Cell PRESS

Neuron
Review

The Expanding Social Network of Ionotropic Glutamate Receptors: TARPs and Other Transmembrane Auxiliary Subunits

Alexander C. Jackson[1,*] and Roger A. Nicoll[1,2,*]
[1]Department of Cellular and Molecular Pharmacology
[2]Department of Physiology
University of California, San Francisco, San Francisco, CA 94143, USA
*Correspondence: ajackson@cmp.ucsf.edu (A.C.J.), nicoll@cmp.ucsf.edu (R.A.N.)
DOI 10.1016/j.neuron.2011.04.007

Ionotropic glutamate receptors (iGluRs) underlie rapid, excitatory synaptic signaling throughout the CNS. After years of intense research, our picture of iGluRs has evolved from them being companionless in the post-synaptic membrane to them being the hub of dynamic supramolecular signaling complexes, interacting with an ever-expanding litany of other proteins that regulate their trafficking, scaffolding, stability, signaling, and turnover. In particular, the discovery that transmembrane AMPA receptor regulatory proteins (TARPs) are AMPA receptor auxiliary subunits that are critical determinants of their trafficking, gating, and pharmacology has changed the way we think about iGluR function. Recently, a number of novel transmembrane proteins have been uncovered that may also serve as iGluR auxiliary proteins. Here we review pivotal developments in our understanding of the role of TARPs in AMPA receptor trafficking and gating, and provide an overview of how newly discovered transmembrane proteins expand our view of iGluR function in the CNS.

Introduction

The control of neuronal excitability is accomplished through the finely tuned spatial and temporal regulation of ion flow across cell membranes. Two broad classes of ion channels are critical determinants of membrane excitability in neurons: voltage-gated and ligand-gated channels. Many if not all voltage-gated channels are associated with smaller auxiliary β subunits, which can affect where, when, and how the channel gets activated. These β subunits are often stable components of the channel complex and can affect every aspect of ion channel biology, including forward trafficking through the ER, surface delivery, targeting to specific subcellular compartments, and gating kinetics (Arikkath and Campbell 2003; Vacher et al., 2008; Dai et al., 2009; Pongs and Schwarz, 2010). Ligand-gated ion channels, or ionotropic receptors, include gamma amino-butyric acid ($GABA_A$) receptors, nicotinic acetylcholine receptors (nAChRs), and a variety of glutamate receptor subtypes, including α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), N-methyl-D-aspartic acid (NMDA), and kainate (KA) receptor subtypes. Until recently, these ligand-gated channels were thought to differ fundamentally from voltage-gated channels because, despite considerable effort, no transmembrane auxiliary subunits had been identified. This picture changed with the discovery that the small transmembrane protein stargazin or γ-2 (Letts et al., 1998) is critical for the functional expression of AMPA receptors (AMPARs) in cerebellar granule neurons (CGNs) (Chen et al., 1999, 2000; Hashimoto et al., 1999). Stargazin has since been shown to be the founding member of a family of transmembrane AMPAR regulatory proteins (TARPs) (Tomita et al., 2003), which exhibit remarkable phylogenetic conservation (Wang et al., 2008). The discovery of TARPs provided a missing link in relating the behavior of AMPARs expressed alone in heterologous systems to that in native neurons, and has inspired the search for other transmembrane auxiliary subunits. Recent database mining and various proteomic and screening methods have unearthed several unrelated transmembrane proteins that may also serve as auxiliary AMPAR subunits. Furthermore, emerging work indicates that KA receptors (KARs), and perhaps also NMDA receptors (NMDARs), are similarly regulated by transmembrane auxiliary subunits. Thus ligand-gated ion channels may now rival voltage-gated ion channels with regard to the extraordinary diversity and richness that auxiliary subunits impart to their function.

Before going any further, it is worth asking how we define a bona fide transmembrane auxiliary subunit. Is any transmembrane protein that interacts with iGluRs an auxiliary subunit? What about a protein that interacts with iGluRs as a chaperone during the early stages of biogenesis, but plays no role in the function of the mature protein? Our working definition of an iGluR transmembrane auxiliary subunit is that it avidly and selectively binds to mature iGluRs as part of a stable complex at the cell surface, that it can modulate the functional characteristics of iGluRs, and that it may also mediate surface trafficking and/or targeting to specific subcellular compartments, such as synapses. The rapidly expanding host of candidate iGluR transmembrane auxiliary subunits raises fascinating questions about the broad role of auxiliary subunits in ion channel function, and specifically about the biology of iGluRs. For example, why are there so many TARP family members with largely redundant roles in trafficking and gating? How do the TARPs interact with newly discovered transmembrane proteins—do they play unique roles within supramolecular complexes or are they involved in different phases of the lifecycle of iGluRs? In what way do these often structurally unrelated transmembrane proteins display

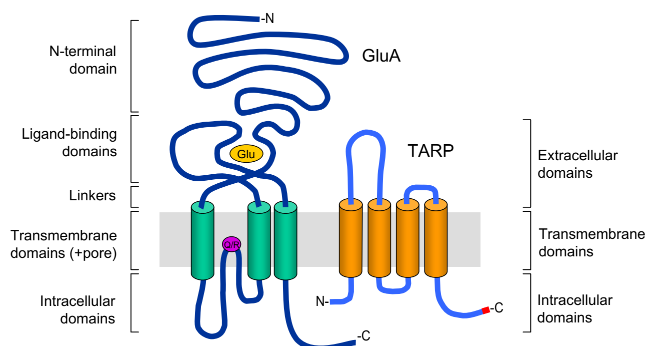

Figure 1. Major Structural Domains of AMPARs and TARPs
Illustration of the structural features of a closely apposed individual GluA subunit (left) and a canonical TARP auxiliary subunit (right). The GluA subunit of a tetrameric AMPAR is composed of a large extracellular N-terminal domain (NTD), the ligand-binding core, transmembrane domains, linker regions, and several intracellular domains including the C-terminal tail (CTD). Agonists such as glutamate (yellow) bind within the ligand-binding core to mediate channel opening. The Q/R site (magenta) is the narrowest constriction of the AMPAR pore and is an important determinant of its functional properties. The TARP auxiliary subunit (right) consists of four transmembrane domains with a large extracellular loop that is essential for TARP modulation of AMPAR gating. The tip of the TARP CTD contains a PDZ binding motif (red), which is known to bind to PDZ domain-containing proteins such as PSD-95, and which is essential for the synaptic targeting of AMPARs.

similar effects on iGluR trafficking and gating? With an eye to some of these broader questions, this review will summarize key developments in our understanding of the TARP family before moving on to a discussion of recent work on TARPs and the ever-growing list of other AMPAR, NMDAR, and KAR transmembrane auxiliary subunits. Interested readers are also directed to several excellent reviews on the *stargazer* mouse (Letts, 2005; Osten and Stern-Bach, 2006) and TARP modulation of AMPAR trafficking and gating (Nicoll et al., 2006; Sager et al., 2009a; Payne, 2008; Coombs and Cull-Candy, 2009; Milstein and Nicoll, 2008; Kato et al., 2010; Tomita, 2010; Díaz, 2010b).

The iGluR Family and Its Regulation by Intracellular Protein-Protein Interactions

Fast excitatory neurotransmission in the CNS is primarily mediated by three classes of tetrameric iGluRs: AMPARs (GluA1–4), NMDARs (GluN1, GluN2A–D, GluN3A–B), and KARs (GluK1–5), along with a fourth, less well-characterized, class, the δ receptors (GluD1–2) (Collingridge et al., 2009). Sequence homology between and within classes suggests that the general architecture of iGluRs is modular and shares several common features (Figure 1). Aside from sequence and structural differences, iGluRs are distinguished by their differential pharmacology, unique activation, deactivation and desensitization kinetics, selective permeability, single-channel properties, and the unique roles they play in different forms of both neuronal and glial signaling (Wollmuth and Sobolevsky, 2004; Mayer, 2005; Traynelis et al., 2010). To a large extent, iGluRs determine the shape of synaptic currents at glutamatergic synapses. For AMPARs, the kinetics of deactivation and desensitization, in addition to other factors including subunit composition, RNA editing, and alternative splicing, are key regulators of the amplitude and kinetics of synaptic currents and determine their role in synaptic integration, signaling, and plasticity (Jonas, 2000). Yet, rigorous comparisons of AMPAR gating kinetics found recombinant AMPARs (Mosbacher et al., 1994) to be faster than those of native receptors (Colquhoun et al., 1992). In addition, the gating properties analyzed at the single-channel level in heterologous systems (Swanson et al., 1997) failed to match those recorded from native receptors (Wyllie et al., 1993). A similar lack of congruence existed between the kinetics of native (Castillo et al., 1997) and heterologously expressed KARs (Swanson and Heinemann, 1998). These findings suggested that additional proteins might associate with native receptors and alter their gating.

Over the past 20 years, tremendous progress has been made toward identifying proteins that interact with iGluRs, thus unraveling the molecular machinery that regulates the trafficking and function of iGluRs. The picture that emerges is that iGluRs are but one component of larger-scale, multimeric complexes. This is of particular interest in the context of the postsynaptic density (PSD) of excitatory synapses—a vast web of interacting proteins that comprise large and dynamic supramolecular assemblies (Scannevin and Huganir, 2000; Grant et al., 2005; Yamauchi, 2002: Feng and Zhang, 2009). The C-terminal tails (CTDs) of iGluRs have been a particular focus of attention in this regard, because they exhibit a great deal of diversity in length and sequence, and display numerous consensus sites for phosphorylation and a variety of protein-protein interactions. A myriad of cytosolic proteins have been identified that interact with the CTDs of iGluRs and regulate their membrane trafficking, anchoring at synapses, and involvement in intracellular signaling cascades. Depending on the particular class of iGluR, such cytoplasmic proteins include postsynaptic density-95/discs large/zona occludens-2 (PDZ) domain-containing proteins (such as GRIP/ABP, PICK1, and a variety of membrane-associated guanylate kinase or MAGUK proteins), cytoskeleton-interacting or scaffolding proteins (such as α-actinin, protein 4.1, and spectrin), and the ATPase NSF (Song and Huganir, 2002; Malinow and Malenka, 2002; Bredt and Nicoll, 2003; Collingridge et al., 2004; Kim and Sheng, 2004; Derkach et al., 2007; Lau and Zukin, 2007; Elias and Nicoll, 2007). The CTDs of iGluRs are also subject to phosphorylation by a variety of kinases such as protein kinase C (PKC), protein kinase A (PKA), and calcium-calmodulin kinase II (CaMKII), and by tyrosine kinases such as src and fyn (Boehm and Malinow, 2005; Lee, 2006).

The *stargazer* Mutant Mouse and a New Family of AMPAR Regulatory Proteins

The first bona fide transmembrane auxiliary subunit of an iGluR was discovered through the characterization of *stargazer*, a spontaneous mutation in an inbred mouse line, originally distinguished by its striking behavioral phenotype—dyskinesia, severe ataxia, characteristic head-tossing, and frequent spike-wave discharges (SWDs), reminiscent of absence epilepsy in humans (Noebels et al., 1990). Genetic mapping revealed that the *stargazer* mutation is attributable to a single recessive mutation on mouse chromosome 15 (Letts et al., 1997). Subsequent positional cloning showed that the locus of the mutation encodes stargazin—a novel, brain-specific, low-molecular weight,

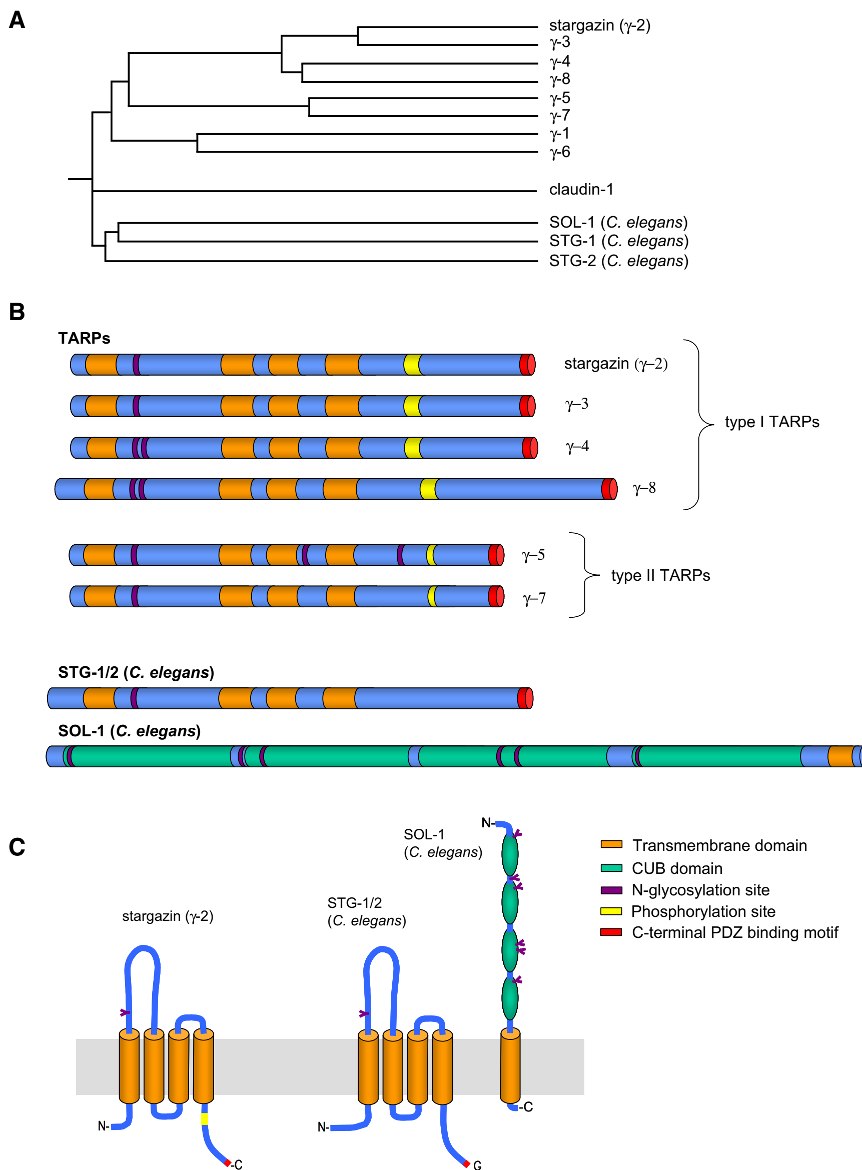

Figure 2. The TARP Family of Transmembrane AMPAR Auxiliary Subunits and Their Relatives

(A) Dendrogram illustrating the approximate phylogenetic relationships between all known TARP auxiliary subunits and several related proteins. The TARPs include stargazin (γ-2), γ-3, γ-4, γ-5, γ-7, and γ-8. The TARPs are homologous to the skeletal muscle voltage-gated calcium channel auxiliary subunit γ-1, as well as γ-6. Claudin-1 is a member of the claudin family of tight-junction proteins. The more distantly related proteins from *C. elegans*, SOL-1, STG-1, and STG-2, are necessary for the function of GLR-1, the AMPAR homolog in *C. elegans*. The dendrogram is based on sequence alignment of amino acid sequences using ClustalW. All protein sequences are from mouse unless otherwise noted (*C. elegans*).

(B) Bar diagrams showing the predicted domain structures of the TARPs (both type I and type II), in addition to *C. elegans* STG-1/2 and SOL-1 for comparison. Noteworthy are the four large extracellular CUB domains of SOL-1 and the approximate locations of sites of posttranslational modification.

(C) Illustration of the proposed secondary structures of the proteins shown in (B). Top is extracellular, bottom is intracellular.

tetraspanning membrane protein with homology to the voltage-gated calcium channel (VGCC) subunit γ-1, hence its alternative name, γ-2 (Letts et al., 1998) (Figure 2). Despite the role of γ-1 in modulating the functional properties of VGCCs in skeletal muscle (Jay et al., 1990), stargazin displays only subtle changes in the voltage dependence of activation and inactivation of VGCCs when coexpressed in heterologous systems (Letts et al., 1998; Klugbauer et al., 2000; Kang et al., 2001, 2006; Rousset et al., 2001). Instead, the weight of evidence is in favor of stargazin being essential for the regulation of AMPARs, first demonstrated in the cerebellum. In the *stargazer* mouse, AMPAR-mediated synaptic currents at the glutamatergic synapse between mossy fibers and CGNs, as well as extrasynaptic currents, are largely absent. NMDAR-mediated responses are normal, however, indicating that excitatory synapses generally develop properly and are capable of releasing glutamate (Chen et al., 1999, 2000; Hashimoto et al., 1999). Chen and colleagues subsequently transfected *stargazer* CGNs with full-length recombinant stargazin and found that both synaptic and extrasynaptic AMPAR-mediated responses could be reconstituted, suggesting that stargazin plays a critical role in the trafficking and ultimate synaptic targeting of AMPARs (Chen et al., 2000).

Stargazin is neither confined to the cerebellum nor alone in its ability to modulate AMPAR-mediated transmission. Database mining revealed that stargazin is a member of an extended family of tetraspanning proteins that includes γ-3, γ-4, γ-5, γ-6, γ-7, γ-8, and members of the claudin protein family. These homologous proteins exhibit widespread expression within the CNS (Burgess et al., 1999, 2001; Klugbauer et al., 2000; Moss et al., 2002). Phylogenetic analyses of the primary sequences showed that the family of γ subunit proteins can be divided into subgroups based on homology, with stargazin, γ-3, γ-4, and γ-8 forming one highly homologous group, γ-5 and γ-7 forming another, and γ-1 and γ-6 being yet another (Klugbauer et al., 2000; Burgess et al., 2001; Tomita et al., 2003) (Figure 2A). Does the clustering on the basis of sequence alignment have functional implications? Indeed, stargazin, γ-3, γ-4, and γ-8 can rescue AMPAR-mediated surface currents in *stargazer* CGNs, whereas γ-1, γ-5, and claudin-1 fail to do so. As such, stargazin, γ-3, γ-4, and γ-8 were initially classified as TARPs (Tomita et al., 2003). With the discovery

that γ-5 and γ-7 also exhibit a more limited ability to modulate AMPAR trafficking and gating (Kato et al., 2007, 2008; Soto et al., 2009), the TARP family was later expanded and subclassified into canonical or type I TARPs (stargazin, γ-3, γ-4, and γ-8) and type II TARPs (γ-5 and γ-7) (Kato et al., 2010) (Figure 2B and Table 1). The basis for this subclassification as well as the differential expression patterns and roles of these various TARP family members will be explored later in this review.

