Mechanisms of splicing-dependent trans-synaptic adhesion by PTPδ-IL1RAPL1/IL-1RAcP for synaptic differentiation

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Synapse formation is triggered through trans-synaptic interaction between pairs of pre- and postsynaptic adhesion molecules, the specificity of which depends on splice inserts known as ‘splice-insert signaling codes’. Receptor protein tyrosine phosphatase δ (PTPδ) can bidirectionally induce pre- and postsynaptic differentiation of neurons by trans-synaptically binding to interleukin-1 receptor accessory protein (IL-1RAcP) and IL-1RAcP-like-1 (IL1RAPL1) in a splicing-dependent manner. Here, we report crystal structures of PTPδ in complex with IL1RAPL1 and IL-1RAcP. The first immunoglobulin-like (Ig) domain of IL1RAPL1 directly recognizes the first splice insert, which is critical for binding to IL1RAPL1. The second splice insert functions as an adjustable linker that positions the Ig2 and Ig3 domains of PTPδ for simultaneously interacting with the Ig1 domain of IL1RAPL1 or IL-1RAcP. We further identified the IL1RAPL1-specific interaction, which appears coupled to the first-splice-insert-mediated interaction. Our results thus reveal the decoding mechanism of splice-insert signaling codes for synaptic differentiation induced by trans-synaptic adhesion between PTPδ and IL1RAPL1/IL-1RAcP.

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Mammalian brains are composed of at least a few hundred billions of neurons, which are connected by synapses in a spatiotemporally organized manner to establish higher-order brain functions such as learning, memory and emotion. Synapse formation is triggered through trans-synaptic interactions between selective pairs of pre- and postsynaptic adhesion molecules known as ‘synaptic organizers’ or ‘synaptogenic proteins’, many of which are associated with neurodevelopmental disorders such as intellectual disability and autism [1–5]. Recent studies have highlighted a major role of type-IIa receptor protein tyrosine phosphatases (RPTPs) as synaptic organizers in pre- and postsynaptic differentiation during neural development [5,6]. Vertebrate type-IIa RPTPs comprise LAR, PTPσ and PTPδ, which share common domain architecture with a single transmembrane (TM) helix flanked by a large extracellular domain (ECD) and cytoplasmic tandem PTP domains: the membrane-proximal PTP domain (D1) is catalytically active, whereas the membrane-distal PTP domain (D2) is inactive. The ECD consists of three immunoglobulin-like (Ig) domains and four to five fibronectin type-III (Fn) domains. In mammals, presynaptic type-IIa RPTPs form trans-synaptic adhesion by specifically binding to their cognate postsynaptic organizers through their ECDs for inducing synaptic differentiation of neurons. To date, five different types of postsynaptic organizers for type-IIa RPTPs have been reported: interleukin-1 receptor (IL-1R) accessory protein (IL-1RAcP) [7], IL-1RAcP-like-1 (IL1RAPL1) [8], neurotrophin receptor tyrosine kinase C (TrkC) [9], netrin-G ligand-3 (NGL-3) [10,11] and Slit- and Trk-like (Slitrk) family proteins [12,13]. For example, co-culture with either IL-1RAcP- or IL1RAPL1-expressing non-neuronal HEK293T cells can stimulate presynaptic differentiation in contacting axons of cultured cortical neurons, however, IL1RAPL1- and IL-1RAcP-induced presynaptic differentiations are completely abolished and decreased by ~70% in the neurons lacking PTPδ, respectively [7,8]. Similarly, PTPδ can induce postsynaptic differentiation of cultured cortical neurons in a manner specific to IL1RAPL1 and IL-1RAcP (more precisely, a central nervous system-restricted IL-1RAcP isoform, which differs only in the carboxy (C)-terminal from a widely distributed isoform). On the other hand, Slitrk family proteins induce presynaptic differentiation through the interaction with type-IIa RPTPs [12,13]. Among Slitrk family proteins, Slitrk3 selectively induces inhibitory synapse formation, suggesting potential roles of synapse organizers in balancing excitatory and inhibitory synapses [12]. Various combinations of trans-synaptic adhesion between pre- and postsynaptic organizers might play an important role in generating extremely diverged but highly organized synaptic connections of neurons.

Varieties of the combinations between pre- and postsynaptic organizers rely upon structural diversities of their ECDs, which can be drastically increased by multiple isoforms and splice variants. In fact, several isoforms and splice variants of type-IIa RPTPs are expressed in mouse brain, and bind selectively to the corresponding postsynaptic organizers [5,6]. Type-IIa RPTPs commonly have three Ig domains (Ig1–Ig3) in their amino (N)-terminal regions. The first splicing site is located within Ig2, whereas the second one is in the junction between Ig2 and Ig3. These two splicing sites generate variants of the type-IIa RPTPs that lack or contain short-peptide inserts termed mini-exon peptides. A recent study has shown that Slitrk family members bind to all type-IIa RPTP members, depending on the second mini-exon peptide (meB) [14]. On the other hand, IL1RAPL1 and IL-1RAcP specifically bind to PTPδ but not to LAR or PTPσ. Furthermore, our previous cell-surface binding assays showed that both the first mini-exon peptide (meA) and meB can alter their binding properties [7,8]. MeB comprises four residues, Glu-Leu-Arg-Glu, whereas there are three variations in meA: a three-residue peptide Gly-Ser-Ile (meA3), a six-residue peptide Gly-Gly-Thr-Pro-Ile-Arg (meA6) and their tandem combination (Glu-Ser-Ile-Gly-Thr-Pro-Ile-Arg) (meA9). Binding to IL-1RAcP is significantly enhanced by the presence of both meA and meB, independently of the meA variation. In contrast, the length and sequence of meA are critically important for binding to IL1RAPL1. Only PTPδ variants containing meA9 and meA6 can bind to IL1RAPL1. The meB insertion increases binding of the meA9-containing variant, whereas the meA6-containing variant absolutely requires the combination with meB. These findings indicate that information of synaptogenic postsynaptic ligands for the type-IIa RPTPs is encoded in their splice inserts. Similarly, in another presynaptic organizer neurexin, the insert in the splice site 4 regulates the specificity to postsynaptic organizers Cbln1-GluR2 and LRRTM1/2 (refs 15,16). The splice-insert signaling code concept for specific synaptic connections was proposed originally for trans-synaptic adhesion between neurexin and neurelin, but appears likely more general for synaptogenic adhesion [3,7–9,13–18]. However, structural mechanisms underlying this concept remain elusive. To elucidate the structural basis for decoding the ‘splice-insert signaling code’ in the type-IIa RPTPs, we determined the crystal structures of PTPδ-ECD in complex with IL-1RAcP-ECD and IL1RAPL1-ECD. Together with structure-based mutational analyses by surface plasmon resonance (SPR) spectroscopy and synaptogenic assays, we reveal the structural basis of the splicing-dependent trans-synaptic adhesion by PTPδ and IL1RAPL1/IL-1RAcP for synaptic differentiation.

