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Epigenetic reprogramming and development: a unique heterochromatin organization in the preimplantation mouse embryo

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Abstract

Fertilization of the oocyte by the sperm results in the formation of a totipotent zygote, in which the maternal and paternal chromatin is enclosed in two pronuclei undergoing distinct programmes of transcriptional activation and chromatin remodelling. The highly packaged paternal chromatin delivered by the sperm is decondensed and acquires a number of specific epigenetic marks, but markedly remains devoid of those usually associated with constitutive heterochromatin. During this period the maternal chromatin remains relatively stable except for marks associated with transcription and/or replication such as arginine methylation and H3/H4 acetylation. The embryo then undergoes a series of mitotic divisions without significant additional growth but differentiation, resulting in the formation of a blastocyst containing distinct cell types. The chromatin remodelling events during these stages are likely to be important in establishing the nuclear foundations required for later triggers of differentiation. Overall, we summarize three important points during these earliest reprogramming events: (i) relatively stable maternal chromatin after fertilization, (ii) rapid acquisition of specific histone marks by the paternal chromatin during the hours that follow fertilization and (iii) rapid remodelling of constitutive heterochromatic marks and modifications in the core of the nucleosome from the first mitotic division. These features are likely to be required for the creation of a chromatin environment compatible with cellular reprogramming and plasticity.

Keywords: *Mouse embryo; epigenetic reprogramming; cell plasticity; totipotency; chromatin; heterochromatin; methylation*

INTRODUCTION

During the life cycle of a mammal from a newly fertilized egg to a fully differentiated adult with over 200 different cell types, major changes in cellular specification must occur through differentiation and reprogramming events. Throughout this cycle the genetic information itself remains largely constant while epigenetic modifications undergo extensive changes. Upon fertilization in mammals, the haploid genomes of two highly differentiated cells; the sperm and oocyte combine and the first of two major reprogramming events during the life cycle occurs, resulting in the production of a totipotent zygote. This unique cell, by definition, is capable of differentiating into every specialized cell type in the organism. The zygote undergoes a series of cleavage divisions resulting in the formation of the blastocyst, by which time the first differentiation event has occurred separating the outer trophectoderm that will go on to form the placenta and

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extra-embryonic tissues and the inner cell mass that comprises pluripotent embryonic cells that will develop into the embryo proper.

The timescale of these events varies considerably among mammalian species. In the mouse, in which the majority of studies have been conducted and will therefore form the focus of this review, the first two cycles take one and a half days in total, with consecutive divisions occurring ~12 h apart until implantation of the blastocyst takes place 4.5 days after fertilization [1]. During this time, the embryo develops first under the control of maternally inherited factors during the early stages and later by embryonic gene expression, which begins at a low level in S-phase of the one-cell stage, and to a greater extent during the two-cell stage [2, 3]. The mechanisms controlling chromatin remodelling and gene expression during reprogramming and early differentiation events are the subject of ongoing research but a significant role for epigenetics is likely.

Epigenetic mechanisms have attracted much interest due to their ability to regulate and interpret the DNA sequence, in a fluid yet potentially heritable manner [4]. The epigenetic dynamics during mammalian pre-implantation development are characterized by major changes in DNA methylation, histone modifications and the incorporation of histone variants [5–7]. Furthermore, the genetic inactivation (knockout) of many chromatin modifying enzymes results in early developmental defects, even when maternal genes are present [7]. Collectively these observations suggest that such modifications play a crucial, but still poorly understood role in the processes of reprogramming and differentiation during development.

We will cover two different aspects concerning chromatin changes during the earliest phases of mouse development. The first part collects our current knowledge on the changes that characterize the epigenetic asymmetry in paternal and maternal chromatin in the hours following fertilization. The second section deals with the global changes on the chromatin during the subsequent cleavage stages and up to blastocyst formation. Although there remains significant gaps in our knowledge, the asymmetries between the paternal and maternal chromatin in the pronuclear stages have been relatively well studied compared to the epigenetic dynamics during subsequent developmental stages. There is a second major reprogramming event later in development during the formation of the germline, but we will not deal with this topic here.

