

p39, the Primary Activator for Cyclin-dependent Kinase 5 (Cdk5) in Oligodendroglia, Is Essential for Oligodendroglia Differentiation and Myelin Repair*

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Background: Cyclin-dependent kinase 5 (Cdk5) is crucial for brain development.

Results: In contrast to neurons that utilize p35 as the primary Cdk5 activator, oligodendroglia employ p39-dependent Cdk5 activation to advance differentiation and myelin repair.

Conclusion: p39 is the primary Cdk5 activator in oligodendroglia, essential for oligodendroglia development.

Significance: Our study revealed distinct mechanisms controlling Cdk5 activity in neurons and oligodendroglia.

Cyclin-dependent kinase 5 (Cdk5) plays key roles in normal brain development and function. Dysregulation of Cdk5 may cause neurodegeneration and cognitive impairment. Besides the well demonstrated role of Cdk5 in neurons, emerging evidence suggests the functional requirement of Cdk5 in oligodendroglia (OL) and CNS myelin development. However, whether neurons and OLs employ similar or distinct mechanisms to regulate Cdk5 activity remains elusive. We report here that in contrast to neurons that harbor high levels of two Cdk5 activators, p35 and p39, OLs express abundant p39 but negligible p35. In addition, p39 is selectively up-regulated in OLs during differentiation along with elevated Cdk5 activity, whereas p35 expression remains unaltered. Specific knockdown of p39 by siRNA significantly attenuates Cdk5 activity and OL differentiation without affecting p35. Finally, expression of p39, but not p35, is increased during myelin repair, and remyelination is impaired in p39^{-/-} mice. Together, these results reveal that neurons and OLs harbor distinct preference of Cdk5 activators and demonstrate important functions of p39-dependent Cdk5 activation in OL differentiation during *de novo* myelin development and myelin repair.

Normal brain function relies upon proper development and integrity of neurons and oligodendroglia (OLs),³ the myelinat-

ing glia that ensheath neuronal axons and enable rapid information flow in the CNS. OL impairment has become an increasingly recognized pathophysiological feature of numerous neurological disorders originally believed to result from neuronal defects alone, including Alzheimer disease and schizophrenia (1–4). Moreover, key components of cell signaling pathways traditionally thought to control neuronal development and function have now been found expressed and playing critical roles in OLs as well. Thus, whether neuronal and OL lineages share common molecular pathways that govern their development and cooperation is an intriguing question and a growing area of research in neuroscience.

One such signaling molecule is Cyclin-dependent kinase 5 (Cdk5), an unconventional Cdk member that does not control cell cycle progression but primarily functions in post-mitotic cells (5–9). Despite the ubiquitous expression and increasingly recognized broad function of Cdk5 in numerous cell types, the highest activity of this kinase is detected in the brain (5, 10). Earlier studies demonstrated essential functions of Cdk5 in neuronal migration, neural network formation, and synaptic plasticity in mammalian brains (5). Besides the traditional view of neuronal centric roles of Cdk5, emerging evidence suggests that Cdk5 also governs the development of oligodendroglia progenitor cells (OPCs) (11–13). Similar to brain neurons, OPCs migrate to distant brain areas and become mature OLs to myelinate neuronal axons (14). However, unlike neuronal development in the embryonic brains, the most vigorous OPC development occurs postnatally (14). Loss of Cdk5 function affects OPC migration and differentiation in culture and results in CNS hypomyelination (12, 13, 15, 16). However, molecular mechanisms that regulate Cdk5 function in neurons and OLs remain elusive.

The activity of Cdk5 is controlled by the available amounts of two activator homologs, p35 and p39 (9, 17, 18). Mice lacking both p35 and p39 displayed nearly identical defects in embryonic brain development and perinatal lethality as those found in

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³ The abbreviations used are: OL, oligodendroglia; Cdk5, cyclin-dependent kinase 5; OPC, oligodendroglia progenitor cell; PLP, proteolipid protein; eIF5 α , translation initiation factor 5 α ; MBP, myelin basic protein; DIV, days *in vitro*; EGFP, enhanced GFP; qRT, quantitative real-time; DM, demyelination; RM, remyelination.

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the Cdk5-null mice (19, 20), indicating that p35 and p39 together are solely responsible for Cdk5 activity and function in the brain. However, the Cdk5 activators display distinct temporal expression profiles, suggesting non-redundant roles for each activator over the course of brain development that have yet to be identified (9, 21–24). p35 is abundantly expressed in embryonic neurons and is essential for Cdk5 activation in these cells, which govern corticogenesis (17, 20). Conventional p35 knockout mice display impaired neuronal migration and cortical organization during embryonic development, similar to but less severe than that in Cdk5 null mice (20, 25). In contrast to the early function of p35 in the embryonic brain, p39 levels are elevated and most prominently expressed in the postnatal brain (26). Nonetheless, mice lacking p39 alone showed no overt neuronal abnormalities (19). Thus, p35 is considered the most important Cdk5 activator in neurons, whereas the functional importance of p39 remains undetermined.

We report here that neurons and OLs display distinct profiles of Cdk5 activator expression and regulation. In contrast to the major role of p35 in activating Cdk5 in neurons, p39 is the primary Cdk5 activator in OLs, where p35 expression is negligible. In addition, selective up-regulation of p39, but not p35, accompanies the increased Cdk5 activity during normal OPC differentiation in culture and CNS myelin development *in vivo*. Up-regulation of p39 is also detected in OLs during myelin repair upon induced demyelination. Finally, we show that p39 plays essential roles in advancing OPC differentiation, and the loss of p39 leads to impairment in myelin repair in the brain. Together, these studies suggest that p39 is the primary activator responsible for Cdk5 function in CNS myelinogenesis in normal and diseased brains.

EXPERIMENTAL PROCEDURES

Animal Care—All animal procedures adhere to NIH regulations under the approval of IACUC by Emory University, University of Kentucky, and UCLA. The p39^{-/-} mice were generated in a previous study (19). Mice expressing EGFP under the oligodendrocyte-specific 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNP) and proteolipid protein (PLP) promoters were also previously described (27, 28). Sprague-Dawley rats were purchased from The Jackson Laboratory.

FACS Isolation of Oligodendrocytes—FACS isolation of EGFP⁺ OLs from PLP-EGFP mice at postnatal days 1, 10, and 20 was performed as previously described (27). Briefly, finely minced brain tissues (6–10 brains) were digested with papain in Hanks' balanced salt solution (15 units/ml) at 37 °C for 15 min to produce cell suspensions. The cell suspensions were then dissociated by sequential passages through gauge 18, 21, and 23 needles. Dissociated cell suspensions were sorted on a MoFlo (Beckman Coulter) using a 100- μ m nozzle tip at 30 psi. The FACS sorting scatter profile was Forward Scatter for relative size *versus* Side Scatter for granularity. Apoptotic cells appeared as smaller and more granular and were excluded from further sorting process. The EGFP⁺ cells were sorted based on fluorescence intensity and stored at -80 °C before being subjected to RNA and protein quantification.

Cuprizone-induced Demyelination—The cuprizone demyelination-remyelination paradigm and tissue dissection were

performed as previously reported (29). Briefly, male PLP-EGFP mice at 8 weeks of age were fed with 0.2% cuprizone (Sigma) mixed into milled chow (Harlan Tekland). Mice were fed the cuprizone diet for 3 weeks to induce demyelination (3 weeks DM). To analyze remyelination, a parallel group of mice was returned to normal chow for an additional 3 weeks after cuprizone treatment (3 + 3 weeks RM). Control mice demonstrating normal myelination (normal) were maintained on normal pellet chow.

Lysolecithin-induced Demyelination—Focal demyelination was induced by unilateral lysolecithin injection as described (30). Briefly, 2 μ l of 1% lysolecithin (Sigma) in 0.9% NaCl was injected into the corpus callosum of WT and p39^{-/-} PLP-EGFP mice at 6 weeks of age. Stereotactic coordinates of 1.1 mm anterior and 1 mm lateral to the bregma and 1.8 mm deep from the skull surface were used for injection. Control mice received saline injections. To minimize reflux along the needle track, the needle was not removed until 5 min after injection. Brains were harvested at 5 and 21 days post lysolecithin injection (days 5 and 21) to evaluate demyelination and remyelination, respectively. The demyelinated lesion was marked by the loss of EGFP signal.

Cell Culture and Treatment—Primary cortical neurons were raised from rat brains at embryonic day 18 as described (31). Dissociated neurons were plated in poly-D-lysine-coated 35-mm² dishes (300,000 cells/dish) in B27/Neurobasal medium supplemented with 1 \times GlutaMax.

Primary rat OPCs were isolated by shaking off from a confluent glia monolayer prepared from Sprague-Dawley rats at postnatal day 2 following a well established procedure (32). OPCs are purified by differential plating and maintained in a defined serum-free medium supplemented with PDGF (10 ng/ml) and basic fibroblast growth factor (20 ng/ml) overnight before being induced for differentiation in defined medium lacking growth factors. The remaining astrocytes were trypsinized, and subcultures were allowed to grow for 3 days before harvest.

