Polish Journal of Veterinary Sciences Vol. 18, No. 3 (2015), 549–556

DOI 10.1515/pjvs-2015-0071

Original article

Dynamic expression profile of DNA methyltransferases in rat testis development

H.X. Xu[1,2,a], J.Z. Qin[1,a], K.Y. Zhang[1], W.X. Zeng[1]

[1] College of Animal Science and Technology, Northwest A&F University, 22 Xinong Road, Yangling, Shaanxi 712100, P. R. China
[2] Institute of Genetics, College of Life Sciences, Xinyang Normal University, 237 Nanhu Road, Xinyang, Henan, 464000, P. R. China

Abstract

DNA methyltransferases (Dnmts) are unique and perform specific functions during male germ cell development. To further characterize the significance of Dnmts in the events leading to production of spermatozoa, we investigated whether the expression patterns in Dnmt1, Dnmt3a, Dnmt3b and Dnmt3l were apparent in rat testes at different time points during development. The qRT-PCR results showed that expression levels of *Dnmt3a* and *Dnmt3l* were abundant before birth and were present at the highest levels in testes tissue at 18.5 days postcoitus (dpc), and gradually decreased from day 0 postpartum (dpp) to 90 dpp. Expression of *Dnmt1* and *Dnmt3b* reached a peak after birth (P <0.01), and then gradually reduced until adulthood. Western blotting and immunolocalization analysis of Dnmt3a and Dnmt3b further confirmed the differential expression and localization of the two proteins during rat testis development. The dynamic expression profile of Dnmts implies specific and potentially nonredundant roles for each of these enzymes in the developing rat testis.

Key words: DNA methyltransferases, testis development, spermatogenesis, rat

Introduction

Spermatogenesis is a dynamic, complex process, tightly regulated by the precise control of a variety of factors, which occurs within the seminiferous tubules in the testis. This process involves not only the spermatogenic cells but also several types of somatic cells in testicular tissue, such as the Sertoli cell and Leydig cell (Costa et al. 2013, Urriola-Munoz et al. 2014). Sertoli cells play a key role in protection, support and nutrition of germ cells, while Leydig cells secrete testosterones, stimulate testis development, and regulate germ cell differentiation and spermatogenesis. Spermatogenesis depends not only on unique processes such as specialized transcription, meiosis and histone-to-protamine replacement, but also on various epigenetic events, for instance DNA methylation, which affects turn-on and turn-off of gene expression. Methylation of the mammalian genomic DNA at the 5 loci in cytosine residues via DNA methyltransferases (Dnmts) is an important epigenetic modification, which plays central regulatory roles in the control of cellular physiology, including embryonic development, cellular reprogramming, spermatogenesis,

Correspondence to: W.X. Zeng e-mail: zengwenxian2013@126.com
[a] These authors contributed equally to this work

X chromosome inactivation, genomic imprinting and transposon silencing (Li 2002, Pontier and Gribnau 2011, Cedar and Bergman 2012, Smith and Meissner 2013). DNA methylation patterns which established in the germ line during gametogenesis are largely erased at early stage of embryogenesis and are reset after implantation (Cedar and Bergman 2012). The Dnmts mainly includes the Dnmt3 family and Dnmt1. The Dnmt3 consists of two catalytically active members (Dnmt3a and Dnmt3b), and a catalytically inactive member called Dnmt3-like (Dnmt3l) (Jurkowska et al. 2011, Neri et al. 2013). Previous research showed that Dnmt3a and Dnmt3b mainly take part in catalyzing de novo methylation at the cytosine of unmethylated CpG sites during embryonic development; both of them have similar biochemical properties but distinct functions (Okano et al. 1998, Okano et al. 1999, Chen et al. 2003). In addition, the Dnmt3a genomic locus produces two transcripts giving rise to two proteins, the longer Dnmt3a1 and the shorter Dnmt3a2 (here collectively referred to as Dnmt3a), which differ in that a 219-amino-acid (aa) amino (N)-terminal tail is only present in Dnmt3a1. Dnmt3l may not possess DNA methylation activity, but plays a regulatory role in DNA methylation by enhancing or inhibiting the activity of Dnmt3a and Dnmt3b in different chromatin contexts (Bourc'his et al. 2001, Hata et al. 2002, Neri et al. 2013). Dnmt1 functions as a major maintenance methyltransferase in vivo, has a preference for hemimethylated CpGs and is critical for the maintenance of methylation patterns during DNA replication (Schaefer et al. 2007). In male mice, Dnmt1 is not detected in fetal gonocytes when methylation patterns are initially set up, but is detected in proliferating spermatogonia, as well as spermatocytes and round spermatids (Sakai et al. 2001, Goossens et al. 2011).

Although DNA methylation and Dnmts expression during murine germ cell development have been demonstrated (Reik and Dean 2001, La Salle and Trasler 2006, Schaefer et al. 2007), such information in rats is largely unknown. Sprague -Dawley rats have served as an important animal model for research in reproduction with excellent reproductive performance and maternal characteristics. Therefore, the goal of this study was to describe the dynamic expression of the Dnmts for identifying candidate methyltransferases which are likely to be involved in the DNA methylation process during testis development in rats.

