New *Olig1* null mice confirm a non-essential role for Olig1 in oligodendrocyte development

Joana Paes de Faria¹², Nicoletta Kessaris¹, Paul Andrew¹, William D Richardson¹* and Huiliang Li¹

**Abstract**

**Background:** *Olig1* and *Olig2*, encoding closely related basic helix-loop-helix transcription factors, were originally identified in screens for glial-specific genes. *Olig1* and *Olig2* are both expressed in restricted parts of the neuroepithelium of the embryonic spinal cord and telencephalon and subsequently in oligodendrocyte lineage cells throughout life. In the spinal cord, *Olig2* plays a crucial role in the development of oligodendrocytes and motor neurons, and both cell types are lost from *Olig2* null mutant mice. The role of *Olig1* has been more cryptic. It was initially reported that *Olig1* null mice (with a *Cre-Pgk-Neo* cassette at the *Olig1* locus) have a mild developmental phenotype characterized by a slight delay in oligodendrocyte differentiation. However, a subsequent study of the same line following removal of *Pgk-Neo* (leaving *Olig1-Cre*) found severe disruption of oligodendrocyte production, myelination failure and early postnatal lethality. A plausible explanation was proposed, that the highly expressed *Pgk-Neo* cassette in the original line might have up-regulated the neighbouring *Olig2* gene, compensating for loss of *Olig1*. However, this was not tested, so the importance of *Olig1* for oligodendrocyte development has remained unclear.

**Results:** We generated two independent lines of *Olig1* null mice. Both lines had a mild phenotype featuring slightly delayed oligodendrocyte differentiation and maturation but no long-term effect. In addition, we found that *Olig2* transcripts were not up-regulated in our *Olig1* null mice.

**Conclusions:** Our findings support the original conclusion that *Olig1* plays a minor and non-essential role in oligodendrocyte development and have implications for the interpretation of studies based on *Olig1* deficient mice (and perhaps *Olig1-Cre* mice) from different sources.

**Keywords:** Oligodendrocyte, Olig1, Olig2, Myelin, Knockout mice, Spinal cord, Forebrain

---

**Background**

*Oligodendrocyte lineage* genes *Olig1* and *Olig2* encode basic helix-loop-helix (bHLH) transcription factors. *Olig2* is a master regulator of oligodendrocyte (OL) lineage development [1-3]. *Olig2* is also required for generation of some neurons, notably spinal motor neurons (MNs) [1-3]. MNs are generated from neural stem/progenitor cells in a specialized region of the ventral ventricular zone (VZ) of the spinal cord known as pMN. Around embryonic day 12 (E12) in mice, the same group of progenitors stops producing MNs and switches to production of OL precursors (OPs), which proliferate and migrate away from the VZ in all directions before associating with axons and differentiating into myelin-forming OLs (reviewed in reference [4]).

*Olig1* and *Olig2* (referred to here as Oligs) are involved at multiple stages of this developmental sequence. *Olig2* is also required for specifying oligodendrocytes and some types of neurons in the brain – some ventrally-derived interneurons and cholinergic projection neurons in the forebrain, for example [5].

*Olig1* can compensate for *Olig2* in some regions including the hindbrain and parts of the forebrain, because OPs still form there in *Olig2* null mice but not in *Olig1/Olig2* double nulls [1,3]. *Olig1* also plays a later role in the differentiation of OPs into myelinating OLs, although there is disagreement about whether there is an absolute requirement for *Olig1* during normal development [1,6]. The original *Olig1* null allele, made by inserting a *Cre-frt-Pgk-Neo-frt* cassette into the mouse *Olig1* locus [1] caused a...
delay in the appearance of differentiated OLs but no long-term myelin deficit. However, a subsequent study by Xin et al. [6], who crossed the original line with FLP-expressing mice to remove the Pgk-Neo selection cassette (leaving behind Olig1-Cre), found a severe myelination defect leading to early postnatal lethality. Apart from this contested role in OL lineage development, Olig1 is known to be required for remyelination of experimentally-induced demyelinated lesions in the mouse spinal cord [7].

