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# Extensive and orderly reprogramming of genome-wide chromatin modifications associated with specification and early development of germ cells in mice

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**Abstract**

Induction of mouse germ cells occurs from the proximal epiblast at around embryonic day (E) 7.0. These germ cells then migrate to, and enter the gonads at about E10.5 after which they undergo epigenetic reprogramming including erasure of parental imprints. However, the epigenetic properties acquired by nascent germ cells and the potential remodeling of these epigenetic marks in the subsequent migratory period have been largely unexplored. Here we have used immunohistochemistry to examine several genome-wide epigenetic modifications occurring in germ cells from their specification to their colonization of the genital ridges. We show that at around E8.0, germ cells concomitantly and significantly reduce H3-K9 dimethylation and DNA methylation, two major repressive modifications for gene expression. These events are preceded by the transient loss of all the DNA methyltransferases from their nuclei. By contrast, germ cells substantially increase the levels of H3-K27 trimethylation, another repressive modification with more plasticity, at E8.5–9.0 and maintain this state until at least E12.5. H3-K4 methylation and H3-K9 acetylation, modifications associated with transcriptionally permissive/active chromatin, are similar in germ and surrounding somatic cells but germ cells transiently increase these marks sharply upon their entry into the genital ridge. H3-K9 trimethylation, a hallmark of centromeric heterochromatin, is kept relatively constant during the periods examined. We suggest that this orderly and extensive epigenetic reprogramming in premigratory and migratory germ cells might be necessary for their reacquisition of underlying totipotency, for subsequent specific epigenetic remodeling, including the resetting of parental imprints, and for the production of gametes with an appropriate epigenotype for supporting normal development.


*Keywords:* Germ cells; Epigenetics; Histone modification; DNA methylation; Reprogramming; Transcriptional repression

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## Introduction

Germ line cells are exclusively programmed for the creation of a new organism, allowing transmission of original genetic information across generations. The unique capacity of germ cells to fulfill this function relies on their innate epigenetic reprogramming ability and their maintenance of underlying genomic totipotency (Surani, 2001). This is particularly challenging in mammals in which the germ line is derived from epiblast cells and is not predetermined at fertilization as in many other organisms (Saitou et al., 2003). In mice, the germ line epigenome seems to be remodeled in a temporally and spatially controlled manner throughout its development, which ultimately leads to the formation of haploid gametes that upon fusion initiate a new life. Perturbation of these processes leads to abnormal embryonic development, demonstrating the importance of epigenetic reprogramming in the germ line (Li, 2002). However, the precise mechanisms regulating these processes remain largely unknown.

Methylation of cytosine on the CpG dinucleotide is a key epigenetic modification involved in the imposition of differential epigenetic states on the genome, functionally associated with parental imprints, control of gene expression and genome structure (Bird, 2002). Generally, methylated DNA acts as a repressive element for transcription by creating a local heterochromatic structure (Li, 2002). CpG methylation is highly concentrated at centromeric heterochromatin and widely distributed throughout repressed regions within euchromatin. DNA methyltransferases (Dnmts: Dnmt1, Dnmt3a, Dnmt3b) are differentially responsible for establishing and maintaining methyl-CpG and disruption of these genes significantly perturbs genome-wide DNA methylation patterns, leading to aberrant development of homozygously null animals (Li et al., 1992; Okano et al., 1999). Remarkably, recent molecular, biochemical and genetic studies have identified another major epigenetic determinant of gene expression, i.e., the covalent modification of specific residues of histone N terminal tails (Jenuwein and Allis, 2001). These modifications include acetylation, phosphorylation, methylation, ubiquitination, and ADP ribosylation. Amongst these, methylation of lysine (K) residues of histone H3 has been shown to be important in the creation of distinct chromatin domains and a family of enzymes that have SET (Suv39, Enhancer of zeste, Trithorax) domains responsible for histone methyltransferase (HMTase) activity has been identified (Lachner et al., 2003). In general, dimethylation of H3-K9 mediated by G9a is associated with repressed genes/regions in euchromatin, and trimethylation of H3-K9 by Suv39h is concentrated at centromeric heterochromatin (Peters et al., 2001, 2003; Tachibana et al., 2002). In contrast, methylation of H3-K4 and acetylation of H3-K9 represent transcriptionally active/permissive chromatin (Jenuwein and Allis, 2001). Enhancer of zeste 2 (Ezh2) catalyzes repressive H3-K27 trimethylation on the inactive X chromosome and is also required for the derivation and propagation of embryonic stem (ES) cells (O'Carroll et al., 2001; Plath et al., 2003; Silva et al., 2003). H3-K27 trimethylation is thus reasoned to be important for the epigenetic plasticity of the pluripotent ES cell genome. Importantly, it has been reported that disruption of H3-K9 methylation in *Neurospora* abolishes DNA methylation as well, indicating a mechanistic interplay between these two key epigenetic marks (Tamaru and Selker, 2001). By contrast, in some cases, methylated DNA acts as a core element to recruit histone modification complexes (Hashimshony et al., 2003), and a mutation of a DNA methyltransferase in *Arabidopsis* resulted in the reduced levels of H3-K9 dimethylation (Soppe et al., 2002). Combined these findings point to a presence of complex molecular network that determines certain epigenetic states of particular cell types.

Early development in mammals culminates in the establishment of a blastocyst that consists of extraembryonic trophectoderm and pluripotent epiblast, from which all the somatic cell lineages as well as the germ line arise. Upon fertilization, the paternal genome is known to undergo active genome-wide DNA demethylation that is followed by passive replication-dependent demethylation of both the paternal and maternal genomes after the 2-cell stage, which continues until the morula stage, by which time the genome-wide DNA methylation level is very low (Li, 2002; Mayer et al., 2000; Oswald et al., 2000; Rougier et al., 1998). Genome-wide H3-K9 dimethylation decreases in a similar manner, reaching its lowest state at the 8-cell stage (Arney et al., 2002; Liu et al., 2004). Thereafter, in the pluripotent epiblast, Dnmt3a and 3b become active and the methylation levels in the epiblast cells increase rapidly (Li, 2002; Watanabe et al., 2002). Germ cells in mice are recruited from the proximal epiblast cells in response to signaling molecules at around embryonic day (E) 7.0 (Ginsburg et al., 1990; Lawson and Hage, 1994; Lawson et al., 1999; Saitou et al., 2002). Established germ cells start to migrate in the hindgut and mesentery (Anderson et al., 2000), eventually colonizing the differentiating genital ridges after E10.5. Between E10.5 and E12.5, germ cells undergo unique and extensive epigenetic reprogramming which appears to be equivalent in both sexes (Hajkova et al., 2002; Molyneaux et al., 2001; Sato et al., 2003; Tam et al., 1994). This reprogramming includes the erasure of parental imprints, reactivation of inactive X chromosome, and genome-wide CpG demethylation. However, the precise cellular and molecular mechanisms involved in these events remain largely obscure. Moreover, epigenetic properties that germ cells initially acquire upon specification and their potential remodeling in the migratory stage have been poorly resolved.

To address these issues, we have explored genome-wide epigenetic modifications in mouse germ cells from their specification to their entry into genital ridges using whole mount immunohistochemistry. Our data show that in germ cells the level of H3-K9 dimethylation decreases greatly at around E8.0, and is temporally closely coupled to genome-