Structural basis of the intracellular interaction between type IIa receptor protein tyrosine phosphatases and Liprin-α

In a mammalian brain, billions of neurons are connected and form circuits for brain functions. Neurons are composed of three parts: an axon, dendrites, and a cell body (Fig. 1(a)). The axon terminal of one neuron is connected to the dendrites or cell bodies of another neuron. Neuronal signals are transmitted from the axon terminal to the attached dendrites or cell bodies. The connection between neurons is mediated by a cell-cell adhesion termed a synapse, which is specialized for neuronal signal transmission (Fig. 1(b)). The axon terminal side of the synapse is termed a presynapse, while the dendritic or cell-body side is termed a postsynapse. The pre- and postsynapses face each other across a synaptic cleft. At the presynapse, synaptic vesicles (SV) containing neurotransmitters are pooled, and the neurotransmitters are released to the synaptic cleft upon action potential-evoked Ca^{2+} influx. At the postsynapse, ion-channel-type neurotransmitter receptors generate action potentials upon binding to the neurotransmitters released to the synaptic cleft. Dense molecular assemblies or condensates are formed at both pre- and postsynapses, which are termed an active zone (AZ) and postsynaptic synaptic density (PSD), respectively. The AZ is an ultrastructure for Ca^{2+}-dependent neurotransmitter release, while the PSD is an ultrastructure for the neurotransmitter-dependent generation of action potentials. Understanding the molecular mechanisms underlying the formation of the AZ and PSD will help to understand how synapses are formed to establish a neural circuit.

Synaptic organizers are a family of cell adhesion molecules that can induce synapse formation through extracellular interaction across the synaptic cleft (Fig. 1(b)). Pre- and postsynaptic organizers interact with each other and stimulate the accumulation of pre- and postsynaptic proteins to induce the formation of the AZ and SV pools at the presynapse and the PSD at the postsynapse, respectively. Type IIa receptor protein tyrosine phosphatases (IIa RPTPs) function as presynaptic organizers. Mammalian IIa RPTPs consist of three members, LAR, PTPσ, and PTPδ. The large extracellular domain (ECD) of IIa RPTPs interacts with the ECD of various partner postsynaptic organizers. Functional defects in synaptic organizers have been reported to be associated with neurodevelopmental disorders such as autism and intellectual disability. We and other research groups have extensively studied the mechanism of the extracellular interaction between IIa RPTPs and their postsynaptic partners by crystallography and other biophysical techniques in combination with structure-based mutational analyses *in vitro* and *in cellulo* (see our latest review [1]). On the other hand, the structural basis of the intracellular interaction with downstream effectors of IIa RPTPs remained unknown.

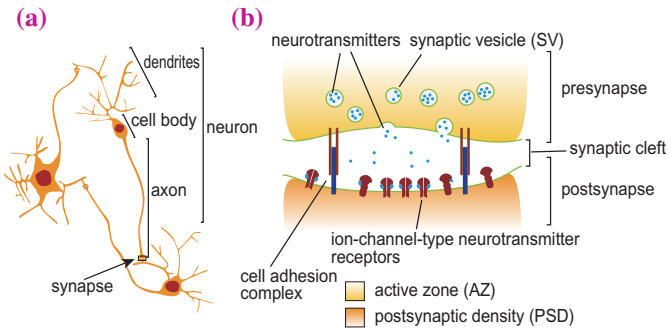

Fig. 1. Schematic of neurons **(a)** and a synapse **(b)**.

The ECD of IIa RPTPs is followed by a single transmembrane helix and a cytoplasmic domain, which is composed of two protein tyrosine phosphatase domains (Fig. 2(a)). The membrane-proximal domain (D1) is catalytically active, whereas the membrane-distal one (D2) is inactive. The D2 domain of IIa RPTPs has been reported to directly interact with several synaptic proteins including Liprin-α. The intracellular interactions of IIa RPTPs with synaptic proteins via the D2 domain are critical to the synaptogenic activity. Liprin-α is the first protein identified as an intracellular binding partner of IIa RPTPs and is localized in the AZ. Among four mammalian Liprin-α isoforms (Liprin-α1–Liprin-α4), Liprin-α2 and -α3 are predominant in the brain. All isoforms share a similar domain organization and contain an N-terminal coiled-coil domain and three tandem sterile alpha motifs (SAM1–SAM3; tSAM; Fig. 2(a)). The N-terminal coiled-coil domain binds to other AZ proteins including CAST/ELKS and RIM, whereas the tSAM domain interacts with IIa RPTPs, CASK, and Liprin-β. Liprin-α may serve as a hub for synaptic function.

