nature reviews genetics

https://doi.org/10.1038/s41576-025-00831-4

Review article

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Epigenome dynamics in early mammalian embryogenesis

Adam Burton[1] & Maria-Elena Torres-Padilla[1,2]

Abstract

During early embryonic development in mammals, the totipotency of the zygote — which is reprogrammed from the differentiated gametes — transitions to pluripotency by the blastocyst stage, coincident with the first cell fate decision. These changes in cellular potency are accompanied by large-scale alterations in the nucleus, including major transcriptional, epigenetic and architectural remodelling, and the establishment of the DNA replication programme. Advances in low-input genomics and loss-of-function methodologies tailored to the pre-implantation embryo now enable these processes to be studied at an unprecedented level of molecular detail in vivo. Such studies have provided new insights into the genome-wide landscape of epigenetic reprogramming and chromatin dynamics during this fundamental period of pre-implantation development.

Sections

[1]Institute of Epigenetics and Stem Cells (IES), Helmholtz Zentrum München, München, Germany. [2]Faculty of Biology, Ludwig-Maximilians Universität, München, Germany. e-mail: torres-padilla@helmholtz-muenchen.de

Introduction

During early embryonic development, the differentiated gametes are reprogrammed into a totipotent cell (the zygote) after fertilization that is capable of forming an entire organism. By the pre-implantation blastocyst stage in mammals, the generation of the inner cell mass and the trophectoderm marks the first cell fate decision and the concomitant emergence of pluripotency. As almost all cells in an organism share the same DNA sequence, cell plasticity must be controlled by the epigenome. Indeed, it has long been appreciated that the epigenetic landscape during early mammalian embryonic development is highly dynamic, characterized by global DNA demethylation, atypical patterns of histone post-translational modifications (PTMs), and the incorporation of histone variants[1–6]. Moreover, the knockout or depletion of several chromatin regulators results in early developmental defects, which confirms that chromatin-based epigenetic strategies are essential for the regulation of cellular reprogramming during early embryonic development[7].

Until recently, the limited cellular material available from pre-implantation embryos hindered the in-depth molecular study of chromatin at these stages. Now, low-input sequencing-based methods have become available that enable genome-wide mapping of epigenetic features such as nucleosome occupancy, histone PTMs, DNA methylation, transcription factor binding, the spatial organization of chromatin, and DNA replication timing (Table 1). These approaches have provided high-resolution maps of the epigenome of mammalian pre-implantation embryos, confirming that the pre-implantation epigenome is highly dynamic and has unique features[8–10]. Functional approaches, including knockdown of gene expression with small interfering RNA or antisense oligonucleotides, modification of the epigenome at specific sites (epigenetic editing) and acute protein depletion using Trim-Away[11], combined with low-input gene expression analysis, have started to uncover the regulators of zygotic genome activation (ZGA; also known as embryonic genome activation) in this context.

In this Review, we summarize recent major findings on chromatin-related processes in the early, pre-implantation, mammalian embryo and how they relate to the developmental process of ZGA. We then discuss how chromatin is globally remodelled upon fertilization, with a focus on chromatin dynamics at two key genomic features: *cis*-regulatory elements and heterochromatin. We also discuss how the 3D organization of the genome is established after fertilization. Lastly, and considering the major role of DNA replication and replication timing in nuclear organization and epigenetic inheritance, we highlight recent findings on the emergence of the DNA replication programme after fertilization and the association with chromatin regulation. Most of these studies have been carried out in mice, but where available, we discuss studies in other mammalian models, including bovine, pig and human embryos. Although cell culture model systems of early mammalian development have been introduced that recapitulate certain molecular features of embryos (Box 1), we focus this Review on studies of embryos in vivo.

Zygotic genome activation

Broad changes in the epigenome and in cellular potency take place during the switch from maternal to embryonic control of development, that is, the maternal-to-zygotic transition (MZT; also known as the oocyte-to-embryo transition). The MZT involves degradation of maternal RNA and proteins and activation of the embryonic genome (ZGA). Early studies using radiolabelled proteins and bromouridine triphosphate labelling of RNA have indicated that ZGA in mice occurs in two main waves (minor and major)[12] (Fig. 1).

