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Review

Establishment of male-specific epigenetic information

Sophie Rousseaux*, Cécile Caron, Jérôme Govin, Cécile Lestrat, Anne-Karen Faure, Saadi Khochbin

Unite INSERM U309, Institut Albert Bonniot, Domaine de la Merci, 38706 La Tronche Cedex, France

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Abstract

The setting of male-specific epigenetic information is a complex process, which involves a major global re-organisation, as well as localized changes of the nucleus structure during the pre-meiotic, meiotic and post-meiotic stages of the male germ cell differentiation. Although it has long been known that DNA methylation in targeted regions of the genome is associated with male-specific genomic imprinting, or that most core histones are hyperacetylated and then replaced by sperm-specific proteins during the post-meiotic condensation of the nucleus, many questions remain unanswered. How these changes interact, how they affect the epigenetic information and how the paternal epigenetic marks contribute to the future genome are indeed major issues remaining to be explored.

Keywords: Chromatin remodelling; Histone acetylation; Mammalian; Spermatogenesis; Spermatid; Sperm nucleus; Protamines; Transition proteins

1. Introduction

Many cases of male infertility associated with a severe impairment of spermatogenesis are now successfully overcome by the technique of intracytoplasmic sperm injection (ICSI), where a spermatozoa of the patient is directly injected into the cytoplasm of his partner's oocyte. Hence, ICSI is nowadays a widely used method, and has allowed many azoospermic or severely oligozoospermic males to become biological fathers. However, very little is known on the risks of an abnormality in the ICSI offspring of such patients (Winston and Hardy, 2002). These include a genetic risk due to the possible transmission of a genetic defect associated with the paternal infertility, as well as a risk linked to potential "epigenetic" consequences on the development of the embryo and the future individual. Concerning the latter, it is worth noticing that successful fertilizations have been obtained by injecting spermatozoa with an abnormal nuclear structure, or immature spermatozoa, or even their precursors, the spermatids (Ogura and Yanagimachi, 1995; Tesarik et al., 1995; Vanderzwalmen et al., 1998; Ogura et al., 1999; Yanagimachi, 1999). Moreover, recent studies have pointed out possible epigenetic abnormalities in children conceived by ICSI (Cox et al., 2002; De Rycke et al., 2002; DeBaun et al., 2003). A recent study suggests an association between abnormal sperm epigenetic information and altered spermatogenesis (Marques et al., 2004).

Therefore, understanding the events affecting the onset of paternal epigenetic information during spermatogenesis has become a crucial issue. It is now established that, during differentiation of the male gamete, the genome undergoes major changes not only affecting the DNA sequence and genetic information (homologous recombination), but also altering its nuclear structure and epigenetic information. The resulting sperm nucleus is packaged into a highly condensed

Abbreviations: BORIS, brother of the regulator of imprinted sites; BRDT, bromodomain testis-specific; CDYL, chromodomain Y-like; CIS cells, carcinoma in situ cells; CTCF, CCCTC binding factor; DMR, differentially methylated region; Dnmt, DNA methyltransferase; DSB, double stand break; H3K9, lysine 9 of histone H3; HAT, histone acetyl transferase; HDAC, histone deacetylase; HP1, heterochromatin protein 1; ICR, imprinting control region; ICSI, intracytoplasmic sperm injection; Prm, protamine; TNP, transition protein.

* Corresponding author. Tel.: +33 476 54 95 12; fax: +33 476 54 95 95.
E-mail address: sophie.rousseaux@ujf-grenoble.fr (S. Rousseaux).

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and specialized chromatin structure. The knowledge of the events and molecular mechanisms involved in chromatin re-organization during the main steps of spermatogenesis will provide a basis for a better understanding of important epigenetic modifications in the maturing male germinal cell, which might be altered in cases of defective spermatogenesis and therefore create a wrong environment for appropriate epigenetic information to be transmitted via the sperm nucleus.