To elucidate the mechanism of the interaction between IIa RPTPs and Liprin-α, we determined the crystal structure of the complex between the D2 domain of mouse PTPδ and the tSAM domain of mouse Liprin-α3 at 1.91 Å resolution (Fig. 2(b)) [2]. X-ray diffraction data were collected at 100 K at SPring-8 **BL41XU**. The D2 domain of PTPδ adopts an α/β structure similar to the reported D1 and D2 structures of IIa RPTPs [3,4]. The tSAM domain of Liprin-α3 has

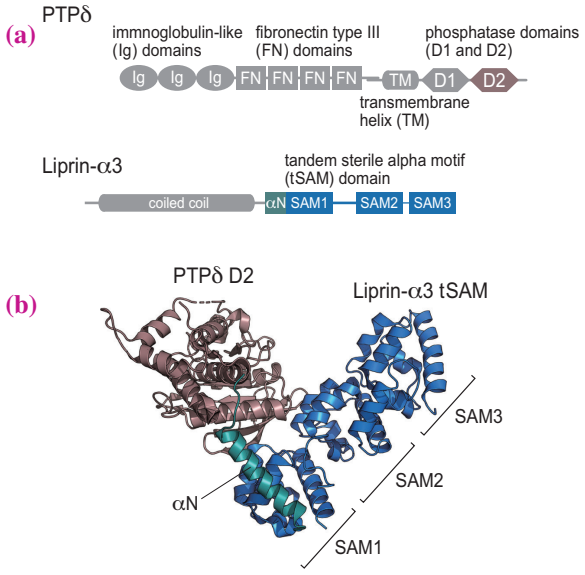

Fig. 2. **(a)** Domain organizations of PTPδ and Liprin-α3. The regions analyzed in this study are colored in brown (PTPδ D2), green (Liprin-α3 tSAM αN), and blue (Liprin-α3 tSAM SAM1–SAM3). **(b)** Overall structure of the complex between PTPδ D2 and Liprin-α3 tSAM. The coloring scheme is the same as that in (a).

an additional helix located at the N-terminal end of SAM1 (αN). The individual SAM structures and their relative configurations of the PTPδ D2-bound Liprin-α3 are similar to those of the previously reported CASK-bound Liprin-α2 [5]. Three SAMs in the tSAM domain of Liprin-α seem to function as a single structural unit.

The D2 domain of PTPδ interacts with αN, SAM1, and SAM2 of Liprin-α3 but not with SAM3. Further intermolecular interaction analysis using surface plasmon resonance (SPR) spectroscopy of site-directed mutants of PTPδ D2 or Liprin-α3 showed that the interfaces with SAM1 and SAM2 are critical to the binding between PTPδ D2 and Liprin-α3 tSAM. Tyr1373, Leu1380, Phe1399, and Phe1430 of PTPδ form a hydrophobic pocket that accommodates Trp856 of Liprin-α3 at the interface with SAM1 (Fig. 3(a)). Phe1430 of PTPδ hydrophobically interacts with Leu978 of Liprin-α3, and Arg1397 and Asp1433 of PTPδ form hydrogen bonds with Glu976 and Arg971 of Liprin-α3, respectively, at the interface with SAM2. These structural features well explain the specificity between the D2 domain of IIa RPTPs and the tSAM domain of Liprin-α proteins. The effects of the PTPδ mutations that impair the interaction with either or both SAM1 and SAM2 domains of Liprin-α on presynaptic differentiation were further examined by artificial synaptogenic assays. Briefly, single point mutations of PTPδ at the interface with Liprin-α SAM1 (Y1373A) or with SAM1/SAM2 (F1430A) disturbed the synaptogenic activity, which was observed in wild-type PTPδ and a triple mutant of PTPδ at the interface with Liprin-α αN (F1503A D1504A Y1506A). The docking analyses using other Liprin-α-binding proteins and *in vitro* binding assay suggested the tripartite assembly of IIa RPTPs, Liprin-α, and CASK (Fig. 3(b)). The predicted geometry of the tripartite complex raises the possibility that this complex can form a larger complex by binding to other AZ proteins and may function as a platform of the AZ. Further studies on this molecular assembly may help us gain a deeper understanding of the molecular mechanism of synapse formation.

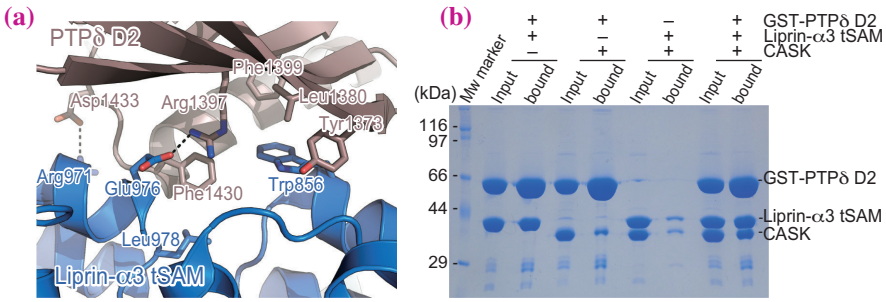

Fig. 3. **(a)** Close-up view of the interaction between PTPδ D2 and Liprin-α3 tSAM. The interacting residues are shown as sticks. Dotted lines indicate hydrogen bonding. The coloring scheme is the same as that in Fig. 2. **(b)** GST pulldown assays to test the tripartite assembly of PTPδ, Liprin-α3, and CASK. Samples were analyzed by SDS-PAGE with Coomassie Brilliant Blue staining.

Shuya Fukai

Department of Chemistry, Kyoto University

Email: fukai@kuchem.kyoto-u.ac.jp

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