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Protein-tyrosine Phosphatase α Acts as an Upstream Regulator of Fyn Signaling to Promote Oligodendrocyte Differentiation and Myelination*

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The tyrosine kinase Fyn plays a key role in oligodendrocyte differentiation and myelination in the central nervous system, but the molecules responsible for regulating Fyn activation in these processes remain poorly defined. Here we show that receptor-like protein-tyrosine phosphatase α (PTPα) is an important positive regulator of Fyn activation and signaling that is required for the differentiation of oligodendrocyte progenitor cells (OPCs). PTPα is expressed in OPCs and is up-regulated during differentiation. We used two model systems to investigate the role of PTPα in OPC differentiation: the rat CG4 cell line where PTPα expression was silenced by small interfering RNA, and oligosphere-derived primary OPCs isolated from wild-type and PTPα-null mouse embryos. In both cell systems, the ablation of PTPα inhibited differentiation and morphological changes that accompany this process. Although Fyn was activated upon induction of differentiation, the level of activation was severely reduced in cells lacking PTPα, as was the activation of Fyn effector molecules focal adhesion kinase, Rac1, and Cdc42, and inactivation of Rho. Interestingly, another downstream effector of Fyn, p190RhoGAP, which is responsible for Rho inactivation during differentiation, was not affected by PTPα ablation. *In vivo* studies revealed defective myelination in the PTPα$^{-/-}$ mouse brain. Together, our findings demonstrate that PTPα is a critical regulator of Fyn activation and of specific Fyn signaling events during differentiation, and is essential for promoting OPC differentiation and central nervous system myelination.

Myelination is an essential feature of the vertebrate nervous system. The myelin sheath provides electrical insulation to axons and facilitates transmission of nerve impulses. Other important roles of myelin are to contribute to neuronal survival and development, as well as neurotransmission and synaptic activity (1). Deficiencies in myelination during development, or demyelination that can occur following injury or in diseases such as multiple sclerosis, lead to neurological disorders (2–4).

The formation of the highly specialized multilamellar myelin sheath by oligodendrocytes (OLs)[3] in the CNS occurs early in development, following proliferation and migration of oligodendrocyte progenitor cells (OPCs) to their final axonal targets (5). The molecular mechanisms that regulate OPC differentiation and OL maturation and myelination remain poorly understood. Consistent with the physical juxtaposition of axons and enwrapping oligodendroglia, axonal signals have been identified that influence OPC differentiation and/or myelination, such the axonal ligands Jagged1 and contactin that engage the glial receptor Notch (6, 7). Other signals, such as those described below that are provided by components of the extracellular matrix or the presence or absence of growth factors, are also important in these processes.

The Src family tyrosine kinase (SFK) Fyn is an essential participant and central coordinator of OL differentiation, maturation, and myelination. Although mice null for the SFKs Src, Yes, or Lyn do not exhibit defects in CNS myelination, mice with mutant Fyn or lacking Fyn exhibit hypomyelination (8, 9). *In vitro* studies have linked Fyn activation or inhibition to several stimuli that, respectively, induce or inhibit OL differentiation and maturation. Fyn is required for and activated in OL differentiation by serum withdrawal, IGF-1, β1 integrin stimulation (for example, by laminin binding to α6β1 integrin), netrin-1 interaction with the receptor Dcc, and antibody-mediated cross-linking of MAG or FcRγ (8, 10–15). Recently, co-stimulation of an integrin-contactin complex in OLs was found to amplify Fyn activation and promote myelination (16). Conversely, inhibition of OL differentiation by LINGO-1 or myelin protein extract mimicking the myelin debris generated by demyelination results in reduced Fyn activity (17, 18). Fyn signals to several molecules that are important for OL morphological changes that require cytoskeletal rearrangement and process extension and elaboration, such as focal adhesion kinase

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[3] The abbreviations used are: OL, oligodendrocyte; bFGF, basic fibroblast growth factor; CNTF ciliary, neurotrophic factor; CNPase, 2′,3′-cyclic nucleotide 3′-phosphodiesterase; FAK, focal adhesion kinase; GFAP, glial fibrillary acidic protein; GAP, GTPase activating protein; IGF, insulin-like growth factor; MAG, myelin-associated glycoprotein; MBP, myelin basic protein; OPC, oligodendrocyte progenitor cell; PBD, PAK-binding domain; PDL, poly-D-lysine; PDLO, poly-DL-ornithine; PTP, protein-tyrosine phosphatase; RBD, rhotekin-binding domain; SFK, Src family kinase; PBS, phosphate-buffered saline; siRNA, small interfering RNA; GST, glutathione *S*-transferase; WT, wild type; CNS, central nervous system; ANOVA, analysis of variance; P10 and P18, postnatal day 10 and postnatal day 18.

(FAK), the Rho GTPases Rho, Rac1, and Cdc42, the Rho regulators p190 and p250 RhoGAP, Tau protein, and possibly via the kinase Cdk5 to paxillin (13, 19–23). It also controls myelin production transcriptionally and post-transcriptionally (24, 25).

SFK activation in various cell types and model systems is regulated by catalytic and non-catalytic mechanisms, and is manifested by altered SFK tyrosine phosphorylation. Dephosphorylation of the C-terminal tail inhibitory tyrosine residue by protein-tyrosine phosphatases (PTPs) is an important event in SFK activation (26). The potential roles of PTPs in coupling upstream signals, many of which involve engagement of catalytically inactive receptors, to Fyn activation in OL differentiation have not been extensively investigated. Indeed, although several PTPs have been implicated in OL differentiation and myelination, little is known of their specific actions in these processes. Investigation of regulated PTP expression during differentiation of the CG4 OL cell line revealed the most abundant of the 11 transcripts identified to be those of 4 receptor-type PTPs: PTPα, PTPε, PTPζ, and PTPγ (27). Roles for several of these receptor PTPs in *in vivo* myelination have been described. PTPα and PTPε are involved in peripheral nervous system myelination (28, 29). CNS myelination appears normal in PTPζ-null mice, although myelin stability may be reduced (30). However, remyelination is impaired in PTPζ-null mice after experimental autoimmune encephalomyelitis-induced demyelination (31). Mice null for another receptor PTP, CD45, have reduced numbers of MAG-positive myelinating OLs and exhibit general, mild dysmyelination (32). FcRγ-stimulated $CD45^{-/-}$ OPCs show impaired morphological differentiation and a lack of MBP up-regulation. In normal OPCs, CD45 is complexed with Fyn and the Fyn negative regulatory kinase Csk, suggesting that CD45 is a candidate activator of Fyn and Fyn-MBP signaling in response to FcRγ engagement. Myelination defects are also detected in mice lacking the cytosolic PTP SHP-1, and MBP expression is reduced in OLs from the mice (33).

Although PTPα mRNA is up-regulated in OL differentiation, a role for PTPα in this process and in CNS myelination has not been described. PTPα is a well characterized activator of SFKs (34). It is an essential component of several SFK-dependent signaling systems, and acts in physical and functional conjunction with various ligand-activated receptors; such as integrins, contactin, NCAM, and c-Kit, to catalyze Fyn dephosphorylation and activation (35–38). We therefore investigated whether PTPα is required for OL differentiation and Fyn activation and signaling in this process using two model systems: the cultured CG4 OL cell line in which PTPα expression was ablated by siRNA-mediated silencing, and primary OPCs derived from wild-type and $PTP\alpha^{-/-}$ mouse embryos. We report that PTPα is required for OL differentiation and morphological changes. It acts as an essential but not the only regulator of Fyn in differentiating OLs, and is required for Fyn signaling to several effectors that includes a distinct mechanism of Rho inhibition. Furthermore, we find that forebrain myelination is impaired in PTPα-deficient mice.

EXPERIMENTAL PROCEDURES

Materials—Reagents were obtained from Sigma, unless otherwise indicated. Anti-PTPα antiserum has been described previously (39). Antibodies to A2B5, O4, NG2, MBP, and phosphotyrosine (4G10) were purchased from Millipore (Billerica, MA). Antibodies to phospho-Tyr^{527}-Src and phospho-Tyr^{576}-FAK were purchased from BIOSOURCE (Camarillo, CA). Antibodies to Fyn, FAK, Rac1, Cdc42, and p190 RhoGAP were purchased from BD Transduction Laboratories. Antibody for the immunoprecipitation of Fyn was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Secondary antibodies conjugated with Alexa Fluor 488 or 594 (Molecular Probes) were purchased from Invitrogen. Human recombinant platelet-derived growth factor-AA, bFGF, and epidermal growth factor were purchased from PeproTech (Rocky Hill, NJ). Human recombinant IGF-1 was purchased from BioVision (Mountain View, CA).

