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Embryonic expression of GINS members in the development of the mammalian nervous system

Weizhen Jia, Han-Yun Hsieh, Hiroyasu Kidoya*, Nobuyuki Takakura**

Department of Signal Transduction, Research Institute for Microbial Disease, Osaka University, 3-1 Yamada-oka, Suita, Osaka, 565-0871, Japan

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ABSTRACT

The GINS (Go, Ichi, Nii, and San) complex contains four protein subunits (PSF1, PSF2, PSF3, and SLD5) and has been identified as a factor essential for the initiation and elongation stages of the DNA replication process. A previous study indicated that PSF2 participated in the developing central nervous system (CNS) of *Xenopus laevis*. However, the expression and function of GINS members in the mammalian developing nervous system remains unclear. Here, we examined the expression of GINS members in mice during nervous system development via immunofluorescence staining. At the beginning of neural development, PSF1 and SLD5 were highly expressed in neuroepithelial stem cells (NSCs) of the inner surface of neural tube (NT) and overlapped with proliferation marker Ki67. After entering the mid- and late-phase of neural development, PSF1 and SLD5 changed their regions of expression. These genes were highly expressed in dorsal root ganglion (DRG) progenitors, but they showed no overlap with Ki67 positive cells. Instead, a reduction of SLD5 expression promoted neuronal differentiation and maturation in the late-phase. PSF2 and PSF3 showed no tissue-specificity. PSF2 was constitutively and highly expressed whereas PSF3 was expressed at very low levels during neural development. In this study, we demonstrated variations in proteins and expression regions of the GINS members during mammalian CNS development and revealed a correlation between GINS expression and cell proliferation. Furthermore, we have suggested a novel function of GINS member SLD5, which regulates the differentiation of neural stem/progenitors.

1. Introduction

During embryo neural development in mammals, neurulation is a key step in the development of the central nervous system (CNS). To ensure the complete development of the neural system, the neural plate (NP) is generated and the neural tube (NT) is precisely closed, a process termed neurulation. After NT closure, the neuroepithelium becomes a critical region in which neuroepithelial stem cells (NSCs) are formed. Then, the CNS is completely established through NSC proliferation, migration, and differentiation (Saade et al., 2018; McKay, 1997; Copp et al., 2003; Nikolopoulou et al., 2017). This complicated mechanism is followed by a series of conserved genes, which are expressed specifically during embryo development. However, the regulation of gene expression in mammalian neural development is still unknown. Previous research had shown that PSF2, a member of the GINS (Go, Ichi, Nii, and San) complex, regulated the CNS development of *Xenopus laevis* (Walter et al., 2008). But in mammalian neural development, alterations in the expression and function of the GINS complex remain undisclosed. In this study, we investigated the GINS components expression region, density, and potential functions during development of the mammalian nervous system by immunohistochemical and gene expression analyses.

The GINS protein complex is highly conserved in eukaryotes, and participates in the initiation and elongation of DNA replication. It contains four protein subunits (PSF1, PSF2, PSF3, and SLD5) (Kubota et al., 2003; Kamada, 2012; Kanemaki et al., 2003). During replication initiation, after MCM2-7 helicase is loaded onto DNA, the GINS complex and CDC45 are recruited to the origin to form the CDC45-MCM2-7-GINS (C-M-G) complex. This active C-M-G helicase unwinds the origin DNA and starts the process of DNA replication (Onesti and MacNeill,

Abbreviations: GINS, Go Ichi Nii and San (5, 1, 2 and 3 respectively, in Japanese); CNS, central nervous system; NSCs, neuroepithelial stem cells; DRG, dorsal root ganglion; NP, neural plate; NT, neural tube; HSCs, hematopoietic stem cells

* Corresponding author. Department of Signal Transduction, Research Institute for Microbial Diseases, Osaka University, 3-1 Yamada-oka, Suita, Osaka, 565-0871, Japan.

** Corresponding author.

E-mail addresses: kidoya@biken.osaka-u.ac.jp (H. Kidoya), ntakaku@biken.osaka-u.ac.jp (N. Takakura).

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0197-0186/

Table 1
Mouse primer list.

