived membrane fractions from the mammalian brain (with only minor ‘contaminations’ from other (intra)cellular membrane compartments): They demonstrated that (i) GluA1–4 proteins are part of several complexes with distinct molecular mass(es) and that (ii) these complexes represent co-assemblies of the GluAs with distinct interaction partners (Schwenk et al. 2019). Importantly, these interaction partners, predominantly proteins FRRS1l, carnitine palmitoyltransferase 1c (CPT1c), and α/β-hydrolase domain-containing protein 6 (ABHD6) (and in more rare cases porcupine (PORCN)) were exclusively found in ER-located GluA complexes, but were not detected in AMPARs at the plasma membrane (Brechet et al. 2017; Schwenk et al. 2019). And, while FRRS1l and CPT1c are predominantly found in the CNS, ABHD6 and PORCN exhibit a more ubiquitous expression pattern across cell-types and tissues.

Subsequent co-expression of GluAs with the identified ER-interactors in heterologous expression systems combined with native gel-electrophoresis and recordings of AMPAR-mediated currents finally identified the biogenesis of functional (core) AMPARs in the ER as a stepwise process reminiscent of an industrial ‘assembly line’ (Fig. 1, lower part; Schwenk et al. 2019). In this line the GluA1–4 proteins pass through discrete ‘production stages’ determined by the distinct ER-interactors and thereby assemble from monomers to tetramers. Biochemically, the assembly line should

![Figure 1. Biogenesis of AMPAR cores in the ER](image-url)

Schematic representation of the assembly line of AMPA core receptors as occurs in native ER membranes. The individual stages of the assembly line are numbered (1) to (5) and together represent an equilibrium reaction as detailed in the text. The depicted stages are: (1) ABHD6-associated GluA monomers; (2) formation of GluA dimers driven by co-assembly of bimolecular FRRS1l–CPT1c complexes; (3) dimer-of-dimer formation and dissociation of ABHD6; (4) binding of CNHs and TARPs and dissociation of FRRS1l–CPT1c complexes; and (5) initiation of ER export via induction of transport vesicles. Hetero-octameric AMPA core receptors are finally transported to synaptic and extrasynaptic sites of the plasma membrane.

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be envisaged as an equilibrium reaction with multiple intermediate states that are closely interlinked and thus influence each other’s occupancy.

At the first stage in this production line, the GluA monomer, after release from the translocon, is grasped by protein ABHD6, a single-span transmembrane protein with a short extracellular (or ER-luminal) and a large cytoplasmic domain. The resulting bi-molecular GluA–ABHD6 complexes (Fig. 1, state 1) are rather stable and have two important consequences for the GluA protein: first, it is effectively protected from ER-associated degradation (ERAD; Wu & Rapoport, 2018) and, second, it is locked in a monomeric state that is unable to form dimers with other GluAs (Schwenk et al. 2019). This is in contrast to heterologous (over)expressions (also in neurons and neuronal cultures), where effective dimerization between GluA proteins (and subsequent receptor assembly) occurs readily, most likely driven by high-affinity interaction(s) between both their TM domains (Kim et al. 2010; Gan et al. 2015) and their N-terminal domains (NTDs or ATDs; Ayalon & Stern-Bach, 2001; Rossmann et al. 2011; see also section below). And it is also different from the action of the two classical J-domain-containing chaperones (DNAJBs 12 and 14) that were recently reported to bind to the nascent chain(s) of some voltage-gated K+ channel subunits and, in cooperation with heat shock protein 70 (HSP70), promote their assembly into tetramers in the ER (Li et al. 2017). How ABHD6 stabilizes GluA monomers and/or inhibits their dimerization is currently unclear, but, due to the topology of ABHD6 (with most parts of the protein located cytoplasmically opposite to the NTDs), inhibition may originate from the interaction site(s) with the GluA protein in the membrane plane. In addition, this

Figure 2. Structural determinants of channel gating and ER export differ between auxiliary subunits of the receptor core

