Structures of the AMPA receptor in complex with its auxiliary subunit cornichon

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In the brain, AMPA-type glutamate receptors (AMPARs) form complexes with their auxiliary subunits and mediate the majority of fast excitatory neurotransmission. Signals transduced by these complexes are critical for synaptic plasticity, learning, and memory. The two major categories of AMPAR auxiliary subunits are transmembrane AMPAR regulatory proteins (TARPs) and cornichon homologs (CNIHs); these subunits share little homology and play distinct roles in controlling ion channel gating and trafficking of AMPAR. Here, I report high-resolution cryo–electron microscopy structures of AMPAR in complex with CNIH3. Contrary to its predicted membrane topology, CNIH3 lacks an extracellular domain and instead contains four membrane-spanning helices. The protein-protein interaction interface that dictates channel modulation and the lipids surrounding the complex are revealed. These structures provide insights into the molecular mechanism for ion channel modulation and assembly of AMPAR/CNIH3 complexes.

AMPAs form complexes with various structurally unrelated auxiliary subunits, which are membrane proteins that regulate AMPAR trafficking or gating (and in some cases both) (7). The two major classes of AMPAR auxiliary subunits belong to the claudin homolog and cornichon homolog (CNIH), which share little homology (8). Transmembrane AMPAR regulatory proteins (TARPs) are members of the claudin homolog family and are the most extensively studied (7–9). Among the cornichon family, CNIH2 and -3 (CNIH2/3) are AMPAR auxiliary subunits (10). Unlike the TARPs, CNIH2/3 function at the endoplasmic reticulum where, in mammals, they may control assembly of heteromeric AMPARs (11, 12). CNIH2/3 remain associated with the synaptic AMPAR complex and, in many cases, coassemble with TARPs (11, 13, 14). Both TARPs and CNIH2/3 slow ion channel desensitization to varying degrees (7). The role of auxiliary subunits in tuning ion channel gating kinetics is predicted to have a substantial impact on circuit dynamics (7). Knowledge of the modulation mechanisms of AMPAR gating and trafficking used by various auxiliary subunits could guide rational design of new therapeutic compounds. Currently, our structural knowledge of AMPAR auxiliary subunit complexes has been limited to those that contain either TARPs or GSGIL, which are both claudin homologs (15, 16).

I investigated cryo–electron microscopy (cryo-EM) structures of complexes composed of GluA2 and CNIH3 (hereafter referred to as A2-C3) bound to the antagonist ZK200775 (280 μM). Detailed methods on sample preparation and data collection are given in the supplementary materials. In the first step of the analyses, I obtained full-length structures at an overall resolution of 4.4 Å (Figs. S3 and S4). The low flexibility between NTD and LBD interfered with further high-resolution analyses, and therefore in the second step the NTD layer and the rest of the complex (hereafter referred to as LBD-TMD-C3) were analyzed separately by focused classification and refinement (supplementary text and Figs. S3 to S10). High-resolution maps, whose overall resolutions ranged from 3.0 to 3.5 Å, and their molecular models (for statistics used, see table S1) were placed into the 4.4 Å-resolution full-length map to reconstruct the complete structure (Fig. 1).

I observed two global conformations. The pseudosymmetric conformation (PS) resembles the canonical Y shape (Fig. 1C), whereas the asymmetric conformation (AS) exhibits a tilted NTD layer relative to the LBD layer and makes interlayer contact at one corner (Fig. 1, B to D). Similar numbers of particles (AS, 218,413 particles; PS, 190,470 particles) contributed to each conformation. The maps of PS and AS revealed four extra densities in the micelle attached to the TMD of GluA2; these detected densities are CNIH3, bound at a GluA2:CNIH3 stoichiometry of 4:4 (Fig. 1, B to D).