Gene name	Sequence
PSF1	F: 5′-CCGGTTGCTTCGGATTAGAG-3′ R: 5′-CTCCCAGCGACCTCATGTAA-3′ (Nagahama et al., 2010)
PSF2*	F: 5′-GGCCGCCTACTCTTCAACAT-3′ R: 5′-CCAGTTTCTCCACATCCATCCA-3′
PSF3*	F: 5′-GGCTCACAACTCCTGCATTT-3′ R: 5′-GCGGAACCGTCCAATAAAAG-3′
SLD5*	F: 5′-TTGAGGGCAGTTCCCAAACC-3′ R: 5′-GCCCACCTCCAAGTCAATCA-3′
Cdt1*	F: 5′-CTGGGACCTCTACTCCACCA-3′ R: 5′-AGCTGCTTCTGGACCTCCTT-3′
Cdc6*	F: 5′-CAGCAAAGGGCAGGATGTAT-3′ R: 5′-AGCTTCAAGTCTCGGCAGAA-3′
Mcm2*	F: 5′-CCCAAGAACCCAGGTGGAAA-3′ R: 5′-GATGGCACGGCTAGACACTT-3′
Mcm3*	F: 5′-CTCCTCTGGAGTGGGTCTCA-3′ R: 5′-CAGGACCATAGCACCAGCTT-3′
Mcm4*	F: 5′-TGGGCAGACACCTCACACTA-3′ R: 5′-TTCACGTTGCTCACTCTTGG-3′
Orc1*	F: 5′-CGTCGGTCAGGACTAGAGGA-3′ R: 5′-ACATGGTTGGGTATGGCAGT-3′
Orc2*	F: 5′-CTCGAGAGTTACAGGTCACTGGATG-3′ R: 5′-TTCTCCAACTCACAGTCTGCC-3′
Cdc45*	F: 5′-CCGGCAACAAGGAACCAATC-3′ R: 5′-CAAGCACACAAGGCATCCAC-3′
Rad51*	F: 5′-GCCGGTTACCATACAGTGGA-3′ R: 5′-GTGGTGAAACCCATTGGAAC-3′
Bcl2*	F: 5′-TCGCAGAGATGTCCAGTCAG-3′ R: 5′-CCTGAAGAGTTCCTCCACCA-3′
Bcl2l1*	F: 5′-TGACCACCTAGAGCCTTGGA -3′ R: 5′-AGAACCACACCAGCCACAGT-3′
Casp3*	F: 5′-ATGGGAGCAAGTCAGTGGAC-3′ R: 5′-CGTACCAGAGCGAGATGACA-3′
Bad*	F: 5′-GAAGGATGAGCGATGAGTT-3′ R: 5′-GCTTTGTCGCATCTGTGTTG-3′
Bak1*	F: 5′-AGAACAGCTTCAGCCCACAG-3′ R: 5′-GGTAGACGTACAGGGCCAGA-3′
Bax*	F: 5′-GAAGCTGAGCGAGTGTCTCC-3′ R: 5′-GAAGTTGCCATCAGCAAACAT-3′
Casp1*	F: 5′-GGCACATTTCCAGGACTGAC-3′ R: 5′-GAGGGCAAGACGTGTACGAG-3′
Casp8*	F: 5′-CCTAGACTGCAACCGAGAGG-3′ R: 5′-TCGCTCACTTCTTCTGAGAGC-3′
p53	F: 5′-AAAGGATGCCCATGCTACAG-3′ R: 5′-TATGGCGGGAAGTAGACTGG-3′ (Yamakawa et al., 2018)
p15	F: 5′-AGATCCCAACGCCCTGAAC-3′ R: 5′-CCCATCATCATGACCTGGATT-3′ (Yamakawa et al., 2018)
p16	F: 5′-CGTACCCCGATTCAGGTGAT-3′ R: 5′-TTGAGCAGAAGAGCTGCTACGT-3′ (Yamakawa et al., 2018)
p18	F: 5′-TTTCAAAGACCGATGCGTATCC-3′ R: 5′-CTATGTCAGTATCAGCCAGCAAA-3′ (Yamakawa et al., 2018)
p19	F: 5′-GCCGCACCGGAATCCT-3′ R: 5′-TTGAGCAGAAGAGCTGCTACGT-3′ (Yamakawa et al., 2018)
p21	F: 5′-TAGGGGAATTGGAGTCAGGC-3′ R: 5′-AGAGTGCAAGACAGCGACAA-3′ (Yamakawa et al., 2018)
p27	F: 5′-AGATACGAGTGGCAGGAGGT-3′ R: 5′-TCTTAATTCGGAGCTGTTTACGTC-3′ (Yamakawa et al., 2018)
CDK1*	F: 5′-CTCCACTCCGGTTGACATCT-3′ R: 5′-GGCCACACTTCGTTGTTAGG-3′
Cyclin B1*	F: 5′-ATGGTGCATTTTGCTCCTTC-3′ R: 5′-TAGCCAGGTGCTGCATAACA-3′
Cyclin D1*	F: 5′-AGTGCGTGCAGAAGGAGATT-3′ R: 5′-AGGAAGCGGTCCAGGTAGTT-3′
Cyclin E1*	F: 5′-GGTCTGAGTTCCAAGCCCAA-3′ R: 5′-GTCTTGCAAAAACACGGCCA-3′
c-Myc*	F: 5′- CCTGTACCTCGTCCGATTCC-3′ R: 5′-GGTGTCTCCTCATGCAGCAC-3′
Pax6*	F: 5′-CTTGGGAAATCCGAGACAGA-3′ R: 5′-CTAGCCAGGTTGCGAAGAAC-3′
Sox2*	F: 5′-CAGCTCGCAGACCTACATGA-3′ R: 5′-CCTCGGACTTGACCACAGAG-3′
Hes1*	F: 5′-CTACCCCAGCCAGTGTCAAC-3′ R: 5′-TATTCTTGCCCTTCGCCTCT-3′
Nes*	F: 5′-GGAAGAAGTTCCCAGGCTTC-3′ R: 5′-TGAGGACAGGGAGCACAGAT-3′
GAPDH	F: 5′-TGGCAAAGTGGAGATTGTTGCC-3′ R: 5′-AAGATGGTGATGGGCTTCCCG-3′ (Jia et al., 2013)

*This work, F: forward; R: reverse.

2013; Abid et al., 2016; Yuan et al., 2016). Recent studies have indicated that GINS components participate in several physiological functions, for example, eukaryotic DNA replication and immature cell development. Other studies have indicated that PSF1 and SLD5 are highly expressed in the bone marrow and gonads, which contain abundant progenitor and stem cells (Ueno et al., 2005; Mohri et al., 2013; Han et al., 2009). The GINS complex is highly conserved in mammals, and has a critical role in embryogenesis. Previously, our study reported that PSF1 was highly expressed in the inner cell mass during embryogenesis, and that the loss of PSF1 caused embryonic lethality. In a transplantation study, heterozygous PSF1$^{+/-}$ mice showed the delayed proliferation of hematopoietic stem cells (HSCs) during bone marrow reconstitution (Ueno et al., 2008). SLD5 is also essential for early embryogenesis, and the deletion of SLD5 in mice not only disrupted cell proliferation but also caused embryonic lethality (Mohri et al., 2013). PSF1 and SLD5 have similarities in the early mouse embryo.

Our results demonstrated that the PSF1 and SLD5 expression were correlated with NSC proliferation at the beginning of neural development, and after entering the mid- and late-phase, the reduction of SLD5 expression correlated with differentiation of dorsal root ganglion (DRG) progenitors. Based on current findings, suggested that importance of GINS family proteins in the development of the mouse CNS.

2. Material and methods

2.1. Animals

C57BL/6 pregnant mice (E9.5, E10.5, E11.5, E13.5, E15.5, E17.5) were purchased from Japan SLC (Shizuoka, Japan). Animals were housed in environmentally controlled rooms of the animal experimentation facility at Osaka University. All experiments were performed in accordance with the guidelines of Osaka University Committee for Animal and Recombinant DNA Experiments. Mice were handled and maintained according to Osaka University guidelines for animal experimentation.