TARPs Form an Integral Component of Native AMPAR Complexes

Multiple lines of compelling evidence suggest that TARPs avidly and selectively bind to native AMPAR subunits in the brain and are key components of the AMPAR complex at every point in its life cycle. Initial coimmunoprecipitation experiments found that stargazin associates with multiple GluA subunits in both heterologous cells (Chen et al., 2000) and brain extracts (Tomita et al., 2003, 2004; Fukata et al., 2005). Vandenberghe and coworkers analyzed cerebellar extracts using blue native gel electrophoresis and found that AMPAR complexes migrate as two distinct bands—a low and a high molecular weight band. Stargazin comigrates exclusively with the heavier band, which is absent in cerebellar extracts from *stargazer* mice. These data suggest that stargazin is stably associated with tetrameric AMPARs, and not monomers or dimers. Under these conditions, it is noteworthy that other AMPAR CTD-interacting proteins, including GRIP, PICK1, and NSF, are undetectable in native AMPAR complexes, suggesting that their interactions may be less stable and/or more transient than AMPAR-stargazin interactions. On the basis of these biochemical data, stargazin was designated as a bona fide AMPAR auxiliary subunit (Vandenberghe et al., 2005a). Furthermore, mass spectrometric analyses revealed the presence of multiple TARP family members within native AMPAR complexes from solubilized rodent brain preparations (Fukata et al., 2005; Nakagawa et al., 2005; Schwenk et al., 2009).

A longstanding, and as yet unresolved, question remains regarding the structural basis for AMPAR-TARP interactions. Single-particle electron microscopic approaches have been valuable in showing that TARP family members substantially contribute to the transmembrane density seen in 3D reconstructions of individual complexes, isolated from whole rat brain (Nakagawa et al., 2005, 2006). Such close apposition of the transmembrane domains of AMPARs and TARPs indirectly suggests a transmembrane interaction, but it could also be a consequence of more specific conjunctions at the level of the intracellular and extracellular domains. Mutagenesis and domain swapping experiments revealed specific regions of stargazin that interact with AMPARs. The first extracellular loop and regions within the CTD are especially important for AMPAR binding (Tomita et al., 2004) (Figure 1). The first extracellular loop of stargazin is essential for the modulation of AMPAR gating, but not trafficking. Conversely, the stargazin CTD is critical for AMPAR trafficking and aspects of gating (Tomita et al., 2004, 2005b, Turetsky et al., 2005, Bedoukian et al., 2006; Sager et al., 2009b; Milstein and Nicoll, 2009). Subsequent work showed that regions within the AMPAR ligand-binding core, but not the amino terminal domain (NTD), are essential for TARP modulation of gating (Tomita et al., 2007a). Furthermore, TARP effects on gating kinetics depend on the Q/R site within the AMPAR pore, suggesting an indirect role for the pore, and possibly the transmembrane domains, in determining TARP binding (Körber et al., 2007a). Together, these studies suggest that TARPs bind to AMPARs in a complex and distributed fashion, with a special role for the first extracellular loop, likely through a direct interaction with the AMPAR ligand-binding core. Although the crystallization of AMPAR structural domains, such as the ligand-binding core (Armstrong et al., 1998), as well as the full AMPAR tetramer (Sobolevsky et al., 2009), represented quantum leaps in our understanding of iGluR structure and function, the exact nature of the interaction between AMPARs and TARPs awaits either the crystal structure of a TARP or the cocrystallization of an AMPAR-TARP complex.

Aside from determining the structural basis for AMPAR-TARP interactions, persistent questions remain regarding TARP stoichiometry. How many TARP molecules are associated with single-AMPAR complexes in native systems? Can the trafficking and gating effects of TARPs be tuned by differences in stoichiometry? The dose dependence of TARP gating effects, reflected in miniature excitatory postsynaptic current (mEPSC) decay, provided the first tantalizing hint that AMPAR-TARP interactions may exhibit variable stoichiometry (Milstein et al., 2007). Since then, TARP modulation of KA efficacy has been a valuable metric for TARP stoichiometry in both heterologous and native systems. Fusion proteins, in which GluA subunits are bound to various TARP family members through linker domains, provide AMPAR-TARP complexes with defined stoichiometry. Using these constructs to calibrate KA efficacy in heterologous cells, AMPARs are estimated to associate with either two or four TARPs, suggesting a degree of cooperativity in TARP binding. TARP stoichiometry, suggested by KA efficacy, was subsequently found to differ among hippocampal cell types, suggesting that gating effects could be modulated by differential TARP expression (Shi et al., 2009). However, biochemical data has shown that AMPARs are capable of associating with one, two, three, or four stargazin molecules depending on its expression level, contradicting the notion of cooperative binding. In addition, AMPARs in CGNs were estimated to associate with only one stargazin molecule, which is sufficient to modulate KA efficacy (Kim et al., 2010). These contrasting results may be attributed, in part, to cell-type-specific differences in TARP subtypes and expression level. Clearly, further quantitative work will be required to clarify the possible TARP subtype and cell-type-specific regulation of stoichiometry. More broadly, there remains the possibility that TARP stoichiometry is not fixed throughout the lifecycle of an AMPAR, but rather that it can be dynamically regulated. Evidence that AMPAR-TARP complexes can undergo acute, agonist-dependent dissociation (Tomita et al., 2004), and can modify paired-pulse ratio (PPR) in hippocampal neurons (Morimoto-Tomita et al., 2009), suggests that dynamic regulation is possible, but its ultimate impact on postsynaptic AMPAR function remains to be elucidated.

TARPs and AMPAR Biogenesis

The AMPAR lifecycle begins in the ER through the sequential assembly of homodimers or heterodimers followed by the dimerization of dimers. Tetramers are subsequently exported from the

ER and passed through the Golgi network, during which they are subjected to posttranslational modification in the form of phosphorylation and glycosylation (Greger et al., 2007; Ziff, 2007). From early work on stargazin, it was unclear whether the lack of surface and synaptic AMPARs observed in *stargazer* CGNs (Chen et al., 2000) could be attributable to a role for stargazin as a chaperone during these early biosynthetic events, specific effects on surface expression and synaptic targeting of AMPARs, or both. In *stargazer* CGNs, despite only a minor reduction in total GluA2 protein in whole cerebella, GluA2 surface expression is dramatically reduced. A large proportion of the remaining GluA2 exhibits immature ER-type glycosylation, implying that GluA2 is unable to exit the ER and fully mature in *stargazer* CGNs. This result suggested that stargazin is involved in the early stages of GluA biosynthesis (Tomita et al., 2003). In fact, previous work showed that the majority of GluA protein, expressed in heterologous cells in the absence of TARPs, is also incompletely glycosylated and accumulates in intracellular pools, presumably corresponding to the ER (Hall et al., 1997). Fluorescence resonance energy transfer (FRET) experiments suggested that TARPs may facilitate ER export by blocking ER-retention sites on the AMPAR (Bedoukian et al., 2006), although later work demonstrates that the stargazin CTD contains a region that is essential for forward traffic through the ER and Golgi. Furthermore, the stargazin CTD can be tacked onto unrelated receptors, and not only mediates their ER export, but directs their localization to specific membrane compartments (Bedoukian et al., 2008). Additional evidence that stargazin has a role to play in AMPAR biosynthesis and ER export are experiments showing that induction of the unfolded protein response (UPR), a homeostatic response to the accumulation of unfolded or misassembled protein in the ER, can boost GluA1 surface expression in heterologous cells in a way that mimics and occludes the effect of stargazin. In addition, *stargazer* CGNs exhibit enhanced UPR, compatible with the notion that, in the absence of stagazin, AMPAR subunits may be incompletely folded or assembled and stuck in the ER (Vandenberghe et al., 2005b). Consistent with stargazin being exclusively associated with tetrameric AMPARs (Vandenberghe et al., 2005a; Shanks et al., 2010), TARPs are likely to be incorporated into nascent AMPAR complexes at some point between tetramerization and ER export. The role that TARPs play, if any, in protein folding, RNA editing, and subunit assembly at an earlier stage in AMPAR biogenesis remains to be determined. However, given that there is no clear enhancement in the levels of GluA monomers and dimers in cerebellar extracts from *stargazer* mice, the possibility that TARPs influence dimerization or tetramerization in the ER seems unlikely (Vandenberghe et al., 2005a). An intriguing possibility is that members of the newly characterized AMPAR auxiliary proteins, the Cornichon homologs (CNIHs), also have an important role to play in early steps in AMPAR biogenesis, considering their well-established role in ER export in other systems (Roth et al., 1995; Schwenk et al., 2009; Shi et al., 2010).

The Role of TARPs in AMPAR Surface Trafficking and Synaptic Targeting

In both heterologous systems and neurons, TARPs dramatically, selectively, and dose-dependently enhance the surface expression of AMPARs. In *stargazer* CGNs, both synaptic and extrasynaptic AMPARs are essentially absent (Chen et al., 1999; Hashimoto et al., 1999) but can be restored by transfection with full-length stargazin (Chen et al., 2000). Other members of the type I TARPs, γ-3, γ-4, and γ-8, but not γ-7 and γ-5, are able to rescue AMPAR surface expression when expressed in *stargazer* CGNs (Tomita et al., 2003). This effect was further characterized in heterologous systems where coexpression of various TARP family members along with GluA subunits greatly enhanced AMPAR surface expression as measured by the amplitude of agonist-evoked currents and a surface biotinylation assay. This effect is specific to AMPARs because TARPs are unable to traffic structurally related KARs (Chen et al., 2003; Yamazaki et al., 2004; Tomita et al., 2004, 2005b; Priel et al., 2005). Furthermore, the enhancement of surface expression by stargazin is not the result of inhibition of constitutive AMPAR endocytosis (Vandenberghe et al., 2005b). The type II TARP γ-7, but not γ-5, was later shown to enhance glutamate-evoked AMPAR currents in HEK293 cells in a subunit-specific manner (Kato et al., 2007, 2008), but had a very limited ability to do so in *stargazer* CGNs (Kato et al., 2007) (Table 1).

Importantly, TARPs direct AMPAR trafficking in neurons by specifically targeting them to synapses through PDZ binding motifs located in the last four residues of their cytosolic CTDs. Transfection of *stargazer* CGNs with a construct encoding a mutant stargazin with the last four residues missing (stargazinΔC) results in the reconstitution of AMPAR surface expression, but not synaptic trafficking (Chen et al., 2000). The PDZ binding motif of stargazin binds to PDZ domain-containing scaffolding proteins like PSD protein-95 (PSD-95) and related members of the MAGUK protein family (Chen et al., 2000; Schnell et al., 2002; Dakoji et al., 2003), which are pivotal components of the PSD and essential for AMPAR synaptic targeting (Kim and Sheng, 2004; Elias and Nicoll, 2007). Because PSD-95 and PDS-93 do not directly bind to AMPARs, TARPs play an essential intermediary role in anchoring and stabilizing AMPARs at synapses. Furthermore, TARP-dependent AMPAR clustering is dependent on PSD-95 palmitoylation (El-Husseini et al., 2002). Bats and coworkers showed, using single-particle quantum dot and fluorescence recovery after photobleaching (FRAP) imaging in cultured hippocampal neurons, that TARPs regulate the lateral diffusion of AMPARs between extrasynaptic and synaptic sites. They demonstrated that the disruption of stargazin-PSD-95 interactions prevents clustering of freely diffusible AMPAR-stargazin complexes at PSDs (Bats et al., 2007). Furthermore, a recent chemical-genetic approach demonstrated that the introduction of biomimetic ligands, which compete for both stargazin CTDs and PSD-95 binding sites, can acutely disrupt stargazin-PSD-95 interactions in cultured hippocampal neurons and enhance the surface mobility of AMPARs (Sainlos et al., 2011).

The modulatory influence of TARPs on AMPAR trafficking is itself subject to modulation through posttranslational modification. In particular, the CTDs of type I TARPs are studded with serine, threonine, and tyrosine residues that are substrates for phosphorylation. The threonine within the PDZ binding motif of stargazin can be phosphorylated by cAMP-dependent PKA, which disrupts its ability to bind to PSD-95. Furthermore,

Table 1. Modulation of AMPA Receptor Surface Trafficking and Synaptic Targeting and Gating by Mammalian Transmembrane Auxiliary Proteins

	Type I TARPs				Type II TARPs		Candidate AMPAR Auxiliary Proteins		
	stargazin (γ-2)[a]	γ-3[b]	γ-4[c]	γ-8[d]	γ-5[e]	γ-7[f]	CNIH-2/3[g]	CKAMP44[h]	SynDig1[i]
PDZ binding motif	Class I-TTPV	Class I-TTPV	Class I-TTPV	Class I-TTPV	Atypical-SSPC	Atypical-TSPC	none	Class II-EVTV	none
Surface trafficking of AMPARs	↑	↑	↑↑	↑↑	↔	↑	↔	↓↓	unknown
Enriched at synapses/PSD fractions	↑↑	↑↑	↑↑	↑↑	↔	↑	↑	↑↑	↑↑
Synaptic targeting of AMPARs	↑↑	↑↑	↑↑	↑↑	↔	↑	↔	unknown	↑↑
Desensitization and deactivation rates	↓	↓	↓↓	↓↓	↑	↓	↔	↑ desensitization ↓ deactivation	unknown
mEPSC decay	↓	↓	↓↓	↓	↔	unknown	↔	no effect	unknown
Resensitization	no effect	no effect	↑	↑	no effect	↑	no effect	unknown	unknown
Mean channel conductance	↑	↑	↑	↑	↑	↑	↑	unknown	unknown
Peak open probability	↔	no effect	no effect	no effect	↓	no effect	no effect	unknown	unknown
Intracellular polyamine affinity	↓↓	↓↓	↓↓	↓↓	↓	↓↓	↓↓	unknown	unknown
Glutamate affinity	↑↑	↑↑	↑	↑	↓	no effect	unknown	↑↑	unknown
Kainate efficacy	↑↑	↑↑	↑↑	↑↑	no effect	↑	no effect	unknown	unknown
CNQX efficacy	↑	↑	↑	↑	unknown	unknown	↔	unknown	unknown
Polyamine toxin efficacy	↑	↑	↑	↑	unknown	unknown	unknown	unknown	unknown

Notation: ↑, increase; ↓, decrease; ↔, variable effects/conflicting reports. Note: this chart represents our best approximation of data in the literature. Experimental caveats and conflicting results are discussed in the text.

[a] Hashimoto et al., 1999; Chen et al., 1999, 2000; Schnell et al., 2002; Tomita et al., 2003, 2005b; Yamazaki et al., 2004; Priel et al., 2005; Turetsky et al., 2005; Bedoukian et al., 2006; Kott et al., 2007, 2009; Soto et al., 2007, 2009; Körber et al., 2007b; Milstein et al., 2007; Cho et al., 2007; Menuz et al., 2007; Suzuki et al., 2008; Jackson et al., 2011

[b] Tomita et al., 2003; Kott et al., 2007, 2009; Kato et al., 2007, 2010; Milstein et al., 2007; Cho et al., 2007; Menuz et al., 2007; Suzuki et al., 2008; Soto et al., 2009; Shi et al., 2009; Jackson et al., 2011

[c] Tomita et al., 2003; Kott et al., 2007, 2009; Kato et al., 2007, 2010; Körber et al., 2007b; Milstein et al., 2007; Cho et al., 2007; Menuz et al., 2007; Suzuki et al., 2008; Soto et al., 2009; Shi et al., 2009; Jackson et al., 2011

[d] Tomita et al., 2003; Rouach et al., 2005; Kott et al., 2007; Milstein et al., 2007; Cho et al., 2007; Menuz et al., 2007; Suzuki et al., 2008; Soto et al., 2009; Kott et al., 2009; Shi et al., 2009, 2010; Kato et al., 2010; Jackson et al., 2011

[e] Tomita et al., 2003, 2005b; Kato et al., 2007, 2008, 2010; Soto et al., 2009

[f] Priel et al., 2005; Kato et al., 2007, 2008, 2010; Soto et al., 2009

[g] Schwenk et al., 2009; Shi et al., 2010; Kato et al., 2010

[h] von Engelhardt et al., 2010

[i] Kalashnikova et al., 2010

Cell PRESS

expression of a stargazin construct with a phosphomimic residue at this site greatly reduces AMPAR-mediated synaptic transmission in hippocampal neurons (Choi et al., 2002; Chetkovich et al., 2002). Interestingly, activation of PKA with forskolin fails to alter the synaptic localization of transfected stargazin (Chetkovich et al., 2002), and forskolin actually increases synaptic AMPAR currents (Carroll et al., 1998). The same threonine residue is also phosphorylated through the mitogen-activated protein kinase (MAPK) pathway. Paradoxically, phosphorylation of this site is associated with diametrically opposing effects on synaptic AMPAR clustering and plasticity, depending on the kinase that phosphorylates it (Stein and Chetkovich, 2010). Clearly, the physiological role of this phosphorylation site remains to be determined.

The CTD of stargazin also has a series of nine conserved serines common to all type I TARPs that, under basal conditions, are the only detectable phosphorylated residues in cultured cortical neurons (Tomita et al., 2005a). These serines, found within a highly basic region of the CTD, are substrates for phosphorylation by CaMKII and/or PKC (Tomita et al., 2005a; Tsui and Malenka, 2006). The physiological significance of this poly-serine region of the CTD is suggested by evidence that induction of NMDAR-dependent long-term depression (LTD) in the hippocampal CA1 region is dependent on dephosphorylation of stargazin through a protein phosphatase 1 (PP1) and PP2B-mediated pathway. Expression of a phosphomimic stargazin construct, in which all nine serines are phosphorylated, enhances synaptic delivery of AMPARs (Tomita et al., 2005a; Kessels et al., 2009) and prevents LTD. On the other hand, expression of a phosphonull stargazin construct prevents the induction of long-term potentiation (LTP) (Tomita et al., 2005a). Additional evidence suggests that TARPs γ-2 and γ-8 are differentially regulated by CaMKII and PKC (Inamura et al., 2006).