A, structure of the GluA tetramer (3KG2) indicating the distinct domains of the AMPAR pore subunit(s) in the membrane plane (channel pore) and on the extracellular (or ER-luminal) side of the membrane (NTD/ATD, N-terminal domain; LBD, ligand-binding domain). B, structures of CNIII-3 (PDB database entry: 6PEQ, in ribbon representation), TARP-8 (6QK) and GSG1l (5WEK) illustrate the distinct domains responsible for the distinct actions of the three types of auxiliary subunits on channel gating and initiation of ER-export. CNIII-3 may impact channel gating via the cytoplasmic extension formed by TMs 1 and 2 (shown in orange, N-terminal part of helical TM2 (in red) is unique to CNIII-3 and 3), and binding of Sec proteins should occur at the TM3,4-linker (in green) that is conserved among all four mammalian CNIII proteins. The claudin homologues TARP and GSG1l impact channel gating through their extensions on the extracellular side (orange), and initiation of ER-export may involve cytoplasmic domain(s) (in green).
Table 1. GluA1-4 interactome of the rodent brain. Summary of all constituents together with notions on the database entry (accession number of the UniProt database), membrane topology and current understanding of their function(s) related to AMPARs

| ID                  | Acc. No. | Name                                                               | Topology | Function/localization                      |
|---------------------|----------|--------------------------------------------------------------------|----------|-------------------------------------------|
| **AMPAR core, pore-forming subunits** | | | | |
| GluA1               | P23818   | AMPA-type glutamate receptor 1                                     | 3 TMs    | Receptor ion channel                      |
| GluA2               | P23819   | AMPA-type glutamate receptor 2                                     | 3 TMs    | Receptor ion channel                      |
| GluA3               | Q9ZW9    | AMPA-type glutamate receptor 3                                     | 3 TMs    | Receptor ion channel                      |
| GluA4               | Q9ZWW8   | AMPA-type glutamate receptor 4                                     | 3 TMs    | Receptor ion channel                      |
| **AMPAR core, auxiliary subunits** | | | | |
| TARP-2              | O88602   | Transmembrane AMPA-regulatory protein γ-2                         | 4 TMs    | Modulates trafficking and gating (Tomita et al. 2005; Tomita et al. 2003) |
| TARP-3              | Q9JJV5   | Transmembrane AMPA-regulatory protein γ-3                         | 4 TMs    | Modulates trafficking and gating (Tomita et al. 2003) |
| TARP-4              | Q9JJV4   | Transmembrane AMPA-regulatory protein γ-4                         | 4 TMs    | Modulates trafficking and gating (Tomita et al. 2003; Milstein et al. 2007) |
| TARP-5              | Q8VHW4   | Transmembrane AMPA-regulatory protein γ-5                         | 4 TMs    | Modulates trafficking and gating (Kato et al. 2008; Soto et al. 2009) |
| TARP-7              | P62956   | Transmembrane AMPA-regulatory protein γ-7                         | 4 TMs    | Modulates trafficking and gating (Kato et al. 2007) |
| TARP-8              | Q8VHW2   | Transmembrane AMPA-regulatory protein γ-8                         | 4 TMs    | Modulates trafficking and gating (Tomita et al. 2003; Fukaya et al. 2006; Kato et al. 2010) |
| CNIH-2              | Q35089   | Cornichon homolog 2                                               | 4 TMs    | ER exit, modulates gating (Kato et al. 2010; Boudkakki et al. 2014; Herring et al. 2013; Schwenk et al. 2019) |
| CNIH-3              | Q6ZWS4   | Cornichon homolog 3                                               | 4 TMs    | modulates trafficking and gating (Hawken et al. 2017; Nakagawa, 2019) |
| GSG1-like           | D3Z7H4   | Germ cell-specific gene 1-like                                     | 4 TMs    | Modulates gating (Schwenk et al. 2012; Shanks et al. 2012) |
| **ER-interactors, biogenesis of AMPAR cores** | | | | |
| FRRS1I              | B1AXV0   | Intellectual-disability-related protein FRRS1I                    | 1 TM/1GPI-anchor | Biogenesis of AMPARs (Schwenk et al. 2019) |
| CPT1c               | Q8BGD5   | Carnitine O-palmitoyltransferase 1, brain isoform                  | 2 TMs    | Biogenesis of AMPARs (Schwenk et al. 2019; Brechet et al. 2017; Fado et al. 2015) |
| ABHD6               | Q8R2Y0   | Monoacylglycerol lipase ABHD6                                     | 1 TM     | Biogenesis of AMPARs (Schwenk et al. 2019; Brechet et al. 2017) |
| ABHD12              | Q9LR1    | Lysophosphatidylserine lipase ABHD12                               | 1 TM     | Biogenesis of AMPARs (Schwenk et al. 2019; Brechet et al. 2017) |
| PORCN               | Q9JJ7    | Protein-serine O-palmitoleoyltransferase porcupine                | 8 TMs    | Biogenesis of AMPARs (Schwenk et al. 2019; Brechet et al. 2017; Erlenhardt et al. 2016) |
| SAC1                | Q9EP69   | Phosphatidylinositol phosphate phosphatase SAC1                    | 3 TMs    | Biogenesis of AMPARs (Schwenk et al. 2019; Brechet et al. 2017; Erlenhardt et al. 2016) |
| **AMPAR periphery, intracellular/transmembrane interactors** | | | | |
| PRRT1               | O35449   | Proline-rich transmembrane protein 1                               | 2 TMs    | Modulates gating, preferred localization of AMPARs to extrasynaptic sites (Kirk et al. 2016; Matt et al. 2018) |
| PRRT2               | E9PUL5   | Proline-rich transmembrane protein 2                               | 2 TMs    | Modulates gating (von Engelhardt et al. 2010) |
| CKAMP44             | Q9CZN4   | Cysteine-knot AMPAR modulating protein of 44 kDa                  | 1 TM     | Modulates gating (Klaassen et al. 2016) |
| CKAMP52             | Q3UH99   | Cysteine-knot AMPAR modulating protein of 52 kDa                  | 1 TM     | Synapse organizing protein (Siddiqui, et al. 2013) |
| LRRTM4              | Q80XG9   | Leucine-rich repeat transmembrane neuronal protein 4               | 1 TM     | Synapse organizing protein (Siddiqui, et al. 2013) |
| DLG1                | Q81100   | Disks large homologue 1, SAP97                                    | Cytoplasm | Component of the post-synaptic density |

