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Asymmetry in Histone H3 variants and lysine methylation between paternal and maternal chromatin of the early mouse zygote

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Abstract

In mammalian fertilization, the paternal genome is delivered to the secondary oocyte by sperm with protamine compacted DNA, while the maternal genome is arrested in meiotic metaphase II. Thus, at the beginning of fertilization, the two gametic chromatin sets are strikingly different. We elaborate on this contrast by reporting asymmetry for histone H3 type in the pre-S-phase zygote when male chromatin is virtually devoid of histone H3.1/3.2. Localization of the histone H3.3/H4 assembly factor Hira with the paternal chromatin indicates the presence of histone H3.3. In conjunction with this, we performed a systematic immunofluorescence analysis of histone N-tail methylations at position H3K4, H3K9, H3K27 and H4K20 up to the young pronucleus stage and show that asymmetries reported earlier are systematic for virtually all di- and tri-methylations but not for mono-methylation of H3K4 and H4K20, the only marks studied present in the early male pronucleus. For H4K20 the expanding male chromatin is rapidly mono-methylated. This coincides with the formation of maternally derived nucleosomes, a process which is observed as early as sperm chromatin decondensation occurs. Absence of tri-methylated H3K9, tri-methylated H4K20 and presence of loosely anchored HP1-β combined with the homogenous presence of mono-methylated H4K20 suggests the absence of a division of the paternal chromatin in eu- and heterochromatin. In summary the male, in contrast to female G1 chromatin, is uniform and contains predominantly histone H3.3 as histone H3 variant.

Keywords: Zygote; Fertilization; Protamines; Pre-implantation development; Chromatin; H3.3; Hira; Histone methylation; Epigenetics

1. Introduction

In mammalian reproduction, the gametes of the two sexes have very different cell biological roles. After sperm entry, the cell cycle of the dormant metaphase II secondary oocyte is reactivated to proceed into telophase and subsequent female pronucleus (PN) formation, while at the same time the sperm nucleus is reshaped into the male PN (see for reviews Wright, 1999; Sutovsky and Schatten, 2000).

The major function of the male sperm is to transfer genetic information to the future zygote. Therefore, the sperm chromatin is compacted at a degree of condensation six-fold higher compared to a somatic cell nucleus (Ward and Coffey, 1991). This condensed state is reached through step-wise transformation of nucleosome-based chromatin into sperm-specific protamine-based chromatin during spermiogenesis. Protamine deposition takes place after spermatid elongation (see for time table Baarends et al. (1999)) whereas functions such as transcription and DNA repair slowdown at the onset of spermatid nuclear elongation (Sega et al., 1978; Monesi, 1965). When the two gametes fuse, the sperm-specific chromatin is actively

Abbreviations: PN, pronucleus; pi, post-insemination; ab, antibody; IF, immunofluorescence; IVF, in vitro fertilisation; H3.1, histone H3.1 and histone H3.2; H3.3, histone H3.3; HMTase, histone methyl transferase.

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transformed into nucleosomal chromatin. The literature is not specific on the timing of the protamine–nucleosome transition and often contradictive (Rodman et al., 1981; Nonchev and Tsanev, 1990; Kopecny and Pavlok, 1975).

Thus, the two gametic chromosome sets at the beginning of fertilization are strikingly different. It is this notion that might be at the basis of several biological, for instance epigenetic, differences observed between the parental nuclei in the zygote.