Materials and Methods

Isolation of testes tissue

Sprague-Dawley rats were obtained from the Fourth Military Medical University (Xian, P. R. China). The animals were allowed to mate naturally. In the morning of a day when vaginal plugs were observed were considered to be 0.5 dpc and the day of delivery as 0 dpp. Testes were collected in fetal (15.5 dpc and 18.5 dpc) and postnatal (0 dpp, 7 dpp, 14 dpp, 21 dpp and 90 dpp) stages, three of them from each stage from three different individuals were fixed in carbazotic acid buffer solutions (saturation picric acid: acetic acid: methanol = 75 : 20 : 5) for 8-12 hours and the others were decapsulated and rinsed in sterile DEPC-treated saline, pooled, and frozen in liquid nitrogen for RNA extraction. Among them thirty male rats were collected at embryonic stage, and ten at each postnatal time point. The entire experimental procedure was approved by the Animal Care Commission of the College of Animal Science, Northwest A&F University, China.

RNA isolation, cDNA synthesis and qRT-PCR analysis

Total-RNA extractions were carried out from pooled testes from different developing stages (57 testes from each embryonic stages, and 27 testes from each postnatal time point) using Trizol (Takara, Japan). The first strand cDNA was synthesized by reverse transcription of 1-5 μg total RNA using M-MLV Reverse Transcriptase and Oligo$(dT)_{15}$ (Invitrogen, USA). qRT-PCR was performed on the IQ5 (Bio-Rad, USA) using the GoTaq® qPCR Master Mix (Promega, USA). Gene-specific primers (including each transcript) were used to determine the relative expression levels of *Dnmt1*, *Dnmt3a, Dnmt3b* and *Dnmt3l* according to the standard curve method, the primer sequences that were used are listed in Table 1. Gene expression levels were quantified relative to the expression of the β-*actin* gene, using Gene Expression Macro software (Bio-Rad, USA) by employing an optimized comparative Ct (ΔΔCt) value method. The expression level was calculated as 2^ (-ΔΔCt) to compare the relative expression, and one-way ANOVA was conducted to identify genes differing in expression; $P < 0.05$ was considered as significant. Data are presented as mean±SEM.

Western Blotting

Rat testes were prepared by homogenization in 0.15 M NaCl, 0.05M Tris.HCl (pH 7.5), 2 mg/ml leupeptin, 2 mg/ml aprotinin and 100 mg/ml PMSF. 30 fg proteins were denatured by heating at 100°C, separated by electrophoresis on 10% SDS polyacrylamide gels, and transferred to PVDF membranes. The primary antibodies used were polyclonal rabbit

Table 1. Primer sequences for the specific genes.

Gene	Primer sequence	Primer length	Product size	GeneBank sequence No.
Dnmt3a	F: GGTGTGTGTCGAGAAGCTCA R: CCAAGGGCCCACTCAATCAT	20 bp 20 bp	222 bp	NM_001003958.1
Dnmt3b	F:GGGCCGCTACCACGTTCAGG R:AGGGCCGTCCTGGCTCAAGT	20 bp 20 bp	178 bp	NM_001003959.1
Dnmt3l	F:GTATGCCCGGCCTCGCCAAG R:CAGGTCCGCGTGCTTGCTCT	20 bp 20 bp	208 bp	NM_001003964.1
Dnmt1	F:GGTTCTGCGCGGGGACAGAC R:CCGGCAACATGGCCTCAGGG	20 bp 20 bp	183 bp	NM_053354.3
β-actin	F:GCGTCCACCCGCGAGTACAA R:ACATGCCGGAGCCGTTGTCG	20 bp 20 bp	118 bp	NM_031144.3

anti-Dnmt3a (sc-20703) and polyclonal goat anti-Dnmt3b (sc-10236) (Santa Cruz, USA). The membrane was incubated in the primary antibody solution(1:1000) overnight at 4°C with agitation. Anti-rabbit IgG and anti-goat IgG were used as secondary antibodies (Santa Cruz, USA). The membrane was incubated in the secondary antibody reagent(1:1000) for 2 hours at room temperature. The membrane was washed 3 times for 5 min each time with TBS containing 0.05% Tween 20. The detection of proteins was performed using enhanced chemiluminescence (ECL), and then the blot was imaged using the FluorChem M multicolor fluorescence Western blot imaging system (ALPHA, USA).