CG4 cells were propagated and differentiated as previously described (33, 34). Briefly, proliferating CG4 cells were cultured in Dulbecco's modified Eagle's medium containing 1% heat-inactivated fetal bovine serum, insulin (5 μ g/ml), transferrin (50 μ g/ml), PBPS (100 mM putrescine, 10 ng/ml biotin, 20 nM progesterone, and 30 nM selenium). Platelet-derived growth factor AA (PDGFAA, Sigma) and basic fibroblast growth factor (Promega) were added to the proliferation medium (final, 10 ng/ml each). To induce differentiation, proliferation medium was replaced with differentiation medium, which contained Dulbecco's modified Eagle's medium, insulin, PBPS, transferrin, tri-iodothyronine (50 nM), and 0.5% fetal bovine serum.

For p39, p35, or Cdk5 knockdown, CG4 cells were transfected with control-, p39-, p35-, Cdk5-siRNA using Lipofectamine 2000. The p39-siRNA (Invitrogen catalog #RSS366369) was predesigned. siRNA duplexes targeting the nucleotide sequence 5'-AAGCUGUACUCCACGUC-CAUC-3' of rat Cdk5 mRNA were synthesized by Invitrogen. Previously described siRNA duplexes targeting the nucleotide sequence 5'-GAUGCUGCAGAUAUGCU-3' of rat p35 mRNA were synthesized by Thermo Scientific.

Morphological Analysis—CG4 cells were cotransfected with an EGFP plasmid together with either control-, p39-, or Cdk5-siRNA. At 0, 24, and 48 h of differentiation, live images of randomly selected GFP⁺ cells were captured using the Olympus IX-51 inverted fluorescent microscope followed by quantification of primary, secondary, and tertiary processes as previously described (34). Cells were categorized into the following groups; 1) only harboring primary processes, 2) the most complex processes were secondary processes, and 3) the most complex processes were tertiary processes. The percentages of cells in each category from three independent transfections were calculated and statistically compared. More than 50 randomly selected cells were analyzed from each transfected culture.

Cdk5 Kinase Assay—CG4 cells were co-transfected with p39-specific siRNA or negative control-siRNA (Invitrogen). To exclude potential off-target effects, a p39-FLAG construct was used to rescue p39 knockdown. Twenty-four hours after transfection, cells were lysed in a buffer containing 50 mM Tris-HCl (pH 7.5), 250 mM NaCl, 1 mM EDTA, 5 mM sodium orthovanadate, 5 mM sodium fluoride, 0.5% Nonidet P-40, and protease inhibitors. After preabsorption with Protein A beads, lysates containing equal amounts of total protein were incubated with 2 μ g of anti-Cdk5 antibody (Santa Cruz, C-8) for 3 h before capture by Protein A beads. After washing with lysis buffer and kinase buffer (20 mM MOPS (pH 7.0), 5 mM MgCl₂, 100 μ M EDTA, 100 μ M EGTA, and protease inhibitors), beads were resuspended in 25 μ l of kinase buffer plus 25 μ M cold ATP, 10 μ Ci [γ -³²P]ATP, and 100 ng/ μ l histone H1. After incubation at 30 °C for 30 min, proteins from each reaction were resolved by SDS-PAGE and transferred to PVDF membranes. From the same membrane, phosphorylation of H1 was detected by autoradiography, and immunoprecipitated Cdk5 was detected by immunoblot analysis and quantified based on densitometry. For each experiment, the relative level of phosphorylated H1 to Cdk5 protein was quantified as percentage of levels in immunoprecipitates from control-siRNA-transfected cells. The average relative level of phosphorylated H1 to Cdk5 protein was determined from three independent experiments.

Linear Sucrose Gradient Fractionation—Cytoplasmic extracts from CG4 cells lysed with gradient buffer (20 mM Tris, pH 7.5, 100 mM KCl, 5 mM MgCl₂) containing 1% Triton X-100 were loaded onto linear sucrose gradients (15–45%, w/v), centrifuged at 39,000 rpm in a SW41 rotor for 60 min at 4 °C, and fractionated as previously described (15). To disassociate polyribosomes, parallel cytoplasmic extracts from CG4 cells were prepared in the presence of 20 mM EDTA and no MgCl₂ followed by fractionation on a gradient containing 1 mM EDTA but lacking MgCl₂.

mRNA Quantification—For tissue and cells, total RNA was extracted using TRIzol (Invitrogen) according to the manufacturer's instructions, and mRNAs were quantified by qRT-PCR. After linear sucrose gradient fractionation (35), RNA was isolated by phenol/chloroform followed by semiquantitative RT-PCR. The following primers were used: p39-specific primers (5'-AACCTGGTGTTCGTGTACCTGCT-3' (forward) and 5'-AGATCTCGTTGCCCATGTAGGAGT-3' (reverse)), p35-specific primers (5'-AACAGCAAGAACGCCAAGGAC-AAG-3' (forward) and 5'-ATGTTGCTCTGGTAGCTGCTG-

TTG-3'), Cdk5-specific primers (5'-ATGATGACGATGAG-GGTGTGCCAA-3' (forward) and 5'-GGTCACCATTGCAG-CTGTCGAAAT-3' (reverse)), and GAPDH primers (5'-GGT-GAAGGTCGGTGTGAAC-3' (forward) and 5'-CCTTGACT-GTGCCGTTGAA-3' (reverse)).

Immunoblot Analysis—Whole cell lysates were prepared by sonication in 1 \times Laemmli buffer for SDS-PAGE. Resolved proteins were transferred to PVDF membranes, which were blocked in 10% nonfat dry milk in phosphate-buffered saline with 0.1% Tween 20. The membranes were then probed with primary antibodies. Anti-p39 (1:1000) was kindly provided by Dr. Nancy Ip (Hong Kong University of Science and Technology) or purchased from Santa Cruz (F-4), anti-Cdk5 (1:1000) and anti-p35 (1:1000) were from Cell Signaling, anti-eIF5 α (1:10000) was from Santa Cruz, and anti-MBP (1:9000) was from Millipore.

Immunohistochemistry—Mice were deeply anesthetized with isoflurane and intracardially perfused with 4% paraformaldehyde in phosphate-buffered saline (PBS). Brains were removed and postfixed in 4% paraformaldehyde. Cryostat brain sections were permeabilized with PBS containing 0.1% Triton X-100 for 20 min followed by blocking with PBS containing 1% BSA or 10% normal goat serum for 1 h. Sections were incubated with the following primary antibodies according to the manufacturer's suggestions: anti-p39 (Santa Cruz), anti-MBP, and anti-Olig2 (Millipore), and anti-CC1 (Abcam). After washes, sections were incubated with secondary antibodies and nuclear stain DAPI (2 ng/ml; Molecular Probes) in PBS containing 2% normal goat serum at room temperature for 1 h, washed 3 times with PBS, and mounted with either Vectashield mounting medium (Vector Laboratories) or Fluoromount-G (Southern Biotech).

MBP⁺ Cell Quantification—Immunofluorescent staining for myelin basic protein (MBP), a well accepted marker for OL differentiation, was performed on three adjacent brain sections from four pairs of WT and p39^{-/-} mice at postnatal day 6. The total numbers of MBP⁺ cells in the corpus callosum from all three adjacent sections for each mouse were counted in a blinded manner and graphically displayed.

Statistical Analysis—All bar graphs in the manuscript are presented as the means \pm S.E. Student's *t* test was used for two sample comparisons. For multiple sample comparisons, one-way ANOVA was carried out followed by Tukey's post test, and two-way ANOVA analysis was performed followed by Bonferroni's post test as indicated in the corresponding figure legends.

RESULTS

Distinct Expression Profiles of p35 and p39 in Neurons and OLs—Previous studies have demonstrated the essential function of Cdk5 in the development of neuronal and glial lineages. However, whether neurons and glia harbor similar or distinct expression profiles of p35 and p39 has not been defined. To address this question, we performed immunoblot analysis to detect Cdk5, p35, and p39 proteins using lysates derived from primary cultured rat neurons, astrocytes, and OLs (Fig. 1A). The housekeeping protein translation initiation factor 5 α (eIF5 α) was detected as a loading reference. On the same blot, lysates of p39^{-/-} and WT mouse brains were loaded as speci-

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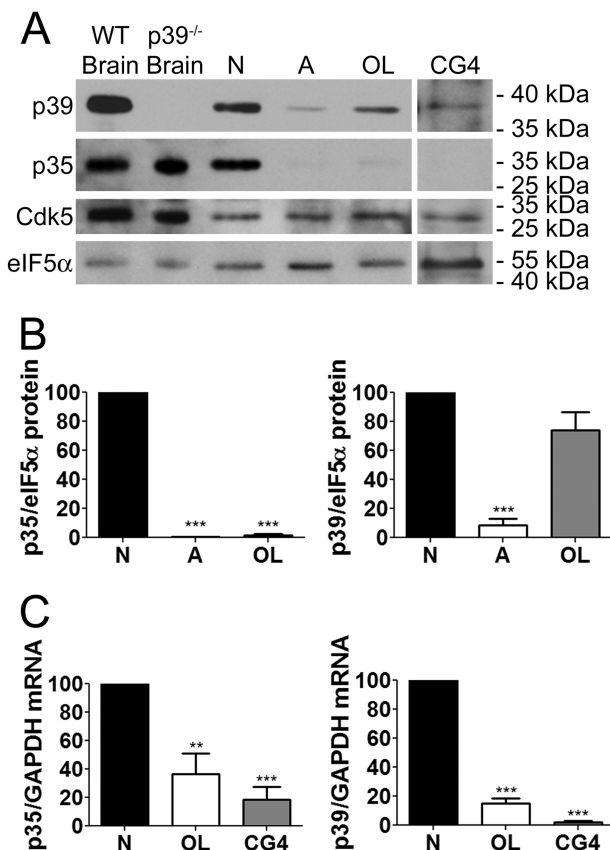


