Dynamic chromatin modifications characterise the first cell cRe search Institu

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Received for publication 2 September 2004, revised 16 December 2004, accepted 21 January 2005

Abstract

On fertilisation, gametes undergo epigenetic reorganisation and re-establish totipotency. Here, we investigate links between chromatin remodelling and asymmetric maintenance of DNA methylation in the early mouse embryo. Using antibodies for lysine specific H3 methylation reveals that the male pronucleus is negative for di- and trimethyl H3-K9 yet the female is positive for these residues. However, the male is positive for monomethyl H3-K9 and H3-K27 and these signals increase during pronuclear maturation. Non-histone chromatin proteins of the Polycomb group are found in the paternal compartment as early as sperm decondensation. However, trimethyl H3-K27 is not observed in the male until the completion of DNA replication. Heterochromatin protein 1 beta (HP1β) is abundant in the male pronucleus, despite the absence of di- and trimethyl H3-K9, and co-localises with monomethyl H3-K9. Recent evidence identifies monomethyl H3-K9 as the preferred substrate of Suvar39h, the histone methyl transferase (HMT) responsible for heterochromatic H3-K9 trimethylation. The association of HPIβ with monomethyl H3-K9 may assist in preventing further modification of H3-K9. Association of dimethylation but not trimethylation of H3-K9 with DNA methylation, in the female pronucleus, suggests a mechanistically significant link. These differences begin to provide a chromatin based explanation for paternal-specific active DNA demethylation and maternal specific protection in the mouse.

Epigenetics; DNA methylation; Histone methylation; Reprogramming; Mouse

Introduction

On fertilisation, highly specialised gametes undergo remodelling, restoring totipotency and reforming the diploid zygote. This event brings together the meiotically arrested MII oocyte and the fully matured sperm with chromatin organised in protamine based toroidal structures (Balhorn et al., 2000; Braun, 2001). Despite the similar genetic content of the gametes, differences in epigenetic information reinforce their distinctive nature.

During the first cell cycle, remodelling of maternal and paternal components re-establishes transcriptional activation of zygotic gene expression (Latham and Schultz, 2001; Ram and Schultz, 1993). Epigenetic alterations are central to achieving this end, involving chromatin and DNA methylation changes. DNA methylation is reprogrammed during development influencing genome stability and gene regulation, especially differential DNA methylation associated with imprinted gene expression (Reik and Walter, 2001). However, DNA methylation does not influence transcription in isolation; rather, it exerts its influence through interactions with other epigenetic features of chromatin (Bird, 2002; Li, 2002) which are competent to create stable and heritable changes associated with cellular differentiation and development.

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0012-1606/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.ydbio.2005.01.025

Epigenetic regulation of gene silencing includes non-histone chromosomal proteins such as the polycomb gene group (Pc-G)/trithorax (trx) complexes (

of the branched antibody with K27 none is observed (Supplementary Fig. 3).

Fertilised oocytes generated by IVF and natural matings were washed in PBS, fixed for 15 min in 4% paraformaldehyde in PBS and permeabilised with 0.2% Triton X-100 in PBS for 30 min at room temperature. Fixed materials were blocked in 0.05% Tween-20 in PBS containing 1% BSA overnight at 4°C prior to the application of primary antibodies. For the experiments described in Fig. 4B, Triton X-100 was replaced in all solutions with Saponin (S-4521, Sigma) at incremental concentrations between 0.1% and 3%. Samples were incubated either for 1 h at room temperature or overnight at 4°C with immunoaffinity-purified rabbit IgG antibodies (all diluted 1:500) against mono-(α-2x-monomethH3-K9), di-(α-2x-di-methH3-K9) (Peters et al., 2003) and tri-(α-2x-tri-methH3-K9) (Lehnertz et al., 2003) methylated histone H3 lysine 9; α-4x-dimethH3-K9 antibody raised against a branched peptide (Peters et al., 2001) and mono-(α-2x-mono-methH3-K27) or trimethylated (α-2x-tri-methH3-K27) histone H3 lysine 27 (Peters et al., 2003). In order to establish continuity of results, we have compared the staining pattern of the previously used α-dimethyl-H3-K9 (Upstate 07-212) and the new α-2x-di-methH3-K9 (Peters et al., 2003

in the RGB) of two 8-bit images (threshold of channels 100; ratio setting value 50%).