Cell Line and Primary Cell Cultures—CG4 cells were kindly provided by Dr. Y. Feng (Emory University School of Medicine) and maintained in CG4 proliferation medium (Dulbecco's modified Eagle's medium, 1% FBS, 5 μg/ml of insulin, 50 μg/ml of transferrin, 30 nM sodium selenite, 100 μM putrescine, 20 nM progesterone, 10 ng/ml of biotin, 10 ng/ml of platelet-derived growth factor, and 10 ng/ml bFGF). To promote differentiation, cells were seeded on poly-D-lysine (PDL, 10 μg/ml)-coated dishes at a density of $1.5 \times 10^4/cm^2$. After attachment (~3 h), cells were gently washed and subsequently cultured in CG4 differentiation medium (Dulbecco's modified Eagle's medium, 0.5% FBS, 5 μg/ml of insulin, 50 μg/ml of transferrin, 30 nM sodium selenite, 50 nM triiodothyronine) for various times. Primary mouse OPCs were generated from neurospheres as described previously (40, 41) with some modifications. In brief, after removal of meninges and cerebellum, cerebral cortex tissue from E14.5–E17.5 mouse embryos was mechanically triturated with a 1-ml Gilson pipette until the cell suspension had no or very few small clumps, filtered through a 70-μm cell strainer, and plated at 5×10^4 cells/ml in a six-well plate (4 ml/well of neural culture medium supplemented with 20 ng/ml of bFGF and 20 ng/ml of epidermal growth factor). Neural culture medium contains Dulbecco's modified Eagle's medium/F-12, 25 μg/ml of insulin, 100 μg/ml of apo-transferrin, 20 nM progesterone, 60 μM putrescine, and 30 nM sodium selenite. After 3–4 days, floating neurospheres were passaged at a 1:3 ratio in the same medium every 3–4 days. Passage 2–6 neurospheres were mechanically dissociated into a single cell suspension and resuspended in neural culture medium supplemented with 20 ng/ml of platelet-derived growth factor-AA and 20 ng/ml of bFGF (oligosphere medium) to induce oligosphere formation. After 72 h, cell aggregates were passaged at a 1:2 ratio every 4–6 days. Oligospheres (passage 2–6) were dissociated using the NeuroCult Chemical Dissociation kit (mouse) (StemCell Technologies, Alberta, Canada) and plated on poly-DL-ornithine (PDLO, 50 μg/ml)-coated chamber slides or dishes at a density of $3 \times 10^4/cm^2$ in oligosphere medium for 2 days. To induce differentiation, medium was changed to neural culture medium supplemented with 5 μg/ml of *N*-acetyl-L-cysteine and 50 nM triiodothyronine for 2–4 days.

Immunofluorescence Labeling of Cells and Tissues—Cells grown on PDL- or PDLO-coated coverslips or chamber slides (Nalgene Nunc International, Rochester, NY) were fixed with 4% paraformaldehyde for 15 min at room temperature and then

washed three times with PBS. For other experiments, animals were anesthetized and perfused intracardially with 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.2. The brain was removed, post-fixed in the same solution overnight at 4 °C, and then cryopreserved in 30% sucrose in 0.1 M phosphate buffer and embedded in OCT media. Cells and tissue sections (10 μm) were incubated with blocking buffer (0.1 M phosphate buffer, 0.1% gelatin, 1% bovine serum albumin, 0.02% sodium azide, 10% goat serum) for 30 min (0.5% Triton X-100 was added to the blocking buffer if permeablization was required), followed by incubation with primary antibodies overnight at 4 °C. After washing three times with PBS, cells and tissues were incubated with secondary antibodies for 2 h at room temperature. The slides were washed three times with PBS followed by mounting in Prolong Gold Antifade Reagent (Invitrogen) with 4′,6-diamidino-2-phenylindole and viewed using an Axioplan2 fluorescence microscope (Carl Zeiss MicroImaging, Thornwood, NY).

Immunoblotting—Cells were harvested by washing twice with ice-cold PBS on ice. For preparation of lysates, cells were lysed on ice by adding RIPA lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.5% sodium deoxycholate, 1% Nonidet P-40, 0.1% SDS, 1 mM EDTA, 2 mM sodium orthovanadate, 50 mM sodium fluoride, 10 μg/ml of aprotinin, 10 μg/ml of leupeptin, and 1 mM phenylmethylsulfonyl fluoride) or Nonidet P-40 lysis buffer (RIPA lysis buffer without sodium deoxycholate and SDS) directly onto the cells. Cell lysates were then transferred to microtubes and incubated for 30 min on ice, centrifuged at 12,000 × *g* for 10 min at 4 °C, and the supernatants collected to obtain protein extracts. Protein concentration was determined with the BCA Protein Assay kit (Pierce). Protein extracts were resolved by SDS-PAGE and transferred to a polyvinylidene difluoride membrane, which was then blocked with 3% bovine serum albumin in PBS with 0.1% Tween 20 for 1 h at room temperature. The membranes were probed overnight at 4 °C with the relevant antibodies, washed, and probed again with species-specific secondary antibodies coupled to horseradish peroxidase. Chemiluminescent reagents were then added for signal detection.

Immunoprecipitation—Cell lysates (50–100 μg of protein) prepared with RIPA buffer were immunoprecipitated with the indicated antibody at 4 °C overnight, followed by incubation with 40 μl of protein A/G-agarose (Santa Cruz Biotechnology) at 4 °C for 3 h. After washing three times with lysis buffer, the immunoprecipitates were analyzed by immunoblotting.

siRNA Transfection—The following siRNAs (Dharmacon, Chicago, IL) were used: Control (siCONTROL Non-Targeting siRNA Pool number 2 D-001206-14-20), PTPα (ON-TARGETplus SMARTpool L-080089-01-0050), and rat PTPRA, NM_012763). CG4 cells were seeded in CG4 proliferation medium ($3 \times 10^4/cm^2$). After overnight attachment, cells were incubated with 20 nM siRNA and Lipofectamine RNAiMax (Invitrogen) for 24 h. Cells were trypsinized and seeded on PDL-coated chamber slides or dishes in CG4 proliferation medium. After 3 h, cells were gently washed and incubated in CG4 differentiation medium for various times.

Rho Family GTPase Activities—Rho activity was measured by GST-RBD (rhotekin-binding domain) pulldown assays performed using the Rho activation assay kit (Upstate, Temecula, CA). Rac1 and Cdc42 activities were measured by GST-PBD (PAK-binding domain) pulldown assays (42). Cells were lysed on ice by adding RIPA lysis buffer directly onto the cells. Cell lysates (50–100 μg) were incubated with 10 μg of GST-PBD bound to glutathione-Sepharose beads. Samples were washed with lysis buffer and then immunoblotted with anti-Rac1 and Cdc42 antibodies. Lysates were directly immunoblotted to determine the total amount of Rho, Rac1, or Cdc42 proteins. Levels of active Rho, Rac1, and Cdc42 were normalized to those of total Rho, Rac1, and Cdc42.

Data Analysis—Densitometric quantification of immunoblots and cell differentiation data were statistically analyzed using ANOVA (single factor).

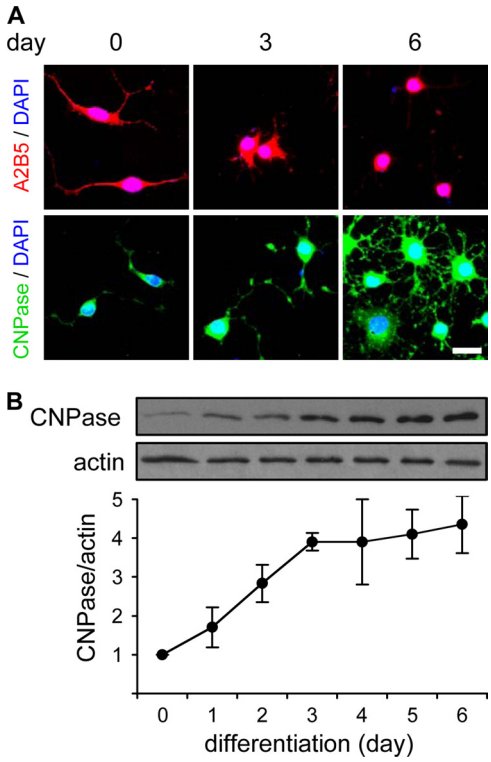

FIGURE 1. **Characterization of CG4 cell differentiation.** CG4 cells were placed in CG4 differentiation medium for the indicated times. *A*, cells were immunostained with antibodies against A2B5 and CNPase. *Scale bar*, 10 μm. *B*, CNPase and actin protein amounts were determined by immunoblotting cell lysates. The band intensities were quantified from three independent experiments. CNPase was normalized to actin and is shown in the graph as mean ± S.D. *DAPI*, 4′,6-diamidino-2-phenylindole.

