

DNMT1, DNMT3A and DNMT3B proteins are differently expressed in mouse oocytes and early embryos

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Abstract DNA methylation is one of the epigenetic mechanisms and plays important roles during oogenesis and early embryo development in mammals. DNA methylation is basically known as adding a methyl group to the fifth carbon atom of cytosine residues within cytosine–phosphate–guanine (CpG) and non-CpG dinucleotide sites. This mechanism is composed of two main processes: de novo methylation and maintenance methylation, both of which are catalyzed by specific DNA methyltransferase (DNMT) enzymes. To date, six different DNMTs have been characterized in mammals defined as DNMT1, DNMT2, DNMT3A, DNMT3B, DNMT3C, and DNMT3L. While DNMT1 primarily functions in maintenance methylation, both DNMT3A and DNMT3B are essentially responsible for de novo methylation. As is known, either maintenance or de novo methylation processes appears during oocyte and early embryo development terms. The aim of the present study is to investigate spatial and temporal expression levels and subcellular localizations of the DNMT1, DNMT3A, and DNMT3B proteins in the mouse germinal vesicle (GV) and metaphase II (MII) oocytes, and early embryos from 1-cell to blastocyst stages. We found that there are remarkable differences in the expressional levels and subcellular localizations of the DNMT1, DNMT3A and DNMT3B proteins in the GV and MII oocytes, and 1-cell, 2-cell, 4-cell, 8-cell, morula, and blastocyst stage embryos. The fluctuations in the expression of DNMT proteins in the analyzed oocytes and early embryos are largely compatible with

DNA methylation changes and genomic imprint establishment appearing during oogenesis and early embryo development. To understand precisely molecular biological meaning of differently expressing DNMTs in the early developmental periods, further studies are required.

Keywords DNA methyltransferase · DNA methylation · Oocyte · Early embryo

Introduction

DNA methylation is an epigenetic mechanism and plays critical roles in transcriptional repression or activation, X-chromosome inactivation, cell differentiation, and tumorigenesis (Uysal et al. 2015). DNA methylation is specifically catalyzed by various types of DNA methyltransferase (DNMT) enzymes, and there are two different DNA methylation processes: maintenance and de novo. In the maintenance methylation, previously established DNA methylation patterns are continued at the end of each round of DNA replication. By contrast, new DNA methylation marks in the genomic DNA are created in the de novo methylation process.

The DNMT enzymes basically add a methyl group to the fifth carbon atom of cytosine residues commonly in the cytosine–phosphate–guanine (CpG) and rarely in the non-CpG islands such as cytosine–phosphate–thymine (CpT), cytosine–phosphate–adenine (CpA), and cytosine–phosphate–cytosine (CpC) by using S-adenosyl methionine (AdoMet) as a methyl donor (Turek-Plewa and Jagodzinski 2005). To date, structurally and functionally six different DNMTs have been characterized in mammals: DNMT1, DNMT2, DNMT3A, DNMT3B, DNMT3C, and DNMT3L. The DNMT1 protein primarily functions in maintenance methylation by adding methyl groups to

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the hemi-methylated DNA strands during DNA replication (Bestor 2000), and also it partially contributes to de novo methylation process (Fatemi et al. 2002). And, the DNMT2 protein can methylate cytosine 38 in the anticodon loop of aspartic acid transfer RNA instead of methylating genomic DNA (Goll et al. 2006). On the other hand, both DNMT3A and DNMT3B essentially implicate in the de novo methylation process, commonly being appeared in the unmethylated DNA strands at the CpG islands (Turek-Plewa and Jagodzinski 2005). DNMT3C is recently identified de novo methyltransferase which protects male germ cells from retrotransposon activity and is essential for mouse fertility (Barau et al. 2016). DNMT3L does not have any catalytic domain; however, it is capable of inducing DNMT3A and DNMT3B activity, and thereby contributes to de novo methylation indirectly (Deplus et al. 2002; Margot et al. 2003).

In mammals, female germ cells are originated from primordial germ cells (PGCs) arising in the endoderm of yolk sac. When female PGCs reach to the gonadal ridges at the end of long migration during which they undergo many mitotic divisions, the PGCs ultimately differentiate into oogonia. The oogonia are defined as oocytes after entering meiotic division, and the oocytes are arrested at prophase I (PI) stage of first meiotic division in the developing ovary before birth. The oocytes at the PI stage in the ovarian follicles from primordial to antral follicles are immature oocytes also described as germinal vesicle (GV) stage. In the ovulatory follicles, the GV oocytes fulfill the first meiotic division, enter into second meiotic division, and become arrested at the metaphase II (MII) stage known as mature oocytes (Verlhac and Terret 2016). Once the MII oocytes are fertilized by mature spermatozoa, 1-cell embryos (zygotes) including female and male pronuclei are formed. Then, the 1-cell embryos undergo consecutive mitotic divisions (cleavage) and develop into 2-cell, 4-cell, 8-cell, morula and blastocyst stage embryos, respectively. The early embryo development from 1-cell to blastocysts includes exclusive events such as cleavage, embryonic genome activation (EGA), compaction, and cavitation. EGA is shortly defined as synthesis of the required RNAs from newly formed embryonic genome and occurs at the 2-cell stage in mice (Bolton et al. 1984). In the eight-cell stage embryo, the blastomeres enable to create tight junctions with each other so that compaction is partially commenced. The compaction is thoroughly completed in the morula stage embryos in which the cellular borders become fuzzy and cannot be distinguished easily. Following morula stage, cavitation known as formation of the blastocoel cavity filled with fluid take place in the blastocyst stage embryos. The blastocoel cavity facilitates division of the embryonic cells into two different layers in the blastocysts: trophoblast cells (TE) and inner cell mass (ICM) (Fujimori 2010).

