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# Comparative functional genomics of mammalian DNA methyltransferases

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Identifying molecular mechanisms regulating mammalian embryonic development has been an exciting quest for Erdogan Memili. He has a passion for uncovering mysteries behind molecular reprogramming events, including embryonic genome activation, at the onset of development. His current research focuses on identifying molecular determinants of high developmental competency and fertility in mammalian gametes and preimplantation embryos using functional genomics approaches. He has earned his PhD in Endocrinology and Reproductive Physiology from the University of Wisconsin-Madison, and had post-doctoral training in embryonic stem cells and epigenetics at the Harvard Medical School.

**Abstract** DNA methylation involves biochemical modification of DNA by addition of methyl groups onto CpG dinucleotides, and this epigenetic mechanism regulates gene expression in disease and development. Mammalian DNA methyltransferases, DNMT (DNMT1, DNMT3A and DNMT3B), together with the accessory protein DNMT3L establish specific DNA methylation patterns in the genome during gametogenesis, embryogenesis and somatic tissue development. The present study addresses the structural and functional conservation of the DNMT in humans, mice and cattle and the patterns of mRNA abundance of the different enzymes during embryogenesis to improve understanding of epigenetic regulation in early development. The findings showed a high degree of structural and functional conservation among the human, mouse, and bovine DNMT. The results also showed similar patterns of transcript abundance for all of the proteins at different stages of early embryo development. Remarkably, all of the DNMT with an important role in DNA methylation (DNMT1, DNMT3A, DNMT3B, and DNMT3L) show a greater degree of structural similarity between human and bovine than that between human and mouse. These results have important implications for the selection of an appropriate model for study of DNA methylation during early development in humans.


**KEYWORDS:** Bovine, embryogenesis, DNA methylation, epigenetics

## Introduction

Regulation of gene expression without any actual modification of DNA sequence, or epigenetics, is a topic that has garnered increasing attention in the post-genomic era. Epigenetic regulation causes differential expression of genes depending on the type of tissue and stage of development. DNA methylation is a well-studied epigenetic process with a variety of key roles in gene repression (Bird, 2002; Meehan and Stancheva, 2001), control of cellular differentiation (Ehrlich, 2003a; Li, 2002), gene regulation during embryonic development (Ng and Bird, 1999; Okano et al.,

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1999; Reik et al., 2001), X chromosome inactivation, and genomic imprinting (Chow and Brown, 2003; Murphy and Jirtle, 2003). Other important roles of DNA methylation include silencing of endogenous retroviruses, suppression of homologous recombination, and protection from the mutagenic effects of the abundant transposable elements in mammalian genomes (Bestor, 2000; Yoder et al., 1997).

DNA methylation results from the activity of a family of enzymes called DNA methyltransferases (DNMT) that catalyse the addition of a methyl group to the cytosine residues at CpG dinucleotides (Bird, 1986). Four different DNA methyltransferases have been identified and their structure as well as functions have been extensively reviewed (Bestor, 2000; Hermann et al., 2004; Kumar et al., 1994). These DNA methyltransferases are widely conserved among different species. Mammalian DNMT contain at least three structural regions: the N-terminal regulatory domain, which is responsible for the localization of DNMT in the nucleus, the C-terminal catalytic domain, which is responsible for the methyltransferase activity, and the central linker, consisting of repeated glycyl lysine dipeptides (Araujo et al., 2001). The regulatory N-terminal domain contains a proliferating cell nuclear antigen-binding domain (PBD), a nuclear localization signal (NLS), a cysteine-rich zinc finger DNA-binding motif (ATRX), a polybromo homology domain (PHD), and a PWWP (Pro-Trp-Trp-Pro) tetrapeptide chromatin-binding domain (Bestor, 2000). The C-terminal DNMT catalytic domain contains 10 different characteristic sequence motifs, six of which are evolutionally conserved: I, IV, VI, VIII, IX and X (Turek-Plewa and Jagodzinski, 2005).