It should be noted that the majority of studies concerning the dynamics of histone modifications during these stages are based upon immunofluorescent labelling of specifically modified histone residues in the mouse embryo. Although the antibodies utilized are in general believed to be specific, the possibility of epitope exclusion cannot be ruled out in such experiments, particularly for modifications occurring in close vicinity to others such as the hot-spot of modifications on the histone H3 K9/S10 N-terminal tail. There are also a number of known histone modifications that have not been studied in the mouse embryo, such as histone H3K36 methylation, H3K56 acetylation and methylation and H2A ubiquitination. Finally, it should be mentioned that the generality of the mouse as a model for all mammalian species should not be assumed, particularly during these crucial early developmental events.

PARENTAL CHROMATIN DYNAMICS: MARKED DISCREPANCIES AND SIMILARITIES

During the first reprogramming event at the zygote stage, the two parental genomes remain physically separated as two pronuclei, and although in theory both have access to the same maternal factors they go through very distinct programmes of chromatin remodelling [8]. Indeed, it is the paternal pronucleus that experiences the most extensive reprogramming at this early stage, as the highly packaged chromatin of the sperm head undergoes decondensation cycles, likely resulting in a more permissive structure for remodelling [9]. The sperm-specific packaging protamines are replaced with histones, which are hypomethylated and hyperacteylated. As acetylation of histone H4 lysines 5 and 12 has been correlated with deposition [10], this suggests that their initial hyperacetylation is a function of their incorporation itself. The paternal DNA subsequently undergoes 'active' global DNA demethylation, in a process that resembles a resetting of the epigenetic landscape before DNA replication begins.

DNA demethylation of the paternal genome occurs extremely rapidly after fertilization, prior to DNA replication and is thus referred to as active demethylation [11–13]. However, this demethylation of the paternal DNA is perhaps not as global

as originally thought and is composed of pre- and post-replication demethylation, at least on some repetitive elements [14]. The role of this process is intuitively likely to be a part of the wider genomic reprogramming that accompanies fertilization, but the reason why the paternal pronucleus must undergo such dramatic and active demethylation before the passive demethylation of both genomes during subsequent divisions is not clear [15–17]. Interestingly, the paternal pronucleus is supportive of a greater transcriptional activity, which is first observed during S-phase of the zygotic stage and increases progressively thereafter [2, 18]. It has been suggested that this is at least partially due to epigenetic events including higher levels of histone acetylation in the male pronucleus [2, 3]. A link to DNA demethylation is supported by the correlation that species with earlier zygotic gene activation, including mouse and human show a more extensive zygotic demethylation compared to those where zygotic genome activation occurs later [5, 19, 20]. However despite this genome wide remodelling, it is yet to be established and remains imperative whether and, if so which, specific genes in the paternal genome must undergo DNA demethylation at this stage to allow their expression at later embryonic stages for correct pre-implantation development to occur.

The susceptibility of the paternal genome to DNA demethylation in the fertilized zygote is due to an intrinsic difference in the paternal genome, as no demethylation is observed in parthenogenetically activated embryos, with exclusively maternal components [21]. A potential explanation for this emerges from the consideration of the dissimilar chromatin environments of the two parental genomes. Histone modifications including lysine methylation show a dramatic asymmetry in the pronuclei, with enrichment in the maternal pronucleus throughout the zygotic stage, in contrast to H4 acetylation (Figure 1) [22–27]. The paternal chromatin is incorporated in an overall hypomethylated state and remains devoid of all detectable histone modifications normally associated with heterochromatin, including H3K9me2/3, H4K20me2/3 and H3K64me3 as well as H3K79me2/3 [22–24, 27–30]. In addition, H3K4me3 and H3K27me2/3 are also absent until after replication of the paternal DNA at the late pronuclear stage [24, 26–28].

