The C-terminal domains of TARPs
Unexpectedly versatile domains

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AMPAR receptors mediate the majority of fast synaptic transmission in the central nervous system and are therefore among the most intensively studied ligand-gated ion channels over the last decades. However, the recent discovery that native AMPAR receptor complexes contain auxiliary subunits classified as transmembrane AMPAR receptor regulatory proteins (TARPs) was quite a surprise and dramatically changed the field of AMPAR receptor research. TARPs regulate trafficking as well as synaptic localization of AMPAR receptors, and alter their pharmacological and biophysical properties, generally resulting in strongly elevated receptor-mediated currents. In this regard, unravelling the mechanisms by which TARPs modulate AMPAR receptor function is an intriguing challenge. Studying the functional importance of the carboxy-terminal domain (CTD) of TARPs for receptor modulation, we found that the increased trafficking mediated by the two TARPs γ2 and γ3 is attributable to their CTDS. Furthermore, we demonstrated that the CTD additionally determines the differences between TARPs regarding their modulation of AMPAR receptor function. As a case in point, we showed a unique role of the CTD of γ4, suggesting that TARPs modulate AMPAR receptor function via individual mechanisms.

The protein stargazin was the first transmembrane protein found to modulate AMPAR receptors and was originally termed γ2 because of its homology to the voltage-dependent calcium channel subunit γ1.1,3 In the following years five additional, homologous stargazin-like proteins (γ3, γ4, γ5, γ7 and γ8) were identified and collectively referred to as transmembrane AMPAR receptor regulatory proteins.3-5

Based on functional differences the family of TARPs was recently subdivided into two subfamilies referred to as “type I TARPs” (γ2, γ3, γ4 and γ8) and “type II TARPs” (γ5 and γ7).5 Although research on TARPs has boomed lately, the mechanisms whereby they modulate AMPAR receptors are still poorly understood and have thus become our focus of research. The CTD is the largest domain of a TARP, containing a conserved type I PDZ-binding motif and nine conserved serine residues, which have been implicated in mechanisms of synaptic plasticity.2,6 This prompted us to study this region in more detail using a domain transplantation approach: We replaced the CTDS of the four type I TARPs by that of γ1, which lacks TARP properties, to investigate whether the sequence differences among the CTDS account for the functional differences between individual TARPs. We found that replacing the CTDS markedly reduced the potentiation of agonist-induced AMPAR receptor current responses by TARPs.7 In this addendum we take the opportunity to examine the mechanisms for this reduction of TARP function more closely. We demonstrate that our chimeric approach discloses hidden functions of the TARP CTDS, highlighting the importance and versatility of this domain.
The C-Terminal Domain as an Accelerator of Trafficking

The first characterization of our TARP chimeras carrying the CTD of γ2, its C-terminal chimera with γ1, and a wild-type γ1 control. Confocal sections of Xenopus oocytes representative of 4–5 oocytes each are shown demonstrating plasma membrane expression of γ2-EGFP, γ2-(CT)γ1-EGFP and γ1-EGFP 1–5 days after injection of 10 ng of the respective cRNA. As a control, uninjected oocytes were used and in every picture shown, such a control had been placed adjacent to injected oocytes. Note that all image acquisition settings were identical for all samples over all days to enable comparison of fluorescent signal intensities.

|         | 1 day | 2 days | 3 days | 4 days | 5 days |
|---------|-------|--------|--------|--------|--------|
| γ2-EGFP |        |        |        |        |        |
| uninjected |       |        |        |        |        |
| γ2-(CT)γ1-EGFP |    |        |        |        |        |
| uninjected |       |        |        |        |        |
| γ1-EGFP |        |        |        |        |        |
| uninjected |       |        |        |        |        |

Replacing the CTD of γ2 by that of γ1 changes the time course of trafficking such that the chimera reaches the plasma membrane later than the wild type (Fig. 1). However, we observed robust surface expression of γ2-(CT)γ1-EGFP starting from day 2, which became virtually indistinguishable from wild-type γ2-EGFP on days 4 and 5 (Fig. 1). A very similar time course of membrane delivery was found for γ1, the donor for CTD transplantation (Fig. 1). Hence, although the γ2-(CT)γ1 chimera reaches the plasma membrane, it does not enhance surface expression of GluR1. From these data we conclude that the CTD of γ2 is essential for increasing AMPA receptor trafficking but not critical for trafficking of γ2 itself.