During oogenesis and preimplantation embryo development, DNA methylation plays critical roles in strictly regulating stimulation or repression of the development-related genes and in timely establishing maternal and paternal imprints (Bartolomei and Ferguson-Smith 2011). The global DNA methylation in the growing GV oocytes begins to progressively increase after birth, and reaches to the highest levels in the MII oocytes at puberty (Saitou et al. 2012; Smallwood and Kelsey 2012) (Fig. 4). During early embryo development, the global DNA methylation gradually reduces from 1-cell embryos to 16-cell embryos, and it starts to increase in the blastocyst stage embryos onward (Smallwood and Kelsey 2012) (Fig. 4). Although DNA methylation profiles during oogenesis and early embryo development are well-known, the spatial and temporal expression levels and subcellular localizations of the DNMTs in the oocytes and early embryos are not characterized in detail. We hypothesized that the DNMT1, DNMT3A, and DNMT3B proteins display different expression patterns and subcellular localizations during oocyte and early embryo development. The aim of this study was to determine the subcellular localizations and relative expression levels of the DNMT1, DNMT3A, and DNMT3B proteins in the mouse oocytes and early embryos.

Materials and methods

Animals

The experimental protocol was approved by the Animal Care and Usage Committee of Akdeniz University (Protocol no: B.30.2.AKD.0.05.07.00/30). The female Balb/C mice at 4–6 weeks and male mice at 8–10 weeks of age were purchased from Research Animal Laboratory Unit of Akdeniz University Medical Faculty. All mice were housed with free access to food and water, and kept in a 12 h light/dark cycle.

Collection of oocytes and early embryos

The GV and MII oocytes, and 1-cell, 2-cell, 4-cell, 8-cell, morula and blastocyst stage embryos were obtained from 4 to 6 week-old female Balb/C mice superovulated with 5 IU pregnant mare's serum gonadotropin (PMSG; Intervet, Milton Keynes, UK) and 5 IU human chorionic gonadotropin (hCG; Sigma-Aldrich, St. Louis, MO, USA). It is important to note that each oocyte and early embryo type was recruited at least three different mice. To obtain GV oocytes, the female mice were injected intraperitoneally (i.p.) with 0.1 mL 5 IU of PMSG. Twenty hours after PMSG injection, the female mice were killed and then ovaries were punctured with a 23-gauge needle in human tubal fluid (HTF) medium (Vitrolife, Gothenburg, Sweden), and we collected GV

oocytes having a visible germinal vesicle by using a mouth-controlled pipette under a dissecting microscope (Zeiss, Oberkochen, Germany) as described previously (Akkoynlu et al. 2007). For collection of MII oocytes, the female mice were injected i.p. with 5 IU PMSG, and subsequently 5 IU hCG was administered 48 h after PMSG injection. We obtained the cumulus–oocyte complexes (COCs) from oviducts' ampulla region of the mice 14 h after hCG injection. The COCs were treated with HTF medium including 1 mg/mL hyaluronidase (Sigma-Aldrich) to remove cumulus cells, and the MII oocytes in normal morphology and having visible polar bodies are pooled.

To collect early embryos, female mice at 4–6 weeks old were injected i.p. with 5 IU PMSG. Following 48 h PMSG treatment, 5 IU hCG were injected to the PMSG-primed female mice immediately mated overnight with mature male mice at a rate of 2 female:1 male. The next morning, presence of vaginal plug has been controlled, and the female mice including vaginal plug were used to obtain 1-cell, 2-cell, 4-cell, 8-cell and morula stage embryos from oviducts and blastocysts from uterus. The early embryos were collected at the following time points after hCG injection: 1-cell embryos at 20 h, 2-cell embryos at 42 h, 4-cell embryos at 60 h, 8-cell embryo at 68 h, morula at 72 h, and blastocyst at 96 h. Notably, the cumulus cells surrounding the 1-cell embryos were removed by using hyaluronidase at a concentration of 1 mg/mL as described above.

Immunofluorescence staining

Immunofluorescence staining has been applied to characterize the subcellular localizations and relative expression profiles of the DNMT1, DNMT3A, and DNMT3B proteins in the GV and MII oocytes, and 1-cell, 2-cell, 4-cell, 8-cell, morula, and blastocyst stage embryos obtained from superovulated female mice ($n \geq 10$ oocytes or early embryos stained per group). All oocytes and early embryos were fixed in 3% paraformaldehyde (Sigma-Aldrich), and then permeabilized with 1% tween-20 (Sigma-Aldrich) prepared in $1 \times$ PBS at room temperature. The fixed oocytes and early embryos were blocked with blocking solution including 20% normal goat serum (Vector Laboratory, Burlingame, CA, USA). Then, we incubated the oocytes and embryos overnight at 4 °C with primary antibodies specific for DNMT1 [Abcam, Cambridge, USA; ab87654, reactive with either oocyte-specific (DNMT1o) or somatic (DNMT1s) isoforms], DNMT3A (Abcam, ab23565) or DNMT3B (Abcam, ab2851). After washing three times for 10 min with $1 \times$ PBS including 2% bovine serum albumin (BSA) (PBS–BSA; Sigma-Aldrich), the oocytes and early embryos were incubated with anti-rabbit Alexa 488 secondary antibody (Invitrogen) for 1 h at room temperature in the dark. After incubation with secondary antibody, we washed the oocytes and