The epigenetic information is conveyed by inherited modifications of the chromatin. In somatic cells, chromatin is organised in a nucleohistone structure, of which the basic unit of chromatin is the nucleosome. It consists of an octamer formed by two of each of the four core histones, H2A, H2B, H3 and H4 (MW 10,000–16,000), around which 146 base pairs of DNA are wrapped in 1.75 turns. The central portion of the fifth histone, H1 or linker histone, binds to the DNA as it enters and exits the nucleosome (Wolffe, 1995). In many processes requiring access to DNA, such as transcription or replication, the chromatin has to undergo a very complex and regulated remodelling of its structure. Several mechanisms can actively remodel localised regions of chromatin in somatic cells (Featherstone, 2002), involving factors capable of chromatin remodelling in an ATP-dependent fashion (Lusser and Kadonaga, 2003), enzymes chemically modifying some specific residues of the core histones by acetylation, methylation, phosphorylation or ubiquitination (Berger, 2002), or the incorporation of histones with different primary sequences, named histone variants, instead of conventional histones (Malik and Henikoff, 2003). Interestingly, two of the chromatin remodelling mechanisms described in somatic cells are involved in the global chromatin re-organisation during spermatogenesis: first the incorporation of histone variants and second a hyperacetylation of the core histones, which, unlike that observed in somatic cells, initially affects the whole spermatid nucleus (Lewis et al., 2003a).

Another well-known modification affecting chromatin structure and function is the methylation of the cytosines in CpG-rich regions of the genome. Generally, genes located downstream a highly methylated CpG promoter region are inactive, whereas demethylated CpG-rich promoter regions correspond to active genes. There are many CpG islands (regions enriched with CpGs) with tissue-dependent and differentially methylated regions (Shiota et al., 2002; Shiota, 2004). Differential DNA-methylation is involved in the regulation of transcription in several cellular processes, including parental imprinting, where one parental allele of the gene is expressed and the other is actively silenced (Kierszenbaum, 2002), and early embryonic development (Bourc'his et al., 2001a). During early foetal development, parental imprints are erased in primordial germ cells and then re-established during male and female gametogenesis.

This review aims to summarize our current knowledge on global and local chromatin events associated with the differentiation of the male gametes.

2. Overview of chromatin re-organization during spermatogenesis

During spermatogenesis, the genome undergoes major changes, including meiotic recombination and chromosome segregation directly affecting genetic information, as well as its post-meiotic packaging within a specific highly condensed chromatin structure. Indeed, meiotic recombination and random segregation of the paternal and maternal chromosomes of each pair into the new haploid germinal cells will ensure the genetic diversity of the species. They will also be specifically packaged for a safe transfer to the next generation. Important changes in chromatin structure are involved in the successive steps of this process, including the establishment of paternal imprints, meiotic recombination and post-meiotic chromatin re-organisation and condensation.

Spermatogenesis is the differentiation of germinal cells from spermatogonia to spermatozoa. Beginning at puberty, spermatogenesis is a continuous process, characterized by three major stages: pre-meiotic (or spermatogoniogenesis), meiotic and post-meiotic (or spermiogenesis) (for review, see Holstein et al., 2003). Spermatogonia divide by mitosis. They then enter meiosis by the formation of preleptotene primary spermatocytes, which replicate DNA and subsequently go through the leptotene, zygotene, pachytene and diplotene stages of the first meiotic division prophase. In pachytene spermatocytes, homologous chromosomes are paired and exchange DNA segments through a process of homologous recombination (or meiotic crossing-overs). This is helped by a number of proteins, which are localized in the sites of recombination along the paired chromosomes, in structures called synaptonemal complexes. The partial pairing of the X and Y chromosomes in their pseudo-autosomal region and their inactivation is a poorly understood specific feature of male meiosis, which involves some specific localized modifications of chromatin, including a "heterochromatinization", in the so-called "sex-vesicle" (or X–Y body). Meiosis is also characterized by the replacement of somatic histones by variants, among which some are testis-specific. Meiotic I division yields secondary spermatocytes which then rapidly go through meiotic II division, generating haploid round spermatids (Hess, 1999). During its post-meiotic maturation, the spermatid undergoes a global remodelling of its nucleus, which elongates and compacts into the very unique nucleus structure of the spermatozoa (Balhorn et al., 1977; Balhorn, 1982; Ward and Coffey, 1991; Ward, 1994; Ward and Zalensky, 1996).

Hence, chromatin undergoes several dramatic changes during spermatogenesis, ranging from localized modifications related to imprinting and regulation of expression of specific genes, to heterochromatinization of specific regions such as the sex vesicle in spermatocytes, and global modifications, including the final histone to protamine exchange and condensation.