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Protein Tyrosine Phosphatase Receptor Type Z Dephosphorylates TrkA Receptors and Attenuates NGF-dependent Neurite Outgrowth of PC12 Cells

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Protein tyrosine phosphatase receptor type Z (Ptprz/Ptpζ/RPTPβ) is a receptor-like protein tyrosine phosphatase (RPTP) which is predominantly expressed in the central nervous system. Tropomyosin-related kinases (Trks) are single-pass transmembrane molecules that are highly expressed in the developing nervous system. Upon the ligand binding of neurotrophins, Trk receptors are activated through autophosphorylation of tyrosine residues; however, the PTPs responsible for the negative regulation of Trk receptors have not been fully elucidated. Here, we identified Ptprz as a specific PTP that efficiently dephosphorylates TrkA as a substrate. Co-expression of Ptprz with Trk receptors in 293T cells showed that Ptprz suppresses the ligand-independent tyrosine phosphorylation of TrkA, but not of TrkB or TrkC, and that Ptprz attenuates TrkA activation induced by nerve growth factor (NGF). Co-expression analyses with TrkA mutants revealed that Ptprz dephosphorylates phosphotyrosine residues in the activation loop of the kinase domain, which are requisite for activation of the TrkA receptor. Consistent with these findings, forced expression of Ptprz in PC12D cells markedly inhibited neurite extension induced by a low dose of NGF. In addition, an increment in the tyrosine phosphorylation of TrkA was observed in the brain of *Ptprz*-deficient mice. Ptprz thus appears to be one of the PTPs which regulate the activation and signalling of TrkA receptors.

Key words: Ptprz, TrkA, NGF, dephosphorylation, neurite extension.

Abbreviations: CNS, central nervous system; FRS2, fibroblast growth factor receptor substrate 2; Grb2, growth factor receptor-bound protein 2; ICR, intra-cellular region; NGF, nerve growth factor; PLC-γ, phospholipase C-γ; PSD-95, postsynaptic density-95; PTK, protein tyrosine kinase; PTP, protein tyrosine phosphatase; Ptprg, protein tyrosine phosphatase receptor type G; Ptprz, protein tyrosine phosphatase receptor type Z; RPTK, receptor PTK; RPTP, receptor-like PTP; Shc, Src-homology and collagen; Trk, tropomyosin-related kinase.

Activation of a receptor protein tyrosine kinase (RPTK) by a given extra-cellular stimulus, such as a growth factor, initiates a highly orchestrated signalling cascade. On binding a ligand, a RPTK becomes phosphorylated at multiple tyrosine residues, which serve as docking sites for distinct signalling proteins (*1*). The spectrum of signalling molecules that associate with the RPTK determine the nature of the response following stimulation by the ligand (*1*). On the other hand, protein tyrosine phosphatases (PTPs) are assumed to be key regulatory molecules in kinase cascades (*2*). In some studies including ours, receptor-like PTPs (RPTPs) have been shown to dephosphorylate a particular phosphorylated tyrosine residue in a RPTK and thereby determine the specificity of a signalling event rather than simply acting as an 'off-switch' (*3*, *4*). Identifying PTPs that regulate (attenuate) RPTKs should reveal novel mechanisms by which extra-cellular stimuli and intrinsic programs regulate RPTK function.

Neurotrophin signalling is critical for normal nervous system development and function, including survival, differentiation, axonal and dendritic growth, neurotransmitter secretion and neuronal plasticity (*5*). In general, each neurotrophin binds to a specific tropomyosin-related kinase (Trk): nerve growth factor (NGF) to TrkA; brain-derived neurotrophic factor (BDNF) and neurotrophin-4/5 (NT-4/5) to TrkB; and neurotrophin-3 (NT-3) to TrkC (*5*). The intra-cellular pathways stimulated by Trk receptors share many common substrates with other receptor tyrosine kinases, such as Src-homology and collagen (Shc), growth factor receptor-bound protein 2 (Grb2), fibroblast growth factor receptor substrate 2 (FRS2) and phospholipase Cγ (PLC-γ) (*6*). While the signal transduction pathways used by the Trk receptors are well understood, the PTPs responsible for the regulation of Trk receptors have not been fully elucidated.