early embryos three times for 10 min with PBS–BSA. After counterstained with 4',6-diamidino-2-phenylindole (DAPI; Sigma-Aldrich, D8417) for 2 min at room temperature in the dark, the oocytes and early embryos were washed three times for 10 min with PBS–BSA. All staining steps were performed using mini well trays (VWR-Thermo Scientific, Radnor, PA, USA) in a humidified chamber. We mounted the oocytes and early embryos onto slides using mounting solution (Vector Laboratory). All immunostaining experiments for the oocytes and early embryos were performed at the same time periods under the same conditions. Fluorescence signals originating from oocytes or early embryos were detected with a motorized fluorescence microscope at 400x magnification (Olympus BX61, Tokyo, Japan). Then, we have evaluated the micrographs with Image J software (National Institutes of Health, Bethesda, Maryland, USA), and the relative expression levels and subcellular localizations of the DNMT proteins have been characterized in detail. All negative controls involving no primary antibody were also analyzed, but no signal was detected. It is important to note that we carried out all experiments at least three times.

Statistical analysis

All experiment results were analyzed by one-way analysis of variance (one-way ANOVA) followed by Dunn's post hoc test. We conducted statistical calculations by using SigmaStat for Windows, version 3.5 (Jandel Scientific Corp). For all tests, $P < 0.05$ was considered to be statistically significant.

Results

DNMT1 expression in the oocytes and early embryos

In the current study, subcellular localizations and relative expression levels of the DNMT1 protein have been analyzed in the GV and MII oocytes, and 1-cell, 2-cell, 4-cell, 8-cell, morula, and blastocyst stage embryos. DNMT1 protein was abundantly localized in the cytoplasm of the oocytes and early embryos, but it was at weak levels in the nuclear regions (Fig. 1a). When we have evaluated the relative DNMT1 expression from GV oocytes to blastocysts by using ImageJ software, it reached to the highest levels in both MII oocytes and 1-cell embryos, whereas it was at the lowest levels in the 8-cell embryos (Fig. 1b). Additionally, MII oocytes, 1-cell embryos and blastocysts exhibited significantly higher DNMT1 protein expression than the remaining oocytes and early embryos ($P < 0.05$; Fig. 1b). Importantly, DNMT1 expression gradually decreased from 2-cell embryos to 8-cell embryos, and it