**Results**

**Structure of the complex between PTPδ and IL1RAPL1.** To elucidate the structural basis of the PTPδ–IL1RAPL1 interaction for trans-synaptic adhesion, we determined the crystal structures of IL1RAPL1-ECD in complex with PTPδ Ig1-Ig2 and the full-length PTPδ-ECD at 2.7 and 4.4 Å resolutions, respectively (Fig. 1a,b, Table 1). Description of PTPδ Ig1-Ig2 will be based on the 2.7-Å complex structure, whereas that of the other regions of PTPδ-ECD will be based on the 4.4-Å complex structure. For crystallography, we used a PTPδ isoform containing four Fn domains and both meA9 and meB, which is the most abundant in the developing brain and has the highest affinity for IL1RAPL1 (K<sub>d</sub> 0.15 μM) among all the PTPδ variants (Table 2). Hereafter, ‘PTPδ’ indicates this isoform, unless otherwise noted. The Ig1-Ig2 domains of PTPδ retain the binding ability for IL1RAPL1-ECD, as shown in our serial deletion analysis of PTPδ (Supplementary Fig. 1a). PTPδ-ECD binds to IL1RAPL1-ECD at a ratio of 1:1 and the asymmetric unit contains one PTPδ•IL1RAPL1 complex (Fig. 1b and Supplementary Fig. 1b). IL1RAPL1-ECD consists of three Ig domains, which are arranged in an L-shape (Fig. 1a,b). PTPδ-ECD exhibits an elongated shape with a length of 180 Å along the longest axis. The Ig1 and Ig2 domains of PTPδ tightly interact with each other to form a compact V-shaped unit, which is almost identical to the isolated Ig1-Ig2 structures of PTPδ [19] (PDB:2YD6, 2YD7) (rmsd values of 0.8–0.9 Å for 195 Cz atoms). No substantial change in the backbone structure of PTPδ Ig1-Ig2 occurred upon binding to IL1RAPL1 (Supplementary Fig. 1c). The Ig3 domain is spatially separated by the meB-containing linker and positioned apart from the Ig1-Ig2 unit. The following Fn1 and Fn2 domains are aligned in a straight line, similarly to the isolated PTPδ Fn1-Fn2 structure, which we determined at 2.0 Å resolution (Supplementary Fig. 1d). The Fn3 domain of PTPδ is oriented perpendicular to the Fn1-Fn2 domains, as observed in the PTPδ Ig1-Fn3 structure (Supplementary Fig. 1e). Electron density of the Fn4 domain and subsequent C-terminal
The region was not visible, likely due to the disorder. The length of the PTPδ-ECD/IL1RAPL1-ECD complex along the longest axis is 206 Å, which is equivalent to the average length of excitatory synaptic clefts, implying that PTPδ is kinked at the junction between the Fn2 and Fn3 domains in synapses.

**Interactions between IL1RAPL1 Ig1 and PTPδ Ig2 domains.** The Ig1 domain of IL1RAPL1 interacts with both Ig2 and Ig3 domains of PTPδ (Fig. 1b). The interface between the IL1RAPL1 Ig1 and PTPδ Ig2 domains comprises both hydrophobic and hydrophilic interactions with a buried surface area of 699 Å². These interactions occur on two β-strands comprising Arg181–Ser187 and Arg196–Glu202 in the Ig2 domain of PTPδ, between which meA9 is inserted (Fig. 1c). These two β-strands are hereafter referred to as the IL1RAPL1/IL1RAPL1-Ig1-interacting β-strands, because they also mediate the interaction with the Ig1 domain of IL1RAPL1 as described later. The first eight residues of meA9 (188Glu-Ser-Ile-Gly-Gly-Thr-Pro-Ile195) form a loop connecting these two β-strands, whereas the last residue, Arg196, extends one of the two β-strands. Trp34 of IL1RAPL1 hydrophobically interacts with Leu153, Ala198 and Leu185 of PTPδ (Fig. 1c). The affinity of the IL1RAPL1 W34A mutant for PTPδ could not be measured by surface-plasmon resonance (SPR) spectroscopy, due to substantially low signals, indicating that this hydrophobic interaction is essential for binding between PTPδ and IL1RAPL1. The aliphatic portion of PTPδ Arg196 also participates in this hydrophobic interaction, which enables the Ig2 domain of PTPδ to more tightly interact with the Ig1 domain of IL1RAPL1 (Fig. 1c). The Arg196 side chain forms a hydrogen bond with Asp37 of IL1RAPL1, which is further stabilized by a hydrogen bond with Tyr59 of IL1RAPL1. Concomitantly, the side chain of Ser187 and the main-chain N atom of Glu188 in meA9 hydrogen bond with the main-chain O atoms of IL1RAPL1 Tyr59 and Gly58, respectively. The interactions mediated by Arg196 of PTPδ are critically important: the R196A mutation of PTPδ and the D37A mutation of...
IL1RAPL1 decreased the affinity 11- and 16-fold, respectively (Table 3). The meA6-containing PTP0 variant also contains Arg196 but has a fivefold reduced affinity for IL1RAPL1 (Table 2). Therefore, this affinity reduction of the meA6-containing variant might be due to a lack of the concomitant hydrogen bonding. The atomic-level meA-specific interactions described here illustrate how the meA insertion contributes to the affinity between PTP0 and IL1RAPL1.