Immunocytochemistry

The cross-sections were deparaffinized in xylene for 10 min and rehydrated in a descending alcohol series. Sections were washed for 5 min in phosphate-buffered saline (PBS). Next, tissue sections were performed antigen retrieval step by keeping them in boiling Tris-EDTA Buffer for 10 minutes, and then were pretreated after being cooled to room temperature, incubated with 3% H_2O_2 for blocking of endogenous peroxidases, the followed is the blocking step with 5% BSA for blocking non-specific binding. The specific primary antibodies for Dnmt3a and Dnmt3b were added and incubated overnight at 4°C. For the negative controls, PBS was added instead of primary antibody. The next day, sections were washed three times with PBS after which the biotin labeling secondary antibody (Beyotime, China) was added for one hour at room temperature, and then with HRP-Avidin working liquid (Beyotime, China). The sections were again washed three times with PBS. The visualization of the staining was done with Diaminobenzidine (DAB) (Beyotime, China). The preparations were immersed in PBS and counterstained with hematoxylin. The sections were dehydrated in a mounting series of alcohol and finally in xylene. Glycerol jelly mounting medium (Beyotime, China) was added before placing the cover slip. The images were visualized using Nikon 80i with NIS-Elements software (Nikon, Japan).

Results

Dynamic expression of DNA methyltransferases in the developing testes

As the establishment and maintenance of DNA methylation pattern are ongoing during gametogenesis, we examined the dynamic gene expression of *Dnmts* in the prenatal (15.5, 18.5 dpc) and postnatal (0, 7, 14, 21 and 90 dpp) testes using qRT-PCR. Expression of *Dnmt3a* was abundant before birth and was present at the highest levels at 18.5 dpc, which was much higher than that at any other time points (P <0.01), and gradually decreased from 0 dpp to 90 dpp (Fig. 1). *Dnmt3l* had a similar expression pattern with *Dnmt3a*, but also with minor differences, such as a dramatic drop of *Dnmt3l* expression detected after birth, about 54 times lower at 14 dpp than that at 18.5 dpc (Fig. 1). However, the expression pattern of *Dnmt3b* was different. Before birth, *Dnmt3b* expression was low, only increased most notably on 7 dpp and 14 dpp, whereas it was 5 times higher than that at the embryonic period. Compared with the *Dnmt3* family, the expression of *Dnmt1* was relatively low from 15.5 dpc to 90 dpp, while the expression pattern was very similar to *Dnmt3b* (Fig. 1). In general, the four genes were all present at the lowest levels at 90 dpp.

