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# **Histone post-translational modifications in preimplantation mouse embryos and their role in nuclear architecture**

Nathalie BEAUJEAN<sup>1,2</sup>

1) INRA, UMR1198 Biologie du Développement et Reproduction, F-78350 Jouy-en-Josas, France

2) ENVA, F-94700 Maisons Alfort, France

Correspondence : Dr Nathalie BEAUJEAN, UMR 1198 Biologie du Développement et Reproduction, INRA Domaine de Vilvert, 78 350 Jouy-en-Josas, France  
Tèl. : +33 1 (0)1 34 65 29 03, Fax : +33 1 (0)1 34 65 29 09  
Nathalie.Beaujean@jouy.inra.fr

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## **Abbreviations:**

PTMs: post-translational modifications  
HATs and HDACs: histone acetylases and deacetylases  
HMTs: histone methyltransferases  
KDMs: lysine demethylases  
pPN and mPN: paternal and maternal pronuclei respectively  
EGA: embryonic genome activation  
ChIP: chromatin immunoprecipitation  
TE: trophectoderm cells  
ICM: inner cell mass  
IVF: in vitro fertilization  
ART: assisted reproductive technology  
ROSI: round spermatid injection  
CENP-A: centromere protein-A  
HP1: heterochromatin protein 1  
NPBs : Nucleolar Precursor Bodies

## Abstract

In mammals, epigenetic modifications are globally rearranged after fertilization, when gametes fuse to form the embryo. While gametes carry special epigenetic signatures and a unique nuclear organization, they attain embryo-specific patterns after fertilization. This reprogramming is promoted by the intimate contact between the parental inherited genomes and the oocyte cytoplasm over the first cell cycles of development. Although the mechanisms of this reprogramming remain poorly understood, it appears that the particular epigenetic landscape established after fertilization is essential for further development. In this review we will first introduce histone post-translational modifications, their functions in chromatin organization and their role in nuclear architecture during mouse embryonic development. We will also consider epigenetic changes linked to the use of assisted reproductive technologies. .

## Introduction

It is widely thought that epigenetics play key role in cellular identity and lineage determination (Keenen and De La Serna, 2009; Roper and Hemberger, 2009; Bernstein et al., 2007). Epigenetics refers to heritable processes regulating gene expression without alteration of gene sequences. Epigenetic control is mainly achieved by chemical modifications, which can be propagated through mitosis, and in some cases through meiosis (Bonasio et al., 2010). It involves several mechanisms such as DNA methylation, histone post-translational modifications (PTMs) as well as chromatin structure and nuclear architecture (Schneider and Grosschedl, 2007; Bernstein et al., 2007). Indeed, epigenetic modifications represent constraints to the acquisition of a specific nuclear organization. Whereas the genetic information provides the framework for the manufacture of RNAs & proteins, chromatin structure and nuclear architecture control the accessibility of proteins to the DNA, especially transcription factors and RNA polymerase, and thereby gene expression (Schneider and Grosschedl, 2007). Epigenetics has also been recently extended to small non coding RNAs which mainly downregulate gene expression but may also activate gene expression. This includes microRNAs (miRNA), small interfering RNAs (siRNA) and Piwi-interacting RNA (Bourçhis and Voinnet, 2010).

The purpose of this review is to introduce histone post-translational modifications to scientists unfamiliar with the topic, highlight their functions in chromatin organization and their role in nuclear architecture. We will then list the histone post-translational modifications analysed so far in early mouse embryos. Indeed, the mouse (taxon-*mus*) has always been a good embryological model, easy to generate, giving around 8-20 litters and with a quick gestation period of 21 days. On the other hand, mouse embryogenesis is much slower than other model organisms such as *Drosophila* and *Xenopus*: 24 hours after fertilization, the mouse embryo is still at the 2-cell stage; development then continues slowly, the embryo moving along from the oviduct into the uterus to implant after 4.5 days (Wang and Dey, 2006). This biological feature allows precise studies on the preimplantation period after fertilization.

Furthermore, the advantage of gene  $\delta$ knock-out and  $\delta$ knock-in technologies in the mouse has been instrumental for the functional dissection of key epigenetic mechanisms. We will report some of the results gained with this approach, suggesting that histone post-translational modifications may influence gene expression in embryos and that they most probably play a key role in mouse development.

Finally, we will consider alterations of the histone post-translational modifications in embryos generated by assisted reproductive technologies. It is indeed through epigenetic modifications that environmental elements can appose marks on genes which are passed from one generation to the next (Jammes et al., 2011; Feil and Fraga, 2011; Dupont et al., 2012).

## Defining histone post-translational modifications

Histone proteins directly interact with DNA to form the fundamental unit of the chromatin, the nucleosome, which consists in 146bp of DNA wrapped around an octamer of histone core:: two copies of H3-H4, and two dimers of H2A-H2B; enclosed in 146 bp of DNA. The structure of the core histones is similar, each consisting of a globular, hydrophobic internal region and are highly conserved N-terminal histone tail, which emanates out of the nucleosome. These tails can be targeted by various enzymes that allow post-translational modifications (PTMs) : lysine residues for example can be acetylated, methylated or coupled to ubiquitin; arginine residues can be methylated; and serine or threonine residues phosphorylated (Peterson and Laniel, 2004; Kouzarides, 2007). Moreover, some residues can be mono-, di-, or trimethylated, adding complexity to histone PTMs. To identify these modifications, the common nomenclature indicates: 1) the name of the histone (e.g., H3); 2) the single-letter amino acid abbreviation with the amino acid position in the N-terminal tail (e.g., K4 for lysine 4); 3) the type of modification such as ac, acetylation/ me1, mono-methylation/ me3, tri-methylation etcí .