These findings demonstrate that TARPs are an important target of CaMKII and PKC and may play a central role in the bidirectional regulation of synaptic plasticity. How might the phosphorylation state of TARP CTDs control AMPAR trafficking? Conceivably, the basic residues within this region of the CTD interact strongly with the acidic phosphate head groups of surrounding membrane lipids, and this interaction is disrupted by poly-serine phosphorylation. As a consequence, stargazin would become more mobile for recruitment to the PSD. This idea has been explored by generating knockin mice containing either phosphomimic or phosphonull stargazin constructs. The phosphomimic stargazin enhances cerebellar mossy fiber/CGN AMPAR EPSCs, while the phosphonull construct reduces, but does not eliminate, EPSCs (Sumioka et al., 2010). Thus, stargazin appears to interact with negatively charged lipid bilayers in a phosphorylation-dependent manner, and this lipid interaction inhibits the binding of stargazin to PSD-95. A similar mechanism had been proposed for the PKC phosphorylation of the MARCKS protein family (Arbuzova et al., 2002). These results suggest that the regulation of the synaptic delivery of AMPARs is dependent on the phosphorylation state of stargazin and its interaction with membrane lipids. Additional work suggests that CaMKII phosphorylation of stargazin CTDs promotes the trapping and synaptic stabilization of laterally diffusing AMPARs (Opazo et al., 2010), which may have important implications for the role of CaMKII in synaptic plasticity (Hayashi et al., 2000; Merrill et al., 2005; Derkach et al., 2007).

Finally, through biochemical means, stargazin has been shown to be S-nitrosylated at a cysteine residue in its CTD, which results in an enhancement of GluA1 surface expression. This represents a potential pathway through which nitric oxide (NO) signaling could influence AMPAR trafficking (Selvakumar et al., 2009).

TARP Modulation of AMPAR Gating and Pharmacology

On the basis of initial experiments in heterologous systems and cerebellar CGNs, it was reasonable to imagine that the entirety of stargazin's role in AMPAR function was limited to that of a receptor chaperone—trafficking receptors to the cell surface and subsequently mediating their synaptic targeting, clustering, and turnover. Later quantitative biochemical and biophysical experiments made clear, however, that an increase in the cell surface expression of AMPARs alone was insufficient to account for the observed enhancement of steady-state agonist-evoked currents (Yamazaki et al., 2004, Priel et al., 2005; Tomita et al., 2005b). It was suggested, therefore, that stargazin, in addition to its role in trafficking, could also be augmenting the functional properties of AMPARs. Indeed, both type I and II TARPs modulate AMPAR gating and pharmacology to varying degrees in a number of interrelated ways (Figure 3 and Table 1).

Gating Kinetics

In heterologous expression systems, coexpression of stargazin with either GluA1 or GluA2 slows the rate of desensitization and enhances the amplitude of steady-state currents in response to glutamate, as compared with GluA1 or GluA2 alone. In addition, coexpression with stargazin slows the rate of deactivation and hastens recovery from desensitization (Priel et al., 2005, Tomita et al., 2005b; Turetsky et al., 2005; Bedoukian et al., 2006). These effects of stargazin on AMPAR kinetics could, in part, be explained by the behavior of GluA4-mediated currents at the single-channel level, which show that stargazin enhances single-channel conductance and channel burst duration (Tomita et al., 2005b). These molecular and biophysical studies demonstrate that stargazin allosterically augments AMPAR currents independent of its role in receptor trafficking. Furthermore, the dual roles of stargazin could be ascribed to specific domains of the stargazin protein and are functionally dissociable (Tomita et al., 2005b). Subsequent work showed that TARPs not only modulate the gating kinetics of AMPARs but do so in a TARP subtype-dependent manner. The expression of different type I TARPs along with AMPAR subunits in heterologous cells results in differential effects on rise time, deactivation, and desensitization kinetics. For example, γ-4 and γ-8 both slow the deactivation of glutamate-evoked currents to a greater extent than γ-2 or γ-3 (Milstein et al., 2007; Cho et al., 2007). Differential effects of type I TARPs on the gating kinetics of heterologously expressed AMPARs are also shown in other studies (Kott et al., 2007; Körber et al., 2007b; Soto et al., 2007, 2009; Suzuki et al., 2008). In addition, some TARPs confer a peculiar component of desensitization kinetics referred to as "resensitization." First observed with GluA1 coexpressed with γ-7, resensitization manifests as the slow increase in steady-state current following rapid desensitization, in the sustained presence of

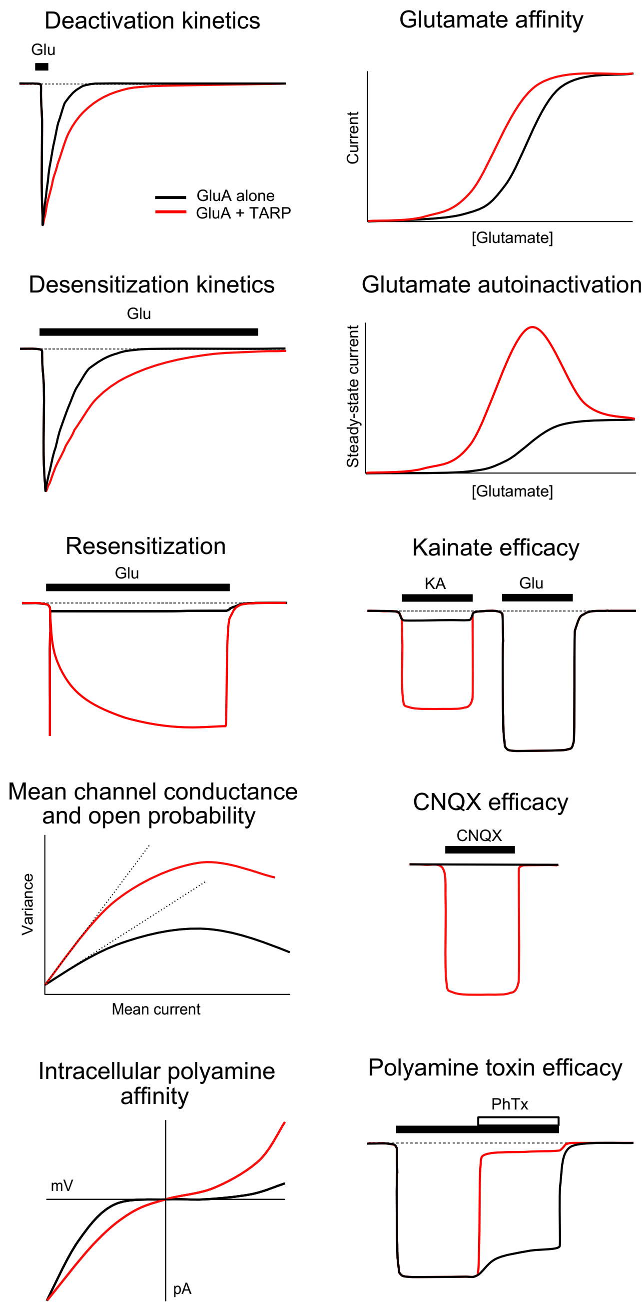

Figure 3. TARP Modulation of AMPAR Gating and Pharmacology
Schematic summary of the myriad ways in which TARP association can modulate the gating and pharmacology of AMPARs. Note that not every TARP effect on AMPAR function is illustrated here. TARP modulation (red) is shown relative to GluA alone (black). Represented are TARP-dependent changes in deactivation and desensitization kinetics, as well as the phenomenon known as resensitization. TARPs are known to modulate other channel properties such as mean channel conductance, open probability, and intracellular polyamine affinity. TARPs also modulate AMPAR pharmacology in the form of changes in glutamate affinity, kainate efficacy, CNQX efficacy, and sensitivity to polyamine toxins such as philanthotoxin (PhTx). These functional properties vary in a combinatorial manner depending on TARP subtype and AMPAR subunit composition.

agonist (Kato et al., 2007, 2008) (Figure 3). Subsequent work showed that only TARPs γ-4, γ-7, and γ-8 confer resensitization kinetics (Kato et al., 2010). Although the physiological significance of resensitization is unclear, determining its molecular underpinnings would be of interest because it may inform the structural basis of TARP subtype-dependent interactions with AMPARs.

TARPs clearly modulate the kinetics of agonist-evoked AMPAR currents in heterologous systems, but what are the effects of TARPs on the kinetics of synaptic responses in neurons? Viral infection of hippocampal slice cultures with a chimeric construct designed to dissociate stargazin's roles as trafficking chaperone and allosteric modulator of gating show that stargazin can modulate the amplitude and kinetics of native AMPAR-mediated mEPSCs (Tomita et al., 2005b). Subsequently, TARP subtype-dependent effects observed in heterologous systems were largely mirrored in differential effects of TARP expression on mEPSC amplitude, rise-time, and decay in CGNs (Milstein et al., 2007; Cho et al., 2007). For example, γ-4 and γ-8 slow the rise-time of mEPSCs to a greater extent than γ-2 or γ-3, whereas γ-4 slows the decay to a far greater extent than γ-2, γ-3, or γ-8 (Milstein et al., 2007). Domain swapping experiments demonstrated that the TARP subtype-dependent effects on gating kinetics could be largely attributed to unique characteristics of the first extracellular domains (Milstein et al., 2007; Cho et al., 2007). However, the TARP intracellular domains (N-terminal, intracellular loop, and C-terminal) also have unexpected roles to play in AMPAR gating kinetics (Milstein and Nicoll, 2009). What is the physiological significance of TARP-dependent modulation of deactivation and desensitization kinetics? Clearly the most straightforward effect would be an enhancement in charge transfer associated with synaptic glutamate release, which, when combined with other important variables that determine the kinetics of AMPAR-mediated synaptic currents (Jonas and Spruston, 1994; Edmonds et al., 1995; Conti and Weinberg, 1999; Jonas, 2000), would be predicted to have important functional ramifications on dendritic integration, calcium entry, coincidence detection, and spike-timing-dependent plasticity.

Channel Pharmacology

The presence of stargazin potentiates the affinity of AMPARs to glutamate, evidenced by the leftward shift in the glutamate dose-response curve (Yamazaki et al., 2004; Tomita et al., 2005b, Priel et al., 2005; Turetsky et al., 2005). However, the degree of enhancement of glutamate affinity by the type I TARPs depends on GluA subunit composition, GluA splice variant (flip versus flop), and TARP subtype (Kott et al., 2007, 2009; Tomita et al., 2007a, 2007b). Interestingly, AMPARs exhibit a bell-shaped glutamate concentration-response curve when steady-state instead of peak current is measured in some neuronal preparations, a phenomenon referred to as autoinactivation (Vlachová et al., 1987; Raman and Trussell, 1992; Kinney et al., 1997) (Figure 3). Recent work suggests that autoinactivation may be explained by the rapid dissociation of TARPs from AMPARs at glutamate concentrations above ~10 μM (Morimoto-Tomita et al., 2009).

KA is a glutamate analog that acts as a partial agonist of AMPARs, meaning that even at saturating concentrations, it

only induces submaximal channel activation in the form of small, nondesensitizing current (Zorumski and Yang, 1988; Patneau and Mayer, 1991). The structural basis for partial agonist action lies in its failure to induce complete cleft closure of the AMPAR ligand-binding core (Jin et al., 2003). The presence of TARPs greatly enhances KA efficacy to the point that it behaves as a full agonist in both heterologous cells and neurons (Tomita et al., 2005b; Turetsky et al., 2005) (Figure 3 and Table 1). The ratio of KA-evoked current and glutamate-evoked current, or KA/Glu ratio, has since been shown to be an invaluable tool in determining the presence or absence of TARPs and in estimating AMPAR-TARP stoichiometry (Shi et al., 2009).

Derivatives of quinoxaline such as 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) have been commonly used as competitive antagonists of AMPARs. Paradoxically, CNQX enhances the excitability of some cell types (Maccaferri and Dingledine, 2002; Menuz et al., 2007). Furthermore, bath application of CNQX can induce a steady-state inward current in neurons that can be enhanced by allosteric AMPAR potentiators such as tichloromethiazide (TCM) and blocked by selective, noncompetitive AMPAR antagonists such as GYKI53655. These data suggest that in neurons, CNQX behaves as a partial agonist of AMPARs. Using heterologous cells with AMPARs coexpressed with any one of the type I TARPs, it was revealed that CNQX can only behave as a partial agonist when AMPARs are TARP-associated (Menuz et al., 2007). Furthermore, TARP subtypes can differentially affect CNQX efficacy (Kott et al., 2009) (Figure 3 and Table 1). This effect of TARPs is generally consistent with the notion that TARPs influence the degree to which ligand binding translates into cleft closure and channel opening, possibly through a direct interaction with the linker domains (Milstein and Nicoll, 2008).

TARP association also modulates the action of so-called allosteric AMPAR potentiators, like the commonly used compound cyclothiazide (CTZ), which blocks desensitization in a splice-variant-dependent manner (Partin et al., 1994) by acting at the AMPAR dimer interface (Sun et al., 2002). Consistent with the role of TARPs in generally enhancing AMPAR function, stargazin association boosts AMPAR affinity for AMPAR potentiators while modulating their splice variant specificity (Tomita et al., 2006). TARPs also modulate the affinity of negative allosteric AMPAR modulators like GYKI53655 (Cokić and Stein, 2008; Schober et al., 2011).

Pore Properties

TARPs have effects on AMPAR pore properties that are likely secondary to direct modulation of the ligand-binding core and/or linker domains. Single-channel analysis has shown that individual AMPARs can traverse any of several distinct subconductance states (Jahr and Stevens, 1987; Cull-Candy and Usowicz, 1987; Ascher and Nowak, 1988). Single-channel recordings from heterologously expressed GluA2-lacking AMPARs show that the presence of stargazin favors the probability of channels occupying the highest of these subconductance states and enhancing channel burst-duration during prolonged agonist application (Tomita et al., 2005b). Furthermore, ultrafast agonist application and subsequent nonstationary fluctuation analysis (NSFA) has been a valuable means of determining that TARP association dramatically enhances AMPAR mean channel conductance with variable effects on peak open probability (Figure 3 and Table 1). Thus far, TARPs have not exhibited any subtype-dependent differences in the enhancement of mean channel conductance of GluA2-lacking AMPARs (Soto et al., 2007, 2009; Suzuki et al., 2008). However, recent evidence shows that TARP subtypes can differentially modulate the mean channel conductance of heteromeric, GluA2-containing AMPARs (Jackson et al., 2011). Even the type II TARP γ-5 enhances the mean channel conductance of both homomeric and heteromeric AMPARs (Soto et al., 2009).

GluA2-lacking, calcium-permeable AMPARs are subject to voltage-dependent block by endogenous intracellular polyamines such as spermine and spermidine, resulting in characteristic inwardly rectifying current-voltage (I-V) relationships (McBain and Dingledine, 1993; Bochet et al., 1994; Jonas et al., 1994; Geiger et al., 1995; Kamboj et al., 1995; Koh et al., 1995; Bowie and Mayer, 1995). The degree of rectification of both synaptic and agonist-evoked AMPAR-mediated current is frequently used as a metric for GluA2 content (Isaac et al., 2007). TARP association dramatically diminishes the affinity of the AMPAR pore for intracellular spermine, thus enhancing charge transfer and calcium entry (Bowie and Mayer, 1995; Soto et al., 2007; 2009) (Figure 3 and Table 1). TARP-dependent effects on I-V shape may account for rectification being a misleading measure of synaptic and extrasynaptic GluA2 content (Jackson and Nicoll, 2011). Moreover, recent evidence suggests that TARP association enhances the efficacy of externally applied polyamine toxins such as philanthotoxins (PhTx) in a subunit-dependent and agonist-dependent manner (Jackson et al., 2011).

Modulation of AMPAR Gating by Type II TARPs

The effects of the type II TARPs on AMPAR gating are complex and sometimes contradictory. TARP γ-7, but not γ-5, was shown to display modest slowing of both the deactivation and desensitization kinetics of GluA1 homomers (Kato et al., 2007), although in another study neither γ-7 nor γ-5 had any effect on the desensitization kinetics of GluA4 homomers, but had differential effects on other gating parameters (Soto et al., 2009). And while γ-5 does nothing to unedited GluA subunits, Kato and coworkers showed that it can modulate the gating of edited GluA2(R)-containing, calcium-impermeable AMPARs, seeming to have a more pronounced effect on GluA2/3 heteromers than GluA1/2 heteromers, by accelerating both deactivation and desensitization. Furthermore, γ-5 association lowers the affinity of GluA2-containing AMPARs for glutamate (Kato et al., 2008). TARP γ-5, therefore, appears to be a contrarian TARP that does not participate in AMPAR trafficking but modulates AMPARs of a specific composition, in a way that is opposite to that of other TARPs. The eccentric functional behavior of γ-5 is all the more remarkable when compared with that of γ-7, with which it exhibits a high degree of sequence homology. A subsequent study from Soto and coworkers showed that γ-5 indeed exhibits irregular behavior, but in an entirely different way than that described by Kato et al. (2008). Both γ-7 and γ-5 enhance the mean channel conductance and have a modest effect on the rectification of Glu4 homomers. In striking contrast to Kato et al. (2008), γ-5 was found to preferentially modulate the mean channel conductance of AMPARs composed of "long-form" subunits, which are

predominantly GluA2 lacking and calcium permeable (Soto et al., 2009) (Table 1). Further study will be required to reconcile these contradictory findings. Nevertheless, the unique characteristics of type II TARPs add a degree of functional diversity, and possibly bidirectional control, to AMPAR trafficking and gating.

TARP Mutant Mice and the Role of TARP Subtypes in Specific Brain Regions

TARPs exhibit widespread and extensively overlapping expression patterns throughout the brain as assessed by in situ hybridization. Type I and II TARPs are found in both neurons and glia and display complex, cell-type-specific expression that varies over the course of development (Tomita et al., 2003; Fukaya et al., 2005; Lein et al., 2007). Given their apparent functional redundancy, why are there so many TARP family members? Why do some cell types appear to only express one TARP subtype while another expresses a multitude? A great deal can be learned about the subtype-specific role of TARPs in brain function by examining their differential expression patterns and complex effects on AMPAR trafficking and gating following their genetic deletion. A useful way of unpacking these questions is to consider TARP subtype-specific effects in well-characterized cell types in the hippocampus, cerebellum, neocortex, and thalamus (Table 2).