Relationship of meB with IL1RAPL1 Ig1–PTP0 Ig3 interaction. On the opposite side to the IL1RAPL1 Ig1–PTP0 Ig2 interface, the Ig1 domain of IL1RAPL1 interacts with the Ig3 domain of PTP0 with a buried surface area of 694 Å². The IL1RAPL1 Ig1–PTP0 Ig3 interface is stabilized by hydrophobic interactions (Fig. 1d). The side chains of IL1RAPL1 Tyr77 and PTP0 Tyr273 are aligned in an antiparallel manner to form a hydrophobic core, which is surrounded by Met75, Pro88 and Phe91 of IL1RAPL1 and Pro270, Met271, Ile291 and Thr314 of PTP0. The Y273A mutation of PTP0 decreased the affinity for IL1RAPL1 sixfold, whereas the M75A/Y77A/P88A/F91A quadruple mutation of IL1RAPL1 decreased the affinity for PTP0 19-fold (Table 3).

The meB insertion of PTP0 variant also contains Arg196 but has a fivefold reduced affinity for IL1RAPL1 (Table 2). Therefore, this affinity reduction of the meA6-containing variant might be due to a lack of the concomitant hydrogen bonding. The atomic-level meA-specific interactions described here illustrate how the meA insertion contributes to the affinity between PTP0 and IL1RAPL1.

Table 1 | Data collection and refinement statistics.

| Data collection | PTP0 Ig1-Ig2+ IL1RAPL1-ECD | PTP0-ECD+ IL1RAPL1-ECD | PTP0 Fn1-Fn2 | PTP0A3B+ Ig1-Fn2 | PTP0 Ig1-Fn2+ IL1AcP-ECD | IL1RAPL1-ECD |
|-----------------|-----------------------------|------------------------|--------------|-----------------|------------------------|--------------|
| Space group     | C2                          | I4                     | P2(1,2,1)    | P1              | P2                      | P4212        |
| Cell dimensions | a, b, c (Å)                 | α, β, γ (°)             | α, β, γ (°)  | α, β, γ (°)     | α, β, γ (°)             | α, β, γ (°)  |
| Resolution (Å)  | 50-2.70                     | (2.75-2.70)             | 50-4.40      | (4.48-4.40)     | 50-0.19                 | (2.00-1.97)  |
| Rwork/Rfree    | 0.12 (0.43)                 | 0.12 (0.43)             | 0.12 (0.33)  | 0.12 (0.33)     | 0.12 (0.33)             | 0.12 (0.33)  |
| I/σ/I           | 12.2 (2.0)                  | 17.7 (1.7)              | 26.6 (4.4)   | 4.8 (1.6)       | 10.7 (2.0)              | 10.3 (2.3)   |
| Completeness (%)| 98.7 (95.9)                 | 95.6 (86.0)             | 99.8 (99.7)  | 91.4 (84.2)     | 97.3 (92.5)             | 97.5 (93.4)  |
| Redundancy      | 51.3 (3.2)                  | 4.2 (2.2)               | 10.7 (7.7)   | 2.5 (1.9)       | 4.7 (2.9)               | 7.1 (3.3)    |

Rwork, Rfree, Rwork/Rfree, I/σ/I, Completeness, Redundancy

Table 2 | Affinities (Kd) between the splicing variants of PTP0 and IL1RAPL1/IL1RAPL1.

| PTP0 (meA/meB) | IL1RAPL1 (µM) | IL1AcP (µM) |
|---------------|---------------|-------------|
| meA9/+        | 0.15 ± 0.011  | 0.68 ± 0.013 |
| meA9/-        | 0.80 ± 0.11   | 2.0 ± 0.085  |
| meA6/+        | 0.81 ± 0.14   | 0.73 ± 0.013 |
| meA6/-        | ND            | 2.3 ± 0.016  |
| meA3/+        | ND            | 0.51 ± 0.010 |
| meA3/-        | ND            | 2.8 ± 0.053  |
| –/+           | ND            | 2.2 ± 0.075  |
| –/-           | ND            | 2.4 ± 0.075  |
| ND, not detectable. Data are presented as mean ± s.d.