As mentioned above histones can undergo different post-translational modifications through their N-terminal tails, which are catalyzed by different histone modifier enzymes that overwrite or erase the modification. There are several chromatin-modifying enzymes such as histone acetylases (HATs) and deacetylases (HDACs) which acetylates/deacetylates specific lysine residues in histone substrates; the histone kinase family and phosphatases which phosphorylates/dephosphorylates specific serine or threonine residues; the histone methyltransferases (HMTs) and lysine demethylases (KDMs) which in turn add/remove methyl groups (Peterson and Laniel, 2004; Tsukada et al., 2006; Black et al., 2012).

Histone PTMs are found at functionally distinct regions of the genome such as coding regions, promoters and enhancers. These histone PTMs may then function as chromatin based on/off switches which can modulate chromatin structure and drive gene expression. They also participate in the distinction of chromatin domains such as euchromatin, which is lightly packed, and heterochromatin, which is tightly packed (as described in more details hereafter). Finally, by orchestrating the unfolding of chromatin, histone PTMs also play essential roles in DNA repair, DNA replication or chromosome condensation (Kouzarides, 2007; Peterson and Laniel, 2004).

Histone PTMs modulate the contacts between the nucleosomes and the chromatin , leading to either compaction or relaxation of the DNA fiber. The most accepted idea is that the degree of compaction of the chromatin fiber varies locally according to the need for access: it is less compact in regions undergoing transcription and replication which need a local and transient decondensation, whereas it is more compact in transcriptionally silent regions and during mitosis, when dense packing is required for accurate segregation of DNA (Schneider and Grosschedl, 2007). Acetylation has the highest potential to unravel chromatin, since it neutralizes the basic charge of lysine residues, decreasing their affinity for DNA (Hasan and Hottiger, 2002). Moreover, histone PTMs function by recruitment of non-histone proteins to chromatin, which mediate downstream effects on chromatin compaction and accessibility (Kutateladze, 2011). Recruitment of these regulatory proteins, such as the chromodomain-

containing CBX family proteins, will depend on the histone PTMs present on a given histone (e.g. HP1/CBX1 binds to H3K9me2/3 whereas CBX2 binds to H3K27me3).

From these modifications the first to be described and most well-characterized are acetylation and methylation. Generally speaking, lysine acetylation of histones H3 and H4 is often associated to gene expression, whereas histones in condensed chromatin areas are relatively hypoacetylated but marked by the presence of H3K9me3 (Schneider and Grosschedl, 2007). In certain cases it can however vary depending on the residue and histones which are being modified. For example, H3K9me3 and H3K4me which are both methylation present different effects on gene expression: H3K9me3 correlates to gene silencing while H3K4me initiates gene expression (Kouzarides, 2007). As mentioned above, some histone PTMs are involved in the orchestration of fundamental biological tasks: histone ubiquitination in particular is critical in mammalian meiosis (Baarends et al., 2005). It should be mentioned however that some histone PTMs, such as histone phosphorylation, present contradictory biological functions. In interphase cells, phosphorylation of histone H3 at Ser10 (H3S10P) has turned out to be an important phosphorylation site for transcription from yeast to humans, whereas during mitosis and meiosis H3S10P has an opposite role, being correlated to chromosome condensation (Figure 1) (Kouzarides, 2007).

## **Epigenetic modifications occurring in mouse preimplantation embryos**

During the last few years, many studies focusing on epigenetic modifications have shown that, immediately after fertilization and during early embryonic stages, parental genomes are characterized by an epigenetic asymmetry and extensive reprogramming (Table).

After fertilization, full maturation of the oocyte, including completion of the second meiotic division and extrusion of the second polar body, is completed within 4 hours. The newly formed embryo then enters the first mitotic cell cycle and nuclear membranes form around the separate haploid paternal and maternal pronuclei (pPN and mPN respectively) that remain physically separated (Adenot et al., 1997). DNA replication takes place during the migration of the two pronuclei to the center of the oocyte, from 9 to 14 hours post-fertilization (Adenot et al., 1997; Bouniol-Baly et al., 1997). During this first cell cycle, the pronuclei do not fuse and it is not before the next stage that the maternal and paternal genomes are enclosed into a single nucleus in each blastomere of the 2-cell embryo.