2.2. Immunohistochemistry

Immunostaining analysis was performed on 10 μm O.C.T. frozen (PSF2 and PSF3) and paraffin (PSF1 and SLD5) sections of embryos. Immunohistochemistry of frozen section and paraffin sections, and procedures for tissue fixation and staining of sections with antibodies were previously described (Kidoya et al., 2015; Matsui et al., 2013). The primary antibodies used were as follows: rat anti-PSF1 [GeneStem, Osaka, Japan (Ueno et al., 2008; Matsui et al., 2013)], rabbit anti-PSF2 [Proteintech, Rosemont, IL (Xu et al., 2016)], rabbit anti-PSF3 [Abcam, Cambridge, UK (Yamane et al., 2016)], rat anti-SLD5 [GeneStem (Mohri et al., 2013)], rabbit anti-SLD5 [Gene Tex, Irvine, CA (Li et al., 2018)], rabbit anti-Sox2 (Abcam), rabbit anti-β3-Tubulin/Tuj1 (Cell Signaling Technology, Danvers, MA), rabbit anti-Ki67 (Cell Signaling Technology), and rat anti-Ki67 (eBioscience, San Diego, CA). The activity of all primary antibodies is guaranteed by the individual manufacturer's, and the specificity of antibodies for GINS were previously validated as cited above. The following secondary antibodies were used: anti-rat IgG Alexa Fluor 488 (Invitrogen, Carlsbad, CA), anti-

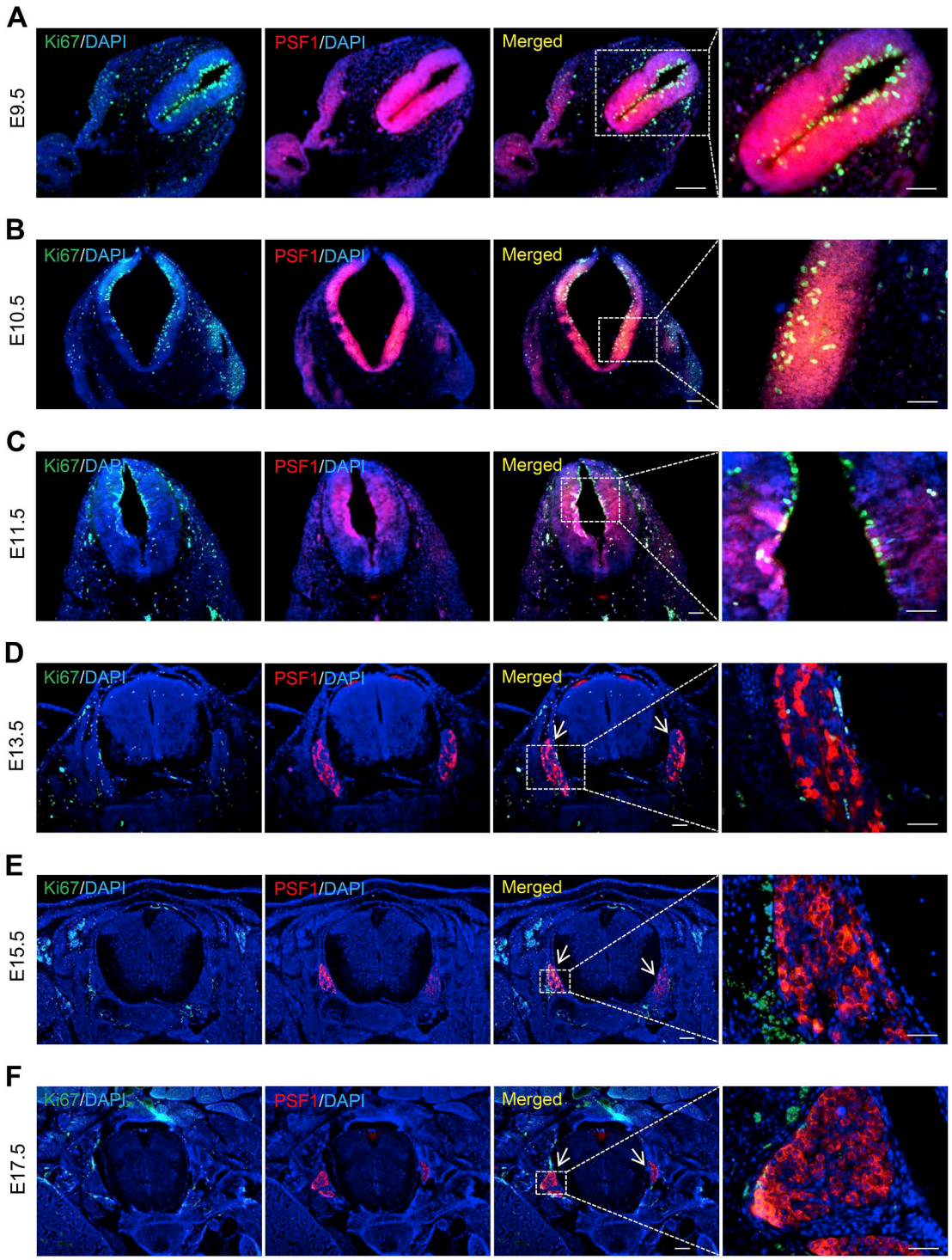

Fig. 1. Expression of PSF1 during development of the nervous system. Immunofluorescence of Ki67 (green) and PSF1 (red) in tissue sections from E9.5 (A), E10.5 (B), E11.5 (C), E13.5 (D), E15.5 (E) and E17.5 (F) embryos. DAPI (blue) was used to detect nuclei and the dashed box indicates areas shown at higher magnification. Arrows indicate the focal DRG area. Data are representative of three independent experiments. Scale bar, 100 μm (E9.5, E10.5, E11.5, E13.5), 200 μm (E15.5, E17.5) and 50 μm (inset).

rabbit IgG Alexa Fluor 488 (Invitrogen), anti-rabbit IgG Alexa Fluor 546 (Invitrogen), and anti-rat IgG secondary biotin (Invitrogen). We used streptavidin Cy-3 (Invitrogen) with biotin as a tertiary antibody. Cell nuclei were visualized with DAPI (Invitrogen). Sections were observed by conventional microscopy (brightfield) (DM5500 B; Leica, Wetzlar, Germany) or confocal microscopy (TCS/SP5; Leica), and images were acquired with a digital camera (DFC500; Leica). In all analyses, an isotype-matched control Ig was used as a negative control to confirm that the positive signals were not derived from nonspecific background staining. Images were processed using Photoshop CS6 software (Adobe Systems, San Jose, CA).