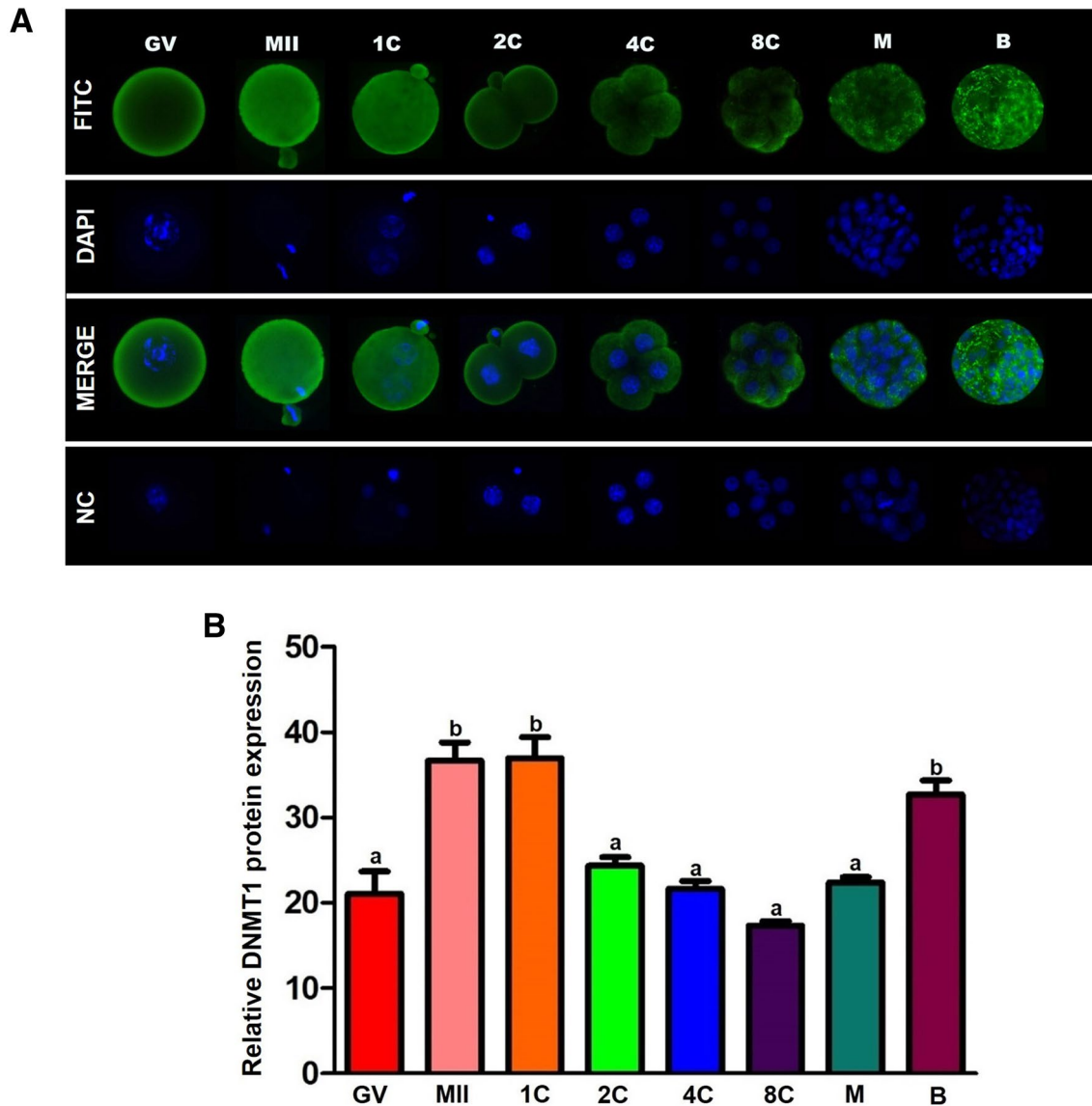


Fig. 1 Immunostaining of the DNMT1 protein in the mouse oocytes and early embryos. **a** The immunostaining results of the DNMT1 protein in the germinal vesicle oocytes (GV), MII oocytes (III), 1-cell embryos (1C), 2-cell embryos (2C), 4-cell embryos (4C), 8-cell embryos (8C), morulae (M) and blastocysts (B) have been shown. The negative control (NC) included no primary antibody was used to identify whether there was any non-specific staining. The DNMT1 protein expression exhibited spatial and temporal differences from GV oocytes to blastocyst stage embryos. In the micrographs, FITC (green) staining for DNMT1; DAPI (4',6-diamidino-2-phenylindole; blue) staining for the nuclei; Merge, combination of

the DAPI-stained nucleus with the green fluorescence signal. **b** The relative expression levels of the DNMT1 protein in the GV and MII oocytes, and 1-cell, 2-cell, 4-cell, 8-cell, morula and blastocyst stage embryos have been analyzed by using ImageJ software. The oocytes and early embryos expressed the DNMT1 protein at different levels showing gradual decreases and progressive increases. The statistical significance among groups has been determined by using one-way ANOVA test. The letters on the columns denote statistical significance, and $P < 0.05$ is considered as statistically significant. (Color figure online)

progressively increased from GV oocytes to MII oocytes and similarly from morula to blastocysts (Fig. 1b). As a result, we revealed here that DNMT1 protein expression was differently expressed in the oocytes and early embryos and displayed remarkable fluctuations from GV oocytes to blastocysts.

DNMT3A expression in the oocytes and early embryos

When subcellular localizations of the DNMT3A protein in the GV and MII oocytes, and 1-cell, 2-cell, 4-cell, 8-cell, morula and blastocyst stage embryos were analyzed, we found that DNMT3A was strongly localized in the nucleus of the oocytes and early embryos at all stages (Fig. 2a).

