Brief Communications

Antagonistic Regulation of PAF1C and p-TEFb Is Required for Oligodendrocyte Differentiation

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Oligodendrocytes are myelinating glial cells in the CNS and are essential for proper neuronal function. During development, oligodendrocyte progenitor cells (OPCs) are specified from the motor neuron precursor domain of the ventral spinal cord and differentiate into myelinating oligodendrocytes after migration to the white matter of the neural tube. Cell cycle control of OPCs influences the balance between immature OPCs and myelinating oligodendrocytes, but the precise mechanism regulating the differentiation of OPCs into myelinating oligodendrocytes is unclear. To understand the mechanisms underlying oligodendrocyte differentiation, an *N*-ethyl-*N*-nitrosourea-based mutagenesis screen was performed and a zebrafish *leo1* mutant, *dalmuri* (*dal*^{knu6}) was identified in the current study. Leo1 is a component of the evolutionarily conserved RNA polymerase II-associated factor 1 complex (PAF1C), which is a positive regulator of transcription elongation. The *dal*^{knu6} mutant embryos specified motor neurons and OPCs normally, and at the appropriate time, but OPCs subsequently failed to differentiate into myelinating oligodendrocytes and were eliminated by apoptosis. A loss-of-function study of *cdc73*, another member of PAF1C, showed the same phenotype in the CNS, indicating that PAF1C function is required for oligodendrocyte differentiation. Interestingly, inhibition of positive transcription elongation factor b (p-TEFb), rescued downregulated gene expression and impaired oligodendrocyte differentiation in the *dal*^{knu6} mutant and Cdc73-deficient embryos. Together, these results indicate that antagonistic regulation of gene expression by PAF1C and p-TEFb plays a crucial role in oligodendrocyte development in the CNS.

Introduction

Oligodendrocytes are glial cells which myelinate axons in the CNS. In the developing neural tube, oligodendrocyte progenitor cells (OPCs) are generated from the ventral motor neuron precursor (pMN) domain after motor neurons are produced. After birth, OPCs exist as proliferative progenitors and differentiate into myelinating oligodendrocytes after migration to the white matter of the neural tube (Park et al., 2002; Rowitch, 2004). However, the precise mechanisms that regulate the differentiation of OPCs into myelinating oligodendrocytes are unclear.

Eukaryotic transcription begins with the assembly of a preinitiation complex and recruitment of RNA polymerase II (Pol II) to the promoter, followed by initiation, elongation and termination of RNA synthesis. In many cases, recruitment of Pol II to the promoter is necessary and sufficient for the activation of gene transcription. However, recent genome-wide studies have revealed the existence of the promoter-proximal pausing of Pol II for the expression of numerous genes that respond to specific stimuli and developmental signals (Muse et al., 2007; Zeitlinger et al., 2007; Core et al., 2008). In the promoter-proximal pausing model, transcription elongation factor, DRB sensitivity-inducing factor (DSIF), collaborates with negative elongation factor (NELF) to inhibit Pol II-mediated elongation shortly after transcription initiation, and positive transcription elongation factor b (p-TEFb) is required to release paused Pol II (Price, 2008; Chiba et al., 2010). Pol II-associated factor 1 complex (PAF1C) is a positive regulator of transcription elongation and is commonly composed of five components: Paf1, Rtf1, Cdc73, Ctr9, and Leo1 (Hager et al., 2009; Jaehning, 2010). Interestingly, a recent study has shown that antagonistic regulation by p-TEFb and PAF1C is required for the relief of Pol II pausing in erythropoiesis (Bai et al., 2010).

In the current study, we describe the function of PAF1C in the regulation of oligodendrocyte development using the ze-