2.3. Quantitative real-time PCR

Total RNA was extracted from tissues using RNeasy-plus mini kits (Qiagen, Hilden, Germany) and was reverse-transcribed using the

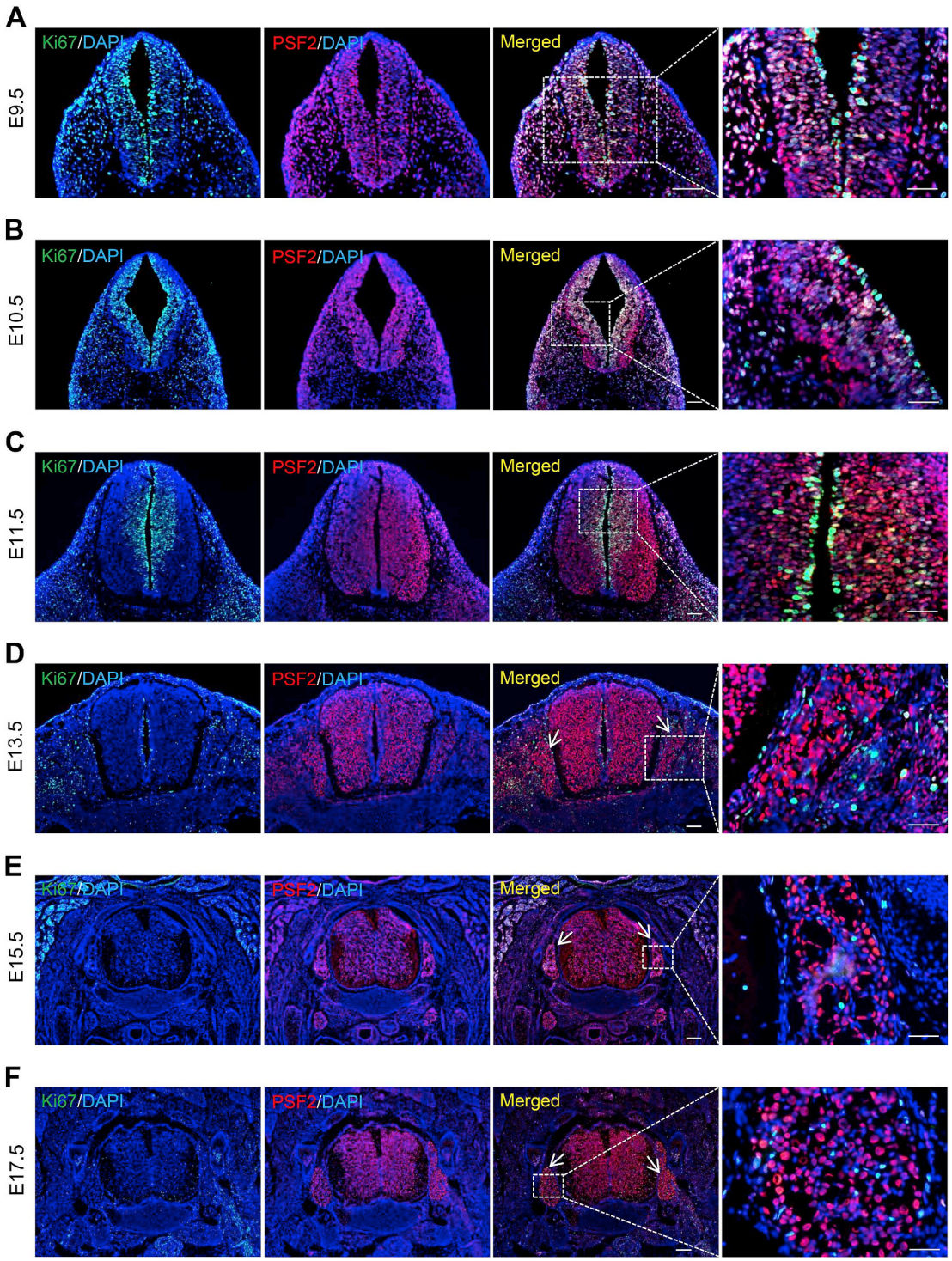

Fig. 2. Expression of PSF2 during development of the nervous system. Immunofluorescence of Ki67 (green) and PSF2 (red) in tissue sections from E9.5 (A), E10.5 (B), E11.5 (C), E13.5 (D), E15.5 (E) and E17.5 (F) embryos. DAPI (blue) was used to detect nuclei and the dashed box indicates areas shown at higher magnification. Arrows indicate the focal DRG area. Data are representative of three independent experiments. Scale bar, 100 μm (E9.5, E10.5, E11.5, E13.5), 200 μm (E15.5, E17.5) and 50 μm (inset).

PrimeScript RT reagent kit (Takara, Kyoto, Japan) according to the manufacturer's protocol. Real-time PCR analysis was performed using Platinum SYBR Green qPCR SuperMix-UDG (Invitrogen) and a Mx3000p QPCR System (Stratagene, La Jolla, CA). The baseline and threshold were adjusted according to the manufacturer's instructions. The level of target gene expression was normalized to that of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in each sample. We used the primer sets described in Table 1.

2.4. Isolation of DRG neurons

Isolation of mouse DRG neurons was performed as previously described (Zhao et al., 2009) with slight modifications. Briefly, pregnant mice at 13.5 or 17.5 days post-coitus were anesthetized prior to cervical dislocation. The DRG were isolated from embryos, washed once in PBS, and minced with scissors. The cells were placed in RNA lysis buffer (buffer RLT from a RNeasy-plus mini kit).