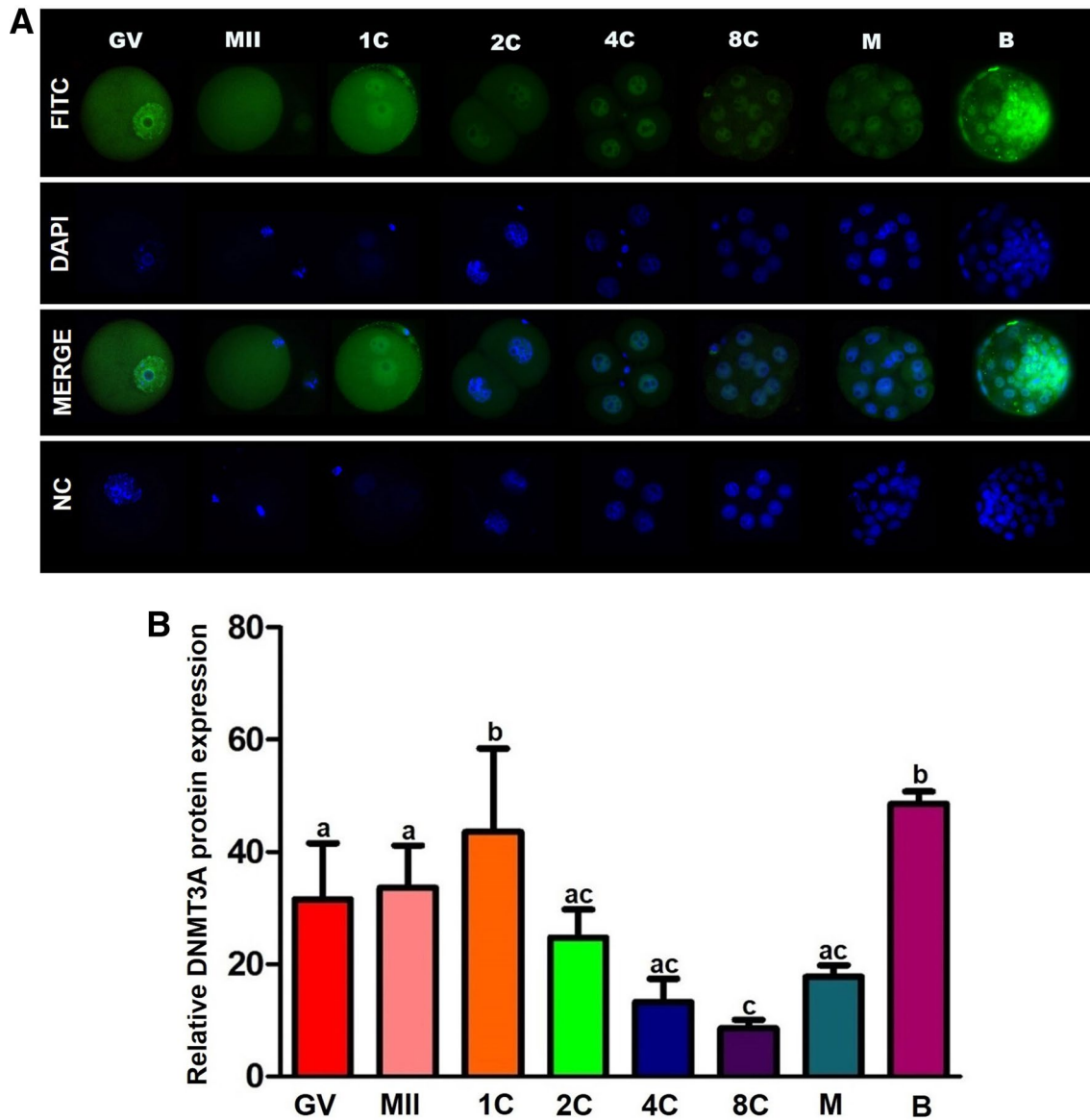


Fig. 2 Immunoeexpression of the DNMT3A protein in the mouse oocytes and early embryos. **a** The immunostaining of the DNMT3A protein in the germinal vesicle oocytes (*GV*), MII oocytes (*MI*), 1-cell embryos (*1C*), 2-cell embryos (*2C*), 4-cell embryos (*4C*), 8-cell embryos (*8C*), morulae (*M*) and blastocysts (*B*) have been presented. The DNMT3A protein was expressed at the higher levels in the nuclear region than that of the cytoplasm in the oocytes and early embryos. The negative control (*NC*) included no primary antibody has been used to identify whether there is any non-specific staining. In the micrographs, FITC (green) staining for DNMT3A; DAPI

(4',6-diamidino-2-phenylindole; blue) staining for the nuclei; Merge, combination of the DAPI-stained nucleus with green fluorescence signal. **b** The relative expression levels of the DNMT3A protein in the *GV* and *MI* oocytes, and 1-cell, 2-cell, 4-cell, 8-cell, morulae and blastocyst stage embryos have been analyzed by using ImageJ software. And, we found that the oocytes and early embryos differently expressed the DNMT3A protein. The statistical significance among groups was tested by using one-way ANOVA. The letters on the columns depict statistical significance, and $P < 0.05$ is considered as statistically significant. (Color figure online)

However, there was a weak DNMT3A expression in the cytoplasm of the oocytes and early embryos (Fig. 2a).

In addition to subcellular localization analysis, we have further evaluated the relative DNMT3A protein expression from *GV* oocytes to blastocyst stage embryos. The blastocyst stage embryos had the highest, but the 8-cell embryos possessed the lowest DNMT3A expression levels ($P < 0.05$;

Fig. 2b). Importantly, both 1-cell and blastocyst stage embryos expressed the DNMT3A significantly higher than the remaining oocytes and early embryos ($P < 0.05$; Fig. 2b). Remarkably, DNMT3A protein expression progressively increased from *GV* oocytes to 1-cell embryos, and it gradually decreased from 1-cell embryos to 8-cell stage embryos (Fig. 2b). Similarly, a progressive increase from 8-cell to

blastocyst stage embryos has been observed. Taken together, DNMT3A expression distributes either in the cytoplasm or nucleus of the oocytes and early embryos at different levels. Also, it exhibits remarkable fluctuations from GV oocytes to blastocyst stage embryos.

DNMT3B expression in the oocytes and early embryos

The subcellular localizations and relative expression levels of the DNMT3B protein in the GV and MII oocytes, and 1-cell, 2-cell, 4-cell, 8-cell, morula and blastocyst stage embryos have been examined. The DNMT3B protein was highly expressed in the nuclear regions of the GV oocytes, 1-cell, 2-cell and morula stage embryos. On the other hand, it was localized in either nuclear or cytoplasm regions of the MII oocytes, 4-cell and 8-cell embryos. In the blastocysts, DNMT3B was more highly expressed in the cytoplasm than that of in the nucleus (Fig. 3a).

When we evaluated the relative expression levels of DNMT3B from GV oocytes to blastocysts, it reached the highest levels in the blastocysts, but it was at the lowest profile in the 8-cell embryos (Fig. 3b). Both MII oocytes and blastocysts had significantly higher DNMT3B protein expression than that of the remaining oocytes and early embryos except for the 1-cell embryos ($P < 0.05$; Fig. 3b). Following predominant increase of the DNMT3B protein expression from GV oocytes to MII oocytes, it began to be gradually reduced from MII oocytes to 8-cell embryos. Then, the DNMT3B expression progressively increased from 8-cell embryos to blastocyst stage embryos (Fig. 3b).

Overall, DNMT3B intensively located to the nuclear region of the oocytes and early embryos. Besides, the relative expression of the DNMT3B protein exhibited gradual decrease from MII oocytes to 8-cell embryos, and progressively increase from 8-cell to blastocyst stage embryos except for the GV oocytes remained at low level. It is important to note that the expression profiles of DNMT3B protein in the oocytes and early embryos coincide to a large extent with that of the DNMT3A.

