A complex between contactin-1 and the protein tyrosine phosphatase PTPRZ controls the development of oligodendrocyte precursor cells

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The six members of the contactin (CNTN) family of neural cell adhesion molecules are involved in the formation and maintenance of the central nervous system (CNS) and have been linked to mental retardation and neuropsychiatric disorders such as autism. Five of the six CNTNs bind to the homologous receptor protein tyrosine phosphatases gamma (PTPRG) and zeta (PTPRZ), but the biological roles of these interactions remain unclear. We report here the cocrystal structure of the carbonic anhydrase-like domain of PTPRZ bound to tandem Ig repeats of CNTN1 and combine these structural data with binding assays to show that PTPRZ binds specifically to CNTN1 expressed at the surface of oligodendrocyte precursor cells. Furthermore, analyses of glial cell populations in wild-type and PTPRZ-deficient mice show that the binding of PTPRZ to CNTN1 expressed at the surface of oligodendrocyte precursor cells inhibits their proliferation and promotes their development into mature oligodendrocytes. Overall, these results implicate the PTPRZ/CNTN1 complex as a previously unknown modulator of oligodendrogenesis.

crystal structure | knockout mouse | receptor/ligand complex | glial cell differentiation

In the central nervous system (CNS), oligodendrocytes produce the layered myelin sheath surrounding axons, which is critical to the propagation of saltatory impulse and the long-term integrity of axons during the lifetime of an organism (1). Furthermore, myelination has been implicated with neurological conditions such as multiple sclerosis, leukodystrophies, and demyelinating neuropathies as well as psychiatric disorders. The development of oligodendrocytes and the communication between oligodendrocytes and axons relies on multiple classes of interactions that regulate cell proliferation and differentiation and coordinate initial contact, wrapping, control of axon size, trophic support, and differential clustering of ion channels. In turn, these bidirectional processes are underpinned by interactions between receptors on the cell surface or in the extracellular matrix (ECM) that belong to several families of cell adhesion molecules (CAMs), receptor tyrosine kinases, receptor protein tyrosine phosphatases (RPTPs), and secreted ECM proteins. However, even though the specific nature of the signals that underlie oligodendrocyte maturation has been the focus of recent investigations, there is still a significant gap in knowledge that hinders the design of molecules that would repair demyelinating lesions.

The protein tyrosine phosphatase PTPRZ is expressed primarily by astrocytes, and oligodendrocyte precursor cells (OPCs), immature and mature oligodendrocytes in the developing and adult nervous systems (2–4). This receptor binds to a variety of cell adhesion and matrix molecules expressed during neurogenesis and in particular to contactin-1 (CNTN1) (5, 6), which is expressed at the surface of OPCs and is involved in their proliferation and differentiation (7). Interestingly, PTPRZ has been strongly suggested to be implicated in oligodendrogenesis because the recovery of *PTPRZ*$^{-/-}$ mice from demyelinating lesions is impaired (8). Furthermore, PTPRZ is aberrantly distributed in the brain of *CNTN1*$^{-/-}$ mice, suggesting that PTPRZ and CNTN1 might interact functionally (9). Taken together, these lines of evidence suggest that a complex of PTPRZ and CNTN1 may be involved in the maturation of oligodendrocytes.

The extracellular domain (ECD) of PTPRZ includes, in its full-length form, an N-terminal carbonic anhydrase-like (CA-like) domain followed by a fibronectin type III (FNIII) repeat, a spacer region, and a large insert with attachment sites for chondroitin sulfate proteoglycan (10) (Fig. 1*A*). The cytoplasmic portion of PTPRZ is composed of two tyrosine phosphatase domains. PTPRZ is also expressed in a soluble form, phosphacan, which is one of the most abundant proteoglycans in the brain and is expressed during myelination (3). CNTN1 is a glycophosphatidyl-anchored protein composed of six Ig repeats and four FNIII domains (Fig. 1*A*) and is the first identified member of the CNTN family of neural cell adhesion molecules, which are involved in the construction of neural networks (11). Recent work has shown that Ig repeats 2 and 3 of CNTN1 interact with the CA-like domain found at the N terminus of the PTPRZ extracellular domain (12), but the atomic details of these interactions and their significance during neural development remain unknown. Likewise, the functional roles played by the recently identified interactions between protein tyrosine phosphatase receptor type gamma (PTPRG), a homolog of PTPRZ expressed in neurons, and CNTN3 to 6 are poorly understood (12). Here, we provide a structural basis for the binding between PTPRZ and CNTN1 and demonstrate that the formation of this complex regulates the proliferation and differentiation of OPCs.

Results

Structural Basis for PTPRZ/CNTN1 Interactions. We determined the cocrystal structure of PTPRZ and CNTN1 as a first step to characterize their function. A complex of the CA-like domain of human PTPRZ bound to the Ig2–Ig3 fragment of human CNTN1 was obtained by incubation of the purified components followed by purification by size exclusion chromatography. The crystal struc-

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Data deposition: The atomic coordinates and structure factors have been deposited in the Protein Data Bank, (accession no. 3S97).