Given the central role of the Oligs in OL lineage development, it is important to try to settle the controversy over the developmental requirement for Olig1. This might have added significance because the Olig1 null locus [1,6] contains an expressed Cre cassette under Olig1 transcriptional control and these Olig1(+/Cre) mice are being used to delete floxed genes specifically in OL lineage cells. For example, conditional deletion of Dicer1 (flox/flox) using Olig1(+/Cre) [6] caused severe impairment of myelination and death around P21 [8], whereas analogous experiments using Olig2(+/Cre) or Csp(+/Cre) resulted in only slightly delayed myelination with full recovery by P60 [9]. In another example, constitutively activating the Wnt signaling pathway by conditional deletion of exon 3 of β-catenin [10] using Olig1(+/Cre) completely prevented OL lineage specification, judging by the complete absence of OP markers such as Pdgfra [11], whereas similar experiments using Olig2(+/Cre) did not affect OP specification but only their subsequent differentiation into OLs [12]. While there might be a simple explanation for these differences, such as earlier or more complete recombination by Olig1(+/Cre) than by Olig2(+/Cre), the possibility remains that the Olig1 null allele generated by Xin et al. [6] might carry some additional, unidentified defect that can amplify the phenotype of other deleterious mutations.

To attempt to throw some light on these matters we undertook a study of two independent Olig1 null lines generated in our own laboratory. We found that loss of Olig1 causes a transient delay in OL development and myelination. We quantified Olig2 mRNA in our Olig1 mutant mice and found no increase relative to wild type controls. The mild phenotype we observe is therefore likely to be a genuine consequence of Olig1 loss, not moderated by cis regulatory effects on Olig2.

**Methods**

**Mice**

Mice were maintained on a 12 hour light–dark cycle. For timed mating, male and female mice were caged together overnight (from ~6 pm) and 12 noon the following day was designated embryonic day 0.5 (E0.5). All mouse work was approved by the Home Office of the UK Government, and conformed to the Animals (Scientific Procedures) Act 1986. New Olig1 null lines, Olig1(−/−) and Olig(−/−), Olig2(Tg) were generated as described previously [13] (also see Results).

**Embryonic Stem (ES) cell targeting**

We generated a new Olig1(−/−) line by ES cell targeting. Briefly, Olig1 targeting vector (see Results) was linearized and electroporated into R1 ES cells (129 background) [14]. After 10 days’ selection in 150 μg/ml G418 (Invitrogen), 200 colonies were picked and expanded in 96-well plates. Targeted ES clones were identified by Southern blotting using a 700 bp NcoI—EcoRI fragment as probe (Figure 1B). Positive ES clones were confirmed by Southern blotting using a 200 bp PstI—NcoI probe (Figure 1C). Five correctly targeted ES cell clones were expanded for karyotyping; two clones with normal karyotype were used for C57/B6 blastocyst injection to produce chimeric mice. Male chimeras were bred to C57/B6 females to produce Olig1 heterozygotes.

**Tissue preparation and histochemistry**

Embryonic and postnatal spinal cords or brains were immersion-fixed in 4% (w/v) paraformaldehyde in phosphate-buffered saline (PBS) overnight at 4°C. The tissue was cryo-protected overnight at 4°C in 20% (w/v) sucrose and stored at −80°C. Tissue was cryo-sectioned (nominal thickness 30 μm) in a Bright cryotome and sections collected on Superfrost Plus slides. Sections were treated with blocking solution [10% (v/v) fetal calf serum in 0.1% (v/v) Triton X-100 in PBS] at 20-25°C for one hour before immuno-labeling. Primary antibodies were anti-Sox10 (guinea pig, 1:4,000 dilution; a gift from M. Wegner, University of Erlangen) and anti-Olig1 (rabbit, 1:4,000 dilution; a gift from Charles Stiles, Dana Farber Cancer Institute, Harvard Medical School). Secondary antibodies were Alexa Fluor 488 conjugated goat anti-rabbit and Cy3-conjugated goat anti-guinea pig IgG (Chemicon, 1:500 dilution). Sections were counterstained with Hoechst 33258 dye (Sigma, 1000-fold dilution), for 10 minutes at 20-25°C after the secondary antibody and mounted under coverslips in fluorescence mounting medium (Dako).