Minor ZGA in mice occurs already in the zygote, coinciding with the onset of S phase of the first cell division[13,14]. Minor ZGA is characterized by prevalent transcription of intergenic regions and widespread loading of RNA polymerase II (Pol II), a phenomenon referred to as 'pre-configuration'[15,16]. Genes that are transcribed during minor ZGA may not always initiate at precise transcription start sites (TSSs) and are unspliced[17,18]. The exact number of genes that are transcribed during minor ZGA is difficult to determine, owing in part to the abundance of maternal RNAs in the zygote and the differential poly-adenylation of RNAs from oocyte to zygote, which interferes with poly-A-based RNA sequencing (RNA-seq) methods. Reversible inhibition of minor ZGA leads to developmental arrest and failure to initiate major ZGA, which suggests that minor ZGA is essential for developmental progression[19].

Major ZGA in mouse embryos initiates during S phase of the two-cell stage and involves the activation of several thousands of genes, many of which are expressed only at this stage of development[20]. The use of Pol II inhibitors to prevent major ZGA results in arrest at the two-cell stage, and thus, major ZGA is essential for development[21]. The transcriptional control during major ZGA is more canonical than that during minor ZGA and involves typical promoter-proximal TSSs, splicing and regulation by typical elongation factors such as SPT5 (refs. 16,17,22,23).

In other mammalian species, major ZGA occurs later in embryonic development and the distinction between minor and major ZGA is less clear. For example, in human embryos, the major wave of ZGA occurs at the four-cell to eight-cell stage, although transcriptional activity is detected as early as the zygote stage, producing correctly spliced transcripts, which suggests that the distinction between minor and major waves of genome activation in terms of splicing regulation is not conserved from mice to humans[24,25]. The distinction between minor and major ZGA has also been questioned recently in mice as nascent RNA-seq has revealed that many 'major ZGA genes' are transcribed earlier than previously thought[23]. This is most probably owing to the increase in sensitivity of emerging low-input RNA-seq approaches, but potentially also owing to the incomplete removal of steady-state or nonspecific RNAs.

A distinctive feature of pre-implantation embryonic development across mammalian species is the widespread transcription of the majority of transposable element families[26–28]. However, the regulatory effects of chromatin remodelling on the expression of transposable elements are not fully understood, and the transcription factors involved in their activation are only now beginning to emerge. In mouse, human and pig embryos, the most abundant transcribed transposable elements belong to the long terminal repeat (LTR) family of endogenous retroviruses. Their LTRs often function as alternative promoters and first exons, leading to the generation of novel protein isoforms, particularly in mouse oocytes and pre-implantation embryos[29–31]. For example, the MT2_Mm LTR of the mouse endogenous retrovirus MERV-L, which is highly and specifically expressed at the two-cell embryonic stage, functions as a promoter for a large proportion of the transcriptome at the two-cell stage[29,30,32–34]. Expression of MERV-L, but not the encoded retroviral proteins, is essential for pre-implantation development[34–36]. Interestingly, the MERV-L Gag protein has also been implicated in regulating the totipotency-to-pluripotency transition of the embryo by preventing the stabilization of pluripotency factors by the prefoldin protein URI[37]. ZGA also involves the prevalent transcription of non-coding RNAs, including long non-coding RNAs and small

Table 1 | Low-input genomics techniques to study chromatin in mammalian oocytes and pre-implantation embryos