Hippocampus

Because the expression of synaptic plasticity at Schaffer collateral-CA1 pyramidal neuron synapses depends on the activity-dependent regulation of postsynaptic AMPARs (Malenka and Bear, 2004; Kerchner and Nicoll, 2008), a compelling issue since the discovery of TARPs has been discerning their role in modulating AMPAR trafficking and plasticity in these neurons. CA1 pyramidal neurons are known to express multiple TARP family members, including stargazin, γ-3, γ-4, γ7, and γ-8. However, a striking and unique feature of the hippocampus is the selective enrichment of γ-8 (Tomita et al., 2003; Fukaya et al., 2005; Lein et al., 2007). The generation of the γ-8 knockout (KO) mouse revealed that AMPAR expression and distribution are selectively diminished in the hippocampus, as evidenced by the dramatic reduction in hippocampal GluA subunit protein expression without a corresponding change in amounts of mRNA. At the subcellular level, immunogold electron microscopy showed that both synaptic and extrasynaptic AMPARs are severely diminished. Interestingly, CA1 pyramidal neurons from γ-8 KO mice exhibit relatively modest reductions in field EPSC (fEPSC) slope, AMPA/NMDA ratio, and mEPSC amplitude, but do exhibit the near-complete loss of extrasynaptic AMPARs. The impact of this pattern of AMPAR deficit on hippocampal synaptic plasticity is impairment in LTP without a significant effect on LTD (Rouach et al., 2005). In contrast to the role of stargazin in CGNs, where the absence of functional stargazin results in the loss of both synaptic and extrasynaptic AMPARs, γ-8 seems to have a specialized role in delivering AMPARs to extrasynaptic sites in hippocampal neurons. Whether or not the impairment in LTP is the direct result of losing γ-8, or whether it is secondary to the loss of the extrasynaptic pool of AMPARs, remains to be determined. The impact of losing γ-8 is likely mitigated by the presence of other TARP family members in CA1 pyramidal neurons. Initial experiments using *stargazer*/γ-8 double KO mice suggested that AMPAR-mediated transmission in CA1 pyramidal neurons is further reduced, but not eliminated (Rouach et al., 2005). Additional biochemical and anatomical evidence suggests that γ-8 and stargazin may be present in separate but overlapping subcellular compartments in hippocampal neurons (Inamura et al., 2006). *Stargazer* (Hashimoto et al., 1999), *stargazer*/γ-3 double KO (Menuz et al., 2008), and γ-3/γ-4 double KO mice (Menuz et al., 2009) all fail to exhibit any significant impairment in synaptic transmission in CA1 pyramidal neurons. Only γ-3/γ-4/γ-8 triple KO mice display defects in synaptic transmission that are similar to the loss of γ-8 by itself. It is enticing to speculate that in a *stargazer*/γ-3/γ-4/γ-8 quadruple KO pyramidal neuron, AMPAR-mediated transmission would be entirely eradicated, but so far this goal has remained out of reach, owing to some KO combinations being embryonically lethal (Menuz et al., 2009) (Table 2). Single-cell deletion strategies would be required for future investigation. Taken together, these data suggest that at least in CA1 pyramidal neurons, multiple type I TARPs are largely redundant and that any one TARP, to varying degrees, can compensate for the loss of the others in mediating AMPAR synaptic targeting. However, γ-8 appears to have a unique role in regulating the pool of extrasynaptic AMPARs. In addition, the stoichiometry of AMPAR-TARP γ-8 interactions, as measured by the KA/Glu ratio, appears to vary between distinct cell types within the hippocampus (Shi et al., 2009).

Another striking TARP expression pattern in the hippocampus is the robust expression of γ-5 in the CA2 region (Fukaya et al., 2005; Lein et al., 2007). Consistent with the contrarian nature of γ-5, glutamate-evoked currents from acutely dissociated CA2 pyramidal neurons exhibit faster desensitization kinetics and smaller steady-state currents than those from CA3 (Kato et al., 2008). Curiously, γ-8 is also robustly expressed in CA2, as it is throughout the hippocampus (Fukaya et al., 2005; Lein et al., 2007), yet the channel kinetics appear to be more in line with those of γ-5 than γ-8. Do γ-5 and γ-8 compete for control of AMPAR gating? Are γ-5-associated AMPARs concentrated at the soma while γ-8-associated AMPARs are at synapses? Further work is required to answer these intriguing questions. Finally, the function of the CA2 region itself has been a longstanding mystery; however, recent characterization of the unique role that CA2 pyramidal neurons play within the hippocampal microcircuit (Chevaleyre and Siegelbaum, 2010) may point the way for a physiological role for γ-5 in hippocampal function.

Cerebellum

The cerebellum is another powerful model system for studying glutamatergic transmission and synaptic plasticity (Hansel et al., 2001; Ito, 2006), and is another brain region where TARP KO mice have shed light on the role of TARP subtype-specific AMPAR trafficking and gating (Coombs and Cull-Candy, 2009). CGNs from *stargazer* mice are virtually devoid of both synaptic and extrasynaptic AMPARs (Hashimoto et al., 1999; Chen et al., 2000), suggesting that stargazin accounts for the entirety of type I TARP function in this cell type. This is somewhat surprising given the central importance of TARPs in AMPAR function and that most cell types examined thus far express multiple, largely redundant TARP subtypes.

Interestingly, AMPAR-mediated synaptic transmission in cerebellar Golgi cells (GoCs), which reside in the granule cell layer and appear to be unique in the cerebellum in robustly expressing TARP γ-3 in addition to stargazin (Fukaya et al., 2005; Lein et al.,

Table 2. Summary of Behavioral and AMPAR Trafficking Phenotypes Observed in TARP Mutant Mice

Mutant Mouse	Targeting	Viability/ Survival	Behavioral Phenotype	Cell-Type Specific AMPAR Trafficking Phenotype
stargazer (γ-2)[a]	spont	viable	dyskinesia, head-tossing, severe ataxia, spike-wave discharges (seizures), low body weight	CGN: severe loss of synaptic and extrasynaptic AMPARs; PC: reduction in CF and PF synaptic AMPARs with no loss of extrasynaptic AMPARs; SC: severe reduction in PF synaptic AMPARs, with no loss of extrasynaptic AMPARs; CA1: normal; nRT: reduction in synaptic AMPARs; TRN: normal
γ-3[b]	KO	viable	normal	GoC: normal; CA1: normal
γ-4[c]	KO	viable	normal	MSN: loss of synaptic AMPARs in neonates (P5–6), normal in juveniles (P14–16)
γ-7[d]	flox (global)	viable	normal	PC: normal
γ-8[e]	KO	viable	normal	CA1: modest reduction in synaptic AMPARs but severe loss of extrasynaptic AMPARs
γ-2/γ-3[f]	spont/KO	failure to thrive	more severe ataxic phenotype than *stg*, low bodyweight	GoC: reduction in PF synaptic AMPARs; CA1: normal
γ-2/γ-4[g]	spont/KO	failure to thrive/viable (see references)	enhancement in seizures in *waggler* and *stargazer3J* mutants	N/A
γ-2/γ-7[d]	flox/flox (global)	viable	more severe ataxic phenotype than *stg*, low bodyweight	PC: severe loss of CF synaptic AMPARs
γ-2/γ-8[h]	spont/KO	failure to thrive		CA1: more severe reduction in synaptic AMPARs than γ-8 KO alone
γ-3/γ-4[i]	KO/KO	viable	normal	CA1: normal
γ-2/γ-3/γ-4[i]	spont/KO /KO	lethal	newborns do not breathe or move	CTX: normal synaptic and extrasynaptic AMPARs in cultured embryonic neurons; SpC: normal synaptic and extrasynaptic AMPARs in embryonic slices
γ-2/γ-3/γ-8[i]	spont/KO /KO	lethal	N/A	N/A
γ-3/γ-4/γ-8[i]	spont/KO /KO	viable	normal	CA1: modest reduction in synaptic AMPARs, similar to loss of γ-8 alone

Abbreviations: spont, spontaneous mutation; KO, knockout; flox, conditional knockout; CGN, cerebellar granule neurons; PC, cerebellar Purkinje cells; SC, cerebellar stellate cells; GoC, cerebellar Golgi cells; CF, cerebellar climbing-fiber pathway; PF, cerebellar parallel-fiber pathway; CA1, hippocampal CA1 pyramidal neurons; nRT, thalamic nucleus reticularis neurons; TRN, thalamic relay neurons; MSN, striatal medium spiny neurons; CTX, cortical neurons; SpC, spinal cord neurons.

[a] Noebels et al., 1990; Letts et al., 1998; Hashimoto et al., 1999; Chen et al., 1999, 2000; Menuz et al., 2008; Menuz and Nicoll, 2008, Jackson and Nicoll, 2011
[b] Menuz et al., 2008
[c] Letts et al., 2005, Milstein et al., 2007
[d] Yamazaki et al., 2010
[e] Rouach et al., 2005
[f] Menuz et al., 2008, 2009
[g] Letts et al., 2005, Menuz et al., 2009
[h] Rouach et al., 2005; Menuz et al., 2009
[i] Menuz et al., 2009

2007), is unaffected in *stargazer* mice. Likewise, GoCs from γ-3 KO mice are indistinguishable from those of wild-type. However, GoCs in the *stargazer*/γ-3 double KO mouse exhibit severe defects in AMPAR-mediated synaptic transmission. Consistent with the notion that type I TARPs are largely redundant in many cell types, stargazin and γ-3 are capable of compensating for the loss of the other. Another interesting observation in this study is that GoC synaptic AMPARs, which have linear I-Vs in wild-type mice, are moderately rectifying in the *stargazer*/γ-3 double KO mouse, implicating TARPs in determining subunit composition.

Phenotypically, *stargazer*/γ-3 double KO mice are sickly, consistently fail to thrive, and exhibit ataxia that is more severe than that in *stargazer* mice (Menuz et al., 2008).

Cerebellar Purkinje cells (PCs) are the primary output of the cerebellar cortex and are innervated by both CGNs in the form of parallel fibers and brainstem neurons in the form of powerful climbing fiber inputs. PCs are a useful illustration of a cell type that clearly expresses one type I TARP, stargazin, and one type II TARP, γ-7 (Fukaya et al., 2005; Lein et al., 2007). PCs from *stargazer* mice exhibit reductions in both parallel fiber (~70% loss) and climbing fiber (~50% loss) -evoked synaptic transmission, which likely contributes to *stargazer*'s prominent ataxia. Interestingly, *stargazer* PCs do not exhibit any defect in agonist-evoked currents from outside-out patches (Menuz and Nicoll, 2008). If stargazin is indeed the only type I TARP expressed in PCs, this suggests that in its absence, either γ-7 can compensate for a portion of the synaptic targeting and the entirety of the extrasynaptic trafficking, or that there are TARP-independent trafficking mechanisms at play. The recent generation of a conditional KO mouse in which both stargazin and γ-7 are deleted shows that the additional removal of γ-7 further reduces PC climbing fiber responses to ~10% of wild-type, thus implicating γ-7 in mediating some synaptic targeting in the absence of stargazin. Phenotypically, the stargazin/γ-7 double KO appears to exhibit more severe ataxia than stargazin KOs (Yamazaki et al., 2010). The impact that these various TARP deletions may have on forms of cerebellar synaptic plasticity, such as LTD at parallel fiber-PC synapses, remains to be seen.

Cerebellar stellate cells (SCs) and basket cells (BCs) are small interneurons that reside in the molecular layer, receive parallel fiber input, and mediate feedforward inhibition onto PCs. Recent work has shown that SCs from *stargazer* mice exhibit a profound loss in synaptic AMPARs but preservation of extrasynaptic receptors (Jackson and Nicoll, 2011), underscoring a possible role for different TARP family members in the subcellular compartmentalization of AMPARs in neurons (Rouach et al., 2005; Inamura et al., 2006; Menuz and Nicoll, 2008; Ferrario et al., 2011). In addition, parallel fiber-SC synapses exhibit a unique form of synaptic plasticity (Liu and Cull-Candy, 2000) that is compromised in *stargazer* mice (Jackson and Nicoll, 2011).

Thus far, Bergmann glial cells (BGCs) are the only glial cells that have been studied in any detail in the context of TARPs. BGCs are essential for the development and function of the cerebellar cortex (Bellamy, 2006) and expression of calcium-permeable AMPARs (Iino et al., 2001). Interestingly, BGCs express both TARP γ-4 and TARP γ-5 (Tomita et al., 2003; Fukaya et al., 2005; Lein et al., 2007). Although γ-4 is the predominant TARP expressed in the brain during development, its expression persists in adult BGCs (Tomita et al., 2003). BGCs have been used as a model system for examining AMPAR subunit-specific trafficking and gating by γ-5. The AMPAR properties of BGCs closely match those of heterologous cells in which GluA4 is coexpressed with γ-5, suggesting that γ-5 has a functional role in modulating glutamatergic transmission in BGCs (Soto et al., 2009).

Neocortex and Thalamus

In addition to profound ataxia and dyskinesia, *stargazer* mice exhibit seizure activity characterized by SWDs, qualitatively similar to human absence epilepsy (Noebels et al., 1990). To investigate the cellular mechanisms that account for this aspect of the *stargazer* phenotype, several studies have focused on the neocortex and thalamus. Dysregulation of excitability and synchrony within recurrent corticothalamic loops has been implicated in the origin of absence seizures (Huguenard and McCormick, 2007; Beenhakker and Huguenard, 2009). In early characterizations of *stargazer* mice, defects in neocortical excitability were thought to account for the occurrence of seizures and frequent SWDs. Specifically, layer V pyramidal neurons from *stargazer* mice are hyperexcitable, and exhibit spontaneous giant depolarizing EPSPs, a reduction in the postburst afterhyperpolarization, and an enhancement in the hyperpolarization-activated cation current, or I_h (Noebels et al., 1990; Di Pasquale et al., 1997). Interestingly, *stargazer*/γ-3/γ-4 triple KO mice, despite not surviving past birth, do not exhibit any defect in AMPAR-mediated transmission in late embryonic neocortical neurons (Menuz et al., 2009).

Subsequent work on TARP mutants focused on neurons in the thalamus, in particular the activity of thalamic nucleus reticularis (nRT) neurons and thalamocortical relay neurons (TRNs), which have pivotal roles to play in the generation of absence seizures (Huguenard and McCormick, 2007; Beenhakker and Huguenard, 2009; Chetkovich, 2009). Menuz and coworkers found that glutamatergic synapses onto inhibitory nRT neurons, but not onto excitatory TRNs, were disrupted in *stargazer* mice. These data suggest that disinhibition in the thalamus may contribute to seizure activity, characteristic of the *stargazer* mouse (Menuz and Nicoll, 2008). In addition, CNQX and the related quinoxaline-derived compound DNQX, but not NBQX, selectively depolarize nRT neurons, but not TRNs (Lee et al., 2010), pointing to possible cell-type-specific differences in TARP expression or function within the thalamus. Finally, TARP γ-4 has also been shown to have a role to play in the generation of SWDs and absence seizures when crossed with hypomorphic *stargazer* alleles such as *waggler* and *stargazer3J* (Letts et al., 2005). Future work will be required in order to dissect the functional roles of various TARP family members in regulating glutamatergic transmission, and ultimately, the balance of excitation and inhibition between specific cell types within corticothalamic networks.

TARPs and Human Disease

Defects in glutamatergic synaptic transmission have been implicated in the pathogenesis of numerous neurodegenerative and psychiatric diseases. Emerging human genetic evidence suggests that TARPs may play a role in the etiology of disorders as diverse as epilepsy, schizophrenia, and neuropathic pain. Homozygosity analysis of a consanguineous family exhibiting a high frequency of epilepsy, schizophrenia, and/or hearing loss revealed a link to a region of chromosome 22 that includes the human stargazin gene (CACNG2) (Knight et al., 2008). The human γ-3 gene (CACNG3) on chromosome 16 has been implicated as a susceptibility locus in a subpopulation of patients suffering from childhood absence epilepsy (CAE) (Everett et al., 2007), whereas another study of consanguineous families showed that CACNG2 is not linked with CAE (Abouda et al., 2010). In a genetic study of families with a high incidence of

schizophrenia, stargazin was linked to susceptibility in a subpopulation of patients (Liu et al., 2008). Furthermore, postmortem analyses of gene expression in the dorsolateral prefrontal cortices of patients with schizophrenia, bipolar disorder, and major depression revealed aberrant expression of stargazin (Beneyto and Meador-Woodruff, 2006; Silberberg et al., 2008). Interestingly, certain stargazin polymorphisms were shown to be associated with enhanced responsiveness to lithium, a common treatment for bipolar disorder (Silberberg et al., 2008). Finally, polymorphisms in human stargazin have been linked to susceptibility to chronic pain in a subset of cancer patients (Nissenbaum et al., 2010). These human genetic and histological data are complex, and in some cases contradictory, but when taken together, point to a plausible link between TARPs and the pathophysiology of several neurological and psychiatric disorders. TARPs may therefore serve as novel pharmacological targets and/or markers for a variety of human diseases.

TARPs and iGluR Trafficking and Gating in *C. elegans*

As the role of TARPs in mammalian systems was being worked out, Maricq and colleagues identified an unrelated auxiliary subunit for GLR-1, the AMPAR homolog in *C. elegans*, using an elegant genetic screen (Zheng et al., 2004). In brief, they made a transgenic worm expressing a GLR-1 subunit containing the same mutation that occurs in *lurcher* mutant mice. This results in a constitutively active GLR-1, a gain-of-function mutation that causes a marked "hyper-reversal" movement phenotype. They then screened for mutations that suppressed this behavior and identified suppressor of lurcher (*sol-1*). SOL-1 is predicted to be a type 1 transmembrane protein with a single transmembrane domain and four extracellular N-terminal complement subcomponents (C1r/C1s), urchin embryonic growth factor (Uegf), and bone morphogenetic protein (Bmp1), comprising CUB (C1r/C1s/Uegf/Bmp1) domains (Figures 2B and 2C). CUB domains are conserved, developmentally regulated, structural modules present in the extracellular domains of a diverse set of membrane proteins (Bork and Beckmann, 1993). SOL-1 colocalizes with GLR-1 at synaptic puncta, but is not necessary for the surface expression of GLR-1. Coimmunoprecipitation studies in COS-7 cells show that antibodies to SOL-1 coprecipitate GLR-1. Despite the seemingly normal synaptic targeting of GLR-1 in the absence of SOL-1, electrophysiological recordings from neurons expressing GLR-1 demonstrate that SOL-1 is essential for GLR-1 function. On the other hand, NMDAR function remains intact. Further studies indicate that SOL-1 controls the gating of GLR-1 and that the extracellular CUB domain 3 is required for this action (Zheng et al., 2006). Interestingly, and in striking contrast to vertebrate AMPARs, expression of GLR-1 in heterologous cells fails to elicit currents, indicating that a functional GLR-1 requires one or more additional proteins. Surprisingly, expression of SOL-1 together with GLR-1 in heterologous cells also fails to restore GLR-1 function. However, expression of a GLR-1 variant with mutations that greatly enhance gating, but is incapable of generating glutamate-evoked responses on its own, can generate substantial current in the presence of SOL-1. This indicates that GLR-1 is on the surface and that SOL-1 can interact with GLR-1.