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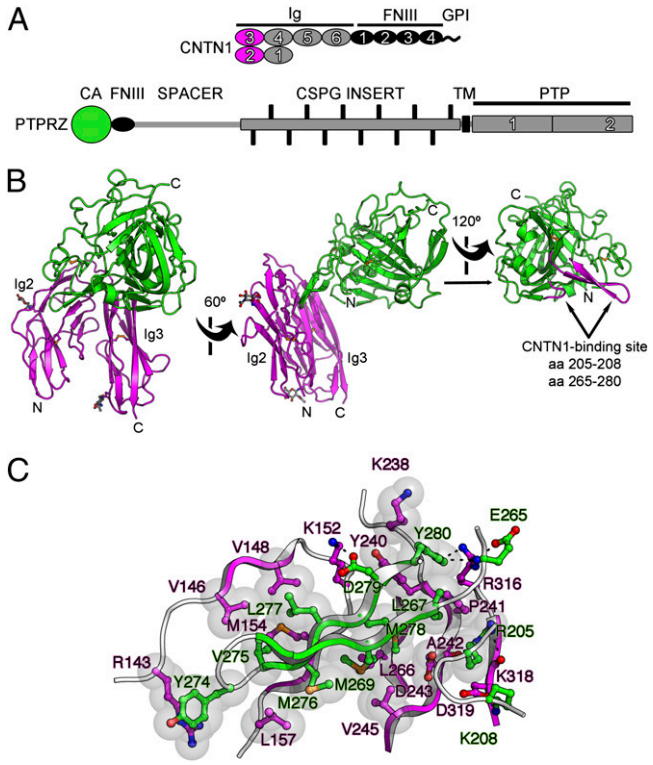

Fig. 1. Crystal structure of a PTPRZ/CNTN1 complex. (*A*) Schematic representation of PTPRZ and CNTN1. (*B*) Ribbon diagram of the PTPRZ/CNTN1 complex. The letters N and C indicate the N- and C termini, respectively. Disulfide bonds are shown as orange ball-and-stick models. Asparagine-linked *N*-acetylglucosamine residues are depicted as gray ball-and-stick models along with the asparagine side chain. The CA domain of PTPRZ is colored green and Ig domains 2 and 3 of CNTN1 are colored magenta. (*C*) Contacts at the PTPRZ/CNTN1 interface. This view is in the same orientation as the view shown on the *Left* in *B*. Residues are shown as ball and sticks with transparent gray spheres for those involved in van der Waals contacts. Dashed lines indicate potential hydrogen bonds and salt bridges. Residues from PTPRZ and CNTN1 are colored green and magenta, respectively.

ture was determined by molecular replacement and refined to 2.3 Å limiting resolution (R_{work}/R_{free} = 19.8/25.6%, Table S1). The Ig2–Ig3 tandem repeats of CNTN1 adopt a horseshoe-like conformation in which apolar contacts between Ig2 and Ig3 bury 1,355 Å^2 with a shape complementarity coefficient of 0.77 (13) (Fig. 1*B*). This arrangement is similar to the one adopted by CNTN2 and CNTN4, which strongly suggests that this structural motif is a hallmark of CNTN family members (12, 14, 15).

The interface between the CA domain of PTPRZ and repeats Ig2–Ig3 of CNTN1 occludes 1,658 Å^2 with a shape complementarity coefficient of 0.68 (Fig. 1*B*), both of which compare favorably to those of known biological interfaces and are similar to the values obtained for the complex between PTPRG and CNTN4 (12, 13, 16). In fact, the two complexes are closely related and superimpose with a rmsd of 1.7 Å over 454 shared residues. As is the case for the PTPRG/CNTN4 complex, the interface between PTPRZ and CNTN1 consists of a β-hairpin loop contacting both the Ig2 and Ig3 domains of CNTN1 (PTPRZ residues 265–280) and a short loop (PTPRZ residues 205–208) that contacts the Ig3 domain of CNTN1 exclusively (Fig. 1*B*). Despite the overall resemblance between the two complexes, the interfaces are distinct. The PTPRZ/CNTN1 binding site is largely apolar and includes residues V146, V148, M154, L157, Y240, P241, V245, and L266 in CNTN1 and L267, M276, L277, M278, and Y280 in PTPRZ (Fig. 1*C*). In addition, K152, R316, K318, and D319 in CNTN1 mediate potential hydrogen bonds and salt bridges with R205, K208, E265, D279, and Y280 in PTPRZ. Finally, R143 in CNTN1 and Y274 in PTPRZ are involved in a cation–pi interaction. This interaction replaces the hydrogen bond between the main chain nitrogen atom of S130 in CNTN4 and the side chain of D294 in PTPRG. Furthermore, PTPRG residues that are found in place of PTPRZ residues E265, M269, Y274, and Y280 do not interact with CNTN4. These amino acid substitutions are likely to account for the distinct binding specificities of PTPRG and PTPRZ toward members of the CNTN family (12).

