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Dynamic expression of combinatorial replication-dependent histone variant genes during mouse spermatogenesis

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ABSTRACT

Nucleosomes are basic chromatin structural units that are formed by DNA sequences wrapping around histones. Global chromatin states in different cell types are specified by combinatorial effects of post-translational modifications of histones and the expression of histone variants. During mouse spermatogenesis, spermatogonial stem cells (SSCs) self-renew while undergo differentiation, events that occur in the company of constant re-modeling of chromatin structures. Previous studies have shown that testes contain highly expressed or specific histone variants to facilitate these epigenetic modifications. However, mechanisms of regulating the epigenetic changes and the specific histone compositions of spermatogenic cells are not fully understood. Using real time quantitative RT-PCR, we examined the dynamic expression of replication-dependent histone genes in post-natal mouse testes. It was found that distinct sets of histone genes are expressed in various spermatogenic cells at different stages during spermatogenesis. While gonocyte-enriched testes from mice at 2-dpp (days post partum) express pre-dominantly thirteen histone variant genes, SSC-stage testes at 9-dpp highly express a different set of eight histone genes. During differentiation stage when testes are occupied mostly by spermatocytes and spermatids, another twenty-two histone genes are expressed much higher than the rest, including previously known testis-specific *hist1h1t*, *hist1h2ba* and *hist1h4c*. In addition, histone genes that are pre-dominantly expressed in gonocytes and SSCs are also highly expressed in embryonic stem cells. Several of them were changed when embryoid bodies were formed from ES cells, suggesting their roles in regulating pluripotency of the cells. Further more, differentially expressed histone genes are specifically localized in either SSCs or spermatocytes and spermatids, as demonstrated by *in situ* hybridization using gene specific probes. Taken together, results presented here revealed that different combinations of histone variant genes are expressed in distinct spermatogenic cell types accompanying the progression of self-renewal and differentiation of SSCs, suggesting a systematic regulatory role histone variants play during spermatogenesis.

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Chromatin remodeling is a dynamic process that changes the landscape of chromatin in cells during animal development. Various degrees of chemical modifications of DNA and complexity of DNA binding proteins set regions of chromatin into either transcriptional active state (ON, euchromatin) or transcriptional repressive state (OFF, heterochromatin). It has been known that DNA methylation, as well as histone methylations (H3K27me3) can mark in-active genes, while other modifications of DNA and histones (H3K4me3) enable genes to be transcriptionally active (Reik et al., 2001; Santos-Rosa et al., 2002; Schuettengruber et al., 2007). The overall structural features of chromatin are determined by the combinatorial effects of DNA modifications, DNA binding proteins and their modifying enzymes, of which their own expressions are temporally and spatially controlled (Henikoff and Ahmad, 2005; Greer and Shi, 2012). The dynamic modeling of chromatin thus regulates global gene expression profiles that determine the cellular properties and developmental potentials of cells beyond DNA sequences. This epigenetic regulation plays a pivotal role during animal development (Banaszynski et al., 2010). Gene deletion/mutation studies in Xenopus and mouse have shown that several epigenetic modifiers are essential regulators for the development of germ cells and embryos (Roberts et al., 2002; Szenker et al., 2012; Shih et al., 2012). However, molecular mechanisms of epigenetic regulation that govern the animal development are not fully understood.

The fundamental DNA binding proteins are histones. An octamer of core histones, which contains dimers of H2A, H2B, H3

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and H4, is wrapped around by 146 nucleotides in two helical turns and generates the basic unit of the 30-nm fiber of chromatin: nucleosome (Malik and Henikoff, 2003). The linker DNA sequences between nucleosomes are bound by linker histone H1, which determine the distances between nucleosomes and fold chromatin into higher order structures. Various post-translational modifications (PTMs) have been found for histones, including acetylation, phosphorylation, methylation and ubiquitination, which render their DNA binding affinities differently causing either active or repressive effects on gene expression. It was found that hyperacetylation of histones is a pre-requisite of histone replacement during germ cell development (Hazzouri et al., 2000; Gaucher et al., 2012; Qian et al., 2013). Recent proteomic analyses further extended post-translational modifications of histones with 67 more PTM sites and novel lysine crotonylation and tyrosine hydroxylation (Tan et al., 2011). Combinations of various post-translational modifications can make up specific epigenetic code (the histone code) and facilitate the determination of overall chromatin structure of a particular cell type and thus give rise to specific gene expression profiles (Jenuwein and Allis, 2001). In addition, each histone family is composed of variant forms that are encoded by numerous genes (Marzluff et al., 2002). The histone variants often differ in only a few amino acids at the amino- and carboxy-termini that are the potential sites for post-translational modifications. During S phase of the cell cycle, histones are synthesized to accommodate the nucleosome formation of newly replicated DNA (replication-dependent histone variants). Researches in the past decades have also discovered histone variants that are expressed ubiquitously independent of cell cycles (replication-independent histone variants) and cell-type specific histones. They are deposited into chromatin at specific regions (such as centromere and telomere) and facilitate the formation of specific chromatin structures (Howman et al., 2000; Watanabe et al., 2013). Similar histone compositions help the inheritance of epigenetic information from parental cells, while differential replacement by other histone variants leads to changes of gene expression and cell properties (Turner, 2002). Thus the dynamic chromatin structure and gene expression are under the control of combinatorial effects from both differential expression of histone variants and their post-translational modifications.