FIGURE 1. Differential Cdk5 activator expression in neurons and OLs. A, lysates from WT and p39^{-/-} brain, primary cultured rat neurons (N), astrocytes (A), OLs, and CG4 cells were subjected to immunoblot analysis of p39, p35, Cdk5, and eIF5α. All cells underwent 5 days of differentiation in culture. p39 antibody specificity was confirmed by the absence of signal in p39^{-/-} lysates. B, densitometer readings of p35 (left) and p39 (right) proteins are normalized to the housekeeping protein eIF5α as a loading reference. The normalized level of p35 and p39 protein in neurons on the same immunoblot was set as 100%, respectively, for estimation of relative abundance of these proteins in neurons and glia. C, total RNA was extracted from cultured neurons (N), OLs, and CG4 cells at DIV5, and qRT-PCR was performed for p35, p39, and GAPDH. The levels of p35 (left) and p39 (right) mRNAs relative to the GAPDH mRNA are graphically displayed. The relative mRNA level in neurons was set as 100% in each replicate experiment for normalization. For B and C, three independent batches of cultures were used for immunoblots ($n = 3$). The data are reported as the mean \pm S.E. $p < 0.0005$, one-way ANOVA; **, $p < 0.01$; ***, $p < 0.001$, Tukey's post test.

ficiency controls (Fig. 1A). Densitometry of p35 and p39 in neurons from the same blot were used to assess the relative abundance of these proteins in OLs and astrocytes. Cdk5 was detected in all lysates examined. Both p35 and p39 were abundantly expressed in primary cultured neurons and the WT brain. In contrast, negligible amounts of p35 were detected in cultured OLs, whereas p39 levels were $74 \pm 12\%$ that in neurons (Fig. 1B). A similar pattern of Cdk5 activator expression was also observed in the OPC cell line CG4, which closely mimics molecular and cellular properties of primary OPCs in early differentiation (36). Among the examined neural cell types, astrocytes harbored the lowest levels of Cdk5 activators, with negligible amounts of p35 and p39 levels at $9 \pm 4\%$ that expressed in neurons (Fig. 1, A and B). The function of Cdk5 in normal astrocytes still remains elusive, although Cdk5 activities are clearly detectable in glioma cells (37, 38). These data suggest

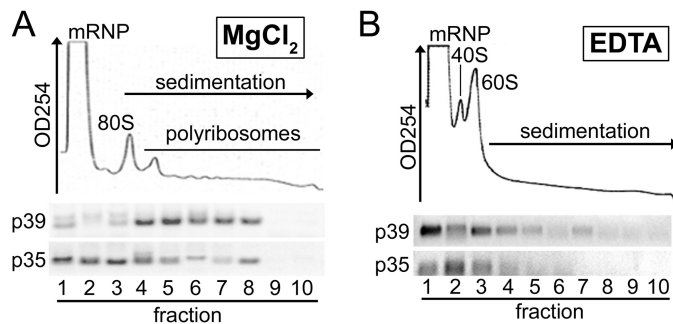


FIGURE 2. Active translation of p39 mRNA and translation suppression of p35 mRNA in CG4 cells. A and B, cytoplasmic RNA was isolated from CG4 cells and fractionated on a linear sucrose gradient in the presence of MgCl₂ (A) or EDTA (B). Top, shown are plots of absorption at 254 nm (OD₂₅₄) of linear sucrose gradients fractions. The direction of sedimentation as well as absorption peaks for messenger ribonucleoprotein complexes, 40 S and 60 S ribosomal subunits, 80 S monoribosome, and translating polyribosomes are indicated. Bottom, shown are representative images of semi-quantitative RT-PCR products of p39 and p35 mRNA in each gradient fraction on agarose gel after electrophoresis. mRNP, messenger ribonucleoprotein.

that in contrast to the more crucial role of p35 in neurons, p39 may function as the primary Cdk5 activator in OLs.

To explore molecular mechanisms that underlie the distinct protein levels of Cdk5 activators in neurons and OLs, we examined the expression levels of p35 and p39 mRNAs in neurons, OLs, and CG4 cells by qRT-PCR using specific primers. As shown in Fig. 1C, the levels of p35 and p39 mRNAs in primary OPCs are 36 ± 15 and $15 \pm 4\%$ of the corresponding mRNA levels expressed in neurons, respectively. Similar levels of p35 and p39 mRNAs to those in OPCs were also detected in CG4 cells. Thus the mRNA levels could not explain the distinct expression profiles of p39 and p35 proteins in neurons and OLs. One possibility is that p39 and p35 mRNAs are differentially translated in OLs, leading to preferential p39 expression.

To test this hypothesis, we performed linear sucrose gradient fractionation, a common assay for determining endogenous mRNA translation efficiency based on their association with translating polyribosomes (35). CG4 cells that recapitulate the preferential expression of p39 protein in primary OLs (Fig. 1A) were used to obtain sufficient amounts of extracts for biochemical fractionation, which would be difficult to derive from primary cultured OLs. As shown in Fig. 2A, 84.4% of p39 mRNA co-sediments with translating polyribosomes (fractions 4–10). The remaining p39 mRNA was detected in ribosome-free messenger ribonucleoprotein particles (mRNP, fractions 1–3). In contrast, the majority of p35 mRNA was sequestered into messenger ribonucleoproteins that are not engaged in translation. Moreover, when polyribosomes are dissociated by EDTA treatment, p39 mRNA is shifted into messenger ribonucleoproteins fractions (Fig. 2B), confirming the association of p39 mRNA with polyribosomes. These data suggest that active translation of p39, in contrast to translational suppression of p35, is an underlying mechanism for the preferential expression of p39 protein in OLs.

p39, but Not p35, Is Up-regulated during OL and Myelin Development—During OL differentiation, Cdk5 activity is markedly up-regulated (11, 12). To explore which activator is responsible for the enhanced Cdk5 activity, we analyzed the expression levels of Cdk5 and its activators during OL differen-

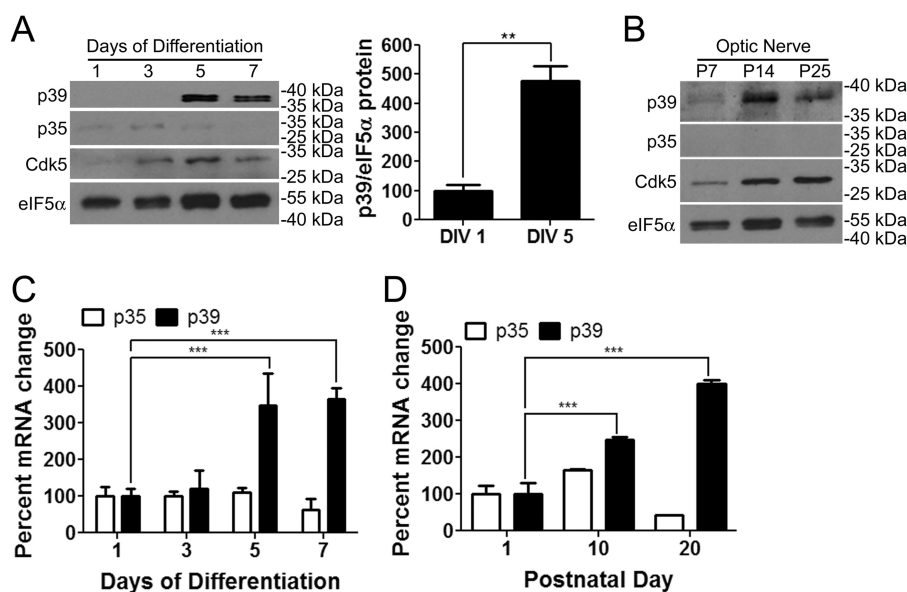


FIGURE 3. Selective up-regulation of p39 during OL differentiation and myelin development. *A*, lysates from primary cultured rat oligodendrocytes at the indicated days of differentiation were subjected to immunoblot analysis of p39, p35, Cdk5, and eIF5 α . A representative blot is shown (*left*). Band intensities were quantified by densitometry, and the levels of p39 relative to eIF5 α from immunoblots in three batches of cultured oligodendrocytes at DIV1 and DIV5 ($n = 3$) are shown (*right*). The densitometer reading in DIV1 cells are set as 100% to assess percent changes from independent experiments. The data are reported as the mean \pm S.E. ******, $p < 0.01$ by two-tailed *t* test. *B*, lysates from optic nerves, highly enriched of myelinating oligodendrocytes, were isolated at the indicated postnatal days during myelin development and subjected to immunoblot analysis of p39, p35, Cdk5, and eIF5 α . *C* and *D*, total RNA was extracted from primary cultured rat oligodendrocytes harvested at the indicated days of differentiation (*C*) and FACS-isolated oligodendrocytes from mouse brains during myelin development at the indicated postnatal days (*D*). Three independent samples were collected ($n = 3$). qRT-PCR was performed for p35, p39, and GAPDH. The levels of p35 and p39 relative to GAPDH are shown. qRT-PCR reading in day 1 samples are set as 100%. The data are reported as the mean \pm S.E. $p < 0.01$, two-way ANOVA; *******, $p < 0.001$, Bonferroni's post test.

tiation (Fig. 3A, *left*). A notable increase of p39 protein was detected by immunoblot analysis accompanied by increased Cdk5 expression during OPC differentiation in culture. Densitometric analysis revealed a 5 ± 0.5 -fold increase of p39 protein in cells that had undergone 5 days of differentiation *in vitro* (DIV5) as compared with DIV1 (Fig. 3A, *right*). To assess whether p39 protein is up-regulated during OL and myelin development *in vivo*, optic nerves, highly enriched in OLs, were isolated from WT mice during the most vigorous myelinogenesis at postnatal days 7, 14, and 25. As shown in Fig. 3B, a concomitant up-regulation of Cdk5 and p39 proteins during myelin development was detected by immunoblot analysis in the optic nerves. Conversely, no appreciable changes of p35 protein levels were detected during OPC differentiation in culture (Fig. 3A) or myelination of the optic nerve (Fig. 3B).