Protein tyrosine phosphatase receptor type Z and type G (Ptprz/PTPζ/RPTPβ and Ptprg/PTPγ, respectively) structurally resemble each other and form the R5 subfamily of RPTPs: Both molecules contain a carbonic anhydrase (CAH)-like domain and a fibronectin type III-like domain extra-cellularly, and two

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phosphatase domains intracellularly (*7*). Three and four splicing variants are known for Ptprz (Ptprz-A, -B and -S) and Ptprg (Ptprg-A, -B, -C and -S), respectively (*8*, *9*). Ptprz and Ptprg distribute predominantly in the nervous system from the early embryonic stage through adulthood, suggesting their involvement in neuronal cell migration, differentiation, circuit formation and regulation of neuronal plasticity (*10*–*15*). Ptprz is known to bind several extra-cellular matrix proteins and cell-adhesion molecules such as contactin, tenascin, L1, NCAM and TAG1 (*16*). In addition, we found that heparin-binding growth factors, pleiotrophin/HB-GAM and midkine, bind to the extra-cellular region of Ptprz (*17*, *18*) and inactivate its PTP activity through oligomerization (*19*).

For elucidation of the physiological functions of Ptprz, we attempted to identify its specific substrates by developing a novel screening system for PTP substrates, named the 'yeast substrate-trapping system'. We successfully identified several intra-cellular substrates for Ptprz, such as G protein-coupled receptor kinase-interactor 1 (Git1), p190 RhoGAP, golgi-associated PDZ and coiled-coil motif containing (GOPC/PIST) and MAGI-1 (*20*, *21*). *Ptprz*-deficient mice exhibit abnormalities in hippocampus-dependent memory formation (*22*, *23*), in that aberrant phosphorylation of p190 RhoGAP was observed (*23*). Very recently, we identified ErbB4 as a novel substrate for Ptprz by pull-down experiments using a carboxyl terminal region of Ptprz, and revealed that the tyrosine phosphorylation level of ErbB4 is increased in *Ptprz*-deficient mice (*24*). Both Ptprz and ErbB4 bind to post-synaptic density-95 (PSD-95) through the PDZ-binding motif at their C-terminal ends (*20*, *24*, *25*).

We previously reported that Ptprg but not Ptprz strongly inhibits NGF-induced neurite outgrowth in PC12D cells (*26*). Although we revealed modulation of the $p13^{suc1}$ complex by Ptprg, it remained to be explored whether activation of TrkA is directly affected by Ptprg or Ptprz. In the present study, we examined this possibility, and unexpectedly found that Ptprz but not Ptprg efficiently dephosphorylates the TrkA receptor. Ptprz preferentially dephosphorylated the receptor at Y674 and Y675, which are responsible for the regulation of Trk tyrosine kinase activity. Consistent with these findings, the tyrosine phosphorylation of TrkA was increased in *Ptprz*-deficient mice. In addition, we demonstrated that Ptprz indeed modulates the sensitivity of cells to NGF by neurite extension assays using PC12D cells.

MATERIALS AND METHODS

Antibodies—Anti-Ptprz-S was described previously (*27*). Anti-pan-Trk (C-14) and anti-TrkA (763) antibodies were purchased from SantaCruz. Anti-phosphotyrosine antibody (4G10) was from UBI. Anti-Myc and anti-HA antibodies were from Sigma. Anti-GFP antibody was from Nacalai. Alexa-conjugated secondary antibodies were from Invitrogen.

Cell Culture—293T cells were maintained in DMEM/F-12 medium (Sigma) supplemented with 10% fetal bovine serum (FCS). PC12D cells were maintained in DMEM supplemented with 5% fetal bovine serum and 10% heat-treated horse serum (HS). Transfection was performed using LipofectAMINE PLUS (Invitrogen) according to the manufacturer's protocol.

DNA Constructs—Expression constructs encoding the full-length rat Ptprz-B (pZeo-PTPζ) and its PTP-inactive DA mutant (pZeo-PTPζD1902A) were described previously (*19*, *20*). HA-Ptprg(WT), an expression construct encoding a fusion protein comprising the signal peptide sequence of murine Ig κ-chain (amino acid residues, 1–21), a HA-epitope tag (SYPYDVPDYAS) and the full-length form of mouse Ptprg (residues, 34 to the end), was prepared with the expression vector pDisplay (Invitrogen) using *Bgl*II and *Not*I sites. The full-length mouse TrkA, TrkB and TrkC cDNAs were cloned using the total RNA from the embryonic Day 18 mouse brain as a template by RT-PCR. These cDNA fragments were then cloned into the expression vector pcDNA3.1 (Invitrogen) using *Eco*RI and *Xho*I sites. Point mutants of TrkA were also generated using PCR-based site-directed mutagenesis.