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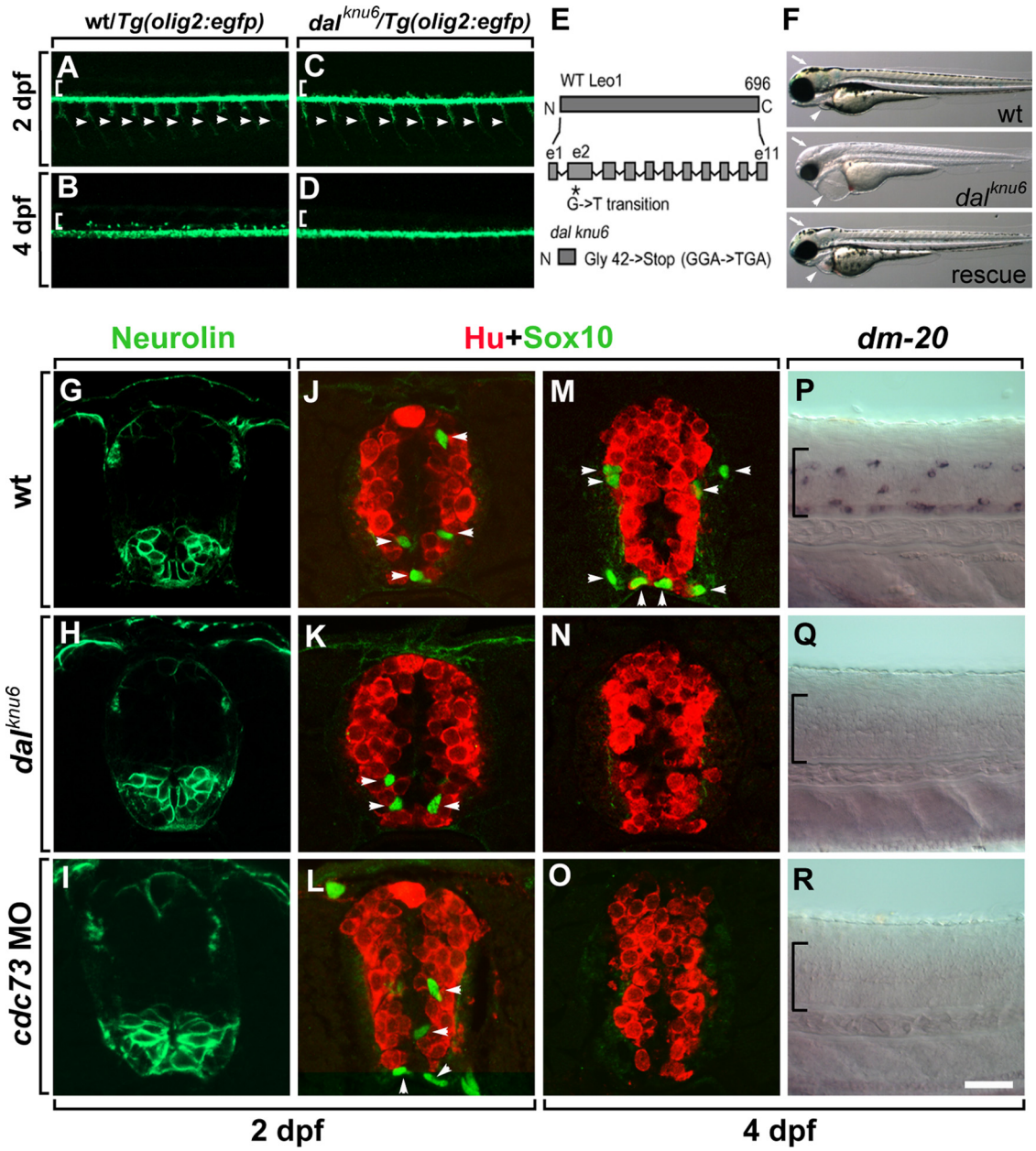

Figure 1. PAF1C is required for oligodendrocyte differentiation. ***A–D***, Lateral views of the spinal cord of wild-type (wt) and *dal*^{knu6} mutant *Tg(olig2:egfp)* embryos, anterior to the left. Arrowheads indicate motor axon bundles and bracketed areas indicate dorsal spinal cord. ***E***, Schematic representation of wt and mutant Leo1. ***F***, Phenotypes of wt and *dal*^{knu6} mutant embryos at 4 dpf. Arrows indicate pigment cells in the brain and arrowheads indicate heart. The *dal*^{knu6} mutation was rescued by injection of WT *leo1* mRNA. ***G–O***, Transverse sections of spinal cord of wt (***G***, ***J***, ***M***), *dal*^{knu6} mutant (***H***, ***K***, ***N***) and *cdc73* MO-injected embryos (***I***, ***L***, ***O***), orientated with dorsal to the top. Antibodies and stages are indicated on each panel. ***P–R***, Lateral views of the spinal cord of wt (***P***), *dal*^{knu6} mutant (***Q***) and *cdc73* MO-injected embryos (***R***) hybridized with a *dm-20* RNA probe, orientated with anterior to the left. Bracketed area indicates spinal cord. Scale bars: ***A–D***, 80 μm; ***G–R***, 20 μm.

brafish *dalmuri* (*dal*^{knu6}) mutant, which has a mutation in Leo1 (a component of the PAF1C), and a *cdc73* morphant. The *dal*^{knu6} mutant embryos and *cdc73* morphants specified OPCs normally, and at the appropriate time, in the CNS but OPCs subsequently failed to differentiate into myelinating oligodendrocytes and were eliminated by apoptosis, indicating that PAF1C function is required for oligodendrocyte differentiation. Interestingly, inhibition of p-TEFb function rescued downregulated gene expression and impaired oligodendrocyte differentiation in *dal*^{knu6} mutant embryos and *cdc73* morphants, indicating that functional antagonism between PAF1C and p-TEFb plays a crucial role in regulating oligodendrocyte development.

Materials and Methods

Fish lines. Wild-type AB, *Tg(olig2:egfp)* (Shin et al., 2003), *Tg(nkx2.2a:megfp;olig2:dsred)* (Kucenas et al., 2008) and *dalmuri*^{knu6} (*dal*^{knu6}) zebrafish mutant of either sex were used for this study.

Morpholino injection and rescue experiments. For knock-down of Leo1 and Cdc73, *leo1* exon1 splicing blocking morpholinos (MOs; *leo1* MO), *cdc73* exon 2 splicing blocking MOs (*cdc73* MO) were purchased from Gene-Tools: *leo1* MO, 5′-TATGAATGTACCTCGTTGCTCATTG-3′; *cdc73* MO, 5′-TTACTTACTGCAGCTCTCCGCACAT-3′. The specificity of *leo1* and *cdc73* MO were verified by Western blot analysis with anti-Leo1 antibody (ab33157, Abcam) and reverse transcription PCR (RT-PCR), respectively (data not shown). For rescue experiments, *leo1* mRNA (150 pg) were injected into one-cell embryos.