RESULTS

Characterization of CG4 Cell Differentiation—To study OL differentiation, we used the rat-derived CG4 cell line (A2B5-positive OPCs) (43). When the cells were plated on PDL-coated dishes and incubated in CG4 differentiation medium for up to 6 days, they underwent differentiation from A2B5-positive bipolar OPCs into CNPase-positive OLs that extend multiple branched processes (Fig. 1*A*). To confirm that specific OL markers were up-regulated in these cells after differentiation was induced, cell lysates were subjected to immunoblotting with anti-CNPase antibody. CNPase protein levels were up-regulated in CG4 cells during differentiation (Fig. 1*B*).

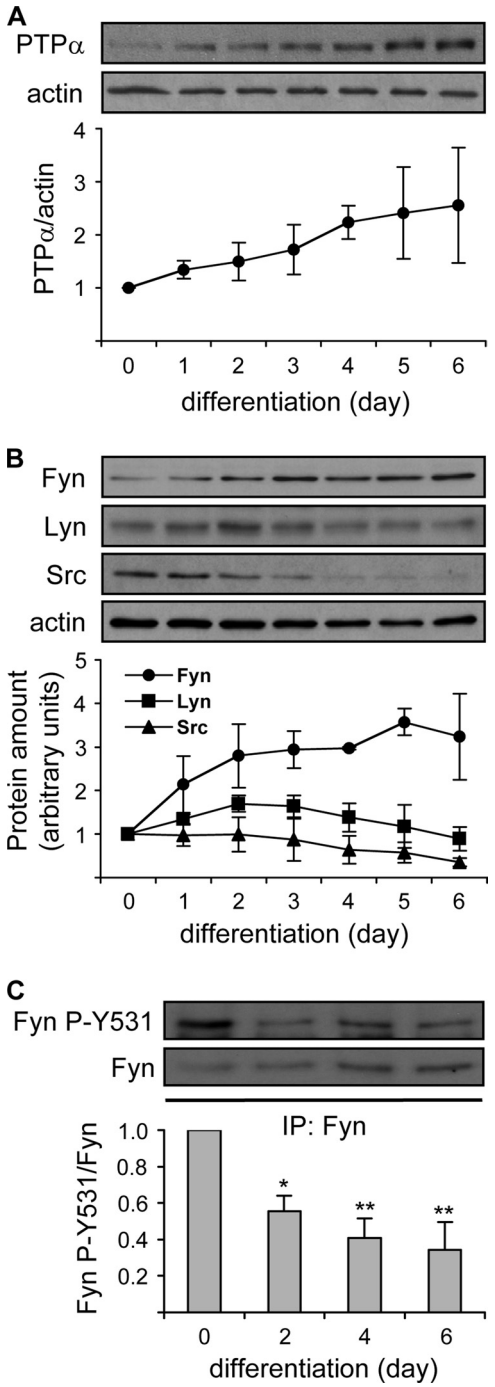

FIGURE 2. **Protein expression of PTPα and SFKs and activity of Fyn in CG4 cells during differentiation.** CG4 cells were placed for the indicated times in CG4 differentiation medium. Lysates were probed for PTPα and actin (*A*), or Fyn, Lyn, Src, and actin (*B*). The band intensity of each protein was normalized to that of actin. *C*, Fyn phosphorylation at Tyr^{531} was analyzed by immunoprecipitation (*IP*) followed by immunoblotting with anti-Src P-Tyr^{527} antibody. Membranes were stripped and reprobed with anti-Fyn antibody. The band intensity of Fyn P-Tyr^{531} was normalized to that of Fyn. Data obtained from three independent experiments are shown in the graph as the mean ± S.D. *, $p < 0.05$; **, $p < 0.01$, ANOVA test.

It has been reported that PTPα mRNA expression increases during OL differentiation (27). As shown in Fig. 2*A*, we found that the PTPα protein level was up-regulated ~1.5-fold in CG4 cells after differentiation for 2 days and was gradually up-regulated to ~2.5-fold during differentiation days 3–6, indicating that PTPα may play a role in this process.

Because PTPα can dephosphorylate and activate SFKs (34), we characterized the protein expression of the three SFKs present in OLs, Fyn, Lyn, and Src (25), in differentiating CG4 cells. Among these SFKs, Fyn plays a unique role in myelination, because myelin deficits are only found in $Fyn^{-/-}$ mice and not in $Lyn^{-/-}$ or $Src^{-/-}$ mice (9). As shown in Fig. 2*B*, Fyn protein level rapidly increased (2.8-fold) over the first 2 days of CG4 differentiation, and then increased slightly further and was maintained for the remainder of the 6-day differentiation period. Consistent with previous studies (10, 12, 24), we found that Lyn protein level increased by 1.7-fold over the first 2 days of differentiation, and then decreased over subsequent days to return to the starting level or somewhat lower by days 5–6. Src protein continually decreased during differentiation, and by day 6 was reduced to 35% of the starting level. In conjunction with these findings, and as Fyn is reported to be the only SFK with significant kinase activity in either cultured OPCs or OLs (10), we investigated Fyn activity in differentiating CG4 cells by determining its phosphorylation status. Phosphorylation of Fyn at the negative regulatory C-terminal tail residue Tyr^{531} was significantly reduced during CG4 differentiation, especially over the first 2 days (Fig. 2*C*), indicative of Fyn activation. Overall, Fyn Tyr^{531} phosphorylation per unit of Fyn protein decreased to 34% of the starting level by day 6.

PTPα Is Required for CG4 Differentiation—To investigate the role of PTPα in OL differentiation, we generated PTPα-deficient CG4 cells using siRNA. Cells were cultured in proliferation medium and transfected, 24 h later they were seeded on PDL-coated plates. After 3 h of attachment in proliferation medium, the medium was changed to CG4 differentiation medium (differentiation day 0). Lysates of control siRNA- and PTPα siRNA-treated CG4 cells were prepared from cells maintained for 0–3 days in differentiation medium, and examined by immunoblotting to determine the effectiveness of siRNA-mediated knockdown (Fig. 3*A*). PTPα expression was reduced by more than 90% during the 24–72 h following siRNA transfection (differentiation day 0–2). To evaluate the differentiation of control siRNA- and PTPα siRNA-treated CG4 cells after 2 days in differentiation medium, they were immunostained with anti-A2B5 and anti-CNPase antibodies for microscopic visualization and quantitative measurements. The numbers of A2B5-positive cells were counted, and about 4 times more cells in the PTPα-knockdown CG4 population were found to remain A2B5-positive (progenitor-like) compared with the control siRNA-treated cell population (Fig. 3, *B* and *C*). CNPase immunofluorescence revealed multiple branched processes that were formed by the control siRNA-treated cells, but that were lacking in the PTPα-directed siRNA-treated cells (Fig. 3*B*, *bottom panels*). As cell process extension is an indicator of differentiation, we counted the number of processes per cell with a length greater than that of the cell body. After 2 days in differentiation medium, significantly more PTPα-knockdown CG4 cells had a low number (2 or less) of extended processes per cell, and fewer PTPα-knockdown CG4 cells had a high number (4 or 5) of extended processes per cell, compared with control siRNA-treated cells (Fig. 3, *B* and *D*). Moreover, CNPase protein

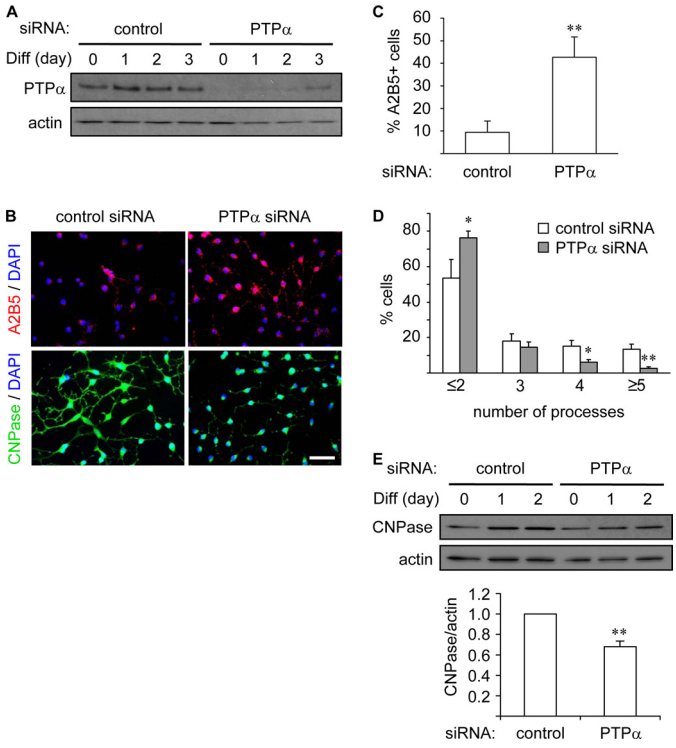

FIGURE 3. **PTPα is required for CG4 differentiation.** *A*, PTPα expression in siRNA-treated CG4 cells. CG4 cells were transfected with control siRNA or PTPα siRNA overnight and then seeded on PDL-coated dishes. Cells were placed for the indicated times in CG4 differentiation medium (day 0) and maintained for the indicated times. Cell lysates were probed with anti-PTPα and anti-actin antibodies. *B*, CG4 cells were induced to differentiate on PDL-coated chamber slides for 2 days and then immunostained with anti-A2B5 and anti-CNPase antibodies. *Scale bar*, 20 μm. *C*, the number of A2B5-positive cells was counted (at least 100 cells were counted for each group). Data obtained from three independent experiments are expressed as the mean ± S.D. **, $p < 0.01$, ANOVA test. *D*, the number of processes (longer than one cell body) per cell were counted (at least 100 were counted for each group). Data obtained from three independent experiments are expressed as the mean ± S.D. *, $p < 0.05$; **, $p < 0.01$, ANOVA test. *E*, CNPase expression in siRNA-treated CG4 cells. Control siRNA and PTPα siRNA-treated CG4 cells were induced to differentiate on PDL for 0–2 days, and lysates were probed with anti-CNPase and anti-actin antibodies. The band intensity of CNPase was normalized to that of actin. Data obtained from three independent experiments for CNPase expression per unit of actin on differentiation day 2 are shown as the mean ± S.D. **, $p < 0.01$, ANOVA test. *DAPI*, 4′,6-diamidino-2-phenylindole.