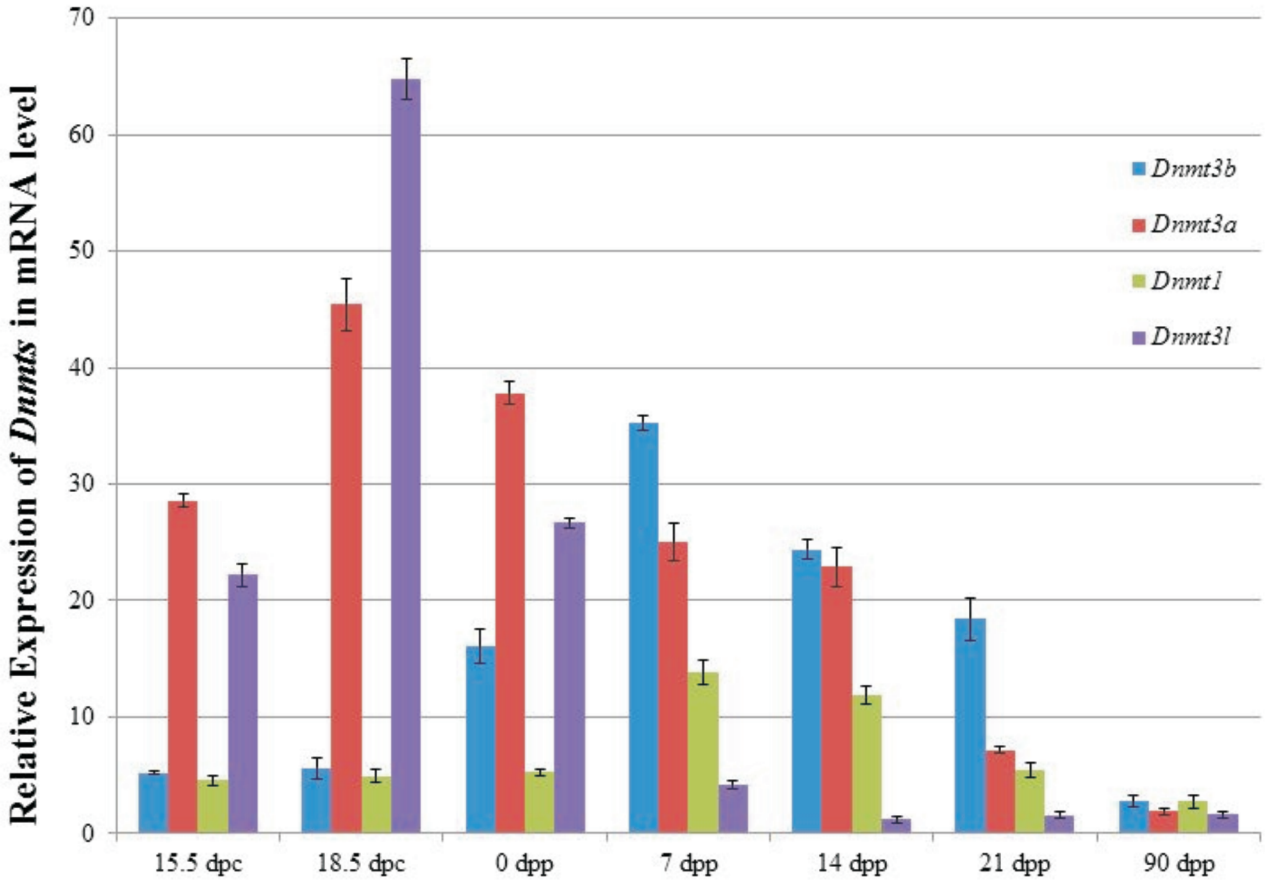

Fig. 1. Expression pattern of DNA methyltransferases in rat developing testes. Relative quantification of *Dnmt1*, *Dnmt3a*, *Dnmt3b* and *Dnmt3l* in testis mRNA populations was done in triplicate from pooled testes at the indicated developmental stages via qRT-PCR. For each DNA methyltransferase, the expression level was normalized to β-actin and measured with 2^ (-ΔΔt) value. Results were averaged from three independent replicates during all stages. Data are shown as the mean±SE of 3 independent replicates. Day postcoitus (dpc); day postpartum (dpp).

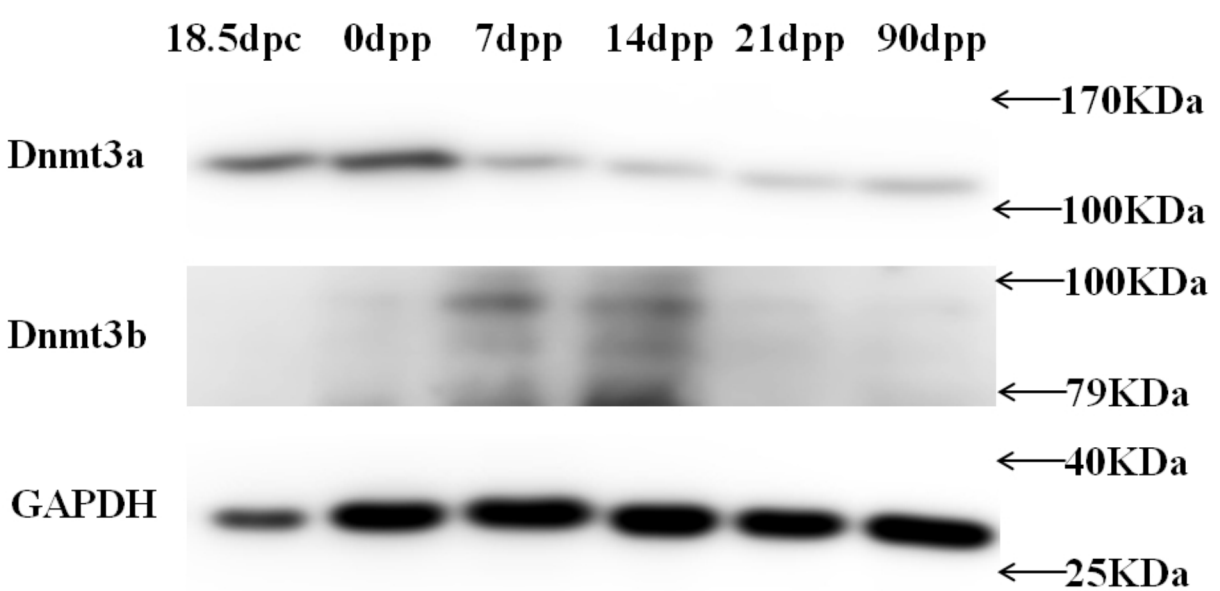

Fig. 2. Protein expression of Dnmt3a and Dnmt3b during rat testis development. Expression was analyzed by western blotting, and GAPDH expression levels were used as controls. Day postcoitus (dpc); day postpartum (dpp).