To further investigate molecular mechanisms that underlie the selective up-regulation of p39 during OL development, we performed qRT-PCR to quantify the levels of p39 and p35 mRNA during OL development in culture and *in vivo*. As shown in Fig. 3C, p39 mRNA, but not p35 mRNA, was significantly up-regulated by 3.5 ± 0.9 -fold in cultured OPCs after 5 days of induced differentiation. To determine whether p39 mRNA is up-regulated in developing OLs *in vivo*, we FACS-isolated brain OLs from mice, which express EGFP driven by the OL-specific 2',3'-cyclic nucleotide 3'-phosphodiesterase promoter (27), at postnatal days 1, 10, and 20 of myelinogenesis. A similar extent of p39 mRNA up-regulation, 2.5 ± 0.06 -fold at P10 and 4 ± 0.1 -fold at P20, was detected in OLs isolated from the brain during *de novo* myelin development (Fig. 3D). Thus, the developmentally programmed up-regulation of p39 mRNA

leads to increased production of p39 protein, which underlies the increase of Cdk5 activity during OL and myelin development reported by previous studies (11, 12).

p39-dependent Cdk5 Activation Is Essential for OL Differentiation—To directly test whether p39 is primarily responsible for Cdk5 activity in OPCs, we specifically knocked down p39 mRNA in CG4 cells ($69 \pm 4\%$ reduction) using an siRNA that does not affect p35 (Fig. 4A). To assess the role of p39 in Cdk5 activation, the Cdk5-activator complex was immunoprecipitated from p39 siRNA- and control-siRNA-treated cells. Cdk5 activity was then determined by a well established kinase assay using histone H1 as substrate, in which ^{32}P -H1 signal on autoradiography was normalized to the level of immunoprecipitated Cdk5 protein detected by immunoblot analysis of the same membrane. As shown in Fig. 4B, p39-specific siRNA significantly reduced Cdk5 activity, which was largely rescued by expression of exogenous p39 (p39-FLAG). These data suggest that p39 plays a major role in Cdk5 activation in OLs.

Previous studies have established the essential role of Cdk5 in OPC development (12). We next asked whether p39-mediated activation of Cdk5 advances OPC differentiation. CG4 cells can be induced for morphological differentiation, during which increasingly sophisticated processes develop over time, recapitulating primary OPCs. We treated CG4 cells with control- or p39-siRNA before induction of differentiation. The complexity of process arborization, a well established hallmark of OL differentiation, was analyzed based on primary, secondary, and tertiary processes (Fig. 5A). At the start of differentiation, comparable percentages of cells harboring primary, secondary, or tertiary processes were observed in control- and

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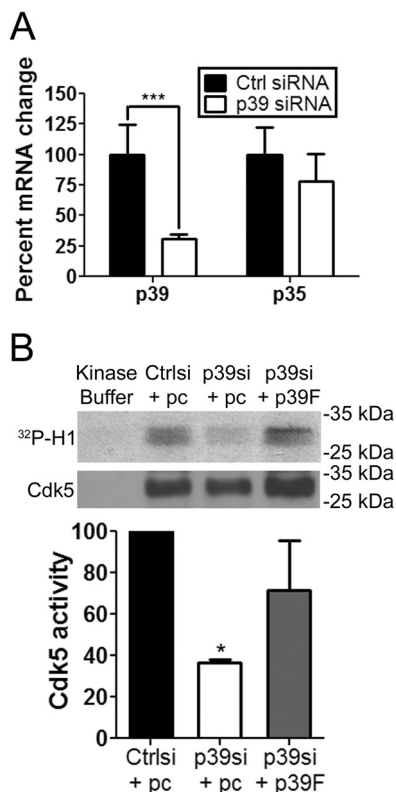


FIGURE 4. p39 activates Cdk5 in OLs. *A*, total RNA was extracted from three independent experiments in which CG4 cells were transfected with p39 siRNA or a negative control-siRNA ($n = 3$). qRT-PCR was performed for p35, p39, and GAPDH mRNA. The levels of p35 and p39 mRNA relative to GAPDH mRNA are shown. qRT-PCR reading in control-siRNA-treated cells were set as 100%. The data are reported as the mean \pm S.E. $p < 0.001$, two-way ANOVA; $***$, $p < 0.001$, Bonferroni's post test. *B*, Cdk5 was immunoprecipitated from lysates of CG4 cells cotransfected with negative control-siRNA and empty vector (*pc*), p39-siRNA and empty vector, or p39-siRNA and p39-FLAG (*p39F*). The immunoprecipitated Cdk5 or kinase reaction buffer alone was incubated with histone H1 and [γ - 32 P] ATP in a kinase reaction. Reactions were resolved on SDS-PAGE and transferred to PVDF membranes. *Top*, the same membrane was used to detect H1 phosphorylation by autoradiography and immunoprecipitated Cdk5 by immunoblot analysis. *Bottom*, band intensities were quantified by densitometry, and the levels of H1 phosphorylation relative to Cdk5 protein are shown. The normalized Cdk5 activity in control-siRNA-treated cells was set as 100% for each experiment. The data are reported as the mean \pm S.E. $n = 3$; $p < 0.05$, one-way ANOVA; *, $p < 0.05$, Tukey's post test.

p39-siRNA-treated cultures. Although control-siRNA-treated cells progressively develop complex secondary and tertiary processes over 2 days of differentiation, p39-siRNA treatment resulted in a significantly increased percentage of cells that harbored only primary processes as compared with control-siRNA-treated cells (Fig. 5A). This was accompanied by a reciprocal reduction of cells that carry tertiary processes upon p39 siRNA treatment, suggesting that p39 knockdown blocked initial differentiation.

A previous study reported that a siRNA that targets p35 also impaired OPC differentiation (12). However, we were surprised to find that this siRNA caused a comparable reduction of both p35 and p39 mRNAs when transfected into CG4 cells ($59 \pm 15\%$ reduction of p35 and $77 \pm 11\%$ reduction of p39 by qRT-PCR, $p < 0.0005$, two-way ANOVA; $p < 0.01$ for p35 and p39, Bonferroni's post test; data not shown). Further examination revealed that this siRNA contains high levels of sequence complementarity against p39 mRNA as well. Thus the previ-

ously reported effects of this siRNA on OL development likely involved suppression of p39.

To further discern if the impaired morphogenesis of CG4 cells by the p39 siRNA is due to suppression of Cdk5 function, we transfected CG4 cells with an siRNA that significantly knocked down Cdk5 ($65 \pm 6\%$ reduction of Cdk5 mRNA by qRT-PCR, $p < 0.01$, two-tailed t test; data not shown). Indeed, we observed a similar profile of impairment in morphological differentiation as seen with p39-siRNA treatment (Fig. 5B). Together, these data suggest that p39-dependent Cdk5 activation is essential for OPC morphogenesis in culture.

To determine whether p39 functions in the OL lineage *in vivo*, we examined OL development in p39 $^{-/-}$ mice (19). MBP is an important hallmark for OL development, and deficiency of Cdk5 in OPCs was previously reported to cause reduced MBP $^{+}$ cells during OPC differentiation in culture (12). Thus, we first analyzed the expression levels of MBP in the brains of WT and p39 $^{-/-}$ at postnatal day 14 when p39 levels peak during normal OL development *in vivo* (Fig. 3B). As shown in Fig. 6A, the p39 $^{-/-}$ brain harbors reduced expression of MBP that can be visualized by immunoblot analysis. To minimize the potential influence of p39 deficiency in neurons, which may in turn affect OLs through axo-myelin interactions, we performed immunofluorescence staining of MBP to examine OLs in the neonatal brain at postnatal day 6 before the onset of rigorous myelination. Reduced MBP immunofluorescence and process complexity, both indicative of impaired OL differentiation, were detected in p39 $^{-/-}$ OLs in the corpus callosum that is composed of myelin-enriched axon tracts connecting the left and right cerebral hemispheres (Fig. 6B). In addition, the loss of p39 resulted in reduced numbers of MBP $^{+}$ cells in the neonatal corpus callosum (Fig. 6C).