Discussion

In the present study, we characterized for the first time that DNMT1, DNMT3A, and DNMT3B proteins exhibited spatial and temporal expressional differences. And, they were differently localized in the mouse oocytes and early embryos. The DNMT1 protein was predominantly resided in the cytoplasm of the oocytes and early embryos, and showed gradual decrease and progressive increase from GV oocytes to blastocyst stage embryos. Unlike DNMT1 subcellular localization, DNMT3A was abundantly localized in the nucleus of the oocytes and early embryos, and it exhibited

expressional fluctuations from GV oocytes to blastocysts. Similarly, DNMT3B was more intensively expressed in the nucleus when compared to its cytoplasmic localization in the oocytes and early embryos. The DNMT3B expression levels gradually decreased and progressively increased from GV oocytes to blastocyst stage embryos, largely coinciding with the expression distributions of the DNMT3A protein at the same developmental terms.

DNA methylation is one of the epigenetic mechanisms and plays critical roles in the expressional regulation of the development-related genes, which are required during oogenesis and early embryos. During oogenesis, global DNA methylation begins to progressively increase in the growing GV oocytes at the time period of from birth to puberty, and it reaches the highest levels in the MII oocytes in mouse (Saitou et al. 2012). Following fertilization, the global DNA methylation levels gradually decreases from 1-cell to 16-cell or morula stage embryos, and the paternally and maternally imprinted genes are also demethylated late in embryogenesis (Yang et al. 2007). In blastocysts, global DNA methylation reaches the highest levels because new genomic imprints are established by activating de novo methylation process as well as maintenance methylation establishments (Saitou et al. 2012).

In the present study, we demonstrated that the expression levels of either DNMT1 or DNMT3B proteins were remarkably increased from GV oocytes to MII oocytes except for DNMT3A which remained at the same levels in both oocyte-types (Fig. 4). This predominant increase of either DNMT1 or DNMT3B expression is largely compatible with the increasing global DNA methylation levels from GV oocytes to MII oocytes. Both proteins most likely implicate in establishing maintenance and de novo methylation processes in both GV and MII oocytes. On the other hand, DNMT1, DNMT3A, and DNMT3B protein expression were gradually decreased from 1-cell embryo to morula, and they were progressively increased from morula to blastocysts (Fig. 4). The expression patterns of the DNMT proteins parallel with the gradually decreasing DNA methylation levels from 1-cell to morula stage embryos because there is no need for highly expressed DNMTs at this time points of early development. Since new genomic imprints, de novo and maintenance methylation begin to be reestablished in the blastocyst stage embryos, consistent with this we found that the expression levels of the DNMT1, DNMT3A and DNMT3B proteins are at the highest levels in the blastocysts.

It has been reported in the previous studies (Mertineit et al. 1998; Howell et al. 2001; Hirasawa et al. 2008) that DNMT1 is intensively localized in the cytoplasm of oocytes and early embryos as is detected in our current study. Ratnam et al. (2002) found that *Dnmt1* mRNA is present in the postnatal oocytes obtained from days 1, 5, 10, 15, 21 and 35 in mouse (Ratnam et al. 2002). In bovines, DNMT1 is also