What additional protein or proteins are required for GLR-1 function? This could be another unidentified GLR subunit or an additional auxiliary protein. Based on weak sequence identity to vertebrate stargazin (~25%), a *C. elegans* stargazin-like protein was identified (*Ce* STG-1) (Walker et al., 2006a). Expression of STG-1 together with GLR-1 and SOL-1 reconstitutes glutamate-evoked currents from GLR-1 in *Xenopus* oocytes. Although expression of GLR-1 and STG-1 produces little current in response to bath-applied glutamate in oocytes, ultrafast application of glutamate indicates that, in the presence of STG-1, GLR-1 produces currents that rapidly and completely desensitize in several milliseconds (Walker et al., 2006b). Thus, SOL-1 is actually not required for the gating of GLR-1; rather, SOL-1 modulates GLR-1 function by greatly slowing its desensitization and enhancing steady-state currents. Is STG-1 necessary for GLR-1 function in *C. elegans* neurons? To answer this question STG-1 was deleted from *C. elegans*, but GLR-1 function remained intact (Wang et al., 2008). Based on the possibility that another STG-1-like protein might exist and mask the loss of STG-1, this mutant was crossed to worms expressing the *lurcher* mutant and the progeny was screened for mutants that could suppress the abnormal behavior. Wang et al. identified STG-2 and found that a worm lacking both STG-1 and STG-2 is entirely devoid of GLR-1 function, despite the normal surface/synaptic trafficking of GLR-1. Why is it that GLR-1 requires STGs for function while vertebrate AMPARs are functional on their own in heterologous expression systems? One possibility, given the low amino acid identity among STG-1, STG-2, and stargazin, is that additional TARPs with more limited identity might exist. Alternatively the heterologous systems used to study AMPARs might have endogenous TARPs given the surprising finding that *Xenopus* oocytes endogenously express numerous iGluR subunits (Schmidt et al., 2009). Also, CNS neurons other than CGNs are likely to express other TARPs, which could account for the inability of Menuz et al. (2009) to silence AMPAR function with multiple TARP KOs. Interestingly, GluA1 expressed in *C. elegans* muscles, which lack glutamate receptors, is unresponsive to glutamate, but coexpression of vertebrate stargazin rescues function (Wang et al., 2008).

Taken together these findings indicate that GLR-1 in *C. elegans* requires, in addition to the pore-forming subunit, two distinct auxiliary subunits for normal function. The finding that auxiliary subunits are essential for the function of the pore-forming subunits of either ligand- or voltage-gated channels is unprecedented. It is of interest that while the effects of stargazin on AMPAR gating have largely been preserved throughout evolution, stargazin has acquired an additional critical role as a chaperone for the trafficking of AMPARs to the surface and to synapses in vertebrates. The sequence of GLR-1 is only slightly more similar to vertebrate AMPARs than to vertebrate KARs. However, the ability of vertebrate and invertebrate TARPs to function interchangeably with the two receptors indicates that GLR-1 is, in fact, functionally an AMPAR.

Transmembrane AMPAR Auxiliary Subunits beyond TARPs

TARPs appear to be associated with most neuronal AMPARs (Tomita et al., 2003, Menuz et al., 2007). However, recent

proteomic screens and/or genome mining have identified, in addition to TARPs, unrelated transmembrane proteins that exhibit similar effects on AMPAR trafficking and/or gating, and are therefore candidate auxiliary subunits. These exciting recent findings provide us with a bewildering and daunting level of combinatorial possibilities when we consider how this host of proteins may interact with AMPARs and with each other.

Cornichon Homologs-2 and -3

Recent proteomic analyses identified transmembrane proteins Cornichon homologs-2 and -3 (CNIH-2 and CNIH-3) as binding to AMPARs (Schwenk et al., 2009). CNIHs are highly conserved evolutionarily with Cornichon (Cni) and Erv14p, the *Drosophila* and yeast homologs, respectively, serving as chaperones that aid in the forward trafficking of epidermal growth factor receptor (EGFR) ligands from the ER to the Golgi (Roth et al., 1995; Powers and Barlowe, 1998; Hwang et al., 1999; Bökel et al., 2006; Castro et al., 2007; Hoshino et al., 2007). Using antibody shift assays with solubilized membrane fractions from whole rat brain, Schwenk and coworkers report the surprising finding that AMPARs associate primarily with CNIHs and that AMPARs associated with TARPs represent a smaller and largely nonoverlapping population. When expressed in heterologous cells, CNIHs were found to enhance AMPAR surface expression and slow the deactivation and desensitization kinetics of agonist-evoked currents to an even greater extent than stargazin (Schwenk et al., 2009; Tigaret and Choquet, 2009; Jackson and Nicoll, 2009; Brockie and Maricq, 2010). Further studies, mostly focusing on CNIH-2, have found that CNIHs and TARPs share a number of other properties. They both can immunoprecipitate GluA1, although considerably more GluA1 is pulled down with TARPs. In addition, they both promote the forward trafficking of GluA1 in the ER as measured by the glycosylation state of the receptor. Expression of a GluA1 construct that is covalently linked to γ-8 generates an AMPAR associated with the full complement of four γ-8 molecules where overexpression of γ-8 causes no further slowing of deactivation. However, expression of CNIH-2 does cause further slowing, strongly suggesting the presence of two nonoverlapping binding sites for these two proteins. CNIH-2 increases the mean channel conductance with no change in the channel open probability, similar to TARPs. However, in contrast to TARPs, CNIH-2 only has a modest effect on the efficacy of AMPARs to the partial agonist KA. Furthermore, CNIH-2 and –3 decrease spermine affinity for GluA2-lacking receptors, similar to the effect of stargazin (Shi et al., 2010). Finally, CNIH-2 has an antagonistic effect on TARP-dependent resensitization. As described previously, when GluA subunits are expressed with γ-4, γ-7, or γ-8, glutamate-evoked currents slowly recover in the continued presence of glutamate with a time constant of about 3 s. This phenomenon is not seen with GluA1 alone or coexpressed with stargazin, γ-3, or γ-5. Interestingly, coexpression of CNIH-2 prevents this resensitization (Kato et al., 2010).

What role might CNIHs play in neurons? *Stargazer* CGNs provide an ideal preparation for addressing this question because they express little CNIH-2 and surface AMPARs are essentially absent in the *stargazer* mouse. Expression of CNIH-2 fails to rescue synaptic currents in CGNs, although it is able to rescue a small component of glutamate-evoked whole-cell currents. The decay time constant of synaptic currents, as well as glutamate-evoked currents from nucleated patches, in CGNs from the *stargazer* heterozygote, which has reduced AMPAR/γ-2 stoichiometry, is also unaltered by the expression of CNIH-2. These results suggest that CNIH-2 is not associated with surface AMPARs even when overexpressed (Shi et al., 2010). In contrast, another study reported that CNIH-2 can indeed slow the synaptic currents rescued by γ-8 in *stargazer* CGNs (Kato et al., 2010). There is also some disagreement concerning the cellular distribution of CNIH-2. Shi and coworkers found that although CNIH-2 could be detected on the surface of HEK293 cells, it is undetectable on the surface of hippocampal neurons. Furthermore, immunocytochemical experiments found that FLAG-tagged CNIH-2 largely colocalizes with the *cis*-Golgi marker GM130 in both hippocampal neurons and CGNs (Shi et al., 2010). In contrast, Kato and coworkers found that CNIH-2 could not only be detected on the surface of hippocampal neurons, but also colocalizes with both GluA1 and TARPs (Kato et al., 2010). Expression of CNIH-2 in hippocampal pyramidal neurons fails to slow the deactivation or desensitization kinetics of glutamate responses from outside-out patches (Shi et al., 2010), even though the kinetics are considerably faster than what would be expected if these receptors were associated with endogenous CNIH-2. Yet there is evidence suggesting that CNIH-2 can interact with AMPAR/γ-8 complexes in the hippocampus (Kato et al., 2010). First, the level of CNIH-2 is dramatically reduced in the γ-8 knockout. Second, AMPAR responses to the continuous application of glutamate do not show resensitization unless γ-8 is overexpressed, and coexpression of CNIH-2 prevents this resensitization. These data suggest that the lack of resensitization of AMPAR/γ-8 complexes in hippocampal pyramidal neurons is attributable to the presence of CNIH-2.

These results raise a number of questions. The results from Shi et al. (2010) suggest that the role of CNIH-2 in neurons is more consistent with that of an ER chaperone rather than a bona fide auxiliary subunit. If so, it raises the intriguing question of why CNIH-2 has such profound effects on the gating of AMPARs. One possibility is that the salutary effects that glutamate-induced conformational changes have on the biogenesis of AMPARs (Coleman et al., 2009; Penn et al., 2008) may be enhanced by CNIH-2, and the same could hold for TARPs. In contrast to this model, Kato et al. (2010) present evidence that a primary effect of CNIH-2 is to counteract the resensitization of AMPAR/γ-8 complexes. If this latter model is correct, then AMPARs must normally be associated with both γ-8 and CNIH-2, contrary to the findings of Schwenk et al. (2009). This model then raises a number of questions. If CNIH-2 is, in fact, associated with AMPARs in hippocampal neurons, why are the kinetics of native neurons much faster than would be expected judging from data in heterologous cells? What is the mechanism underlying resensitization and how does CNIH-2 prevent it? What is the physiological role for resensitization, which requires the continued application of glutamate for many seconds? In addition, how is it that TARPs and CNIHs are so divergent structurally and yet have common effects on AMPAR kinetics? Hopefully many of these perplexing issues will be clarified by

quantitative structure-function analysis and the use of mice deficient in CNIH-2.

Cystine-Knot AMPAR Modulating Protein

Cystine-knot AMPAR modulating protein (CKAMP44) was identified by a proteomic approach in which immunoprecipitation and mass spectrometry of AMPAR complexes were used to search for previously unknown AMPAR-interacting proteins (von Engelhardt et al., 2010). CKAMP44 is a brain-specific type I transmembrane protein that contains a cysteine-rich N-terminal domain, likely forming a cystine knot similar to that in many peptide toxins (Norton and Pallaghy, 1998) and the extracellular domains of a diverse set of membrane proteins (Vitt et al., 2001). It is widely expressed, though at modest levels, throughout the brain with particularly robust expression in hippocampal dentate granule cells. CKAMP44 interacts with all GluA subunits, and AMPARs immunoprecipitated by CKAMP44 also contain stargazin, suggesting that CKAMP44 and stargazin are present within the same complexes. Furthermore, flag-tagged CKAMP44 localizes to dendritic spines. Surprisingly, coexpression of CKAMP44 with GluA1–3 in *Xenopus* oocytes results in a prominent *reduction* in glutamate-evoked currents without any change in the amount of GluA protein measured by biotinylation. A series of experiments in both oocytes and neurons reach the remarkable conclusion that CKAMP44 prolongs deactivation but accelerates desensitization. In addition, it slows the rate of recovery from desensitization. These findings are in striking contrast to those of both TARPs and CNIHs, which both prolong deactivation and desensitization and accelerate, or have no effect on, recovery from desensitization (von Engelhardt et al., 2010; Farrant and Cull-Candy, 2010; Guzman and Jonas, 2010) (Table 1).

What consequences might the unique properties of CKAMP44 have on hippocampal function? To discern this, the authors used overexpression of CKAMP44 in combination with CKAMP44 KO mice. They first examined CA1 pyramidal neurons, which express low levels of CKAMP44. They show that overexpression slows the decay of mEPSCs and reduces PPR, consistent with the slowing of recovery from desensitization. Interestingly, in contrast to the effects of overexpression, the CKAMP44 KO has no effect on EPSC kinetics, as might be predicted by the low expression level. The authors repeated these experiments in dentate granule neurons where CKAMP44 is expressed at high levels. Overexpression of CKAMP44 has no effect on PPR, but in the KO, PPR is enhanced. It would be of interest to know whether the decay of EPSCs in KO granule neurons is accelerated as would be expected. These findings are of considerable interest because, except for a few types of synapses where the probability of release is high and/or multiple active zones are present, desensitization is not thought to play a prominent role in PPR (Silver and Kanichay, 2008). How widespread might the role of CKAMP44 in the CNS be? CKAM44 expression is especially high in the dentate gyrus compared to many other regions of the brain, raising the possibility that its role could be more restricted than that of TARPs. It is not clear what advantage may be conferred by having TARPs and CKAMP44 interacting with the same AMPAR, given that their actions are antagonistic, at least in terms of their effects on desensitization.

Synapse Differentially Induced Gene 1

Synapse differentially induced gene 1 (SynDIG1) is a candidate AMPAR auxiliary subunit that was identified through application of a microarray approach to the expression profile of the cerebella of *lurcher* mice, which show defects in neuronal differentiation. One of the most highly differentially expressed genes was SynDIG1 (Díaz et al., 2002), which is upregulated during postnatal development in wild-type, but not *lurcher*, cerebella. SynDIG1 is a type II transmembrane protein that regulates AMPAR content at developing hippocampal synapses (Kalashnikova et al., 2010). Immunocytochemical experiments in cultured hippocampal neurons show that, while SynDIG1 clusters at excitatory synapses, most clusters are nonsynaptic, but are nonetheless associated with GluA2, suggesting that it might bind to GluA2. Indeed, anti-SynDIG1 antibodies coimmunoprecipiate GluA2 from brain extracts and the two proteins cluster on the surface of heterologous cells. This clustering requires an intact extracellular C terminus of SynDIG1. Overepression of SynDIG1 increases synapse density and increases the size and fluorescent intensity of GluA1 puncta, but not NR1 puncta. These anatomical changes are accompanied by an increase in both the frequency and amplitude of AMPAR mEPSCs, without a change in NMDAR mEPSCs. Thus both the immunocytochemical and electrophysiological results suggest that SynDIG1 selectively augments synaptic AMPAR content (Table 1).

What do these overexpression experiments tell us about the function of endogenous SynDIG1? To examine this, the authors used short hairpin RNA (shRNA)-mediated knockdown of endogenous SynDIG1. Indeed, SynDIG1 shRNA decreases the density of GluA-containing synapses, and both the size and fluorescent intensity of GluA clusters are also decreased. These changes are accompanied by a reduction in AMPAR mEPSC frequency and a dramatic reduction in mEPSC amplitude, but again without a change in NMDAR mEPSCs. Interestingly, the distribution of SynDIG1 at excitatory synapses is regulated by activity. These intriguing findings indicate that SynDIG1 plays an important function in the trafficking of AMPARs, but not NMDARs, to synapses during development (Kalashnikova et al., 2010; Díaz, 2010a, 2010b). It will be of great interest to determine if SynDIG1 shares other properties commonly attributed to auxiliary subunits—most importantly, modulation of AMPAR gating. In addition, SynDIG1 has been proposed to define a family of four genes in the mouse, and it will be of interest to see if these other family members act similarly to SynDIG1.

A Novel Transmembrane NMDAR Auxiliary Subunit

Neuropilin Tolloid-like 1

It has been reported that neuropilin tolloid-like 1 (NETO1), a single-pass transmembrane protein with two extracellular CUB domains (Stöhr et al., 2002; Michishita et al., 2003) (Figures 4A and 4B), interacts with NMDARs and is a candidate NMDAR auxiliary subunit (Ng et al, 2009). NETO1 was found to coimmunoprecipitate with GluN2A, GluN2B, and PSD-95 and is expressed in the CA1 region of the hippocampus in addition to other brain regions. Although the overall abundance of GluN1, GluN2A, and GluN2B in synaptosomal fractions is unchanged in the NETO1 KO mouse, as are the surface protein levels, there is a selective reduction in the

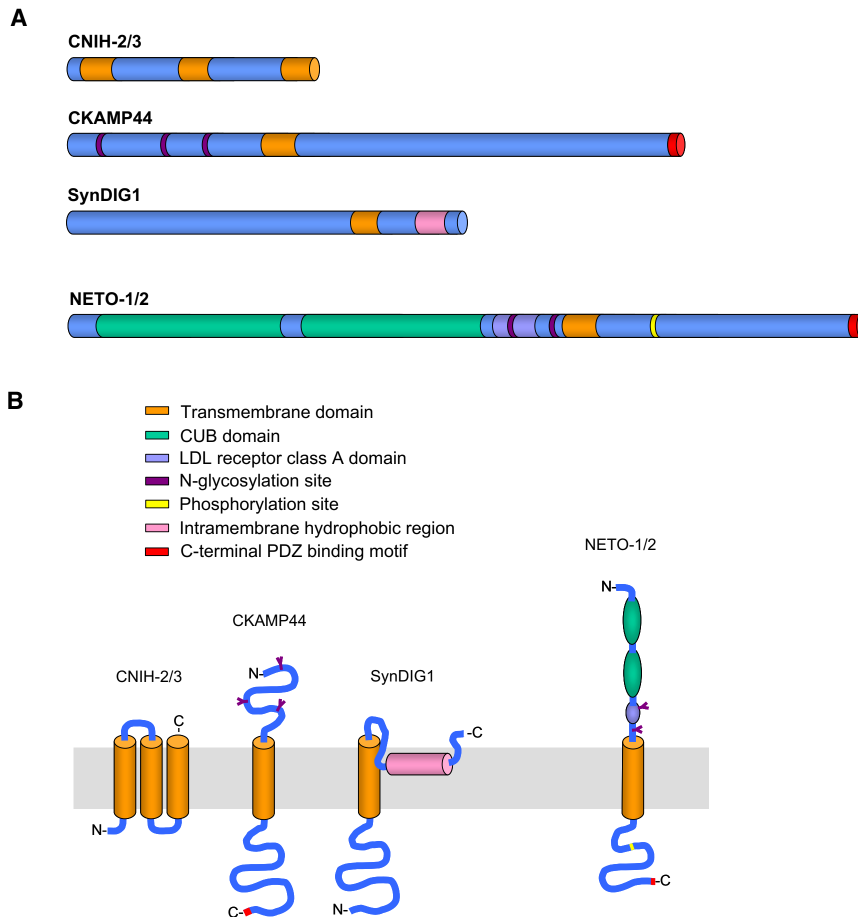

Figure 4. Candidate iGluR Transmembrane Auxiliary Subunits
(A) Bar diagrams showing the predicted domain structures of four candidate iGluR transmembrane auxiliary subunits. CNIH-2/3, CKAMP44, and SynDIG1 have been shown to bind to and influence the trafficking and/or gating of AMPARs. CNIH-2/3 is a small protein with three predicted transmembrane domains. CKAMP44 has a single transmembrane domain, a cysteine-rich extracellular N-terminal domain (likely forming a cystine knot), and a long intracellular CTD ending in a PDZ binding motif. SynDIG1 also has only one predicted transmembrane domain with a C-terminal hydrophobic region. NETO1 is a candidate NMDAR auxiliary subunit, while NETO2 is a candidate KAR auxiliary subunit. These homologous proteins are represented by one bar diagram, which highlights the two large extracellular CUB domains and a long CTD ending in a PDZ binding motif.
(B) Illustration of the proposed secondary structures of the proteins shown in (A). Top is extracellular, bottom is intracellular.

amount of GluN2A in the PSD fraction. In addition, there is a reduction in the amplitude of synaptic NMDAR currents, which was accompanied by a decrease in the contribution of GluN2A-containing receptors. Furthermore, LTP at Schaffer collateral-CA1 synapses and spatial learning are both impaired in the NETO1 KO mouse. Thus it is proposed that NETO1 is a component of the NMDAR complex and is involved in the delivery and/or stability of GluN2A-containing NMDARs at CA1 synapses (Ng et al., 2009).