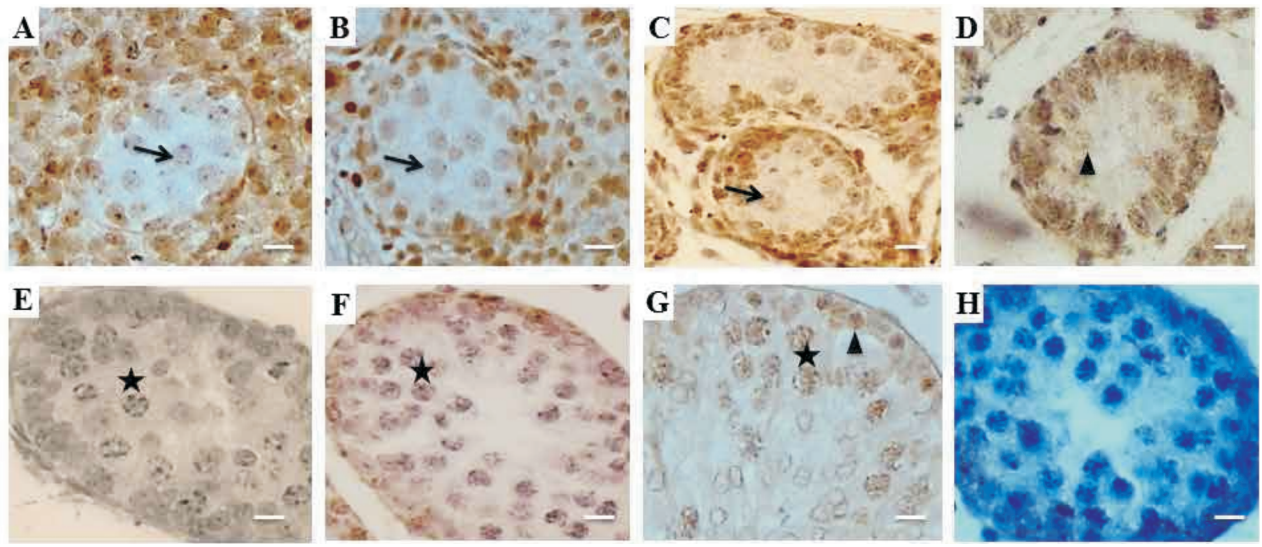

Fig. 3. Immuno-histochemistry of Dnmt3a in rat developmental testes. The positive signal was revealed by diaminobenzidine (DAB) and appears as a brownish precipitate. Panels A, B, C, D, E, F and G show sections of seminiferous tubules at 15.5 dpc, 18.5 dpc, 0 dpp, 7 dpp, 14 dpp, 21 dpp, 90 dpp of rat testis respectively. Day postcoitus (dpc); day postpartum (dpp), and gonocytes are indicated by black arrows; spermatocytes are indicated by asterisks; triangles indicate spermatogonias. No signal was obtained when PBS was used instead of the primary antibody as a negative control (Panel H), and all panels were counterstained with hematoxylin. Bars indicate 10 μm.

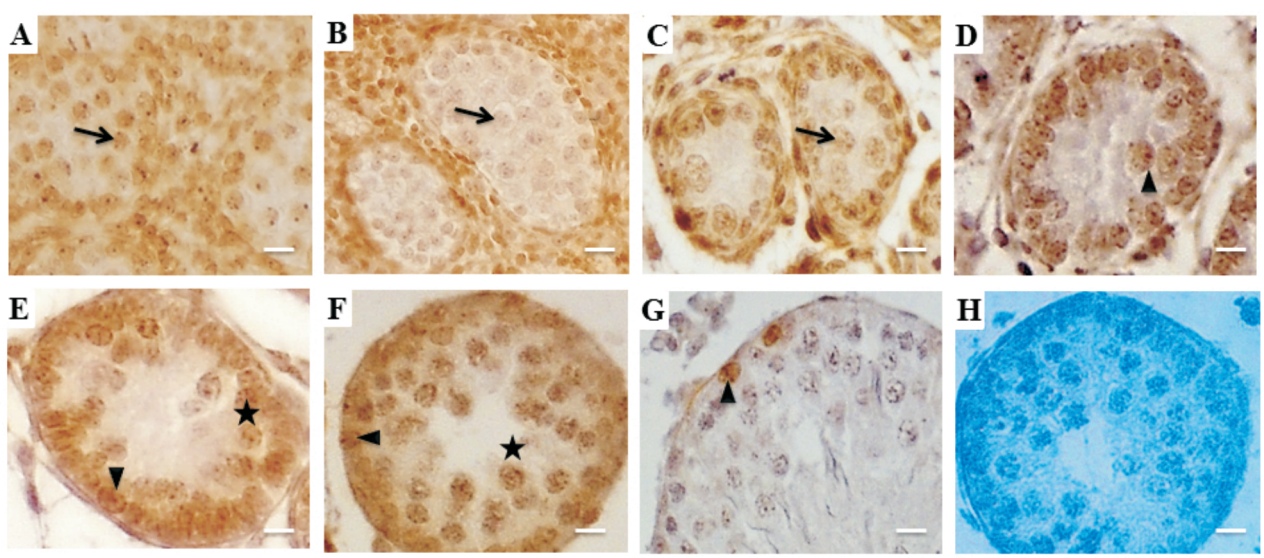

Fig. 4. Immuno-histochemistry of Dnmt3b in rat developmental testes. The positive signal was revealed by diaminobenzidine (DAB) and appears as a brownish precipitate. Panels A, B, C, D, E, F and G show sections of seminiferous tubules at 15.5 dpc, 18.5 dpc, 0 dpp, 7 dpp, 14 dpp, 21 dpp, 90 dpp of rat testis respectively.Day postcoitus (dpc); day postpartum (dpp), and gonocytes are indicated by black arrows; spermatocytes are indicated by asterisks; triangles indicate spermatogonias. No signal was obtained when PBS was used instead of the primary antibody as a negative control (Panel H), and all panels were counterstained with hematoxylin. Bar = 10 μm.