(Continued)
intra-membranous interaction may also be the decisive feature behind the ABHD6-mediated protection from ERAD envisaged as a result of shielding the ‘hydrophilic pore-lining’ face(s) of the GluA protein from the lipid phase of the membrane environment (Deutsch, 2003; Schwappach, 2008). Interestingly, both shielding from ERAD and prevention of dimerization are also achieved by PORCN, a multi-span ER-membrane protein (Schwenk et al. 2019), which seems to replace or cooperate with ABHD6 in the rodent brain in some cases. And similar to with ABHD6, the enzymatic activity of the protein is not required for its role in the assembly line as has been verified by respective loss-of-function mutants (Schwenk et al. 2019). In contrast, enzymatic activities are fundamental for the processes that have been reported for either protein in the mammalian brain. Accordingly, ABHD6 serves as a regulator in endocannabinoid signalling via its hydro-lase activity (Blankman et al. 2007; Marrs et al. 2010; Lord et al. 2013), while PORCN controls Wnt signalling through palmitoylation of distinct Wnt isoforms in the ER lumen (Galli et al. 2007).

For successful receptor assembly, the stabilized GluA monomers must proceed to the second stage of the assembly line, which is promoted by binding of the dimeric FRRS1l–CPT1c complex to ABHD6–GluAs (Fig. 1, state 2). Both FRRS1l and CPT1c are integral membrane proteins with one and two TMs, respectively, that extend to distinct sides of the membrane (FRRS1l into the ER lumen, CPT1c into the cytoplasm) and combine for stable binding to the ABHD6–GluA monomers (via interactions with both N- and C-terminal domains of the GluA protein; Brechet et al. 2017). As a consequence of their binding, the inhibitory effect of ABHD6 is released, most likely via its partial (or complete) dissociation from the GluAs, and two GluA proteins are assembled into GluA–FRRS1l–CPT1c dimers. Subsequently, two of these dimers are co-assembled into ‘dimers-of-dimers’, thus forming GluA tetramers associated with up to four FRRS1l–CPT1c complexes as could be derived from their apparent molecular mass (Fig. 1, state 3). While dimer-of-dimer formation is a common theme in symmetric ion channels (Deutsch, 2003; Greger & Esteban, 2007; Schwappach, 2008; Isacoff et al. 2013), FRRS1l–CPT1c complexes may impact receptor assembly beyond the sheer catalysis of GluA tetramerization. In this sense, it is particularly tempting to speculate that FRRS1l–CPT1c complexes may induce the preferred positioning of distinct GluA subtypes in heteromeric channels as has been reported for GluA2 and its almost exclusive appearance in the BD position (versus the AC position) in recent structural work (Herguedas et al. 2019; Zhao et al. 2019). It is important to note, ABHD6 proteins presumably are no longer part of these GluA tetramers as they must dissociate from the pore-lining surface(s) of the GluAs in order to enable formation of the channel pore.