While various post-translational modifications of histones and their accompanying enzymes have been uncovered, how histone variants (including both replication-dependent and replication-independent forms) define distinct chromatin landscape typical of a particular cell type, how they mark chromatin regions that affect developmental programs remain largely unclear. Several studies have been carried out to identify the expression patterns of histone variants in various mouse tissues or cells, including testes and early embryos (Nishida et al., 2005; Teng et al., 2010; Liu et al., 2011; Medrzycki et al., 2012). Recent work have suggested that variants of H1 are differentially expressed in embryonic stem cells and differentiated cells (Terme et al., 2011). During Drosophila male germ-line stem cell division, the daughter cells that inherit stem cell properties contain asymmetrically segregated histone variants that are different from the differentiated cells (Tran et al., 2012). These work pointed out that histone variants are also important for the regulation of cell potential and animal development.

One of the most dynamic chromatin remodeling processes occurs during mammalian spermatogenesis. To support life-long generation of spermatozoa, spermatogonial stem cells (SSCs) undergo both self-renewal and differentiation. Differentiating SSCs undergo meiosis and spermiogenesis to give rise to mature spermatozoa. Mammalian testis is comprised of developing spermatogenic cells that are heterologous in gene expression, cellular components and developmental potentials. Part of the reasons for these to occur is the constantly changing chromatin structure and gene expression profiles that accompany the developing cells. During spermiogenesis, chromatin bound histones are gradually replaced by basic proteins transient proteins (TNPs) and protamines (PRMs). The overall chromatin structure becomes highly condensed and gene transcription is completely turned off during late stage of spermiogenesis. Previous researches have suggested that testes highly or specifically express histone variants in different compositions in a step-wise manner in order to accommodate the chromatin structure changes (Brock et al., 1980; Meistrich et al., 1985; Churikov et al., 2004; Govin et al., 2004). Most of the testis-specific histone variants found are expressed in differentiated spermatocytes and spermatids, including H1.6 (H1t), H1.9 (H1LS1), TH2A, H2B.1 (TH2B), H3.4, H3.5 and H4t (Drabent et al., 1996; Yan et al., 2003; Huh et al., 1991; Trostle-Weige et al., 1982, 1984; Choi and Chae, 1991; Witt et al., 1996; Schenk et al., 2011; Grimes et al., 1987; Talbert et al., 2012). Functional roles of these histone variants during spermatogenesis are not fully understood. Gene targeting experiments in mouse showed that testis H2A.X is important for sex body (condensed un-paired XY chromosomal regions) formation during meiosis (Fernandez-Capetillo et al., 2003). Acetylations of H3 and H4 appear to be important for proper chromatin condensation and histone replacement and have profound long-term effects on gene expression and cell properties (Nair et al., 2008; Loyola et al., 2006). However, deletion of H1t gene in mouse did not exhibit immediate effects on sperm development and male fertility (Drabent et al., 2000), where as H1T2 and TH2B (H2B.1) play crucial roles during nuclear condensation and histone-to-protamine transition, respectively (Martianov et al., 2005; Montellier et al., 2013). Nonetheless, these studies indicated that the histone variants play important regulatory roles during mammalian spermatogenesis. What compositions of histone variants different spermatogenic cell types contain and how they function during male germ cell development require further investigation.

In the present study, we used real time quantitative RT-PCR and *in situ* hybridization to examine the expression of replication-dependent histone genes in mouse testis during post-natal development. The results showed that different spermatogenic cell types contain distinct groups of histone genes that are expressed pre-dominantly over others. Histone genes that pre-dominantly express in gonocytes and SSCs are also highly expressed in embryonic stem cells. During differentiation stage, spermatogenic cells express a different set of histone genes, including previously known testis-specific ones. These dynamic changes of histone gene expression may underlie the epigenetic regulation of gene expression and cell fate determination during spermatogenesis.

1. Results

1.1. Differential expression of canonical replication-dependent (RD) histones in mouse testis

In previous gene expression profiling of mouse spermatogonial stem cells, we found that several histones are differentially expressed in gonocytes, SSCs and differentiated spermatogenic cells (Yang et al., 2013). They included not only testis-specific histone (*hist1h1t*) but also the replication-dependent histone variants. *In situ* hybridization of *hist1h3a* showed that it is localized in the outer-most layer of seminiferous tubules where the SSCs reside. To examine whether other RD type histones are differentially expressed in spermatogeneic cells, *in situ* hybridizations of five histones, including linker histone H1 and four core histones, were performed on testis sections of adult mice. There are about 65 genes encoding RD type histones in the mouse genome, mostly

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