The first identified DNA methyltransferase, DNMT1, plays a key role in maintenance of DNA methylation by restoring the methylation pattern on newly synthesized hemi-methylated DNA strands during replication (Bestor et al., 1992; Pradhan et al., 1999). An interesting DNMT1 isoform lacking 118 amino acids from the N-terminal domain (DNMT1o) is exclusively active in oocytes and preimplantation embryos and is later replaced by the regular DNMT1 (Bestor, 2000). DNMT2, the smallest mammalian DNMT, contains only the methyltransferase motifs of the C-terminal domain, and although it is highly conserved, its biological function has been enigmatic (Dong et al., 2001; Yoder and Bestor, 1998). Some studies show that DNMT2 acts as a DNA methyltransferase (Kunert et al., 2003), while other studies have detected little DNA methylation activity. Recent research has demonstrated that DNMT2 methylates $tRNA^{Asp}$ in the cytoplasm (Goll et al., 2006; Rai et al., 2007). DNMT3a and DNMT3b are similar proteins that have been identified as de-novo DNA methyltransferases acting upon hemi-methylated and unmethylated DNA with equal efficiency during early embryonic development (Okano et al., 1998, 1999). The DNA cytosine-like 5-methyltransferase (DNMT3L) protein lacks the most important C-terminal methyltransferase motifs, but possesses an active nuclear localization signal sequence (NLS) and the ATRX zinc finger motif (identical to the ones in DNMT3A and DNMT3B enzymes), which enable nucleus translocation and DNA binding. DNMT3L has a plant homeodomain (PHD)-like motif that activates histone deacetylase 1 (HDAC1) (Deplus et al., 2002; Turek-Plewa and Jagodzinski, 2005) and has recently also been shown to recognize histone H3 tails that are unmethylated at lysine 4 (Ooi et al., 2007) and induce *de novo* DNA methylation by recruitment or activation of DNMT3A2. Thus, DNMT3L has a dual role in de-novo DNA methylation, interacting with unmethylated lysine 4 of histone H3 through its PHD-like domain, while interacting and activating DNMT3A through its carboxy-terminal domain (Jia et al., 2007).

Modulation of DNA methylation during early embryogenesis is a dynamic and developmentally regulated process. Genome-wide DNA demethylation, with the exception of methylation marks at imprinted genes, occurs during the first embryonic stages (Oswald et al., 2000; Reik et al., 2001). The paternal genome is significantly and actively demethylated within hours of fertilization, before the onset of DNA replication, whereas the maternal genome is demethylated after several cleavage divisions (Mayer et al., 2000). This demethylation is followed by de-novo DNA methylation, which establishes a new embryonic methylation pattern. The DNA of blastocysts is thus relatively undermethylated. The exact biological function of this dynamic reprogramming of DNA methylation in early development is unknown. Several studies support the hypothesis that DNA methylation is crucial for the establishment of gene expression during embryonic development (Eden and Cedar, 1994; Jones et al., 1998). However, recent data suggest that DNA methylation may only affect genes that are already silenced by other mechanisms in the embryo, indicating that DNA methylation could be a consequence rather than a cause of gene silencing during development (Bestor, 2000; Bird, 2002; Walsh and Bestor, 1999).

Genomic imprinting is the best example of epigenetic control of gene expression. It is established during gametogenesis and early embryonic development (Edwards, 2003). For a group of genes only the maternal copy is expressed while the paternal copy remains silenced through DNA methylation. For other genes, the paternal copy is the only transcriptionally active one, while the maternal allele is not expressed (Murphy and Jirtle, 2003; Swales and Spears, 2005). In humans, genomic imprinting errors have been associated with several disorders including Prader-Willi, Angelman, Beckwith-Wiedemann, and Russell—Silver syndromes, Wilm's tumour and retinoblastoma (Edwards and Ludwig, 2003; Prescott and Wilkie, 2007). Animal studies have also shown a direct link between genomic imprinting disorders and the use of assisted reproduction. The large offspring syndrome (LOS) is one of the best studied imprinting disorders in cattle linked with IVF and culture of embryos (Young et al., 1998). It consists of unusually large offspring that can also exhibit a number of organ defects caused by imprinting errors in the IGF2R gene (Lazzari et al., 2002; McEvoy et al., 2000).

In the last decade, several reports have shown a higher risk of genomic imprinting disorders for children conceived by means of assisted reproductive technologies, namely IVF and intracytoplasmic sperm injection (ICSI) (Allen and Reardon, 2005; Bertelsmann et al., 2008; Georgiou et al., 2006; Horsthemke and Ludwig, 2005; Maher, 2005; Maher et al., 2003; Niemitz and Feinberg, 2004; Paoloni-Giacobino, 2006). It is important to consider that the increased frequency of imprinting disorders among children conceived by different assisted reproduction techniques cannot solely be attributed to the reproductive technologies since patients requesting assisted reproduction have a lower fertility rate