Due to growing evidence that DNA methylation may act downstream of H3K9 methylation [31–33], the lack of H3K9me2/3 could allow global DNA demethylation to occur specifically in the male pronucleus. Some particular regions of the paternal genome including paternally imprinted genes, repeat sequences such as IAP retrotransposons and centromeric regions remain methylated at this stage [16, 17, 34]. Apart from a few exceptions [35, 36], the mechanism for the specific protection of these regions is largely unknown, although centromeric methylation is likely to be required for the maintenance of genome stability and chromosome segregation during mitosis in the absence of paternal H3K9me3-labelled centromeric heterochromatin. It is interesting to note that uniquely in the paternal pronucleus, H3K9me1 is localized to pericentromeric regions that are protected from DNA demethylation, where it may be responsible for the observed weak binding of HP1β and provide a marker of these regions for further H3K9 methylation at later embryonic stages [28]. Additionally, alternative mechanisms for recruiting HP1β might be in place [37]. H3K27me3 and maternally provided PRC1 components accumulate at constitutive heterochromatin in the mature paternal pronucleus, which may also play an important compensatory role in the absence of H3K9me3 [28, 38]. Strikingly, despite these dramatic differences in heterochromatin marks, the mature paternal pronucleus is able to acquire a similar chromocenter organization to that of the maternal one by the end of the zygotic stage and before the first mitotic division of the embryo [37].

In contrast to the corresponding di- and trimethylation, monomethylation of H3K4, 9 and 27 and H4K20 do appear relatively early in the decondensing paternal chromatin but not immediately upon histone incorporation (Figure 1) [25, 26, 28, 39], suggesting that DNA demethylation, or at least decondensation must first take place before these modifications can occur. The precise enzymes responsible for placing these histone H3 methylation marks in the zygote have not been well defined. The variable localizations of these marks imply that, although their temporal dynamics appear closely correlated, their functional roles are likely to be distinct. These observations also suggest that the mono-, di- and tri-methyl activities are controlled by the temporal regulation of distinct protein complexes. Indeed, it seems that delayed H3K9 di- and tri-methylation in the male pronucleus might be caused by a maternally provided inhibitory factor rather than the lack of the enzyme itself [23] and in

Figure 1: Summary of the histone modifications occurring on maternal and paternal chromatin in germinal vesicle oocyte (GV) and pronuclear stages (PN 0-5) in the zygote. For color code and grade please refer to the online version of the manuscript, which contains a color version of this figure. The outer circle represents the nuclear

continued

addition Suvar3-9h2 mRNA is detected in fertilized oocyte and two-cell stage libraries (Unigene clusters Mm.128273). Similarly, H3K27 methylation shows delayed progression to trimethylation in the paternal pronucleus despite the presence of the Eed/Ezh2 protein complex in the zygote cytoplasm [26]. However, in this case the mechanism is due to a preferential recruitment of the complex to the maternal genome during early pronuclear stages and only at later pronuclear stages around S-phase it is apparent in the paternal pronucleus, concomitant with the appearance of di- and tri- methylation of H3K27 [26]. How this preferential targeting of the Eed/Ezh2 complex is regulated is unknown but intriguing.

RELATIVELY STABLE EPIGENETIC LANDSCAPE OF THE MATERNAL PRONUCLEAR CHROMATIN

The epigenetic modifications of the oocyte chromatin that will form the maternal pronucleus are more stable in general, while dynamic changes take place in the paternal pronucleus. The chromatin of the mature oocyte is organized such that histone modifications are already associated with the chromatin of the stalled metaphase II plate, undergoing relatively little changes during the zygotic stage. In particular, methylation of lysines characteristic of constitutive heterochromatin (such as H3K9me3, H3K64me3 and H4K20me3) are enriched in the regions surrounding the precursor nucleolar bodies in the maternal pronuclei only (Figure 1) [24, 27–29, 39]. These structures are uniquely apparent and very distinct in both pronuclei from the second pronuclear stage onwards and are surrounded by centromeric and pericentromeric satellite DNA [37]. Interestingly, in the female pronucleus H3K9me2 and not the H3K9me3 perinucleolar ring was shown to colocalize with regions enriched with DNA methylation [28]. The equalization of chromosomal organization between the paternal and maternal pronuclei is likely to be required for proper chromosome alignment and segregation during subsequent mitotic cycles in the developing embryo. Although this remains to be experimentally tested, this organization has also been suggested to be required for transcriptional silencing of pericentric satellite regions, in the zygote in particular. The paternal precursor nucleolar bodies have a similar organization to that of the maternal pronucleus but lack the characteristic constitutive heterochromatic marks (except HP1β) and this may therefore be relevant for the establishment of new heterochromatin domains in the paternal genome.