However, by postnatal day 20, we observed a 2.5-fold increase of p35 protein in p39 $^{-/-}$ optic nerves compared with that of the WT controls (Fig. 7A). Considering the enriched OL soma on the optic nerve, the compensatory up-regulation of p35 likely occurs in OLs. In the same samples, MBP expression in p39 $^{-/-}$ mice is normal (Fig. 7B). Moreover, by the age of 1 month, electron microscopy detected normal compact myelin in p39 $^{-/-}$ corpus callosum (Fig. 7C, *upper panel*). The g-ratio (ratio of axon diameter/axon + myelin diameter), a well established method for quantifying myelin thickness, in the WT and p39 $^{-/-}$ corpus callosum was nearly identical (Fig. 7C, *bottom panel*). Thus, despite the delay of OL development in mice lacking p39, myelination is largely normal by the age of 1 month, likely due to compensatory mechanisms including p35 up-regulation.

p39 Is Up-regulated during Remyelination and Essential for Repair of Myelin Lesions—Failure in repairing myelin lesions is a prevailing issue in demyelination diseases (39). Thus, an important question is whether p39-dependent Cdk5 activation is functionally important for myelin repair besides *de novo* OL development. We first questioned whether p39 expression is dynamically changed during induced demyelination and/or myelin repair. To answer this question, we employed the cuprizone paradigm that induces demyelination primarily in the corpus callosum, which can be readily dissected for molecular and biochemical quantification of changes occurring during demy-

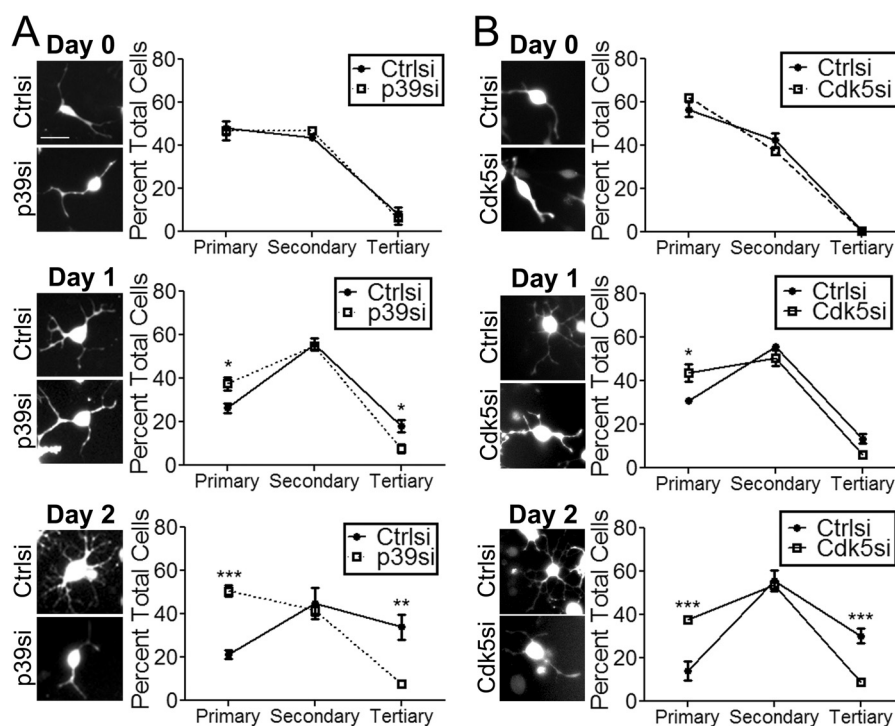


FIGURE 5. p39-dependent Cdk5 activation is essential for OL differentiation. A, p39-siRNA or control-siRNA were cotransfected with a GFP construct into CG4 cells in three independent experiments ($n = 3$). At 0, 1, and 2 days of differentiation, images of GFP⁺ cells (left) were captured from more than 50 randomly selected cells in each transfected culture, and the percentage of total cells harboring process complexity up to primary, secondary, or tertiary processes were determined and plotted for each day of differentiation (right). B, Cdk5-siRNA or control-siRNA were cotransfected with a GFP construct into CG4 cells. Analysis was performed as described for (A). For A and B, the data are reported as the mean \pm S.E. $n = 3$; $p < 0.01$, two-way ANOVA; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$, Bonferroni's post test. Scale bar, 50 μ m.

elination and the myelin repair process. Young adult mice that express EGFP specifically in cells of the OL lineage under the control of the PLP promoter were fed a cuprizone diet for 3 weeks to induce demyelination (3 weeks DM) and then returned to a normal diet for remyelination (3 + 3 weeks RM) (29). Demyelination was indicated by the reduced EGFP signal and reduced MBP immunofluorescence (Fig. 8A, 3 wk DM compared with *Normal*). In this model, both demyelination and impaired axonal conductance are largely recovered 3 weeks after returning animals to normal diet (3 + 3 weeks RM) (29). In fact, remyelination already initiates by the end of 3 weeks of cuprizone treatment, indicated by repopulation of the demyelinated corpus callosum with OPCs expressing Olig2 (Fig. 8A, bottom panel). Enhanced p39 expression after 3 weeks of cuprizone treatment was evident in cells of the OL lineage expressing EGFP (Fig. 8B, b and c, compared with Ba), including mature OLs marked by CC1 immunofluorescence (Fig. 8Bd). Moreover, p39 protein was increased by 3.1 ± 0.3 -fold in the dissected corpus callosum starting from the earliest phases of remyelination and remained above basal levels even after remyelination was largely completed (Fig. 8C). We next investigated the mechanisms that underlie the increase of p39 protein during myelin repair by measuring p39 mRNA levels by qRT-PCR. Similar to *de novo* OL differentiation, p39 mRNA expression was up-regulated by 3.5 ± 1.6 -fold during myelin repair (Fig. 8C, bottom panel). In contrast, p35 protein was unchanged during either the demyelination or the remyelination phase (Fig. 8D). These data suggest an increased functional requirement for p39 during myelin repair.

To directly assess whether p39 is required for myelin repair, acute demyelination was induced by unilateral lyssolecithin injection into the corpus callosum of WT and p39^{-/-} mice that carry the PLP-EGFP transgene. Unlike the cuprizone model where demyelination occurs over weeks, lyssolecithin injection produces a focal myelin lesion, clearly marked by the lack of EGFP-expressing OLs and myelin membrane, within 5 days (30). The reappearance of EGFP⁺ cells in the lesion area during myelin repair can be visualized. We chose to inject lyssolecithin at 6 weeks of age because p39^{-/-} mice have developed normal myelin by that age (Fig. 7), which is also indicated by the similar intensity of EGFP-labeled myelin on the contralateral side comparable with that in the WT control (Fig. 8E, top panels). Successful demyelination was achieved in both WT and p39^{-/-} mice (Fig. 8E, arrows in top panels). However, although WT mice almost fully repaired the demyelinated lesion 21 days post-injection, remyelination was severely impaired in p39^{-/-} mice, as indicated by the obvious lesion that remained at the injection site (Fig. 8E, arrowheads in the bottom panels). Furthermore, MBP staining and EGFP⁺ cells were reduced in the p39^{-/-} lesion as compared with the WT lesion (Fig. 8F). Together, these data provide the first evidence for selective up-regulation of p39, but not p35, during remyelination and the functional requirement of p39-dependent Cdk5 activity for myelin repair.

DISCUSSION

In this study we demonstrated for the first time that neurons and glia employ distinct molecular mechanisms to control

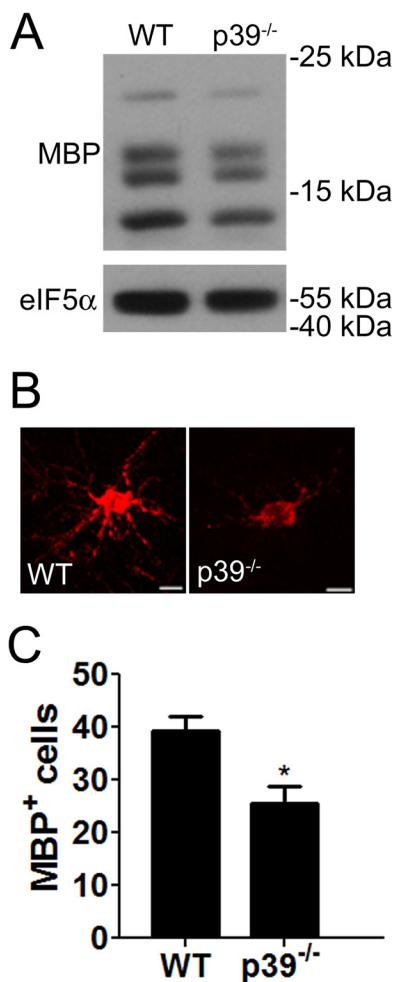


FIGURE 6. Loss of p39 delays oligodendroglia development *in vivo*. *A*, lysates from WT and p39^{-/-} mouse brains at postnatal day 14 were subjected to immunoblot analysis of MBP and eIF5α. *B*, brain slices from WT and p39^{-/-} corpus callosum at postnatal day 6 were stained for MBP. Representative images of MBP⁺ cells are shown. Scale bar, 10 μm. *C*, brain slices from WT and p39^{-/-} mice at postnatal day 6 were stained for MBP. The total number of MBP⁺ cells in the corpus callosum from three adjacent slices at comparable anatomic position from each brain was quantified. Four pairs of WT and p39^{-/-} mice were examined. The data are reported as the mean ± S.E. (*n* = 4; *, *p* < 0.05 by two-tailed *t* test).