Our fluorescence in situ hybridization procedure has been described before; detailed protocols are available at http://www.ucl.ac.uk/~ucbwdr/Richardson.htm. Briefly, digoxigenin (DIG)-labelled RNA probes were transcribed in vitro from cloned cDNAs of Mbp or Plp. After hybridization, the DIG signal was detected using horse-radish peroxidase (HRP)-conjugated anti-DIG (Roche) followed by developing in fluorescein-tyramide reagent (NENTM Life Science Products, Boston).
Quantitative PCR

Quantitative PCR (qPCR) was performed using forebrain and spinal cord tissue collected from Olig1 null mice and control littermates that carried either one or two endogenous copies of Olig1 at embryonic day 13.5 (E13.5) and/or E18.5. The tissue was homogenized in the presence of Trizol reagent (Invitrogen), and total RNA was purified and used for cDNA synthesis following the manufacturer’s instructions. Oligonucleotides 5′-att gta caa aac ggc cac aa 3′ and 5′-agt gct ctg cgt ctc gtc ta 3′ were used for Olig2 cDNA amplification. Oligonucleotides 5′-aca act ttg gca ttg tgg aa 3′ and 5′-agt gct cgt ctc gtc ta 3′ were used for Olig2 cDNA amplification. Oligonucleotides 5′- ’ac aca act ttg gca ttg tgg aa 3′ and 5′-gat gca ggg atg atg ttc tg 3′ were used to amplify Gapdh as an internal control. qPCR values were calculated using the relative standard curve method. At least three embryos of each genotype were analyzed at each age.

Mouse embryonic fibroblast (MEF) culture and Western blotting

Mouse embryos (E13.5-E15.5) were placed in PBS (without Mg or Ca) and the head, vertebral column, dorsal root ganglia, and inner organs were removed. The remaining tissue was digested in 0.25% (w/v) trypsin, finely minced with a razor blade and incubated at 37°C for 15 minutes to make a single-cell suspension. Cells were then plated in 35 mm dishes coated with 0.1% (w/v) gelatin and grown at 37°C in 5% (v/v) CO₂ in MEF medium (DMEM-Glutamax, 10% FBS, 1:100 MEM non-essential amino acids and 1:1000 2-mercaptoethanol, Invitrogen). A plasmid encoding Cre under the control of the PGK promoter (pPGKcreSV40) was used for transfection with Fugene 6 (Promega). Proteins from transfected MEFs and mouse spinal cord tissue were separated by SDS-PAGE and transferred to polyvinylidene difluoride membranes. Rabbit anti-Myc antibody was purchased from Abcam and used at a 1:10,000 dilution. Protein bands were visualized by chemi-luminescence (ECL reagent; GE Healthcare).

Results

Generation of new Olig1 null mouse lines

To try to resolve the discrepancy between the reported phenotypes of two different Olig1 null mouse lines [1,6] we generated two new Olig1 null strains, using different approaches. For one, we replaced the Olig1 open reading frame (ORF) with a DNA fragment including an inverted phosphoglycerate kinase promoter -neomycin resistance cassette (Pgk-Neo) flanked by loxP sites in mouse embryonic stem (ES) cells, line R1 [14] (Figure 1A). We refer to this line as Olig1(-/-). For purposes unrelated to the work described here, the modified locus also included a Myc-tagged Notch intracellular domain (NICD) coding sequence downstream of the floxed Pgk-Neo cassette; in the absence of Cre recombinase this NICD cassette is not expressed (Additional file 1: Figure S1) and is phenotypically neutral. Targeted clones were identified by Southern blot analysis of genomic DNA using a 700 bp NcoI–EcoRI fragment as a 5′ probe (Figure 1B).
Correct targeting was confirmed using a 200 bp PstI-NcoI fragment as a 3′ probe (Figure 1C). One karyotypically normal ES cell line was selected for blastocyst injection and germline transmission.