In contrast to lysine methylation, methylation of arginines H3R17 and H4/H2AR3 is not observed on the metaphase plate of the fertilized oocyte and H4/H2AR3me in particular is dramatically reduced upon fertilization and remains at low levels in both pronuclei throughout the zygotic stage, suggesting that some as yet unidentified arginine demethylase activity is active upon fertilization [39]. Since H4/H2AR3me methylation has been correlated with transcriptional repression [40] its removal may play a role in enabling gene activation to occur at later zygotic stages. Interestingly H4/H2AR3me is also lost during the reprogramming of germ cells, the other major reprogramming event during the life cycle of an organism, due to translocation of PRMT5 to the cytoplasm [41].

Histone acetylation, which is present at low levels in the mature oocyte also shows a delayed incorporation into the maternal chromatin after fertilization, which may be due to out competition by the decondensing paternal DNA, at least for hyperacetylated histone H4 [39, 42, 43]. However, by the third pronuclear stage, concomitant to DNA replication, the levels of acetylation of H4K5 appear similar in both pronuclei, colocalizing with areas enriched in chromatin as well as peripherally, while contrasting reports describe its presence or absence on the metaphase chromosomes at mitosis [42, 43]. Acetylation of histones H3 and H4 at a number of other lysine residues were observed in

Figure I: Continued
membrane of the germinal vesicle (GV stage) or the pronucleus (PN stage) and the nucleolar-like bodies (NLBs) are shown as just one inner circle for simplicity. Histone modifications associated with active transcription are coloured in green, repressive in red and for those with no clear distinct correlation in blue. The intensity of shading represents the relative intensity of the labelling throughout stages. Those marks that are reported to localize to the perinucleolar (NLB) ring(s) are highlighted on the inner circle in the appropriate colour. A question mark is shown for the modifications that have not been reported at the particular stage. Associated references are also shown on the right.

both interphase and mitosis in the zygote and at later stages [43]. Phosphorylation of the conserved N-terminal serine residues of histones H2A and H4 is observed stably and at equal levels in both pronuclei and appears to be more abundant in nuclear cortical regions [39]. Very little is known about the function of this mark, although it may play a role in mitosis [44].

The importance of these changes during normal development is not clear, although genetic studies have indicated that knockouts of many epigenetic modifiers result in embryonic lethality at early developmental stages, generally post-implantation [7]. However, such knockout studies typically do not take into account the maternal gene contribution, which controls events in the zygotic and to a lesser extent, the early cleavage divisions. Therefore, targeted knockouts or knockdowns in the mature oocyte or zygote need to be performed to analyse the role of the dynamic epigenetic changes in the first reprogramming event following fertilization. For example, depletion of maternally provided Ezh2, even when restoration of the protein occurs by the paternally provided allele results in severe long-term developmental effects [26].

Overall, it is clear that the parental chromatin undergoes distinct programmes and are epigenetically marked very differently upon fertilization. The female chromatin is enrobed in a myriad of relatively stable inherited chromatin modifications acquired during oocyte growth and only some changes occur, particularly of euchromatic marks that are directly linked to replication or transcriptional activation such as H3 and H4 acetylation and arginine methylation. In contrast, the decondensed paternal chromatin gradually acquires most histone modifications, with the exceptions of those located in the globular domain of histone H3 or marks characteristic of constitutive heterochromatin. Lack of the latter might be relevant to ensure a plastic state necessary for reprogramming.