expression was lower in PTPα-knockdown CG4 cells than in control siRNA-treated cells after differentiation (Fig. 3*E*). These results indicate that PTPα is required for the differentiation of CG4 cells into OLs.

PTPα Is Required for Activation of Fyn and Fyn Effectors FAK, Rac1, and Cdc42 during CG4 Differentiation—To determine whether PTPα is required to dephosphorylate and activate Fyn in differentiating CG4 cells, the phosphorylation of Fyn at Tyr^{531} was determined in control siRNA- and PTPα siRNA-treated CG4 cells that were induced to differentiate for 2 days. Fyn immunoprecipitates from PTPα-directed siRNA-treated cells contained higher levels of phospho-Tyr^{531}-Fyn than those from control siRNA-treated cells (Fig. 4*A*), indicating that PTPα is required to dephosphorylate this tyrosine residue and activate Fyn in differentiating CG4 cells.

The effect of silencing PTPα on the activation of several downstream effectors of Fyn was examined. FAK activation involves the phosphorylation of FAK Tyr^{576} (44), and this is reported to be up-regulated in a Fyn-dependent manner during differentiation of CG4 cells (19). We confirmed that phosphorylation of FAK Tyr^{576} increases during CG4 differentiation (Fig. 4*B*, *upper panels*). This required PTPα, because compared with control siRNA-treated CG4 cells, the PTPα siRNA-treated cells displayed significantly reduced (by ~40%) phospho-Tyr^{576}-FAK after induction of differentiation (Fig. 4*B*, *lower panels*).

The Rho family GTPases Rac1 and Cdc42 play important roles in cytoskeleton rearrangement and are crucial for morphological differentiation of OLs and myelination (13, 45). It has also been reported that activation of Rac1 and Cdc42 is dependent on the activity of Fyn and FAK in differentiating OLs (13, 19). We therefore investigated whether the activities of Rac1 and Cdc42 were affected by PTPα silencing in CG4 cells following the induction of differentiation. Using GST-PBD pulldown assays to measure the levels of active GTP-bound Rac1 and Cdc42, we found that both Rac1 and Cdc42 were activated in CG4 cells during differentiation (Fig. 4*C*, *upper panels*). However, the differentiation-induced activity of both Rac1 and Cdc42 was significantly reduced by more than 50% in PTPα-knockdown CG4 cells placed in differentiation medium for 2 days (Fig. 4*C*, *lower panels*). These results indicate that PTPα is required for Fyn-mediated signaling to FAK, Rac1, and Cdc42 in differentiating CG4 cells.

PTPα Is Not Required for Fyn-mediated Signaling to p190RhoGAP, but Is Required for Rho Inactivation during CG4 Differentiation—During OL differentiation, the Fyn-interacting protein and substrate p190RhoGAP is tyrosine phosphorylated, resulting in increased p190RhoGAP activity that promotes Rho inhibition and OL differentiation (13, 20). In accord with these findings, we observed that p190RhoGAP co-immunoprecipitated with Fyn in both progenitor and differentiating CG4 cells and that tyrosine phosphorylation of p190RhoGAP increased after differentiation for 2 days (Fig. 5*A*). To investigate if PTPα is an upstream regulator of differentiation-induced Fyn signaling to p190RhoGAP, we determined Fyn and p190RhoGAP association and p190RhoGAP tyrosine phosphorylation in CG4 cells that were treated with control siRNA or PTPα-directed siRNA and induced to differentiate for 2 days. Surprisingly, both the Fyn-p190RhoGAP interaction and tyrosine phosphorylation of p190RhoGAP were not affected by PTPα siRNA treatment (Fig. 5*B*). These results suggest that PTPα does not act upstream of Fyn-mediated regulation of p190RhoGAP, and that PTPα thus regulates specific aspects of Fyn signaling in differentiating CG4 cells.

The Rho family GTPase Rho plays important roles in controlling cellular morphology. Overexpression of constitutively active Rho inhibits process extension in OLs, whereas overexpression of dominant-negative Rho results in a hyperextension of OL processes (20). Because the primary function of p190RhoGAP is to inactivate Rho, we determined Rho activities to determine whether, like p190RhoGAP, this was unaffected upon PTPα silencing. Using GST-RBD pulldown assays to measure the levels of active GTP-bound Rho, we found that Rho was inactivated in CG4 cells during differentiation (Fig. 5*C*). Compared with control CG4 cells, Rho activity was significantly increased by ~3-fold in PTPα-