Our second Olig1 KO was generated by crossing Olig1/Olig2 double-null mice [3] with a phage artificial chromosome (PAC) transgenic line that contains a single copy of mouse Olig2 [13] (Figure 1E). We refer to this line as Olig (−/−), Olig2(Tg). We confirmed that this line does not express Olig1 protein (Additional file 2: Figure S2).

**Lack of compensatory up-regulation of Olig2 in Olig1-null mice**

The Olig1 and Olig2 genes are located about 40 kb apart on mouse chromosome 16 and there is a large degree of overlap in their expression patterns in vivo [15]. Xin et al. [6] suggested that the Pgk-Neo cassette introduced by Lu et al. [1] to disrupt the Olig1 ORF might have exerted a cis-activating effect on the neighbouring Olig2 locus, resulting in over-expression of Olig2 which compensated for loss of Olig1. Since our own Olig1(−/−) mice also contain a Pgk-Neo cassette at the Olig1 locus (but in the opposite orientation to the mice described in reference 1), we compared Olig2 mRNA levels in our Olig1(−/−) mice and Olig1(+/−) controls. We collected forebrain tissue at two embryonic stages (E13.5 and E18.5) and quantified Olig2 transcripts by PCR, using total cellular RNA as substrate. We could not detect a significant difference in the brain or spinal cord between Olig1(−/−) and Olig1(+/−), indicating that Olig2 was not mis-regulated by the Pgk-Neo cassette at the Olig1 locus in our mice (Figure 1D and Additional file 3: Figure S3).

Note that although two Pgk-Neo cassettes are present at the disrupted Olig1/Olig2 locus in our Olig(−/−), Olig2 (Tg) mice (Figure 1E) they are almost certainly physically remote from the randomly-integrated Olig2 PAC transgene and therefore are not expected to impose cis-regulation on Olig2.

**Oligodendrocyte precursors are specified normally in Olig1 null CNS**

We analyzed the expression of both platelet-derived growth factor receptor-alpha (Pdgfra), a marker of OPs, and Sox10, which marks all stages of the OL lineage, by immunofluorescence microscopy of E15.5 spinal cord and P2 forebrain sections. Neither Pdgfra nor Sox10 expression were noticeably altered in our two Olig1 null lines, relative to Olig1(+/−) controls (not shown). This is as expected, given that Olig1 protein does not appear until after OP specification [16], and is consistent with the phenotypes of the two previously-described Olig1 null lines [1,6].

**OL differentiation is delayed in Olig1 null spinal cord**

To investigate OL differentiation in our two new Olig1 null lines, we visualized mRNAs encoding mature OL markers myelin basic protein (MBP) and myelin proteolipid protein (PLP) by in situ hybridization. At E17.5, Mbp and Plp transcripts were absent from spinal cord in both Olig1 null lines, in contrast to littermate controls that carried one good copy of endogenous Olig1 (Figure 2). At E18.5, Mbp and Plp transcripts were present but in lower numbers of cells relative to Olig1 heterozygotes (Figure 3); by postnatal day 3 (P3), there were normal numbers of Mbp and Plp-positive cells in the Olig1 null spinal cord (Figure 4). These results indicate that Olig1 is involved in, but is not critically important for OL differentiation in the developing spinal cord, consistent with the original study by Lu et al. [1].