A Novel Transmembrane KAR Auxiliary Subunit

Neuropilin Tolloid-like 2

To identify novel transmembrane proteins that interact with KARs, Tomita and colleagues carried out coimmunoprecipitation experiments with cerebellar extracts followed by mass spectrometry (Zhang et al., 2009). They identified neuropilin tolloid-like 2 (NETO2), which, like NETO1, is a single-pass transmembrane protein with two extracellular CUB domains (Stöhr et al., 2002; Michishita et al., 2004) (Figures 4A and 4B). In heterologous cells, NETO2 greatly enhances current through GluK2 receptors, but not GluA1 receptors. NETO2 also increases the efficacy of KA compared to glutamate in activating GluK2. The enhancement occurs without any change in surface GluK2 protein. However, expression of GluK2 does enhance the surface expression of NETO2. In cerebella from mice lacking GluK2, the levels of NETO2 are reduced by 60%, and much of this decrease is attributable to the loss of surface NETO2. Similar to the action of TARPs on AMPARs, NETO2 slows deactivation and desensitization and speeds the recovery from desensitization of GluK2. To examine the possible effects of NETO2 on synaptically evoked KAR-mediated currents, a mutant of GluK2 with reduced desensitization was expressed in *stargazer* CGNs. When NETO2 is coexpressed with this mutant, the frequency of mEPSCs increases and their time course is slowed. Finally, to determine if NETO2 is normally associated with KARs, the authors used shRNA to knock down endogenous NETO2 in hippocampal neurons. They found that the KA/Glu ratio of currents evoked by KARs is reduced with the knockdown of NETO2.

These results raise a number of interesting questions. There are a number of subunits that are involved in KAR function in the brain. Does NETO2 have similar effects on the other types of KARs? Does the related protein NETO1 also serve as a KAR auxiliary subunit? Although the authors show that NETO2 can slow the kinetics of synaptic currents generated by a mutated GluK2, it will be of interest to know what happens to well-characterized KAR-mediated EPSCs when NETO2 is deleted. Furthermore, it is remarkable that NETO1 and NETO2, which are homologous to each other, act on entirely separate classes of iGluR. Can NETO2 also act on NMDARs? Is it possible that NETO proteins are auxiliary subunits for both KARs and NMDARs? Clearly there is much to be resolved in this rapidly evolving area.

Concluding Remarks and Future Directions

Early studies on fast excitatory synaptic transmission in the brain emphasized the stereotyped nature of excitatory synapses whereby information is transmitted faithfully from one neuron

to another. However, the discovery of synaptic plasticity and the cloning of the various AMPAR subunit genes put this simplistic view to rest. Importantly, receptors assembled from different subunits have strikingly different biophysical properties. Add to this the discovery that subunits exist as splice variants and can undergo RNA editing, both of which control receptor gating, and one begins to reach a daunting level of complexity. Given this background one can reasonably wonder why AMPARs and other iGluRs should need various auxiliary subunits and the mind-boggling combinatorial possibilities that come with these newly discovered proteins. Only further studies will shed light on this general question.

There are, however, a number of specific and perhaps more tractable questions that arise from this research. (1) Are all surface iGluRs associated with auxiliary subunits? Although most neuronal AMPARs studied thus far appear to be associated with TARPs and perhaps other auxiliary subunits, it is unclear if this association is required for functional surface receptors. For *C. elegans*, auxiliary subunits are essential for functional receptors, but this remains an open question for vertebrate AMPARs. (2) How dynamic is the association of iGluRs and auxiliary subunits? Although there is some evidence that prolonged agonist application can dissociate TARPs from AMPARs, can this occur under physiological conditions and with other iGluRs and their auxiliary subunits? (3) How are so many proteins with such little amino acid identity capable of modifying AMPAR gating? Given this seeming lack of stringency, how many more proteins remain to be discovered that can control AMPAR gating? Do they all act on the same site or sites? Do they all impose the same conformational changes in the receptor? Only X-ray crystallographic studies of AMPAR/auxiliary subunit complexes will shed light on this problem. (4) What is the advantage of a neuron expressing multiple auxiliary subunits? Can single iGluRs assemble with multiple types of auxiliary subunit? (5) How does the modulation of iGluR gating kinetics by auxiliary subunits tune spatial and temporal integration in dendrites and action potential timing? And is this modulation homeostatically regulated in parallel with other mechanisms that determine EPSC time course? (6) Might auxiliary subunits provide a target for synaptic plasticity? Although considerable work suggests that the C termini of AMPARs are important for plasticity, there is still limited evidence that activity directly targets the AMPARs themselves. The key role auxiliary subunits play in controlling the shuttling of AMPAR from extrasynaptic to synaptic sites makes them ideal targets for the activity-dependent control of AMPAR trafficking. (7) Might auxiliary subunits play a role in neurological and psychiatric disease? Genetic studies have provided tantalizing hints, but thus far direct linkage is lacking. As is clear from all the questions posed above, we are just beginning to appreciate the importance of this exciting and rapidly expanding field.

ACKNOWLEDGMENTS

We wish to thank Sabine Schmid, Alexander Chesler, Avi Priel, Anna Lisa Lucido, Wei Lu, and Anastasios Tzingounis for valuable comments on the manuscript; and all members of the Nicoll lab for thoughtful discussions. A.C.J. is supported by a Ruth L. Kirschstein National Research Service Award from the NIMH (F32MH081430), and R.A.N. is supported by grants from the NIMH.

REFERENCES

Abouda, H., Hizem, Y., Gargouri, A., Depienne, C., Bouteiller, D., Riant, F., Tournier-Lasserve, E., Gourfinkel-An, I., LeGuern, E., and Gouider, R. (2010). Familial form of typical childhood absence epilepsy in a consanguineous context. Epilepsia *51*, 1889–1893.

Arbuzova, A., Schmitz, A.A., and Vergères, G. (2002). Cross-talk unfolded: MARCKS proteins. Biochem. J. *362*, 1–12.

Arikkath, J., and Campbell, K.P. (2003). Auxiliary subunits: essential components of the voltage-gated calcium channel complex. Curr. Opin. Neurobiol. *13*, 298–307.

Armstrong, N., Sun, Y., Chen, G.Q., and Gouaux, E. (1998). Structure of a glutamate-receptor ligand-binding core in complex with kainate. Nature *395*, 913–917.

Ascher, P., and Nowak, L. (1988). Quisqualate- and kainate-activated channels in mouse central neurones in culture. J. Physiol. *399*, 227–245.

Bats, C., Groc, L., and Choquet, D. (2007). The interaction between Stargazin and PSD-95 regulates AMPA receptor surface trafficking. Neuron *53*, 719–734.

Bedoukian, M.A., Weeks, A.M., and Partin, K.M. (2006). Different domains of the AMPA receptor direct stargazin-mediated trafficking and stargazin-mediated modulation of kinetics. J. Biol. Chem. *281*, 23908–23921.

Bedoukian, M.A., Whitesell, J.D., Peterson, E.J., Clay, C.M., and Partin, K.M. (2008). The stargazin C terminus encodes an intrinsic and transferable membrane sorting signal. J. Biol. Chem. *283*, 1597–1600.

Beenhakker, M.P., and Huguenard, J.R. (2009). Neurons that fire together also conspire together: is normal sleep circuitry hijacked to generate epilepsy? Neuron *62*, 612–632.

Bellamy, T.C. (2006). Interactions between Purkinje neurones and Bergmann glia. Cerebellum *5*, 116–126.

Beneyto, M., and Meador-Woodruff, J.H. (2006). Lamina-specific abnormalities of AMPA receptor trafficking and signaling molecule transcripts in the prefrontal cortex in schizophrenia. Synapse *60*, 585–598.

Bochet, P., Audinat, E., Lambolez, B., Crépel, F., Rossier, J., Iino, M., Tsuzuki, K., and Ozawa, S. (1994). Subunit composition at the single-cell level explains functional properties of a glutamate-gated channel. Neuron *12*, 383–388.

Boehm, J., and Malinow, R. (2005). AMPA receptor phosphorylation during synaptic plasticity. Biochem. Soc. Trans. *33*, 1354–1356.

Bökel, C., Dass, S., Wilsch-Bräuninger, M., and Roth, S. (2006). Drosophila Cornichon acts as cargo receptor for ER export of the TGFalpha-like growth factor Gurken. Development *133*, 459–470.

Bork, P., and Beckmann, G. (1993). The CUB domain. A widespread module in developmentally regulated proteins. J. Mol. Biol. *231*, 539–545.

Bowie, D., and Mayer, M.L. (1995). Inward rectification of both AMPA and kainate subtype glutamate receptors generated by polyamine-mediated ion channel block. Neuron *15*, 453–462.

Bredt, D.S., and Nicoll, R.A. (2003). AMPA receptor trafficking at excitatory synapses. Neuron *40*, 361–379.

Brockie, P.J., and Maricq, A.V. (2010). In a pickle: is cornichon just relish or part of the main dish? Neuron *68*, 1017–1019.

Burgess, D.L., Davis, C.F., Gefrides, L.A., and Noebels, J.L. (1999). Identification of three novel Ca(2+) channel gamma subunit genes reveals molecular diversification by tandem and chromosome duplication. Genome Res. *9*, 1204–1213.

Burgess, D.L., Gefrides, L.A., Foreman, P.J., and Noebels, J.L. (2001). A cluster of three novel Ca2+ channel γ subunit genes on chromosome 19q13.4: evolution and expression profile of the γ subunit gene family. Genomics *71*, 339–350.

Carroll, R.C., Nicoll, R.A., and Malenka, R.C. (1998). Effects of PKA and PKC on miniature excitatory postsynaptic currents in CA1 pyramidal cells. J. Neurophysiol. *80*, 2797–2800.

Castillo, P.E., Malenka, R.C., and Nicoll, R.A. (1997). Kainate receptors mediate a slow postsynaptic current in hippocampal CA3 neurons. Nature *388*, 182–186.

Castro, C.P., Piscopo, D., Nakagawa, T., and Derynck, R. (2007). Cornichon regulates transport and secretion of TGFalpha-related proteins in metazoan cells. J. Cell Sci. *120*, 2454–2466.

Chen, L., Bao, S., Qiao, X., and Thompson, R.F. (1999). Impaired cerebellar synapse maturation in waggler, a mutant mouse with a disrupted neuronal calcium channel gamma subunit. Proc. Natl. Acad. Sci. USA *96*, 12132–12137.

Chen, L., Chetkovich, D.M., Petralia, R.S., Sweeney, N.T., Kawasaki, Y., Wenthold, R.J., Bredt, D.S., and Nicoll, R.A. (2000). Stargazin regulates synaptic targeting of AMPA receptors by two distinct mechanisms. Nature *408*, 936–943.

Chen, L., El-Husseini, A., Tomita, S., Bredt, D.S., and Nicoll, R.A. (2003). Stargazin differentially controls the trafficking of alpha-amino-3-hydroxyl-5-methyl-4-isoxazolepropionate and kainate receptors. Mol. Pharmacol. *64*, 703–706.

Chetkovich, D. (2009). Thalamic reticular neurons are unexcited by new stargazer seizure mechanism. Epilepsy Curr. *9*, 59–61.

Chetkovich, D.M., Chen, L., Stocker, T.J., Nicoll, R.A., and Bredt, D.S. (2002). Phosphorylation of the postsynaptic density-95 (PSD-95)/discs large/zona occludens-1 binding site of stargazin regulates binding to PSD-95 and synaptic targeting of AMPA receptors. J. Neurosci. *22*, 5791–5796.

Chevaleyre, V., and Siegelbaum, S.A. (2010). Strong CA2 pyramidal neuron synapses define a powerful disynaptic cortico-hippocampal loop. Neuron *66*, 560–572.

Cho, C.H., St-Gelais, F., Zhang, W., Tomita, S., and Howe, J.R. (2007). Two families of TARP isoforms that have distinct effects on the kinetic properties of AMPA receptors and synaptic currents. Neuron *55*, 890–904.

Choi, J., Ko, J., Park, E., Lee, J.R., Yoon, J., Lim, S., and Kim, E. (2002). Phosphorylation of stargazin by protein kinase A regulates its interaction with PSD-95. J. Biol. Chem. *277*, 12359–12363.

Cokić, B., and Stein, V. (2008). Stargazin modulates AMPA receptor antagonism. Neuropharmacology *54*, 1062–1070.

Coleman, S.K., Möykkynen, T., Jouppila, A., Koskelainen, S., Rivera, C., Korpi, E.R., and Keinänen, K. (2009). Agonist occupancy is essential for forward trafficking of AMPA receptors. J. Neurosci. *29*, 303–312.

Collingridge, G.L., Isaac, J.T., and Wang, Y.T. (2004). Receptor trafficking and synaptic plasticity. Nat. Rev. Neurosci. *5*, 952–962.

Collingridge, G.L., Olsen, R.W., Peters, J., and Spedding, M. (2009). A nomenclature for ligand-gated ion channels. Neuropharmicology *56*, 2–5.

Colquhoun, D., Jonas, P., and Sakmann, B. (1992). Action of brief pulses of glutamate on AMPA/kainate receptors in patches from different neurones of rat hippocampal slices. J. Physiol. *458*, 261–287.

Conti, F., and Weinberg, R.J. (1999). Shaping excitation at glutamatergic synapses. Trends Neurosci. *22*, 451–458.

Coombs, I.D., and Cull-Candy, S.G. (2009). Transmembrane AMPA receptor regulatory proteins and AMPA receptor function in the cerebellum. Neuroscience *162*, 656–665.

Cull-Candy, S.G., and Usowicz, M.M. (1987). Multiple-conductance channels activated by excitatory amino acids in cerebellar neurons. Nature *325*, 525–528.

Dai, S., Hall, D.D., and Hell, J.W. (2009). Supramolecular assemblies and localized regulation of voltage-gated ion channels. Physiol. Rev. *89*, 411–452.

Dakoji, S., Tomita, S., Karimzadegan, S., Nicoll, R.A., and Bredt, D.S. (2003). Interaction of transmembrane AMPA receptor regulatory proteins with multiple membrane associated guanylate kinases. Neuropharmacology *45*, 849–856.

Derkach, V.A., Oh, M.C., Guire, E.S., and Soderling, T.R. (2007). Regulatory mechanisms of AMPA receptors in synaptic plasticity. Nat. Rev. Neurosci. *8*, 101–113.

Di Pasquale, E., Keegan, K.D., and Noebels, J.L. (1997). Increased excitability and inward rectification in layer V cortical pyramidal neurons in the epileptic mutant mouse Stargazer. J. Neurophysiol. *77*, 621–631.

Díaz, E. (2010a). SynDIG1 regulation of synaptic AMPA receptor targeting. Commun. Integr. Biol. *3*, 347–349.

Díaz, E. (2010b). Regulation of AMPA receptors by transmembrane accessory proteins. Eur. J. Neurosci. *32*, 261–268.

Díaz, E., Ge, Y., Yang, Y.H., Loh, K.C., Serafini, T.A., Okazaki, Y., Hayashizaki, Y., Speed, T.P., Ngai, J., and Scheiffele, P. (2002). Molecular analysis of gene expression in the developing pontocerebellar projection system. Neuron *36*, 417–434.

Edmonds, B., Gibb, A.J., and Colquhoun, D. (1995). Mechanisms of activation of glutamate receptors and the time course of excitatory synaptic currents. Annu. Rev. Physiol. *57*, 495–519.

El-Husseini, Ael.-D., Schnell, E., Dakoji, S., Sweeney, N., Zhou, Q., Prange, O., Gauthier-Campbell, C., Aguilera-Moreno, A., Nicoll, R.A., and Bredt, D.S. (2002). Synaptic strength regulated by palmitate cycling on PSD-95. Cell *108*, 849–863.

Elias, G.M., and Nicoll, R.A. (2007). Synaptic trafficking of glutamate receptors by MAGUK scaffolding proteins. Trends Cell Biol. *17*, 343–352.