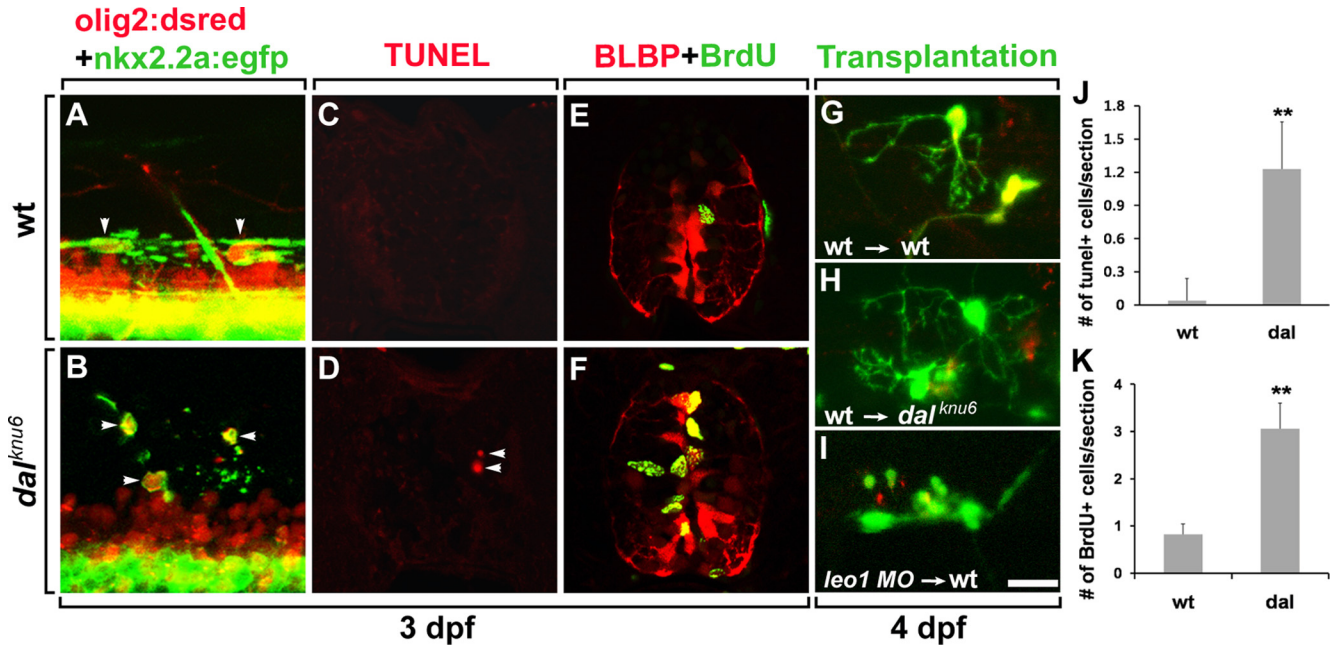

Figure 2. Cell autonomous functioning of Leo1 is required for the survival and differentiation of OPCs. ***A***, ***B***, Lateral views of wt (***A***) and *dal*^{knu6} mutant (***B***) *Tg(nkx2.2a:megfp::olig2:dsred)* spinal cord, orientated with anterior to the left. Arrowheads indicate *olig2*$^{+}$/nkx2.2a^{+} myelinating oligodendrocytes. ***C–F***, Transverse sections of spinal cord, orientated with dorsal to the top. TUNEL staining of wt (***C***) and *dal*^{knu6} mutant (***D***) embryos. Arrowheads indicate TUNEL^{+} cells. BrdU treatment and labeling of wt (***E***) and *dal*^{knu6} mutant (***F***) embryos with anti-BrdU and anti-BLBP antibodies. ***G–I***, Lateral views of the spinal cord of wt (***G***) and *dal*^{knu6} mutant (***H***) embryos transplanted with *Tg(olig2:egfp)* cells, and wt (***I***) embryos transplanted with *leo1* MO-injected *Tg(olig2:egfp)* cells. ***J***, ***K***, Quantification of TUNEL^{+} (***J***) and BrdU^{+} (***K***) cells (**$p < 0.001$). Scale bar: 20 μm.

BrdU labeling, immunohistochemistry and in situ *RNA hybridization.* Dechorionated embryos were labeled with BrdU (Roche) by incubating them for 20 min on ice in a solution of 10 mM BrdU and 15% DMSO in embryo medium (EM) at 3 dpf. The embryos were then placed in EM, incubated for 20 min at 28.5°C, and fixed using 4% paraformaldehyde in PBS. Embryos were processed for immunohistochemistry, treated for 1 h with 2 M HCl, and then processed for anti-BrdU immunohistochemistry. For immunohistochemistry, we used the following primary antibodies: a mouse anti-BrdU (G3G4, 1:1000, DSHB, IA), a rabbit anti-Sox10 (1:1000) (Park et al., 2005), a mouse anti-Neurolin (zn-8, 1:1000, DSHB), a rabbit anti-BLBP (1:100, Abcam), rabbit anti-MBP (1:100) (Lyons et al., 2005), and a mouse anti-HuC/D (16A11, 1:20, Invitrogen). Alexa 488-, 568-conjugated secondary antibodies were used for fluorescence detection (1:500, Invitrogen). *In situ* RNA hybridization was performed as previously described (Hauptmann and Gerster, 2000), and *dm20* RNA probe was used to detect oligodendrocytes (Park et al., 2002).