Table 3 | Affinities (Kd) of PTP0 or IL1RAPL1 mutants for binding between PTP0 and IL1RAPL1.

| PTP0 (µM) | IL1RAPL1 (µM) |
|-----------|---------------|
| WT        | 0.15 ± 0.011  |
| R75A      | 2.8 ± 0.34    |
| R196A     | 1.7 ± 0.45    |
| Y273A     | 0.86 ± 0.11   |
| meB       | 0.14 ± 0.022  |
| GSSG      | 0.16 ± 0.0026 |
| QLEQ      | 0.15 ± 0.052  |
| ELREEIRE  | 0.42 ± 0.038  |
| ELREEIREL |               |

| IL1RAPL1 (µM) |
|---------------|
| WT            | 0.15 ± 0.011 |
| W34A          | ND            |
| D37A          | 2.4 ± 0.016  |
| M75A/Y77A/P88A/F91A | 2.8 ± 0.48 |
| D292A         | 2.2 ± 0.075  |

ND, not detectable. WT, wild type. Data are presented as mean ± s.d.
and orientation between the Ig2 and Ig3 domains of PTPδ (Fig. 1b). When the apo structure of PTPδ Ig1-Fn2 containing meA3 and lacking meB (PTPδA3B−) (Supplementary Fig. 1f), which we determined at 3.5 Å resolution, is superposed onto the IL1RAPL1-ECD-bound structure of PTPδ Ig1-Fn2 (Supplementary Fig. 1g) using the Ig1–Ig2 unit of PTPδ as the reference, the Ig3 domain of the apo PTPδA3B− is oriented in the opposite direction from that of the IL1RAPL1-ECD-bound PTPδ and cannot interact with IL1RAPL1, even if structural flexibility of the linker between the Ig2 and Ig3 domains of PTPδ is assumed (Supplementary Fig. 1g). Replacement of the native meB (Glu-Leu-Arg-Glu) by two tandem units of meB or a Gly-Ser-Ser-Gly or Gln-Leu-Glu-Gln tetrapeptide hardly affected the affinity, whereas that by three tandem units reduced the affinity threefold (Table 3). Insertions of four or more residues at the meB position are sufficient for binding to IL1RAPL1, but the longer insertion prevents the efficient binding. Therefore, the meB insertion likely acts as an adjustable linker to locate the Ig3 domain of PTPδ in the appropriate position for interacting with the Ig1 domain of IL1RAPL1, representing how the meB insertion contributes to the affinity between PTPδ and IL1RAPL1.

Interactions between IL1RAPL1 Ig3 and PTPδ Ig1 domains. The Ig3 domain of IL1RAPL1 interacts with the Ig1 domain of PTPδ with a buried surface area of 433 Å². The IL1RAPL1 Ig3–PTPδ Ig1-Fn2 interactions are shown in Fig. 2c–e.

**Figure 2 | Structure of the complex between PTPδ and IL-1RAcP.** (a) Domain organizations of PTPδ and IL-1RAcP. The meA and meB insertion sites of PTPδ are indicated by magenta and orange triangles, respectively. (b) Overall structure of the PTPδ Ig1-Fn2/IL-1RAcP-ECD complex. PTPδ and IL-1RAcP are coloured green and blue, respectively. The meA9 and meB insertions are highlighted in magenta and orange, respectively. N-linked glycans are shown as sticks. (c) Interactions between the Ig1 domain of IL-1RAcP and the Ig2 domain of PTPδ. The colouring scheme is the same as that in b, except that IL1RAPL1/IL-1RAcP-Ig1-interacting β-strands of PTPδ are coloured brown. Residues involved in the interactions are shown as sticks. Hydrogen bonds are indicated by dotted lines. (d) Hydrophobic interactions between the Ig1 domain of IL-1RAcP and the Ig3 domain of PTPδ. The representation scheme is the same as that in c. (e) Hydrophilic interactions between the Ig1 domain of IL-1RAcP and the Ig3 domain of PTPδ. The representation scheme is the same as that in c.
PTPα Ig1 interface is overlapped with the putative binding pocket for glycosaminoglycan chains of heparan sulfate and chondroitin sulfate proteoglycans (Supplementary Fig. 1b), which mediate axonal growth control though the interaction with the type-IIa RPTPs. Similarly to the PTPα•IL1RAPL1 complex, interactions between the Ig1 domain of IL-1RAcP and the Ig2 domain of PTPα occur on the IL1RAPL1/IL-1RAcP-Ig1-interacting β-strands (Fig. 2c). The interface between the IL-1RAcP Ig1 and PTPα Ig2 domains comprises both hydrophobic and hydrophilic interactions with a buried surface area of 726 Å². Trp27 of IL1RAPL1 hydrophobically interacts with Leu153, Ala198 and Leu185 of PTPα (Fig. 2c). The W27A mutation of IL-1RAcP reduced the affinity sevenfold (Table 4). Although Arg196 of PTPα is positioned in close proximity to Asp30 of IL-1RAcP, no observed electron density of its side chain guanidino group indicates that the interaction between Arg196 of PTPα and Asp30 of IL-1RAcP is substantially weak (Supplementary Fig. 2f). Thus, the D30A mutation of IL-1RAcP and the R196A mutation of PTPα hardly affected the affinity (Table 4). Basically, meA does not extensively interact with IL-1RAcP in the PTPα Ig1-Fn2•IL-1RAcP-ECD complex, consistent with the finding that the variation of meA does not affect the affinity for IL-1RAcP (Table 2). Only one meA-mediated interaction is a hydrogen bond between the main-chain N atom of PTPα Glu188 and the main-chain O atom of IL-1RAcP Phe53, which could be formed in the shorter mini-exon variants, meA6 and meA3, but not in the variant lacking meA. The complete deletion of meA shortens the IL1RAPL1/IL-1RAcP-Ig1-interacting β-strands, which might disturb and weaken the hydrophobic interaction of IL-1RAcP Trp27 with Leu153, Ala198 and Leu185 of PTPα, resulting in the threefold reduced affinity to IL-1RAcP (Table 2; compare meA9+/− with −/+). These structural features may reflect that the meA insertion increases the affinity between PTPα and IL-1RAcP, independently of its variation.

### Relationship of meB with IL-1RAcP Ig1–PTPα Ig3 interaction.

The position and orientation of the PTPα Ig3 domain relative to the IL-1RAcP Ig1 domain are similar to those relative to the IL1RAPL1 Ig1 domain (Figs 1b and 2b). The interface between the Ig1 domain of PTPα is not conserved in IL-1RAcP (Supplementary Fig. 2e).