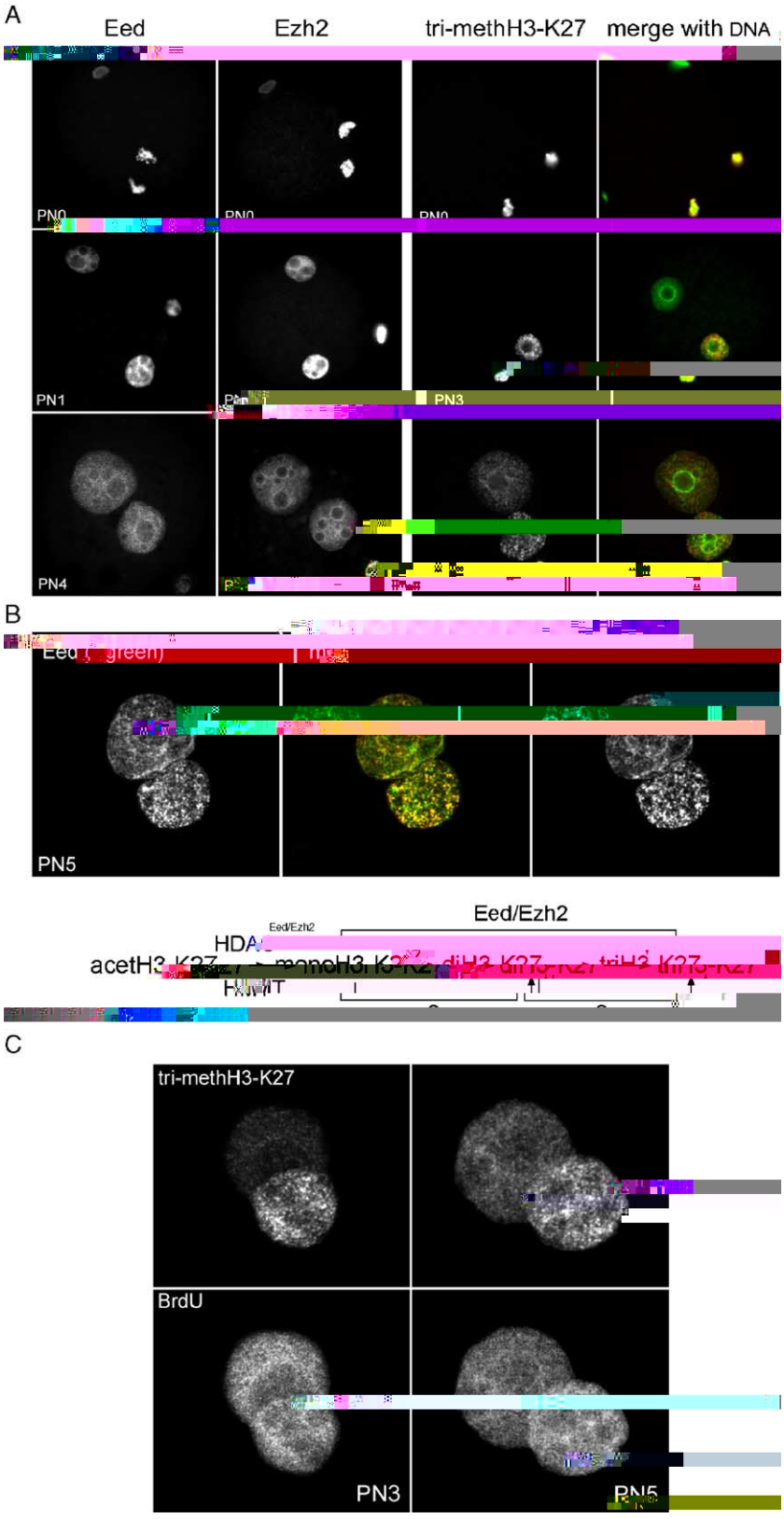
Results

Epigenetic marking by DNA methylation and histone modification has been reported in a number of model systems (Fuks et al., 2003; Jackson et al., 2002; Tamaru and Selker, 2001; Tamaru et al., 2003). A vast body of literature indicates that chromatin modifications dictate transcriptional states (Adenot et al., 1997; Santos-Rosa et al., 2002; Schubeler et al., 2004; Zhang and Reinberg, 2001; reviewed in Fischle et al., 2003a). These modifications may in turn rely on DNA methylation to formalise configurations and establish heritable memory patterns essential for cell differentiation and development (Bachman et al., 2003; Jaenisch and Bird, 2003). Therefore, we were interested in examining the potential relationship of DNA methylation to the organisation of chromatin components in the early mouse embryo.