PTPRZ Binds to CNTN1 Expressed at the Surface of OPCs. Because PTPRZ and CNTN1 have been implicated in the development of oligodendrocytes (7, 8), we investigated their interactions at the surface of these cells. We analyzed the binding of the CA domain of PTPRZ on glial cells using a fluorescein isothiocyanate (FITC)-labeled soluble protein (CA-Fc) incubated with mixed primary cultures (MPCs) of neonatal cortex (Fig. 2 *A* and *B*). CA-Fc binding was detected at the surface of oligodendroglial cells identified by the expression of the transcription factor Olig2 and specific markers of the oligodendrocyte lineage (Fig. S1), but not on microglial cells or astrocytes (Fig. S2). The binding of CA-Fc could be inhibited by an untagged wild-type form of PTPRZ (CA-WT), but not by an untagged mutant form of PTPRZ (CA-Mut) lacking most of the β-hairpin loop that binds CNTN1 (Fig. 2 *C* and *D*). This finding is consistent with the binding mode described above for PTPRZ and CNTN1. The pattern of CA-Fc staining overlapped with the sites of CNTN1 staining at the surface of OPCs (Fig. 2*G* and Fig. S1*B*), suggesting that PTPRZ colocalizes with CNTN1. Addition of either an excess of CNTN1 antiserum or repeats Ig2–Ig3 of CNTN1 blocked CA-Fc binding to OPCs (Fig. 2 *F* and *J*), indicating that CA-Fc binds CNTN1. In addition, PTPRZ/CNTN1 interaction was not inhibited after treatment with Ig2–Ig3 repeats from CNTN6, another CNTN family member expressed by oligodendroglial cells (17) (Fig. 2*I*). The specific binding of PTPRZ to CNTN1 was further confirmed by the similar binding of CA-Fc at the surface of OPCs isolated from *CNTN6* or *TAG1(CNTN2)*-deficient mice (Fig. S3). This observation is consistent with earlier work indicating that PTPRZ binds to CNTN1 but not to other CNTN family members (12). Finally, using an Fc fusion of the CA and FNIII domains of PTPRZ, we were able to isolate CNTN1 from OPCs and from oligodendrocytes, albeit to a lesser extent (Fig. 2*K*). Conversely, we were able to purify phosphacan bound to CNTN1 from the conditioned media of wild-type MPCs (Fig. 2*L*). Taken together, these results indicate that PTPRZ binds specifically to CNTN1 expressed at the surface of OPCs.

Altered Proliferation and Differentiation of Oligodendroglial Cells in PTPRZ-Deficient Mice. Because the PTPRZ/CNTN1 complex was detected strongly at the surface of OPCs, we examined the proliferation and differentiation of OPCs in *PTPRZ*$^{-/-}$ mice during early postnatal development. Stages of oligodendroglial development can be distinguished by the expression of distinct markers (1): platelet-derived growth factor receptor α (PDGFRα) and O4 for OPCs (2), 2′,3′-cyclic nucleotide 3′ phosphodiesterase (CNP) and O1 for immature oligodendrocytes and myelin basic protein (MBP) and myelin-associated glycoprotein (MAG) for mature myelinating oligodendrocytes. We first focused on the optic nerve of *PTPRZ*$^{-/-}$ mice at postnatal day 10 (P10) when myelination is occurring. We found a 40% increase in the number of cells expressing the oligodendrocyte markers Olig2 and Sox10 compared with controls (Fig. 3 *A* and *B*). In particular, we detected an excess of OPCs expressing PDGFRα (30% compared with controls) in *PTPRZ*$^{-/-}$ mice (Fig. 3*C*). Results from a BrdU incorporation assay in vivo also indicated that the loss of *PTPRZ* resulted in a 25% increase in the number of proliferating BrdU$^+$Olig2$^+$ OPCs compared with controls (Fig. 3*B*). Finally, the aberrant proliferation of oligodendroglial cells in *PTPRZ*$^{-/-}$ mice