### Interactions between IL-1RAcP Ig1 and PTPα Ig2.

Similarly to the PTPα•IL1RAPL1 complex, interactions between the Ig2 domain of IL-1RAcP and the Ig2 domain of PTPα occur on the IL1RAPL1/IL-1RAcP-Ig1-interacting β-strands (Fig. 2c). The interface between the IL-1RAcP Ig1 and PTPα Ig2 domains comprises both hydrophobic and hydrophilic interactions with a buried surface area of 726 Å². Trp27 of IL-1RAcP hydrophobically interacts with Leu153, Ala198 and Leu185 of PTPα (Fig. 2c). The W27A mutation of IL-1RAcP reduced the affinity sevenfold (Table 4). Although Arg196 of PTPα is positioned in close proximity to Asp30 of IL-1RAcP, no observed electron density of its side chain guanidino group indicates that the interaction between Arg196 of PTPα and Asp30 of IL-1RAcP is substantially weak (Supplementary Fig. 2f). Thus, the D30A mutation of IL-1RAcP and the R196A mutation of PTPα hardly affected the affinity (Table 4). Basically, meA does not extensively interact with IL-1RAcP in the PTPα Ig1-Fn2•IL-1RAcP-ECD complex, consistent with the finding that the variation of meA does not affect the affinity for IL-1RAcP (Table 2). Only one meA-mediated interaction is a hydrogen bond between the main-chain N atom of PTPα Glu188 and the main-chain O atom of IL-1RAcP Phe53, which could be formed in the shorter mini-exon variants, meA6 and meA3, but not in the variant lacking meA. The complete deletion of meA shortens the IL1RAPL1/IL-1RAcP-Ig1-interacting β-strands, which might disturb and weaken the hydrophobic interaction of IL-1RAcP Trp27 with Leu153, Ala198 and Leu185 of PTPα, resulting in the threefold reduced affinity to IL-1RAcP (Table 2; compare meA9+/− with −/+). These structural features may reflect that the meA insertion increases the affinity between PTPα and IL-1RAcP, independently of its variation.

### Interactions between IL-1RAcP and PTPα.

The position and orientation of the PTPα Ig3 domain relative to the IL-1RAcP Ig1 domain are similar to those relative to the IL1RAPL1 Ig1 domain (Figs 1b and 2b). The interface between

### Table 4 | Affinities (Kd) of PTPα or IL-1RAcP mutants for binding between PTPα and IL-1RAcP.

| PTPα (μM) | IL-1RAcP (μM) |
|-----------|----------------|
| WT        | 0.68 ± 0.013   |
| R75A      | 1.1 ± 0.023    |
| R196A     | 0.89 ± 0.17    |
| Y273A     | ND             |
| E286A     | 5.3 ± 0.52     |
| meB       | 0.64 ± 0.036   |
| G555      | 0.91 ± 0.019   |
| QLEQ      | 1.5 ± 0.038    |
| ELREELERE | 3.8 ± 0.21     |

| IL-1RAcP (μM) | PTPα (μM) |
|---------------|-----------|
| WT            | 0.68 ± 0.013 |
| W27A          | 4.6 ± 0.076  |
| D30A          | 8.2 ± 0.033  |
| K94A          | ND          |
| I69A/YY71A/P82A/F85A | ND          |

ND, not detectable; WT, wild type.

Data are presented as mean ± s.d.
the IL-1RACp Ig1 and PTPö Ig3 domains buries a surface area of 906 Å². The inside of the interface is stabilized by more tightly packed hydrophobic interactions than that of the IL1RAPL1 Ig1–PTPö Ig3 interface (Figs 1d and 2d). The side chains of IL-1RACp Tyr71 and PTPö Tyr273 are stacked in an antiparallel manner to form a hydrophobic core, which is surrounded by the side chains of IL-1RACp Phe85, Pro82 and Ile69 and PTPö Pro270 and Ile291 and the aliphatic portion of PTPö Thr314. The side chains of IL-1RACp Tyr71 and PTPö Tyr273 are stabilized by hydrogen bonds with the main-chain O atoms of PTPö Met271 and IL-1RACp Trp70, respectively. Furthermore, the Ig1 domain of IL-1RACp recognizes a negatively charged loop comprising G卢86, Asp287 and Asp288 of PTPö. Tyr58 and Arg86 of IL-1RACp hydrogen bond with G卢86 and Asp288 of PTPö, respectively, whereas Lys94 of IL-1RACp hydrogen bonds with both G卢86 and Asp287 of PTPö (Fig. 2e). Conformation of this negatively charged loop is supported by Met289 of PTPö. In addition, Thr67 of IL-1RACp hydrogen bonds with Lys275 of PTPö. Mutations of the IL-1RACp Ig1–PTPö Ig3 interface impaired the binding affinity more drastically than those of the IL1RAPL1 Ig1–PTPö Ig3 interface: the Y273A mutation of PTPö and the I69A/Y71A/R82A/F85A quadruple mutation of IL-1RACp completely eliminated the binding ability, whereas the K94A mutation of IL1RAPL1 and the E286A mutation of PTPö decreased the affinity 12- and 8-fold, respectively (Table 4). Therefore, the interface mediated by PTPö Ig3 is more critically important for binding to IL-1RACp than for binding to IL1RAPL1, consistent with the finding that PTPö Ig3 is essential for binding to IL-1RACp but not to IL1RAPL1 (Supplementary Figs 1a and 2a).