During spermiogenesis, sperm chromatin is compacted at a degree of condensation six-fold higher compared to a somatic cell nucleus through the deposition of protamines replacing most of the histones. After fertilization, following the entry of the sperm into the oocyte, the sperm-specific chromatin is actively transformed into nucleosomal chromatin. Protamines are actively exchanged with maternally inherited histones resulting in a rapid decondensation of the paternal chromatin (Adenot et al., 1991; Heijden et al., 2008). During this decondensation, the mouse paternal genome is associated with hyperacetylation: histone H4 acetylated at K8 and K12 are directly transmitted by the spermatozoon. The histones then become acetylated on lysines 5 and 16 of histone H4 and lysines 9, 14, 18 and 27 of histone H3 (Figure 2), with a more rapid increase in the paternal pronucleus for most of them (Adenot et al., 1997; Heijden

et al., 2006; Santenard et al., 2010). Conversely, the maternal genome is more methylated than the paternal one. H3K4 and K27 methylation is for example predominant in the maternal genome as compared to the paternal one that becomes methylated progressively (Figure 3) (Lepikhov and Walter, 2004; Santos et al., 2005). Methylation of H3K9 also occurs extensively in the mouse female pronucleus (Figure 4), whereas only monomethylation of H3K9 can be clearly detected in the male pronucleus (Lepikhov and Walter, 2004; Santos et al., 2005; Yeo et al., 2005). Recently, H3K36me3 was found enriched in the maternal, but not the paternal pronucleus following fertilization (Bo-kovi et al., 2012). Similarly, H4K20me3, H3K64me3 and H3K9me3S10P were also found to be inherited exclusively maternally, in the perinucleolar rings that correspond to pericentromeric heterochromatin (Figure 3) (Kourmouli et al., 2004; Wongtawan et al., 2011; Daujat et al., 2009; Ribeiro-Mason et al., 2012a). Other histone PTMs are present equivalently in both pronuclei ó e.g. H3S10P ó or completely absent from the pronuclei (Ribeiro-Mason et al., 2012b; Kan et al., 2012; Teperek-Tkacz and Meglicki, 2010; Sarmiento et al., 2004; Ooga et al., 2008; Wongtawan et al., 2011).

These different histone PTM profiles create an asymmetry between the two parental genomes. The functional importance of this asymmetry remains unclear. However, it is hypothesized that reprogramming of histone PTMs established in the gametes may be required for proper embryonic development, especially during the onset of embryonic gene expression (i.e. EGA: embryonic genome activation; Nothias et al., 1995). Indeed, the acquisition of a hyperacetylated and hypomethylated chromatin state in the paternal genome may increase the remodeling of the paternal genome and allow the minor wave of transcription seen at the late 1-cell stage during the transition from the late S to the G2 phase (Aoki et al., 1997; Bouniol-Baly et al., 1997) (Table).

The asymmetry between the two parental genomes can be detected up to the 4-cell stage, especially with H3K9me3 modification (Figure 4). As this modification is present only in the maternally derived genome, staining with antibodies directed against H3K9me3 clearly shows that maternal and paternal chromosomes do not intermingle during the first mitosis or in the nuclei of two-cell embryos (Ribeiro-Mason et al., 2012a; Hayashi-Takanaka et al., 2009). On the other hand, some modifications such as methylation of H3K27 or H3K4 are equalized by the two-cell stage (Santenard et al., 2010; Wongtawan et al., 2011).

Thereafter, histone PTMs levels are either maintained up to the blastocyst stage (Figure 4) or completely disappear from interphase blastomeres (Table) (Wongtawan et al., 2011; Ooga et al., 2008; Daujat et al., 2009; Bo-kovi et al., 2012; Yeo et al., 2005; Kan et al., 2012; Sarmiento et al., 2004). Some of these modifications however reappear later, usually after EGA, by the blastocyst stage or at implantation (Table). Finally, some histone PTMs have very specific dynamics during the preimplantation period: 1) H3S10P for example is present in interphase until the 4-cell stage but it becomes more specific to mitosis as in somatic cells after the 8-cell stage ; 2) another peculiar example is H4K20me1 for which the staining is intense up to 8-cell stage but then becomes significantly reduced from morula to blastocyst stage (Ribeiro-Mason et al., 2012b; Wongtawan et al., 2011).

Interestingly, these reprogramming steps correlate with the major activation of the embryonic genome taking place at the end of the 2-cell stage (Nothias et al., 1995; Aoki et al., 1997).

Moreover, it has been shown that increasing histone acetylation with trapoxin, a histone deacetylase inhibitor (HDACi), stimulated global transcription by 60% in embryos at the end of the 2-cell stage (Aoki et al., 1997). Similarly, inhibition of H3K4 demethylation by  $\alpha$ -bisguanidine 1cö resulted in aberrant expression of Oct4 by the 2-cell stage and inhibited the second embryonic cleavage to 4-cell (Shao et al., 2008). Recently, we also showed that components of the PRC1 complex, that binds H3K27me<sub>3</sub>, serve transcriptional functions during oogenesis that are essential for proper EGA and developmental progression beyond the two-cell stage (Posfai et al., 2012). Altogether these results suggest that histone acetylation and methylation may be closely correlated with the formation of a transcriptionally active or repressive state respectively during EGA and that they participate in the establishment of appropriate gene expression patterns required for further development.

Remarkably, it has been shown by chromatin immunoprecipitation (ChIP) of H3K4me<sub>3</sub> and H3K9me<sub>3</sub> for LINE-1 and IAP retrotransposons that H3K4me<sub>3</sub> decrease at the 8-cell stage coincides with the reduced transcriptional activity observed by RNA-FISH for these sequences (Fadloun et al., 2013). However, epigenetic changes in preimplantation embryos do not always correlate with the transcriptional status of specific genes. It was shown by use of carrier ChIP (CChIP) that the presence of H4K8ac, H3K4me<sub>3</sub>, and H3K9me<sub>2</sub> at the promoters of *Nanog*, *Oct4*, *Cdx2*, and *Gapdh* do not correlate with their transcriptional status at the 8-cell and morula stages (Vermilyea et al., 2009).