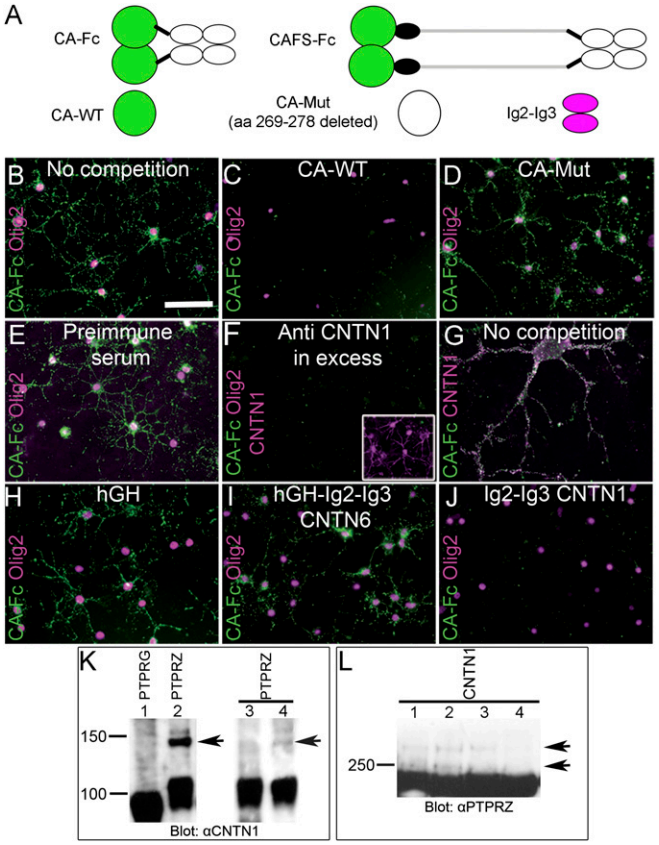

Fig. 2. PTPRZ binds to CNTN1 expressed at the surface of OPCs. (*A*) Cartoon depicting the molecules used in vitro binding assays. (*B–K*) Mixed primary cells incubated with an Fc fusion of the CA domain of PTPRZ labeled with fluorescein isothiocyanate (CA-Fc-FITC, green) and oligodendrocytes were labeled with anti-Olig2 antibodies (magenta). (*B–D*) The labeling of oligodendrocyte membranes by CA-Fc-FITC is inhibited by the presence of the CA domain of PTPRZ (*C*), but not by a mutant lacking most of the CNTN1-binding site (*D*). (*E* and *F*) The binding of CA-Fc to oligodendrocytes is blocked by rabbit anti-CNTN1 antibodies (*F*), but not by preimmune serum (*E*). CA-Fc-FITC and anti-CNTN1(Cy5) stain oligodendrocyte membranes similarly as seen in the higher magnification picture (*F*). (*G*) CA-Fc-FITC and CNTN1 staining colocalize at the surface of oligodendrocytes. Cells were labeled with anti-CNTN1 antibodies (magenta) and a FITC conjugate of CA-Fc (green). (*H–J*) The labeling of oligodendrocyte membranes by CA-Fc-FITC is not blocked by the presence of hGH (*H*), a fusion of hGH and domains Ig2–Ig3 of the CNTN1-homolog CNTN6 (*I*), but is inhibited by the presence of domains Ig2–Ig3 of CNTN1. (Scale bar, 50 μm.) (*K*) An Fc fusion of the CA and FNIII domains of PTPRZ, but not PTPRG, coprecipitated CNTN1 from transfected cells (lanes 1 and 2) and lysates of oligodendrocytes (lane 3) and OPCs (lane 4). (*L*) An Fc fusion of CNTN1 coprecipitated purified phosphacan (lane 1, positive control) and from the conditioned media of rat cells (lane 2), mouse wild-type cells (lane 3), but not *PTPRZ*$^{-/-}$ cells (lane 4). Arrows at 250 kDa and 350 kDa indicate the position of phosphacan.

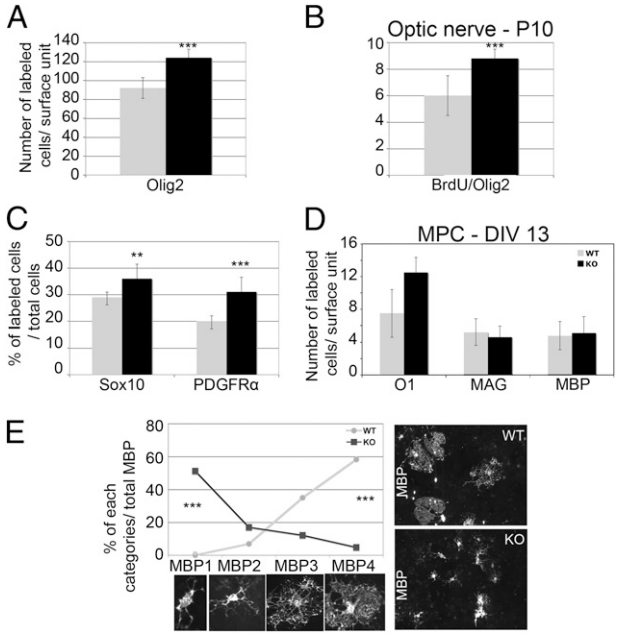

Fig. 3. The proliferation and differentiation of oligodendroglial cells are altered in *PTPRZ*$^{-/-}$ mice. (*A–C*) The proliferation of OPCs is elevated in PTPRZ-deficient mice in vivo. Cells at stage P10 derived from the optic nerves of BrdU-injected pups from wild-type and *PTPRZ*$^{-/-}$ mice were labeled with the oligodendrocyte markers Olig2 (*A*), PDGFRα, and Sox10 (*C*). In each case, the number of labeled cells is increased in the absence of PTPRZ. Likewise, the number of Olig2^{+}BrdU^{+} is elevated in *PTPRZ*$^{-/-}$ pups (*B*). (*D*) The increased proliferation detected in *PTPRZ*$^{-/-}$ OPCs does not correlate with an increased yield of MBP^{+} cells in vitro. MPCs at division 13 were labeled with anti-O1, anti-MAG, and anti-MBP antibodies. The number of immature O1^{+} cells is increased in *PTPRZ*$^{-/-}$ mice, whereas the numbers of mature MAG^{+} and MBP^{+} cells are unchanged. (*E*) MBP^{+} oligodendrocytes from *PTPRZ*$^{-/-}$ mice show reduced morphological maturity. Typical morphologies of wild-type and *PTPRZ*$^{-/-}$ MBP^{+} cells are shown on the *Right*. MBP^{+} cells were categorized according to their morphologic maturity (graph and *Lower* panels). $^{**}P < 0.01$; $^{***}P < 0.001$ (Student's *t* test). (Scale bar, 50 μm.) Data are shown as mean ± SD.

was confirmed by additional counting of O4-expressing OPCs in MPCs and of both O4^{+} and CNP^{+} OPCs in the spinal cord at embryonic stage E14.5 (Fig. S4). Overall, these data demonstrate that the absence of PTPRZ results in an increase of proliferating OPCs, thus suggesting that PTPRZ influences OPC proliferation (Fig. S4).