Cdk5 activity by differential regulation of Cdk5 activator expression. Moreover, using multiple experimental paradigms, our studies clearly established that in contrast to the predominant function of p35 in Cdk5 activation in neurons and many other peripheral cell types (40–47), p39 is the primary Cdk5 activator in OLs and plays essential roles to advance normal OL differentiation as well as repair of myelin lesion in the brain.

The critical roles of Cdk5 in controlling normal neuronal development and function have been well established (5, 20). More recent studies have also established a parallel role of Cdk5 in OL development (12, 13, 15, 16). However, molecular mechanisms that regulate Cdk5 function in different neural cell lineages still remain vastly elusive. In particular, because of the lack of overt detrimental phenotypes in adult p39^{-/-} mice (19), the functional importance of p39 is not understood. We show for the first time that OLs primarily express p39 for Cdk5 activation, with scarce levels of p35. Moreover, the selective up-regulation of p39, but not p35, during OL differentiation in

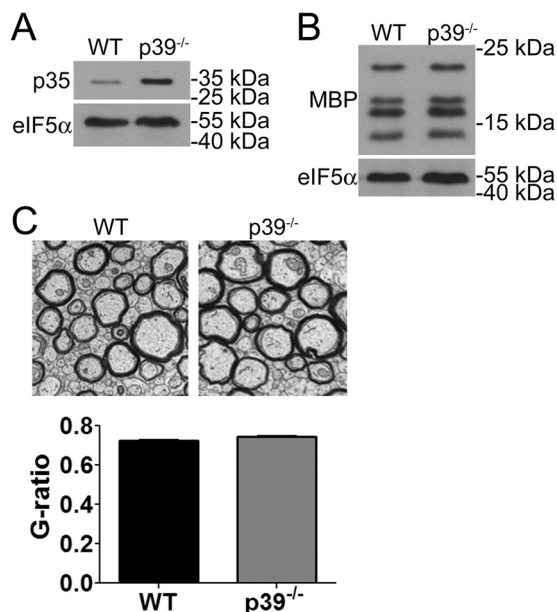


FIGURE 7. p35 is up-regulated in p39^{-/-} optic nerves at the peak of myelination, and p39^{-/-} young adults display normal MBP expression and myelin formation. *A*, optic nerves were isolated from multiple WT and p39^{-/-} mice at postnatal day 20 and combined for each genotype to prepare protein lysates to detect the low levels of p35 in WT. Immunoblot analysis of p35 and eIF5α were carried out. Densitometer reading of p35 band intensity was normalized to that of eIF5α. *B*, lysates from the same preparations used in *A* were subjected to a parallel immunoblot analysis for MBP and eIF5α. *C*, top, shown are representative images of electron micrographs of cross-sections of corpus callosum from 1-month-old WT and p39^{-/-} mice. Bottom, shown is the average *g*-ratio of axons in the corpus callosum of 1-month-old WT and p39^{-/-} mice. The data are reported as the mean ± S.E.

culture and *in vivo* clearly demonstrated that p39 is responsible for the increased Cdk5 activity during OL differentiation (11, 12). Given the fact that the most rigorous OL development occurs in the first few postnatal weeks (14), OL-produced p39 must be an important contributor to the previously reported preferential increase of p39 during neonatal brain development (26). Importantly, selective up-regulation of p39 is also observed during myelin repair in adults, suggesting that similar mechanisms control p39 expression in neonatal and adult OPCs.

We have provided *in vitro* and *in vivo* evidence that p39-dependent Cdk5 activation is crucial for OPC differentiation, which expands the importance of p39-dependent Cdk5 function from the previous report that shRNA knockdown of p39 impairs OPC migration in culture (13). In fact, the reduced numbers of MBP⁺ cells in the corpus callosum of p39^{-/-} mice likely originate from defects in both migration and differentiation, two concurrent events tightly coupled during *in vivo* myelinogenesis. Although the lack of p39 in neurons may also contribute to the delayed OL development *in vivo* in the p39^{-/-} mice, impairment of morphological differentiation of CG4 cells by siRNA-mediated knockdown of p39 and Cdk5 clearly demonstrated the essential role of cell autonomous p39-dependent Cdk5 function in OPCs.

Unlike the conditional Cdk5 knock-out mice that exhibit CNS hypomyelination (15, 16), we found that p39^{-/-} mice only exhibit a delay in early OL development without overt hypomyelination. One likely explanation is the compensatory increase

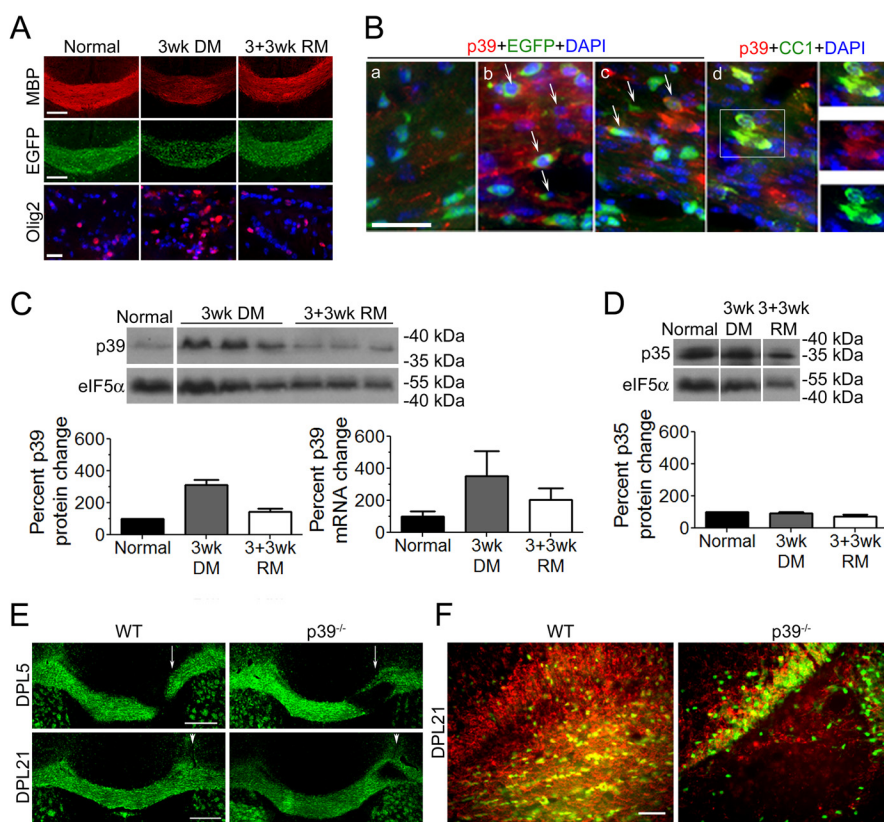


FIGURE 8. p39 is selectively up-regulated during remyelination and required for myelin repair. *A–C*, mice expressing EGFP under the oligodendroglia-specific PLP promoter were subjected to the following diet paradigms: a normal diet for 6 weeks (*Normal*), a cuprizone diet for 3 weeks followed by a normal diet for 3 additional weeks (*3 + 3 wk RM*). *A*, *top*, brain slices containing the corpus callosum were stained for MBP. *Middle*, shown are EGFP signals in OLs and on axonal tracts. *Bottom*, brain slices containing the corpus callosum were stained for the OL precursor marker Olig2. *Scale bar*, 100 μ m for MBP and EGFP images, 20 μ m for Olig2 images. *B*, *a–d*, immunofluorescence of p39 (*red*) in the corpus callosum of normal (*a*), 3 weeks DM (*b*), and 3 + 3 weeks RM (*c* and *d*) mice. DAPI staining (*blue*) marks nuclei. *a–c*, OLs are EGFP⁺, and increased p39 expression (*red*) is indicated by arrows. *d*, shown is immunofluorescence of p39 (*red*) and the mature OL marker CC1 (*green*). *Scale bar*, 20 μ m. *C*, *top*, corpus callosum of normal, 3 weeks DM, and 3 + 3 weeks RM mice were dissected, and lysates were subjected to immunoblot analysis of p39 and eIF5 α . *Bottom left*, protein band intensities were quantified by densitometry, and the levels of p39 were normalized to that of eIF5 α . The level of p39 protein in normal was set as 100% and graphically displayed. *Bottom right*, total RNA was extracted from dissected corpus callosum of normal, 3 weeks DM, and 3 + 3 weeks RM mice, and qRT-PCR was performed for p39 and GAPDH. The levels of p39 mRNA relative to GAPDH mRNA were calculated, and the level of p39 mRNA in normal was set as 100%. The data are reported as the mean \pm S.E. (*n* = 3). *D*, *top*, shown is an immunoblot analysis of p35 and eIF5 α in dissected corpus callosum. *Bottom*, protein band intensities were quantified by densitometry, and the levels of p35 relative to eIF5 α are shown. Normal was set as 100%. The data are reported as the mean \pm S.E. (*n* = 3). *E*, WT and p39^{-/-} mice expressing EGFP under the oligodendroglia-specific PLP promoter were unilaterally injected with lysolecithin to induce demyelination. *Top*, the demyelinated lesion was visualized by the lack of PLP-EGFP signals 5 days post lysolecithin injection (*DPL5*). Arrows indicate the site of injection. *Bottom*, myelin repair was assessed 21 days post lysolecithin injection (*DPL21*). Arrowheads indicate the injection tracks, which are still visible. *Scale bar*, 500 μ m. *F*, brain slices containing corpus callosum from WT and p39^{-/-} mice at 21 days post lysolecithin injection were stained for MBP. High magnification images of MBP staining and EGFP⁺ cells are shown. *Scale bar*, 50 μ m.

of p35 in OLs due to the absence of p39, which may permit delayed myelination in p39^{-/-} adult. In addition, given the facts that many Cdk5 target proteins can be phosphorylated by other kinases (48–52), compensatory cross-talk by other signaling mechanisms may also contribute to myelinogenesis in the absence of p39-dependent Cdk5 function. Nonetheless, the defects in early OPC development in p39^{-/-} mice argue that the function of p39 could not be completely spared despite compensation by various possible mechanisms.

