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Dynamic expression of DNMT3a and DNMT3b isoforms during male germ cell development in the mouse

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Abstract

In the male germ line, sequence-specific methylation patterns are initially acquired prenatally in diploid gonocytes and are further consolidated after birth during spermatogenesis. It is still unclear how DNA methyltransferases are involved in establishing and/or maintaining these patterns in germ cells, or how their activity is regulated. We compared the temporal expression patterns of the postulated de novo DNA methyltransferases DNMT3a and DNMT3b in murine male germ cells. Mitotic, meiotic and post-meiotic male germ cells were isolated, and expression of various transcript variants and isoforms of *Dnmt3a* and *Dnmt3b* was examined using Quantitative RT-PCR and Western blotting. We found that proliferating and differentiating male germ cells were marked by distinctive expression profiles. Dnmt3a2 and Dnmt3b transcripts were at their highest levels in type A spermatogonia, decreased dramatically in type B spermatogonia and preleptotene spermatocytes and rose again in leptotene/zygotene spermatocytes, while Dnmt3a expression was mostly constant, except in type B spermatogonia where it increased. In all cases, expression declined as pachynema progressed. At the protein level, DNMT3a was the predominant isoform in type B spermatogonia, while DNMT3a2, DNMT3b2, and DNMT3b3 were expressed throughout most of spermatogenesis, except in pachytene spermatocytes. We also detected DNMT3a2 and DNMT3b2 in round spermatids. Taken together, these data highlight the tightly regulated expression of these genes during spermatogenesis and provide evidence that DNMTs may be contributing differentially to the establishment and/or maintenance of methylation patterns in male germ cells.

Keywords: DNA methyltransferase; Dnmt1; Dnmt3a; Dnmt3b; Germ line; DNA methylation; Spermatogenesis; Male germ cells; Epigenetic

Introduction

Methylation of genomic DNA is an epigenetic regulatory mechanism involved in controlling the transcriptional activity of genes and establishing higher order chromatin structures to preserve genome integrity (reviewed by Goll and Bestor, 2004). In mammals, DNA methylation patterns are initially reprogrammed during germ cell development. The patterns differ markedly between male and female gametes, especially at imprinted loci where methylation differences have important implications for allele-specific gene expression in the offspring (reviewed by Reik and Walter, 2001). In the mouse, a major demethylation event takes place in both germ lines between embryonic day (E) 10.5 and E12.5, around the time when primordial germ cells (PGCs) enter the gonads (Hajkova et al., 2002; Kato et al., 1999; Lane et al., 2003; Lee et al., 2002; Szabo and Mann, 1995; Szabo et al., 2002).

Following erasure, DNA methylation patterns are then reestablished in a sex- and sequence-specific manner during gametogenesis. In the male germ line, methylation acquisition begins before birth, in prospermatogonia (Davis et al., 1999, 2000; Kafri et al., 1992; Lees-Murdock et al., 2003; Li et al., 2004; Ueda et al., 2000; Walsh et al., 1998). Between E15 and E19 germ cells begin to stain strongly with an antibody directed against methylated cytosine, indicating the timing of increases in overall global methylation; further methylation changes occur in the few days after birth (Coffigny et al., 1999). At the

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individual sequence level, the majority of methylated CpGs in the genome are found in repetitive DNA sequences. For repetitive elements such as intracisternal A particles (IAPs), long interspersed nuclear elements (LINEs) and satellite sequences, methylation acquisition is for the most part complete by E17.5 (Lees-Murdock et al., 2003; Walsh et al., 1998). Remethylation of imprinted genes also begins around E15.5, but the process is only completed after birth (Davis et al., 1999, 2000; Li et al., 2004). Developmental studies have shown that the imprinted gene *H19* begins to acquire its methylation marks between E15.5 and E18.5, but only becomes fully methylated postnatally by pachynema (Davis et al., 1999, 2000). Similarly, Chaillet et al. (1991) have demonstrated that an imprinted transgene initially gains its methylation before birth, but the process is only completed postnatally. Assessing the methylation status of a few testis-specific genes has further substantiated methylation acquisition to be continual during spermatogenesis; while some genes are demethylated prior to their expression in the testis, others become de novo methylated (reviewed by Maclean and Wilkinson, 2005). A feature unique to the male germ line is that, in parallel to their establishment, methylation marks must be maintained during DNA replication in spermatogonia and preleptotene spermatocytes; these cells are therefore capable of de novo and maintenance methylation. Conversely in the female germ line, methylation patterns are acquired postnatally during the oocyte growth phase, after the pachytene phase of meiosis is completed and DNA has been replicated (Kono et al., 1996; Lucifero et al., 2002; Walsh et al., 1998).