To investigate these epigenetic marks in context, we devised a method of antibody staining whereby the DNA methylation status and histone modification, i.e. methylation, could be evaluated in the same embryo (see Materials and methods). Natural mating and in vitro fertilised (IVF) oocytes were collected and analysed for a selection of chromatin modifications thought to operate in concert with DNA methylation. Particular emphasis has been placed on the lysine residues of histone H3, largely owing to positioning of the amino terminal tail in the major groove of the DNA helix (Luger et al., 1997).

each of the methylation modifications of K9 and K27 (Table 1). On decondensation, the sperm nucleus acquired H3-K9 acetylated histones and stained positive for mono-methylated H3-K9 (monoK9) and K27 (monoK27) (Fig. 1). During this time, paternal DNA methylation is specifically lost (Santos et al., 2002). Remarkably, in contrast, no di- (diK9) or trimethylated H3-K9 (triK9) (including the heterochromatin specific branched configuration) signal was observed in decondensing sperm (Fig. 1; di-, tri-methK9). However, anaphase chromosomes of maternal origin stained very intensely for DNA methylation and all H3-K9 methylation modifications (Fig. 1; PN_1). Intense staining in perinucleolar regions confirm that di- and triK9 and monoK27 are associated to pericentromeric and centromeric satellites (Arney et al., 2002; Dillon and Festenstein, 2002). Immediately on fertilisation, monoK27 was observed in both the metaphase chromosomes of the oocyte and the decondensing sperm nucleus. Co-incident staining with DAPI indicates an association with the AT

To further elaborate on the parental asymmetry observed for K27, we investigated the status of Eed and Ezh2 during these earliest stages of the first cell cycle. Polycomb (Pc) proteins are known to be associated with heritable epigenetic regulation of gene expression in development. Eed/Ezh2 form complexes that methylate K27 via the SET domain containing histone methyl transferase (HMT) function associated to Ezh2 (reviewed in Cao and Zhang, 2004).



The MII oocyte is positive for both proteins but it is not seen in the male pronucleus until PN_1. Despite the presence of Eed/Ezh2 and monoK27 in the male pronucleus, it does not become positive for triK27 until PN_3, after replication has occurred (Figs. 3A, C). Co-localisation of Eed, Ezh2 and K27 reinforces their functional association (Fig. 3B; Eed/triK27) although the patterns of distribution are markedly different between the male and female pronuclei.

The pattern of DNA and histone H3-K9 methylation suggests that other components associated with silent chromatin configurations may also be present in one but not in both of the mature pronuclei in the fertilised oocyte. We have tested this hypothesis using an antibody to HP1β

pronucleus from demethylation and raising the question of when and how the paternal chromatin acquires these modifications.

Polycomb group proteins have been described to function in heritable epigenetic regulation of gene expression. In particular, the Eed/Ezh2 complex is significant since it has HMT activity on H3-K27. The very early appearance of both proteins in the decondensed sperm nucleus coincident with methylated K27 (Fig. 3A) is consistent with the proposed HMT function of the SET domain of Ezh2. Erhardt et al. demonstrated that this relationship holds true in the early fertilised embryo as conditional deletion of Ezh2 impairs asymmetric distribution of Eed between pronuclei and resulted in the absence of triK27. Following histone assembly in the decondensing sperm nucleus, K27 needs to be deacetylated, perhaps

These observations suggest a model in which the differential modifications of lysine residues in the male and female pronuclei are regulated by the absence or presence of specific HMTs, and more importantly by the availability of their substrates (Fig. 5). The presence of monomethylation discounts previous models which proposed that the differential chromatin structure observed could be attributed to the absence or exclusion of HDACs in the male pronucleus (Adenot et al., 1997). On the basis that Eed/Ezh2 is present from a very early stage, we propose that the HDACs associated with the Eed/Ezh2

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