The ability of supernumerary OPCs to differentiate was then analyzed in MPCs derived from *PTPRZ*$^{-/-}$ mice. Quantification of cells expressing markers of differentiating oligodendrocytes showed an increased number of O1^{+} immature oligodendrocytes, whereas the population of mature MBP^{+} or MAG^{+} cells was not changed (Fig. 3*D*). Oligodendrocyte differentiation was further assessed by analyzing their cellular morphology. MBP^{+}-cells were classified into four categories from cells with poorly branched processes (MBP1) to cells with prominent myelin membranes (MBP4) (Fig. 3*E*, *Lower*). In *PTPRZ*$^{-/-}$-derived cultures, half of the cells remained undifferentiated and only 5% of MBP^{+} oligodendrocytes developed typical membrane sheets, in contrast to 60% in wild-type cultures (Fig. 3*E*, graph). Therefore, the supernumerary OPCs generated in *PTPRZ*$^{-/-}$ mice are unable to undergo proper differentiation.

The Ectodomain of PTPRZ Is Necessary for the Proper Development of Oligodendrocytes. As phosphacan, a soluble form of PTPRZ encompassing its entire ECD, is expressed abundantly during myelination (6, 18), we hypothesized that it could be released from the surrounding tissues and have a paracrine effect in repressing OPC proliferation. Thus, we quantified BrdU^{+}Olig2^{+} cells derived from wild-type or *PTPRZ*$^{-/-}$ mice, after incubation with conditioned medium of MPCs. The enhanced proliferation of *PTPRZ*$^{-/-}$ OPC was reduced by conditioned medium from *PTPRZ*$^{+/+}$ mice. In contrast, wild-type conditioned medium immunodepleted by anti-PTPRZ antibodies failed to decrease the number of *PTPRZ*$^{-/-}$ OPCs, indicating that phosphacan likely mediates the inhibition of OPC proliferation. Likewise, both CA-Fc and the soluble PTPRZ ectodomain (CA, FNIII, and Spacer) lacking the proteoglycan stretch (CAFS-Fc, Fig. 2*A*) reduced the proliferation of OPCs from *PTPRZ*$^{-/-}$ mice in a dose-dependent manner (Fig. 4*C*). Conversely, the mutant form of PTPRZ that does not bind to CNTN1 did not inhibit mitosis of *PTPRZ*$^{-/-}$ OPCs

