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Dynamic transition of Dnmt3b expression in mouse pre- and early post-implantation embryos

Ryutaro Hirasawa [a,b,c,*], Hiroyuki Sasaki [a,b]

[a] Division of Human Genetics, Department of Integrated Genetics, National Institute of Genetics, Research Organization of Information and Systems, Mishima 411-8540, Japan
[b] Department of Genetics, School of Life Science, The Graduate University for Advanced Studies, Mishima 411-8540, Japan
[c] Institute of Molecular Genetics, CNRS UMR-5535, 34293 Montpellier, France

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ABSTRACT

The *de novo* DNA methyltransferases, Dnmt3a and Dnmt3b, are responsible for the creation of DNA methylation patterns in mouse development. Dnmt3b is more highly expressed in early developmental stages than Dnmt3a, and is thought to have an important role in the epigenetic gene regulation during early embryogenesis. Previous reports suggest that Dnmt3b is expressed preferentially in the embryonic lineage, but less in the extra-embryonic lineage, in early post-implantation embryos. However, it is unclear when this lineage-specific differential expression is established. Here we demonstrate that Dnmt3b shows a dynamic expression change during pre- and early post-implantation development. Contrary to the expectation, Dnmt3b is preferentially expressed in the trophectoderm rather than the inner cell mass at the mid blastocyst stage. Subsequently, the spatial Dnmt3b expression gradually changes during pre- and early post-implantation development, and finally Dnmt3b expression is settled in the embryonic lineage at the epiblast stage. The findings are consistent with the role for Dnmt3b in cell-lineage specification and the creation of lineage-specific DNA methylation patterns.

1. Results and discussion

In mammalian development, the first cell-lineage-allocation occurs in the morula stage, giving rise to the inner cell mass (ICM, embryonic lineage) and trophectoderm (TE, extra-embryonic lineage) of the blastocyst. Differential gene expression and epigenetic modifications are observed between the two lineages (Morgan et al., 2005). One such differential modification is ICM-specific *de novo* DNA methylation (Dean et al., 2001; Santos et al., 2002), but it is unknown which DNA methyltransferase is responsible for this methylation. During pre-implantation development, Dnmt3a is present as an oocyte-derived protein and disappears before the blastocyst stage (Hirasawa et al., 2008). By contrast, Dnmt3b is produced only after fertilization and accumulates in cell nuclei of pre-implantation embryos (Hirasawa et al., 2008). It was also reported that Dnmt3b is present more abundantly than Dnmt3a in early post-implantation embryos (Okano et al., 1999; Watanabe et al., 2002). We therefore examined by immunostaining whether Dnmt3b is differentially expressed between the ICM and TE.

1.1. Dnmt3b is preferentially expressed in the TE of blastocysts

To reveal the lineage-specific Dnmt3b expression, embryos were co-stained for Dnmt3b and Oct4, the latter of which is a marker for the ICM. Dnmt3b was expressed in almost all blastomeres of morulae at embryonic day 3.0 (E3.0) (Fig. 1A, top). In E3.5 blastocysts, strong Dnmt3b expression was observed in cell nuclei with weak Oct4 signals (surrounding the ICM and blastocele) but not in those with strong Oct4 signals (Fig. 1A, middle), indicating that Dnmt3b is expressed preferentially in TE cells. This was surprising because *de novo* DNA methylation occurs in the ICM rather than the TE (Dean et al., 2001; Santos et al., 2002) and because Dnmt3b is reported to be expressed in the ICM at least at a more developed stage (Watanabe et al., 2002). The TE is subdivided into two regions: the polar TE overlying the ICM and mural TE surrounding the blastocoel (see Figs. 1B and 2B). In E4.0 blastocysts, Dnmt3b signals were more restricted within the TE and observed predominantly in the polar TE (Fig. 1A, bottom). Although Dnmt3b and Oct4 signals overlapped in some regions, many Oct4-positive ICM nuclei were still negative for Dnmt3b. Even in the overlapped regions, one could follow the contours of individual nuclei and find that many Oct4-positive nuclei did not coincide with Dnmt3b-positive nuclei.

* Corresponding author. Address: Institute of Molecular Genetics, CNRS UMR-5535, 34293 Montpellier, France. Tel.: +33 (0)467613661; fax: +33 (0)467040231.
E-mail address: ryutaro.hirasawa@igmm.cnrs.fr (R. Hirasawa).