Western blotting analysis of Dnmt3a and Dnmt3b expression during testis development

Having established that the mRNA expression profiles of the *Dnmts*, *Dnmt3a* and *Dnmt3b* have distinct patterns in developing rat testes, they can be chosen as the two representative proteins for further analysis. The immunoreactive results showed that Dnmt3a was detected in all stages of testes development with its expression peaking at newborn, and then dropped gradually after birth (Fig. 2). Dnmt3b was expressed faintly in newborn rat testes, and was higher at 7 dpp and 14 dpp, then decreased in testes at 90 dpp. Most surprisingly, Dnmt3b was not detec-

	Germ cell type	15.5 dpc (%)	18.5 dpc (%)	0 dpp (%)	7 dpp (%)	14 dpp (%)	21 dpp (%)	90 dpp (%)
Dnmt3a	gonocyte	9	15	20				
	spermatogonia				0	0	0	62
	spermatocyte					0	0	35
Dnmt3b	gonocyte	89	10	91				
	spermatogonia				90	81	75	40
	spermatocyte					87	78	0

cell types exist with staining
cell types exist without staining
cell types not existing

Fig. 5. Quantitative results from germ cells expressing Dnmt3a and Dnmt3b in rat testes. For each stage (15.5 dpc, 18.5 dpc, 0 dpp, 7 dpp, 14 dpp, 21 dpp, 90 dpp), the percentage of germ cells expressing Dnmt3a or Dnmt3b was determined by choosing several sections from three testes of each developing stage and counting randomly the numbers of each cell type (gonocyte, spermatogonia, spermatocytes) to 200 as well as how many of these cells are positive for Dnmt3a or Dnmt3b expression.

ted at 18.5 dpc, indicating that Dnmt3b was either expressed at levels too low to be detected in these conditions or the mRNA remained untranslated.

Immunolocalization of Dnmt3a and Dnmt3b in testis cells

We further investigated the expression and localization of Dnmt3a and Dnmt3b in specific cell types in fetal and postnatal developing testes by immunohistochemistry. Dnmt3a was already present in the testis, but did not express intensely in prepuberty germ cells; not only was the percentage of gonocytes expressing Dnmt3a small, but the positive staining was also weaker than the Sertoli cells and Leydig cells that surround them (Fig. 3 A-C, Fig. 5). In addition, a positive reaction could not be detected in spermatogonia and spermatocyte from 7 dpp,14 dpp and 21 dpp (Fig. 3 D-F, Fig. 5). Compared with Dnmt3a, except for somatic cells, Dnmt3b showed a weak but distinct positive signal in gonocytes at 15.5 dpc, and the signal of Dnmt3b staining was decreased at 18.5 dpc (Fig. 4 A-B, Fig. 5), but after birth, the staining signals became relatively stronger again in spermatogonia and spermatocytes (Fig. 4 C-F, Fig. 5). It was noted that in the adult testes Dnmt3a could be detected in spermatogonia and spermatocytes, but Dnmt3b was expressed only in spermatogonia (Fig. 3 G-H, 4 G-H, Fig. 5).

Discussion

Spermatogenesis is an intricate and highly specialized process, and proper establishment of DNA methylation patterns in the dinucleotide sequence CpG during male germ cell development are crucial for gamete integrity and transmission of epigenetic information to the next generation (Bourc'his and Bestor 2004, Kaneda et al., 2004, Neri et al. 2013). Recent research in mice has clearly shown that Dnmts are involved in these important processes, and play a vital role in establishment and maintenance of DNA methylation patterns (Kaneda et al. 2004, La Salle and Trasler 2006, Shovlin et al. 2007). In rats, an earlier research reported that DNA MTase expression was developmentally regulated during the initiation of spermatogenesis (Jue et al. 1995). However, the four members of Dnmts were not distinguished. So it is still unclear if all Dnmts contribute equally to the establishment and the maintenance of DNA methylation patterns throughout germ cell development and spermatogenesis in rats. Rat gonocytes occur about 13 dpc-5 dpp (Culty 2013). At 6-7 dpp, the testis contains more spermatogonia cells; by 13-14 dpp, leptotene spermatocytes appear; by 19-23 dpp, pachytene spermatocytes are seen, and adult testes tissue contains multiple types of germ cells; development of any one generation of spermatogonia, spermatocytes or spermatids are closely integrated with that of other generations present in the same area of the seminiferous

tubules (Leblond and Clermont 1952, Malkov et al. 1998, Lagos-Cabre and Moreno 2008).

This study was the first to reveal that the expression levels of the *Dnmts* varies with the developmental stage undergoing transition of germ cell types in rats through qRT-PCR. The trends of *Dnmts* expression are similar between rat and mouse although a slight deviation was observed (La Salle et al. 2004, La Salle and Trasler 2006, Shovlin et al. 2007). However, western blotting and immunohistochemistry detection produced discordances from the qRT-PCR results, although the general trend was consistent. For example, *Dnmt3a* had relatively abundant expression in mRNA levels, but did not express, or had weak expression, in prepuberty germ cells under immunohistochemistry detection. This disagreement may be explained by the specific primer including Dnmt3a1 and Dnmt3a2 used in qRT-PCR, and the commercial Dnmt3a antibody against the amino-terminal portion of Dnmt3a only recognizing Dnmt3a1. Studies have reported that Dnmt3a2 may be the de novo DNA methyltransferase responsible for the global methylation of the genome, and strongly stains the germ cells (Sakai et al. 2004), and that Dnmt3a1 positively affects transcription of specific genes at the promoter level and targets chromosomal domains to play silence (Kotini et al. 2011). However, the specific roles for either Dnmt3a1 or Dnmt3a2 in de novo DNA methylation and transcription regulation from epigenetics are not yet known, and further investigations are urgently needed.