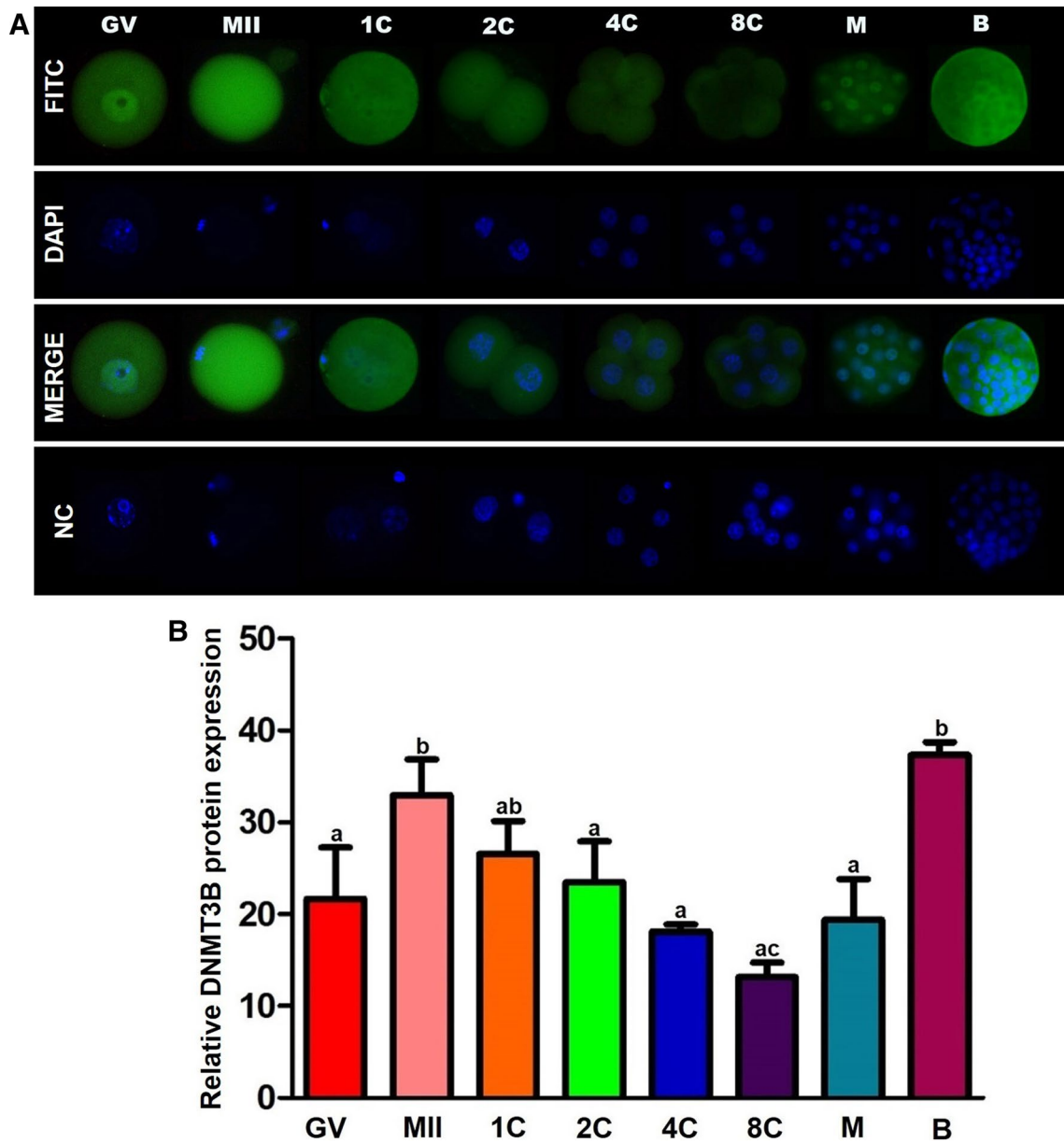


Fig. 3 Immunofluorescence of the DNMT3B protein in the mouse oocytes and early embryos. **a** The immunostaining of DNMT3B protein in the germinal vesicle oocytes (GV), MII oocytes (MII), 1-cell embryos (1C), 2-cell embryos (2C), 4-cell embryos (4C), 8-cell embryos (8C), morulae (M) and blastocysts (B) have been presented. The DNMT3B protein expression differs in the oocytes and early embryos. The negative control (NC) included no primary antibody has been used to identify whether there is any non-specific staining. In the micrographs, FITC (green) staining for DNMT3B; DAPI (4',6-diamidino-2-phenylindole; blue) staining for the nuclei; Merge,

combination of the DAPI-stained nuclei with green fluorescence signal. **b** The relative expression levels of the DNMT3B protein in the GV and MII oocytes, and 1-cell, 2-cell, 4-cell, 8-cell, morula and blastocyst stage embryos have been analyzed by using ImageJ software. We observed that DNMT3B exhibited gradual decreases and progressive increases from GV oocytes to blastocysts. The statistical significance between groups has been determined by using one-way ANOVA test. The letters on the columns depict statistical significance, and $P < 0.05$ is considered as statistically significant. (Color figure online)

identified at all GV oocyte stages (from GV0 to GV3) and in the MII oocytes (Lodde et al. 2009). Similar to the findings from mouse and bovines, *DNMT1* is expressed in human GV and MII oocytes (Huntriss et al. 2004). Although DNMT1 is predominantly localized in the nucleus of GV oocytes,

it is intensively resided in the cytoplasm of MII oocytes in humans (Petrucci et al. 2014). These results are moderately comparable with our findings, and the differences former and present studies are most likely originates from use of different species samples, analyzing transcript level other than

