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TRANSITION OF MOUSE DE NOVO METHYLTRANSFERASES EXPRESSION FROM Dnmt3b TO Dnmt3a DURING NEURAL PROGENITOR CELL DEVELOPMENT

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Abstract—Dnmt3a and Dnmt3b, which are known as functional de novo methyltransferases, are responsible for creating genomic methylation patterns during mammalian development. Recently, we have shown that specific expression of Dnmt3b in epiblast, embryonic ectoderm, hematopoietic progenitor cells and spermatogonia cells is followed by Dnmt3a expression (Watanabe D, Suetake I, Tada T, Tajima S (2002) Stage- and cell-specific expression of Dnmt3a and Dnmt3b during embryogenesis. Mech Dev 118:187–190; Watanabe D, Suetake I, Tajima S, Hanaoka K (2004) Expression of Dnmt3b in mouse hematopoietic progenitor cells and spermatogonia at specific stages. Gene Expr Patterns 5:43–49). In this study, we analyzed the expression of mouse de novo methyltransferases during development of the nervous systems. In the embryonic olfactory epithelium (OE), Dnmt3b was specifically expressed in Mash1 positive globose basal cells (i.e. transiently amplifying neural progenitor cells), while Dnmt3a was expressed in immature olfactory receptor neurons. Dnmt3b-positive cells were rarely observed in the adult OE, but were increased in regenerating OE with intranasal $ZnSO_4$ administration. Dnmt3b was also detected in the E8.5 neural plate, E10.5 spinal cord and retina cells, while Dnmt3a was expressed in postmitotic young neurons. Furthermore, Dnmt3b was specifically expressed in ES cells, while Dnmt3a was transiently expressed during neural cell differentiation of ES cells. Dnmt3b is specifically expressed in progenitor cells during hematopoiesis, spermatogenesis and neurogenesis, suggesting an important role in the initial steps of progenitor cell differentiation. Dnmt3a is expressed in postmitotic young neurons following the Dnmt3b expression. Dnmt3a may be required for the establishment of tissue-specific methylation patterns of the genome. The coordinated expression of de novo methyltransferases from Dnmt3b to Dnmt3a suggests conserved mechanisms of de novo methylation of the genome and different functions for Dnmt3b and Dnmt3a during progenitor cell development.

Key words: DNA methylation, neural progenitor cells, ES cells, olfactory epithelium, GBC, neurogenesis.

In the mammalian genome, C residues of the CpG dinucleotide sequence are modified post-replication by DNA methylation; about 70–80% of CpG dinucleotides are methylated (Ng and Bird, 1999). During mammalian development, the methylation levels of genomic DNA are not stable and vary during embryogenesis and tissue differentiation. De novo DNA methylation starts after implantation, following genome-wide demethylation after fertilization.

Until now, two major independently encoded de novo methytransferases, Dnmt3a and Dnmt3b, and one classical maintenance methyltransferase, Dnmt1, have been identified in mammals (Bestor et al., 1988; Yen et al., 1992; Okano et al., 1998; Xie et al., 1999). Dnmt1 preferentially acts on hemimethylated CpG dinucleotides and is necessary for maintenance of specific methylation patterns during DNA replication, while Dnmt3a and Dnmt3b contribute to the establishment of de novo methylation patterns during early embryogenesis. These enzymes are essential for normal mouse development; targeted disruption analysis showed lethality during embryogenesis or postnatal development. Dnmt1 and Dnmt3b null mice die at the postgestation stage, while Dnmt3a null mice die 4 weeks after birth. Dnmt3b null embryos have multiple developmental defects, including growth impairment and neural tube defects (Okano et al., 1999). In addition, condition knockout analysis of Dnmt3a and Dnmt3b in germ cells showed distinct phenotypes. Dnmt3a-conditioned mice showed lack of methylation at the imprinting locus, impaired spermatogenesis and embryonic death of offspring from Dnmt3a-conditioned females, while Dnmt3b-conditioned mutant mice did not display any apparent phenotype (Kaneda et al., 2004). Furthermore, *in vitro* experiments using Dnmt3a and Dnmt3b null ES cells demonstrated that some gene loci are preferentially methylated by Dnmt3a but not by Dnmt3b (Oka et al., 2006). These results suggest Dnmt3a and Dnmt3b have distinct functions during mammalian development.

It has been shown that there is direct causality between DNA methylation and tumorigenesis, and disruptions of the normal DNA methylation pattern are frequently observed in cancer cells (Jones and Baylin, 2002). In addition, naturally occurring mutations in genes that control DNA methylation patterns have been linked to mental retardation disorders such as Rett syndrome, immunodeficiency-centromeric instability-facial anomalies (ICF) syndrome, fragile X syndrome and X-linked alpha thalassemia (ATR-X) syndromes, and Dnmt3a is highly expressed in neural cells, suggesting important roles for DNA methylation, particularly in the developmental of the CNSs (Fan et al., 2001; Feng et al., 2005; Robertson and Wolffe, 2000).