From the overall expression trend of *Dnmts*, we showed here that *Dnmt3a* and *Dnmt3l* have a high expression during the perinatal period (15.5 dpc-0 dpp), at which time a global methylation occurs in male germ cells. De novo methylation begins in the male germ line before birth at the gonocytes stage and finishes after birth and before the end of pachytene. Once established, methylation patterns must be maintained during DNA replication that takes place in spermatogonia and spermatocytes (Lees-Murdock et al. 2003, La Salle et al. 2004). Previous studies have identified that Dnmt3l cooperates with Dnmt3a to establish maternal imprints in mice, and the two members interact with each other to stimulate DNA methylation activity of Dnmt3a (Chedin et al. 2002, Hata et al. 2002). A crystallography study also showed that Dnmt3l forms a heterotetrameric complex with Dnmt3a, and this tetramerization prevents Dnmt3a oligomerization and localization in heterochromatin (Jia et al. 2007, Jurkowska et al. 2011). More interestingly, Dnmt3a-deficient mice have similar phenotypes with Dnmt3l-deficient mice, which exhibit infertility associated with abnormal chromosomal structures in germ cells (Kaneda et al. 2004, Oakes et al. 2007). These findings suggest that Dnmt3a may have a synergistic effect with Dnmt3l in the de novo methylation process during prenatal testis development. Dnmt3b, another de novo methylation member of the Dnmt3 family, had different expression patterns compared to *Dnmt3a* and *Dnmt3l*, but very similar to *Dnmt1*. *Dnmt3b* and *Dnmt1* had the highest expressed levels in postnatal rat testes, at times when the predominant cells are either mitotic spermatogonia (7 dpp) or spermatocytes entering meiotic differentiation (14 dpp) (Malkov et al. 1998, Lagos-Cabre and Moreno 2008). It is possible that Dnmt3b and Dnmt1 plays a key role at these important stages undergoing transition of germ cell types in rats to ensure proper pairing and recombination between homologous chromosomes. However, these results have some variance with the previous research that Dnmt3a and Dnmt3b have similar biochemical properties, and mainly play a key role in catalyzing de novo methylation at the cytosine of unmethylated CpG sites during embryonic development (Okano et al. 1998, Okano et al. 1999, Chen et al. 2003). Further investigations are needed to evaluate whether the different expression levels of Dnmt3a and Dnmt3b in rat testis development are associated with their role in spermatogenesis.

The results presented in this study showed that Dnmts are expressed in a defined, spatiotemporal manner during rat testis development. Testicular levels of specific transcripts vary according to the rat developmental stages. Our data support the idea that each DNA methyltransferase is expressed in a specific manner at different times during spermatogenesis, which implies specific and potentially nonredundant roles for each of these enzymes.

Acknowledgements

This study was supported by a grant from National Natural Science Foundation of China (31072029, 31272439 and 31230048).

References

Bourc'his D, Bestor TH (**2004**) Meiotic catastrophe and retrotransposon reactivation in male germ cells lacking Dnmt3L. Nature 431: 96-99.

Bourc'his D, Xu GL, Lin CS, Bollman B, Bestor TH (**2001**) Dnmt3L and the establishment of maternal genomic imprints. Science 294: 2536-2539.

Cedar H, Bergman Y (**2012**) Programming of DNA methylation patterns. Annu Rev Biochem 81: 97-117.

Chedin F, Lieber MR, Hsieh CL (**2002**) The DNA methyltransferase-like protein DNMT3L stimulates de novo methylation by Dnmt3a. Proc Natl Acad Sci USA 99: 16916-16921.

Chen T, Ueda Y, Dodge JE, Wang Z, Li E (**2003**) Establishment and maintenance of genomic methylation patterns in mouse embryonic stem cells by Dnmt3a and Dnmt3b. Mol Cell Biol 23: 5594-5605.

Costa DS, Faria FJ, Fernandes CA, Silva JC, Auharek SA (**2013**) Testis morphometry and kinetics of spermatogenesis in the feral pig (Sus scrofa). Anim Reprod Sci 142: 63-70.

Culty M (**2013**) Gonocytes, from the fifties to the present: is there a reason to change the name? Biol Reprod 89: 46.

Goossens E, Bilgec T, Van Saen D, Tournaye H (**2011**) Mouse germ cells go through typical epigenetic modifications after intratesticular tissue grafting. Hum Reprod 26: 3388-3400.

Hata K, Okano M, Lei H, Li E (**2002**) Dnmt3L cooperates with the Dnmt3 family of de novo DNA methyltransferases to establish maternal imprints in mice. Development 129: 1983-1993.

