Structural basis of the extracellular interaction between receptor-type tyrosine-protein phosphatase δ and neuroligin 3

Billions of neurons connected in a mammalian brain form circuits to realize brain functions. The neuronal synapse is an intercellular adhesion specialized for neuronal signal transmission and is formed between the axon terminal of one neuron and the dendrites or cell bodies of another neuron (Fig. 1(a)). During neurodevelopment, synapse formation is induced by a family of cell adhesion molecules termed synaptic organizers. Synaptic organizers on the axon terminal membrane (presynaptic organizers) and those on the dendritic or cell body membrane (postsynaptic organizers) extracellularly interact with each other in the synaptic cleft and then induce the formation of subcellular structures specialized for neurotransmitter release in the presynapse and for responding to neurotransmitters in the postsynapse (Fig. 1(b)). Functional defects in synaptic organizers potentially disturb the formation of neural circuits and play a causative role in neurodevelopmental disorders such as autism spectrum disorders (ASDs).

Receptor-type tyrosine-protein phosphatase δ (PTPδ) is a member of type IIa receptor protein tyrosine phosphatases (IIa RPTPs) and serves as a presynaptic organizer along with the two other members, LAR and PTPσ. The extracellular domain of IIa RPTPs consists of three immunoglobulin-like domains (Ig1–Ig3) and four or eight fibronectin type-III domains (FN1–FN8; Fig. 2(a)). Ig1–Ig3 of IIa RPTPs are responsible for the interaction with the partner postsynaptic organizers such as IL-1 receptor accessory protein (IL-1RAcP), IL-1RAcP-like 1 protein (IL1RAPL1), Slit- and Trk-like proteins 1–6, and synaptic adhesion-like molecules 3 and 5. The interaction with these postsynaptic organizers depends on the two splice inserts derived from the two short exons termed mini-exon A (meA) and mini-exon B (meB), which are positioned within Ig2 and at the junction between Ig2 and Ig3, respectively, in IIa RPTPs. We and other groups have extensively studied such splice-insert-dependent interaction mechanisms by crystallography in combination with structure-based mutational analyses [1].

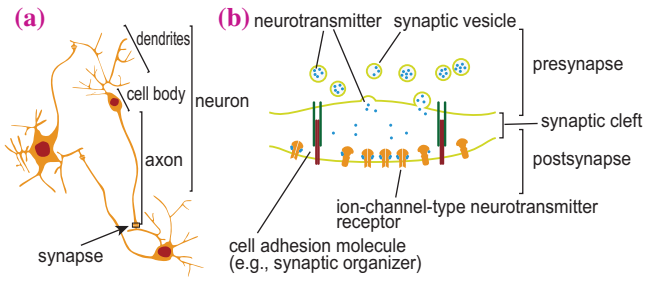

Fig. 1. Schematic of **(a)** neurons and **(b)** a synapse.

Recently, we have identified neuroligin (NLGN) 3 as a novel postsynaptic ligand for PTPδ [2]. NLGN3 is a postsynaptic adhesion molecule and interacts with presynaptic neurexins (NRXNs) to organize synaptogenesis. The NLGN3 gene is one of the best-characterized genes associated with ASDs. The esterase-like domain (ELD) in the extracellular region of NLGN3 is responsible for binding to Ig1–Ig3 of the PTPδ variant lacking the meB insert (PTPδB$^-$). To elucidate the mechanism of the extracellular interaction between PTPδ and NLGN3, we determined the crystal structure of the apo mouse NLGN3 ELD and the mouse NLGN3 ELD–mouse PTPδB$^-$ Ig1–FN1 complex at resolution of 2.76 Å and 3.85 Å, respectively [2]. X-ray diffraction data were collected at 100 K at SPring-8 **BL41XU**. The NLGN3 ELD adopts an α/β-hydrolase fold and forms a 2-fold symmetric homodimer, similarly to other NLGN family proteins [3,4]. In the complex, one PTPδ molecule binds to each protomer of the NLGN3 dimer and assembles into a W-shaped heterotetramer with a 2:2 stoichiometry (Fig. 2(b)).

Ig1, Ig2, and Ig3 of PTPδ interact with the core region of NLGN3 ELD. PTPδ Ig2 also interacts with the tail region following the core region of NLGN3 ELD. Phe170 and the aliphatic portions of Arg75, Glu77, Arg90, and Gln92 in PTPδ Ig1 form a hydrophobic pocket, which accommodates the side chain of Leu320 in NLGN3 (Fig. 3(a)). Leu141, Pro221, and Tyr225 of PTPδ Ig2 form a hydrophobic patch, which binds to Tyr302, Val305, and Ile355 of NLGN3 (Fig. 3(b)). The tail region of NLGN3 ELD lies on the 3-residue meA insertion (meA3) in PTPδ Ig2. Met614 and Phe615 of NLGN3 appear to hydrophobically interact with a hydrophobic patch formed by Leu153, Leu185, Ile190, and the aliphatic portions of Thr151 and Ser187 in PTPδ Ig2 (Fig. 3(c)). The involvement of Ile190, which is included in meA3, in this interaction is consistent with the fact that the PTPδ variant containing meA3 binds to NLGN3 with a higher affinity than the variant lacking meA3. Val257, Met261, and Ile281 of PTPδ Ig3 form a hydrophobic patch that interacts with Gly371, Leu374, and Tyr474 of NLGN3 (Fig. 3(d)). Ser236 and Arg283 of PTPδ appear to form hydrogen bonds with Asn375 and the main chain of Gly475 in NLGN3, respectively. Arg234 of PTPδ also appears to form a hydrogen bond with Glu372 of NLGN3. The spatial arrangement of Ig2 and Ig3 of PTPδ is substantially different between the ligand-free and NLGN3-bound forms (Fig. 3(e)). Upon binding to NLGN3,

PTPδ Ig3 is rearranged so that both PTPδ Ig2 and Ig3 can simultaneously interact with NLGN3. The relative positions of PTPδ Ig2 and Ig3 depend on the length of the meB-lacking linker connecting these two domains; the meB-containing linker tends to be extended and more flexible, as expected from the structure of the PTPδ variant containing meB in complex with IL1RAPL1 (Fig. 3(e)) [5].