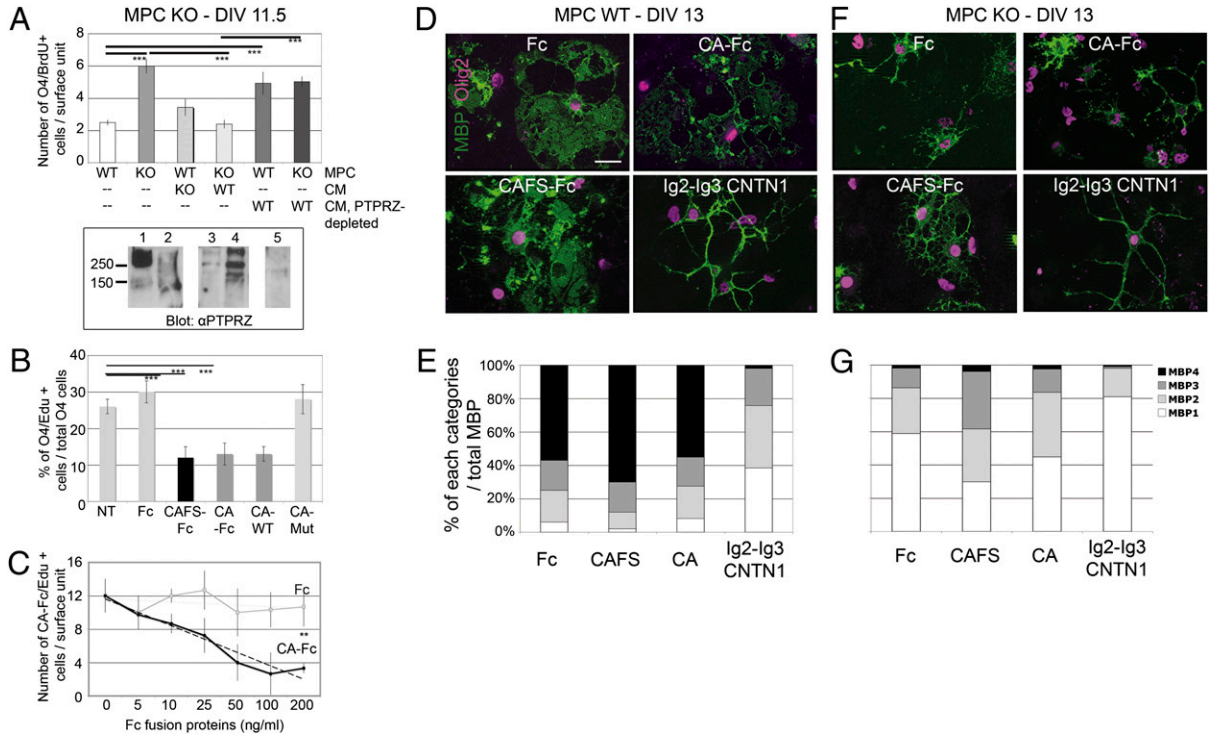

Fig. 4. The extracellular region of PTPRZ controls the maturation of oligodendrocytes. (*A*) Soluble form of PTPRZ represses the proliferation of oligodendrocytes. Number of $O4^+/BrdU^+$ cells is increased in PTPRZ-deficient mice. This increased proliferation of *PTPRZ*$^{-/-}$ OPCs is reversed by incubation with WT-conditioned medium (CM), but not by WT-CM depleted by PTPRZ antibodies. MPC at DIV11 were incubated for the last 16 h with BrdU. The framed box shows a phosphacan immunoblot of TCA-precipated CM of wild-type (lane 1) and KO cells (lane 2). This immunoblot confirms that phosphacan is absent from the CM of *PTPRZ*$^{-/-}$ MPCs. Phosphacan is also present in the optic nerve of wild-type (lane 4), but not *PTPRZ*$^{-/-}$ mice (lane 5). Purified phosphacan is shown in lane 3 for comparison. (*B*) CA domain of PTPRZ is sufficient to modulate the proliferation of *PTPRZ*$^{-/-}$ OPCs. Incubation of *PTPRZ*$^{-/-}$ MPCs with soluble forms of PTPRZ (CAFS-Fc, CA-Fc, or CA-WT) reduces the number of $O4^+/Edu^+$ cells compared with controls (NT, not treated and Fc, human IgG Fc only). In contrast, treatment with a PTPRZ mutant that does not bind CNTN1 does not reduce the number of OPCs, which suggests that OPC proliferation depends on PTPRZ/CNTN1 interactions. (*C*) CA domain of PTPRZ modulates OPC proliferation in a dose-dependent manner. Incubation of PTPRZ-deficient OPCs at DIV11 with increased amounts of CA-Fc results in a robust decrease in the number of OPCs. (*D*–*G*) Ectodomain of PTPRZ, but not its CA domain, promotes the differentiation of WT and *PTPRZ*$^{-/-}$ OPCs. (*D* and *F*) MPCs at DIV13 from WT and *PTPRZ*$^{-/-}$ OPCs were labeled with anti-MBP (green) and anti-Olig2 (magenta) antibodies to highlight the morphology of these cells in the presence of the CA domain of PTPRZ (CA-Fc), the soluble ectodomain of PTPRZ (CAFS-Fc) and domains Ig2–Ig3 of CNTN1 (Ig2–Ig3 CNTN1). Cells were treated with an Fc fragment of human IgG as a control (Fc). The presence of the ectodomain of PTPRZ promotes the differentiation of OPCs. In contrast, the presence of Ig2–Ig3 of CNTN1 prevents OPCs from differentiating, thus suggesting that interactions between CNTN1 and PTPRZ are critical for the maturation of oligodendrocytes. (*E* and *G*) MBP^+ cells were categorized as depicted in the graph in Fig. 3*E*. As is the case in *D* and *G*, the results of these experiments show that the maturation of oligodendrocytes is favored in the presence of the ectodomain of PTPRZ, but is blocked in the presence of domains Ig2–Ig3 of CNTN1.

(Fig. 4*B*). Furthermore, treatment with CAFS-Fc reduced the elevated proliferation of purified *PTPRZ*$^{-/-}$ OPCs (Fig. S5*A*). These results indicate that the proliferation of OPCs is inhibited by the binding of a soluble form of PTPRZ to CNTN1 expressed on their surface.

Because inhibition of proliferation is an essential signal for OPC differentiation, we reasoned that formation of the PTPRZ/CNTN1 complex could inhibit the proliferation of OPCs to permit their differentiation. Mature myelinating oligodendrocytes expressing MBP formed in similar numbers in all types of MPCs derived from wild-type or *PTPRZ*$^{-/-}$ mice, suggesting that PTPRZ was not required for oligodendroglial differentiation. In contrast, the rate of terminal maturation of oligodendrocytes was delayed in the absence of PTPRZ/CNTN1 interactions. Firstly, the number of MBP-expressing cells is reduced in purified *PTPRZ*$^{-/-}$ oligodendrocytes (Fig. S5*B*). Furthermore, fewer immature $O1^+$ cells were detected compared with control cultures when *PTPRZ*$^{-/-}$ cultures were treated with CAFS-Fc (Fig. S6 *A* and *B*). The percentage of $O1^+MBP^+$ cells among $O1^+$ cells was also increased (Fig. S6*C*), indicating that CAFS-Fc favored the maturation of *PTPRZ*$^{-/-}$ oligodendrocytes into MBP^+ myelin-forming cells. Finally, treatment of *PTPRZ*$^{-/-}$ cultures with CAFS-Fc modified the morphology of MBP^+ cells, with more mature-like MBP^+ oligodendrocytes in wild-type (20% for MBP4-class cells) or *PTPRZ*$^{-/-}$ cultures (25% for MBP2-type cells) (Fig. 4 *D* and *E*), and less undifferentiated MBP^+ cells in both genotypes (22% of MBP2-class cells and 37% of MBP1-class cells, respectively) than in controls. Similar results were obtained using purified oligodendrocytes (Fig. S5*C*) although we observed a reduced number of MBP^+ cells in *PTPRZ*$^{-/-}$ OPCs. Interestingly, we found that CA-Fc did not affect the morphological differentiation of MBP^+ cells, indicating that the FNIII and/or the spacer domains of PTPRZ (Fig. 1*A*) were likely implicated in the morphological maturation of oligodendrocytes, whereas the CA domain is sufficient to inhibit the proliferation of OPCs. Finally, addition of the Ig2–Ig3 domain of CNTN1 as a competitor prevented wild-type oligodendrocytes to form myelin sheets (Fig. 4 *D* and *E*), confirming the importance of the PTPRZ/CNTN1 complex in the formation of myelin sheets and maturation of oligodendrocytes.