Similar to the monomeric GluA–ABHD6, FRRS1l–CPT1c-associated GluA tetramers appear rather stable and effectively prevent further processing of these intermediates to functional receptor channels that could be released from the ER at particular exit sites (ERES). This final step only occurs at stage 4 of the production line, when CNIs and members of the TARP family take their places at the two distinct pairs of binding sites on the GluA tetramers and thereby squeeze off the FRRS1l–CPT1c complexes (Fig. 1, state 4). Dissociation of FRRS1l–CPT1c and binding of CNIs and TARPs, finally leads to the hetero-oligomeric/hetero-octameric core-AMPARs that are fully functional and ready for

### Table 1. Continued

| ID     | Acc. No. | Name                                      | Topology         | Function/localization                                                                 |
|--------|----------|-------------------------------------------|------------------|---------------------------------------------------------------------------------------|
| DLG3   | P70175   | Disks large homologue 3, SAP102           | Cytoplasmic      | Component of the post-synaptic density                                                |
| DLG4   | Q62108   | Disks large homologue 4, PSD95            | Cytoplasmic      | Component of the post-synaptic density                                                |
| AMPAR  |          | periphery, extracellular interactors      |                  |                                                                                        |
| Noelin 1 | O88998  | Noelin 1, olfactomedin 1                  | Secreted tetramer| Secreted glycoprotein, may affect motility of AMPARs (Barembaum et al. 2000; Nakaya et al. 2017; Pronker et al. 2015) |
| Noelin 2 | Q8BM13  | Noelin 2, olfactomedin 2                  | Secreted         |                                                                                        |
| Noelin 3 | Q8BM13  | Noelin 3, olfactomedin 3                  | Secreted         | Regulated by synaptic activity, coordinate synaptic maturation/stabilization (Cantallops et al. 2000; Subramanian et al. 2019) |
| Neuritin | Q8CFV4  | Neuritin, CPG15                           | Secreted/ GPI-anchor |                                                                                        |
| Brorin  | Q8C8N3   | Brorin, von Willebrand factor C domain-containing protein 2 | Secreted         |                                                                                        |
| Brorin-like | Q505H4 | Brorin-like, von Willebrand factor C domain-containing protein 2-like | Secreted         |                                                                                        |

TM, transmembrane domain.
ER exit (Schwenk et al. 2019). The latter was shown in experiments that quantitatively monitored ER-exit of functional AMPARs by their appearance at the plasma membrane. And, interestingly, while both CNIH-2 and TARP-2 were able to initiate ER-export, the efficiency of CNIH-2 markedly exceeded that of TARP-2 (Fig. 1, state 5; Schwenk et al. 2019).

How AMPARs exit the ER at ERES, however, has not yet been sorted out. Nonetheless, it appears reasonable to assume that CNIHs 2/3 promote ER exit via formation of COPII vesicles initiated through interaction with the Sec23/24 protein(s) similar to what has been established for the ‘classical’ CNIH (‘cornichon’) protein(s) in flies and yeast (CNIHs 1 and 4 in mammals) (Roth et al. 1995; Bokel et al. 2006; Herzig et al. 2012; Adolf et al. 2019). The latter act as cargo-receptors that recognize their targets, membrane proteins and/or proteins to be secreted, and connect them to the Sec machinery. And in fact, the recently resolved structure of CNIH-3 co-assembled with tetrameric GluA2-receptor pores provided the first view for both cargo recognition and connectivity to COP II vesicles (Nakagawa, 2019; Kamalova & Nakagawa, 2021). Interaction with the GluA cargo is defined by the extended helical TM1 domain, while the connectivity to the Sec proteins should be represented by the cytoplasmic TM3–4 linker (Fig. 2, highlighted in green). This linker is well conserved among the CNIH family and represents the only domain of the protein that is neither buried in the membrane plane nor covered by the GluA protein(s) (Fig. 2; Nakagawa, 2019). Whether TARPs, predominantly subtypes 2 and 3, can act as cargo receptors similar to the CNIHs, or rather trigger ER-export through a distinct mechanism (or mechanisms) must currently remain unresolved. The structural data currently in hand leave both possibilities open, but suggest the long subtype-dependent C-termini (CTD) as the only protein domains suited for interactions with cytoplasmic transport factors (Fig. 2, highlighted in green).