We next designed three distinct types of NLGN3 mutant to dissect the canonical NRXN- and non-canonical PTPδB⁻-mediated NLGN3 pathways, using the structural information from the crystal structures of the NLGN–NRXN and NLGN3–PTPδB⁻ complexes. The designed mutants of NLGN3 exhibited different binding selectivities for PTPδB⁻ and NRXN1β and had potential for use as molecular tools to dissect the canonical and noncanonical transsynaptic signaling of NLGN3 (Fig. 3(f)). Then, we generated mice carrying a NLGN3 mutation that impairs either or both of the canonical and noncanonical NLGN3 pathways by CRISPR-Cas9 genome editing. The analysis of the neurons derived from the mutant mice suggested that either or both of the canonical and noncanonical NLGN3 pathways are indeed impaired in the mutant mice. The results of the analysis also suggested that the canonical and noncanonical pathways appear to counterbalance each other for inhibitory synapse formation. We further assessed the development of social behaviors in the mutant mice. The canonical pathway-impaired mice showed increased sociability, whereas the noncanonical pathway-impaired mice showed impaired social behavior and enhanced motor learning, with an imbalance in excitatory/inhibitory synaptic protein expressions. These findings from behavioral studies with the structure-based design of site-directed mutations suggest that the canonical and noncanonical NLGN3 pathways compete and regulate the development of sociality.

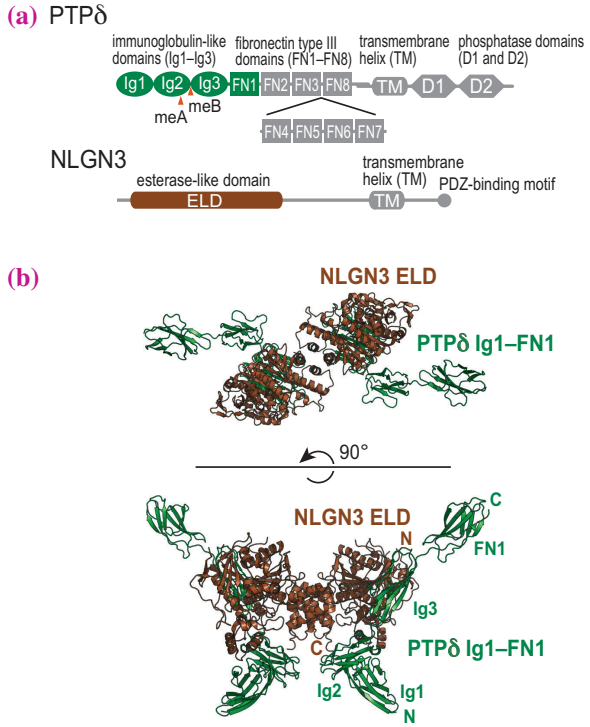

Fig. 2. **(a)** Domain organizations of PTPδ and NLGN3. The regions analyzed in this study are colored green (PTPδ Ig1–FN1) or brown (NLGN3 ELD). **(b)** Overall structure of the complex between PTPδB⁻ Ig1–FN1 and NLGN3 ELD. The coloring scheme is the same as that in (a).

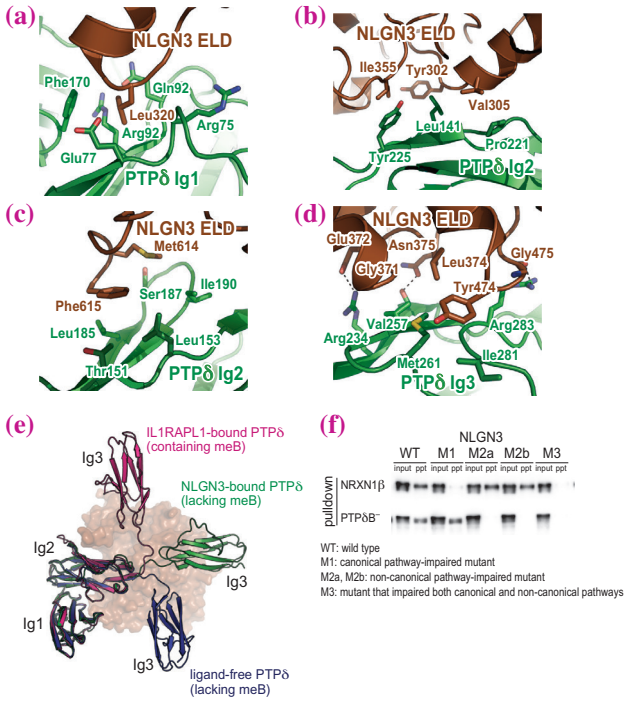

Fig. 3. **(a-d)** Close-up views of the interaction between PTPδ and NLGN3. The interacting residues are shown as sticks. Dotted lines indicate hydrogen bonding. The coloring scheme is the same as that in Fig. 2. **(e)** PTPδ structures in the apo, NLGN3-bound, and IL1RAPL1-bound states. **(f)** Pulldown analysis to test the binding selectivity of NLGN3 mutants for PTPδB⁻ and NRXN1β. Samples were analyzed by SDS-PAGE with immunostaining.

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