CHROMATIN COMPONENTS FURTHER DEFINE PARENTAL ASYMMETRIES

The paternal chromatin also shows selective incorporation of the histone H3.3 replacement variant, while canonical H3.1 is absent until DNA replication [24, 45, 50]. This may play a role in directing or preventing modifications specifically in the paternal pronucleus at the zygotic stage, as the H3.3 variant has been associated predominantly with epigenetic marks associated with transcriptional activation and could also reflect a transient chromatin state during the reprogramming period [46–49]. A distinct role for H3.3 during reprogramming has also emerged with the observation that this variant is, surprisingly, associated with paternal pericentric heterochromatin during the first S-phase [50]. Furthermore, mutation of lysine 27 of H3.3 and not H3.1 leads to developmental arrest. This is likely to be due to a role for H3.3 K27 in the transcription of pericentric repeats and subsequent tethering of HP1β via its hinge region [50].

Variants of the other histones are also important for early mammalian development. In addition, phosphorylation of the H2A.X variant in the C-terminal SQEY motifs, which is enriched at sites of DNA double strand breaks in somatic cells, has been shown to be important during paternal pronuclear formation in Xenopus [51]. This serine phosphorylation is also enriched in the early male pronucleus in mouse embryos and persists in both pronulcei until the two-cell stage, while total H2A.X variant remains constant [14, 52]. Whether the function of this modification is conserved in DNA repair mechanisms or it is only involved in chromatin remodelling at this stage is not clear. Although asymmetry of the H2A.Z variant is not reported in the zygote, deletion of this variant results in failure around the time of implantation [53]. This is probably due to a specialized function of H2A.Z in trophectoderm development and/or differentiation, owing to a preferential expression of H2A.Z in trophoblast cells upon differentiation [54].

The linker histone H1 somatic subtypes are not present during oogeneis and fertilization until the two-cell stage [55]. Instead, an oocyte-specific H1 subtype, H1oo is present in mammals, which has substantial homology to the histone H1 subtypes B4 of Xenopus and cs-H1 of the sea urchin. These are likely trans-species homologs as they show similar developmental restricted incorporation during oogenesis and early developmental stages [51, 56–59]. The basis for the incorporation of these specific histone variants at this stage is unclear, but a role in either transcriptional repression during oogenesis or enabling chromatin remodelling and consequently transcriptional activation following fertilization has been suggested [59, 60].

GLOBAL CHANGES OF THE CHROMATIN IN CLEAVAGE STAGE EMBRYOS AFTER THE FIRST MITOTIC DIVISION

One of the most striking features of the organization of chromatin following completion of the first mitosis is the active and rapid removal of a number of histone modifications, including the methylation of two lysine residues in the globular domain of histone H3, K64 and K79 (Figure 2). H3K64me3, which is strongly enriched in the maternal chromatin throughout the pronuclear phases, is dramatically removed by the two-cell stage independently of DNA replication and remains undetectable until implantation [29]. H3K79me3 however, is gradually removed from the maternal chromatin during pronuclear stages before DNA replication begins and is also undetectable by the two-cell stage until post-implantation [30]. Both of these, (presumably) demethylation events are also observed upon parthenogenetic activation demonstrating that the mechanism for this active removal is sufficiently provided by the oocyte itself (Figure 2). The removal of these hydrophobic marks in the globular domain of histone H3 may unlock the chromatin structure allowing a higher level of plasticity for developmental transitions and transcription to occur during the two-cell and later stages.

During the first mitosis, the chromosomes move together but they are compartmentalized and remain separated even in two-cell stage embryos, indicating that the epigenetic programmes remain distinct. Concordantly, the differences between the maternal and paternal chromosomes' epigenetic marks can still be visualized at syngamy [8]. In particular H3K9me2/3, H4K20me3 and H3K64me3 are strikingly enriched on only half the metaphase plate, presumably the maternal genome, and H4K20me3 and H3K64me3 are then lost rapidly by the two-cell stage (Figure 2) [23, 27, 29]. On the other hand, H3K9me2/3 asymmetry can still be observed in two-cell embryos and decreases passively through the first replication cycles due to the absence of *de novo* methylation [23, 38]. It is likely therefore that this is due to the continued absence of Suv39h activity. Although the genomes remain compartmentalized in the four-cell stage, H3K9me2/3 asymmetry is lost and this is likely to be explained by the observation that methylation levels begin to increase at this stage, possibly due to the expression of the genes responsible from the zygotic genome or the repression of a