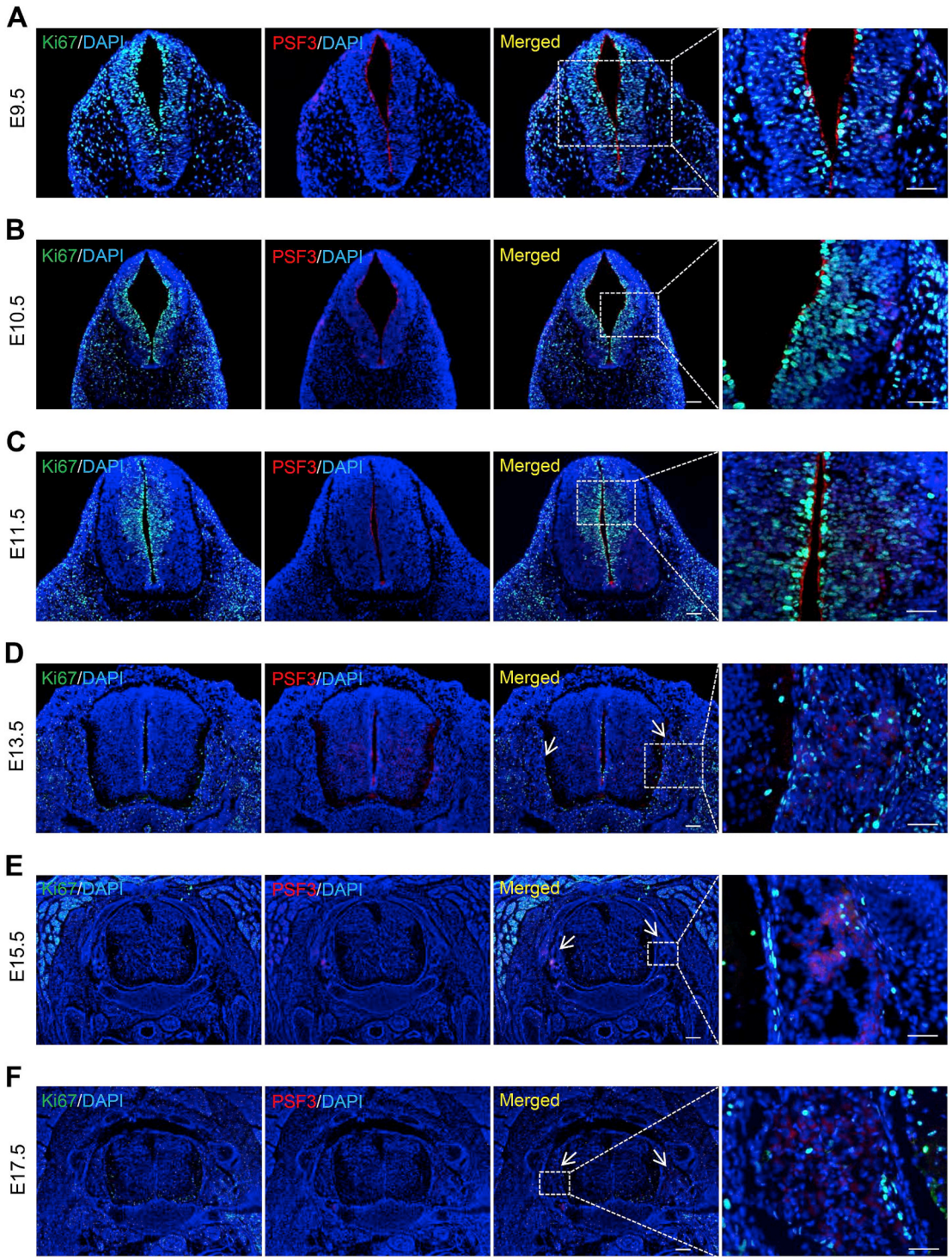

Fig. 3. Expression of PSF3 during development of the nervous system. Immunofluorescence of Ki67 (green) and PSF3 (red) in tissue sections from E9.5 (A), E10.5 (B), E11.5 (C), E13.5 (D), E15.5 (E) and E17.5 (F) embryos. DAPI (blue) was used to detect nuclei and the dashed box indicates areas shown at higher magnification. Arrows indicate the focal DRG area. Data are representative of three independent experiments. Scale bar, 100 μm (E9.5, E10.5, E11.5, E13.5), 200 μm (E15.5, E17.5) and 50 μm (inset).

2.5. Statistical analysis

All data are presented as the means ± standard deviation (SD). Statistical analysis was performed using the Statcel version 2 software package (OMS, Saitama, Japan). Data were analyzed by ANOVA, followed by Tukey-Kramer multiple comparison tests. When only two groups were compared, a two-sided Student's *t*-test was used. A p value less than 0.05 was considered statistically significant.

3. Results

3.1. Expression of PSF1 during development of the mouse nervous system

We analyzed PSF1 expression levels by immunofluorescence during neural development. During initial development (E9.5, E10.5), PSF1 was highly expressed in Sox2 positive (88.4% ± 10.7%) and Tuj1 negative NSCs, and was presented in the inner cell mass of the NT