TUNEL assay. TUNEL assay was performed using In Situ Cell Death Detection Kit (Roche) according to the manufacturer's instructions. TUNEL was done on 10 mm-thick cryosections.

Chemical treatment. Embryos were dechorionated and incubated in EM containing 2.5 μM Flavopiridol (FVP, 10 mg/ml; sc-202157, Santa Cruz Biotechnology), 500 μg/ml 5, 6-Dichloro-1-β-D-ribofuranosylbenzimidazole (DRB, 25 mg/ ml; 287891, Calbiochem), 2.5 μM Purvalanol A (10 mM; P4484, Sigma) from 24 to 72 hpf or from 24 to 96 hpf.

Transplantation. We injected one- to two-cell-stage of *Tg(olig2:egfp)* donor embryos with rhodamine-dextran dye alone or together with *leo1* morpholino. Injected embryos were maintained in the dark at 28.5°C in embryo medium until sphere stage, and thirty to 40 cells were transplanted from donor embryos into wild-type and *dal*^{knu6} mutant embryos, respectively.

FACS and qRT-PCR. Approximately 300 2 dpf *Tg(sox10:egfp); dal*^{knu6} embryos (Dutton et al., 2008) were used to isolate GFP^{+} cells by FACS. Cell dissociation and FACS were performed as previously described (Takada and Appel, 2010) using a FACSAriaII (Becton Dickinson). Cells (5×10^5–1×10^6) were isolated and subsequently homogenized in TRIzol solution (Invitrogen) to purify total RNA. Real-time PCR (qRT-PCR) was performed using a LightCycler R (Roche) in a reaction mixture containing 1 μl of PCR-amplified total cDNA as a template, 0.2 M 5′ and 3′ PCR primer, 0.8 μl of MgCl2, and 1 μl of LightCycler R Fast Start DNA Master SYBR Green I (Roche). The following oligonucleotide primers were used for qRT-PCR: *cdkn1b* F (5′-TGATGATCGTCTTGTCGATGT-3′); R (5′-GCTCTTCATGATCCACCGG-3′); *cdkn1c* F (5′-CAAGAATCCGAGGGAGTCCC-3′); R (5′-GTTCATCCTGCTTCGACTCC-3′); *sox10* F (5′-GCACCACAATCGACACAAAC-3′); R (5′-ATCCGGAGTTCAGGAAGGAT-3′); *quaking* F (5′-ACATTAAAACCCCCGCAGT-3′);R(5′-GTCCTTCCGTACTCGTCCAA-3′); *id2b* F (5′-GCTCAGTCTACTGTACAACATGA-3′); R (5′-GCTCCCAGTGATCTGACAGT-3′); *id4* F (5′-GGTCAACTATCAAACATGCGATG-3′); R (5′-CTGGTCAACACACGTCACCT-3′); *sox5* F (5′-TGAGCCCCTACGCCCAGCACAA-3′); R (5′-TGGCTCGTTCTTGATGAGTTCC-3′); *sox6* F (5′-GCAGAATCATGTCTTCCAAGC-3′); R (5′-GATTGGCTGGAGCTCCTC-3′); *tcf4* F (5′-GGGACGGATAAGGAACTCAGC-3′); R (5′-GTGGCCCGGACTCCATT-3′); *β-actin* F (5′-TAGTCATTCCAGAAGCGTTTACC-3′); R (5′-TACAGAGACACCCTGGCTTACAT-3′).

Results

Zebrafish PAF1C is required for oligodendrocyte differentiation

To screen for mutants with defects in oligodendrocyte development, we used *Tg(olig2:egfp)* zebrafish, which express EGFP under the control of the *olig2* promoter in the pMN domain of the ventral spinal cord (Shin et al., 2003). As was shown in a previous study, EGFP fluorescence is detected in the motor axon bundles at 2 d postfertilization (dpf) (Fig. 1*A*, arrowheads) and in oligodendrocyte lineage cells at 4 dpf in the dorsal spinal cord (Fig. 1*B*, bracketed area) of *Tg(olig2:egfp)* embryos. In this study, *N*-ethyl-*N*-nitrosourea (ENU)-based mutagenesis was performed using *Tg(olig2:egfp)* zebrafish and a mutant, *dalmuri*^{knu6} (*dal*^{knu6}), was identified in which *olig2*-expressing pMN precursors and motor axon bundles were formed normally (Fig. 1*C*, arrowheads) by 2 dpf. However, the pMN precursors failed to generate oligodendrocytes migrated dorsally above the *olig2*-expressing pMN domain at 4 dpf (Fig. 1*D*, bracketed area), indicating that oligodendrocyte development was impaired in the *dal*^{knu6} mutant embryos.