Recently, we studied the expression patterns of mouse de novo methyltransferases during embryogenesis, and

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Abbreviations: E, embryo day; GBC, globose basal cell; OE, olfactory epithelium; ORN, olfactory receptor neuron; SV, subventricular; VNO, vomeronasal organ.

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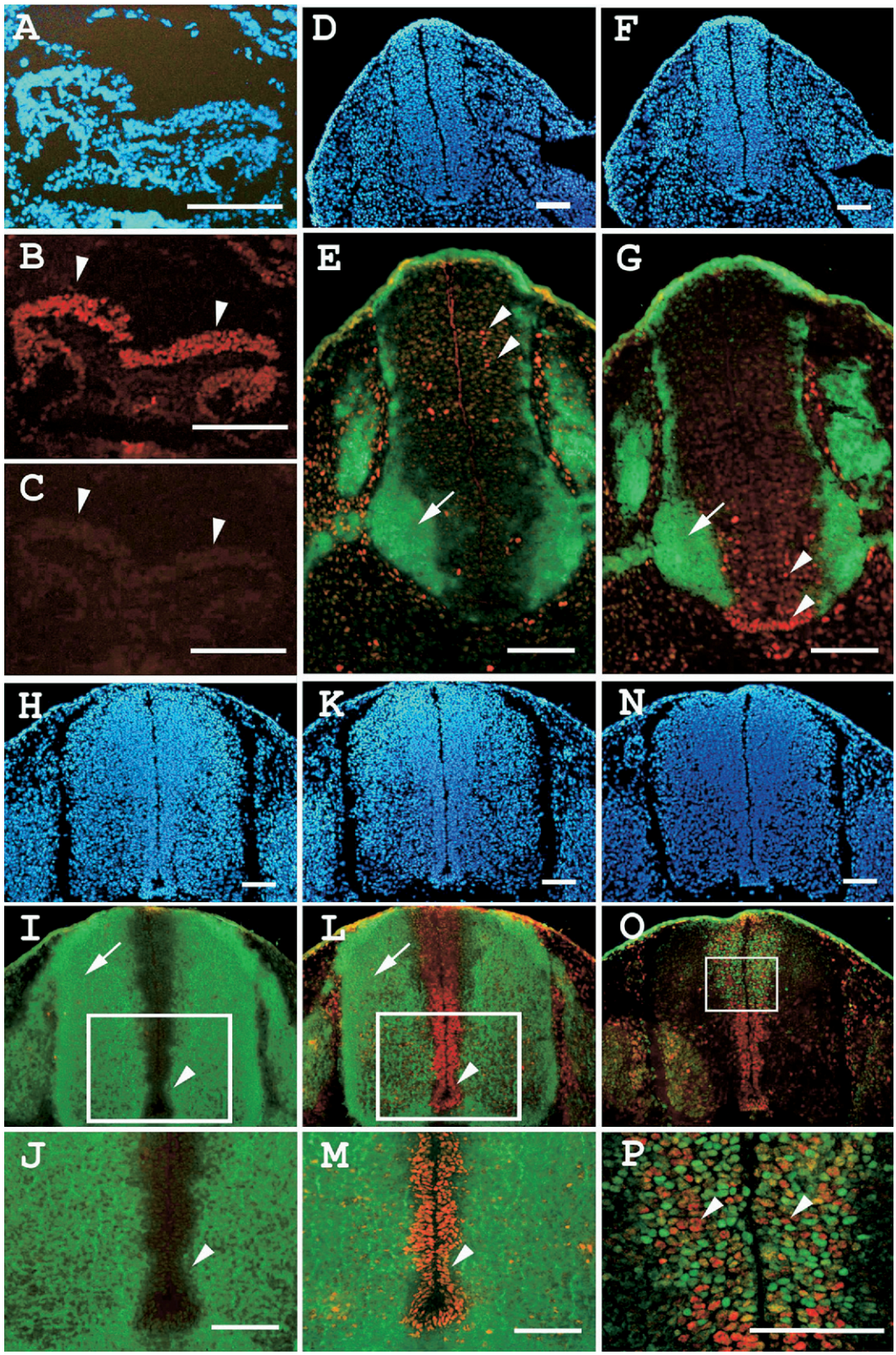

Fig. 1. Dnmt3b and Dnmt3a expression during spinal cord development. Frontal sections prepared from E8.5 neural plate (A–C), E10.5 spinal cord (D–G) and E12.5 spinal cord (H–P) were stained with anti-Dnmt3b (B, E, I, J, red) antibodies or anti-Dnmt3a antibody (C, G, L, M, O, P, red) and further double-stained with anti-Tuj1 (E, G, I, J, L, M, green) or Mash1-antibodies (O, P, green). Hoechst-stained images of each section are shown in A, D, F, H, K and N. High-magnification images of the boxed region of I, L and O are shown in J, M and P, respectively. Dnmt3b was expressed in neural