[Figure 2: No OL differentiation in E17.5 Olig1 null spinal cord. Fluorescence in situ hybridization for myelin basic protein (Mbp) (A,C,E,G) or proteolipid protein (Plp) (B,D,F,H) gene transcripts was performed on sections of E17.5 mouse spinal cords. No Mbp- or Plp-positive cells were detected in the circumferential white matter of Olig1(−/−) or Olig1(−/−),Olig2(Tg) cords (C,D and G,H respectively) compared to cognate Olig1(+/−) controls (A,B and E,F). Scale bar: 80 μm.]
OL differentiation in mouse forebrain does not begin until after birth [17]. On forebrain sections, few Mbp and/or Plp positive cells were detectable by fluorescence in situ hybridization at P4 (not shown). At P7, both Olig1 null lines appeared to have normal numbers of Mbp and Plp positive cells in both the corpus callosum and cortex compared to control mice (Additional file 4: Figure S4).

Discussion
We generated two new Olig1 null mouse lines by different routes - one by homologous recombination in ES cells followed by blastocyst injection, and the other by transgenic rescue of a previously generated Olig1/Olig2 double-null line [3] by pronuclear injection of an Olig2 PAC. Neither of the Olig1 null lines showed any evidence of prenatal lethality and both lines lived and reproduced normally. There was a transient delay in the production of differentiated OLs in the spinal cords of both our Olig1 null lines, as originally reported by Lu et al. [1] but in contrast to Xin et al. [6], who reported a severe myelination block that resulted in death around the third postnatal week. Xin et al. [6] put the discrepancy down to the fact that the original Olig1 null allele retained a Pgk-Neo cassette, speculating that the presence of this highly-transcribed element might have caused compensatory up-regulation of the neighbouring Olig2 gene. Xin et al. [6] removed the Pgk-Neo cassette (which was flanked by frt sites) by crossing the original Lu et al. [1] line with FLP-expressing mice. However, they did not quantify Olig2 expression in either of the Olig1 mutants.

A cis-acting regulatory effect of Pgk-Neo has been implied in previous studies. For example, the initially reported lethal phenotype of a germ line Sur1 deletion [18] was later attributed to the effect of Pgk-Neo on expression of unidentified genes near the Sur1 locus, after a second Sur1 knockout line lacking the Pgk-Neo cassette was found to be unusually long-lived [19]. Another example is the germ line knockout of the zinc finger transcription factor Zfp191, which was initially reported to be embryonic-lethal [20]. Subsequently, an independent line was found to survive after birth, developing a severe dysmyelinating phenotype and dying around P25 [21]. One potential explanation for the difference was that the embryonic-lethal allele contained an expressed Neo selection cassette. We tested the hypothesis that the mild phenotype of our Olig1(-/-) mice might have been due to compensatory up-regulation of the adjacent Olig2 gene by Pgk-Neo, but found no evidence for this. Our data are consistent with a previous study by Samanta et al. [22] who found no evidence for up-regulation of Olig2 when they used the Olig1(+/Cre) line of Lu et al. [1] (which also contains Pgk-Neo) for conditional expression.
deletion of bone morphogenetic protein receptor-1a (BMPR1a). Taken together, the data indicate that the presence or absence of Pgk-Neo cannot easily explain the dramatically different developmental phenotypes of different Olig1 null mice.

Different phenotypic outcomes for the same gene deletion can sometimes result from differences in the genetic backgrounds of the mice. For example, the effect of knocking out Nogo-A, a membrane protein of the adult myelin sheath and an inhibitor of neurite growth and axon regeneration, has a much larger effect on neurite regeneration ability in the 129X1/SvJ background than in the C57BL/6J (C57) background [23]. Our Olig1(−/−) line was generated using R1 ES cells (129 background; reference [14]). Heterozygous nulls were maintained in a 129/C57 mixed background for many (>10) generations with no sign of lethality. They are now maintained on a 129/C57/CBA background, also with no sign of lethality. The Olig1 null of Lu et al. [1] was made using J1 ES cells (129 background; reference [14]). Heterozygous nulls were maintained in a 129/C57 mixed background for many (>10) generations with no sign of lethality. They are now maintained on a 129/C57/CBA background, also with no sign of lethality. The Olig1 null of Lu et al. [1] was made using J1 ES cells (129 background; reference [14]). Heterozygous nulls were maintained in a 129/C57 mixed background for many (>10) generations with no sign of lethality. They are now maintained on a 129/C57/CBA background, also with no sign of lethality. The Olig1 null of Lu et al. [1] was made using J1 ES cells (129 background; reference [14]).