Discussion

Here we show that the formation of a complex between the neural CAM CNTN1 and a soluble form of the tyrosine phosphatase PTPRZ activates a signaling pathway implicated in the

balance between proliferation and maturation of OPCs. We identify the soluble protein phosphacan as regulating this critical step of oligodendrogenesis and as a potential promyelinating factor to repair myelin lesions in the CNS.

A Newfound Role for the Soluble Form of PTPRZ. We have previously shown that repair of demyelinating lesions is impaired in experimental allergic encephalomyelitis-induced *PTPRZ*$^{-/-}$ mice and that PTPRZ is overexpressed in human oligodendrocytes during reparation of lesions to the myelin sheath (8), thus implicating PTPRZ in remyelination. Consistent with these findings, our results demonstrate that the formation of a PTPRZ/CNTN1 complex is a prerequisite first step for the differentiation of OPCs, which leads to remyelination. Interestingly, the soluble form of PTPRZ is responsible for mediating the differentiation of OPCs, indicating that this molecule participates in myelination and thus represents an avenue of treatment for demyelinating lesions. In particular, it will be critical to test the effect of soluble PTPRZ on human white matter glial progenitor cells, as Sim et al. reported an increase in the number of O4^{+} cells in cells treated with PTPRZ siRNA (19). This increase was accompanied by a reduction in the number of proliferating glial cells expressing the A2B5 antigen, indicating that PTPRZ could inhibit human oligodendrocyte differentiation. This discrepancy could be due to differences in the species and developmental stages of the cells that were used (rodent vs. human and embryonic vs. adult) and will warrant further investigation.

The PTPRZ/CNTN1 Complex May Help Recruit Additional Cell Surface Receptors. Our results point to distinct effects of PTPRZ: one that accounts for inhibition of OPC proliferation that is fully rescued by soluble PTPRZ and one that accounts for the maturation of oligodendrocytes that is partially rescued by soluble PTPRZ. These observations suggest that there might be differences in the mechanisms that govern the proliferation and maturation of oligodendroglial cells.

The mechanism that underlies the differentiation of immature oligodendrocytes into mature myelinating cells depends on the presence of the FNIII and spacer regions of PTPRZ (Fig. 5). However, these regions are not necessary to bind to CNTN1 (Fig. 2) (20), which suggests that they may help recruit additional molecules at the surface of OPCs. Interestingly, proteins such as the growth factor pleiotrophin, the extracellular matrix glycoproteins tenascin-C and tenascin-R (TNR), and the cell adhesion molecules L1, NrCAM, and CNTN2, have been shown to interact with the FNIII and the spacer regions of PTPRZ (6). Among these potential binding partners, TNR appears as a promising candidate as it has been implicated in the morphological maturation of oligodendrocytes (21) and is necessary for the proper distribution of phosphacan in the optic nerve (22). Therefore, we propose that a complex formed by CNTN1, PTPRZ, and TNR at the surface of oligodendroglial cells could be responsible for the morphological changes that occur during oligodendrocyte maturation. More generally, these observations indicate that the microenvironment of OPCs plays a critical role in the development of these cells. This notion is consistent with findings that ECM components can regulate the proliferative response of OPCs after treatment with PDGF-AA (23), the main growth factor responsible for OPC proliferation (24, 25).

In contrast, the CA domain of PTPRZ alone is able to inhibit the increased proliferation of PTPRZ-deficient OPCs completely. This result suggests that PTPRZ does not need to recruit an additional molecule to control OPC proliferation. Such control could then be exerted by CNTN1, but this receptor does not have a cytoplasmic region to integrate into the cell signaling machinery and would require another component to inhibit proliferation. For instance, the binding of PTPRZ to CNTN1 could lead to clustering of CNTN1 at the surface of OPCs, which in turn could elicit changes in intracellular signaling using receptor protein tyrosine phosphatase alpha (PTPRA) as a downstream effector. Indeed, PTPRA has been shown to interact *in cis* with CNTN1 (26) and its removal inhibited differentiation and morphological changes of oligodendrocytes and an increase in the OPC population (27).

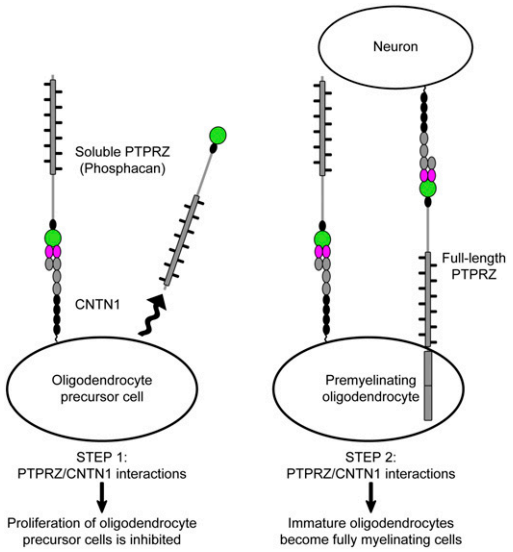

Fig. 5. Model of PTPRZ and CNTN1 function. In the first step, phosphacan inhibits OPC proliferation. As OPCs differentiate into premyelinating oligodendrocytes, the expression of phosphacan and CNTN1 decreases. In the second step, the phosphacan/CNTN1 complex at the surface of OPCs is likely replaced by a PTPRZ/CNTN1 complex that involves CNTN1 expressed on axons and full-length PTPRZ on glial cells, which controls the final morphological and functional differentiation into myelinating oligodendrocytes.