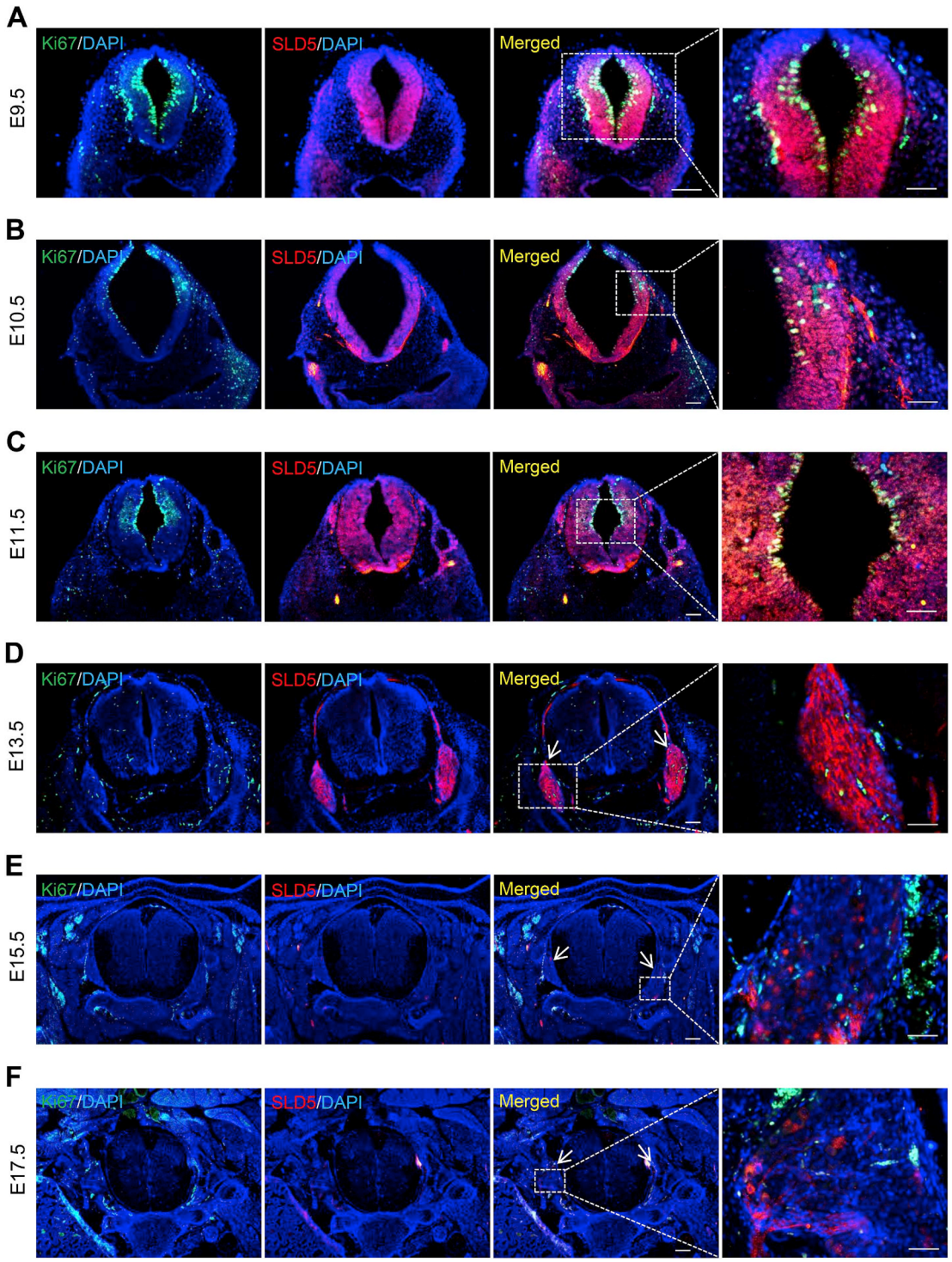

Fig. 4. Expression of SLD5 during development of the nervous system. Immunofluorescence of Ki67 (green) and SLD5 (red) in tissue sections from E9.5 (A), E10.5 (B), E11.5 (C), E13.5 (D), E15.5 (E) and E17.5 (F) embryos. DAPI (blue) was used to detect nuclei and the dashed box indicates areas shown at higher magnification. Arrows indicate the focal DRG area. Data are representative of three independent experiments. Scale bar, 100 μm (E9.5, E10.5, E11.5, E13.5), 200 μm (E15.5, E17.5) and 50 μm (inset).

(Supplemental Fig. 1 A, B and E). Furthermore, PSF1 expressing NSCs significantly overlapped with cell proliferation marker Ki67 (Fig. 1 A and B). These data suggested that PSF1 participated in DNA replication and the proliferation of NT NSCs in the early stage of neural development. This phenomenon was also found in different types of tissue-specific stem cells such as HSCs (Ueno et al., 2008).

Following the end of NSC proliferation in the middle stage of development (E11.5), the expression level of PSF1 gradually decreased (Fig. 1 C). Moreover, we could not detect PSF1 expression in the inner cell mass of the NT at E13.5 (Fig. 1 D). In the mid- and late-phase of the embryonic stage (E13.5–17.5), PSF1 was expressed in different regions. Constitutively and high expression levels of PSF1 were present in the DRG (Fig. 1D and E, and F) and trigeminal ganglion (data not shown). Furthermore, PSF1 expression was detected in the DRG of adult mice (data not shown). We investigated whether highly-expressed PSF1 participated in the regulation of ganglion cell proliferation. Despite the

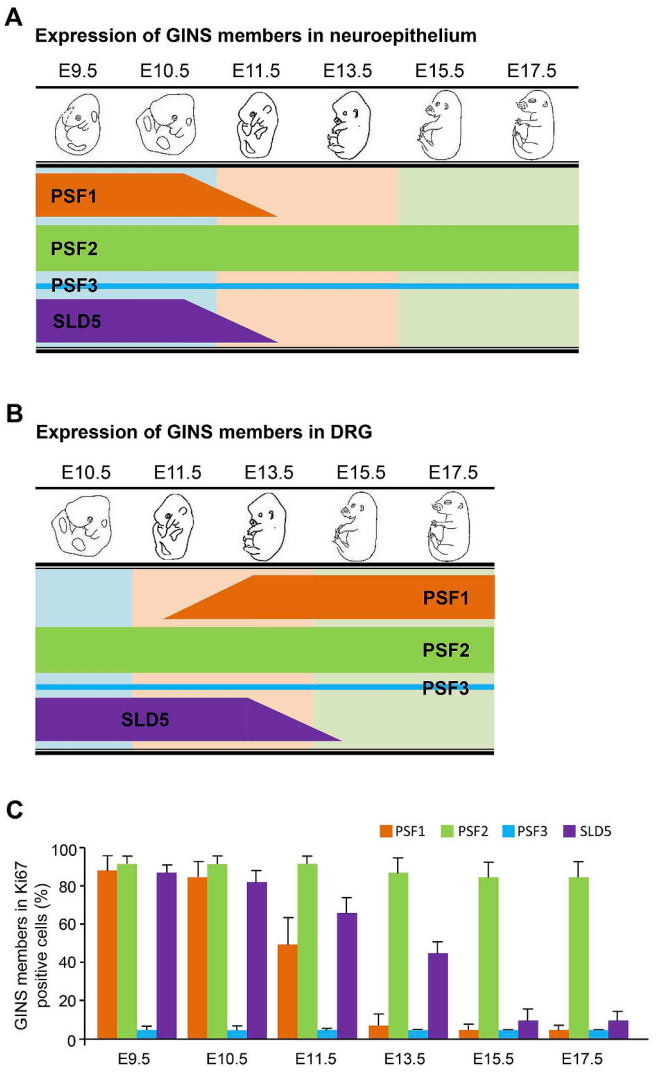

Fig. 5. Summary of GINS expression profiles and correlation analysis with cell proliferation during development of the nervous system. (A and B) Schematic drawing showing the expression of GINS members during the development of neuroepithelium (A) and DRG (B). The thickness of the lines represents the relative amount of protein expression. (C) The percentage of GINS member expressing cells in Ki67 positive cells. Data are representative of three independent experiments, n = 3. Error bars represent the means ± SD.

high expression of PSF1 in ganglion cells, it did not regulate DNA replication (Fig. 1 D, E, and F). Furthermore, PSF1 positive staining of ganglion cells was not associated with the Ki67 positive staining. These results indicated that PSF1 protein did not regulate DNA replication in ganglion cells although it might be involved in the regulation of other cellular functions.

