cells affects information processing in the DCN remains to be elucidated. The authors present a feasible model whereby NA modulation of cartwheel cells may function to filter auditory information during states of attention and wakefulness. Further analysis of the physiological action of NA can be advanced by controlling activity of LC axons and studying the impact of endogenously released NA. It was shown recently that optogenetic approaches can be used to selectively activate LC axons (Carter et al., 2010). The findings by Kuo and Trussell present the opportunity to experimentally address the functional significance of NA modulation by applying these optogenetic tools to investigate how NA release from LC axons impacts the strength of cartwheel cell synapses in vitro and auditory information processing in the DCN in vivo. It is thus safe to predict that in the near future the elegant analysis of NA action accomplished by Kuo and Trussell in vitro will be integrated together with in vivo studies of NA action in intact animals.

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How Glutamate Receptor Subunits Mix and Match: Details Uncovered

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DOI 10.1016/j.neuron.2011.07.008

Until now, the atomic details explaining why certain subunits prefer to coassemble has been lacking in our understanding of glutamate receptor biogenesis. In this issue, Kumar et al. describe the structural basis by which preferential subunit assembly occurs for homomeric and heteromeric kainate-type glutamate receptors.

The requirement for assembly of multiple subunits to form a functional oligomeric complex is a shared property among ligand-gated ion channels. Several different gene products for channel subunits exist within virtually all ion channel families. This subunit multiplicity in theory allows the cell to tailor specific populations of receptors to match the needed physiological roles, a process that is typically considered dynamic. Receptors comprised of different subunit combinations often have strikingly different subcellular localization or trafficking properties and may activate and desensitize differently in response to agonist binding. The potential for cells to fine tune receptor properties through altering subunit combination is a prominent feature of the ionotropic glutamate receptors, which are the primary mediators of excitatory synaptic transmission (Traynelis et al., 2010). Following cloning of the 18 different glutamate receptor subunits almost two decades ago, it soon became apparent that certain combinations of subunits preferred to coassemble to form functional receptors in heterologous expression systems, and groups of subunits that coassembled nicely matched known receptor subfamilies (AMPA-, kainate-, and NMDA-type). This led to the obvious hypothesis that mechanisms must exist to tightly control the specificity and stoichiometry of subunit assembly. The idea that subunit assembly is tightly regulated became more intriguing when it was discovered that some neurons express several different glutamate receptor subunits capable of forming multiple homomeric and heteromeric receptor subtypes, yet only distinct subunit combinations seemed to be functionally expressed (e.g., see Lu et al., 2009). These observations hinted that assembly is not a simple stochastic process and that not all subunits are free to mix and match even within subfamilies of glutamate receptors.

Recent work on a variety of fronts has cast a spotlight on the roles of the extracellular amino-terminal domains (ATDs) of the glutamate receptor subunits (Hansen et al., 2010). These regions form a semiautonomous domain of ~400 amino

acids in all glutamate receptor subunits (Figure 1), which has been hypothesized to play a critical role in subunit assembly (reviewed in Greger et al., 2007), in addition to controlling functional properties and recognizing a host of divergent ligands ranging from ions to organic molecules to proteins (see Hansen et al., 2010). High-affinity ATD dimer formation is likely to occur early on during receptor biogenesis, perhaps even before translation of the subunit polypeptide has been fully completed (Greger et al., 2007), thereby providing a mechanism to facilitate and control the process of subunit assembly. However, even though a role for the glutamate receptor ATD in subunit assembly is well established, detailed information on the structural basis for the manner by which the ATD controls specificity and the energetics of subunit assembly have remained largely unresolved.

In this issue of Neuron, Kumar et al. (2011) use their characteristically careful experimental approaches and multiple lines of investigation to describe in detail the role of the ATD in assembly for the GluR6 and KA2 subunits (also called GluK2 and GluK5, respectively) of the kainate-type glutamate receptors. Although mechanistic details have been lacking until now, it had been recognized for years that GluR6 can form both homomeric and heteromeric receptors, whereas KA2 is an obligate heteromer that requires assembly with other kainate-type subunits to function (Egebjerg et al., 1991; Herb et al., 1992). Kumar et al. evaluate interactions between ATDs of GluR6 and KA2 using analytical size exclusion chromatography coupled with ultraviolet absorbance, refractive index and multiangle light scattering detectors (SEC-UV/RI/MALS), and analytical ultracentrifugation (AUC), providing binding constants for the association of the homomeric and heteromeric ATD combinations. The experiments elegantly demonstrate that the K_d for heteromeric GluR6/KA2 ATD dimer formation is 32,000-fold lower than that for KA2/KA2 ATD dimer formation and 23-fold lower than the K_d for GluR6/GluR6 homodimer formation under their

amino-terminal domain (ATD)

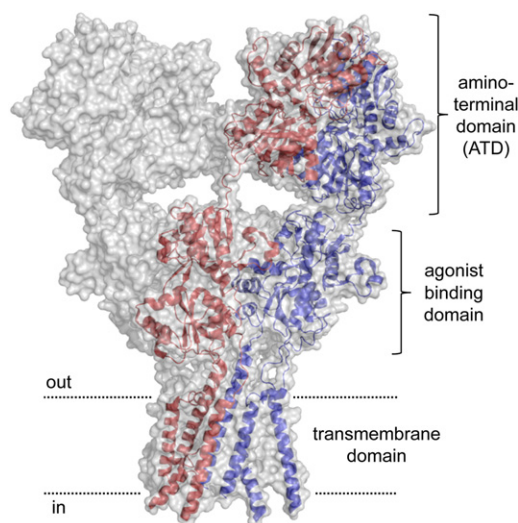agonist binding domain

out

transmembrane domain

in

Figure 1. Domain Organization in Ionotropic Glutamate Receptors
Crystal structure of the tetrameric AMPA-type glutamate receptor (GluA2; intracellular C-terminal domain omitted, PDB code 3KG2). The surface of the tetramer is shown in transparent gray, and the polypeptide backbones of two GluA2 subunits that form an ATD dimer are highlighted in blue and red. The homotetrameric GluA2 AMPA-type receptor is the only glutamate receptor for which a membrane-spanning structure exists (Sobolevsky et al., 2009). This structure provides a detailed view of the entire extracellular domain, which includes the agonist-binding domain and the amino-terminal domain (ATD), as well as the transmembrane domain, forming the ion channel pore. It has been shown that the domain organization observed in the GluA2 structure is conserved between AMPA- and kainate-type receptors (Das et al., 2010).