By the 64- to 128-cell stage a blastocyst with two cell subpopulations forms: an outer layer of epithelial trophectoderm cells (TE) surrounds an inner cluster of cells, the inner cell mass (ICM) located eccentrically within the blastocoelic cavity. In the blastocyst, the trophectoderm will give rise to parts of the extra embryonic tissues and allow the implantation of the embryo in the uterine mucous membrane. The inner cell mass will give rise to the embryo proper (Wang and Dey, 2006). Remarkably, it has been shown that the blastomeres which have a higher level of H3R26me at the 4-cell stage are likely to localize to the ICM (Torres-padilla et al., 2007). Correspondingly, upregulation of H3R26 methyltransferase CARM1 can induce the upregulation of the pluripotency markers NANOG and SOX2 in the mouse blastocysts.

Some histone PTMs have been found to differ between the ICM and TE (Table). H4/H2AS1P for example is more intense in the TE blastomeres when compared to the ICM (Sarmiento et al., 2004). Reversely, ICM shows extensive global methylation of H3K27, whereas in the TE, methylation of H3K27 is only detected on the inactive X chromosome (Xi) (Erhardt et al., 2003). This difference between ICM and TE is in line with the results from a genome-wide survey by use of micro ChIP assay profiling the bivalent histone PTMs, H3K4me<sub>3</sub> and H3K27me<sub>3</sub>, on promoters in mouse ICM and TE (Dahl et al., 2010). The promoters of *Lifr* and *Sox2*, which are highly expressed in ICM but not in the TE, are for example enriched in H3K4me<sub>3</sub> and depleted in H3K27me<sub>3</sub> in the ICM, whereas they are depleted in H3K4me<sub>3</sub> and enriched in H3K27me<sub>3</sub> in the TE. By contrast, expressions of other genes such as *Oct4* do not correlate with their H3K4 and H3K27 methylation states.



Histone PTMs H4K8ac, H3K4me3, and H3K9me2 at the promoters of *Nanog*, *Oct4*, *Cdx2*, and *Gapdh* have also been investigated in the ICM and TE of the mouse blastocyst by use of ChIP (Vermilyea et al., 2009). Interestingly, in the ICM, where *Nanog* and *Oct4* are highly expressed, the promoters of both genes are enriched in H4K8ac and H3K4me3 whereas in the TE, where *Nanog* and *Oct4* are silent, their promoters are enriched in H3K9me2 (Vermilyea et al., 2009; Neill et al., 2006). Remarkably, the opposite epigenetic patterns are observed for *Cdx2* which is silent in the ICM and highly expressed in the TE.

Finally, it should be mentioned that knock-outs of several histone modification enzymes have underlined their importance during preimplantation development: RNAi-mediated reduction of Class I histone deacetylase HDAC1 for example leads to hyperacetylation of histone H4 and developmental delay (Ma and Schultz, 2008). Similarly, deletion of JMJD2C, the H3K9me3 demethylase normally observed in the embryos at the 2- to 8-cell stage, causes arrest of development before the blastocyst stage (Wang et al., 2010). Inactivation of PR-Set7/Set8/KMT5A, the enzyme that catalyzes monomethylation of H4K20, also induces early embryonic lethality prior to the 8-cell stage (Oda et al., 2009). It was also very recently shown that depletion of lysine-specific demethylase 6B (KDM6B) in preimplantation mouse embryos alters incorporation of H3K27Me3, abrogates CDX2 and GATA3 expression in the nascent TE-lineage and results in improper TE development leading to implantation failures (Saha et al., 2013).

## Histone PTMs and their role in global nuclear architecture after fertilization

In embryos, as well as in all mammalian cells, chromatin is organized in two distinct defined domains known as euchromatin and heterochromatin. Euchromatin refers to the gene-rich part of the chromatin, has a more "flexible" arrangement, and is more accessible to the transcriptional machinery thanks to its "open state" configuration. This "permissive" chromatin is not necessarily transcriptionally active but "poised" for gene expression. On the other hand, heterochromatin, known as being gene-poor, has a more "repressive" chromatin structure, is more compact, hard to access and contains mainly silent transcriptional genes (Grewal and Moazed, 2003; Jost et al., 2012; Grewal and Jia, 2007). Remarkably, diverse parts of the genome have different types of chromatin configuration depending on their function and importance (Grewal and Jia, 2007; Grewal and Moazed, 2003). For instance, heterochromatin is found on the centromeres and telomeres, two components of the chromosomes bearing repetitive elements. These elements are thought to have the mission of protecting important structures essential for chromosomal function. Disruption of heterochromatin formation can indeed cause centromeric dysfunction, incorrect chromosome segregation and nuclear disassembly (Peters et al., 2001; Bouzinba-Segard et al., 2006). The heterochromatin formed at these regions was named constitutive heterochromatin because it maintains its highly condensed and largely transcriptionally silent character during the entire cell cycle (Grewal and Jia, 2007). In mammals, the heterochromatic region located in the

centromeres is flanked by large blocks of pericentromeric heterochromatin. In mouse the centromeric heterochromatin corresponds to the minor satellite sequences and the pericentromeric one to the major satellites (Guenatri et al., 2004; Martens et al., 2005). Within the nucleus of interphase somatic cells, the major satellites from different chromosomes gather into foci which form bright regions intensely stained by DNA dyes. The centromeres from the same chromosomes locate around these regions as individual dots characterized by the presence of centromere-specific histone H3-like proteins (CENP) (Figure 5). These sub-nuclear structures are called chromocenters (Guenatri et al., 2004; Alcobia et al., 2000).