Positional cloning of the mutated gene in the *dal*^{knu6} mutant revealed a G to T substitution at nucleotide position 124 in exon 2 of *leo1* on chromosome 18. This mutation resulted in a premature stop codon (TGA) at Gly-42 (GGA), generating a truncated protein of 41 aa (Fig. 1*E*). Leo1 is a member of PAF1C, which is known to be a positive regulator of transcription elongation (Jaehning, 2010). Recently, Nguyen et al. (2010) has identified a mutation in another allele of *leo1*(*leo1*^{LA1186}), which exhibited distinct recessive defects in pigment cells and in heart development (Nguyen et al., 2010). The *dal*^{knu6} mutant identified in the current study showed the same phenotype as *leo1*^{LA1186}, including defects in heart and pigment development (Fig. 1*F*). Knockdown of *leo1* with a targeted morpholino phenocopied the *dal*^{knu6} mutant (data not shown) while ectopic expression of normal *leo1* mRNA rescued the *dal*^{knu6} mutant phenotype (Fig. 1*F*).

Consistent with normal motor axon bundle formation (Fig. 1*C*), the *dal*^{knu6} mutant embryo generated the usual number of Neurolin^{+} motor neurons (Fig. 1*G*,*H*). Labeling of the spinal cord sections with anti-Sox10 and anti-Hu antibodies, which mark oligodendrocyte lineage cells and neurons, respectively, showed that the *dal*^{knu6} mutant embryo generated the normal number of OPCs and neurons in the gray matter of the spinal cord at 2 dpf, similar to the wild-type embryo (Fig. 1*J*,*K*, arrowheads indicate OPCs). However, compared with wild-type, in which OPCs migrate and differentiate into mature oligodendrocytes in the white matter of spinal cord at 4 dpf (Fig. 1*M*, arrowheads), OPCs had disappeared from the *dal*^{knu6} mutant embryo by this stage of development (Fig. 1*N*). Consistent with these phenotypes, whole-mount *in situ* RNA hybridization with *plp/dm20*, a mature oligodendrocyte marker, revealed that there were no *plp/dm20*$^{+}$ mature oligodendrocytes in the spinal cord of the *dal*^{knu6} mutant at 4 dpf (Fig. 1*P*,*Q*), indicating that *leo1* deficiency causes impaired oligodendrocyte differentiation. Knockdown of *cdc73*, another member of PAF1C, by injection of a *cdc73* morpholino, also caused impaired oligodendrocyte differentiation that was similar to that seen in the *dal*^{knu6} mutant embryo (Fig. 1*I*,*L*,*O*,*R*), suggesting that PAF1C function is required for the differentiation of OPCs into myelinating oligodendrocytes in the spinal cord of zebrafish embryos.