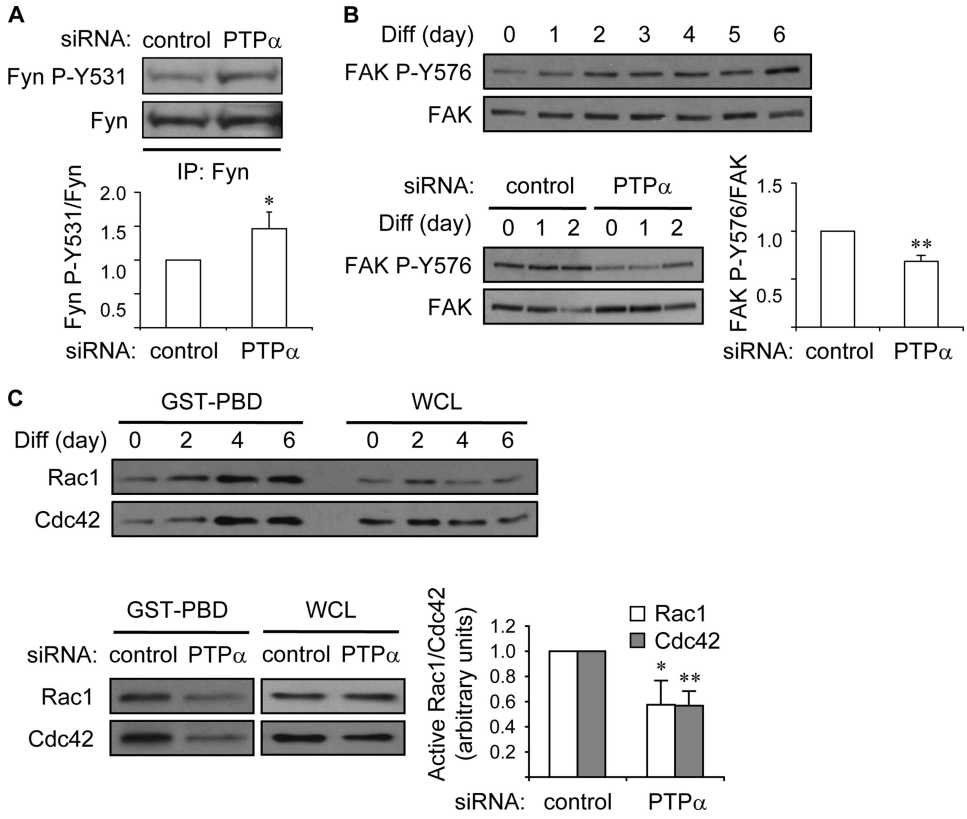

FIGURE 4. **PTPα is required for activation of Fyn and its downstream effectors FAK, Rac1, and Cdc42 in CG4 cells.** *A*, reduced activity of Fyn in PTPα-knockdown CG4 cells at differentiation day 2. Fyn phosphorylation at Tyr^{531} was analyzed by immunoprecipitation (*IP*) with anti-Fyn antibody followed by probing with anti-Src P-Tyr^{527} and anti-Fyn antibodies. The band intensity of Fyn P-Tyr^{531} was normalized to that of Fyn. Data obtained from three independent experiments are expressed as the mean ± S.D. *, $p < 0.05$, ANOVA test. *B*, CG4 cells were differentiated for 0–6 days. Tyrosine phosphorylation of FAK Tyr^{576} and protein expression of FAK were determined by immunoblotting with anti-FAK P-Tyr^{576} and anti-FAK antibodies, respectively (*top panels*). Control siRNA or PTPα siRNA-treated cells were differentiated for 0–2 days. FAK tyrosine phosphorylation at Tyr^{576} and FAK amounts were determined as described above (*bottom panels*). The band intensity of FAK P-Tyr^{576} (differentiation day 2) was normalized to that of FAK. Data obtained from three independent experiments are shown in the graph as the mean ± S.D. **, $p < 0.01$, ANOVA test. *C*, cell lysates from CG4 cells grown on PDL-coated dishes in CG4 differentiation medium for 0–6 days were incubated with a GST-PBD fusion protein to pull down the GTP-bound forms of Rac1 and Cdc42. The amount of Rac1 and Cdc42 in the pulldown assay as well as in the whole cell lysates (*WCL*) were determined by immunoblotting with anti-Rac1 and anti-Cdc42 antibodies, respectively (*top panels*). Control siRNA or PTPα siRNA-treated cells were differentiated for 2 days. Active Rac1 and Cdc42 were determined as described above. The band intensity of GTP-bound Rac1 and Cdc42 was normalized to that of Rac1 and Cdc42 in whole cell lysates. Data obtained from three independent experiments are shown in the graph as the mean ± S.D. *, $p < 0.05$; **, $p < 0.01$, ANOVA test.

knockdown CG4 cells placed in differentiation medium for 2 days (Fig. 5*C*). Taken together, these results indicate that PTPα is required for Rho inactivation in differentiating CG4 cells, but in a manner independent of Fyn-p190RhoGAP signaling.

Isolation and Characterization of OPCs from Mouse Embryonic Neural Progenitors—To extend our findings from CG4 cells where PTPα expression was transiently silenced, we investigated the role of PTPα in OPC differentiation in a different model system where PTPα expression was permanently ablated, using primary OPCs isolated and cultured from PTPα-null mouse embryos. Mouse OPCs are reportedly more difficult to isolate as cultured cells than rat OPCs, because they do not share all of the cell surface antigens with rat, such as A2B5, and they tend to differentiate in *in vitro* mixed glial cultures (40, 46, 47). Several studies have determined that self-renewing OPCs can be generated from neural progenitor/stem cells of different species (40, 41, 47–50), and we utilized these procedures with some modifications (see "Experimental Procedures") to isolate and culture OPCs from wild-type (WT) and PTP$\alpha^{-/-}$ mouse embryos.

Neuronal markers are not detected in OPC cultures derived from oligospheres (41), suggesting that these OPCs are mainly OL type-2 astrocyte (O-2A) progenitors that can differentiate into OLs or type-2 astrocytes. WT mouse OPCs from P2 oligospheres were seeded on PDLO-coated dishes in OPC proliferation medium for 2 days. Cells were fixed and immunostained with antibodies against the progenitor marker NG2, the pre-OL marker O4, and the astrocyte marker GFAP. As shown in Fig. 6*A*, >95% of WT mouse OPCs were positive for NG2 and negative for either O4 or GFAP. The cells were then induced to differentiate by mitogen withdrawal and thyroid hormone (triiodothyronine) exposure (51). In addition, *N*-acetyl-L-cysteine was added to support cell survival (52). Two growth factors, IGF-1 (53, 54) and ciliary neurotrophic factor (CNTF) (55) can promote OPC differentiation and survival. Therefore, we also examined the effects of IGF-1 and CNTF on the differentiation of mouse OPCs. We found that after differentiation for 2 days, the numbers of NG2+ cells decreased and O4+ cells increased in all conditions tested (Fig. 6*A*). In addition, O4 immunostaining revealed that more cells extended highly branched processes in the presence of IGF-1, and that there were less O4+ cells but more GFAP+ cells in the presence of CNTF. Moreover, very few GFAP+ cells were detected in OPC differentiation medium with or without IGF-1. These results are consistent with previous studies showing that IGF-1 induces OPCs to differentiate into OLs (56), whereas CNTF also induces OPCs to differentiate into type-2 astrocytes (57). Therefore, to enrich the OL population after OPC differentiation, we used OPC differentiation medium with or without IGF-1 for further studies.

To characterize the role of PTPα in primary mouse OLs, we first examined PTPα protein expression during WT OPC differentiation. Consistent with our finding in CG4 cells, PTPα protein expression, as well as that of Fyn and CNPase, increased during the differentiation of primary mouse OPCs (Fig. 6*B*).

PTPα Is Required for Primary Mouse OPC Differentiation—To investigate the role of PTPα in the differentiation of primary mouse OPCs, WT and PTP$\alpha^{-/-}$ OPCs were induced to differ-

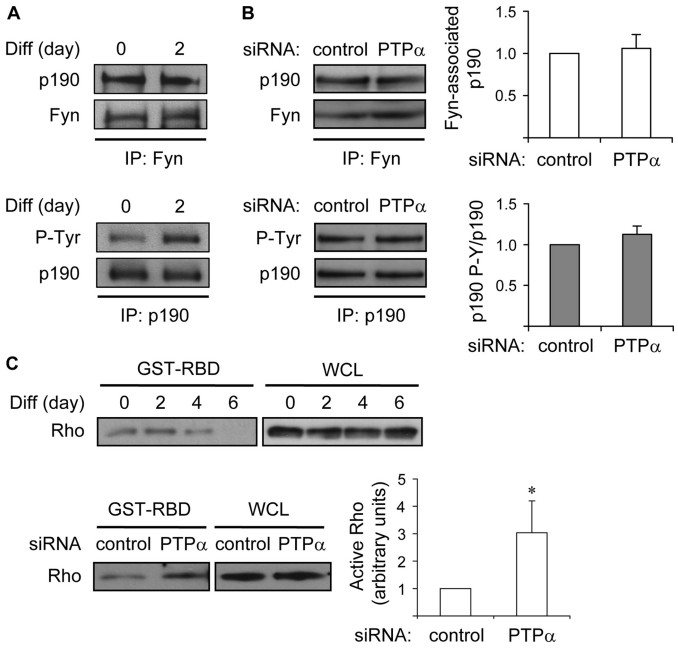

FIGURE 5. **PTPα is not required for tyrosine phosphorylation of p190RhoGAP (p190) and Fyn-p190 interaction in CG4 cells.** *A*, p190-Fyn interaction and tyrosine phosphorylation of p190 in CG4 cells. CG4 cells were induced to differentiate for 0–2 days. Immunoprecipitation (*IP*) complexes isolated using antibodies against Fyn (*top two panels*) or p190 (*bottom two panels*) were evaluated by immunoblotting with anti-p190, Fyn, or P-Tyr (4G10) antibodies. *B*, p190-Fyn interaction and tyrosine phosphorylation of p190 are not altered by PTPα-knockdown in differentiating CG4 cells. Control and PTPα siRNA-treated CG4 cells were induced to differentiate for 2 days. Immunoprecipitation complexes isolated using antibodies against Fyn or p190 were evaluated by immunoblotting with anti-p190, Fyn, or P-Tyr (4G10) antibodies. The band intensity of p190 was normalized to that of Fyn in Fyn immunoprecipitates (*top graph*). The band intensity of P-Tyr (4G10) was normalized to that of p190 in p190 immunoprecipitates (*bottom graph*). Data obtained from three independent experiments are shown in the graph as the mean ± S.D. No significant differences were found between control siRNA and PTPα siRNA-treated cells. *C*, cell lysates from CG4 cells grown on PDL-coated dishes in CG4 differentiation medium for 0–6 days were incubated with a GST fusion containing the RBD to pull down the GTP-bound form of Rho. The amount of Rho in the pulldown assay as well as in the whole cell lysate (*WCL*) was determined by immunoblotting with anti-Rho antibody. Control siRNA or PTPα siRNA-treated cells were differentiated for 2 days. Rho activity was determined as described above. The band intensity of GTP-bound Rho was normalized to that of Rho in WCL. Data obtained from three independent experiments are expressed as the mean ± S.D. *, $p < 0.05$, ANOVA test.