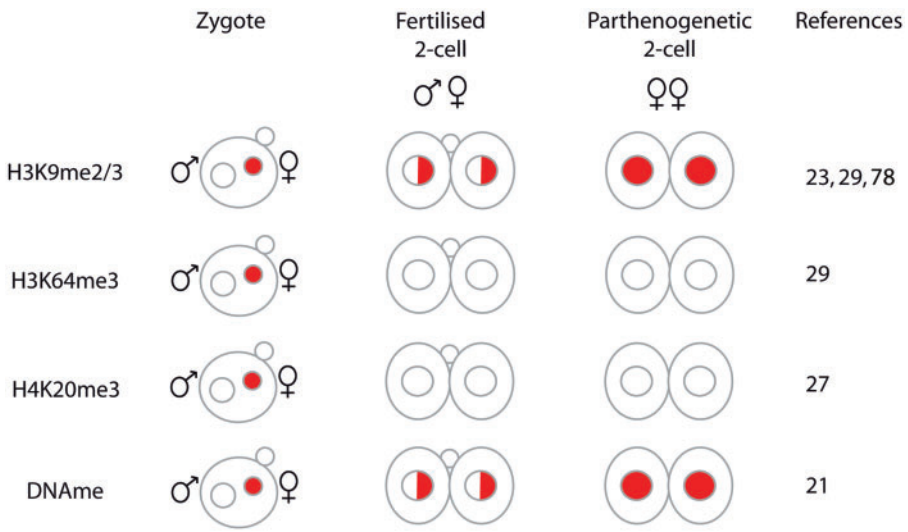

Figure 2: Distribution of heterochromatin marks in two-cell stage embryos from normal fertilization or parthenogenetic activation of oocytes. In two-cell embryos, the maternal and paternal chromosomes remain compartmentalized and therefore the maternally derived H3K9me2/3 and DNA methylation marks are present in only half the nuclear area. In parthenogenetically activated embryos, this asymmetry is not observed, demonstrating that the paternal chromatin is selectively maintained in a hypomethylated state. Other methylation modifications characteristics of heterochromatin (H3K64me3 ad H4K20me) are rapidly removed after the first embryonic mitosis in both types of embryos, suggesting distinct roles in heterochromatin formation/maintenance at these stages of development.

potential inhibitor(s) [23]. It is also possible that only after this stage, the global chromatin configuration of the embryonic chromatin becomes 'receptive' for Suv39h activity. Notably, levels of H3K9 methylation during these early cleavage stages have been correlated with successful development of cloned bovine embryos, where developmental success is low and defects in chromatin structure are often observed [61].

In addition to H3K9me2/3 passive dilution, DNA methylation is lost from the maternal genome across replication cycles due to the lack of maintenance DNA methyltransferase activity, except for certain imprinted genes and repetitive sequences [11, 13, 15–17]. However, maternal and zygotic Dnmt1 are both required for the maintenance of most DNA methylation imprints in the preimplantation embryo [62] in contrast to previous reports suggesting that the maternal Dnmt1o is excluded to the cytoplasm up to the 8-cell stage and the zygotic Dnmt1s is not expressed until post-implantation [63–65]. Therefore, the zygotic Dnmt1s must be present at low levels but specifically targeted to the particular imprinted regions during mitoses, by an unknown mechanism that could be via distinct epigenetic modifications. The maintenance of the methylation of particular genes above a certain threshold is essential for development as knockout embryos for the Dnmt1 methyltransferase arrest at the late gastrulation stage, with hypomethylated repetitive elements and imprinted genes [66–68]. Lineage-specific *de novo* DNA methylation does not begin thereafter until the late morula stage [13] and is also essential for development as knockouts of both the Dnmt3a and b enzymes also results in embryonic lethality at E11.5 [13, 16, 69].