Everett, K.V., Chioza, B., Aicardi, J., Aschauer, H., Brouwer, O., Callenbach, P., Covanis, A., Dulac, O., Eeg-Olofsson, O., Feucht, M., et al. (2007). Linkage and association analysis of CACNG3 in childhood absence epilepsy. Eur. J. Hum. Genet. *15*, 463–472.

Farrant, M., and Cull-Candy, S.G. (2010). Neuroscience. AMPA receptors—another twist? Science *327*, 1463–1465.

Feng, W., and Zhang, M. (2009). Organization and dynamics of PDZ-domain-related supramodules in the postsynaptic density. Nat. Rev. Neurosci. *10*, 87–99.

Ferrario, C.R., Loweth, J.A., Milovanovic, M., Wang, X., and Wolf, M.E. (2011). Distribution of AMPA receptor subunits and TARPs in synaptic and extrasynaptic membranes of the adult rat nucleus accumbens. Neurosci. Lett. *490*, 180–184.

Fukata, Y., Tzingounis, A.V., Trinidad, J.C., Fukata, M., Burlingame, A.L., Nicoll, R.A., and Bredt, D.S. (2005). Molecular constituents of neuronal AMPA receptors. J. Cell Biol. *169*, 399–404.

Fukaya, M., Yamazaki, M., Sakimura, K., and Watanabe, M. (2005). Spatial diversity in gene expression for VDCCgamma subunit family in developing and adult mouse brains. Neurosci. Res. *53*, 376–383.

Geiger, J.R., Melcher, T., Koh, D.S., Sakmann, B., Seeburg, P.H., Jonas, P., and Monyer, H. (1995). Relative abundance of subunit mRNAs determines gating and Ca2+ permeability of AMPA receptors in principal neurons and interneurons in rat CNS. Neuron *15*, 193–204.

Grant, S.G., Marshall, M.C., Page, K.L., Cumiskey, M.A., and Armstrong, J.D. (2005). Synapse proteomics of multiprotein complexes: en route from genes to nervous system diseases. Hum. Mol. Genet. *14 Spec No. 2*, R225–R234.

Greger, I.H., Ziff, E.B., and Penn, A.C. (2007). Molecular determinants of AMPA receptor subunit assembly. Trends Neurosci. *30*, 407–416.

Guzman, S.J., and Jonas, P. (2010). Beyond TARPs: the growing list of auxiliary AMPAR subunits. Neuron *66*, 8–10.

Hall, R.A., Hansen, A., Andersen, P.H., and Soderling, T.R. (1997). Surface expression of the AMPA receptor subunits GluR1, GluR2, and GluR4 in stably transfected baby hamster kidney cells. J. Neurochem. *68*, 625–630.

Hansel, C., Linden, D.J., and D'Angelo, E. (2001). Beyond parallel fiber LTD: the diversity of synaptic and non-synaptic plasticity in the cerebellum. Nat. Neurosci. *4*, 467–475.

Hashimoto, K., Fukaya, M., Qiao, X., Sakimura, K., Watanabe, M., and Kano, M. (1999). Impairment of AMPA receptor function in cerebellar granule cells of ataxic mutant mouse stargazer. J. Neurosci. *19*, 6027–6036.

Hayashi, Y., Shi, S.H., Esteban, J.A., Piccini, A., Poncer, J.C., and Malinow, R. (2000). Driving AMPA receptors into synapses by LTP and CaMKII: requirement for GluR1 and PDZ domain interaction. Science *287*, 2262–2267.

Hoshino, H., Uchida, T., Otsuki, T., Kawamoto, S., Okubo, K., Takeichi, M., and Chisaka, O. (2007). Cornichon-like protein facilitates secretion of HB-EGF and regulates proper development of cranial nerves. Mol. Biol. Cell *18*, 1143–1152.

Huguenard, J.R., and McCormick, D.A. (2007). Thalamic synchrony and dynamic regulation of global forebrain oscillations. Trends Neurosci. *30*, 350–356.

Hwang, S.Y., Oh, B., Zhang, Z., Miller, W., Solter, D., and Knowles, B.B. (1999). The mouse cornichon gene family. Dev. Genes Evol. *209*, 120–125.

Iino, M., Goto, K., Kakegawa, W., Okado, H., Sudo, M., Ishiuchi, S., Miwa, A., Takayasu, Y., Saito, I., Tsuzuki, K., and Ozawa, S. (2001). Glia-synapse interaction through Ca2+-permeable AMPA receptors in Bergmann glia. Science *292*, 926–929.

Inamura, M., Itakura, M., Okamoto, H., Hoka, S., Mizoguchi, A., Fukazawa, Y., Shigemoto, R., Yamamori, S., and Takahashi, M. (2006). Differential localization and regulation of stargazin-like protein, gamma-8 and stargazin in the plasma membrane of hippocampal and cortical neurons. Neurosci. Res. *55*, 45–53.

Isaac, J.T., Ashby, M., and McBain, C.J. (2007). The role of the GluR2 subunit in AMPA receptor function and synaptic plasticity. Neuron *54*, 859–871.

Ito, M. (2006). Cerebellar circuitry as a neuronal machine. Prog. Neurobiol. *78*, 272–303.

Jackson, A.C., and Nicoll, R.A. (2009). Neuroscience: AMPA receptors get 'pickled'. Nature *458*, 585–586.

Jackson, A.C., and Nicoll, R.A. (2011). Stargazin (TARP γ-2) is required for compartment-specific AMPA receptor trafficking and synaptic plasticity in cerebellar stellate cells. J. Neurosci. *31*, 3939–3952.

Jackson, A.C., Milstein, A.D., Soto, D., Farrant, M., Cull-Candy, S.G., and Nicoll, R.A. (2011). Probing TARP modulation of AMPA receptor channel conformation with polyamine toxins. J. Neurosci., in press.

Jahr, C.E., and Stevens, C.F. (1987). Glutamate activates multiple single channel conductances in hippocampal neurons. Nature *325*, 522–525.

Jay, S.D., Ellis, S.B., McCue, A.F., Williams, M.E., Vedvick, T.S., Harpold, M.M., and Campbell, K.P. (1990). Primary structure of the gamma subunit of the DHP-sensitive calcium channel from skeletal muscle. Science *248*, 490–492.

Jin, R., Banke, T.G., Mayer, M.L., Traynelis, S.F., and Gouaux, E. (2003). Structural basis for partial agonist action at ionotropic glutamate receptors. Nat. Neurosci. *6*, 803–810.

Jonas, P. (2000). The time course of signaling at central glutamatergic synapses. News in Phys. Sci. *15*, 83–89.

Jonas, P., and Spruston, N. (1994). Mechanisms shaping glutamate-mediated excitatory postsynaptic currents in the CNS. Curr. Opin. Neurobiol. *4*, 366–372.

Jonas, P., Racca, C., Sakmann, B., Seeburg, P.H., and Monyer, H. (1994). Differences in Ca2+ permeability of AMPA-type glutamate receptor channels in neocortical neurons caused by differential GluR-B subunit expression. Neuron *12*, 1281–1289.

Kalashnikova, E., Lorca, R.A., Kaur, I., Barisone, G.A., Li, B., Ishimaru, T., Trimmer, J.S., Mohapatra, D.P., and Díaz, E. (2010). SynDIG1: an activity-regulated, AMPA- receptor-interacting transmembrane protein that regulates excitatory synapse development. Neuron *65*, 80–93.

Kamboj, S.K., Swanson, G.T., and Cull-Candy, S.G. (1995). Intracellular spermine confers rectification on rat calcium-permeable AMPA and kainate receptors. J. Physiol. *486*, 297–303.

Kang, M.G., Chen, C.C., Felix, R., Letts, V.A., Frankel, W.N., Mori, Y., and Campbell, K.P. (2001). Biochemical and biophysical evidence for gamma 2 subunit association with neuronal voltage-activated Ca2+ channels. J. Biol. Chem. *276*, 32917–32924.

Kang, M.G., Chen, C.C., Wakamori, M., Hara, Y., Mori, Y., and Campbell, K.P. (2006). A functional AMPA receptor-calcium channel complex in the postsynaptic membrane. Proc. Natl. Acad. Sci. USA *103*, 5561–5566.

Kato, A.S., Zhou, W., Milstein, A.D., Knierman, M.D., Siuda, E.R., Dotzlaf, J.E., Yu, H., Hale, J.E., Nisenbaum, E.S., Nicoll, R.A., and Bredt, D.S. (2007). New transmembrane AMPA receptor regulatory protein isoform, gamma-7, differentially regulates AMPA receptors. J. Neurosci. *27*, 4969–4977.

Kato, A.S., Siuda, E.R., Nisenbaum, E.S., and Bredt, D.S. (2008). AMPA receptor subunit-specific regulation by a distinct family of type II TARPs. Neuron *59*, 986–996.

Kato, A.S., Gill, M.B., Yu, H., Nisenbaum, E.S., and Bredt, D.S. (2010). TARPs differentially decorate AMPA receptors to specify neuropharmacology. Trends Neurosci. *33*, 241–248.

Kerchner, G.A., and Nicoll, R.A. (2008). Silent synapses and the emergence of a postsynaptic mechanism for LTP. Nat. Rev. Neurosci. *9*, 813–825.

Kessels, H.W., Kopec, C.D., Klein, M.E., and Malinow, R. (2009). Roles of stargazin and phosphorylation in the control of AMPA receptor subcellular distribution. Nat. Neurosci. *12*, 888–896.

Kim, E., and Sheng, M. (2004). PDZ domain proteins of synapses. Nat. Rev. Neurosci. *5*, 771–781.

Kim, K.S., Yan, D., and Tomita, S. (2010). Assembly and stoichiometry of the AMPA receptor and transmembrane AMPA receptor regulatory protein complex. J. Neurosci. *30*, 1064–1072.

Kinney, G.A., Overstreet, L.S., and Slater, N.T. (1997). Prolonged physiological entrapment of glutamate in the synaptic cleft of cerebellar unipolar brush cells. J. Neurophysiol. *78*, 1320–1333.

Klugbauer, N., Dai, S., Specht, V., Lacinová, L., Marais, E., Bohn, G., and Hofmann, F. (2000). A family of gamma-like calcium channel subunits. FEBS Lett. *470*, 189–197.

Knight, H.M., Maclean, A., Irfan, M., Naeem, F., Cass, S., Pickard, B.S., Muir, W.J., Blackwood, D.H., and Ayub, M. (2008). Homozygosity mapping in a family presenting with schizophrenia, epilepsy and hearing impairment. Eur. J. Hum. Genet. *16*, 750–758.

Koh, D.S., Burnashev, N., and Jonas, P. (1995). Block of native Ca(2+)-permeable AMPA receptors in rat brain by intracellular polyamines generates double rectification. J. Physiol. *486*, 305–312.

Körber, C., Werner, M., Hoffmann, J., Sager, C., Tietze, M., Schmid, S.M., Kott, S., and Hollmann, M. (2007a). Stargazin interaction with alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptors is critically dependent on the amino acid at the narrow constriction of the ion channel. J. Biol. Chem. *282*, 18758–18766.

Körber, C., Werner, M., Kott, S., Ma, Z.L., and Hollmann, M. (2007b). The transmembrane AMPA receptor regulatory protein gamma 4 is a more effective modulator of AMPA receptor function than stargazin (gamma 2). J. Neurosci. *27*, 8442–8447.

Kott, S., Werner, M., Körber, C., and Hollmann, M. (2007). Electrophysiological properties of AMPA receptors are differentially modulated depending on the associated member of the TARP family. J. Neurosci. *27*, 3780–3789.

Kott, S., Sager, C., Tapken, D., Werner, M., and Hollmann, M. (2009). Comparative analysis of the pharmacology of GluR1 in complex with transmembrane AMPA receptor regulatory proteins gamma2, gamma3, gamma4, and gamma8. Neuroscience *158*, 78–88.

Lau, C.G., and Zukin, R.S. (2007). NMDA receptor trafficking in synaptic plasticity and neuropsychiatric disorders. Nat. Rev. Neurosci. *8*, 413–426.

Lee, H.K. (2006). Synaptic plasticity and phosphorylation. Pharmacol. Ther. *112*, 810–832.

Lee, S.H., Govindaiah, G., and Cox, C.L. (2010). Selective excitatory actions of DNQX and CNQX in rat thalamic neurons. J. Neurophysiol. *103*, 1728–1734.

Lein, E.S., Hawrylycz, M.J., Ao, N., Ayres, M., Bensinger, A., Bernard, A., Boe, A.F., Boguski, M.S., Brockway, K.S., Byrnes, E.J., et al. (2007). Genome-wide atlas of gene expression in the adult mouse brain. Nature *445*, 168–176.

Letts, V.A. (2005). Stargazer—a mouse to seize!. Epilepsy Curr. *5*, 161–165.

Letts, V.A., Valenzuela, A., Kirley, J.P., Sweet, H.O., Davisson, M.T., and Frankel, W.N. (1997). Genetic and physical maps of the stargazer locus on mouse chromosome 15. Genomics *43*, 62–68.

Cell
PRESS

Letts, V.A., Felix, R., Biddlecome, G.H., Arikkath, J., Mahaffey, C.L., Valenzuela, A., Bartlett, F.S., 2nd, Mori, Y., Campbell, K.P., and Frankel, W.N. (1998). The mouse stargazer gene encodes a neuronal Ca2+-channel gamma subunit. Nat. Genet. *19*, 340–347.

Letts, V.A., Mahaffey, C.L., Beyer, B., and Frankel, W.N. (2005). A targeted mutation in Cacng4 exacerbates spike-wave seizures in stargazer (Cacng2) mice. Proc. Natl. Acad. Sci. USA *102*, 2123–2128.

Liu, S.Q., and Cull-Candy, S.G. (2000). Synaptic activity at calcium-permeable AMPA receptors induces a switch in receptor subtype. Nature *405*, 454–458.

Liu, Y.L., Fann, C.S., Liu, C.M., Chen, W.J., Wu, J.Y., Hung, S.I., Chen, C.H., Jou, Y.S., Liu, S.K., Hwang, T.J., et al. (2008). RASD2, MYH9, and CACNG2 genes at chromosome 22q12 associated with the subgroup of schizophrenia with non-deficit in sustained attention and executive function. Biol. Psychiatry *64*, 789–796.

Maccaferri, G., and Dingledine, R. (2002). Complex effects of CNQX on CA1 interneurons of the developing rat hippocampus. Neuropharmacology *43*, 523–529.

Malenka, R.C., and Bear, M.F. (2004). LTP and LTD: an embarrassment of riches. Neuron *44*, 5–21.

Malinow, R., and Malenka, R.C. (2002). AMPA receptor trafficking and synaptic plasticity. Annu. Rev. Neurosci. *25*, 103–126.

Mayer, M.L. (2005). Glutamate receptor ion channels. Curr. Opin. Neurobiol. *15*, 282–288.

McBain, C.J., and Dingledine, R. (1993). Heterogeneity of synaptic glutamate receptors on CA3 stratum radiatum interneurones of rat hippocampus. J. Physiol. *462*, 373–392.

Menuz, K., and Nicoll, R.A. (2008). Loss of inhibitory neuron AMPA receptors contributes to ataxia and epilepsy in stargazer mice. J. Neurosci. *28*, 10599–10603.

Menuz, K., Stroud, R.M., Nicoll, R.A., and Hays, F.A. (2007). TARP auxiliary subunits switch AMPA receptor antagonists into partial agonists. Science *318*, 815–817.

Menuz, K., O'Brien, J.L., Karmizadegan, S., Bredt, D.S., and Nicoll, R.A. (2008). TARP redundancy is critical for maintaining AMPA receptor function. J. Neurosci. *28*, 8740–8746.

Menuz, K., Kerchner, G.A., O'Brien, J.L., and Nicoll, R.A. (2009). Critical role for TARPs in early development despite broad functional redundancy. Neuropharmacology *56*, 22–29.

Merrill, M.A., Chen, Y., Strack, S., and Hell, J.W. (2005). Activity-driven postsynaptic translocation of CaMKII. Trends Pharmacol. Sci. *26*, 645–653.

Michishita, M., Ikeda, T., Nakashiba, T., Ogawa, M., Tashiro, K., Honjo, T., Doi, K., Itohara, S., and Endo, S. (2003). A novel gene, Btcl1, encoding CUB and LDLa domains is expressed in restricted areas of mouse brain. Biochem. Biophys. Res. Commun. *306*, 680–686.

Michishita, M., Ikeda, T., Nakashiba, T., Ogawa, M., Tashiro, K., Honjo, T., Doi, K., Itohara, S., and Endo, S. (2004). Expression of Btcl2, a novel member of Btcl gene family, during development of the central nervous system. Brain Res. Dev. Brain Res. *153*, 135–142.

Milstein, A.D., and Nicoll, R.A. (2008). Regulation of AMPA receptor gating and pharmacology by TARP auxiliary subunits. Trends Pharmacol. Sci. *29*, 333–339.

Milstein, A.D., and Nicoll, R.A. (2009). TARP modulation of synaptic AMPA receptor trafficking and gating depends on multiple intracellular domains. Proc. Natl. Acad. Sci. USA *106*, 11348–11351.

Milstein, A.D., Zhou, W., Karimzadegan, S., Bredt, D.S., and Nicoll, R.A. (2007). TARP subtypes differentially and dose-dependently control synaptic AMPA receptor gating. Neuron *55*, 905–918.

Morimoto-Tomita, M., Zhang, W., Straub, C., Cho, C.H., Kim, K.S., Howe, J.R., and Tomita, S. (2009). Autoinactivation of neuronal AMPA receptors via glutamate-regulated TARP interaction. Neuron *61*, 101–112.

Mosbacher, J., Schoepfer, R., Monyer, H., Burnashev, N., Seeburg, P.H., and Ruppersberg, J.P. (1994). A molecular determinant for submillisecond desensitization in glutamate receptors. Science *266*, 1059–1062.

Moss, F.J., Viard, P., Davies, A., Bertaso, F., Page, K.M., Graham, A., Cantí, C., Plumpton, M., Plumpton, C., Clare, J.J., and Dolphin, A.C. (2002). The novel product of a five-exon stargazin-related gene abolishes Ca(V)2.2 calcium channel expression. EMBO J. *21*, 1514–1523.