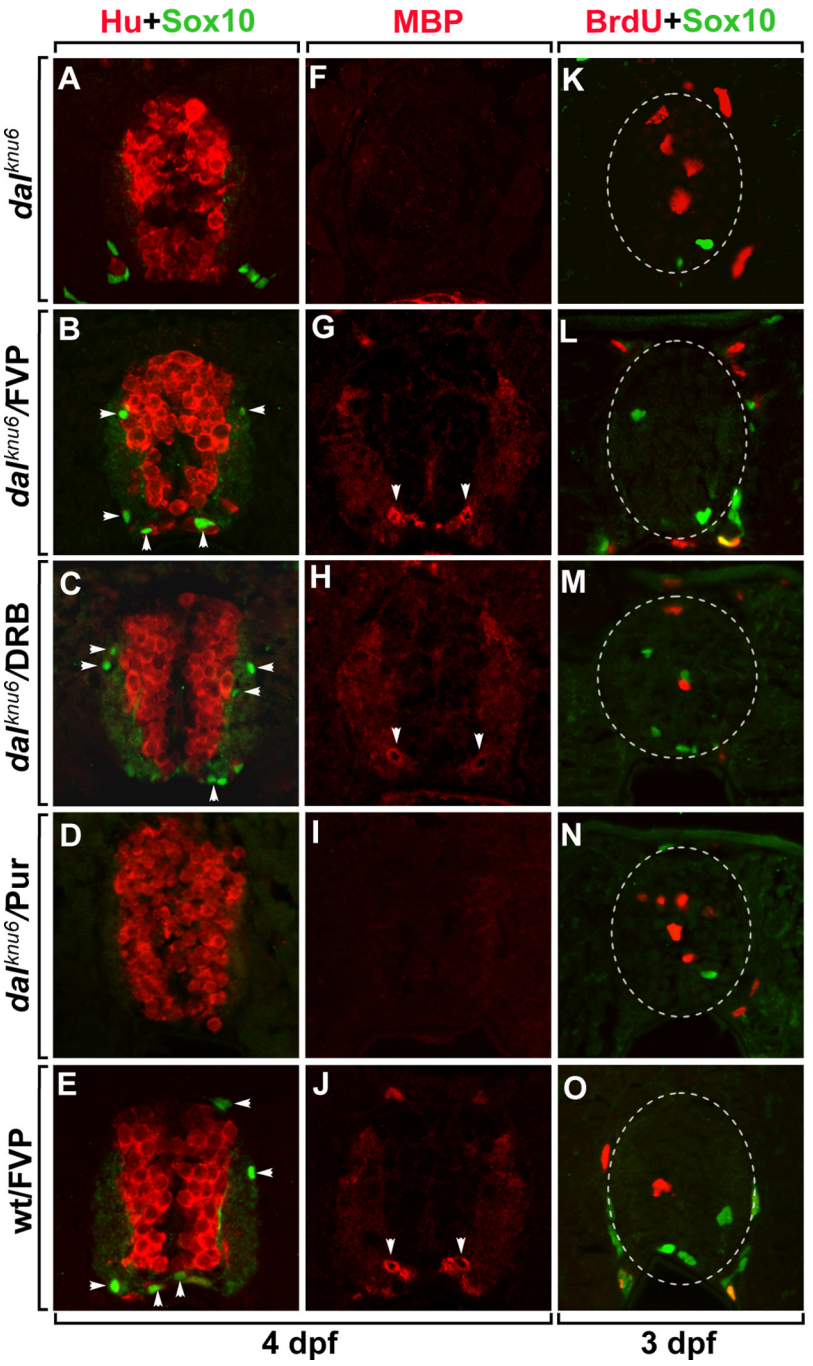

Figure 3. Inhibition of p-TEFb function rescues impaired oligodendrocyte differentiation in *dal*^{knu6} mutant embryos. Transverse sections of spinal cord from wild-type and *dal*^{knu6} mutant embryos treated with chemical inhibitors, oriented dorsal to the top. Chemical inhibitors are indicated on each panel. Embryos were incubated in chemicals from 24 to 96 hpf (***B–E***, ***G–J***) and from 24 to 72 hpf (***L–O***). Control embryos were incubated in EM (***A***, ***F***, ***K***). Antibodies and stages are indicated on each panel. Arrowheads indicate Sox10^{+} myelinating oligodendrocytes in the white matter (***B–E***), and myelin processes labeled with an anti-MBP antibody (***G–J***). Dashed circle outlines spinal cord (***K–O***). Scale bar: 20 μm.

Cell autonomous functioning of Leo1 is required for OPC differentiation and neural precursor cell cycle regulation

Since OPCs disappeared without differentiating into myelinating oligodendrocytes in *dal*^{knu6} mutant embryos, we investigated next whether OPCs were eliminated by apoptosis. To visualize mature oligodendrocytes *in vivo*, *nkx2.2a:megfp* and *olig2:dsred* transgenes were introduced into the *dal*^{knu6} mutant. These transgenes drive the membrane targeting of GFP (mGFP) and Dsred proteins in myelinating oligodendrocytes under control of the *nkx2.2a* and *olig2* promoters, respectively (Kucenas et al., 2008). At 3 dpf, the mGFP^{+}/Dsred^{+} myelinating oligodendrocytes showed numerous processes in the dorsal spinal cord of the wild-type *Tg(nkx2.2a:mgfp;olig2:dsred)* embryo (Fig. 2*A*, arrowheads),

while mGFP$^+$/Dsred$^+$ myelinating oligodendrocytes were rarely observed and showed abnormal morphology in the *Tg(nkx2.2a:mgfp::olig2:dsred);dal^{knu6}* mutant embryo (Fig. 2*B*, arrowheads), suggesting that they had been eliminated by cell death. Consistent with this result, *dal^{knu6}* mutant embryos had increased numbers of TUNEL$^+$ cells compared with the wild-type embryo, indicating that OPCs undergo apoptosis (Fig. 2*C*,*D*,*J*).

Interestingly, *dal^{knu6}* mutant embryos exhibited normal proliferation during neurogenesis at 24 hpf (data not shown), but showed a dramatic increase in the number of BLBP$^+$/BrdU$^+$ proliferating radial glial precursors after neurogenesis was complete at 2 dpf, indicating that the Leo1 deficiency causes defects in cell-cycle regulation in neural precursors (Fig. 2*E*,*F*,*K*). Since OPCs are still proliferating cells and would normally stop cycling before differentiating into myelinating oligodendrocytes, these data suggest that impaired OPC differentiation might be partly due to a failure in OPC cell cycle regulation.

To test whether Leo1 functions in a cell autonomous manner, rhodamine-dextran dye was injected into the one-cell stage of wild-type *Tg(olig2:EGFP)* embryos and cells were transplanted to nontransgenic wild-type and *dal^{knu6}* mutant embryos at the blastula stage. In the wild-type and *dal^{knu6}* mutant host embryos, transplanted *Tg(olig2:EGFP)* cells successfully differentiated into myelinating oligodendrocytes, which showed the typical morphology with multiple processes (Fig. 2*G*,*H*). However, transplanted *leo1*-deficient cells from the *Tg(olig2:EGFP)* embryos injected with *leo1* morpholino together with rhodamine-dextran dye failed to produce mature oligodendrocytes in the wild-type embryos, indicating that the *dal^{knu6}* mutant phenotype is caused by the cell autonomous function of Leo1 (Fig. 2*I*). Altogether, these data indicate that the cell-autonomous function of Leo1 is required for the regulation of neural precursor proliferation and oligodendrocyte differentiation in the spinal cord of zebrafish embryos.