experimental conditions. These quantitative measurements of ATD homo dimer formation nicely correlate with observations of preferred pools of functional receptors in heterologous expression systems. That is, these data explain why GluR6 and KA2 coexpression appears to preferentially form heteromeric receptors. The high affinity of KA2 for GluR6 (K_d 11 nM) ensures that the formation of functional homomeric GluR6 receptors is essentially suppressed whenever KA2 subunits are coexpressed in the same cell.

However, the study by Kumar et al. does not stop simply with this quantification; crystal structures of the GluR6/KA2 ATD heterodimer and the GluR6 ATD homodimer provide a detailed structural view into the mechanism of ATD dimer assembly. The structures reveal local rearrangements at the dimer interface that enable key intersubunit contacts, which are unique to the heteromeric GluR6/KA2 assembly. The tip of loop 3 in the

GluR6 ATD dips down into the heterodimer interface and becomes trapped by residues from KA2; the same trapping of loop 3 is not favorable in the GluR6 homodimer due to loss of a hydrogen bond. Similarly, α-helices B and C of the KA2 ATD are positioned differently in the heterodimer, thereby allowing a series of interactions with GluR6 that are absent in the KA2 homodimer. The authors also describe a crystal structure of the ATD tetramer composed of two GluR6/KA2 dimers with the GluR6 subunits forming the dimer of dimers interface. As opposed to the strong interaction at the interface between GluR6 and KA2 ATDs, the tetrameric assembly reveals weaker interaction at the dimer of dimers interface. This important observation is consistent with the idea that the last dimer-to-tetramer transition does not involve dissociation of the ATD dimer formed initially; a similar mechanism has been proposed for AMPA-type receptors (Shanks et al., 2010).

In addition to the crystal structures, Kumar et al. show by using mutagenesis in combination with sedimentation velocity experiments that the mechanism of dimer formation is complex, involving key interactions at multiple sites in the ATD dimer interface that together govern the specificity and energetics of homomeric versus heteromeric subunit assembly. This experimental approach allows strong conclusions to be drawn regarding the contribution of individual residues to the binding energy of dimer formation. The analysis of changes in K_d for an extensive range of mutants reveals that generation of the heterodimer is mediated by residues in both the upper (R1) and lower (R2) lobes of the KA2 ATD. Furthermore, mutant-cycle analysis shows that the contribution of R1 and R2 of the KA2 ATD to heterodimer formation is additive with little cooperativity. They also show that elements of their hypothesis are compatible with activity in full-length functional receptors using chemical crosslinking of full-length receptors and functional characterization by two-electrode voltage-clamp electrophysiology. These experiments confirm that the

tetrameric ATD assembly observed in the crystal structure also occurs in full-length heteromeric kainate receptors and that the interactions, which enable the high-affinity ATD heterodimer formation, are also required for assembly of functional heteromeric receptors.

This work is timely and accompanies a wave of interest in the ATD and subunit assembly that seems poised to propel our understanding of glutamate receptor biogenesis forward. In addition to the study by Kumar et al., several studies in recent years have tackled the problem of how ATD dimer formation controls receptor assembly using high-resolution techniques (Clayton et al., 2009; Farina et al., 2011; Jin et al., 2009; Kumar and Mayer, 2010; Kumar et al., 2009; Rossmann et al., 2011; Shanks et al., 2010). We have learned how the ATDs of the AMPA-type glutamate receptor subunits (GluR1-4, also called GluA1-4) can direct selective routes of heteromeric and homomeric assembly through a wide spectrum of subunit-specific ATD association affinities (Rossmann et al., 2011). Single-particle electron microscopy has provided glimpses into the structure of stable dimers of AMPA receptor subunits, which are conceivably biosynthetic intermediates that will in turn associate with another subunit dimer to form a functional tetrameric receptor (Shanks et al., 2010). The studies on subunit assembly of AMPA-type receptors and the study by Kumar et al. on kainate-type receptor subunit assembly are consistent with the subunit arrangement observed in the crystal structure of a the membrane-spanning, tetrameric glutamate receptor (Das et al., 2010; Sobolevsky et al., 2009) (see also Figure 1). Furthermore, recent results suggest that glutamate receptors of the AMPA-type assemble via a mechanism that involves initial ATD dimer formation and, subsequently, a dimerization of dimers to form the tetrameric receptor, similar to the observations made by Kumar et al. (2009) for the GluR6/KA2 heterotetramer. Interestingly, the mechanism for subunit assembly of NMDA-type receptors could be different from those of AMPA- and kainate-type receptors (Farina et al., 2011; see also Karakas et al., 2011). The possibility of differences in receptor assembly raises the potential of a striking variation in the domain organization of NMDA- versus AMPA- and kainate-type receptors, underscoring the need for more information on the fundamental process of glutamate receptor assembly. An undeniable axiom of science is that more detail always brings more questions; in this context, the findings presented by Kumar et al. certainly provide an exciting opportunity to think at a new level about questions related to glutamate receptor biogenesis.

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