Replacement of the native meB (Glu-Leu-Arg-Glu) by a Gly-Ser-Ser-Gly or Gln-Leu-Glu-Gln tetrapeptide hardly affected the affinity for IL-1RAcP, whereas that by two or three tandem Gly-Ser-Ser-Gly or Gln-Leu-Glu-Gln tetrapeptide hardly affected the affinity for IL1RAPL1, supporting the notion that the meB insertion acts as an adjustable linker to control relative positions and orientations of the PTPö Ig2 and Ig3 domains for their simultaneous interactions with the Ig1 domain of IL-1RACp.

Synaptogenic activity. Finally, we investigated relationships between the structures and synaptogenic activity (that is, IL1RAPL1/IL-1RACp-induced presynaptic differentiation and PTPö-induced postsynaptic differentiation). IL1RAPL1/IL-1RACp mutations were examined by fibroblast-neuron mixed culture assays (Fig. 3): HEK293T cells expressing IL1RAPL1/IL-1RACp mutants were co-cultured with cortical neurons and then, accumulation of a presynaptic protein Bassoon in the cortical neurons was analysed (Fig. 3a–d). Because IL1RAPL1- and IL-1RACp-induced presynaptic differentiation is completely abolished and decreased by ~70% in cortical neurons prepared from PTPö-deficient mice, respectively,7,8, IL1RAPL1/IL-1RACp-induced presynaptic differentiation is mostly mediated by the mixed, cognate PTPö variants in neurons. On the other hand, to examine postsynapse-inducing activity of PTPö mutants, magnetic beads conjugated with the ECDS of PTPö mutants were co-cultured with cortical neurons, and accumulation of a postsynaptic protein Shank2 around the beads was analysed (Fig. 3e,f). PTPö-stimulating postsynaptic differentiation is mediated by IL1RAPL1, IL-1RACp and possibly other unidentified postsynaptic organizer(s) in neurons.

Synaptogenic activities of IL1RAPL1 and IL-1RACp mutants are basically correlated to their affinities to PTPö, which govern stimulation of the receptor activation and signal transmission inside cells. Synaptogenic activities of PTPö mutants are also correlated with their affinities to IL1RAPL1. Remarkably, the R75A mutation in the Ig1 domain of PTPö reduced the synaptogenic activity to ~7%. This indicates that the interaction between the IL1RAPL1 Ig3 and PTPö Ig1 domains is critically important for inducing postsynaptic differentiation. In contrast to the affinity of IL1RAPL1, that of IL-1RACp is not so consistently correlated with synaptogenic activities of PTPö mutants, possibly due to a lack of the interaction between the IL-1RACp Ig3 and PTPö Ig1 domains. Considering that IL-1RACp knockout neurons retain nearly full activity of PTPö-induced postsynaptic differentiation, we speculate that the PTPö/IL-1RACp complex might function as a unidirectional organizer that is primarily responsible for inducing presynaptic differentiation in vivo, although IL-1RACp can promote postsynaptic differentiation of cultured cortical neurons by binding to the meA3-containing PTPö variant, which is specific to IL-1RACp27. The Ig1 domain of PTPö might be universally important for postsynaptic differentiation in vivo, which involves IL1RAPL1 and possibly other unidentified postsynaptic organizer(s).

Discussion

Recently, crystal structures of two different trans-synaptic type-IIa RPTP complexes have been reported4,25. One is the complex between Ig1–Ig2 of PTPö and leucine-rich repeat (LRR)-Ig1 of TrkC25, while the other is that between Ig1–Ig3 of PTPö and the first LRR (LRR1) of Slitrk1 (ref. 14, Supplementary Fig. 3a). These and our type-IIa RPTP complex structures illustrate that the type-IIa RPTP complexes are structurally distinct architectures. The TrkC- or Slitrk1-interacting surface of the RPTPs is separated from the IL1RAPL1- or IL-1RACp-interacting surface, except that a part of the TrkC-interacting surface is overlapped with the IL1RAPL1-interacting surface. The conserved arginine residues in Ig1 of the RPTPs (that is, Arg95 and Arg98 of PTPö and Arg96 and Arg99 of PTPö) are recognized by both IL1RAPL1 and TrkC through similar hydrogen bond interactions (Supplementary Fig. 3b). Despite the separation of these binding surfaces of the RPTPs, pair-wise superposition between any two of these complex structures shows steric clashes between the bound postsynaptic partners, ruling out their simultaneous binding to the type-IIa RPTPs (Supplementary Fig. 3c). The IL1RAPL1- or IL-1RACp-interacting residues of PTPö are mostly conserved in the type-IIa RPTPs. Their differences in the specificity to IL1RAPL1 or IL-1RACp appear to be dependent exclusively on the variation of meA and meB. Our previous analysis of Ptpsr and Ptpfr CDNA from the developing mouse brain suggested that none of the PTPö variants contains the mini-exon peptide insertion at the meA site8 and that 90% of LR lacks the peptide insertion at the meB site8. Therefore, IL1RAPL1 and IL-1RACp induce synaptogenesis specifically through PTPö, at least in the developing brain. On the other hand, the meB insertion is sufficient for binding to Slitrks. They may regulate synaptogenesis mainly through PTPö or PTPö in the developing brain8,13,14, although they can potentially bind to all the type-IIa RPTP variants containing meB. Both the regulation of meA and meB choice by alternative splicing of the type-IIa RPTPs and the mutually exclusive binding among postsynaptic ligands for the type-IIa RPTPs may contribute to sharpening target specificity of central synaptogenesis.