Differently from somatic cells, the mammalian embryos present a unique organization of the pericentromeric heterochromatin. It is known that in preimplantation mouse embryos this part of the heterochromatin is not organized in clusters but in spherical structures around the Nucleolar Precursor Bodies (NPBs) (Zatsepina, 2003; Fléchon and Kopecný, 1998). The 1-cell stage pronuclei is indeed characterized by a heartwheel organization with the majority of the centromeric and pericentric regions located around the NPBs (Martin et al., 2006; Aguirre-Lavin et al., 2012).

This configuration is rapidly acquired in the maternal pronucleus and more progressively in the paternal one (Figure 6) (Aguirre-Lavin et al., 2012). This difference between the two parental genomes may be related to 1) the specific higher-order chromatin structure in sperm and to the progressive replacement of sperm protamines by histones as well as 2) the specific epigenetic marks present only in male chromatin. However, by the end of the first cell cycle, when minor genome activation occurs, maternal and paternal pericentromeric heterochromatin experience very similar decondensation states, together with a significant tendency to surround NPBs (Figure 6) (Aguirre-Lavin et al., 2012; Probst et al., 2007). It has therefore been suggested that these very early steps of pericentromeric heterochromatin reprogramming were associated with transcriptional activation during pronuclear maturation in the 1-cell stage. During the 2-cell stage, dissociation of pericentromeric heterochromatin from NPBs/nucleoli begins, concomitantly with the major phase of embryonic genome activation (Figure 7) (Martin et al., 2006; Probst et al., 2007). At later stages of the development centromeric and pericentric repeats carry on reorganization, especially at the 4-cell stage, and adopt a somatic-like organization (Aguirre-Lavin et al., 2012). By the blastocyst stage, heterochromatin rearrangements are even more local and accompanied by a switch in replication timing (Martin et al., 2006).

The degree of pericentromeric heterochromatin clustering varies with cell type, cell cycle phase and differentiation stage (Alcobia et al., 2000; Bartova and Kozubek, 2006). It is believed that these chromocenters may behave as structural centers for chromatin organization in interphase, favoring the creation of functional compartments for essential nuclear processes such as gene expression. In some cases, they can indeed induce gene repression either locally (by closing down chromatin access to the transcription machinery) or on neighboring DNA, playing the role of transcription silencer (Jost et al., 2012; Bartova and Kozubek, 2006). Such role has not been demonstrated yet in embryos. In somatic cells, pericentric heterochromatin is characterized by specific histone methylation and hypoacetylation. Remarkably, it contains one of the most studied histone PTMs, the trimethylation of the

histone H3 at Lysine 9 (Figure 5) (Martens et al., 2005; Peters et al., 2001; Richards and Elgin, 2002). This covalent modification together with the heterochromatin protein 1 (HP1) are hallmarks of this type of heterochromatin (Festenstein et al., 2003; Hiragami and Festenstein, 2005). The *in vivo* dynamic of HP1beta was therefore examined by live cell imaging in early embryos, confirming assembly and clustering of chromocenters in 2-cell embryos (Yamazaki et al., 2007a). Photobleaching analyses of HP1beta in embryos also support the idea of more mobile chromatin structures at 1-cell vs. 4-cell stage embryos (Yamazaki et al., 2007a).

It is believed that H3K9me3 and HP1 function together to modulate the chromatin in order to restrict the access of different factors crucial for gene expression (Grewal and Jia, 2007). The SUV39H histone modifying enzyme first trimethylates histone H3 at Lys9 creating the binding site for the chromo domain of HP1 ; HP1 then interacts with other proteins through its chromo-shadow domain to accomplish its function (Richards and Elgin, 2002). However, at the beginning of development, an asymmetry is clearly evident in terms of epigenetic markers within pericentromeric heterochromatin: only the maternal pericentromeric heterochromatin is labeled by H3K9me2/3 marks which recruits HP1 and, whereas the paternal one presents other repressive histone PTMs and/or proteins such as H3K27me2/3 and PRC1 (Polycomb Repressive Complex 1) (Santos et al., 2005; Puschendorf et al., 2008). Conversely, the double modification H3K9me3S10P, that stains chromocenters in G2 phase of somatic cells, could only be detected in the maternal genome, in the pericentromeric rings around the NPBs (Ribeiro-Mason et al., 2012a). Recently, we identified H3S10P as a better marker of pericentromeric heterochromatin in early mouse embryos as it labels both parental genomes (Figure 6) (Ribeiro-Mason et al., 2012b).

Finally, it has also been shown that complementary pericentromeric transcripts which are processed to small RNAs, guide heterochromatin formation and establishment of a transcriptionally silent state in fission yeast and plants. Some evidences in mouse and human argue in favor of a conserved role for centromeric and pericentromeric-derived transcripts across species (Lu and Gilbert, 2007; Muchardt et al., 2002). Moreover, it has been shown that abnormal accumulation of centromeric sequences leads to impaired centromeric architecture and function in human cells (Bouzinba-Segard et al., 2006) and that maintenance of pericentric heterochromatin is disrupted by RNase treatments (Maison et al., 2002). In embryos, it has recently been suggested that transcripts generated by pericentromeric satellite repeats are also involved in chromocenters formation at the 2-cell stage and that interference with the binding of these transcripts results in developmental arrest (Probst et al., 2010; Santenard et al., 2010).