Immunoprecipitation and Immunoblotting—Cells were lysed in a lysis buffer, which consists of 20 mM HEPES, pH 7.0, 120 mM NaCl, 1% NP-40, 50 mM NaF, 0.5 mM Na_3VO_4 and a protease inhibitor mixture (10 μg/ml leupeptin, 1 μg/ml pepstatin A and 1 mM phenylmethylsulphonyl fluoride). Cell lysates were clarified by centrifugation, and subjected to immunoprecipitation with various antibodies bound to protein G-Sepharose CL-4B (Amersham Biosciences). Immunoprecipitates were solubilized with SDS sample buffer and subjected to SDS–PAGE. Proteins were transferred onto Imobilon-P membranes (Millipore), stained with specific primary antibodies and peroxidase-linked secondary antibodies (Amersham Biosciences), and visualized by chemiluminiscence using ECL plus (Amersham Biosciences). For the detection, the lumino-image analyzer LAS-1000plus (Fujifilm) was used.

In Vitro Dephosphorylation Assay—GST-PtprzICR encoding the entire intra-cellular region of rat Ptprz-A was described previously (*20*, *21*). For *in vitro* dephosphorylation, we first prepared the autophosphorylated TrkA proteins as the substrate. Myc-tagged ICR of TrkA was expressed in 293T cells grown on a 35-mm culture dish, and purified by immunoprecipitation with an anti-Myc antibody and protein G-Sepharose CL-4B. The TrkA-ICR was then autophosphorylated *in vitro* with 100 μM of ATP in 100 μl of the PTK buffer: 20 mM Tris–HCl, pH7.2, 10 mM $MgCl_2$, 10 mM $MnCl_2$ and 1 mM DTT. Protein G beads were washed once and re-suspended in 100 μl of 10 mM Tris–HCl, pH 7.0, containing 5 mM DTT, 5 mM EDTA and 100 μg/ml bovine serum albumin (PTP buffer). For the dephosphorylation assay, 10 ng of GST-PtprzICR or GST alone was reacted with 10 μl of Myc-TrkA-ICR at 30°C. At the indicated time, the reaction was stopped by adding SDS sample buffer. The samples were separated by SDS–PAGE followed by immunoblotting with anti-phosphotyrosine antibody.

Morphological Analysis—PC12D cells were seeded in 35-mm dishes precoated with 1 mg/ml of collagen-I (Nitta gelatin) at a density of 5×10^4 cells/dish in DMEM containing 5% FCS and 10% HS. After 24 h, transfection was performed using LipofectAMINE PLUS (Invitrogen)

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according to the manufacturer's protocol. Expression constructs used were pZeo-PTPζ, pZeo-PTPζD1902A and HA-Ptprg(WT). To visualize Ptprz-transfected cells, quarter the amount of EGFP-N1 (Clontech) was cotransfected. At 24 h after transfection, the medium was changed to fresh medium containing 10 or 100 ng/ml of 7S NGF. After another 48 h, cells were fixed with 4% paraformaldehyde in phosphate-buffered saline, and then with anti-GFP antibody and Alexa488-conjugated secondary antibody. EGFP-positive neurites were analyzed with a BX51TRF microscope (Olympus) equipped with a DP-70 digital CCD camera (Olympus). Neurite lengths were measured from 200 cells selected at random in three wells. In colonies, only peripheral cells were examined. Processes of more than 150 μm were scored as long neurites.

Tyrosine Phosphorylation of TrkA in Mouse Brain—The cerebral cortex of mice (5 weeks old) were homogenized with 20 vol of ice-cold 0.32 M sucrose in 4 mM HEPES, pH 7.4, containing 1 mM vanadate and 10 mM NaF. After removal of cell nucleus and debris by centrifugation at 800 *g* for 10 min, crude synaptosomes were prepared by centrifugation at 9,000 *g* for 15 min. Synaptosomes were then subjected to the immunoprecipitation and immunoblotting experiments as above.

RESULTS

Ptprz but not Ptprg Dephosphorylates TrkA Receptors—To test whether Ptprz or Ptprg can dephosphorylate Trk receptors by using a cell culture system, expression constructs for full-length Trk receptors (TrkA, B and C) were co-transfected into 293T cells with the full-length wild-type construct of Ptprz or Ptprg. Immunostaining with anti-Ptprz, anti-HA and anti-Trk antibodies revealed that about 70% of the cells expressed the exogenous genes equally (data not shown). Western blot analysis of immunoprecipitates with anti-pan-Trk antibody showed TrkA proteins (140 and 110 kDa), a TrkB protein (130 kDa) and a TrkC protein (130 kDa) (Fig. 1A–C). Highly tyrosyl-phosphorylated Trk receptor proteins were detected when each Trk receptor alone was expressed (Fig. 1A–C, lane 2). However, when the TrkA, TrkB or TrkC construct was independently co-transfected with wild-type Ptprz [Ptprz(WT)], the tyrosyl-phosphorylation of TrkA was significantly suppressed (Fig. 1A, lane 3; 1D, lane 3). In contrast, the phosphorylation of the TrkA receptor was not affected when the phosphatase-inactive DA mutant of Ptprz [Ptprz(DA)] was cotransfected (Fig. 1D, lane 4), indicating that the suppression of TrkA phosphorylation is attributable to the catalytic activity of Ptprz. On the other hand, Ptprg exerted no effect on the phosphorylation of any Trk receptor when co-expressed (Fig. 1A–C, lanes 4).