Jia D, Jurkowska RZ, Zhang X, Jeltsch A, Cheng X (**2007**) Structure of Dnmt3a bound to Dnmt3L suggests a model for de novo DNA methylation. Nature 449: 248-251.

Jue K, Benoit G, Alcivar-Warren AA, Trasler JM (**1995**) Developmental and hormonal regulation of DNA methyltransferase in the rat testis. Biol Reprod 52: 1364-1371.

Jurkowska RZ, Jurkowski TP, Jeltsch A (**2011**) Structure and function of mammalian DNA methyltransferases. Chembiochem 12: 206-222.

Jurkowska RZ, Rajavelu A, Anspach N, Urbanke C, Jankevicius G, Ragozin S, Nellen W, Jeltsch A (**2011**) Oligomerization and binding of the Dnmt3a DNA methyltransferase to parallel DNA molecules: heterochromatic localization and role of Dnmt3L. J Biol Chem 286: 24200-24207.

Kaneda M, Okano M, Hata K, Sado T, Tsujimoto N, Li E, Sasaki H (**2004**) Essential role for de novo DNA methyltransferase Dnmt3a in paternal and maternal imprinting. Nature 429: 900-903.

Kotini AG, Mpakali A, Agalioti T (**2011**) Dnmt3a1 upregulates transcription of distinct genes and targets chromosomal gene clusters for epigenetic silencing in mouse embryonic stem cells. Mol Cell Biol 31: 1577-1592.

La Salle S, Mertineit C, Taketo T, Moens PB, Bestor TH, Trasler JM (**2004**) Windows for sex-specific methylation marked by DNA methyltransferase expression profiles in mouse germ cells. Dev Biol 268: 403-415.

La Salle S, Trasler JM (**2006**) Dynamic expression of DNMT3a and DNMT3b isoforms during male germ cell development in the mouse. Dev Biol 296: 71-82.

Lagos-Cabre R, Moreno RD (**2008**) Mitotic, but not meiotic, oriented cell divisions in rat spermatogenesis. Reproduction 135: 471-478.

Leblond CP, Clermont Y (**1952**) Definition of the stages of the cycle of the seminiferous epithelium in the rat. Ann N Y Acad Sci 55: 548-573.

Lees-Murdock DJ, De Felici M, Walsh CP (**2003**) Methylation dynamics of repetitive DNA elements in the mouse germ cell lineage. Genomics 82: 230-237.

Li E (**2002**) Chromatin modification and epigenetic reprogramming in mammalian development. Nat Rev Genet 3: 662-673.

Malkov M, Fisher Y, Don J (**1998**) Developmental schedule of the postnatal rat testis determined by flow cytometry. Biol Reprod 59: 84-92.

Neri F, Krepelova A, Incarnato D, Maldotti M, Parlato C, Galvagni F, Matarese F, Stunnenberg HG, Oliviero S (**2013**) Dnmt3L antagonizes DNA methylation at bivalent promoters and favors DNA methylation at gene bodies in ESCs. Cell 155: 121-134.

Oakes CC, La Salle S, Smiraglia DJ, Robaire B, Trasler JM (**2007**) A unique configuration of genome-wide DNA methylation patterns in the testis. Proc Natl Acad Sci U S A 104: 228-233.

Okano M, Bell DW, Haber DA, Li E (**1999**) DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. Cell 99: 247-257.

Okano M, Xie S, Li E (**1998**) Cloning and characterization of a family of novel mammalian DNA (cytosine-5) methyltransferases. Nat Genet 19: 219-220.

Pontier DB, Gribnau J (**2011**) Xist regulation and function explored. Hum Genet 130: 223-236.

Reik W, Dean W (**2001**) DNA methylation and mammalian epigenetics. Electrophoresis 22: 2838-2843.

Sakai Y, Suetake I, Itoh K, Mizugaki M, Tajima S, Yamashina S (**2001**) Expression of DNA methyltransferase (Dnmt1) in testicular germ cells during development of mouse embryo. Cell Struct Funct 26: 685-691.

Sakai Y, Suetake I, Shinozaki F, Yamashina S, Tajima S (**2004**) Co-expression of de novo DNA methyltransferases Dnmt3a2 and Dnmt3L in gonocytes of mouse embryos. Gene Expr Patterns 5: 231-237.

Schaefer CB, Ooi SK, Bestor TH, Bourc'his D (**2007**) Epigenetic decisions in mammalian germ cells. Science 316: 398-399.

Shovlin TC, Bourc'his D, La Salle S, O'Doherty A, Trasler JM, Bestor TH, Walsh CP (**2007**) Sex-specific promoters regulate Dnmt3L expression in mouse germ cells. Hum Reprod 22: 457-467.

Smith ZD, Meissner A (**2013**) DNA methylation: roles in mammalian development. Nat Rev Genet 14: 204-220.

Urriola-Munoz P, Lizama C, Lagos-Cabre R, Reyes JG, Moreno RD (**2014**) Differential expression and localization of ADAM10 and ADAM17 during rat spermatogenesis suggest a role in germ cell differentiation. Biol Res 47: 31.