## **Environmental-related epigenetic changes during early development**

Many studies have shown that changes in environment can alter epigenetic modification which are then passed from one generation to the next (Jammes et al., 2011; Feil and Fraga, 2011; Dupont et al., 2012). Several studies have therefore been looking at the IVF technique (in vitro fertilization) which is a widely used technology for assisted reproduction both in

animal and human. In contrast to embryos fertilized *in vivo*, IVF embryos are exposed to *in vitro* culture medium. Although no difference between IVF and *in vivo* fertilized embryos were observed between fertilization and implantation for H4ac, H3K9me and H3S10P (Figure 8), it was shown that the H3K4me3 level was significantly lower in the IVF embryos (Wu et al., 2012; Huang et al., 2007). In fact, *in vitro* culture on itself already affects the expression of the Axin1Fu sensitive allele through H3K4me2 and H3K9ac changes during early development (Fernandez-Gonzalez et al., 2010). ROSI (round spermatid injection) is another assisted reproductive technology (ART). In this case, it was shown that H3K9me3 present in the round spermatids persisted through the one-cell stage after ROSI and was not removed from the perinucleolar regions of the paternal pronucleus (Kishigami et al., 2006). Remarkably, the presence of numerous pronuclear chromocenters was then correlated with poor rates of 2-cell formation in these ROSI embryos (Yamazaki et al., 2007b). In ART, the preservation of spermatozoa also plays an important role. When the effect of freezing was tested in mouse, it was shown that the dynamic epigenetic reprogramming of H3K4me3 and H4K12ac in embryos derived from frozen sperm (at -20°C) was similar to the reprogramming of embryos derived from fresh sperm from fertilization up to the blastocyst stage (Chao et al., 2012a). The same research group investigated epigenetic reprogramming in preimplantation embryos derived from 65 °C-heated sperm. Although they observed no evident changes for H4K12ac and H3K9me3, significantly lower levels of H3K27me3 were found in the ICM of heated-sperm derived blastocysts (Chao et al., 2012b).

Reversely, oocyte can also be preserved, either by vitrification or cryopreservation. In both cases, epigenetic errors have been observed in the mouse: 1) acH4K12 levels increased significantly in vitrified oocytes compared to controls and the embryos derived from those vitrified oocytes had abnormal acH4K12 patterns upon pronuclear formation (Suo et al., 2010a); 2) similarly, HDAC1 expression in the embryos derived from vitrified-oocytes was significantly lower than in the controls and this correlated with lower developmental rates a negative impact on embryo development (Li et al., 2011). Moreover, it should be remembered that oocyte aging can also alter histone PTMs as shown for H4K12ac levels in mouse (Suo et al., 2010b).

## Conclusion and perspectives

It is very important for developmental biologists to understand how parental inherited genomes are reprogrammed in order to attain embryo-specific chromatin organization which is crucial for normal development. Indeed, it appears that proper scaffolding of the chromatin and the different nuclear sub-structures at these very early stages of development is important to produce healthy embryos. Recent data also provide insight into epigenetic errors that may be associated with the poor development of embryos generated with assisted reproductive technologies.

Amongst the chromatin categories, the one which is quite remarkable is the heterochromatin. Due to all the differences seen between both parental genomes, especially in relation to pericentromeric heterochromatin, it is fundamental to further investigate this heterochromatin

domain in regards to its epigenetic signature and the influence it has on nuclear organization and gene expression. It is indeed likely that pericentromeric heterochromatin rearrangements are correlated with the above mentioned chromatin remodeling and are necessary for proper gene expression and development. As in somatic cells, the role of these rearrangements may be to bring in close proximity different nuclear compartments (NPBs, nuclear periphery, chromosomes territories) in order to activate/repress specific genes yet to be identified.

Interestingly, chromatin organization into specific sub-nuclear domains may participate in cellular differentiation (Keenen and De La Serna, 2009; Roper and Hemberger, 2009). There is increasing evidence that heterochromatin may be a cell fate determinant. Indeed, heterochromatin is qualitatively different in pluripotent cells than in terminally differentiated cells and tissues. Embryonic stem (ES) cells, derived from embryos at the blastocyst stage, are typical pluripotent cells able to generate all cell types, even germ cells, of an adult body. These cells possess a globally open, decondensed chromatin structure compared to differentiated cells, with hyper acetylated histones H3 and H4 and diffused heterochromatic regions (Martens et al., 2005). Fluorescent recovery after photobleaching (FRAP) experiment also shows that chromatin structural proteins such as HP1 indeed bind more loosely in ES cells chromatin as compared to their differentiated progeny (Meshorer et al., 2006).