The architectures of NTD tetramers were virtually identical between AS and PS [root mean square deviation (RMSD) of Cα, 0.374 Å] and similar to previous structures (fig. S11). Glycosylation at N241, which was eliminated by mutation in previous studies (5, 17), points toward LBD, potentially biasing the NTD layer tilted in AS and preventing it from descending vertically (Fig. 1, B, C, F, and G). In AS, K188 is close to I459 and Y469 at the interdomain contact between the NTD and LBD (Fig. 1, B and J). The NTDs might alter dynamics of LBDs through direct interaction, which could potentially result in allosteric gating modulation, such as that seen in N-methyl-D-aspartate receptors (18). The LBDs are nearly identical between AS and PS (RMSD of Cα, 0.542 Å) and also similar to those of GluA2/stargazin and GluA2/GSGIL in the closed state (15, 19) (fig. S12). Consistently, architectures below the NTD layer were virtually identical between AS and PS (RMSD of Cα, 0.526 Å) (fig. S1E). Switching between AS and PS must be a probabilistic process that requires the native NTD-LBD linker. The pore is closed, and the lower part of the channel is more compacted than the GluA2 tetramer with no auxiliary subunit (PDB: 3KG2). This compaction geometrically occludes the M2, which was unresolved (fig. S13) (see supplementary text).

An atomic model of CNIH3 was built de novo and produced the first molecular view of a member of the cornichon family. Previous studies had relied on computational models that predict CNIHs having their N termini in the cytoplasm and spanning the membrane only three times (8, 10, 20–22). Our data, however, redefine the topology of CNIH3 and reveal a geometry resembling four transmembrane segments with extracellular N and
C termi (Fig. 2, A and B). CNIH3 lacks a canonical signal peptide, but the N terminus, which remains uncleaved and buried in the membrane, appears to substitute for it. The first 12 amino acids are almost identical in mammals, flies, and worms, but not in plants or yeast (Fig. 2C). I refer to this fragment as uncleavable membrane inserting peptide (UMIP). UMIP of CNIH2 is intolerant to missense mutations in humans (23) (fig. S14).

The TM1 helix begins within UMIP, and the end of the helix penetrates into the cytoplasm. After a short unresolved loop, the TM2 starts as a cytoplasmic helix, which was misinterpreted as an extracellular loop in previous studies (8, 10, 20–22). The end of TM2 turns 180° in the membrane and connects to TM3, which re-enters the cytoplasm. A tryptophan (W88) at the junction between TM2 and TM3 is conserved among cornichons (fig. S15). The folded jackknife shape of TM2 and TM3 may be a signature of all cornichons. The majority of CNIH3 is embedded in the membrane with a small cytoplasmic domain, and thus a direct interaction between the LBD of GluA2 and CNIH3 in the extracellular space is unlikely. In contrast, TARPs and GSG1L modulate AMPARs by directly contacting the LBD in the extracellular space (24, 25). Based on the structure and the locations of functionally important mutations of CNIH3, receptor modulation must occur via the intramembrane and the cytoplasmic interaction between the two proteins (21, 26) (see supplementary text).