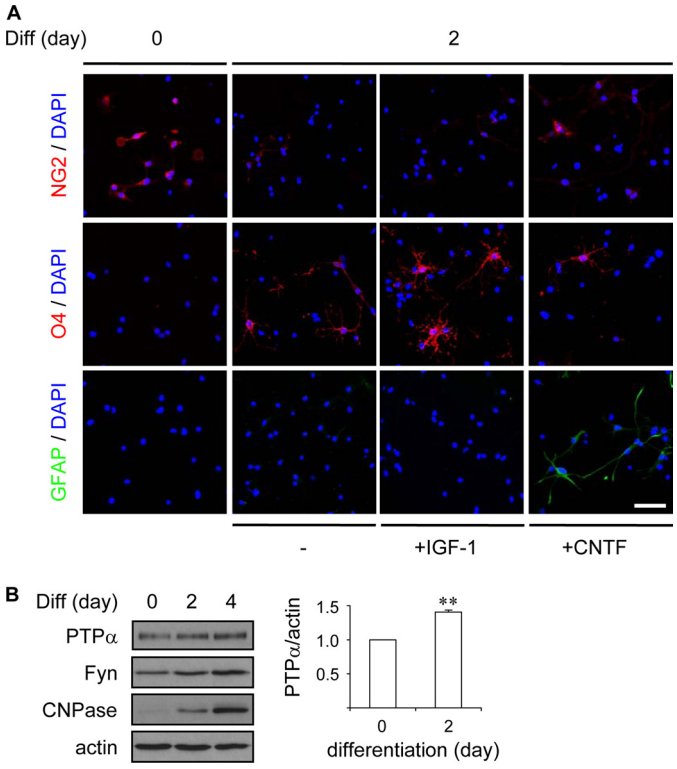

FIGURE 6. **Characterization of primary mouse OPC differentiation.** *A*, WT mouse P2 oligospheres were dissociated and seeded on PDLO-coated chamber slides for 2 days in OPC proliferation medium followed by incubation for the indicated times in OPC differentiation medium alone (−), or with 100 ng/ml of IGF-1 or 10 ng/ml of CNTF. Cells were immunostained with antibodies against NG2, O4, and GFAP. *Scale bar*, 20 μm. *B*, WT mouse P2 oligospheres were dissociated and seeded on PDLO-coated dishes for 2 days in OPC proliferation medium followed by incubation for the indicated times in OPC differentiation medium. The levels of PTPα, Fyn, CNPase, and actin were determined by immunoblotting cell lysates. The band intensity of PTPα (differentiation day 0 and 2) was quantified and normalized to that of actin. Data obtained from three independent experiments are shown in the graph as the mean ± S.D. **, $p < 0.01$, ANOVA test. *DAPI*, 4′,6-diamidino-2-phenylindole.

entiate for 2 days in OPC differentiation medium with or without IGF-1. After differentiation in both conditions, PTPα$^{-/-}$ OLs expressed less of the OL marker CNPase than did WT OLs (Fig. 7*A*), suggesting that their differentiation was impaired. This was confirmed by immunostaining with antibodies against the progenitor marker NG2 and the pre-OL marker O4. As shown in Fig. 7*B*, after differentiation for 2 days in medium with or without IGF-1, fewer WT than PTPα$^{-/-}$ cells were NG2+ (*top panels*), and more WT than PTPα$^{-/-}$ cells were O4+ (*lower panels*). The NG2+ and O4+ cells of each genotype were quantified (Fig. 7*C*), demonstrating that about 3–4-fold higher populations of O4+ WT cells than PTPα$^{-/-}$ cells, and about 3–3.5-fold lower populations of NG2+ WT cells than PTPα$^{-/-}$ cells, were present after 2 days in differentiation medium ± IGF-1. These results further confirm that PTPα is required for OPC differentiation.

PTPα Selectively Regulates Fyn Activation and Signaling in Primary Mouse OPCs—To confirm that PTPα is required for Fyn dephosphorylation at its negative regulatory site, lysates from WT and PTPα$^{-/-}$ progenitors or differentiating OLs were immunoprecipitated with anti-Fyn antibody followed by immunoblotting. Fyn phospho-Tyr^{528} (the equivalent of rat Fyn Tyr^{531}) was enhanced 2-fold in PTPα$^{-/-}$ OPCs and differentiating OLs compared with the WT group, suggesting that PTPα is required for Fyn dephosphorylation at Tyr^{528} in both progenitors and OLs (Fig. 8*A*). Fyn phospho-Tyr^{528} decreased by ~40% in both WT and PTPα$^{-/-}$ cells after differentiation ± IGF-1 for 2 days (Fig. 8*A*), suggesting that there are also other regulators of Fyn activation during OPC differentiation. Despite the decreased phosphorylation of Fyn Tyr^{528} that occurs in both WT and PTPα$^{-/-}$ cells during differentiation, the level of Fyn Tyr^{528} phosphorylation in the PTPα$^{-/-}$ cells after 2 days differentiation was not significantly lower than that in undifferentiated WT cells (Fig. 8*A*). In conjunction with the impaired differentiation of PTPα$^{-/-}$ cells that we observe, this suggests that non-PTPα-mediated Fyn Tyr^{528} activation is insufficient to promote mouse OPC differentiation.

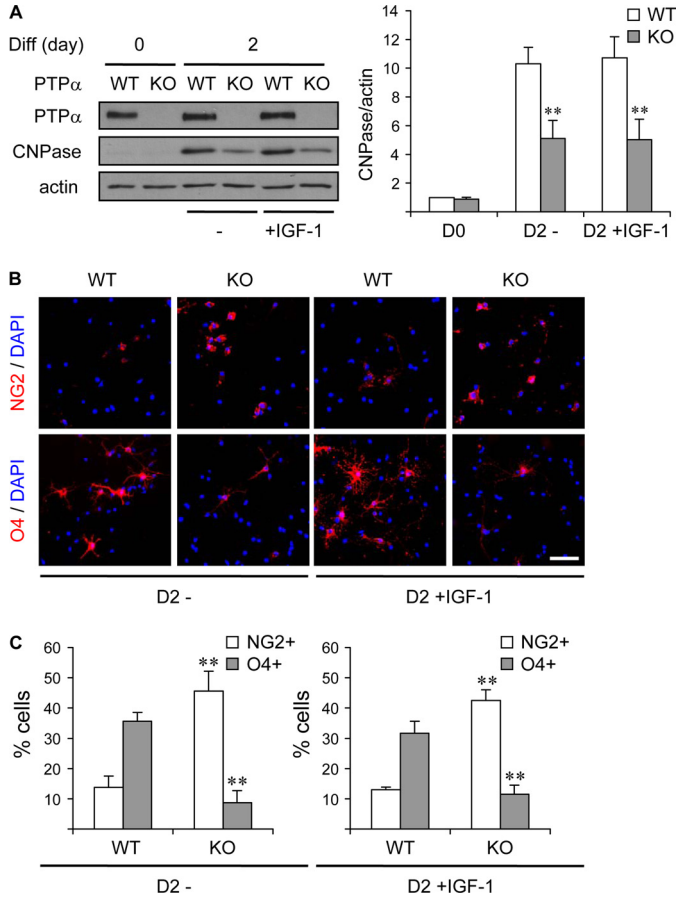

FIGURE 7. **Differentiation of PTPα-null OPCs is impaired.** WT and PTPα$^{-/-}$ mouse oligospheres were dissociated and seeded on PDLO-coated dishes for 2 days in OPC proliferation medium followed by incubation for the indicated times in OPC differentiation medium without (−) or with IGF-1 (+*IGF-1*). *A*, the amounts of PTPα, CNPase, and actin were determined by immunoblotting cell lysates. The band intensity of CNPase was quantified and normalized to that of actin. Data obtained from three independent experiments are shown in the graph as the mean ± S.D. **, $p < 0.01$, ANOVA test. *B* and *C*, cells differentiated for 2 days were immunostained with antibodies against NG2 and O4. *Scale bar*, 20 μm. The numbers of NG2-positive and O4-positive cells were counted. Data obtained from three independent experiments are shown in the graph as the mean ± S.D. **, $p < 0.01$, ANOVA test. *KO*, knockout; *DAPI*, 4′,6-diamidino-2-phenylindole.

To investigate whether PTPα is required for FAK activation in primary mouse OPCs, we examined FAK Tyr^{576} phosphorylation in WT and PTPα$^{-/-}$ progenitors and differentiating OLs. As shown in Fig. 8*B*, FAK phospho-Tyr^{576} was significantly reduced by 60 and 50%, respectively, in PTPα$^{-/-}$ progenitors and differentiating OLs compared with WT cells. Although FAK phosphorylation at Tyr^{576} increased in both WT and PTPα$^{-/-}$ cells after differentiation was induced, the FAK phosphorylation level in the PTPα$^{-/-}$ cells only increased to a level equivalent to that in undifferentiated WT cells (Fig. 8*B*). Thus the differentiation-induced modulation of FAK in both WT and PTPα$^{-/-}$ cells correlated closely with that of Fyn, further indicating that PTPα is required for Fyn-mediated FAK activity in primary mouse OPCs and differentiating OLs.

