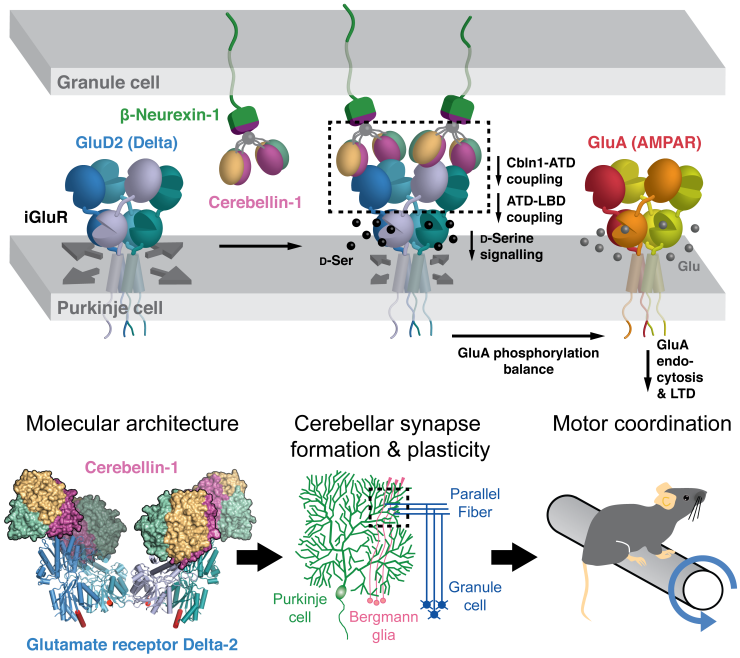
TransSynapticArch – Jonathan Elegheert

Proposal No. 328531 – Final Report

Graphic Abstract;



Synaptic organizing proteins orchestrate the formation and differentiation of excitatory and inhibitory synapses; this process underlies normal development and function of the brain.

With the discovery of the β-NRX1–Cbln1–GluD2 synapse-spanning protein complex, new principles of synaptic organization emerged; it became clear that neurotransmitter receptors embedded in the post-synaptic membrane (GluD2 is a member of the ionotropic glutamate receptor (iGluR) family) could be physically connected to the pre-synaptic neuron *via* soluble synaptic organizer proteins (in this case Cbln, but other iGluRs interact with other C1q-like family members). These latter proteins thus have the potential to modulate receptor signaling; until now however, no structure-based mechanistic understanding of their function was available.

Our research addressed these unknowns for the β-NRX1–Cbln1–GluD2 model system and describes for the first time the structure of an iGluR in complex with a physiological interacting protein. Moreover, the restricted expression pattern of GluD2 in the cerebellar Purkinje cells, and availability of well-characterized *GluD2*-null and *Cbln1*-null mice, allowed us to interrogate our structural predictions in a physiological context. Specifically, we uncovered how the small molecule neurotransmitter D-Serine and the protein ligand Cbln1 cooperate to activate GluD2 signaling.

This work is accepted for publication in the journal *Science*. Reference;

Elegheert J, Kakegawa W, Clay JE, Shanks NF, Behiels E, Matsuda K, Kohda K, Miura E, Rossmann M, Mitakidis N, Motohashi J, Chang VT, Siebold C, Greger IH,

Nakagawa T, Yuzaki M, Aricescu AR. *Science*, accepted. Structural basis for integration of GluD receptors within synaptic organizer complexes.

Below, I summarize in more detail the results obtained on this multidisciplinary project;

- Crystal structures of the extracellular domains that govern the Cbln1–GluD2 interaction; the Cbln1 C1q-like subdomains and the amino-terminal domains (ATDs) of GluD family members (GluD2 and GluD1).
- Single-particle electron microscopic imaging of soluble Cbln1 and the pre-synaptic β-NRX1–Cbln1 complex.
- Crystal structure and mutational validation of a low-affinity Cbln1–GluD2 complex. This structure is the first of a glutamate receptor in complex with a synaptic organizer.
- Full biophysical profiling of the β-NRX1–Cbln1 and Cbln1–GluD2 interactions using various techniques (SPR, ITC, AUC, MALS).
- Correlation of the architecture of the β-NRX1–Cbln1–GluD2 complex with its function at PF-PC synapses, in collaboration with the laboratory of Prof. Michisuke Yuzaki (Keio University, Tokyo). The restricted expression pattern of GluD2 and availability of well-characterized *GluD2*-null and *Cbln1*-null mice allowed us to mechanistically interrogate our structures in a physiological context by genetically introducing engineered protein variants. We showed that the Cbln1 synapse organizer is needed to "anchor" the GluD2 receptor and to allow downstream GluD2 signalling following agonist (D-Serine) binding. This leads to internalisation of post-synaptic AMPA receptors and long-term depression (LTD) of these PF-PC synapses. We validated these findings *in vivo*, where mutation of the Cbln1–GluD2 interface, or of key GluD2 structural features, impaired motor coordination or immature mice.

Our results provide a molecular framework for the large body of studies on the β-NRX1–Cbln1–GluD2 trans-synaptic signaling system, and suggest a three-step model for GluD2 signaling activation. Cbln1 is secreted from cerebellar GCs through yet unidentified mechanisms and remains associated with β-NRX1 on the surface of GC axons. When cerebellar GC axons encounter PC dendritic spines, the β-NRX1–Cbln1 complex "hooks" GluD2, via avidity-enhanced $Cbln1_{C1q}$–$GluD2_{ATD}$ interactions, in a trans-synaptic complex. The biological importance of the binding avidity most likely lies in improving the probability for molecular recruitment of GluD2 by β-NRX1–Cbln1 at PF-PC synapses, given the weak affinity between individual Cbln1 and GluD2 domains. Finally, agonist binding triggers a conformational change of the GluD2 LBD which is transmitted to the transmembrane domain to initiate downstream signaling, resulting in AMPA receptor endocytosis and LTD.

We propose that the concept illustrated here, where small molecule and protein ligands cooperate in order to modulate GluD2 signaling, is likely to be more generally applicable to neurotransmitter receptors.