We then tested whether Ptprz directly dephosphorylates the TrkA receptor *in vitro* using purified proteins. A Myc-tagged intra-cellular region of TrkA (TrkA-ICR) was expressed in 293T cells, and purified by immunoprecipitation with an anti-Myc antibody. The TrkA receptor was autophosphorylated *in vitro*, and then incubated with GST-Ptprz-ICR or GST. GST-Ptprz-ICR

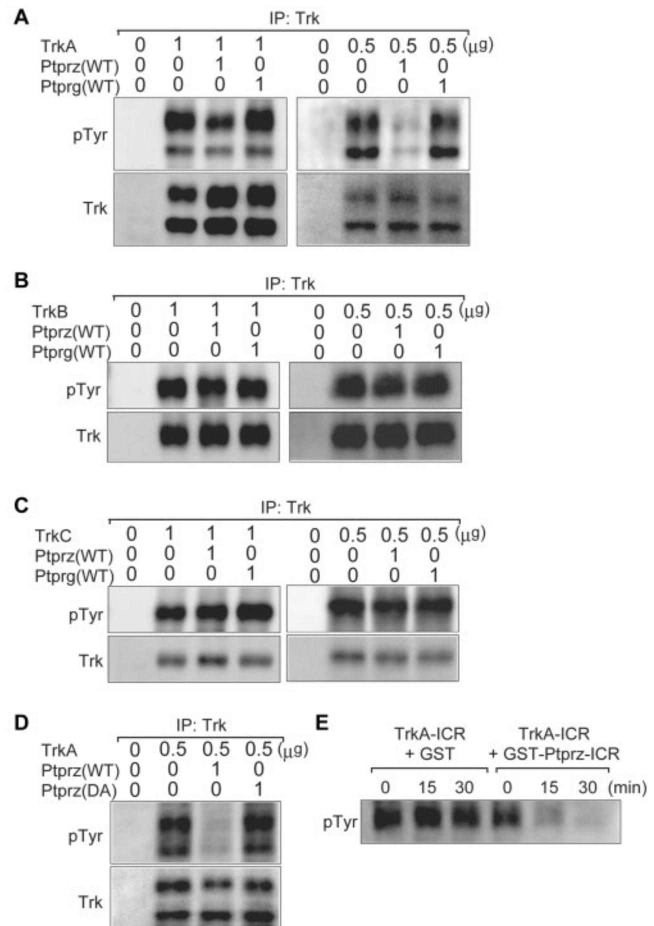

Fig. 1. **Dephosphorylation of TrkA receptors by Ptprz.** (A) Tyrosine dephosphorylation assay of TrkA. 293T cells were co-transfected with the indicated amounts (μg) of expression plasmids. Cell lysates were immunoprecipitated with anti-pan-Trk antibody, and the protein complexes obtained were analysed by SDS–PAGE and immunoblotting using anti-phosphotyrosine antibody (pTyr). Immunoblots were stripped and reprobed with anti-Trk antibody (Trk). The molecular mass of the full-length TrkA was estimated to be 140 and 110 kDa. These two proteins reportedly represent the fully matured form, $gp140^{TrkA}$, and glycosylated precursor form, $gp110^{TrkA}$, respectively (*49*). Lane 1 is a negative control with the empty vectors. (B) Tyrosine dephosphorylation assay of TrkB. The molecular mass of the full-length TrkB was estimated to be 130 kDa. (C) Tyrosine dephosphorylation assay of TrkC. The molecular mass of the full-length TrkC was estimated to be 130 kDa. (D) Dephosphorylation of TrkA by Ptprz(WT), but not Ptprz(DA). (E) *In vitro* dephosphorylation analysis of the TrkA receptor. Tyrosyl-phosphorylated TrkA-ICR was incubated with an equal amount of GST or GST-Ptprz-ICR. After the reaction, proteins were separated by SDS–PAGE and subjected to immunoblotting using anti-phosphotyrosine antibody (pTyr).

efficiently dephosphorylated TrkA, whereas GST alone did not (Fig. 1E), indicating that TrkA is a direct substrate for Ptprz.