PAF1C and p-TEFb regulate oligodendrocyte differentiation antagonistically

A previous study demonstrated that antagonistic regulation of PAF1C and p-TEFb is required for erythroid-specific gene expression by regulating Pol II pausing mechanism (Bai et al., 2010). Therefore, we hypothesized that a functional antagonism between PAF1C and p-TEFb may be required for the regulation of oligodendrocyte development. To test this idea, we used flavopiridol (FVP), a chemical inhibitor of CDK9, to block p-TEFb function (Chao and Price, 2001; Bai et al., 2010). Interestingly, FVP treatment before OPC specification at 24 hpf rescued most of the mutant phenotype in the spinal cord of *dal^{knu6}* mutant embryos. Sox10$^+$ myelinating oligodendrocytes showing numerous processes were observed in the white matter of the spinal cord in *dal^{knu6}* mutant embryos treated with FVP (Fig. 3*B*, arrowheads). Consistent with this, labeling with an anti-Myelin Basic Protein (MBP) antibody, which labels the myelin of mature oligodendrocytes (Lyons et al., 2005), revealed recovery of myelination in the spinal cord of *dal^{knu6}* mutant embryos (Fig. 3*G*, arrowheads). Normal proliferation of spinal precursors in the *dal^{knu6}* mutant embryos was also restored following FVP treatment (Fig. 3*L*). Similarly, treatment with 5, 6-Dichloro-1-β-D-ribofuranosylbenzimidazole (DRB), which blocks CTD kinase activity and transcriptional function of p-TEFb (Dubois et al., 1994), also rescued impaired oligodendrocyte differentiation (Fig. 3*C*,*H*) and precursor proliferation (Fig. 3*M*) in *dal^{knu6}* mutant embryos. However, *dal^{knu6}* mutant embryos treated with

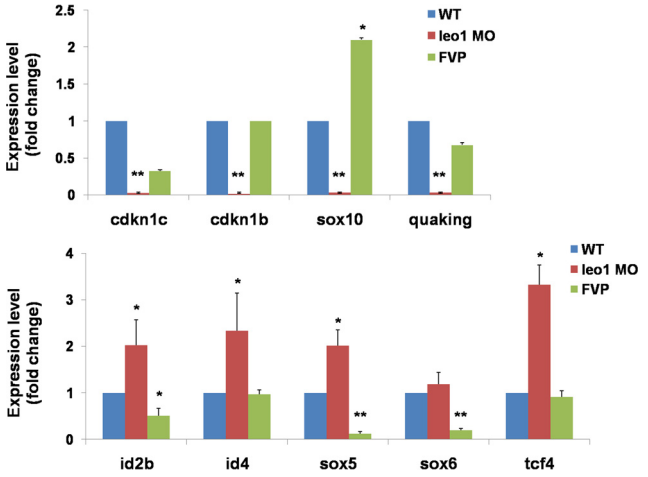

Figure 4. PAF1C and p-TEFb antagonistically regulate gene expression in OPCs. Real-time RT-PCR analyses to compare the expression of OPC genes in GFP$^+$ cells isolated from wild-type (wt), *leo1* morphants and FVP-treated *leo1* morphants. Data are shown as average fold change relative to the wild-type control and normalized to β-actin expression. The results presented as mean $\pm$ SD from three independent experiments (*$p < 0.05$; **$p < 0.001$).

Purvalanol A, which is a protein kinase inhibitor with high selectivity for cell cycle-associated CDKs, including CDK1, CDK2 and CDK4 (Villerbu et al., 2002), failed to rescue impaired oligodendrocyte differentiation and precursor proliferation (Fig. 3*D*,*I*,*N*). Interestingly, oligodendrocyte differentiation and precursor proliferation are not affected in FVP-treated wild-type embryos, indicating that loss of p-TEFb function alone does not affect oligodendrocyte differentiation (Fig. 3*E*–*O*). Next, we demonstrated that treating *cdc73* morpholino-injected embryos (morphants) with FVP also rescued impaired oligodendrocyte differentiation (data not shown). Together, these data indicate that inhibition of p-TEFb function rescues impaired oligodendrocyte differentiation in *dal^{knu6}* mutant embryos, and that PAF1C and p-TEFb antagonistically regulate oligodendrocyte differentiation in the CNS.

Finally, the regulation of gene expression by Leo1 and p-TEFb was examined in OPCs. A morpholino targeted to *leo1* was injected into sox10:EGFP transgenic embryos, in which oligodendrocyte lineage cells were labeled with EGFP (Dutton et al., 2008). EGFP$^+$ OPCs were isolated by fluorescent activated cell sorting (FACS) before undergoing apoptosis. Quantitative Reverse Transcription PCR (qRT-PCR) was then performed to compare the expression levels of the selected genes in wild-type and *leo1* morphants, and *leo1* morphants treated with FVP. We first analyzed expressions of *cdkn1b* (Casaccia-Bonnefil et al., 1997), *cdkn1c* (Park et al., 2005), *quaking* (Larocque and Richard, 2005) and *sox10* (Stolt et al., 2002), which are required for the differentiation of OPCs into myelinating oligodendrocytes. As shown in Figure 4, qRT-PCR analysis showed that expressions of selected genes were downregulated in *leo1*-deficient OPCs, and their expressions were rescued upon FVP treatment (Fig. 4). We next analyzed the expression of negative regulators of myelination, including *id2b, id4, sox5, sox6,* and *tcf4,* which are transcription factors required for the maintenance of OPCs in an undifferentiated state and to repress myelin gene expression (Emery, 2010). Interestingly, expression of these genes was significantly upregulated in the *leo1*-deficient OPCs and was rescued upon FVP treatment (Fig. 4); this suggests that a failure in the repression of negative regulators of myelination due to the loss of PAF1C causes downregulation of the genes required for oligodendrocyte