The first differentiation event occurs before implantation, with the allocation of the trophectoderm and inner cell mass lineages commencing at the 8- to 16-cell stage [70, 71]. It is likely that epigenetic dynamics during the cleavage divisions of the zygote are important for this lineage specification, through their potential to regulate gene expression in a heritable manner [72]. On a global level the ICM displays higher levels of DNA methylation and H3K9me3 and H3K27me3 as well as lower levels of H2A/H4 phosphorylation [13, 26, 39, 73]. Furthermore, embryos lacking methyltransferases responsible for these DNA and histone methylations result in more severely affected embryonic than trophectoderm tissues and often fail in the transition from pre- to post-implantation development, suggesting that these asymmetries are likely to be functionally important [26, 66, 69, 74].

Specific evidence for the hypothesis that epigenetic modifications may play instructive rather than merely consequential roles, derives from the observation that, in a proportion of embryos, the vegetal blastomeres of 4-cell embryos have lower levels of H3R26me2 and are more likely to develop into trophectoderm tissues [75, 76]. Significantly, increasing the levels of H3R26me2 resulted in re-allocation into blastomeres of the ICM, providing evidence for a 'driver' role for an epigenetic modification during development [76]. This also supports a degree of flexibility in the early embryo, as the expression of an epigenetic modifier at the four-cell stage is able to change the allocation of the blastomere. With this in mind it would be interesting to determine whether at later stages this flexibility is lost. It is also important to note that it is likely to be the relationship with the surrounding cells and the relative levels of histone modifications and transcripts between cells rather than absolute levels that governs their eventual fate such that feedback and/or feedforward loops can then be set up to stabilize these effects.

Histone acetylation and other arginine methylations show cell-cycle regulated dynamics in cleavage-stage embryos. Hyperacetylated histone H4 is not observed on metaphase chromatin of blastomeres at the four-cell stage but becomes apparent at later stages suggesting that the appropriate acetyltransferase and deacetylase activities are present at this time. Dimethylation of arginines H3R17 and H4/H2AR3 are not associated with metaphase stage chromatin, although H4/H2AR3 becomes apparent at metaphase of mitotic blastomeres in the blastocyst [39]. H3R2me2 is also abundant during cleavage stages, at least between the two- and the eight-cell stages, although for this mark there is no known relationship to mitosis [76].

The structure and organization of chromatin regions differs fundamentally in zygotes and early embryos compared to somatic cells and this is likely to reflect the distinct plasticities and potencies of the genomes. On a general level chromatin progressively accumulates epigenetic marks during development that are likely to establish a heritable state of gene expression determining lineage allocation. Thus by extension, reprogramming requires the removal of the marks for the specification of the highly specialized sperm and oocyte. However

the situation is clearly more complex, highlighted by the significant asymmetry in the temporal and spatial dynamics of epigenetic modifications in paternal and maternal pronuclei, which is likely coupled to differences in transcription timing and the regulation of chromatin architecture in the pronuclei. The distinction of parental origin of the genomes at the epigenetic level is thus preserved despite the equalization of overall chromatin structure in the mature totipotent zygote and it remains to be determined whether this has functional consequences, for example, in imprinting or early development and differentiation events. It seems likely that histone lysine and arginine methylation may play a particularly crucial role during reprogramming and development due perhaps to their more stable nature than phosphorylation or acetylation and therefore greater potential for heritability, although these marks also clearly undergo dramatic dynamic regulation during reprogramming. Importantly, how all these epigenetic dynamics are achieved mechanistically remains obscure. Therefore the role and regulation of specific chromatin modifications in controlling gene expression during reprogramming and early development and differentiation needs to be addressed by modulation of the enzymatic activities responsible in temporally and spatially higher resolution studies.

Key points

- There is a fundamentally distinct structure and organization of chromatin in mouse zygotes and early embryos, of heterochromatin in particular.
- The paternal and maternal genomes undergo very different epigenetic programmes in the zygote.
- We propose that histone lysine and arginine methylation may play a particularly important role during reprogramming and early differentiation events.
- Higher resolution studies, both temporally and spatially need to be performed to address the role of specific chromatin modifications during pre-implantation development.

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