Fundamentally different from classical cargo receptors, however, both CNIHs and TARPs do not dissociate from their target proteins upon ER-export, but remain associated with the GluA tetramers and travel to the plasma membrane as subunits of the AMPAR core (Fig. 1, upper part; Schwenk et al. 2009; Kato et al. 2010; Schwenk et al. 2012; Herring et al. 2013; Boudkazzi et al. 2014). As such, they largely determine and/or tune the channel properties of the AMPAR complexes in a subtype-specific manner, and also impact their dynamics and subcellular localization. Structurally, the specificity in gating is determined by distinct domains identified in the various high-resolution structures (highlighted in Fig. 2): TARPs and GSG11 influence channel gating via interaction of their extracellular loops with the ligand-binding domain of the GluA subunits (Dawe et al. 2016; Twomey et al. 2016, 2017b, 2019), while CNIHs 2 and 3 most likely act via their helical TM1 and 2 domains on the selectivity filter region located at the cytoplasmic opening of the channel pore (Hawken et al. 2017; Nakagawa, 2019; Schwenk & Fakler, 2019; Fig. 2, gating determinants in orange).

In contrast to TARPs and CNIHs, FRRS1l–CPT1c complexes do not leave the ER after dissociation from the GluA assemblies, CPT1c because it is a ‘bona fide ER protein' (different from the well-known mitochondrial lipid-transferases CPT1a and CPT1b), and FRRS1l because it is captured in the ER through binding to CPT1c (Casals et al. 2016; Brechet et al. 2017). A portion of the FRRS1l pool which is not bound to CPT1c, however, is subjected to processing by the ER-resident GPI-transamidase machinery (Brechet et al. 2017). This enzyme replaces the transmembrane domain of FRRS1l by a lipid anchor and sends the protein to the plasma membrane via the secretory pathway (Schwenk et al. 2019). Whether GPI anchoring is linked to AMPAR biogenesis or occurs independently and whether the GPI-anchored FRRS1l also targets AMPARs or has AMPAR-independent function(s) at the plasma membrane is currently unknown.

Finally, the AMPAR-containing transport vesicles fuse with the plasma membrane, most likely at both synaptic and extrasynaptic sites, and thus get the core AMPARs ready for signal transduction and assembly with additional partner proteins. Interestingly, these partners apparently interact with the AMPAR cores only at the plasma membrane, but are generated and transported to the surface independently of the receptors (summarized GluA interactome in Table 1). For some of these surface interactors, first data related to their cell physiological significance are already in hand (and have been reviewed/reported elsewhere; Haering et al. 2014; Greger et al. 2017; Matt et al. 2018; Bissen et al. 2019; Chen & Gouaux, 2019; von Engelhardt, 2019; see also Table 1); for others, further investigations are necessary.

Impairment and disturbance of receptor assembly

The assembly of functional AMPARs in the ER represents an equilibrium reaction in which several states (1–5, Fig. 1) are closely interlinked and thereby influence the occupancy of, as well as the transition between, each other. Consequently, alterations of the participating proteins are expected to impact the receptor building and, as a result, the molecular appearance of all AMPAR assemblies reflected by the GluA1–4 interactome (Schwenk et al. 2012; Table 1).

As yet, a limited number of such ‘directed alterations in expression’ of assembly line determinants have been performed and experimentally monitored. These are, in particular, (i) overexpression of ABHD6, (ii)
loss-of-function mutations or knock-out, as well as overexpression of FRRS1l, and finally, (iii) knock-out of the two CNIH proteins 2 and 3. In the following, we will briefly review the observed consequences of these alterations for receptor physiology and brain function and provide mechanistic insights derived from AMPAR biogenesis (or receptor assembly).

Overexpression of ABHD6 performed in heterologous expression systems (culture cells, *Xenopus* oocytes) and in neurons of the rodent brain essentially led to the consistent observation of a pronounced decrease or a complete vanishing of AMPARs at or from the surface membrane (Wei et al. 2017; Schwenk et al. 2019); in hippocampal neurons, excitatory synaptic currents (EPSCs) were largely reduced as a result of the AMPAR decrease in synapses (Wei et al. 2016, 2017). In light of the assembly line, these observations may be considered an immediate consequence of state 1 stabilization induced by the excess of free ABHD6 proteins that impairs the binding of FRRS1l–CPT1c and thus reduces progression of GluAs along the assembly line. In fact, such increased occupancy of state 1 could be directly visualized by native gel-electrophoresis and showed similar efficiency for all four GluA proteins (Schwenk et al. 2019).