Nakagawa, T., Cheng, Y., Ramm, E., Sheng, M., and Walz, T. (2005). Structure and different conformational states of native AMPA receptor complexes. Nature *433*, 545–549.

Nakagawa, T., Cheng, Y., Sheng, M., and Walz, T. (2006). Three-dimensional structure of an AMPA receptor without associated stargazin/TARP proteins. Biol. Chem. *387*, 179–187.

Ng, D., Pitcher, G.M., Szilard, R.K., Sertié, A., Kanisek, M., Clapcote, S.J., Lipina, T., Kalia, L.V., Joo, D., McKerlie, C., et al. (2009). Neto1 is a novel CUB-domain NMDA receptor-interacting protein required for synaptic plasticity and learning. PLoS Biol. *7*, e41.

Nicoll, R.A., Tomita, S., and Bredt, D.S. (2006). Auxiliary subunits assist AMPA-type glutamate receptors. Science *311*, 1253–1256.

Nissenbaum, J., Devor, M., Seltzer, Z., Gebauer, M., Michaelis, M., Tal, M., Dorfman, R., Abitbul-Yarkoni, M., Lu, Y., Elahipanah, T., et al. (2010). Susceptibility to chronic pain following nerve injury is genetically affected by CACNG2. Genome Res. *20*, 1180–1190.

Noebels, J.L., Qiao, X., Bronson, R.T., Spencer, C., and Davisson, M.T. (1990). Stargazer: a new neurological mutant on chromosome 15 in the mouse with prolonged cortical seizures. Epilepsy Res. *7*, 129–135.

Norton, R.S., and Pallaghy, P.K. (1998). The cystine knot structure of ion channel toxins and related polypeptides. Toxicon *36*, 1573–1583.

Opazo, P., Labrecque, S., Tigaret, C.M., Frouin, A., Wiseman, P.W., De Koninck, P., and Choquet, D. (2010). CaMKII triggers the diffusional trapping of surface AMPARs through phosphorylation of stargazin. Neuron *67*, 239–252.

Osten, P., and Stern-Bach, Y. (2006). Learning from stargazin: the mouse, the phenotype and the unexpected. Curr. Opin. Neurobiol. *16*, 275–280.

Partin, K.M., Patneau, D.K., and Mayer, M.L. (1994). Cyclothiazide differentially modulates desensitization of alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptor splice variants. Mol. Pharmacol. *46*, 129–138.

Patneau, D.K., and Mayer, M.L. (1991). Kinetic analysis of interactions between kainate and AMPA: evidence for activation of a single receptor in mouse hippocampal neurons. Neuron *6*, 785–798.

Payne, H.L. (2008). The role of transmembrane AMPA receptor regulatory proteins (TARPs) in neurotransmission and receptor trafficking (Review). Mol. Membr. Biol. *25*, 353–362.

Penn, A.C., Williams, S.R., and Greger, I.H. (2008). Gating motions underlie AMPA receptor secretion from the endoplasmic reticulum. EMBO J. *27*, 3056–3068.

Pongs, O., and Schwarz, J.R. (2010). Ancillary subunits associated with voltage-dependent K+ channels. Physiol. Rev. *90*, 755–796.

Powers, J., and Barlowe, C. (1998). Transport of axl2p depends on erv14p, an ER-vesicle protein related to the Drosophila cornichon gene product. J. Cell Biol. *142*, 1209–1222.

Priel, A., Kolleker, A., Ayalon, G., Gillor, M., Osten, P., and Stern-Bach, Y. (2005). Stargazin reduces desensitization and slows deactivation of the AMPA-type glutamate receptors. J. Neurosci. *25*, 2682–2686.

Raman, I.M., and Trussell, L.O. (1992). The kinetics of the response to glutamate and kainate in neurons of the avian cochlear nucleus. Neuron *9*, 173–186.

Roth, S., Neuman-Silberberg, F.S., Barcelo, G., and Schüpbach, T. (1995). cornichon and the EGF receptor signaling process are necessary for both anterior-posterior and dorsal-ventral pattern formation in Drosophila. Cell *81*, 967–978.

Rouach, N., Byrd, K., Petralia, R.S., Elias, G.M., Adesnik, H., Tomita, S., Karimzadegan, S., Kealey, C., Bredt, D.S., and Nicoll, R.A. (2005). TARP gamma-8

controls hippocampal AMPA receptor number, distribution and synaptic plasticity. Nat. Neurosci. *8*, 1525–1533.

Rousset, M., Cens, T., Restituito, S., Barrere, C., Black, J.L., 3rd, McEnery, M.W., and Charnet, P. (2001). Functional roles of gamma2, gamma3 and gamma4, three new Ca2+ channel subunits, in P/Q-type Ca2+ channel expressed in Xenopus oocytes. J. Physiol. *532*, 583–593.

Sager, C., Tapken, D., Kott, S., and Hollmann, M. (2009a). Functional modulation of AMPA receptors by transmembrane AMPA receptor regulatory proteins. Neuroscience *158*, 45–54.

Sager, C., Terhag, J., Kott, S., and Hollmann, M. (2009b). C-terminal domains of transmembrane alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptor regulatory proteins not only facilitate trafficking but are major modulators of AMPA receptor function. J. Biol. Chem. *284*, 32413–32424.

Sainlos, M., Tigaret, C., Poujol, C., Olivier, N.B., Bard, L., Breillat, C., Thiolon, K., Choquet, D., and Imperiali, B. (2011). Biomimetic divalent ligands for the acute disruption of synaptic AMPAR stabilization. Nat. Chem. Biol. *7*, 81–91.

Scannevin, R.H., and Huganir, R.L. (2000). Postsynaptic organization and regulation of excitatory synapses. Nat. Rev. Neurosci. *1*, 133–141.

Schmidt, C., Klein, C., and Hollmann, M. (2009). Xenopus laevis oocytes endogenously express all subunits of the ionotropic glutamate receptor family. J. Mol. Biol. *390*, 182–195.

Schnell, E., Sizemore, M., Karimzadegan, S., Chen, L., Bredt, D.S., and Nicoll, R.A. (2002). Direct interactions between PSD-95 and stargazin control synaptic AMPA receptor number. Proc. Natl. Acad. Sci. USA *99*, 13902–13907.

Schober, D.A., Gill, M.B., Yu, H., Gernert, D.L., Jeffries, M.W., Ornstein, P.L., Kato, A.S., Felder, C.C., and Bredt, D.S. (2011). Transmembrane AMPA receptor regulatory proteins and cornichon-2 allosterically regulate AMPA receptor antagonists and potentiators. J Biol. Chem. *286*, 13134–13142.

Schwenk, J., Harmel, N., Zolles, G., Bildl, W., Kulik, A., Heimrich, B., Chisaka, O., Jonas, P., Schulte, U., Fakler, B., and Klöcker, N. (2009). Functional proteomics identify cornichon proteins as auxiliary subunits of AMPA receptors. Science *323*, 1313–1319.

Selvakumar, B., Huganir, R.L., and Snyder, S.H. (2009). S-nitrosylation of stargazin regulates surface expression of AMPA-glutamate neurotransmitter receptors. Proc. Natl. Acad. Sci. USA *106*, 16440–16445.

Shanks, N.F., Maruo, T., Farina, A.N., Ellisman, M.H., and Nakagawa, T. (2010). Contribution of the global subunit structure and stargazin on the maturation of AMPA receptors. J. Neurosci. *30*, 2728–2740.

Shi, Y., Lu, W., Milstein, A.D., and Nicoll, R.A. (2009). The stoichiometry of AMPA receptors and TARPs varies by neuronal cell type. Neuron *62*, 633–640.

Shi, Y., Suh, Y.H., Milstein, A.D., Isozaki, K., Schmid, S.M., Roche, K.W., and Nicoll, R.A. (2010). Functional comparison of the effects of TARPs and cornichons on AMPA receptor trafficking and gating. Proc. Natl. Acad. Sci. USA *107*, 16315–16319.

Silberberg, G., Levit, A., Collier, D., St Clair, D., Munro, J., Kerwin, R.W., Tondo, L., Floris, G., Breen, G., and Navon, R. (2008). Stargazin involvement with bipolar disorder and response to lithium treatment. Pharmacogenet. Genomics *18*, 403–412.

Silver, R.A., and Kanichay, R.T. (2008). Neuroscience. Refreshing connections. Science *320*, 183–184.

Sobolevsky, A.I., Rosconi, M.P., and Gouaux, E. (2009). X-ray structure, symmetry and mechanism of an AMPA-subtype glutamate receptor. Nature *462*, 745–756.

Song, I., and Huganir, R.L. (2002). Regulation of AMPA receptors during synaptic plasticity. Trends Neurosci. *25*, 578–588.

Soto, D., Coombs, I.D., Kelly, L., Farrant, M., and Cull-Candy, S.G. (2007). Stargazin attenuates intracellular polyamine block of calcium-permeable AMPA receptors. Nat. Neurosci. *10*, 1260–1267.

Soto, D., Coombs, I.D., Renzi, M., Zonouzi, M., Farrant, M., and Cull-Candy, S.G. (2009). Selective regulation of long-form calcium-permeable AMPA receptors by an atypical TARP, gamma-5. Nat. Neurosci. *12*, 277–285.

Stein, E.L., and Chetkovich, D.M. (2010). Regulation of stargazin synaptic trafficking by C-terminal PDZ ligand phosphorylation in bidirectional synaptic plasticity. J. Neurochem. *113*, 42–53.

Stöhr, H., Berger, C., Fröhlich, S., and Weber, B.H. (2002). A novel gene encoding a putative transmembrane protein with two extracellular CUB domains and a low-density lipoprotein class A module: isolation of alternatively spliced isoforms in retina and brain. Gene. *286*, 223–231.

Sumioka, A., Yan, D., and Tomita, S. (2010). TARP phosphorylation regulates synaptic AMPA receptors through lipid bilayers. Neuron *66*, 755–767.

Sun, Y., Olson, R., Horning, M., Armstrong, N., Mayer, M., and Gouaux, E. (2002). Mechanism of glutamate receptor desensitization. Nature *417*, 245–253.

Suzuki, E., Kessler, M., and Arai, A.C. (2008). The fast kinetics of AMPA GluR3 receptors is selectively modulated by the TARPs gamma 4 and gamma 8. Mol. Cell. Neurosci. *38*, 117–123.

Swanson, G.T., and Heinemann, S.F. (1998). Heterogeneity of homomeric GluR5 kainate receptor desensitization expressed in HEK293 cells. J. Physiol. *513*, 639–646.

Swanson, G.T., Kamboj, S.K., and Cull-Candy, S.G. (1997). Single-channel properties of recombinant AMPA receptors depend on RNA editing, splice variation, and subunit composition. J. Neurosci. *17*, 58–69.

Tigaret, C., and Choquet, D. (2009). Neuroscience. More AMPAR garnish. Science *323*, 1295–1296.

Tomita, S. (2010). Regulation of ionotropic glutamate receptors by their auxiliary subunits. Physiology (Bethesda) *25*, 41–49.

Tomita, S., Chen, L., Kawasaki, Y., Petralia, R.S., Wenthold, R.J., Nicoll, R.A., and Bredt, D.S. (2003). Functional studies and distribution define a family of transmembrane AMPA receptor regulatory proteins. J. Cell Biol. *161*, 805–816.

Tomita, S., Fukata, M., Nicoll, R.A., and Bredt, D.S. (2004). Dynamic interaction of stargazin-like TARPs with cycling AMPA receptors at synapses. Science *303*, 1508–1511.

Tomita, S., Stein, V., Stocker, T.J., Nicoll, R.A., and Bredt, D.S. (2005a). Bidirectional synaptic plasticity regulated by phosphorylation of stargazin-like TARPs. Neuron *45*, 269–277.

Tomita, S., Adesnik, H., Sekiguchi, M., Zhang, W., Wada, K., Howe, J.R., Nicoll, R.A., and Bredt, D.S. (2005b). Stargazin modulates AMPA receptor gating and trafficking by distinct domains. Nature *435*, 1052–1058.

Tomita, S., Sekiguchi, M., Wada, K., Nicoll, R.A., and Bredt, D.S. (2006). Stargazin controls the pharmacology of AMPA receptor potentiators. Proc. Natl. Acad. Sci. USA *103*, 10064–10067.

Tomita, S., Shenoy, A., Fukata, Y., Nicoll, R.A., and Bredt, D.S. (2007a). Stargazin interacts functionally with the AMPA receptor glutamate-binding module. Neuropharmacology *52*, 87–91.

Tomita, S., Byrd, R.K., Rouach, N., Bellone, C., Venegas, A., O'Brien, J.L., Kim, K.S., Olsen, O., Nicoll, R.A., and Bredt, D.S. (2007b). AMPA receptors and stargazin-like transmembrane AMPA receptor-regulatory proteins mediate hippocampal kainate neurotoxicity. Proc. Natl. Acad. Sci. USA *104*, 18784–18788.

Traynelis, S.F., Wollmuth, L.P., McBain, C.J., Menniti, F.S., Vance, K.M., Ogden, K.K., Hansen, K.B., Yuan, H., Myers, S.J., and Dingledine, R. (2010). Glutamate receptor ion channels: structure, regulation, and function. Pharmacol. Rev. *62*, 405–496.

Tsui, J., and Malenka, R.C. (2006). Substrate localization creates specificity in calcium/calmodulin-dependent protein kinase II signaling at synapses. J. Biol. Chem. *281*, 13794–13804.

Turetsky, D., Garringer, E., and Patneau, D.K. (2005). Stargazin modulates native AMPA receptor functional properties by two distinct mechanisms. J. Neurosci. *25*, 7438–7448.

Vacher, H., Mohapatra, D.P., and Trimmer, J.S. (2008). Localization and targeting of voltage-dependent ion channels in mammalian central neurons. Physiol. Rev. *88*, 1407–1447.

Cell PRESS

Vandenberghe, W., Nicoll, R.A., and Bredt, D.S. (2005a). Stargazin is an AMPA receptor auxiliary subunit. Proc. Natl. Acad. Sci. USA *102*, 485–490.

Vandenberghe, W., Nicoll, R.A., and Bredt, D.S. (2005b). Interaction with the unfolded protein response reveals a role for stargazin in biosynthetic AMPA receptor transport. J. Neurosci. *25*, 1095–1102.

Vitt, U.A., Hsu, S.Y., and Hsueh, A.J. (2001). Evolution and classification of cystine knot-containing hormones and related extracellular signaling molecules. Mol. Endocrinol. *15*, 681–694.

Vlachová, V., Vyklický, L., Vyklický, L., Jr., and Vyskocil, F. (1987). The action of excitatory amino acids on chick spinal cord neurones in culture. J. Physiol. *386*, 425–438.

von Engelhardt, J., Mack, V., Sprengel, R., Kavenstock, N., Li, K.W., Stern-Bach, Y., Smit, A.B., Seeburg, P.H., and Monyer, H. (2010). CKAMP44: a brain-specific protein attenuating short-term synaptic plasticity in the dentate gyrus. Science *327*, 1518–1522.

Walker, C.S., Brockie, P.J., Madsen, D.M., Francis, M.M., Zheng, Y., Koduri, S., Mellem, J.E., Strutz-Seebohm, N., and Maricq, A.V. (2006a). Reconstitution of invertebrate glutamate receptor function depends on stargazin-like proteins. Proc. Natl. Acad. Sci. USA *103*, 10781–10786.

Walker, C.S., Francis, M.M., Brockie, P.J., Madsen, D.M., Zheng, Y., and Maricq, A.V. (2006b). Conserved SOL-1 proteins regulate ionotropic glutamate receptor desensitization. Proc. Natl. Acad. Sci. USA *103*, 10787–10792.

Wang, R., Walker, C.S., Brockie, P.J., Francis, M.M., Mellem, J.E., Madsen, D.M., and Maricq, A.V. (2008). Evolutionary conserved role for TARPs in the gating of glutamate receptors and tuning of synaptic function. Neuron *59*, 997–1008.

Wollmuth, L.P., and Sobolevsky, A.I. (2004). Structure and gating of the glutamate receptor ion channel. Trends Neurosci. *27*, 321–328.

Wyllie, D.J., Traynelis, S.F., and Cull-Candy, S.G. (1993). Evidence for more than one type of non-NMDA receptor in outside-out patches from cerebellar granule cells of the rat. J. Physiol. *463*, 193–226.

Yamauchi, T. (2002). Molecular constituents and phosphorylation-dependent regulation of the post-synaptic density. Mass Spectrom. Rev. *21*, 266–286.

Yamazaki, M., Ohno-Shosaku, T., Fukaya, M., Kano, M., Watanabe, M., and Sakimura, K. (2004). A novel action of stargazin as an enhancer of AMPA receptor activity. Neurosci. Res. *50*, 369–374.

Yamazaki, M., Fukaya, M., Hashimoto, K., Yamasaki, M., Tsujita, M., Itakura, M., Abe, M., Natsume, R., Takahashi, M., Kano, M., et al. (2010). TARPs gamma-2 and gamma-7 are essential for AMPA receptor expression in the cerebellum. Eur. J. Neurosci. *31*, 2204–2220.

Zhang, W., St-Gelais, F., Grabner, C.P., Trinidad, J.C., Sumioka, A., Morimoto-Tomita, M., Kim, K.S., Straub, C., Burlingame, A.L., Howe, J.R., and Tomita, S. (2009). A transmembrane accessory subunit that modulates kainate-type glutamate receptors. Neuron *61*, 385–396.

Zheng, Y., Mellem, J.E., Brockie, P.J., Madsen, D.M., and Maricq, A.V. (2004). SOL-1 is a CUB-domain protein required for GLR-1 glutamate receptor function in C. elegans. Nature *427*, 451–457.

Zheng, Y., Brockie, P.J., Mellem, J.E., Madsen, D.M., Walker, C.S., Francis, M.M., and Maricq, A.V. (2006). SOL-1 is an auxiliary subunit that modulates the gating of GLR-1 glutamate receptors in Caenorhabditis elegans. Proc. Natl. Acad. Sci. USA *103*, 1100–1105.

Ziff, E.B. (2007). TARPs and the AMPA receptor trafficking paradox. Neuron *53*, 627–633.

Zorumski, C.F., and Yang, J. (1988). AMPA, kainate, and quisqualate activate a common receptor-channel complex on embryonic chick motoneurons. J. Neurosci. *8*, 4277–4286.