Next, we examined the effects of the co-expression of Ptprz on the tyrosyl-phosphorylation of TrkA receptors when stimulated with NGF in living cells. When NGF was applied to serum-starved 293T cells, the tyrosyl-phosphorylation of TrkA was significantly enhanced in a

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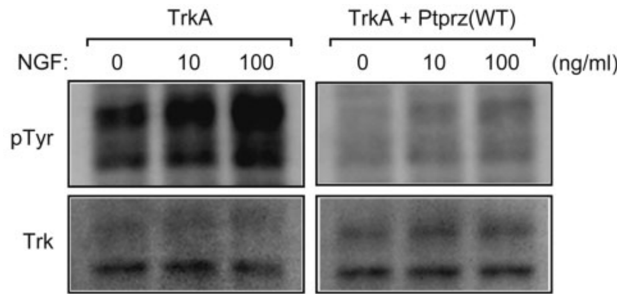

Fig. 2. **Inhibition of NGF-induced tyrosine phosphorylation of TrkA receptors by Ptprz.** 293T cells were transfected with 0.5 μg of TrkA-expression construct and either 1 μg of empty vector or 1 μg of Ptprz(WT)-expression construct. Cells were serum-starved for 24 h. Tyrosine phosphorylation levels of TrkA were analysed after stimulation with NGF. Cells were lysed and immunoprecipitated with anti-Trk. Protein complexes were analysed by immunoblotting using anti-phosphotyrosine (pTyr). Immunoblots were reprobed with anti-Trk (Trk).

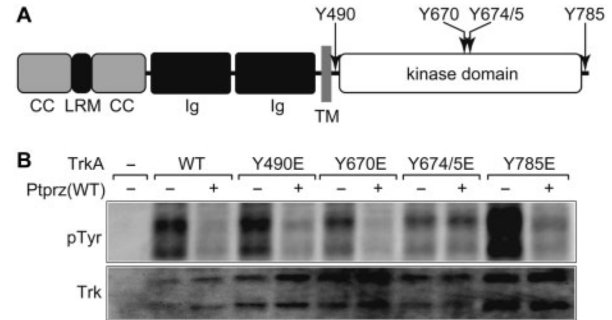

Fig. 3. **Preferential dephosphorylation of Y674/675 in the kinase domain of the TrkA receptor by Ptprz.** (A) Schematic representation of the TrkA receptor. Positions of major phosphorylated tyrosine residues are shown. CC, cysteine cluster; LRM, leucine-rich motif; Ig, immunoglobulin domain; TM, transmembrane region. (B) 293T cells were transfected with the indicated expression plasmids. Cell lysates were subjected to immunoprecipitation with anti-Trk antibody to isolate TrkA receptors. The tyrosine phosphorylation levels on TrkA receptors were analysed by SDS–PAGE and immunoblotting using anti-phosphotyrosine antibody (pTyr). Immunoblots were stripped and reprobed with anti-Trk antibody (Trk). Lane 1 is a negative control with the empty vector.

dose-dependent manner (Fig. 2, lanes 1–3). In contrast, when wild-type Ptprz was co-expressed, the enhancement of the phosphorylation of the TrkA receptor by NGF was significantly suppressed (Fig. 2, lanes 4–6), indicating that Ptprz can attenuate TrkA's activation by NGF.

Ptprz Dephosphorylates TrkA at Specific Sites—Upon binding NGF, TrkA is autophosphorylated at several distinct tyrosine residues (Y490, Y670, Y674, Y675 and Y785) (Fig. 3A) (*6*). We next attempted to identify the tyrosine residue on TrkA that is dephosphorylated by Ptprz. For this purpose, we generated TrkA mutants in which each candidate tyrosine was mutated to glutamate one by one: Glutamate mimics phosphorylated tyrosine but is not dephosphorylated by PTPs. 293T cells were transfected with these mutants, with or without the wild-type Ptprz construct. When wild-type Ptprz was co-expressed, the tyrosyl-phosphorylation of wild-type TrkA, TrkA(Y490E), TrkA(Y670E) and TrkA(Y785) was markedly suppressed, but that of TrkA(Y674/675E) was not (Fig. 3B). These results indicate that Ptprz preferentially dephosphorylates Y674 and/or Y675 in the kinase domain of the TrkA receptor. This finding suggests that Ptprz was functions in inhibiting the activation of TrkA receptors by NGF, because phosphorylation at these residues is implicated in the activation of TrkA receptors (*6*).