Altogether it seems that the concept of a more òdynamicö chromatin structure allowing both early development and emergence of pluripotent cells should be retained. This concept is based at least partially on histone PTMs. However, DNA methylation is another important epigenetic modification, also closely related to pericentric heterochromatin. It has for example been reported that Suv39h histone methyltransferase is required for both H3K9 trimethylation and DNA methyltransferase 3b (DNMT3b)-dependent DNA methylation at pericentromeric repeats (Lehnertz et al., 2003). However, it has been reported in early mouse embryos that the protein PGC7 binding to H3K2me2 in the maternal chromatin protects it from DNA demethylation by Tet3 enzyme (Nakamura et al., 2012). Understanding the complexity of epigenetic regulations in early embryos therefore remains open to further investigations.

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## Legends

**Table:** Summary of the histone modifications occurring on maternal and paternal pronuclei (mPN and pPN respectively) at the 1-cell stage and during the subsequent developmental stages, up to the blastocyst (with the distinction between TE: trophectoderm cells and ICM: inner cell mass cells). The corresponding references are indicated on the right.

**Figure 1.** H3S10P staining in mouse fibroblasts, showing the onset of its detection in late G2 phase of the cell cycle and the labeling on the fully condensed chromosomes at metaphase. (H3S10P in green and DNA in red). Scale bar: 20µm

**Figure 2:** Immunodetection of H3K14ac in *in vivo* fertilized embryos with a standard immunostaining protocol (Ribeiro-Mason et al., 2012a). Z-series were taken for each embryo on an Apotome microscope. Representative images with H3K14ac in green and DNA counterstaining in red are shown here (A-C and F are single z-sections taken in the middle of the embryos; D-E correspond to z-stack projections of the whole embryos). During the whole first embryonic cycle, histone H3 acetylation on lysine 14 was present on both parental pronuclei (A: early 1-cell, B: mid 1-cell). In late 1-cell and upon prophase condensation, H3K14ac clearly stained the whole genome with the exception of the heterochromatin rings around Nucleolar Precursor Bodies - NPBs (C/D). During mitosis staining for H3K14ac covered the whole chromosomes arms but not the pericentric heterochromatic regions (E). At the 2-cell stage, H3K14ac was again first detected in the nucleoplasm and not within perinucleolar heterochromatin rings (F). PB: polar body remaining after fertilization; scale bar: 10µm.

**Figure 3:** Immunodetection of H4K20me3 and H3K4me2/3 in *in vivo* fertilized embryos at the 1-cell stage, with a standard immunostaining protocol (Ribeiro-Mason et al., 2012a). Z-series were taken for each embryo on a confocal microscope. Representative single z-sections are shown here. Paternal (pPN) and maternal (mPN) pronuclei show clear differences with a diffuse/uniform staining H3K4me2/3 and perinucleolar accumulations in the case of H4K20me3 (PB: Polar Body). Scale bar: 10µm.

**Figure 4:** Immunofluorescent staining of H3K9me3 (green) and DNA (red) was performed on preimplantation embryos at different time points from 1-cell to blastocyst stages. Z-series were taken for each embryo on a confocal microscope. Representative single z-sections are shown for 1-cell/ 2-cell stages and Z-series projections for the other stages. Note that only the maternal pronucleus is labeled at 1-cell and that the staining observed at 2-cell is asymmetric. Scale bars: 10 µm.

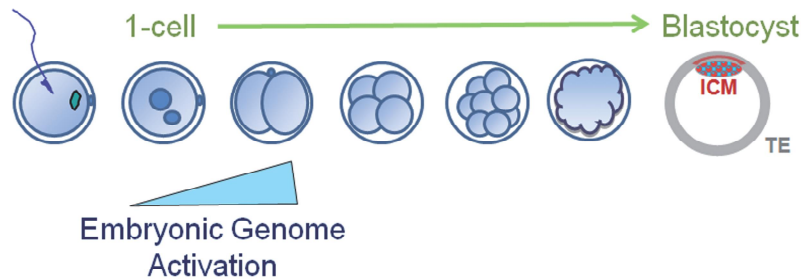
**Figure 5 :** Detection of chromocenters in interphase mouse fibroblasts by immuno-staining with antibodies (green) for centromeric protein CENP-A/B (A) or H3K9me3 (B), and DNA counterstaining (red). Chromocenters can clearly be detected as dense chromatin clusters with associated CENP spots (arrows in A) or co-labelling with H3K9me3 (arrows in B). Images were taken under an Apotome microscope. Scale bar: 5µm.

**Figure 6 :** Pericentromeric heterochromatin distribution in early (A) or late (B) 1-cell stage embryos. 3D-FISH images were obtained with pericentromeric probes (major satellite, red) and DNA counterstaining (green). Z-series were taken for each embryo on a confocal microscope. Single z-sections are shown on this figure. (A) In early 1-cell embryos, while the mPN is just forming, pericentromeric signals begin to accumulate at the nucleolar periphery. In contrast, the pericentromeric heterochromatin is still aggregated in a large central mass in the pPN. (B) In the late 1-cell embryo, pericentromeric heterochromatin is clearly surrounding the NPBs (Nucleolar Precursor Bodies) in both PN. Scale bars = 5µm

**Figure 7:** Three dimensional immuno-FISH with H3S10P antibody (green) and major satellite probes for pericentromeric DNA repeats (red) was performed on 2-cell mouse embryos. Z-series were taken for each embryo on a confocal microscope. Single z-sections are shown on this figure. Note the colocalization of H3S10P and pericentromeric repeats that overlap in the newly formed chromocenters. Scale bar: 10 µm.