Method	Full name	Application	Species	Refs.
ATAC-seq or ATAC-seq+CARM	Assay for transposase-accessible chromatin with sequencing+/−CRISPR–Cas9-assisted removal of mitochondrial DNA	Mapping chromatin accessibility	Mouse	69,104
			Human	103
			Bovine	102,112
li-DNAse-seq	Low-input DNAse I sequencing	Mapping chromatin accessibility	Mouse	75,99
			Human	106
ULI-MNase-seq	Ultra-low-input micrococcal nuclease digestion-based sequencing	Mapping nucleosome positioning	Mouse	92,98
scCOOL-seq	Single-cell chromatin overall omic-scale landscape sequencing	Simultaneous measurements of chromatin accessibility, DNA methylation and copy number variation	Mouse	105
			Human	101
CATCH-seq	Carrier-DNA-assisted chromatin immunoprecipitation and sequencing	Mapping H2AK119Ub	Mouse	73,113
ChIL-seq	Chromatin integration labelling with sequencing	Mapping RNA Pol II serine-2/5 phosphorylation	Mouse	16
CUT&RUN	Cleavage under targets and release using nuclease	Mapping H3K27me3	Mouse	116
		Mapping H3K4me3, H3K27me3, H3K27ac	Human	67
		Mapping H3.1/2	Mouse	90
		Mapping H2AK119Ub, H3K27me3	Mouse	71,72
		Mapping H3K4me3, H3K27me3	Bovine, pig, rat	57
		Mapping H3K9me3, H3K4me3, H3K27me3	Human	122
		Mapping H3K9me3	Mouse	122
		Mapping H3K27me3	Mouse	77
		Mapping H3K27ac	Mouse	69
CUT&Tag	Cleavage under targets and tagmentation	Mapping H3K4me3, H3K27me3, H3K9me3, H3K27ac	Bovine	70
Single-cell DamID	Single-cell DNA adenine methyltransferase identification	Mapping interactions with the nuclear lamina	Mouse	146
Stacc-seq	Small-scale Tn5-assisted chromatin cleavage with sequencing	Mapping RNA Pol II	Mouse	15
STAR ChIP-seq	Small-scale TELP-assisted rapid chromatin immunoprecipitation and sequencing	Mapping H3K27me3	Mouse	74
		Mapping H3K4me3	Mouse	62
		Mapping H3K36me3, H3K4me3, H3K27me3, H3K27ac	Mouse	76
		Mapping H3K36me2, H3K36me3	Bovine, pig, rat, mouse	57
		Mapping H3K27ac	Mouse	96
µChIP-seq	Micro-scale chromatin immunoprecipitation and sequencing	Mapping H3K4me3, H3K27ac	Mouse	61
		Mapping H3K27ac	Mouse, human	68
		Mapping H3K4me3	Human	68
		Mapping H3K9ac, H3K18ac	Mouse	68
		Mapping H3K4me3, H3K9me3, H3K36me3	Mouse	124
ULI-NChIP	Ultra-low-input native chromatin immunoprecipitation and sequencing	Mapping H3K4me3, H3K27me3	Mouse	60
		Mapping H3K4me3, H3K27me3, H3K27ac	Mouse	63
		Mapping H3K9me3, H3K4me3, H3K27me3	Mouse	95
		Mapping H3K27me2	Mouse	117
		Mapping H3K9ac	Mouse	97
		Mapping H3.3	Mouse	90
		Mapping H3K4me3	Bovine	66
		Mapping H3K9me3	Human	123
		Mapping H3K36me2, H3K36me3	Mouse	77
		Mapping H3K4me3, H3K27me3	Pig	65

Table 1 (continued) | Low-input genomics techniques to study chromatin in mammalian oocytes and pre-implantation embryos

Method	Full name	Application	Species	Refs.
Low-input Hi-C	Low-input high-resolution chromosome conformation capture coupled to high-throughput sequencing	Analysis of spatial chromatin organization	Mouse	149
			Human	153
Single-cell Hi-C	Single-cell high-resolution chromosome conformation capture coupled to high-throughput sequencing	Analysis of spatial chromatin organization	Mouse	155,157
Single-nucleus Hi-C	Single-nucleus high-resolution chromosome conformation capture coupled to high-throughput sequencing	Analysis of spatial chromatin organization	Mouse	150
sisHi-C	Small-scale in situ high-resolution chromosome conformation capture coupled to high-throughput sequencing	Analysis of spatial chromatin organization	Mouse	144,152
			Pig	154
scRepli-seq	Single-cell replication timing sequencing	Mapping replication timing	Mouse	162–164
			Mouse, bovine	165

non-coding RNAs, that have important regulatory roles[38]. An intriguing example is U7 small nucleolar RNA, which is itself regulated by a sperm-derived tRNA fragment and in turn regulates ZGA via chromatin compaction[39,40]. Furthermore, early embryonic development is also regulated by RNA modifications that exert post-transcriptional control (Box 2).

The identification of regulators of ZGA in mammals has become an intense area of research[41], which was spearheaded by the discovery that double homeobox (DUX) family members contribute to ZGA in placental mammals[42–44]. Loss of DUX in mice results in severe defects in pre-implantation development ex vivo. The conserved roles of DUX and DUX4 in humans as key drivers of ZGA are, at least in part, associated with direct activation of LTR transposable elements from the MERV-L family in mice and, potentially, HERV-L in humans[43,45]. However, DUX is not essential for embryonic development in vivo, with approximately 13%–17% of major ZGA genes affected by its loss, which suggests that additional transcription factors and/or signalling mechanisms regulate ZGA in vivo[45–48]. Recently, several additional transcription factors that contribute to ZGA in mammals have been identified, including additional homeobox transcription factors from the OBOX family in mice and TPRX family in humans (reviewed in refs. 41,49). Changes to chromatin are thought to function together with sequence-specific transcription factors to drive the gene expression programmes during ZGA, but their precise molecular relationships remain to be determined.