To determine whether PTPα is required for Fyn-p190RhoGAP signaling in the primary mouse cell system, p190RhoGAP immunoprecipitates were prepared from lysates of WT and PTPα$^{-/-}$ progenitors and differentiating OLs and analyzed. Increased amounts of p190RhoGAP immunoprecipitated from both WT and PTPα$^{-/-}$ cells that had been induced to differentiate for 2 days compared with the undifferentiated OPCs (Fig. 8*C*, *middle panel*), but determination of the phosphotyrosine incorporated into the p190RhoGAP protein revealed that there were no differentiation-induced changes in tyrosine phosphorylation of p190RhoGAP in either cell type or between the cell types (Fig. 8*C*, *top panel* and *graph*). It has been reported that p190RhoGAP associates with p120RasGAP in OLs (20), and that Src homology 2 domain of p120RasGAP binds tyrosine-phosphorylated p190RhoGAP (58). We investigated whether PTPα might affect the p190RhoGAP·p120RasGAP complex formation upon differentiation. Although p120RasGAP was detected in p190-RhoGAP immunoprecipitates, no difference in their extent of association was apparent between WT and PTPα$^{-/-}$ cells or between progenitors and OLs of each genotype (Fig. 8*C*, *bottom panel*), indicating that PTPα is not required for Fyn-mediated p190RhoGAP-p120RasGAP signaling in progenitors and OLs.

To determine whether PTPα is required for Rac1 and Cdc42 activation and Rho inactivation in the primary mouse cell system, we examined Rac1, Cdc42, and Rho activities in WT and PTPα$^{-/-}$ OLs. As shown in Fig. 8, *D* and *E*, Rac1 and Cdc42 activities were reduced, and Rho activity was increased in PTPα$^{-/-}$ OLs compared with WT cells.

Ablation of PTPα Results in Decreased MBP Protein Expression in Primary Mouse OLs and Leads to Defective Myelination—Fyn directly stimulates the promoter activity of the *MBP* gene and is involved in post-transcriptional regulation of MBP mRNA (24, 25). Therefore, we investigated if PTPα is required for Fyn-MBP signaling. The WT and PTPα$^{-/-}$ OPCs were induced to differentiate for 2 days in OPC differentiation medium with or without IGF-1. After differentiation in both conditions, PTPα$^{-/-}$ OLs expressed less MBP than WT OLs (Fig. 9*A*), suggesting that PTPα is also required for Fyn-mediated up-regulation of MBP expression.

In support of the above *in vitro* results, myelinated fibers in WT and PTPα$^{-/-}$ mouse brains were examined for MBP immunoreactivity. Fewer myelinated fibers could be observed in the corpus callosum of P18 PTPα$^{-/-}$ mouse brains (Fig. 9*B*) and in the cortex and striatum of P10 and P18 PTPα$^{-/-}$ mouse brains (Fig. 9*C*). Taken together, these results demonstrate that PTPα is involved in regulating MBP expression during OPC differentiation and thus is required for proper myelination in the brain.

DISCUSSION

In this study, we have demonstrated that PTPα is required for OPC differentiation using two distinct model cell systems. The siRNA-mediated silencing of PTPα in the rat CG4 OPC cell line results in impaired differentiation to OLs as evidenced by the prolonged maintenance of a high population of A2B5-positive population of progenitor cells, the inhibition of process extension, and the reduced expression of the maturation marker CNPase that is localized to cell bodies and processes. Oligosphere-derived OPCs isolated from PTPα$^{-/-}$ mouse embryos likewise exhibit an OL differentiation defect as deter-

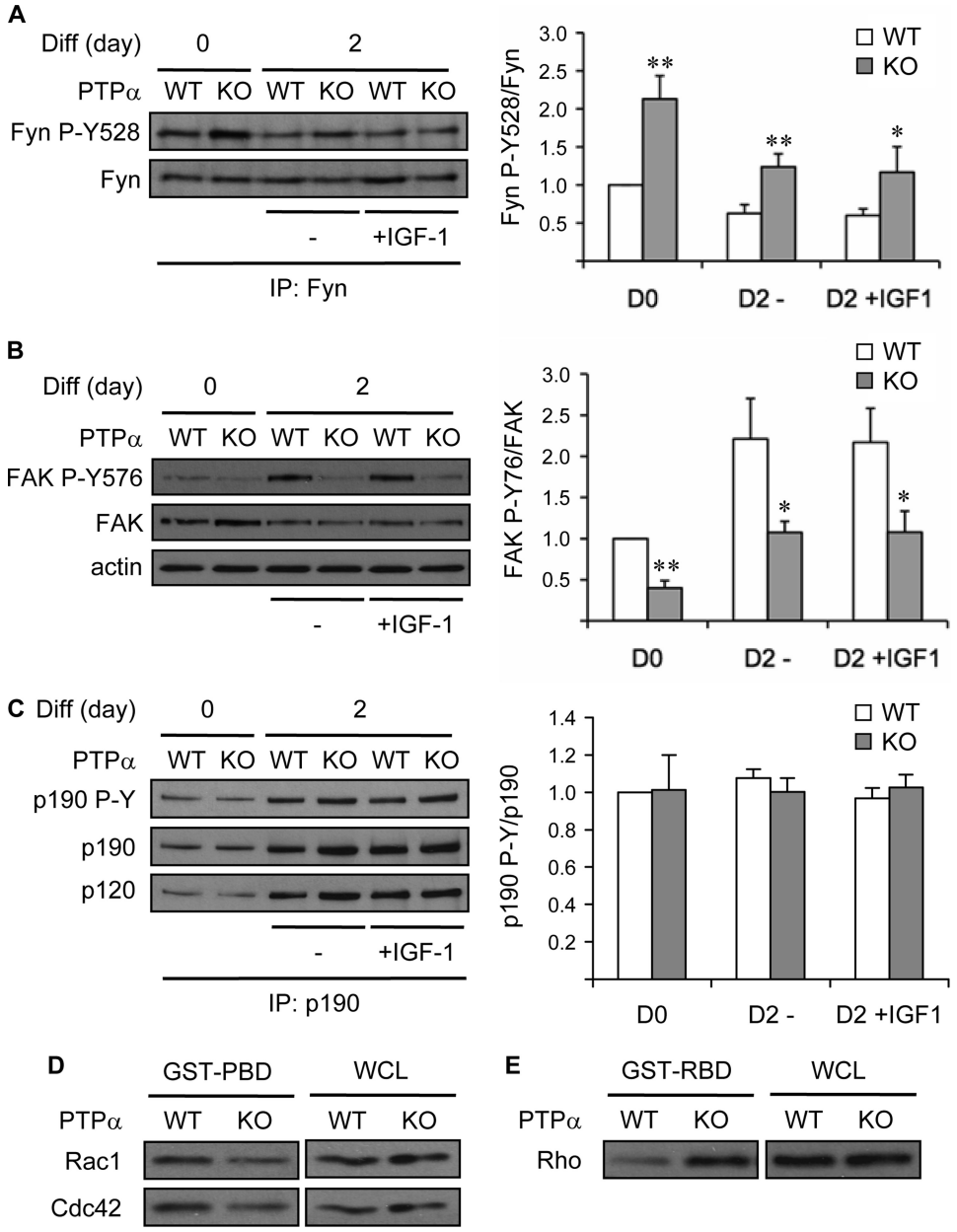

FIGURE 8. **PTPα is a regulator of Fyn-FAK signaling, but not Fyn-p190 signaling in OPCs and OLs.** WT and PTP$\alpha^{-/-}$ mouse oligospheres were dissociated and seeded on PDLO-coated dishes for 2 days in OPC proliferation medium followed by incubation for the indicated times in OPC differentiation medium without (−) or with IGF-1. *A*, Fyn immunoprecipitates were probed with anti-Src P-Tyr^{527} to detect Fyn phospho-Tyr^{528} and with anti-Fyn antibodies. The band intensity of Fyn P-Tyr^{528} was normalized to that of Fyn. Data obtained from three independent experiments are shown in the graph as the mean ± S.D., *, $p < 0.05$; **, $p < 0.01$, ANOVA test. *B*, cell lysates were immunoblotted with anti-FAK P-Tyr^{576} and anti-FAK antibodies, respectively. Actin was used as a loading control. The band intensity of FAK P-Tyr^{576} was normalized to that of FAK. Data obtained from three independent experiments are shown in the graph as the mean ± S.D., *, $p < 0.05$; **, $p < 0.01$, ANOVA test. *C*, p190 immunoprecipitates were probed with anti-p190, anti-p120, or P-Tyr (4G10) antibodies. The band intensity of tyrosine-phosphorylated p190 was normalized to that of p190 protein. Data obtained from three independent experiments are shown in the graph as the mean ± S.D. No significant differences were found between WT and PTP$\alpha^{-/-}$ OPCs and OLs. *D*, OPCs were induced to differentiate for 2 days in the presence of IGF-1. The amounts of activated Rac1 and Cdc42 isolated from cell lysates by GST-PBD pulldowns (*left panels*), or of total Rac1 and Cdc42 in whole cell lysates (*WCL*) (*right panels*), were determined by immunoprobing with anti-Rac1 and anti-Cdc42 antibodies. *E*, as in *D*, but the active Rho isolated by GST-RBD pulldown (*left panel*), and the Rho in whole cell lysates (*left panel*), was detected with anti-Rho antibody. *KO*, knockout.

mined by elevated NG2-positive and reduced O4-positive populations, the appearance of few OLs with a mature morphology of multiple/branched processes, and reduced CNPase and MBP expression as compared with the cells isolated from WT mouse embryos. Furthermore, defective differentiation of PTPα-deficient OPCs correlates with a physiological defect in CNS myelination, because relative to WT mice, PTP$\alpha^{-/-}$ mice have a readily apparent overall reduction in myelin in forebrain sections detected by immunostaining of MBP.