3.2. Expression of PSF2 during development of the mouse nervous system

Next, we investigated PSF2 expression levels during neural development. During embryo development, PSF2 was highly expressed in different tissues, suggesting it had no tissue-specific expression in the CNS (Fig. 2 A-F and Supplemental Figure 3 B). High PSF2 expression was observed in multiple tissues during embryogenesis. Moreover, similar to the expression pattern of PSF1 during initial development (E9.5, E10.5), PSF2 positive NSCs also significantly overlapped with Ki67 positive staining (Fig. 2A and B). This observation suggested that PSF2 might participate in DNA replication and the proliferation of NSCs. However, in the mid- and late-phase of the embryonic stage (E13.5–17.5), high levels of PSF2 were still expressed in the DRG, but were not only limited to Ki67 positive populations (Fig. 2D and E, and F).

Taken together, PSF2 expression is not tissue-specific during neural development. Although its expression overlapped with Ki67 positive populations in the early developmental stage, the importance of PSF2 during cell proliferation requires further study.

3.3. Expression of PSF3 during development of the mouse nervous system

Next, we measured the PSF3 expression level during neural development. Very low levels of PSF3 were presented in a wide range of CNS tissues (Fig. 3 A-F and Supplemental Figure 3 C), and it had no tissue-specific properties. Similar to PSF2 expression, we did not detect overlapping staining between PSF3 expression and Ki67 positive cells (Fig. 3 A-F and Supplemental Figure 3 C). These data suggested that PSF3 has a minor effect during neural proliferation and development.

3.4. Expression of SLD5 during development of the mouse nervous system

Finally, we investigated SLD5 expression level in the neural development. The data indicated that SLD5 expression is essentially the same as PSF1 in the initiation of neural progress (E9.5, E10.5) and both were expressed in the same cells (Fig. 4 A and B, Supplemental Fig. 2). SLD5 expressed high protein levels in Sox2 positive (82.3% ± 11.5%) NSCs, the positive intensity of SLD5 NSCs also overlapped with Ki67 positive populations (Fig. 4 A and B, Supplemental Fig. 1 C, D and E). These results revealed that SLD5 might coordinate with the regulation of DNA replication in NSCs.

However, in the mid-stage of neural development (E11.5), the expression level of SLD5 gradually decline in the NT (Fig. 4 C), in contrast, it enhanced in the DRG (Fig. 4 D and Supplemental Figure 3 D). We could observe SLD5 expression from E10.5, which emerged earlier than PSF1 in DRG neurons (Supplemental Fig. 3 A and D). However, unlike PSF1, expression of SLD5 in DRG was transient and almost undetectable in the late-stage of neural development (E15.5 and E17.5) (Fig. 4 E and F). Furthermore, we investigated whether the expression of SLD5 controlled the neural cell proliferation, the data shared similarity to PSF1 expression, SLD5 positive neural cells did not overlap with Ki67 positive cells.

3.5. Correlation between GINS member expressions and cell proliferation during development of the mouse nervous system

To better understand the functions of GINS members and their effects on CNS cells, we summarized the GINS expression region, density, and correlation with cell proliferation during neural development.

Our data revealed a similar pattern for PSF1 and SLD5 in the neuroepithelium during initial neurulation (E9.5, E10.5). However, their expressions gradually decreased during mid neural development. Next, we found that PSF2 and PSF3 had no tissue-specificity, PSF2 protein was continuously highly expressed during development of the nervous system, whereas PSF3 had a low expression (Fig. 5 A). In the middle stage of neural development (E13.5), after neurulation was completed, both PSF1 and SLD5 showed a change in the region of expression, and they were highly expressed in DRGs. Although SLD5 was expressed earlier in the ganglion cells, its duration of expression was short. SLD5 could not be detected in the late-stage (E15.5) of neural development, which was in contrast to constitutively expressed PSF1 in the DRG (Fig. 5 B and Supplemental Fig. 3 A, D). Moreover, neuroepithelium cells highly expressing PSF1 and SLD5, overlapped with Ki67 positive cells during primary neurulation, but were decreased after entering the mid-stage. Although the expressions of PSF2 and PSF3 cells also overlapped with Ki67 cells, they had no tissue-specific characteristics (Fig. 5 C).