Importantly, in contrast to the largely intact *de novo* myelination in the absence of p39, myelin repair is severely impaired in the p39^{-/-} mice after lysolecithin-induced acute demyelination. On one hand, the much more rapid lesion development may not leave sufficient time to induce compensatory changes. On the other hand, the more challenging environment of lysolecithin-induced lesion may exacerbate the functional defects of lacking p39-dependent Cdk5 signaling, which lead to failures

of myelin repair. Notably, increased p39 expression can also be observed in cells beyond the OL lineage during myelin repair. Thus, whether increased p39 expression from neurons and other types of glia cells together with OL-specific p39 up-regulation are synergistically required for myelin repair is an interesting unanswered question that could be addressed once neural-lineage specific, inducible knock-out of p39 can be achieved.

The low levels of p39 and p35 mRNAs in OLs as compared with that in neurons suggest the existence of neural lineage-specific mechanisms for differential mRNA biogenesis of these Cdk5 activators. We showed that p39 mRNA is active for translation in OLs. Thus, p39 protein levels are largely regulated by the abundance of p39 mRNA, likely involving unidentified OL-specific transcription factors as well as posttranscriptional mechanisms that control the stability of the p39 mRNA. Noticeably, up-regulation of p39 mRNA is observed in *de novo* myelin development as well as myelin repair. Thus, molecular

mechanisms that regulate p39 in neonatal OPCs are likely applicable to myelinating OLs during lesion repair in the adults and warrant rigorous investigation. In contrast, p35 mRNA is translationally repressed in OLs, which is an apparent mechanism that contributes to the scarce expression of p35 protein in OLs. The transacting factors that suppress p35 translation in OLs still remain unknown. Nonetheless, it is worth mentioning that a number of microRNAs are predicted to target the lengthy 3'-UTR of p35 mRNA specifically but not the p39 mRNA (53). Which of these microRNAs are expressed in OLs and responsible for translational suppression of p35 is a challenging question to be addressed by future studies.

In conclusion, our studies have demonstrated differential regulation of Cdk5 activator expression in neurons and OLs. Further delineating molecular mechanisms that control p35 and p39 expression may allow differential and independent manipulation of Cdk5 function in neuronal and glial lineages during normal development as well as in the pathogenesis of brain disorders involving cell type-specific Cdk5 dysregulation, such as neuronal degeneration in Alzheimer disease and glioma tumorigenesis (38, 54, 55). In particular, identification of OL-specific mechanisms that control p39 expression may ultimately help to develop novel strategies to advance CNS myelination in numerous disorders for which the pathogenic impacts of myelin impairment have become increasingly recognized, represented by multiple sclerosis and schizophrenia (1).

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REFERENCES

1. Fields, R. D. (2008) White matter in learning, cognition, and psychiatric disorders. *Trends Neurosci.* **31**, 361–370
2. Ren, Y., Wang, H., and Xiao, L. (2013) Improving myelin/oligodendrocyte-related dysfunction. A new mechanism of antipsychotics in the treatment of schizophrenia? *Int. J. Neuropsychopharmacol.* **16**, 691–700
3. Gold, B. T., Johnson, N. F., Powell, D. K., and Smith, C. D. (2012) White matter integrity and vulnerability to Alzheimer's disease. Preliminary findings and future directions. *Biochim. Biophys. Acta* **1822**, 416–422
4. Bankston, A. N., Mandler, M. D., and Feng, Y. (2013) Oligodendroglia and neurotrophic factors in neurodegeneration. *Neurosci. Bull.* **29**, 216–228
5. Su, S. C., and Tsai, L.-H. (2011) Cyclin-dependent kinases in brain development and disease. *Annu. Rev. Cell Dev. Biol.* **27**, 465–491
6. Lew, J., Beaudette, K., Litwin, C. M., and Wang, J. H. (1992) Purification and characterization of a novel proline-directed protein kinase from bovine brain. *J. Biol. Chem.* **267**, 13383–13390
7. Meyerson, M., Enders, G. H., Wu, C. L., Su, L. K., Gorke, C., Nelson, C., Harlow, E., and Tsai, L. H. (1992) A family of human cdc2-related protein kinases. *EMBO J.* **11**, 2909–2917
8. van den Heuvel, S., and Harlow, E. (1993) Distinct roles for cyclin-dependent kinases in cell cycle control. *Science* **262**, 2050–2054
9. Zheng, M., Leung, C. L., and Liem, R. K. (1998) Region-specific expression of cyclin-dependent kinase 5 (cdk5) and its activators, p35 and p39, in the developing and adult rat central nervous system. *J. Neurobiol.* **35**, 141–159
10. Tsai, L. H., Takahashi, T., Caviness, V. S., Jr., and Harlow, E. (1993) Activity and expression pattern of cyclin-dependent kinase 5 in the embryonic

mouse nervous system. *Development* **119**, 1029–1040

11. Tang, X. M., Strocchi, P., and Cambi, F. (1998) Changes in the activity of cdk2 and cdk5 accompany differentiation of rat primary oligodendrocytes. *J. Cell. Biochem.* **68**, 128–137
12. Miyamoto, Y., Yamauchi, J., Chan, J. R., Okada, A., Tomooka, Y., Hisanaga, S., and Tanoue, A. (2007) Cdk5 regulates differentiation of oligodendrocyte precursor cells through the direct phosphorylation of paxillin. *J. Cell Sci.* **120**, 4355–4366
13. Miyamoto, Y., Yamauchi, J., and Tanoue, A. (2008) Cdk5 phosphorylation of WAVE2 regulates oligodendrocyte precursor cell migration through nonreceptor tyrosine kinase Fyn. *J. Neurosci.* **28**, 8326–8337
14. Miller, R. H. (2002) Regulation of oligodendrocyte development in the vertebrate CNS. *Prog. Neurobiol.* **67**, 451–467
15. He, X., Takahashi, S., Suzuki, H., Hashikawa, T., Kulkarni, A. B., Mikoshiba, K., and Ohshima, T. (2011) Hypomyelination phenotype caused by impaired differentiation of oligodendrocytes in Emx1-cre mediated Cdk5 conditional knockout mice. *Neurochem. Res.* **36**, 1293–1303
16. Yang, Y., Wang, H., Zhang, J., Luo, F., Herrup, K., Bibb, J. A., Lu, R., and Miller, R. H. (2013) Cyclin dependent kinase 5 is required for the normal development of oligodendrocytes and myelin formation. *Dev. Biol.*, in press
17. Tsai, L. H., Delalle, I., Caviness, V. S., Jr., Chae, T., and Harlow, E. (1994) p35 is a neural-specific regulatory subunit of cyclin-dependent kinase 5. *Nature* **371**, 419–423
18. Humbert, S., Dhavan, R., and Tsai, L. (2000) p39 activates cdk5 in neurons and is associated with the actin cytoskeleton. *J. Cell Sci.* **113**, 975–983
19. Ko, J., Humbert, S., Bronson, R. T., Takahashi, S., Kulkarni, A. B., Li, E., and Tsai, L. H. (2001) p35 and p39 are essential for cyclin-dependent kinase 5 function during neurodevelopment. *J. Neurosci.* **21**, 6758–6771
20. Ohshima, T., Ward, J. M., Huh, C. G., Longenecker, G., Veeranna, Pant, H. C., Brady, R. O., Martin, L. J., and Kulkarni, A. B. (1996) Targeted disruption of the cyclin-dependent kinase 5 gene results in abnormal corticogenesis, neuronal pathology, and perinatal death. *Proc. Natl. Acad. Sci. U.S.A.* **93**, 11173–11178
21. Delalle, I., Bhide, P. G., Caviness, V. S., Jr., and Tsai, L. H. (1997) Temporal and spatial patterns of expression of p35, a regulatory subunit of cyclin-dependent kinase 5, in the nervous system of the mouse. *J. Neurocytol.* **26**, 283–296
22. Cai, X. H., Tomizawa, K., Tang, D., Lu, Y. F., Moriwaki, A., Tokuda, M., Nagahata, S., Hatase, O., and Matsui, H. (1997) Changes in the expression of novel Cdk5 activator messenger RNA (p39nck5ai mRNA) during rat brain development. *Neurosci. Res.* **28**, 355–360
23. Honjyo, Y., Kawamoto, Y., Nakamura, S., Nakano, S., and Akiyuchi, I. (1999) Immunohistochemical localization of CDK5 activator p39 in the rat brain. *Neuroreport* **10**, 3375–3379
24. Wu, D. C., Yu, Y. P., Lee, N. T., Yu, A. C., Wang, J. H., and Han, Y. F. (2000) The expression of Cdk5, p35, p39, and Cdk5 kinase activity in developing, adult, and aged rat brains. *Neurochem. Res.* **25**, 923–929
25. Chae, T., Kwon, Y. T., Bronson, R., Dikkes, P., Li, E., and Tsai, L. H. (1997) Mice lacking p35, a neuronal specific activator of Cdk5, display cortical lamination defects, seizures, and adult lethality. *Neuron* **18**, 29–42
26. Humbert, S., Lanier, L. M., and Tsai, L. H. (2000) Synaptic localization of p39, a neuronal activator of cdk5. *Neuroreport* **11**, 2213–2216
27. Zhu, H., Zhao, L., Wang, E., Dimova, N., Liu, G., Feng, Y., and Cambi, F. (2012) The QKI-PLP pathway controls SIRT2 abundance in CNS myelin. *Glia* **60**, 69–82
28. Mallon, B. S., Shick, H. E., Kidd, G. J., and Macklin, W. B. (2002) Proteolipid promoter activity distinguishes two populations of NG2-positive cells throughout neonatal cortical development. *J. Neurosci.* **22**, 876–885
29. Crawford, D. K., Mangiardi, M., and Tiwari-Woodruff, S. K. (2009) Assaying the functional effects of demyelination and remyelination. Revisiting field potential recordings. *J. Neurosci. Methods* **182**, 25–33
30. Aguirre, A., Dupree, J. L., Mangin, J. M., and Gallo, V. (2007) A functional role for EGFR signaling in myelination and remyelination. *Nat. Neurosci.* **10**, 990–1002
31. Meberg, P. J., and Miller, M. W. (2003) Culturing hippocampal and cortical neurons. *Methods Cell Biol.* **71**, 111–127
32. Osterhout, D. J., Wolven, A., Wolf, R. M., Resh, M. D., and Chao, M. V.

