論文の内容の要旨

論文題目：　Studies on GluRδ2-Cbln1-NRXN1β triad for synapse formation
シナプス形成複合体GluRδ2-Cbln1-NRXN1βに関する研究

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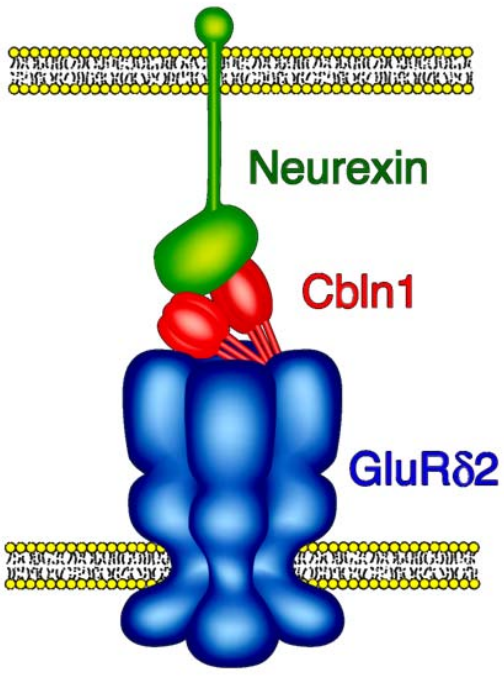
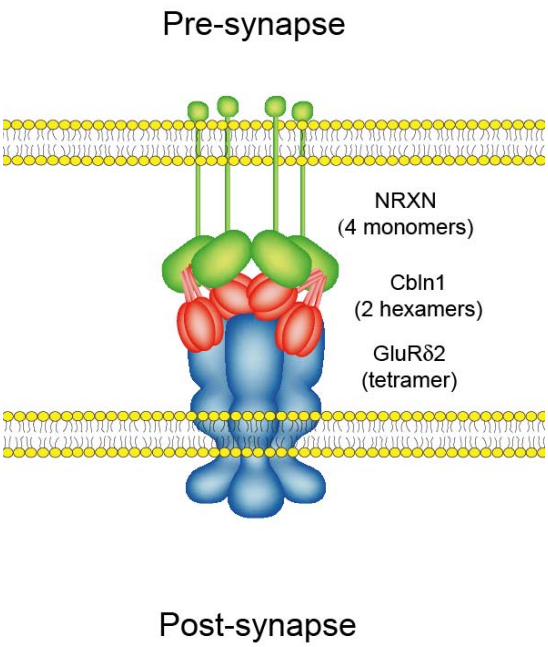
Elucidation of molecular mechanisms that regulate synapse formation is prerequisite for the understanding of neural wiring, higher brain functions and mental disorders. Despite the wealth of information, the fundamental questions about how glutamatergic synapses are formed in the mammalian brain remain unanswered. In cerebellum, there is clear *in vivo* evidence that GluRδ2, a member of the δ-type glutamate receptor (GluR), plays an essential role in cerebellar Purkinje cell (PC) synapse formation (Kashiwabuchi et al., 1995; Kurihara et al., 1997; Takeuchi et al., 2005). The cerebellum receives two excitatory afferents, the climbing fiber (CF) and the mossy fiber-parallel fiber (PF) pathway, both converging onto PCs that are the sole neurons sending outputs from the cerebellar cortex. GluRδ2 is selectively expressed in cerebellar PCs (Araki et al., 1993; Lomeli et al., 1993) and is exclusively localized at PF-PC synapses (Takayama et al., 1996; Landsend et al., 1997). A significant number of PC spines lack synaptic contacts with PF terminals and some of residual PF-PC synapses show mismatching between pre- and postsynaptic specializations in conventional and conditional GluRδ2 knockout mice (Kashiwabuchi et al., 1995; Kurihara et

al., 1997; Takeuchi et al., 2005). These studies indicate that the formation and maintenance of PF-PC synapses are critically dependent on GluRδ2 *in vivo*. The synaptogenic activity of GluRδ2 is reproduced *in vitro* using primary cultures of cerebellar granule cells (GCs). The extracellular N-terminal domain (NTD) of GluRδ2 is essential and sufficient to induce synapse formation *in vitro* (Uemura and Mishina, 2008). Thus, it is likely that GluRδ2 regulates synapse formation by direct interaction between its NTD and presynaptic protein(s). Here, I show that the NTD of GluRδ2 interacts with presynaptic neurexins (NRXNs) through Cbln1. According to the immunocytochemistry, β-NRXNs interacted, in the presence of Cbln1, with GluRδ2 expressed on the surface of HEK 293T cells. And in a protein pull-down assay, Cbln1 was coprecipitated with NTD of GluRδ2 but not with that of GluRα1 or GluRα2 and it was also coprecipitated with extracellular domain (ECD) of NRXN1β. The kinetic analysis by Surface Plasmon Resonance (SPR) binding assay showed both dissociation constant (K_D) values, 16.5 nM for Cbln1-GluRδ2 and 0.17 nM for Cbln1-NRXN1β, indicating that they interact with high affinities. These suggest that Cbln1 directly binds to the N-terminal domain of GluRδ2 and also to the extracelllar domain of NRXN1β with high affinities, acting as a divalent ligand to link postsynaptic GluRδ2 and presynaptic NRXNs in the cerebellar synapse (Figure 1).



Next, I examined the stoichiometry for the assembly of GluRδ2-Cbln1-NRXN1β triad. NTD of GluRδ2 (GluRδ2-NTD) was firstly examined for its own stoichiometry. Native-PAGE and Gel-filtration assay showed that the GluRδ2-NTD exists as a homodimer with non-disulfide interaction. Cbln1 well known

molecule as a hexamer interacted with the GluRδ2-NTD. One molecule of the Cbln1 participated in the interaction with one dimeric GluRδ2-NTD. On the other hand, when the hexameric Cbln1 interacted with NRXN1β which is known as a monomer, it bound to two NRXN1βs. With the mutation assay, I examined which regions of Cbln1 are important for the interaction with each GluRδ2 or NRXN1β. The results indicated that N-terminus of Cbln1 is relatively important to interact with NRXN1β-ECD but its C-terminus is for GluRδ2-NTD, although the interaction properties of Cbln1 to NRXN1β or GluRδ2 seemed to be regulated by its overall structure. GluRδ2 exists as a tetramer form in the plasmamembrane because the GluRδ subfamily belongs to the ionotropic GluR family and positions between the classical AMPA/kainate and NMDA subtypes from the amino acid sequence identity, (Yamazaki et al., 1992; Araki et al., 1993; Lomeli et al., 1993). Thus, the tetrameric GluRδ2, according to the present results, interacts with two Cbln1s and four NRXNs (Figure 2).



Altogether, the present results suggest that GluRδ2 interacts with NRXNs through Cbln1 for synapse formation between Purkinje cell and that parallel fiber in cerebellum and the interac tion stoichiometry of NRXN-Cbln1-GluRδ2 is 4:2:1.