論 文 の 内 容 の 要 旨

論文題目　Structural analysis of the extracellular domain of PTPδ
（PTPδの細胞外ドメインの構造解析）

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Background

Mammalian brains are composed of a few hundred billions of neurons, which are connected by synapses to establish higher-order brain functions such as learning, memory, and emotion. Synapses are specialized cell adhesions that enable cell-cell communications between neurons. Neurotransmitters are released from the axon terminal into the synaptic cleft, and then activate receptors on the dendrite membrane to transmit signals. Synaptic junctions are organized by transsynaptic cell-adhesion molecules (CAMs), which regulate synapse formation, restructuring, and elimination. CAMs are associated with neurodevelopmental disorders such as intellectual disability and autism. Type IIa receptor protein tyrosine phosphatases (RPTPs), also known as LAR subfamily of RPTPs, are among the most-characterized synaptic CAMs. Type IIa RPTPs are originally linked to axon guidance and are recently highlighted as synaptic organizers, which can induce synaptic differentiations during neural development.

The family of type IIa RPTPs in vertebrates is composed of three members: LAR, PTPσ, and PTPδ. Each member contains a large extracellular domain (ECD), a single transmembrane helix, and an intracellular domain. The ECD consists of three immunoglobulin-like (Ig) domains and four or eight fibronectin type-III (FN) domains. The length and function of the ECD are regulated by alternative splicing. The intracellular domain consists of two protein tyrosine phosphatase domains: the membrane-proximal domain D1 has catalytic activity, while the membrane-distal domain D2 is inactive (Figure 1). LAR, PTPσ, and PTPδ can induce synaptic differentiation by interacting with various postsynaptic adhesion molecules including IL-1 receptor

accessory protein-like 1 (IL1RAPL1), IL-1 receptor accessory protein (IL-1RAcP), and neurotrophin receptor tyrosine kinases C (TrkC).

Although the structural mechanism of the interaction between type IIa RPTPs and their postsynaptic ligands has been extensively studied by crystallography and other biophysical techniques, it remains unclear how the extracellular interaction transmits the signal into the intracellular domain, partly due to the lack of the structural information of the extracellular juxtamembrane region (EJR) of type IIa RPTPs. In this study, we isolated a region including the EJR from mouse or zebrafish PTPδ, and investigated the structural feature of the extracellular domain of PTPδ near the cell membrane.

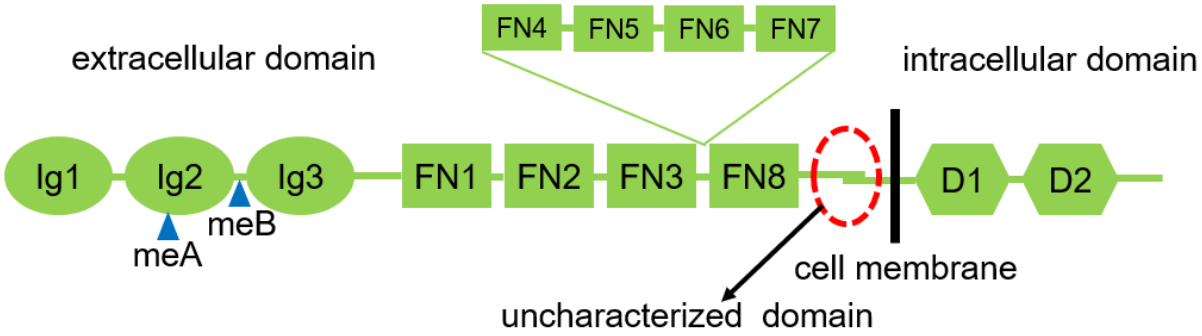

Figure 1. Domain organization of type IIa RPTPs

Methods and results

The gene encoding mouse PTPδ FN3-EJR (520-861) was cloned into pET28aSUMO vector, and B834 (DE3) *Escherichia coli* cells were transformed with the vector for protein expression. Selenomethionine (SeMet)-labeled mouse PTPδ FN3-EJR containing His_6-SUMO tag was purified by Ni-NTA affinity chromatography, followed by anion exchange chromatography and size exclusion chromatography. Finally, the purified sample was subjected to a Ni-NTA column to almost completely remove the cleaved His_6-SUMO tag. Mouse PTPδ FN3-EJR (SeMet) was crystallized by the sitting drop vapor diffusion method at 20° C, and the diffraction data set was collected at the SPring-8 beamline BL45XU (Figure 2).