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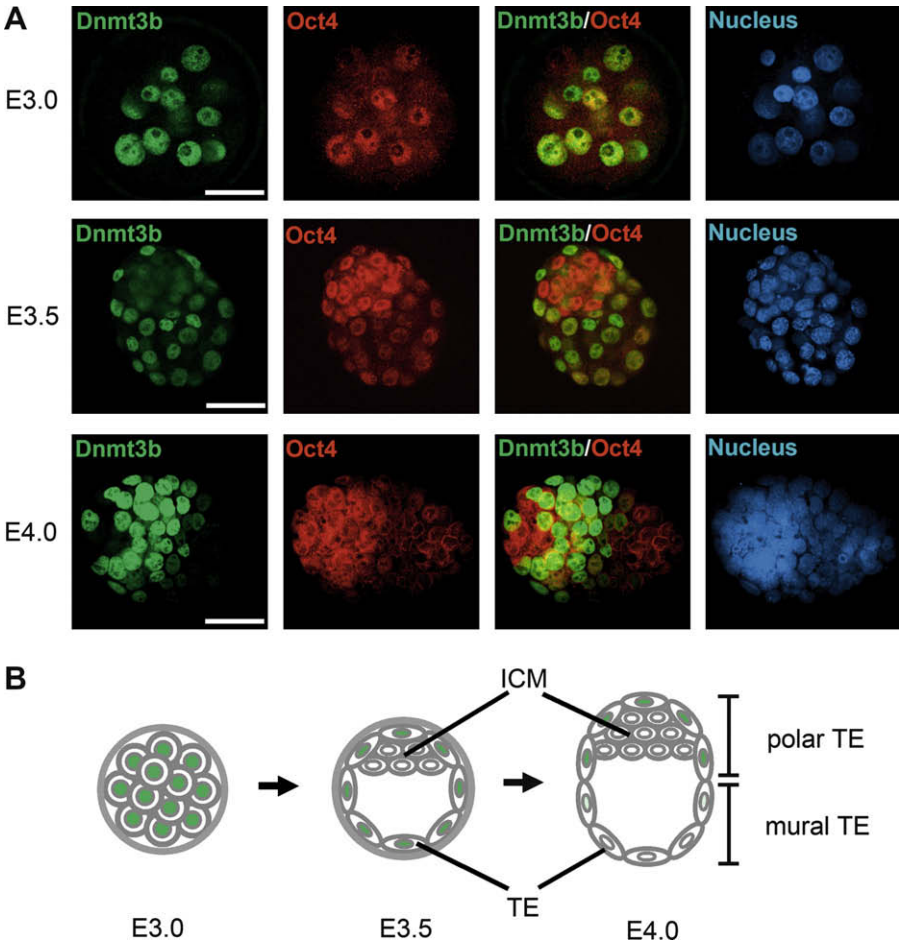

Fig. 1. Expression of Dnmt3b in mouse morulae and blastocysts. (A) Co-immunostaining of Dnmt3b (green) and Oct4 (red) in E3.0 (top), E3.5 (middle) and E4.0 (bottom) pre-implantation embryos. Dnmt3b and Oct4 signals were detectable in each blastomere of E3.0 morulae. Dnmt3b signals were detected in the TE but not in the ICM (Oct4-positive cells) of E3.5 blastocysts. Dnmt3b signals were detected in the polar TE but not in the mural TE and ICM of E4.0 blastocysts. The cell nuclei were counterstained with TOTO-3 (blue). (B) Schematic representation of the expression pattern of Dnmt3b in E3.0, E3.5 and E4.0 embryos. Green color indicates Dnmt3b expression.

1.2. Transient Dnmt3b expression in the primitive endoderm and subsequent expression in the epiblast

In E4.5 embryos, which were flushed out from the uteri, the polar TE cells still strongly expressed Dnmt3b and, in addition, some Oct4-positive ICM cells started to express Dnmt3b (Fig. 2A, top). We co-stained slightly more developed E4.5 embryos for Gata4, which is a marker for the primitive endoderm. All Gata4-positive cells of these E4.5 embryos strongly expressed Dnmt3b, although Dnmt3b signals were also observed in the TE and some ICM cells (Fig. 2A, middle). In post-implantation embryos at E5.5, Dnmt3b signals were no more detected in Gata4-positive primitive endoderm cells (Fig. 2A, bottom). Instead, Dnmt3b expression was clearly detected in the epiblast (embryonic lineage), which is derived from the ICM (Fig. 2A, bottom). No significant Dnmt3b signal was observed in the extra-embryonic lineages at this stage.

1.3. Contribution of Dnmt3b in lineage specification?

In this study, we showed that Dnmt3b expression undergoes dynamic lineage transition during pre- and early post-implantation development (summarized in Fig. 1B and 2B). Dnmt3b is strongly expressed in the TE (notably in the polar TE), but little in the ICM, of mid blastocysts. Strong Dnmt3b expression then occurs in the primitive endoderm of E4.5 embryos and in the epiblast of E5.5 post-implantation embryos. Watanabe et al. (2002) previously reported that Dnmt3b is expressed in the ICM of E4.5 embryos. The discrepancy likely resulted from a mis-identification of the cells in the previous work (the authors did not try double staining for Dnmt3b and Oct4). Our results were reproducible in ICR mice and also in a mixed genetic background of C57BL/6 and 129 strains (Hirasawa et al., 2008 and data not shown).

The Dnmt3b expression pattern in the blastocyst is also apparently inconsistent with the higher DNA methylation of the ICM genome than the TE genome (Dean et al., 2001; Santos et al., 2002). However, such lineage-specific methylation may be established prior to the blastocyst stage and Dnmt3b is indeed present in all blastomeres of morulae (Fig. 1A). Establishment of the role for Dnmt3b in the lineage-specific methylation awaits further studies. Also, it is currently unknown why Dnmt3b is transiently expressed in the TE and primitive endoderm. One possibility is that *de novo* DNA methylation is required to repress genes that are specifically expressed in the ICM. We tested whether Oct4 repression in the TE is dependent on Dnmt3b, because the promoter of this gene is highly methylated in differentiated cells and trophoblast stem cells (Hattori et al., 2004; Li et al., 2007), but Dnmt3b-null E3.5 and 4.0 blastocysts showed no change in Oct4 expression (data not shown). Understanding of the Dnmt3b regulation mechanisms and identification of the target genes regulated by Dnmt3b should clarify the role of this epigenetic modifier in cell-lineage commitment and differentiation.