In summary, PSF1 and SLD5 might be implicated in the regulation

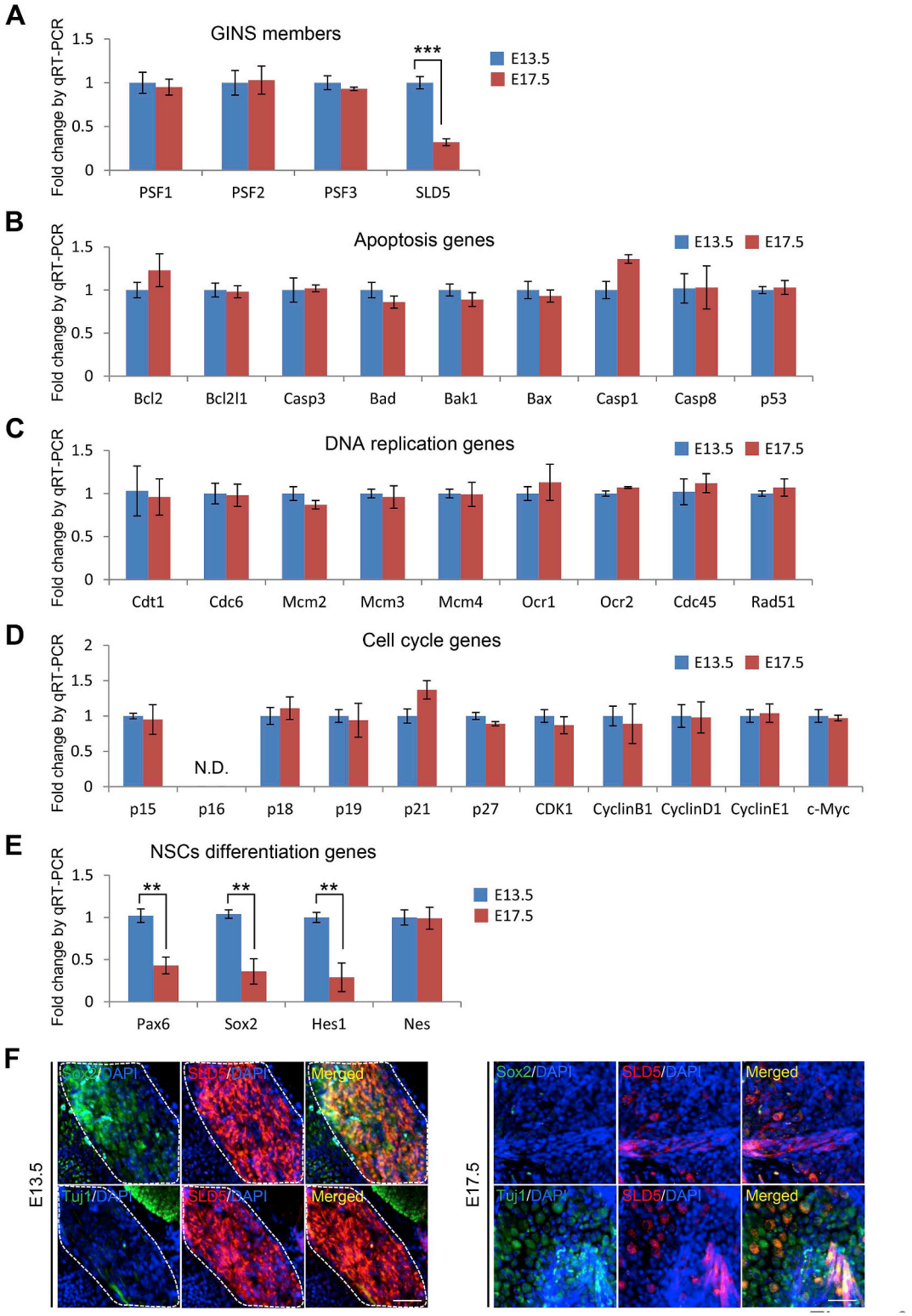

Fig. 6. Reduced expression of SLD5 controls stem/progenitor differentiation in DRG. Quantitative real-time PCR analysis of mRNA expression in DRG neurons from E13.5 and E17.5 embryos. GINS members (A), apoptosis regulatory genes (B), DNA replication genes (C), cell cycle regulatory genes (D), and NSC differentiation genes (E) (n = 3). Error bars represent the means ± SD; **p < 0.01, ***p < 0.001, and N.D., not determined. (F) Immunofluorescence of SLD5 (red) and Sox2 or Tuj1 (green) in the DRG from E13.5 and E17.5 embryos. DAPI (blue) was used to detect nuclei and the dashed box indicates areas shown at DRG. Scale bar, 100 μm and 50 μm (inset).

of NSCs proliferation in the NT neuroepithelium during neurulation. After the end of neurulation, both PSF1 and SLD5 were expressed at high levels in DRG neurons, but were not related to cell proliferation.

3.6. Reduction of SLD5 expression induces DRG stem/progenitor differentiation

To illustrate the role of SLD5 in neural development, we compared

the mRNA expression levels of GINS family genes and neuronal development related genes in the DRG neurons at E13.5 and E17.5. Real-time PCR results indicated that the expression of SLD5 mRNA was significantly decreased at E17.5, yet there was no change in the other GINS member mRNA expressions (Fig. 6 A). Moreover, we analyzed several apoptosis, DNA replication, NSC differentiation and cell cycle-related genes in the DRG neurons at E13.5 and E17.5. There were no marked changes except for NSC differentiation-related genes (Fig. 6B–E). The NSC maintenance genes Pax6, Sox2, and Hes1 were significantly decreased at E17.5 compared with E13.5 in DRG neurons (Fig. 6 E). Immunohistochemical analysis of SLD5 demonstrated its positive expression in Sox2 positive NSCs in the DRG at E13.5 and decreased expression in Tuj1 positive differentiated neurons in the DRG at E17.5 (Fig. 6 F). These results suggested that the reduction of SLD5 in DRG stem/progenitors might be involved in cell differentiation.

4. Discussion and conclusions

The GINS (Go, Ichi, Nii, and San) complex has been reported to regulate the DNA replication process in various types of stem cells including HSCs and gonads stem cells of eukaryotes (Ueno et al., 2005). Our previous research also indicated that a lack of PSF1 or SLD5 caused early embryonic lethality, due to a defect in the regeneration capacity of stem cell proliferation (Ueno et al., 2008; Mohri et al., 2013). However, the role of the GINS complex in neural stem cells of mammals in developmental stages has not been elucidated. Therefore, we investigated alterations in GINS components expression region and investigated the potential functions in both neurulation and mature ganglion cell in the mouse nervous system development via immunohistochemical and gene expression analyses.

The proliferation of NSCs in the inner surface of the NT was induced during the initial neurulation stage at mouse embryonic days 9.5–11.5. At this stage, we observed the robust expressions of PSF1, PSF2 and SLD5 in NSCs and weak expression of PSF3. PSF1 and SLD5 expressions were specifically localized to the inner cell mass of the NT, but PSF2 and PSF3 did not show tissue-specificity. These results are not consistent with a previous report showing that PSF2 was overexpressed in the retina and lens during eye development in *Xenopus laevis* (Walter et al., 2008), and that PSF3 was presented in the posterior and anterior regions of NT (Walter and Henry, 2004). PSF1 and SLD5 expression regions in the NT overlapped with Ki67 positive staining, suggested that the GINS complex is involved in DNA replication and the proliferation of NSCs at the neurulation stage.

In the mid-stage of nervous system development at mouse embryonic days 13.5–17.5, the expression region of GINS family proteins was dramatically changed. Expressions of PSF1 and SLD5 in the inner cell mass of the NT were reduced, whereas high expressions of PSF1 and SLD5 emerged in the DRG region. This indicated that following the end of NSC proliferation in the mid-stage, large amounts of progenitors were recruited in the DRG for differentiation (McKay, 1997). In accordance with this report, Ki67 positive proliferating cells were not presented in the PSF1 and SLD5 expressing DRG region. Expression of PSF1 in the DRG region continued even after mouse embryonic day 13.5, whereas the massive expression of SLD5 instantaneously disappeared at embryonic day 15.5. Gene expression analyses of DRG neurons from E13.5 (SLD5 high expression) and E17.5 (SLD5 low expression) showed that a decrease in SLD5 in DRG progenitors might promote cell differentiation.

In summary, these results suggested that SLD5 affects neuronal differentiation and maturation in addition to DNA replication, indicated the importance of GINS family proteins in neuronal development. GINS family members might have a modulatory role in cell differentiation.

Conflicts of interest

The authors have no conflicting financial interest.

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Appendix A. Supplementary data

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