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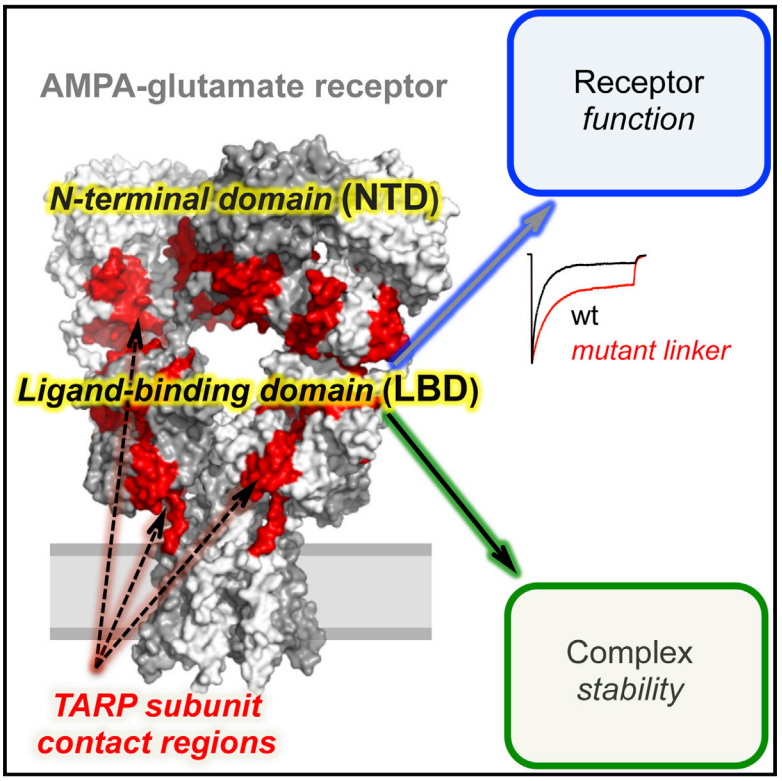
Mapping the Interaction Sites between AMPA Receptors and TARPs Reveals a Role for the Receptor N-Terminal Domain in Channel Gating

Graphical Abstract



Highlights

The NTD linker has a TARP-dependent and TARP-specific impact on AMPAR gating

Peptide arrays reveal binding of TARPs to both extracellular domains of AMPARs

A structural reorganization of AMPARs is triggered by TARP binding

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In Brief

Gating properties of synaptic AMPA-type glutamate receptors (AMPARs) are modulated by the transmembrane AMPAR regulatory proteins (TARPs), yet knowledge about their binding on a molecular level is limited. Here, Cais et al. map this interaction on both partner molecules and reveal a functional role for the receptor N-terminal domain.

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Mapping the Interaction Sites between AMPA Receptors and TARPs Reveals a Role for the Receptor N-Terminal Domain in Channel Gating

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SUMMARY

AMPA-type glutamate receptors (AMPARs) mediate fast neurotransmission at excitatory synapses. The extent and fidelity of postsynaptic depolarization triggered by AMPAR activation are shaped by AMPAR auxiliary subunits, including the transmembrane AMPAR regulatory proteins (TARPs). TARPs profoundly influence gating, an effect thought to be mediated by an interaction with the AMPAR ion channel and ligand binding domain (LBD). Here, we show that the distal N-terminal domain (NTD) contributes to TARP modulation. Alterations in the NTD-LBD linker result in TARP-dependent and TARP-selective changes in AMPAR gating. Using peptide arrays, we identify a TARP interaction region on the NTD and define the path of TARP contacts along the LBD surface. Moreover, we map key binding sites on the TARP itself and show that mutation of these residues mediates gating modulation. Our data reveal a TARP-dependent allosteric role for the AMPAR NTD and suggest that TARP binding triggers a drastic reorganization of the AMPAR complex.

INTRODUCTION

AMPA-type glutamate receptors (AMPARs) mediate fast excitatory transmission and are crucial for various forms of synaptic plasticity (Bredt and Nicoll, 2003; Cull-Candy et al., 2006). Their varied kinetic behavior (Mosbacher et al., 1994), as well as their calcium permeability and voltage-dependent block by polyamines (Cull-Candy et al., 2006; Geiger et al., 1995), varies between brain regions and appear to be adapted to the specific function of a given circuit (Jonas, 2000; Trussell, 1998). These properties depend on the nature and mRNA processing status of the four pore-forming subunits (GluA1–GluA4) (Traynelis et al., 2010; Jonas, 2000) and on the type and stoichiometry of AMPAR auxiliary subunits (Jackson and Nicoll, 2011).

Four families of auxiliary subunits have been identified: transmembrane AMPAR regulatory proteins (TARPs) (Tomita et al., 2005; Turetsky et al., 2005), cornichons (Schwenk et al., 2009), CKAMP44 (von Engelhardt et al., 2010), and GSG1L (Schwenk et al., 2012; Shanks et al., 2012). Most of these alter AMPAR gating and confer effects that can be specific for a given synapse or cell. TARPs were the first identified bona fide AMPAR auxiliary proteins, modifying both AMPAR function and trafficking. Based on their modulatory actions, TARPs have been classified as type 1a (γ-2 and γ-3), type 1b (γ-4 and γ-8), and type 2 (γ-5 and γ-7) (Kato et al., 2010). TARP-like modulation of AMPARs has also been seen in invertebrates (Walker et al., 2006; Wang et al., 2008) and thus appears highly conserved.

The precise nature of the AMPAR/TARP interaction and thus the mechanism underlying gating modulation are poorly understood. Both the AMPAR transmembrane region and the ligand binding domain (LBD) have been implicated in TARP interactions responsible for the modulation of ligand efficacy, pharmacology, gating, and pore properties (Jackson and Nicoll, 2011). Experiments using domain swapping between subtypes have identified TARP regions that are involved in regulating AMPARs. These include the extracellular loop (Ex1), the transmembrane sector, and the C terminus. Specifically, the TARP C tail appears critical for receptor trafficking and mediation of kinetic effects, while Ex1 influences both the efficacy of the partial agonist kainate and AMPAR kinetics (Tomita et al., 2005; Turetsky et al., 2005).

The most distal AMPAR domain, the N-terminal domain (NTD), is expected to be beyond the "reach" of the associated TARP. Apart from a role in subunit assembly, no clear function has been ascribed to this large and most sequence-diverse domain (Hansen et al., 2010; Kumar and Mayer, 2013), although deletion of the NTD slows desensitization kinetics (Bedoukian et al., 2006; Möykkynen et al., 2014; Pasternack et al., 2002). In stark contrast, the NTD of the N-methyl-D-aspartate (NMDA)-type glutamate receptor (NMDAR) mediates allosteric regulation of channel open probability (Paoletti, 2011) in a subunit-specific manner, rendering the NTD an important target for selective NMDAR drugs (Mony et al., 2009). NTD-mediated allostery in NMDARs has been shown to involve the ~16-residue peptide linkers that connect the NTD to the LBD (Gielen et al., 2009; Mony et al., 2011; Yuan et al., 2009).

Here we show that the AMPAR NTD plays a previously unrecognized role in signaling. Shortening of the NTD-LBD linkers altered desensitization rates and recovery from the desensitized state and increased the steady-state response. These gating effects were TARP dependent and TARP specific. Using peptide