Ptprz Attenuates NGF-induced Neurite Extension in PC12D Cells—We next examined whether Ptprz expression affects the outgrowth of neurites induced by NGF in PC12D cells. PC12D cells, a subclone of PC12, extend neurites immediately in response to NGF (*28*, *29*). PC12D cells were transiently transfected with various expression constructs; Ptprz(WT), Ptprz(DA) and Ptprg(WT). In the absence of NGF, none of the transfected cells nor the parental cells extended neurites longer than 150 μm (data not shown). On the other hand, at 48 h after treatment with 10 ng/ml of NGF, the mock-transfected cells extended long neurites vigorously (Fig. 4A). In contrast, neurite outgrowth was considerably attenuated in Ptprz-expressing (Fig. 4B) and Ptprg-expressing (Fig. 4C) cells. Figure 4D summarizes the results on the extension of neurites in the transfected cells. Among mock-transfected cells, ~30% extended neurites longer than 150 μm at 10 ng/ml of NGF. In contrast, only ~10% of Ptprz-expressing cells had long neurites ($P < 0.01$, by Mann–Whitney U-test). This inhibitory effect is dependent on the PTPase activity of Ptprz, because PC12D cells expressing Ptprz(DA) showed significant neurite extension like the mock-transfected cells (Fig. 4C).

As previously reported, a drastic suppression of neurite outgrowth was observed when PC12D cells over-expressed Ptprg(WT) even at a high dose of NGF (100 ng/ml) ($P < 0.01$, by Mann–Whitney U-test). In contrast, at 100 ng/ml of NGF, no significant difference was observed between mock-transfected cells and Ptprz(WT)-expressing cells, indicating that Ptprz under our conditions did not suppress the outgrowth of neurites in PC12D cells at a high dose of NGF. This is probably because the relatively low transfection efficiency and low level of Ptprz caused by the transient expression are not enough to completely suppress the activation of TrkA in PC12D cells as a whole: Immunostaining with an anti-Ptprz antibody revealed that about 3% of the transfected cells expressed the exogenous RPTP gene, and the expression of Ptprz in the individual PC12D cells was markedly small (~1/25) as compared with 293T cells (data not shown).

Increased Tyrosine Phosphorylation of TrkA in Ptprz-Deficient Mice—We examined the tyrosine phosphorylation of TrkA in the synpatosomal fraction of the cerebral cortex of wild-type and *Ptprz*-deficient mice (Fig. 5A). Ptprz-B (250 kDa), a major isoform of Ptprz, was detected in cerebral synaptosomes of the wild-type mice (Fig. 5A, left; see also ref. *24*). Wild-type and *Ptprz*-deficient mice showed a similar expression level of TrkA in the synaptosomal fractions of the cerebral cortex (Fig. 5A, right). However, immunoblotting with anti-phoshotyrosine antibody revealed that the tyrosine

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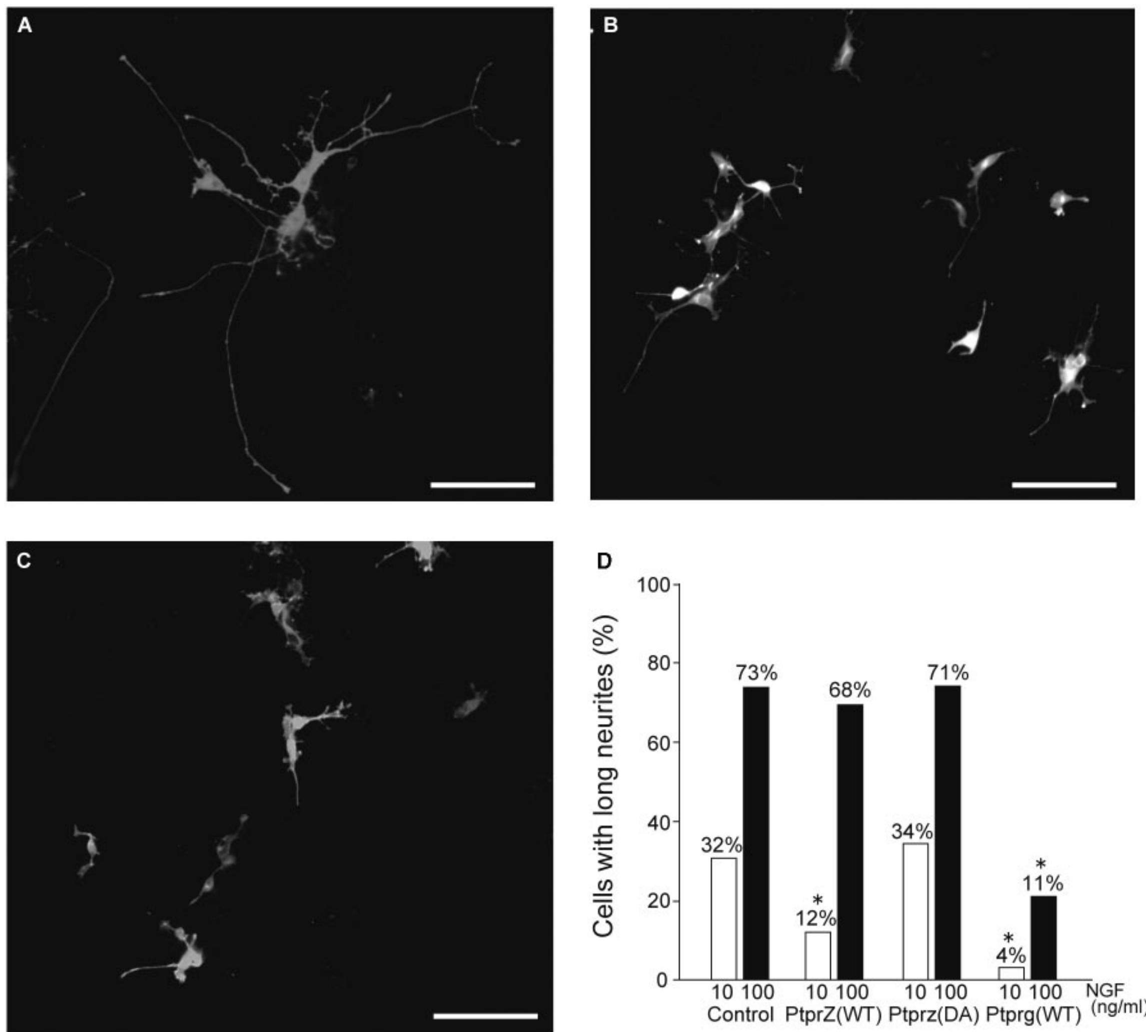