Another possible reason for the divergent phenotypes reported by Lu et al. [1] and Xin et al. [6] might lie in the way in which their mouse lines were generated. Xin et al. [6] made their line by crossing the mice made previously by Lu et al. [1] with a line that expresses FLP recombinase ubiquitously, in order to effect germ line excision of the frt-flanked Pgk-Neo cassette. Given that Olig1 and Olig2 lie close to each other on the chromosome (~40 kb apart) and share significant sequence homologies [24], it is conceivable that an unintended recombination event might have taken place, altering the Olig locus in some way that affects Olig2 expression or structure in addition to disrupting Olig1.

Arnett et al. [7] previously showed that the Olig1 null line of Lu et al. [1] inefficiently remyelinates demyelinated lesions produced either by focal injection of lysolecithin or by systemic administration of cuprizone, despite the nearly normal developmental time course of myelination of these mice [7]. This implied that Olig2 and Olig1 have complementary roles in myelin development and repair, respectively. We have no reason to question this conclusion and have not tested the remyelination abilities of our new Olig1 null mice.

**Figure 4 OL numbers recover by P3.** At P3, the numbers of Mbp- and Plp-expressing cells in Olig1 null spinal cords (C,D and G,H) were indistinguishable from controls (A,B and E,F respectively). Differentiating OLs were quantified based on Mbp and Plp expression (I). Three sections per mouse (n = 3) were counted and the data displayed as mean ± s.e.m. p values (p < 0.05) were calculated by Student's t-test. Scale bar: 100 μm.
OL differentiation is subject to two-tier transcriptional regulation: 1) epigenetic repression of transcriptional inhibitors and 2) direct transcriptional activation of myelin genes [25]. Transcription factors Olig2 [26], Sox10 [27], MRF [28] and Zfp191 [21] are critical for OL differentiation and/or myelination. Ascl1 and Nkx2.2 also play important roles; germ line knockout of either Nkx2.2 or Ascl1 leads to decreased expression of myelin genes in neonatal mice, suggesting that both genes can promote OL maturation [17,29]. In the present study, we have shown that Olig1 can synergize with Sox10 to activate Ascl1 and myelination. Ascl1 and Nkx2.2 also play a role in OL differentiation.

Conclusions
Using two newly-generated Olig1 null lines we show that loss of Olig1 causes a transient delay in OL development and myelination. Our data confirm the original description of a mild phenotypic effect of Olig1 loss [1], but run counter to the subsequent report of a complete myelination block [6]. We have shown that the mild phenotype is unlikely to result from compensatory up-regulation of Olig2, as suggested [6]. We conclude that Olig1 is non-essential for OL development.

Additional file

Additional file 1: Figure S1. No NICD expression in Olig1(+/-) or Olig1 (-/-) mice. Proteins from E18.5 spinal cord were subjected to SDS-PAGE, followed by Western blotting with rabbit anti-Myc antibody. ppGKc-re/v40-transfected MEFs derived from Olig1(+/-) embryos were used as positive control. The NICD band is indicated by an arrow.

Additional file 2: Figure S2. Our new Olig1 null mice do not express Olig1 protein. Co-immunolabeling for Olig1 (green) and Sox10 (red) was performed on sections of E18.5 mouse spinal cords. No Olig1-positive cells were detected in either Olig1(-/-) (B, B') or Olig1(+/-)-Olig2(Tg) spinal cords (D, D') in contrast to in Olig1(+/-) (A, A') or Olig1(+/-)-Olig2(Tg) controls (C, C'). Scale bar: 80 μm for A-D and 20 μm for A'-D'.