A series of recent reports describe the methylation states of several lysine residues in the N-termini of histone H3 and H4 (Cowell et al., 2002; Arney et al., 2002; Roest et al., 2004; Erhardt et al., 2003; Kourmouli et al., 2004; Liu et al., 2004; Sarmento et al., 2004; Santos et al., 2005). Strikingly asymmetry for most positions studied was noted: tri-methylated H3K4; di- and tri-methylated H3K9, di- and tri-methylated H3K27 and tri-methylated H4K20 are initially only present in the maternal chromatin of the 1-cell zygote. Only mono-methylated H3K4, K9 and H3K27 were found to be symmetrical, being present in both PNs (Lepikhov and Walter, 2004; Hewitson et al., 1999; Santos et al., 2005). Furthermore, differences between the parental nuclei at the transcriptional level have been observed. Although full zygotic genome activation takes place in the 2-cell embryo, leaky transcription is observed from early zygote S-phase on. Throughout the first cell cycle, transcription levels in the male PN are 4 to 5 times greater than in the female PN (Aoki et al., 1997). It is also known that the paternal PN exhibits higher levels of hyperacetylated histone H4 compared to the female PN (Adenot et al., 1997). By elevating global acetylation levels of the zygote, transcription can also be observed in the maternal PN, indicating that transcriptional asymmetry is largely due to a more transcription favourable state of the male chromatin as influenced by its hyperacetylation (Wiekowski et al., 1993).

At G1 of the zygotic cell cycle, DNA-methylation marks are actively removed from the paternal genome during the first 6 h after gamete fusion (Santos et al., 2002; Oswald et al., 2000). In contrast, this mark is passively diluted from the maternal DNA over subsequent cleavage divisions by the absence of maintenance methylation (Carlson et al., 1992).

Another area where the two genomes are not each other's equivalent constitutes the reaction to DNA damage by DNA repair. In the early stages after gamete fusion, the vulnerability of the two genomes to irradiation as measured by the yields of chromosome abnormalities at first cleavage is strikingly different. The maternal complement exhibits levels two to three times higher than the paternal one (Matsuda et al., 1989).

To obtain a better conception of the chromatin differences between the sexes, that are particularly apparent at the morphological pre-PN level early after gamete fusion, we have chosen to study the protamine–nucleosome transition in mouse sperm after in vitro fertilization (IVF) in conjuncture with an epigenetic characterisation of the two parental genomes in the early zygote.

We determined chromatin remodeling up to 280 min post-insemination in vitro, i.e. up to the PN stage, using antibodies that are specific for protamines, histones, dsDNA and nucleosomes, respectively. Subsequently, we have followed chromatin development in these early post-gamete fusion stages with histone H3 and H4 N-terminal tail methyl lysine modifications to evaluate one aspect of epigenetic differences between the two genomes. Our results reveal that after gamete fusion, nuclear morphology transition involves immediate chromatin remodeling, especially in the male complement. During this remodeling, paternal nucleosomes predominantly contain the histone H3.3 variant. As already known, epigenetic hallmarks are set differently between the male and female-derived genomes for di- and tri-methylation. However, we show that this is not necessarily the case for the mono-methylation state.

2. Results

2.1. Accessibility of epitopes for immunofluorescence

From penetration up to the formation of the male PN, there is a continuous morphological change of the sperm chromatin. We distinguish three distinct, successive nuclear shapes/states: (1) type a nuclei, which are partially decondensed with a proximal part reminiscent of the initial sperm chromatin; (2) type b nuclei, which are fully decondensed and (3) type c nuclei, which are re-condensing (see Fig. 1 and Section 4.2 for an overview of the morphologies and their moment of appearance). We performed indirect immunofluorescence (IF) with a dsDNA-specific antibody (ab) #36 in combination with DAPI staining to determine whether the paternal chromatin was accessible for antibodies during the process of male PN formation. The staining by dsDNA ab #36 was nearly absent from condensed chromatin whereas decondensed chromatin was homogeneously stained (see Fig. 2A–F). An intense staining by #36 could be observed in the border region where the sperm chromatin transits into decondensed chromatin (see Fig. 2A–C). For the decondensed chromatin, a perfect co-localization of #36 with DAPI was observed for each morphology (see Fig. 2D–F). Thus, antibody #36 (of IgG class) was not able to fully penetrate the sperm nuclei when still condensed. However, directly after decondensation the chromatin was fully accessible. Therefore conclusions presented here are solely based on observations made on decondensed sperm chromatin.