For FRRS1l, a series of loss-of-function mutations have been identified in humans, most of them inducing frameshifts that prevent synthesis of a stable protein, thus leading to a loss or knock-out of the protein (Madeo et al. 2016; Shaheen et al. 2016; Brechet et al. 2017); bona fide knock-out and/or knock-down of FRRS1l has recently become available in rodents and enabled detailed investigation of the respective phenotype(s) (Schwenk et al. 2019; Stewart et al. 2019). In humans, FRRS1l mutations cause a fatal disease phenotype known as 'severe form of intellectual disability' with marked cognitive impairment, strongly restricted speech development, seizures, muscular hypotonia and neuro-regression (finally leading to death) (Madeo et al. 2016; Shaheen et al. 2016; Brechet et al. 2017). Knock-out mice recapitulate several aspects of this disease phenotype, including severe deficits in learning or goal-oriented behaviour (Schwenk et al. 2019; Stewart et al. 2019). Moreover, these animals showed several knock-out-induced alterations on the molecular and cellular level (summarized in Fig. 3): (i) the total amount of GluA1–4 protein(s) in all membranes of the whole brain was reduced to ~50% (of wild-type), without any obvious changes in transcription indicated by the unaltered amounts of the respective mRNAs; (ii) binding of CPT1c to GluA proteins was largely abolished; (iii) the amounts of ABHDs 6, 12 and PORCN bound to GluAs were increased by several-fold; and (iv) the majority of interactors found in AMPAR complexes at the surface (Table 1) were decreased by 30–80% (with the exception of TARP-2) (Schwenk et al. 2019), thus leading to major alterations in the subunit composition of the surface AMPARs. All these observations can be directly derived from the assembly line. First, in the absence of FRRS1l, binding of CPT1c to GluA is lost. Second, impaired dimerization (and tetramer formation) will shift the equilibrium towards state 1, thus leading to the observed increase in GluAs associated with ABHDs 6, 12 and PORCN. Third, after exhausting the pool of available ABHDs and PORCN, newly synthesized, non-protected, GluA proteins will be readily degraded via ERAD (and maybe additional elements of the cellular quality control system), thus resulting in strongly reduced overall amounts of GluAs (Fig. 3A). And fourth, assembly of hetero-octameric AMPARs is strongly impaired, reducing the amount of receptors delivered to the plasma membrane and able to form complexes with the surface constituents (Fig. 3B).

Further investigation of FRRS1l knock-out mice revealed additional changes that may be considered indirect consequences of the altered AMPAR assembly in the ER and the concomitant changes in subunit composition of the receptor complexes (Schwenk et al. 2019). Thus, the remaining surface AMPARs displayed profound asymmetry in their distribution with strong preference for localization to synapses versus extrasynaptic sites (Fig. 3B), EPSCs were decreased by ~50%, activity-dependent recruitment of AMPARs to synapses, the process underlying long-term potentiation (LTP), was entirely abolished (independent of its initiation by pulse-pairing or tetanic stimulation; Fig. 3C), and formation and maturation of synapses, as well as dendritic arborization were severely disturbed (number of immature synapses increased by almost 10-fold; Fig. 3B). But, as profound as these alterations may be, they were all successfully reversed by virus-driven re-expression of the FRRS1l protein (Schwenk et al. 2019). Interestingly, though, virally induced re-expression not only restored normal AMPAR-function and dynamics, but also showed ‘over-compensation’ in all aspects including LTP, thus indicating that AMPAR physiology can be effectively controlled by the assembly line in the ER.

Finally, genetic deletion of the two CNIHs, the most effective drivers of AMPAR export from the ER, revealed a phenotype that partially overlapped with that of the FRRS1l knockout. Thus, investigations in CA1 pyramidal cells indicated strongly decreased EPSCs recorded upon electrical stimulation as a consequence of the reduced number of AMPARs in the Schaffer collateral–CA1 pyramidal cell synapses (Herring et al. 2013). Of course, the reduced number of AMPARs at the synapses may well result from the reduced ER-export expected upon deletion of the CNIH proteins. Whether the reported changes in EPSC kinetics reflect a preference of the CNIHs for selected GluA subtypes as suggested (Herring et al. 2013) needs more detailed investigations by quantitative proteomic analyses although published proteomic data...
have not provided any evidence for such subtype preferences of CNIHs (Schwenk et al. 2012; Boudkkazi et al. 2014; Schwenk et al. 2019).