Additional file 3: Figure S3. No up-regulation of Olig2 expression in Olig1(-/-) spinal cord. Quantitative PCR using cDNA templates prepared from E18.5 spinal cord tissue revealed that there was no appreciable difference in the expression of Olig2 mRNA between Olig1(+/-) and Olig1(-/-) lines or between Olig1(+/-)-Olig2(Tg) and Olig1(-/-)-Olig2(Tg) lines.

Additional file 4: Figure S4. No change in OL numbers in Olig1 null forebrain at P7. In the developing forebrain, OL differentiation starts in the first postnatal week. At P7, coronal sections showed that the numbers of Mbp- and Plp-expressing cells in Olig1 null forebrain (BF and DH respectively) were similar to those in controls (AE and CG respectively). cc, corpus callosum; ctx, cortex. Scale bar: 80 μm.

Competing interests
The authors declare that they have no competing interests.
16. Fu H, Cai J, Clevers H, Fast E, Gray S, Greenberg R, et al: A genome-wide screen for spatially restricted expression patterns identifies transcription factors that regulate glial development. J Neurosci 2009, 29:11399–11408.

17. Qi Y, Cai J, Wu Y, Wu R, Lee J, Fu H, et al: Control of oligodendrocyte differentiation by the Nkx2.2 homeodomain transcription factor. Development 2001, 128:2723–2733.

18. Agostino A, Invernizzi F, Tiveron C, Fagiolori G, Prele A, Lamantea E, et al: Constitutive knockout of Surf1 is associated with high embryonic lethality, mitochondrial disease and cytochrome c oxidase deficiency in mice. Hum Mol Genet 2003, 12:399–413.

19. Dell’agnello C, Lee S, Agostino A, Szabadkai G, Tiveron C, Zulian A, et al: Increased longevity and refractoriness to Ca(2+)-dependent neurodegeneration in Surf1 knockout mice. Hum Mol Genet 2007, 16:431–444.

20. Li J, Chen X, Yang H, Wang S, Guo B, Yu L, et al: The zinc finger transcription factor 191 is required for early embryonic development and cell proliferation. Exp Cell Res 2006, 312:9990–9998.

21. Howry SH, Anila RL, Emery B, Tiveron M, Lin W, Watkins T, et al: ZFP191 is required by oligodendrocytes for CNS myelination. Genes Dev 2010, 24:301–311.

22. Samanta J, Burke GM, McGuire T, Pisarek AJ, Mukhopadhyay A, Mishina Y, et al: BMPR1a signaling determines numbers of oligodendrocytes and calbindin-expressing interneurons in the cortex. J Neurosci 2007, 27:7397–7407.

23. Dimou L, Schnell L, Montani L, Duncan C, Simonen M, Schneider R, et al: Nogo-A deficient mice reveal strain-dependent differences in axonal regeneration. J Neurosci 2006, 26:5591–5603.

24. Li H, Richardson WD: The evolution of Olig genes and their roles in myelination. Neuron Glia Biol 2008, 4:129–135.

25. Li H, He Y, Richardson WD, Casaccia P: Two-tier transcriptional control of oligodendrocyte differentiation. Curr Opin Neurobiol 2009, 19:479–485.

26. Yue T, Xian K, Hurlock E, Xin M, Kernie SG, Parada LF, et al: A critical role for dorsal progenitors in cortical myelination. J Neurosci 2006, 26:1275–1280.

27. Stolt CC, Rehberg S, Ader M, Lommes P, Riethmacher D, Schachner M, et al: Terminal differentiation of myelin-forming oligodendrocytes depends on the transcription factor Sox10. Genes Dev 2002, 16:165–170.

28. Sugimori M, Nagao M, Nishiki M, Nakatani H, Lebel M, Guillemot F, et al: Ascl1 is required for oligodendrocyte development in the spinal cord. Development 2008, 135:1271–1281.

29. Li H, Liu X, Smith HK, Richardson WD: Olig1 and Sox10 interact synergistically to drive myelin basic protein transcription in oligodendrocytes. J Neurosci 2007, 27:14375–14382.