The CTD Determines TARP-Specific Modulation of AMPA Receptor Function

It soon turned out that the CTD of TARPs is not only responsible for the acceleration of AMPA receptor trafficking but also contributes in remarkably complex ways to various effects of TARPs on AMPA receptor function. For instance, our TARP chimeras generally potentiated agonist-induced current responses of GluR1 less dramatically than the respective wild-type TARPs. Moreover, this reduction of potentiation was not uniform but stronger for glutamate-induced than for kainate-induced currents. More detailed electrophysiological studies revealed that some effects of TARPs on AMPA receptor function critically depend on the CTD, while others are only partly affected by the CTD. Of the latter type are the TARP-mediated increases in agonist potencies and in the efficacy of the partial agonist kainate, which we still observed with the TARP chimeras, yet to a considerably lesser extent than we and others had reported for the respective wild-type TARPs. Strikingly, replacing the CTDs with that of γ1 abolished all TARP-specific differences in the modulation of glutamate potency and kainate efficacy, indicating that the CTD accounts for the TARP-specific portion of these effects, while a basal, lower-level modulation is independent of the CTD and thus persists upon its replacement.
A hallmark of TARPs is their ability to decrease the extent and to slow the kinetics of AMPA receptor desensitization.\(^9\,13\,15\) We propose that TARPs decrease AMPA receptor desensitization via destabilization of the desensitized state and an increase in the energy barrier leading to the desensitized state (Fig. 2). Interestingly, the contribution of the CTD to the inhibition of desensitization differs between \(\gamma^2\) and \(\gamma^3\) on the one hand and \(\gamma^4\) and \(\gamma^8\) on the other. While \(\gamma^2\) and \(\gamma^3\) carrying the CTD of GluR1 did not affect desensitization of GluR1 at all anymore, \(\gamma^4\) and \(\gamma^8\) were still able to decrease desensitization to some extent even when their CTDs were replaced. Thus, \(\gamma^4\) and \(\gamma^8\) seem to reduce receptor desensitization via a different mechanism that only partly depends on the CTD, setting them apart from \(\gamma^2\) and \(\gamma^3\), in which the CTD is essential (Fig. 2B and C). Besides reducing receptor desensitization, TARPs enhance AMPA receptor gating, enabling activation by competitive antagonists such as CNQX and DNQX, and increasing the probability of spontaneous gating events in the absence of agonists, probably by reducing the energy barrier for gating (Fig. 2).\(^16\,18\) According to our findings, spontaneous gating appears to be mediated by two distinct mechanisms. While one of these mechanisms is common to all TARPs and renders random movements of the AMPA receptor ligand binding domain sufficient to open the channel, the other mechanism is restricted to \(\gamma^2\) and \(\gamma^3\), which cause additional channel openings probably by a direct interaction with the ion pore or the linkers connecting the ligand binding domain and pore region. Using our domain transplantation approach, we have now demonstrated that the gating enhancement provided by \(\gamma^2\), \(\gamma^3\) and \(\gamma^8\) with all its consequences is dependent on their CTDs, whereas \(\gamma^4\) retains residual capabilities to enhance gating when its CTD is replaced (Fig. 2). This special, outstanding property of \(\gamma^4\) might reflect its special physiological role as the only TARP present throughout the brain and expressed in particularly high amounts during development.\(^4\)

In summary, the data presented provide promising new insights into the mechanisms by which TARPs modulate AMPA receptor function, introducing the carboxy-terminal domain as a determinant of functional modulation of AMPA receptors. However, it is difficult to rationalize how an intracellularly located domain triggers such a diverse set of modulatory effects as enhancement of AMPA receptor gating, reduction of receptor desensitization, and increase of agonist potencies and efficacies, which are usually thought to rely on events occurring on the extracellular side. We suggest that the CTD of TARPs has to interact with AMPA receptors at the intracellular side, as a prerequisite for enabling proper TARP-mediated modulation of AMPA receptor function.

Figure 2. Model of wild-type and chimeric TARP-mediated alteration of AMPA receptor gating and desensitization. Shown are hypothetical plots of changes in free energy during activation and desensitization of GluR1 alone (black line) and in coexpression with wild-type TARPs (blue line) and their respective C-terminal chimeras (black, red and green lines), modified after Sun et al. 2002.\(^19\)

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