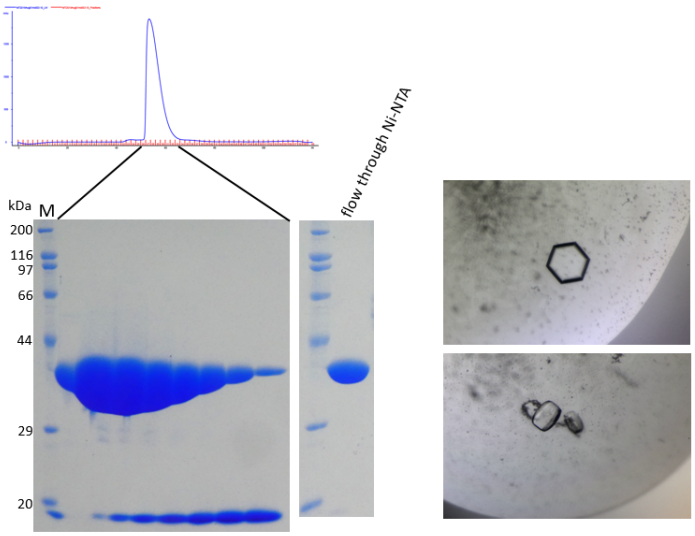

Figure 2. Purification and crystallization of mouse PTP δ FN3-EJR

The gene encoding zebrafish PTP δ FN3-EJR (512-857) was cloned into pET28aSUMO vector, and Rosetta (DE3) *E. coli* cells were transformed with the vector for protein expression. The protein containing His_6-SUMO tag was purified by Ni-NTA affinity chromatography, followed by anion exchange chromatography and size exclusion chromatography. Zebrafish PTP δ FN3-EJR was crystallized by the sitting drop vapor diffusion method at 20° C, and the diffraction data set was collected at the SPring-8 beamline BL45XU (Figure 3).

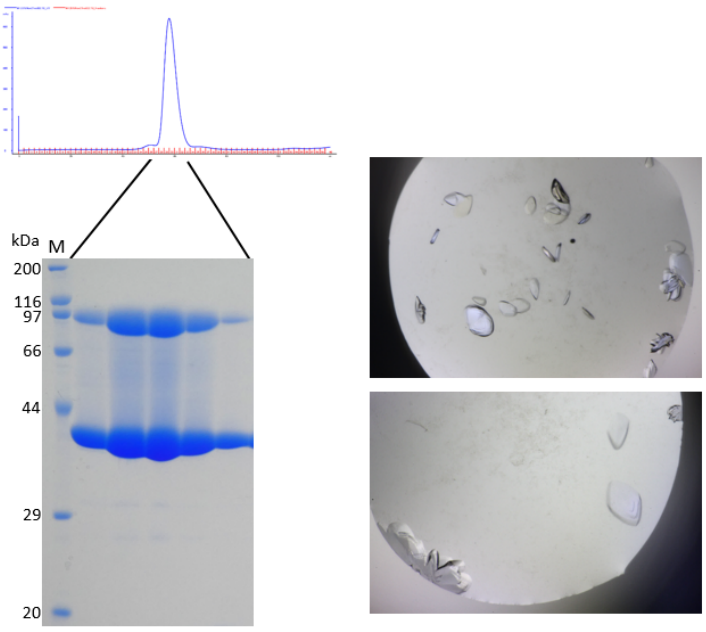

Figure 3. Purification and crystallization of zebrafish PTP δ FN3-EJR

The structure of mouse PTPδ FN3-EJR (SeMet) was solved by the anomalous diffraction method, and the structure of zebrafish PTPδ FN3-EJR was solved by the molecular replacement method, using mouse PTPδ FN3-EJR (SeMet) as the search model (Figure 4). Mouse EJR and zebrafish EJR each consists of one α-helix, several β-strands, and some flexible loop regions, the structures of EJR domains are similar to FN domains. Under physiological conditions, the EJR of PTPδ is cleaved by furin convertase, but the ECD does not dissociate from the remaining region after the processing, suggesting a tight interaction between them. Actually, the furin cleavage site is located within the loop region in EJR, and the cleavage has no effect on the fold of EJR domain.

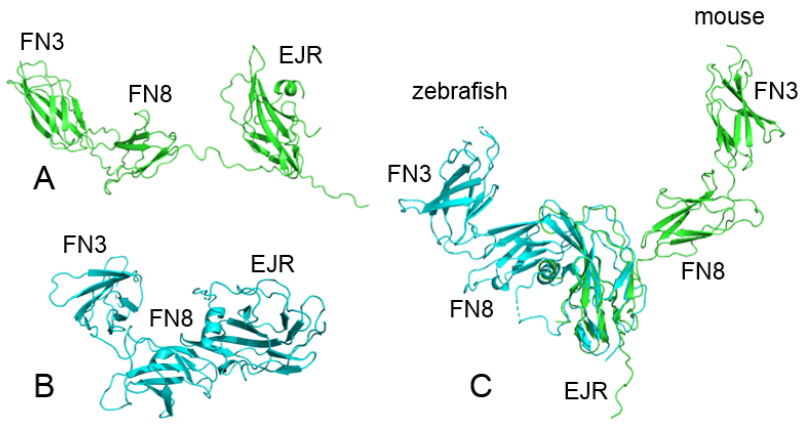

Figure 4. Crystal structures of (A) mouse PTPδ FN3-EJR (green) and (B) zebrafish PTPδ FN3-EJR (cyan). (C) Superposition of mouse PTPδ FN3-EJR and zebrafish PTPδ FN3-EJR by overlapping EJR domain.

Conclusions and perspectives

In this study, we crystallized mouse PTPδ FN3-EJR (520-861) and zebrafish PTPδ FN3-EJR (512-857) and determined their crystal structures. By comparing these two structures, we found that mouse PTPδ FN3-EJR seems to be dimerized in the form of domain swapping while zebrafish PTPδ FN3-EJR remains monomer. This indicates that there may be a dynamic equilibrium between PTPδ FN3-EJR monomer and dimer. To investigate the mechanism of PTPδ FN3-EJR monomer-dimer equilibrium, the structure analysis of the whole PTPδ ECD will be done by cryo-EM.