The Ig–Ig interaction between cell-surface receptors is generally important for cell adhesion and communication in immune and neuronal systems. To our knowledge, binding between Ig-containing adhesion receptors is typically mediated by single, homotypic Ig–Ig interactions26–28 in neuronal systems. In contrast, binding between PTPö and IL1RAPL1/IL-1RACp is mediated by multiple, heterotypic Ig–Ig interactions. The meB insertion of PTPö substantially contributes to these multiple,
heterotypic Ig–Ig interactions; meB is located at the junction between the Ig2 and Ig3 domain of PTPd and adjusts their relative spacing and orientation so that they can simultaneously interact with the Ig1 domain of IL-1RAcP or IL1RAPL1 (Fig. 4a). In the IL-1RAcP/IL1RAPL1-bound state, the Ig2 and Ig3 domains of PTPd exhibits a unique conformation that differs from a linear or V-shaped conformation, which is typically observed in a tandem repeat of Ig domains. Furthermore, this unique conformation of PTPd Ig2–Ig3 also differs from the conformation in a Slitrk-bound state14. Most PTPd variants expressed in the developing mouse brain at postnatal day 11 contain meB and only 4% of variants lack meB8, suggesting that the meB-containing PTPd variants should be mostly utilized for synaptogenesis in the brain. Therefore, the insertion of meB might contribute to allowing PTPd Ig2–Ig3 to form some distinct conformations, depending on the structurally different postsynaptic ligands.

For binding of PTPd to IL1RAPL1, either meA9 or meA6 is essential. Thus, the interface including meA is likely the primary interaction site. Accordingly, the W34A mutation of IL1RAPL1 (at the interface with PTPd Ig2) eliminated binding to PTPd.
Moreover, the interaction with meA appears coupled to the third interface involving PTPδ Ig1 and IL1RAPL1 Ig3, which is critical for binding between IL1RAPL1 and PTPδ and inducing bidirectional synaptic differentiation of cultured cortical neurons, as mentioned above. Comparison between the IL1RAPL1 and IL-1RAcP complexes exhibits a rotational difference in the orientations of the PTPδ Ig1–Ig2 unit, relative to the Ig1 domains of IL1RAPL1 and IL-1RAcP (Fig. 4b). Trp34 of IL1RAPL1 is positioned at the pivot for this rotation, which allows PTPδ Ig1 to interact with IL1RAPL1 Ig3. In this context, meA also contributes to the multiple, heterotypic Ig–Ig interactions between IL1RAPL1 and PTPδ, besides meB, which assists in locating the Ig3 domain of PTPδ in the appropriate position for interacting with the Ig1 domain of IL1RAPL1. Consistent with this, the impact of the lack of meB on binding between PTPδ and IL1RAPL1 is comparable to that of the PTPδ Y273A mutation (deficient in the PTPδ Ig3–IL1RAPL1 Ig1 interaction). In contrast, the impact of the lack of meB on binding between PTPδ and IL-1RAcP is incompatible to that of the PTPδ Y273A mutation, but rather is comparable to that of mutations at the IL-1RAcP Ig1–PTPδ Ig2 interface. The W27A mutation of IL-1RAcP, which is equivalent to the W34A mutation of IL1RAPL1, reduced the affinity sevenfold but did not eliminate the binding. Therefore, for binding to IL-1RAcP, the PTPδ Ig3–IL-1RAcP Ig1 interface is likely the primary interaction site, and the meB insertion may serve as the adjustable linker to locate the Ig2 domain of PTPδ in the appropriate position for interacting with the Ig1 domain of IL-1RAcP (Fig. 4a).

IL-1RAcP functions in both immune and neuronal systems, whereas IL1RAPL1 is exclusively expressed in neuronal systems, suggesting that IL1RAPL1 has evolved to be more specialized for neuronal systems than IL-1RAcP. Correspondingly, IL-1RAcP binds to PTPδ weaker than IL1RAPL1 but has a broader specificity to the meA variants. In contrast, IL1RAPL1 has more strict specificity to meA and can bind to PTPδ more tightly than IL-1RAcP. The affinity difference generated by the splice-insert signaling codes is substantially high for binding between PTPδ and IL1RAPL1 but modest for binding between PTPδ and IL-1RAcP. On the other hand, in our synaptogenic assays, the strength of the induced synaptic differentiation seems non-linearly correlated with the affinity; rather, there seems to be the threshold of affinity for inducing the synaptic differentiation, which might contribute to controlling appropriate synaptic target selection to avoid misconnections to target cells with low affinity ligands. Further functional studies including identification and characterization of additional regulatory factors are awaited to discuss the activation and signaling mechanisms of IL1RAPL1/IL-1RAcP and PTPδ for inducing synaptic differentiation.

Methods

Protein expression and purification. Genes encoding mouse PTPδ-ECD, PTPδ Ig1-Fn4, PTPδ Ig1–Fn3, PTPδ Ig1–Fn2, PTPδ Ig1–Fn1, PTPδ Ig1–Ig3 and PTPδ Ig1–Ig2 (residues 28–861, 28–699, 28–611, 28–518, 28–418, 28–325 and 28–237, respectively) were amplified from cDNA (accession No. NM_011211.3) by PCR and cloned into pEBMulti-Neo vector (Wako Pure Chemical Industries) with N-terminal signal sequences derived from pHsec vector and C-terminal hexahistidine tag. PTPδA3B Ig1–Fn2 gene was also cloned from cDNA in the same manner as the PTPδ constructions. Genes encoding mouse IL-1RAcP-ECD (residues 21–351, gene accession No. NM_008364.2) and IL1RAPL1-ECD (residues 19–352, gene accession No. NM_00116043.1) were cloned into pEBMulti-Neo vector, with N-terminal Ig1 signal sequence and C-terminal hexahistidine tag. All proteins were transiently expressed using Freestyle 293-F cells (Invitrogen). For crystallography, proteins were purified from culture media by Ni-NTA (Qiagen) or Talon metal affinity resin (Clontech) with a standard protocol and dialysed against 20 mM Tris–HCl buffer (pH 7.5) containing 150 mM NaCl. Proteins were concentrated at 5–10 g l−1, flash-frozen in liquid N2 and stored at −80 °C until use. A gene encoding PTPδ (Fn1–Fn2) (residues 328–518) was cloned into pET21a vector (Novagen). PTPδ (Fn1–Fn2) was overexpressed in Rosetta (DE3) Escherichia coli cells (Novagen) and purified by the Ni affinity chromatography followed by the size exclusion chromatography with Superdex 200 16/60 (GE Healthcare).