- (1999) Morphological differentiation of oligodendrocytes requires activation of Fyn tyrosine kinase. *J. Cell Biol.* **145**, 1209–1218
33. Zhao, L., Ku, L., Chen, Y., Xia, M., LoPresti, P., and Feng, Y. (2006) QKI binds MAP1B mRNA and enhances MAP1B expression during oligodendrocyte development. *Mol. Biol. Cell* **17**, 4179–4186
 34. Chen, Y., Tian, D., Ku, L., Osterhout, D. J., and Feng, Y. (2007) The selective RNA-binding protein quaking I (QKI) is necessary and sufficient for promoting oligodendroglia differentiation. *J. Biol. Chem.* **282**, 23553–23560
 35. Lau, A. G., Irier, H. A., Gu, J., Tian, D., Ku, L., Liu, G., Xia, M., Fritsch, B., Zheng, J. Q., Dingleline, R., Xu, B., Lu, B., and Feng, Y. (2010) Distinct 3'-UTRs differentially regulate activity-dependent translation of brain-derived neurotrophic factor (BDNF). *Proc. Natl. Acad. Sci. U.S.A.* **107**, 15945–15950
 36. Louis, J. C., Magal, E., Muir, D., Manthorpe, M., and Varon, S. (1992) CG-4, a new bipotential glial cell line from rat brain, is capable of differentiating *in vitro* into either mature oligodendrocytes or type-2 astrocytes. *J. Neurosci. Res.* **31**, 193–204
 37. Filippova, N., Yang, X., King, P., and Nabors, L. B. (2012) Phosphoregulation of the RNA-binding protein Hu antigen R (HuR) by Cdk5 affects centrosome function. *J. Biol. Chem.* **287**, 32277–32287
 38. Liu, R., Tian, B., Gearing, M., Hunter, S., Ye, K., and Mao, Z. (2008) Cdk5-mediated regulation of the PIKE-A-Akt pathway and glioblastoma cell invasion. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 7570–7575
 39. Fancy, S. P., Kotter, M. R., Harrington, E. P., Huang, J. K., Zhao, C., Rowitch, D. H., and Franklin, R. J. (2010) Overcoming remyelination failure in multiple sclerosis and other myelin disorders. *Exp. Neurol.* **225**, 18–23
 40. Rosales, J. L., and Lee, K. Y. (2006) Extraneuronal roles of cyclin-dependent kinase 5. *Bioessays* **28**, 1023–1034
 41. Liebl, J., Weitensteiner, S. B., Vereb, G., Takács, L., Fürst, R., Vollmar, A. M., and Zahler, S. (2010) Cyclin-dependent kinase 5 regulates endothelial cell migration and angiogenesis. *J. Biol. Chem.* **285**, 35932–35943
 42. Tripathi, B. K., and Zelenka, P. S. (2009) Cdk5-dependent regulation of Rho activity, cytoskeletal contraction, and epithelial cell migration via suppression of Src and p190RhoGAP. *Mol. Cell. Biol.* **29**, 6488–6499
 43. Brinkkoetter, P. T., Wu, J. S., Ohse, T., Krofft, R. D., Schermer, B., Benzinger, T., Pippin, J. W., and Shankland, S. J. (2010) p35, the non-cyclin activator of Cdk5, protects podocytes against apoptosis *in vitro* and *in vivo*. *Kidney Int.* **77**, 690–699
 44. Ubeda, M., Kemp, D. M., and Habener, J. F. (2004) Glucose-induced expression of the cyclin-dependent protein kinase 5 activator p35 involved in Alzheimer's disease regulates insulin gene transcription in pancreatic beta-cells. *Endocrinology* **145**, 3023–3031
 45. Lilja, L., Yang, S.-N., Webb, D.-L., Juntti-Berggren, L., Berggren, P.-O., and Bark, C. (2001) Cyclin-dependent kinase 5 promotes insulin exocytosis. *J. Biol. Chem.* **276**, 34199–34205
 46. Philpott, A., Porro, E. B., Kirschner, M. W., and Tsai, L. H. (1997) The role of cyclin-dependent kinase 5 and a novel regulatory subunit in regulating muscle differentiation and patterning. *Genes Dev.* **11**, 1409–1421
 47. Lazaro, J. B., Kitzmann, M., Poul, M. A., Vandromme, M., Lamb, N. J., and Fernandez, A. (1997) Cyclin dependent kinase 5, cdk5, is a positive regulator of myogenesis in mouse C2 cells. *J. Cell Sci.* **110**, 1251–1260
 48. Wang, J.-Z., Grundke-Iqbal, I., and Iqbal, K. (2007) Kinases and phosphatases and tau sites involved in Alzheimer neurofibrillary degeneration. *Eur. J. Neurosci.* **25**, 59–68
 49. Grant, P., Sharma, P., and Pant, H. C. (2001) Cyclin-dependent protein kinase 5 (Cdk5) and the regulation of neurofilament metabolism. *Eur. J. Biochem.* **268**, 1534–1546
 50. Bibb, J. A., Nishi, A., O'Callaghan, J. P., Ule, J., Lan, M., Snyder, G. L., Horiuchi, A., Saito, T., Hisanaga, S.-i., Czernik, A. J., Nairn, A. C., and Greengard, P. (2001) Phosphorylation of protein phosphatase inhibitor-1 by Cdk5. *J. Biol. Chem.* **276**, 14490–14497
 51. Kansy, J. W., Daubner, S. C., Nishi, A., Sotogaku, N., Lloyd, M. D., Nguyen, C., Lu, L., Haycock, J. W., Hope, B. T., Fitzpatrick, P. F., and Bibb, J. A. (2004) Identification of tyrosine hydroxylase as a physiological substrate for Cdk5. *J. Neurochem.* **91**, 374–384
 52. Bibb, J. A., Snyder, G. L., Nishi, A., Yan, Z., Meijer, L., Fienberg, A. A., Tsai, L.-H., Kwon, Y. T., Girault, J.-A., Czernik, A. J., Haganir, R. L., Hemmings, H. C., Jr., Nairn, A. C., and Greengard, P. (1999) Phosphorylation of DARPP-32 by Cdk5 modulates dopamine signalling in neurons. *Nature* **402**, 669–671
 53. Moncini, S., Salvi, A., Zuccotti, P., Viero, G., Quattrone, A., Barlati, S., De Petro, G., Venturin, M., and Riva, P. (2011) The role of miR-103 and miR-107 in regulation of CDK5R1 expression and in cellular migration. *PLoS ONE* **6**, e20038
 54. Cheung, Z. H., and Ip, N. Y. (2012) Cdk5. A multifaceted kinase in neurodegenerative diseases. *Trends Cell Biol.* **22**, 169–175
 55. An, J. H., Lee, S. Y., Jeon, J. Y., Cho, K. G., Kim, S. U., and Lee, M. A. (2009) Identification of gliotropic factors that induce human stem cell migration to malignant tumor. *J. Proteome Res.* **8**, 2873–2881