**Figure 8:** Immunodetection of H3S10P in *in vitro* fertilized (IVF) embryos was performed using the same immunostaining protocol as in *in vivo* fertilized ones (Ribeiro-Mason et al., 2012b). Z-series were taken for each embryo on an Apotome microscope. Representative images (single z-sections) are shown here. In one-cell embryos the two parental pronuclei can clearly be distinguished (maternal: mPN and paternal pPN) as well as the remaining second polar body (PB). H3S10P presents the same distribution pattern for both types of embryos: very early during the first embryonic cycle (A), histone H3 phosphorylation at Ser10 was already present in the decondensed sperm head and maternal chromatin. During pronuclei formation, both parental complements had a strong signal for H3S10P (B). Both pronuclei then started showing signals for H3S10P in the heterochromatin rings around the NPBs (C). In late 1-cell, the heterochromatin rings showed strong H3S10P staining (D). During mitosis chromosomes were strongly stained for H3S10P (E). At the 2-cell stage, H3S10P was first detected in the heterochromatin rings around the NPBs (F) whereas from middle (G) to late 2-cell (H), H3S10P could be detected on the newly formed chromocenters. Scale bar: 10µm.

## Fertilization



### Histone PTM

### References

|                                |   |  |  |
|--------------------------------|---|--|--|
| H4K12 ac<br>H4K8ac             | Present just after fertilization in both PN   | Enriched at the nuclear periphery at 2-cell  | Worrad et al., 1995  |
| H3K9ac<br>H3K14ac<br>H3K18ac   | Appear during the 1-cell stage in both PN   | Enriched at the nuclear periphery at 2-cell  | Heijden et al., 2008<br>Stein et al., 1997<br>Figure 3   |
| H4K16ac<br>H4K5ac              | First in the pPN, increasing in the mPN during 1-cell stage   | - H4K16ac uniform at 2-cell<br>- H4K5ac enriched at the nuclear periphery at 2-cell  | Adenot et al., 1997<br>Stein et al., 1997  |
| H3K27ac                        | First in the pPN, increasing in the mPN during 1-cell stage   | Almost no staining at 2-cell   | Hayashi-Takanaka et al., 2011  |
| H3cit2+8+17<br>H4cit3          | Both PN   | Present at all stages  | Kan et al., 2012   |
| H3cit26                        | Both PN, on perinucleolar rings   | Disappears at 4-cell   | Kan et al., 2012   |
| H3K4me1<br>____me2<br>____me3  | - Always present in mPN, accumulates in the pPN during 1-cell stage<br>- Always present in mPN, appears in mPN at G2<br>- Always present in mPN, appears in pPN at the very end of 1-cell only                | Present almost uniformly at 2-cell   | Lepikhov et al., 2004<br>Santenard et al., 2010 (supplement)   |
| H3K9me1<br>____me2<br>____me3  | - Both PN<br>- Mainly in the mPN, almost undetectable in the pPN<br>- mPN only  | - Slight asymmetry at 2-cell, increases at 4-cell, maintained up to blastocyst<br>- Asymmetry at 2-cell, present up to the blastocyst stage  | Lepikhov et al., 2004<br>Santos et al., 2005<br>Wongtawan et al., 2011<br>Yeo et al., 2005<br>Figure 5       |
| H3K9me3S10P                    | mPN only, on perinucleolar rings  | Restricted to chromocenters at 2-cell  | Ribeiro-Mason et al., 2012a  |
| H3K27me1<br>____me2<br>____me3 | - Both PN with a stronger signal in the mPN<br>- Always present in mPN, appears on perinucleolar rings in pPN at the end of 1-cell only<br>- Always present in mPN, accumulates in the pPN during replication | - Slight asymmetry at 2-cell, staining always present up to blastocyst, a differential staining then appears between ICM and TE cells  | Erhardt et al., 2003<br>Santenard et al., 2010 (supplement)<br>Santos et al., 2005<br>Wongtawan et al., 2011 |
| H3K36me3                       | mPN only  | Almost undetectable at 2-cell, reappears at 4-cell at low level  | Boskovic et al., 2012  |
| H3K64me3                       | mPN only, on perinucleolar rings  | Undetectable before implantation   | Daujat et al., 2009  |
| H3K79me2/3                     | Undetectable  | Undetectable before the blastocyst stage   | Ooga et al., 2008  |
| H4K20me1<br>____me2<br>____me3 | - Present in both PN with a stronger signal in the pPN<br>- No signal<br>- mPN only   | - Always present with a decrease from morula to blastocyst<br>- Appears at 4-cell and becomes clearly detectable at morula stage<br>- Undetectable for the whole of preimplantation period | Wongtawan et al., 2011<br>Figure 4   |
| H3R17me                        | Both PN quite rapidly   | Always present up to blastocyst  | Sarmento et al., 2004  |
| H4R3me                         | Undetectable  | Staining increasing at all subsequent developmental stages   | Sarmento et al., 2004  |
| H3S10P                         | Both PN with a slight decrease during S-phase   | Present in interphase up to the 4-cell stage, then restricted to mitosis   | Ribeiro-Mason et al., 2012b  |
| H4/H2AS1P                      | Both PN quite rapidly   | Always present up to blastocyst, a differential staining then appears more intense in the TE than in the ICM   | Sarmento et al., 2004  |