Both de novo and maintenance DNA (cytosine-5)-methyltransferases (DNMTs) work in concert to create and propagate genomic methylation patterns. Currently, five DNMTs have been characterized and are classified according to similarities found in their C-terminal catalytic domain: DNMT1, DNMT2, DNMT3a, DNMT3b, and DNMT3L (reviewed by Goll and Bestor, 2004). Of these, only DNMT1, DNMT3a, and DNMT3b have been proven to have catalytic activity in vivo. DNMT1 is the major methyltransferase in somatic tissues; it has a preference for hemimethylated DNA and is critical for the maintenance of methylation patterns after DNA replication (Bestor, 1992; Li et al., 1992; Lei et al., 1996; Yoder et al., 1997). Sex-specific exons control the expression of *Dnmt1* in the mouse germ line (Mertineit et al., 1998). In the male, DNMT1 is not detected in prenatal gonocytes when methylation patterns are initially laid down, but it is detected in proliferating spermatogonia, as well as leptotene/zygotene spermatocytes (Jue et al., 1995; La Salle et al., 2004; Sakai et al., 2001). Complete downregulation of DNMT1 during pachynema is associated with the expression of Dnmt1p, an untranslated pachytene-specific *Dnmt1* transcript (Jue et al., 1995; Mertineit et al., 1998), whereas DNMT1 becomes reexpressed in round spermatids (Jue et al., 1995; Trasler et al., 1992). It is still unclear which role DNMT1 plays during meiotic prophase or in round spermatids in the absence of DNA replication.

In contrast, DNMT3a and DNMT3b have been postulated to function primarily as de novo DNA methyltransferases (Okano et al., 1998). They are expressed at high levels in mouse embryonic stem (ES) cells and during embryonic development (Okano et al., 1998, 1999; Chen et al., 2003; Watanabe et al., 2002). Expression of *Dnmt3a* is controlled by the use of alternate promoters to produce two different isoforms (Chen et al., 2002). DNMT3a is expressed ubiquitously at low levels and localizes to heterochromatin, suggestive of a housekeeping role. In contrast, DNMT3a2 has been suggested to be more important to de novo methylation because it is expressed at high levels in embryonic stem cells and shows restricted expression in tissues known to undergo de novo methylation such as the testis and the ovary, in addition to localizing to euchromatin. All known isoforms of *Dnmt3b* result from alternative splicing of exons 11, 22, and/or 23 in various combinations (Chen et al., 2002; Ishida et al., 2003; Okano et al., 1998; Weisenberger et al., 2004). Of these, only DNMT3b1 (full-length isoform) and DNMT3b2 (shorter isoform missing the amino acids encoded by exon 11) are capable of DNA methylation (Aoki et al., 2001; Okano et al., 1998). Presumably, the other isoforms are incapable of methylating DNA since their catalytic domain is compromised by splicing of exons 22 and 23; however, they could act as regulators of DNA methylation (Aoki et al., 2001; Chen et al., 2002; Okano et al., 1998; Weisenberger et al., 2004). Interestingly, DNMT3b1 and DNMT3b6 appear to be expressed only in ES cells, while DNMT3b2 and DNMT3b3 are expressed in a restricted manner in somatic tissues (Chen et al., 2002; Weisenberger et al., 2004). We have previously shown that expression of *Dnmt3a* and *Dnmt3b* is highly modulated during testis development (La Salle et al., 2004). Recently, Kaneda et al. (2004) have shown that early germ cell-specific inactivation of *Dnmt3a*, but not *Dnmt3b*, impairs the establishment of de novo methylation patterns in male germ cells, more specifically at paternally imprinted loci, without affecting the methylation status of repeat sequences. Interestingly, inactivation of *Dnmt3L*, a member of the DNMT3 family that lacks DNA methyltransferase activity, produces a similar phenotype: deficient males are infertile, and their germ cells show abnormal DNA methylation acquisition of some repeat elements and imprinted loci (Bourc'his and Bestor, 2004; Bourc'his et al., 2001; Hata et al., 2002; Webster et al., 2005). Studies have started to look at the expression of these genes in germ cells (Lees-Murdock et al., 2005; Sakai et al., 2004; Watanabe et al., 2004), but detailed developmental studies monitoring expression of individual isoforms in key cell types and times when de novo and maintenance methylation are occurring during spermatogenesis are still lacking.

The role each DNA methylating enzyme plays in the genome-wide methylation events that take place throughout male germ cell development, as well as the mechanisms governing their expression, are still unclear. Spermatogenesis is a complex process during which diploid spermatogonia divide and mature into spermatocytes that undergo meiosis to produce haploid spermatids; spermatids go through a specialized maturation process termed spermiogenesis in order to become sperm. Establishment of DNA methylation patterns occurs in spermatogonia and in spermatocytes but is not thought to happen in spermatids. In parallel, maintenance methylation has always been thought to take place in the context of DNA