**Assembly of exogenously expressed AMPARs**

Effective assembly of functional AMPARs in the rodent brain requires the concerted action of several biogenesis factors that operate in a consecutive manner and promote protection of GluA monomers, formation of GluA dimers/tetramers and their export from the ER. Disturbance of these actions as induced by removal of FRRS1l leads to prominent degradation of the GluA proteins together with decreased receptor building and surface delivery despite unaltered amounts of mRNAs coding for GluA1–4 and other interactome constituents (Fig. 3; Schwenk et al. 2019). In contrast to this ‘native assembly’, building of functional AMPARs following exogenous expression in neurons and heterologous expression systems (cultured cells, *Xenopus* oocytes) does not require any additional factor(s) prompting the questions of how AMPAR assembly can occur under these conditions and of why evolution established a complex ‘production line’ for AMPARs given that their assembly can also occur spontaneously.

As the key to the first question, highly effective (over)expression of GluA proteins driven by the exogenous cDNAs/cRNAs should be considered that, in the light of the assembly line, exerts several synergistic effects. First, high amounts of GluA protein(s) saturate the protein degradation system(s) and thus render protection by ABHD6 dispensable. Second, the binding capacity of endogenous ABHD6 (present in cultured cells and neurons, but not in *Xenopus* oocytes) is overwhelmed,
which will leave a significant portion of newly synthesized GluA protein in an ABHD-free state ready to form dimers and subsequently dimers-of-dimers driven by several structural determinants including the NTD/ATDs, the LBDs and the transmembrane domains (Ayalon & Stern-Bach, 2001; Kim et al. 2010; Rossmann et al. 2011; Gan et al. 2015). Such ‘spontaneous dimer-formation’ could also be induced with native GluA–ABHD6 complexes after detergent-induced dissociation of ABHD6 (Schwenk et al. 2019). Third, accumulation of GluA tetramers will ‘enforce’ export from the ER using pathway(s) independent of CNHIs and/or TARPs (‘early vs late secretory trafficking’; Tomita et al. 2003, 2005; Schwenk et al. 2009; Harmel et al. 2012) and thus finally traffic to the plasma membrane. In summary, exogenous (over)-expression is able to generate functional receptors at large amounts by effectively bypassing the cellular system(s) of ‘quality control’.

It must be emphasized, though, that addition of the biogenesis factors ABHD6, FRRS1I/CPT1c and CNHIs/TARPs to the exogenously expressed GluA proteins successfully reinstated native quality control in heterologous expression systems and closely recapitulated receptor assembly as detailed above for the mammalian brain (Schwenk et al. 2019). Interestingly, under these conditions some perfectly reproducible observations obtained with sole GluA (over)expression were less prominent. Thus, receptor assembly and ER-export appeared only modestly affected by the editing events at the Q/R site (in the pore loop (Greger et al. 2002, 2003, 2007) or the R/G-site (LBD; Greger & Esteban, 2007; Penn & Greger, 2009)), and, similarly, alternate splicing (flip/flop versions; Penn et al. 2008) failed to prominently impact the biogenesis processes (unpublished results). The mechanistic details behind these differences are currently unknown and their molecular resolution will certainly require a series of combined structural and functional analyses.