Fyn is activated during OPC differentiation, and this is critical for morphological differentiation, maturation, and CNS myelination (9, 10, 25, 59). Several upstream molecules stimulate Fyn activity in this process, including the ligand-receptor interactions of extracellular matrix components like vitronectin and fibronectin with β1 integrins, laminin 2 binding to α6β1 integrin, and the laminin family member netrin 1 and its receptor Dcc (12–14). Other receptors such as FcRγ (upon cross-linking of bound immunoglobulin G) (11) and the PTP CD45 (32) can also promote Fyn activation during OL differentiation/myelination. Our findings identify PTPα as an additional upstream activator of Fyn in OL differentiation. In CG4 cells, the differentiation-associated activation of Fyn, as measured by reduced phosphorylation of its inhibitory tyrosine residue, is reduced by siRNA-mediated silencing of PTPα expression. Likewise, differentiating primary mouse OPCs lacking PTPα contain less activated Fyn than do WT mouse OPCs, irrespective of whether differentiation was induced by platelet-derived growth factor/bFGF withdrawal in the presence or absence of IGF-1. Interestingly, despite the reduced level of activated Fyn in differentiating PTPα-null OPCs, differentiation cues still stimulated some Fyn activation in PTP$\alpha^{-/-}$ cells. Thus, Fyn activation during this process is not exclusively regulated by PTPα but is also controlled by PTPα-independent mechanisms. These could involve inhibition of the C-terminal Src kinase (Csk) that phosphorylates the negative regulatory site of SFKs, or dephosphorylation by other PTPs (32, 60, 61). Nevertheless, in the absence of PTPα-activated Fyn, the Fyn activation that is mediated by these other mechanisms is insuf-

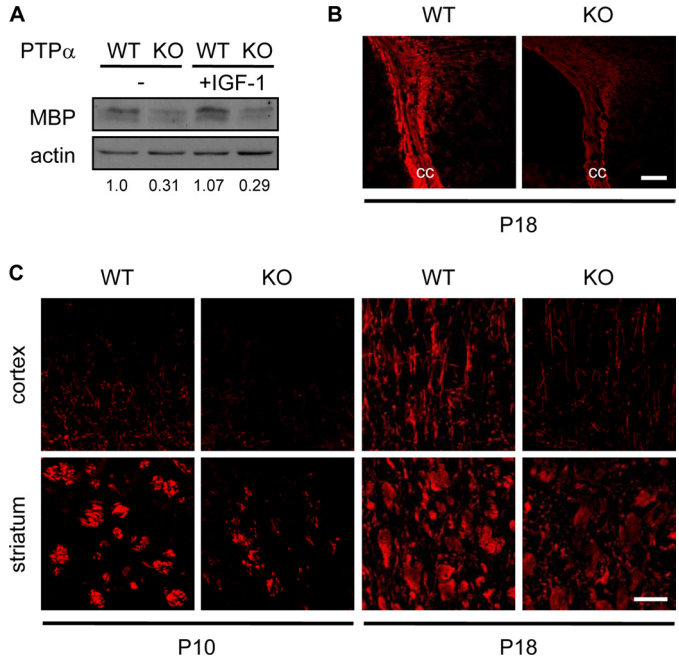

FIGURE 9. **Decreased MBP expression in PTPα$^{-/-}$ OLs and PTPα$^{-/-}$ mouse brain.** *A*, WT and PTPα$^{-/-}$ mouse oligospheres were dissociated and seeded on PDLO-coated dishes for 2 days in OPC proliferation medium followed by incubation for 2 days in OPC differentiation medium without (−) or with IGF-1. Cell lysates were probed for MBP and actin. The *numbers at the bottom* show quantified MBP/actin expression in arbitrary units. *B*, brain coronal sections of P18 WT and PTPα$^{-/-}$ mice were immunostained with anti-MBP antibody. There were fewer myelinated fibers in PTPα$^{-/-}$ corpus callosum (*cc*) compared with WT. *Scale bar*, 100 μm. *C*, there are fewer myelinated fibers in the P10 and P18 cortex and striatum of PTPα$^{-/-}$ mice compared with WT mice, as demonstrated by MBP immunoreactivity. *Scale bar*, 20 μm. *KO*, knockout.

ficient to promote OL differentiation. This may be because other upstream activators cannot stimulate Fyn activity to a level required for differentiation, as supported by our finding that the level of active Fyn detected in PTPα$^{-/-}$ cells after 2 days of differentiation was similar to that in undifferentiated WT OPCS, and/or because PTPα has other unique actions that are required for differentiation.

In accord with the notion of there being insufficient Fyn activation in differentiation-induced PTPα-depleted or -null OPCs to effectively promote differentiation, we observed significantly impaired regulation of the Fyn downstream effectors FAK and the RhoGTPases Rac1, Cdc42, and Rho. FAK has been implicated in CNS myelination, and is proposed to regulate OL process outgrowth and/or remodeling (19, 62). Fyn-mediated signaling through activation of Rac1 and Cdc42 and inhibition of Rho is important for cytoskeletal alterations involved in process extension and branching that occur during morphological differentiation of OLs (13, 20, 45). Diverse Fyn-FAK signaling mechanisms that regulate these RhoGTPases during OL differentiation have been described. For example, laminin stimulation induces Fyn-FAK-Rac1/Cdc42 signaling in OL differentiation (19), whereas netrin-1 stimulates recruitment of Fyn to the netrin-1 receptor Dcc that is complexed with FAK and thereby promotes the inhibition of Rho without affecting Rac1/Cdc42 (14). The ablated Fyn-FAK to RhoGTPase signaling in PTPα-deficient cells, comprising impaired activation of Rac1 and Cdc42 and defective inhibition of Rho, is likely a major defect contributing to their reduced process extension and maturation.

Fyn-mediated inhibition of Rho in differentiating OLs is regulated by Fyn phosphorylating and activating p190RhoGAP, and is essential for process extension and differentiation (13). The differentiation-induced phosphorylation of p190RhoGAP, as well as its interaction with Fyn and p120RasGAP are not altered by the lack of PTPα in differentiation-induced CG4 cells or primary mouse OLs. This indicates that although essential, p190RhoGAP activation is not sufficient to inhibit Rho or promote process extension. Furthermore, our results suggest that OL differentiation involves distinct pathways that regulate Rho: one that appears to involve Fyn-mediated p190RhoGAP activation that is PTPα-independent, and another that requires PTPα and may be Fyn-dependent but is p190RhoGAP-independent. The latter may represent a distinct, possibly specific action of PTPα, and could utilize other Fyn-regulated RhoGAPs, such as the p250RhoGAP implicated in OL differentiation (21). Pending the identification of the specific Rho regulator(s) involved it is, nonetheless, clear that it is critical for optimal PTPα-mediated Rho inhibition during OL differentiation.

The development of OPCs into mature OLs is a complex process that requires exit from the cell cycle, expression of OL-specific genes, and extension of processes and myelin sheets. Another possible role for PTPα in regulating OL differentiation is that PTPα functions in progenitor cells to regulate survival, proliferation, or cell cycle exit. Indeed, we observe an increased number of PTPα$^{-/-}$ primary mouse cells compared with WT cells when they are grown as oligospheres, but not with cells grown as neurospheres (data not shown), suggesting that PTPα functions to control proliferation or survival of OL lineage cells but not cells that are at an earlier stage of development. Whether PTPα is necessary for these or other OPC processes that position the progenitor cells to respond appropriately to differentiation stimuli requires further investigation.

In summary, we have identified PTPα as a novel regulator of OL differentiation and *in vivo* CNS myelination. We propose that the major function of PTPα in promoting these processes is through activation of Fyn, in accord with the well characterized role of PTPα as an activator of SFKs (34) and with the overlapping phenotypes of defective forebrain myelination in PTPα$^{-/-}$ and Fyn$^{-/-}$ mice. This study reveals PTPα to be an essential, but not the sole, regulator of Fyn in differentiating OPCs. Furthermore, we demonstrate that PTPα is required for activation of Fyn effectors FAK, Rac1, and Cdc42 and for Rho inhibition during OL differentiation, and mediates the latter through a p190RhoGAP-independent mechanism. This suggests that upstream regulators such as PTPα are differentially coupled to various Fyn-effector signaling modules to provide stimulus-specific responses that determine aspects of the profound changes in gene expression and morphology that occur during OL differentiation.

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