CNIH3 binds to the M1 and M4 of adjacent subunits of GluA2, where TARPs and GSG1L associate (Fig. 2D) (15, 16). Despite the absence of homology, the bundle of four helices of CNIH3 resembles the geometries of those of TARPs and GSG1L. Geometric conservation extends to the M1 and M4 of GluA2, which interface with the auxiliary subunits. Indeed, the helices of CNIH3 and TARP γ-8 together with the M1 and M4 of GluA2 can be superimposed, when the Cα backbones of M4 of both complexes are
aligned at an RMSD of 0.763 Å (Fig. 2D). The detailed residue contacts are different (see the supplementary text and fig. S16), even though the overall architectures of the complexes appear similar at low resolution (Fig. 2, E and F). Numerous nonprotein densities (L-a to L-h), whose features are characteristic of either lipids or detergents, surround the hydrophobic surface (Fig. 3, A and B). These densities were best resolved in the map LBD-TMD-C3lipid, which was calculated from a smaller number of particles than that used to generate the higher-resolution map LBD-TMD-C3 (fig. S9), indicative of a small structural (or occupancy) variability in the lipids and detergents that are associated with the complex. L-a to L-e and L-h were visible at a wide range of thresholds and modeled as acyl groups. L-h is a component of the inner (cytoplasmic) leaflet of the lipid bilayer and occupies the space where M2 is typically located, making contacts with both GluA2 and CNIH3 (fig. S17). The tips of L-c and L-h contact each other near the center of the membrane (Fig. 3D). L-c, an outer leaflet component, contacts both M1 (Y523 and V530) and M3 (F607) of adjacent subunits (Fig. 3C and fig. S17), and it approaches the center of the ion channel, similar to the lipid density L4 found in the heteromeric AMPAR in complex with TARP γ-8 (25). However, the contact points of L-c and L4 with AMPAR are different, except for F607 of M3. I hypothesize that the contrasting lipid geometries of AMPAR in complex with TARP γ-8 and CNIH3 contribute to their functional differences. A bulkier lipid (L-f), specific to A2-C3 and interpreted as a cholesterol group, sits next to the interaction interface between GluA2 and CNIH3, making contacts with both M4 (Y797) and TM1 (L157 and M153) (Fig. 3D and fig. S17). Within the interface, three phenylalanines (F3, -5, and -8) of CNIH3 make contacts with M4 (L789, A793, and Y797) of GluA2 (Fig. 3E). In particular, Y797 interacts with both L-f and CNIH3. Except for Y797, the residues at the interface are specific to AMPARs and are replaced by different residues in closely related kainate receptors that do not interact with CNIH3, establishing specificity for assembly. A previous study demonstrated that introducing mutations to residues L528, L789, and A793, now shown to be at the interface, destabilizes
Fig. 3. Binding interface and arrangements of lipids. (A) The densities that surround the transmembrane helices have a characteristic appearance of densities derived from lipid and detergents. The map LBD-TMD-C3lipid (see materials and methods and fig. S9) is displayed at 6.02 densities derived from lipid and detergents. The map LBD-TMD-C3lipid (see surrounding text). (B) Magnified view of the area in (A) where the lipid-like densities are attached to the complex (L-a to L-h). The tips of L-c and L-h make contact. L-f is a bulkier density whose contour resembles a cholesterol group. The visualization threshold was set at 6.02. (C) Cross section at the level indicated by the gray line in (A). The map and model are superimposed. Stars (magenta) indicate contacts between L-c and F607(M3). The details within the dashed rectangle are shown in (E). (D) Superimposed density map and a molecular model, showing contacts made between L-f (cholesterol) and side chains of GluA2 and CNIH3. The tips of L-c and L-h make contact at the arrow. The visualization threshold was set at 5.32σ. (E) Molecular architecture of the GluA2/CNIH3 interface. F3, -5, and -8 of CNIH3 (cyan) contact residues in M1 (E524, M527, C528, and F531) and M4 (L789, A793, and Y797) of adjacent subunits of GluA2. Y797 (M4) and M527 (M1) simultaneously contact lipid-like densities L-f and L-b, respectively. Y523 (M1), which does not contact CNIH3, is immediately next to the interface and contacts L-c. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

the complex and bidirectionally alters the magnitude of gating modulation by CNIH3 (26). Replacing A793 of M4 and F3 of CNIH3 with cysteine induced a disulfide cross-link, further supporting the model that two residues interact (fig. S18). I hypothesize that the binding site for the three phenylalanines (F3, -5, and -8 of CNIH3) near the extracellular surface of GluA2 dictates gating modulation and is a potential target for drugs that could be used to control the ion channel activity of AMPARs. The detailed architecture of the interaction interface suggests a possible role for lipids in regulating the assembly or function of the complexes formed by AMPAR and auxiliary subunits. The molecular model of CNIH3 is likely to serve as a reference for future investigations of the biology of the cornichon family.

REFERENCES AND NOTES
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SUPPLEMENTARY MATERIALS

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Materials and Methods
Supplementary Text
Figs. S1 to S18
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References (27–40)

View/request a protocol for this paper from Bio-protocol.

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Regulating synaptic signals

In the brain, AMPA-type glutamate receptors (AMPARs) are ion channels that play key roles in synaptic plasticity, cognition, learning, and memory. Two classes of subunits, the claudin family and the cornichon family, regulate AMPAR gating and trafficking. Previous structures have been presented of AMPAR bound to claudin homologs. Now, Nakagawa reports a high-resolution structure of AMPAR bound to the cornichon homolog CNIH3, determined by cryo–electron microscopy (see the Perspective by Schwenk and Fakler). In contrast to a predicted topology of three transmembrane helices and an intracellular amino terminus, CNIH3 has four transmembrane helices, and both the amino and carboxyl termini are extracellular. The structure reveals the architecture of the interaction interface between AMPAR and CNIH3 and suggests a role for lipids in regulating the assembly and function of the AMPAR-CNIH3 complex.

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