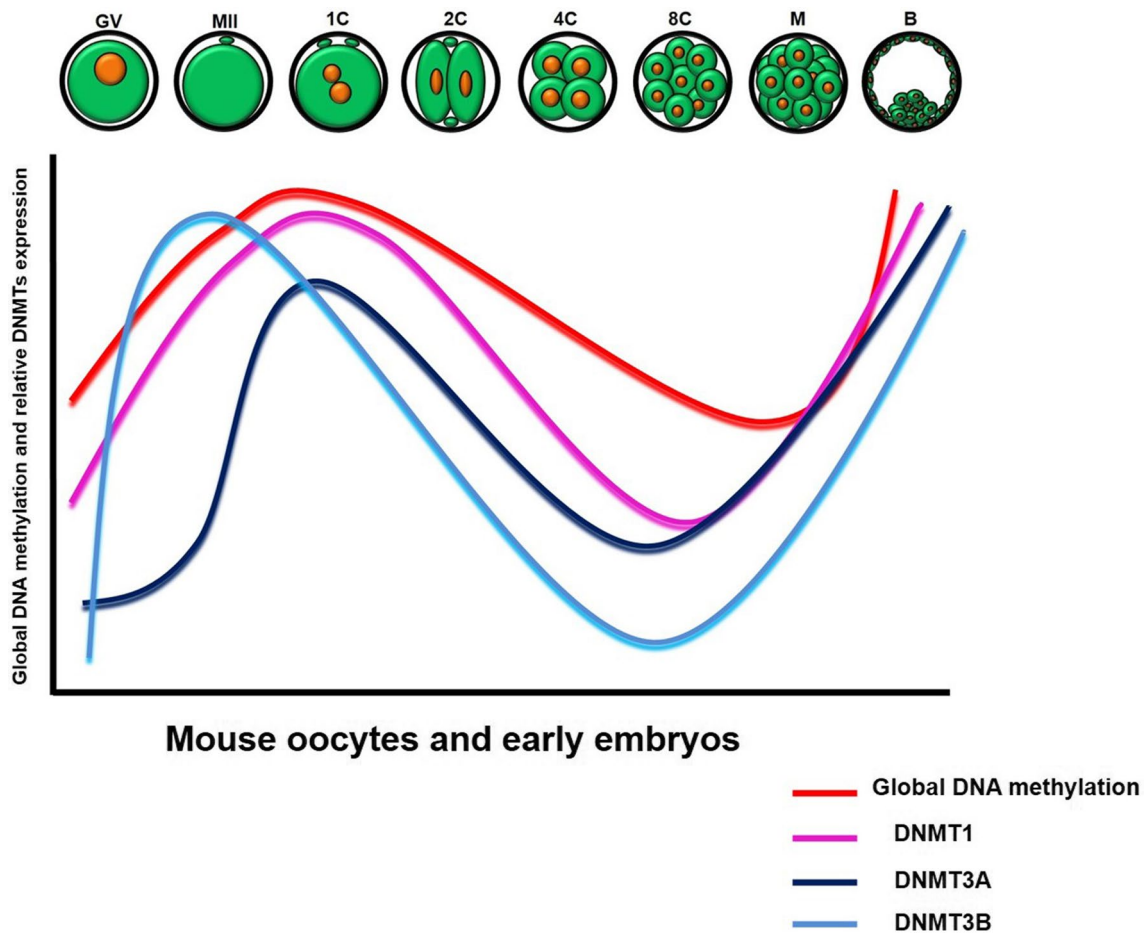


Fig. 4 Global DNA methylation profile and relative expression levels of DNMT1, DNMT3A and DNMT3B proteins in the mouse oocytes and early embryos. Red line, the global DNA methylation levels in the oocytes and early embryos. We have created this line based on the studies by Saitou et al. (2012) and Smallwood and Kelsey (2012). Expression level of DNMT1 gene (pink line), DNMT3A (navy blue

line) and DNMT3B (blue line) in the oocytes and early embryos. The expressional difference has been shown by distinct colour. *GV* germinal vesicle oocyte, *MII* metaphase II oocyte, *1C* 1-cell embryo, *2C* 2-cell embryo, *4C* 4-cell embryo, *8C* 8-cell embryo, *M* morula, *B* blastocyst. (Color figure online)

protein level in the previous investigations and employing distinct techniques to determine DNMT1 expression.

The *Dnmt3a* and *Dnmt3b* mRNAs are also found at detectable levels in mouse, cat and bovine oocytes (Bourc'his et al. 2001; Kaneda et al. 2004; O'Doherty et al. 2012) as well as in human oocytes (Huntriss et al. 2004). Ko et al. (2005) revealed that DNMT3A is highly localized in the cytoplasm of the 8-cell, morula and blastocyst stage embryos, but it is intensively resided in the nuclear regions of the 1-cell, 2-cell and 4-cell embryos in mice (Ko et al. 2005). On the other hand, Hirasawa et al. (2008) demonstrated that DNMT3A is abundantly resided in the nuclear region of the GV oocytes, 1-cell, 2-cell, 4-cell, 8-cell, morula stage embryos and that there is an intensive cytoplasmic expression in the MII oocytes in mouse (Hirasawa et al. 2008). Intriguingly, the blastocyst stage embryos do not exhibit any DNMT3A expression either in the nuclear or cytoplasm regions

(Hirasawa et al. 2008). The DNMT3B expression has not been observed in the GV oocytes and 1-cell embryos, and it is localized in the nuclear region of the 2-cell embryos and onward in mouse (Hirasawa et al. 2008). In contrast, Ko et al. (2005) reported that DNMT3B is mainly localized in the cytoplasm of the early embryos from 1-cell to blastocyst stages (Ko et al. 2005). We have revealed in the present study that both DNMT3A and DNMT3B are intensively detected in the nucleus, and weakly localized in the cytoplasm of the oocytes and early embryos. Among the studies, there are prominent differences about intracellular intensities of the DNMT3A and DNMT3B proteins in the mouse oocytes and early embryos, this may derive from that use of distinct technical applications and/or mouse strains from which oocytes and early embryos are obtained.

In bovines, Dobbs et al. (2013) observed that global DNA methylation is gradually decreased from 2-cell to

6–8 cell stage embryos meaning that the lowest DNA methylation levels has been detected at the 6–8 cell embryos, and the methylation reaches the highest levels in the blastocysts (Dobbs et al. 2013). In accordance with reducing global DNA methylation levels after 2-cell embryos, *DNMT3B* mRNA expression decreases from 2-cell to 6–8 cell embryo stages, and remarkably increases in the blastocyst stage embryos (Dobbs et al. 2013). The expression patterns of the *DNMT3B* mRNA from this study and DNMT3B protein expression from our study argues that DNMT3B likely functions in the genomic imprint and maintenance methylation establishments during early development in mouse and bovines.

As is known, the first lineage differentiation occurs in the 8-cell embryos, and increase of intercellular adhesion, polarization and compaction take place based on the increased cell–cell associations among blastomeres (Albert and Peters 2009). And, DNA methylation is at low levels in the 8-cell embryos (Dobbs et al. 2013). Consistent with this DNMT1, DNMT3A and DNMT3B protein expression is remarkably decreased at this stage. Since many proteins including E-cadherin, catenin, mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PtdIns3K), Wingless (Wnt), and Notch (Zhang et al. 2007) are highly expressed in the 8-cell embryos, the decreased global DNA methylation levels at this stage may be required for facilitating the expression of these genes in time.

In conclusion, this is the first detailed study analyzing subcellular localizations and relative expression levels of the DNMT1, DNMT3A and DNMT3B proteins in the mouse oocytes and early embryos. We found that DNMT1, DNMT3A and DNMT3B exhibit expressional fluctuations from GV oocytes to blastocyst stage embryos. These fluctuations are to large extent compatible with timely establishing global DNA methylation and genomic imprints in the oocytes and early embryos. We suppose that more detailed molecular biological studies are required to clearly understand the potential interactions of the DNMTs in establishing DNA methylation and in the regulation of early development-related genes during oogenesis and early embryo development.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

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