A Model to Explain the Roles of PTPRZ and CNTN1 in CNS Development. Since the discovery of the interactions between PTPRZ and CNTN1 (5), the prevailing notion has been that the transmembrane form of PTPRZ expressed on glial cells interacts *in trans* with CNTN1 at the surface of axons, promoting glial adhesion and neurite outgrowth (20, 28). Our results demonstrate that soluble PTPRZ modulates OPC proliferation via CNTN1 expressed at the surface of OPCs. We propose that PTPRZ and CNTN1 act in two distinct stages during oligodendrocyte development. First, phosphacan inhibits proliferation of OPCs. From stage P0 to P8, OPCs proliferate, migrate, and reach their target axons during which the expression of phosphacan increases gradually to reach its peak at stage P8 (29). At stage P8, the high level of phosphacan inhibits OPC proliferation by interacting with CNTN1 at the surface of these cells and possibly by recruiting additional cell surface receptors. However, after P8, the expression of phosphacan decreases, whereas OPCs differentiate into immature oligodendrocytes that express less CNTN1 (30) (Fig. S1). The transmembrane form of PTPRZ, found primarily at the surface of oligodendrocytes and astrocytes (2), could then interact with CNTN1 expressed on neurons. In the second step, the phosphacan/CNTN1 complex at the surface of OPCs is likely replaced by a PTPRZ/CNTN1 complex that involves CNTN1 expressed on axons and PTPRZ on glial cells. This complex could then recruit additional molecules such as TNR to stimulate the differentiation of oligodendrocytes, thus facilitating the ensheathment of axons. The proposed model is in line with our findings that demonstrate the altered maturation of oligodendrocytes cannot be fully rescued by the ectodomain of PTPRZ (Fig. 5) and is also consistent with the literature describing phosphacan and other chondroitin sulfate proteoglycans as inhibitors of repair of

injuries to the CNS (31–33). The inhibitory action of phosphacan is in this case most likely carried out by its chondroitin sulfate proteoglycan insert, whereas our studies show that the ECD of PTPRZ lacking this insert promotes myelination and thus would favor the regeneration of the CNS after injury.

Concluding Remarks. Our findings provide a structural and biological framework to understand the role of the PTPRZ/CNTN1 complex in oligodendroglial cells. We demonstrate that soluble PTPRZ is a ligand for CNTN1 on OPCs, and that the PTPRZ/CNTN1 complex represses OPC proliferation and promotes oligodendrocyte maturation. Overall, our results pave the way for a more in depth understanding of the biological roles played by interactions between PTPRG, PTPRZ, and members of the CNTN family of neural receptors during CNS development.

Materials and Methods

See *SI Materials and Methods* for additional descriptions.

Primary Culture of Glial Cells. Mixed primary cultures (MPC) of glial cells were prepared from postnatal day 0 cortices dissociated in a papain solution (100 μL/mL; Worthington) also containing L-cysteine (0.48 mg/mL; Sigma) and DNase I (100 μL/mL; Sigma) for 20 min at 37 °C or by mechanical dissociation through 19- and 21-gauge syringes. Cells were washed, resuspended in DMEM containing 10% heat-inactivated FCS and 5% heat-inactivated horse serum, and plated onto poly-D-lysine–coated coverslips. Medium was changed at 5 days in vitro (DIV) and then every other day. At DIV8, medium was changed to defined medium of DMEM/F12 complemented with 1% N2 (Invitrogen) and 400 ng/mL of T3.

Immunocytochemistry and Proliferation Assay. MPC culture or purified OPCs were treated or not with Fc fusion proteins at DIV9 in DMEM/F12 containing 1% N2 and 400 ng/mL T3. At DIV11 Edu or Brdu (1 mM) was added to the medium for the next 16 h. Cells then were fixed in 4% PFA, washed in 0.1 M sodium phosphate buffer (PB) pH 7.4, blocked 2 h in 5% NGS, and incubated overnight at 4 °C with the following primary antibodies: anti-O4 (mouse IgM; R&D), anti-O1 (mouse IgM), anti-MBP (rat IgG; Chemicon), MAG (rabbit; Abcam). Incubation was carried out in PBS supplemented with 3% BSA and 3% NGS. After washing, slides were incubated with the appropriate secondary antibodies, Hoechst stained and mounted. In some experiments, cells were incubated with BrdU and stained with anti-BrdU antibody (Dako) or with Edu following manufacturer's instructions (Invitrogen).

Immunostaining of Optic Nerves. Optic nerves with chiasm were dissected from P10 littermates (n = 3 for each genotype), fixed overnight in PFA 4%, cryoprotected in PBS/20–30% sucrose, and frozen at −40 °C in OCT Tissue Tek. Sections (16 μm each) were prepared by a Microsystems cryostat and collected on Superfrost Plus slides. The sections were then processed either for immunohistochemistry with Olig2 antibody (1/200; Chemicon), followed by antirabbit–488 Alexa (1/1000; Molecular Probes) and Hoechst staining or for in situ hybridization (see below). Photographs of the proximal end of the optic nerve were taken with 10× and 40× objectives in 5–10 consecutive sections per optic nerve. Positive cells for each marker were quantified using Metamorph software (Molecular Devices).

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