Fig. 4. **Attenuation of NGF-induced neurite extension in PC12D cells by Ptprz.** (A) Morphology of mock-transfected cells after treatment with NGF (10 ng/ml). (B) Morphology of Ptprz-expressing cells after treatment with NGF (10 ng/ml). (C) Morphology of Ptprg-expressing cells after treatment with NGF (10 ng/ml). Scale bars in (A)–(C): 100 µm. (D) Summary of neurite outgrowth in PC12D cells. Percentages of cells with neurites longer than 150 µm are shown. $^{*}P<0.01$, when compared with mock-transfected cells (Mann–Whitney U-test).

phosphorylation of TrkA was significantly increased in *Ptprz*-deficient mice (Fig. 5A, right; 1.8-fold increase, $P<0.05$, $n=4$ by unpaired Student's *t*-test). Taken altogether, we concluded that the TrkA receptor is a physiological substrate for Ptprz.

DISCUSSION

It has been postulated that the activation of RTKs is negatively regulated by PTPs (*2*). In this study, we found that Ptprz specifically recognizes the TrkA receptor as a substrate. Our data indicated that Ptprz recognizes particular phosphorylation sites within the activation loop of the kinase domain, and modulates activation of the TrkA receptor. In line with this finding, the expression of Ptprz in PC12D cells attenuated the NGF-induced extension of neurites and the tyrosine phosphorylation of TrkA was significantly increased in *Ptprz*-deficient mice.

TrkA rapidly dimerizes upon binding NGF and becomes phosphorylated at several distinct tyrosine residues (Y490, Y670, Y674, Y675 and Y785) (*6*). This allows the recruitment and activation of a number of specific signalling proteins, such as Shc, FRS2 and phospholipase C-γ (PLC-γ) (*6*). Tyrosine residues Y490 and Y785 are responsible for the direct association with and tyrosine phosphorylation of Shc and PLC-γ, respectively (*30–33*). Activation of the MAP kinase pathway largely depends on the activation of Shc (*6*). Shc has been shown to be essential for mediating the activation of PI3-kinase (*34*), which is critical for the ability of Trk receptors to mediate survival through the activation of Akt (*6*). On the other hand, activation of PLC-γ leads to the release of calcium ion from intra-cellular stores through production of inositol(*1*,*4*,*5*)triphosphate. Phosphorylation at residues Y670, Y674 and Y675 in the activation loop of the kinase domain is implicated in the activation of the tyrosine kinase of TrkA (*35*).

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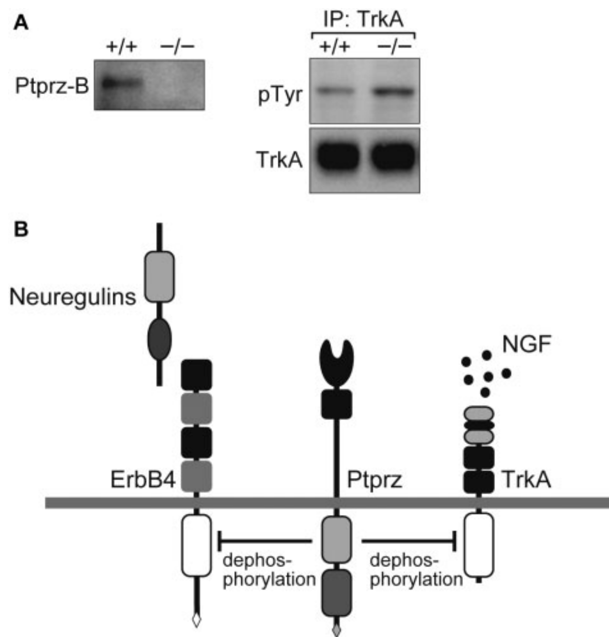