differentiation in *dal*^{knu6} mutant embryos. FVP treatment in *leo1*-deficient OPCs unexpectedly downregulated the expression of sox5 and sox6 to below the level observed in wild-type, and upregulated sox10 expression above wild-type levels. Given that Sox5 and Sox6 both regulate oligodendrocyte development by interfering with the function of Sox9 and Sox10, dramatic upregulation of sox10 appears to be related to downregulation of sox5 and sox6. Altogether, these data show that PAF1C plays a negative role, and p-TEFb a positive role, in the transcriptional regulation of negative regulators of myelination, and functional antagonism between PAF1C and p-TEFb is required for the regulation of oligodendrocyte differentiation.

Discussion

PAF1C is required for oligodendrocyte development

Despite numerous biochemical and genetic studies, the biological roles of PAF1C in multicellular organisms are largely unknown. In the present study, we report that the *leo1* mutation in *dal*^{knu6} mutant embryos causes impaired oligodendrocyte differentiation in the CNS in addition to other known defects. Cdc73 morphants also showed similar CNS defects to those in *dal*^{knu6} mutant embryos, indicating that impaired oligodendrocyte development is caused by a defect in PAF1C function. Interestingly, even though *leo1* is broadly expressed in the CNS, including neurons, neuronal differentiation was normal in *dal*^{knu6} mutants, suggesting a specific role for PAF1C in oligodendrocyte development. However, the mechanisms responsible for the differential roles of PAF1C in specific tissues are largely unknown. Previously, it was shown that PAF1C interacts with the Wnt and Hedgehog signaling pathways in mammalian cells (Mosimann et al., 2006, 2009). Therefore, the specific defect in oligodendrocyte development in the CNS of *dal*^{knu6} mutants could be caused by the loss of interaction with particular genes and signaling pathways important for oligodendrocyte development.

Antagonistic regulation of oligodendrocyte differentiation by PAF1C and p-TEFb

Cell fate specification during development depends upon the precise regulation of tissue-specific gene expression programs. After transcription initiation with tissue-specific transcription factors, promoter-proximal pausing of RNA Pol II is prevalent at developmentally regulated promoters, suggesting that "pausing" mechanisms might facilitate precise, synchronous expression of developmental genes (Muse et al., 2007; Zeitlinger et al., 2007; Core et al., 2008; Price, 2008). Previous genome-wide analysis of Pol II pausing in *Drosophila* embryos revealed two nonexclusive developmental functions of Pol II pausing; it serves as a mechanism for the active transcriptional repression of some genes, and as a mechanism for the rapid induction of other genes at later stages of embryogenesis (Zeitlinger et al., 2007). A recent study shows that the Pol II pausing mechanism mediated by functional antagonism between PAF1C and p-TEFb, which play negative and positive roles in transcriptional activation, respectively, is required for the erythroid gene expression (Bai et al., 2010). In this case, Pol II pausing seems to serve as a mechanism that allows rapid and synchronized activation of erythroid genes. Consistent with this, deficiency of the negative regulator for transcription, PAF1C, in the sunrise mutant did not affect erythroid gene expression because of the existence of the positive transcriptional activator, p-TEFb. However, p-TEFb deficiency in the moonshine mutant causes downregulation of erythroid gene expression, which is rescued by PAF1C deficiency (Bai et al., 2010). Similar to erythropoiesis, our current data demonstrate that antagonistic regulation of PAF1C and p-TEFb is required for the differentiation of OPCs into myelinating oligodendrocytes, suggesting the existence of a Pol II pausing mechanism mediated by PAF1C and p-TEFb. However, unlike erythropoiesis, deficiency in the positive regulator of transcription, p-TEFb, in FVP-treated wild-type embryos does not affect oligodendrocyte differentiation, presumably because the negative regulator of transcription, PAF1C, might successfully downregulate negative regulators of myelination (Fig. 3*E*,*J*). However, PAF1C deficiency in *dal*^{knu6} mutant embryos resulted in upregulation of negative regulators of myelination, and was rescued by p-TEFb deficiency upon FVP treatment (Fig. 4). These data suggest that the Pol II pausing mechanism is required for the repression of negative regulators of myelination to induce oligodendrocyte differentiation. Due to technical limitations, we did not find direct evidence of a role for the Pol II pausing mechanism in the regulation of oligodendrocyte differentiation, but our data show that antagonistic regulation of gene expression by the transcription elongation factors, PAF1C and p-TEFb, is complex, and occurs widely during development.

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