The answer to the question on evolutionary reasoning of a multi-state assembly line is less obvious. But, given its effectiveness in controlling production and surface delivery of core AMPARs, we may speculate about a ‘post-transcriptional regulation mechanism’ that secures operation of excitatory neurotransmission on a safe level. Accordingly, the assembly line may counterbalance both increased and decreased levels of GluA synthesis (based on transcriptional and/or translational activities). Thus, at high GluA levels, the line dampens receptor generation by ‘trapping’ GluAs via binding to free pools of ABHD6, PORCN and FRRS1I/CPT1c, while at low availability of GluAs, the line offers compensation by protecting newly synthesized GluAs from ERAD (which appears rather effective in neurons compared to cell lines). Furthermore, the assembly line may regulate the subunit composition and arrangement of individual GluA sub-units within heteromeric GluA tetramers, and may thereby control the number and the subtype of auxiliary proteins that co-assemble with the GluAs into hetero-oligomeric AMPARs (Lu et al. 2009; Kim et al. 2010; Schwenk et al. 2012; Hastie et al. 2013; Schwenk et al. 2014; Zhao et al. 2019). In this sense, the assembly line can impact the ER-export of the core AMPARs and, thus control the number of the core receptors to be inserted into the plasma membrane under homeostatic and activity-dependent conditions. Moreover, the assembly line exerts control over the assembly of the AMPAR cores with the large set of surface subunits/interactors that form the receptor periphery (Table 1) and that are known to differ between brain regions and/or types of neurons (von Engelhardt et al. 2010; Schwenk et al. 2014; Matt et al. 2018). And finally, the biogenesis factors may be expected to trigger post-translational modification(s) of the GluA subunits and GluA assemblies in the ER that could impact their subsequent processing and/or their ability to interact with the peripheral subunits at distinct subcellular sites of the plasma membrane.

Outlook and future avenues

The assembly of AMPARs in a production line with distinct stages represents the first example that shows how a functional (receptor) ion channel is put together in the ER membrane(s) of a ‘native tissue’ (in contrast to heterologous expression systems). It highlights how profoundly biogenesis in the ER can impact the cell physiology of the receptors at the plasma membrane. The individual stages of the AMPAR production line are determined by proteins that were originally identified by unbiased GluA-targeting high-resolution proteomics and that surprisingly showed complete specificity for GluA proteins (or AMPARs), implying that they do not interact with other ion channels or other ion-handling membrane proteins in the mammalian brain.

According to these distinct aspects, the AMPAR assembly line may be expected to impact future research on (receptor) ion channel biology and their cellular/systemic function(s) in one or more of several directions as follows.

General principle for building membrane protein complexes. Despite the specificity of its key players for GluAs and AMPARs, the assembly line finally provided solutions to long-standing issues in ion channel assembly including protection of newly synthesized pore-forming subunits from ERAD or formation of dimers (and dimers-of-dimers). In this sense, the AMPAR assembly line may represent a blueprint or general procedure for the building of ion channels in native ER membranes. Of course, respective verification requires much more investigations of assembly processes in the ER membranes.
of cells in native tissues. Ideally, such studies should start out from unbiased analyses using quantitative proteomic technologies in combination with native gel separations as first approaches for identification of distinct assembly complexes of given target protein(s) and their respective subunit composition and interaction partners.

**New opportunities for research on AMPARs and AMPAR physiology.** With respect to AMPARs, the assembly line described above opened a new window for both the analysis of the biogenesis of the receptors and studying their role in neuronal signalling transduction, its dynamics and its impact on higher brain function(s). For biogenesis, the assembly line and its determinants provide a molecular framework whose detailed operation should now be unravelled by structural analyses, detailed biochemical investigations and monitoring of protein dynamics applying cryo-EM, proteomics and high-resolution fluorescence and electron microscopy. For investigating AMPAR physiology, the assembly line offers targets for defined manipulation of receptor building and thus for switching on/off synapse maturation, local receptor synthesis and activity-dependent synaptic plasticity. The latter may even be of therapeutic relevance by interfering with the impairments/restrictions enforced via neuro(degenerative) diseases.

**Novel concept for regulation of excitatory synaptic transmission.** As briefly introduced above, the ER assembly line(s) may establish a novel (posttranslational) level or mechanism for the control of synaptic signal transduction beyond the presumed control by transcription of the participating genes. Based on currently available data, the biogenesis line effectively shapes the number and subunit composition of core AMPARs present in the postsynaptic membrane and at extrasynaptic sites under homeostatic conditions, as well as in the reserve pool(s) providing the additional AMPARs that are inserted into the post-synapse upon (increased) synaptic activity. Consequently, biogenesis represents an effective means to determine both the time course of synaptic transmission and its (activity-dependent) dynamics with high accuracy. Furthermore, it appears possible that the assembly line itself is not static, but may rather change during postnatal development or be altered in response to cellular activity or potential feedback loops. In any case, the phenotype observed in humans and rodents upon disturbance of the AMPAR assembly highlights the fundamental importance of the ER-based biogenesis machinery for synaptic signalling and higher brain function(s).

To what extent these outlooks will be corroborated experimentally and in fact impact future research is hard to say, but as of now, the assembly line opens a new field and poses many questions whose answering will require quite a bit of experimental work to be completed.

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