Fig. 5. **Comparison of the tyrosine phosphorylation of TrkA between wild-type and *Ptprz*-deficient mice.** (A) The tyrosine phosphorylation level of TrkA in the synaptosomal fractions of wild-type and *Ptprz*-deficient mice. Expression of Ptprz was analysed by immunoblotting after treatment with chondroitinase ABC as described (*8*). TrkA was immunoprecipiated with anti-TrkA antibody, and the tyrosine phosphorylation level of TrkA was analysed by SDS–PAGE and immunoblotting using anti-phosphotyrosine (pTyr) antibody. Immunoblots were stripped and reprobed with anti-Trk antibody (TrkA). (B) Schematic drawing of regulation of TrkA and ErbB4 by Ptprz. ErbB4 and TrkA are activated by their specific ligands, neuregulins and NGF, respectively. Ptprz attenuates the activation of ErbB4 (*24*) and TrkA through dephosphorylation.

In this study, we found that Ptprz preferentially dephosphorylates Y674 and/or Y675 of TrkA (Fig. 3). This suggests that Ptprz functions in inhibiting the activation of TrkA receptors after they bind NGF. Several PTPs have been reported to dephosphorylate TrkA (*36–38*). Among them, SHP-1, a SH2 domain-containing cytosolic PTP, forms a complex with TrkA at Y490, and dephosphorylates it at Y674 and/or Y675 like Ptprz (*38*). Therefore, Ptprz may regulate the activation of TrkA in coordination with SHP-1 *in vivo*. Here, it is not clear why the substrate-trapping mutant (DA mutant) of Ptprz showed no effect on NGF-induced neurite outgrowth in PC12D cells.

RPTPs have been proposed to be inactivated by ligand-induced dimerization. Since Ptprz is inactivated through oligomerization including dimerization by pleiotrophin/HB-GAM (*21*), it is possible that the activation of TrkA is regulated by pleiotrophin/HB-GAM or Midkine. Thus, the identification of RPTPs as TrkA phosphatases would shed light on the novel mechanisms by which extracellular ligands for RPTPs influence neurotrophic function. From this point of view, the discovery of RPTPs that specifically dephosphorylate Y490 or Y785 is of importance.

Another subfamily member, Ptprg, does not dephosphorylate Trk receptors (Fig. 1), however, Ptprg strongly inhibits NGF-induced neurite outgrowth in PC12D cells (Fig. 4C, see also ref. *26*). This suppression is probably achieved through modulation of the p13^{suc1} complex (*26*), which comprises FRS2 (SNT) and associated proteins (*39*). FRS2 is an important signalling molecule that binds to the NPXY motif on Trks, and considered to play an important role in neuronal differentiation and neurite outgrowth (*39*). Neurotrophin induces phosphorylation of FRS2, and the phosphorylated FRS2 then provides binding sites for adaptor proteins including Grb2, Crk and Src (*6*). We postulated that some of the constituents in this complex are substrates for Ptprg (*26*). Thus, the substrate specificity would be the cause of the functional difference between Ptprz and Ptprg.

In the mature brain, the expression of Ptprz persists in specific regions including the hippocampus and cerebral cortex (*8*, *15*), where the structure and function of synapses are continually modified throughout life. We recently reported that Ptprz forms a complex with ErbB4 through PSD-95 and dephosphorylates it as a substrate (*24*). ErbB4 is a member of the ErbB family of RPTKs activated by neuregulins and other growth factors, and probably modulates synaptic plasticity (*40–42*). NGF is also expressed in the adult CNS, with the highest levels being present in the hippocampus and cerebral cortex (*43–45*). The TrkA receptor is also broadly distributed in the brain including the hippocampus (*46*). The application of anti-NGF antibody inhibits differentiation into the cholinergic phenotype (*47*) and blocks the sprouting of acetylcholinesterase-positive branches in the hippocampus (*48*). In addition, neurotrophic factors and their receptors are implicated in neuronal plasticity (*5*). Consistent with these findings, mutant mice deficient in *Ptprz* exhibited enhanced long-term potentiation (LTP) in the CA1 region in hippocampal slices (*22*), and impaired hippocampus-dependent memory formation (*22*, *23*). Taken altogether, these observations suggest an important role for Ptprz in the development and regulation of synapses through the control of activities of TrkA and ErbB4 (Fig. 5B).

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