Pull-down assay. Binding abilities of serially deleted PTPδ-ECDs were tested by pull-down assay with the C-terminally Fc-tagged IL-1RAcP- or IL1RAPL1-ECD (IL-1RAcP- or IL1RAPL1-Fc, respectively). IL-1RAcP- and IL1RAPL1-Fc were purified by Protein G Sepharose (GE Healthcare) and IL1RAPL1-ECD and PTPδ-ECD were eluted by SDS sample loading buffer, followed by SDS–PAGE analyses.

CrySTALLIZATION. PTPδA3B Ig1–Fn2, PTPδ Ig1–Fn3, PTPδ Ig1–Fn2, PTPδ Ig1–Fn1, PTPδ Ig1–Ig3 and PTPδ Ig1–Ig2 were crystallized by the sitting drop vapour diffusion method. Protein solutions were mixed with the following reservoir solutions: 10% polyethylene glycol (PEG) 3350, 0.1 M ammonium iodide for PTPδA3B Ig1–Fn2, 20% PEG3350, 0.1 M MgCl2 and 0.1M BisTris (pH 5.5) for PTPδ Fn1–Fn2; 2.0–2.4 M ammonium sulfate for IL1RAPL1; 15% PEG3350, 0.1 M ammonium sulfate and 0.1 M tri-sodium citrate (pH 5.5) for PTPδ Ig1–Fn1–Fn2; 2.0–2.4 M ammonium sulfate for IL1RAPL1-ECD complex; 12% PEG4000, 0.1 M lithium sulfate, 0.1 M ADA (pH 6.5) for the PTPδ-ECD/IL1RAPL1-ECD complex; 15% PEG4000, 0.1 M MES (pH 6.0) for the PTPδ Ig1–Ig2/IL1RAPL1-ECD complex. For the crystallization of the IL1RAPL1-ECD complexes, two protein samples were mixed at a molar ratio of 1:1 to a final concentration of ~6 g l−1 before crystallization experiments. All
crystallization experiments except for PT6 F1n–F2n were performed at 20 °C. Temperature shift from 4 to 28 °C drastically improved the crystal size of PT6 F1n–F2n. Crystals were flash-frozen in liquid N₂ followed by soaking in the reservoir solutions supplemented with the following cryo-protectants: 25% glyceral for PT6A3B, 1–F1n–2 and the PT6 Ig1–Ig2/IL1RAPL1-ECD complex; 25% ethylene glycol for PT6 F1n–F2n; 27% ethylene glycol for IL1RAP; 35% xylitol for the PT6 Ig1–F2n/IL-1RAcP-ECD complex; 20% ethylene glycol for the PT6-ECD/IL1RAPL1-ECD complex.

Crystallography. All data were collected at 100 K at BL41XU in SPring-8, and processed with HKL2000 (ref. 30) and CCP4 program suite31. Data collection and refinement statistics were summarized in Table 1. Resolutions were estimated, basically on I/σ(I) values (2 σ). To improve the quality of the atomic models, the resolutions of PT6-ECD/IL1RAPL1-ECD and PT6A3B, Ig1–F2n were set to be as high as possible. The resolution of PT6 F1n–F2n was limited by the size of the detector. We first determined a crystal structure of the PT6 Ig1–Ig2/IL1RAPL1-ECD complex by the molecular replacement method using IL-1RAcP-ECD (PDB:4D4E, 3O4Q)32,33 and PT6 Ig1–Ig2 (PDB:2YD2)19 as the search models with the program MOLREP34. Model building and refinement were carried out using the programs COOT35 and Phenix36, respectively. No residues are in the disallowed regions for all these structures. The stereochemistry of the final model was assessed by the program Procheck. Percentages of residues in the most favoured regions of the NATURE COMMUNICATIONS | DOI: 10.1038/ncomms7926 | www.nature.com/naturecommunications structure of the PTP R and ARTICLE NATURE COMMUNICATIONS | DOI: 10.1038/ncomms7926 | www.nature.com/naturecommunications were determined by the molecular replacement method using IL-1RAcP-ECD complex and refined at 4.4 Å to Rwork and Rfree values of 26.2 and 29.8%, respectively. The structure of the PTP R and ARTICLE NATURE COMMUNICATIONS | DOI: 10.1038/ncomms7926 | www.nature.com/naturecommunications

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Author contributions

T.Y., A.M., T.S. and A.Y. performed pull-down assays and crystallization. A.Y., S.F., Y.S. and S.G.-I. collected diffraction data. A.Y. and S.F. analysed the collected data and determined the structures. T.Y., T.S. and S.I.-O. performed cell biological experiments. A.Y., T.Y. and S.F. wrote the paper with editing by T.U., H.M. and M.M. T.Y., M.M. and S.F. designed and supervised the study.

Additional information

Accession codes: Atomic coordinates and structure factors have been deposited with the accession codes 4YFC (PTPδ-Ig1–Ig2/IL1RAPL1-ECD), 4YFD (PTPδ-Ig1–Ig2/IL1RAPL1-EC), 4YER (PTPδ-Ig1–Ig2/IL1RAPL1-ECD), 4YFG (PTPδA3B-Ig1–Ig2), 4YH6 (IL1RAPL1-ECD) and 4YH7 (PTPδ-ECD/IL1RAPL1-ECD).

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