Epigenetic reprogramming upon fertilization

The information content of chromatin is multi-layered, consisting of nucleosome (dis)assembly and positioning along the DNA; incorporation of distinct histone variants, chromatin-associated proteins, and RNAs; histone PTMs; and DNA methylation. All of these 'layers' undergo considerable changes during pre-implantation embryonic development, and different genomic features (in particular, *cis*-regulatory elements and heterochromatin, as discussed here) have distinct stage-specific chromatin conformations (Fig. 2). Upon fertilization, the zygote harbours the two parental genomes from sperm and oocyte in physically segregated pronuclei. The parental genomes are initially transcriptionally silent and have distinct chromatin configurations that undergo marked reprogramming at fertilization[10].

Chromatin configuration of sperm

Here, we briefly summarize chromatin remodelling during spermatogenesis (further details can be found in recent reviews[50,51]). The paternal genome of the sperm features the widespread replacement of histones by protamines. The histone-to-protamine transition in mammals involves multiple germline-specific histone H1, H2A, H2B and H3 variants and histone PTMs, which are thought to incorporate transiently into the chromatin of elongating spermatids to increase chromatin accessibility, thereby facilitating the replacement of histones by transition proteins and then by protamines[50]. Although it was long thought that protamine replacement enables tighter packaging of the paternal genome into the small nucleus of the sperm, recent work in *Drosophila* has shown that aberrant histone retention is compatible with fertilization but results in misidentification and loss of the paternal chromosomes in the zygote[52]. Whether the same applies to mammals remains to be determined. In mature mammalian sperm, some histones are retained, with the proportion of histones to protamines ranging from 2% to 15% depending on the species. However, the sites of histone retention and their PTM status are currently unclear[50,51] owing to technical difficulties in studying the largely compacted sperm chromatin[50,51,53]. As such, the extent to which epigenetic information in sperm histones is transmitted to the fertilized embryo is unclear. Mammalian sperm DNA is hypermethylated, except at CpG islands, a process that has been shown to depend, in mice, on NSD1-mediated dimethylation of histone H3 lysine 36 (H3K36me2)[54]. Although genome-wide DNA demethylation occurs upon fertilization, the retention of DNA methylation at precise but differential regions in both parental genomes contributes to epigenetic imprinting.

Chromatin configuration of oocytes

The oocyte genome is arrested at the diplotene stage of meiosis I and transitions to metaphase of meiosis II during maturation and ovulation. Although oocyte chromatin contains nucleosomes, it is characterized by numerous histone variants and atypical distributions of histone PTMs and DNA methylation (Fig. 2a). In contrast to the DNA hypermethylation observed in sperm, oocytes have a highly distinctive distribution of large (10–50 kbp) domains that are hypermethylated, hypomethylated or partially methylated, a pattern that seems to be conserved across mammals[55–57]. DNA hypermethylated domains are generally associated with actively transcribed regions during oocyte growth; however, in pig and bovine oocytes (and to an intermediate extent in human oocytes), in which a greater proportion of the genome is methylated compared with mice, hypermethylated domains are also found at non-transcribed regions, with the exception of CpG continents, which are hypomethylated[57–59]. DNA hypomethylated

domains and partially methylated domains are located at intergenic regions but also at non-transcribing gene bodies and promoters, including genes associated with ZGA, and are occupied by broad domains of H3K4me3 spanning more than 10 kb, which cover ~20% of the genome in mouse oocytes[60–62]. These H3K4me3 broad domains (also known as non-canonical H3K4me3 (ncH3K4me3)) are established by KMT2B (also known as MLL2) during oocyte growth[63,64]. Interestingly, the presence of ncH3K4me3 coincides with the time of transcriptional silencing of the oocyte genome, and removal of H3K4me3 in mouse oocytes through expression of the demethylase KDM5A leads to global transcriptional activation[62,64], which suggests that H3K4me3 may be linked to transcriptional silencing in the mouse germline. Broad ncH3K4me3 domains are also detected in pig and bovine oocytes, wherein they also correlate with regions with low levels of DNA methylation, but human oocytes contain exclusively canonical H3K4me3, which is markedly enriched at promoters[57,65–67]. Broad non-canonical domains of H3K27 acetylation (ncH3K27ac) that colocalize with ncH3K4me3 are also detected in mouse and bovine, but not human, oocytes[68–70]. However, human embryos, in addition to the enrichment of canonical H3K4me3 at promoters, are characterized by broad ncH3K4me3 and ncH3K27ac domains present at distal regulatory regions before ZGA[67,68]. These results suggest that although broad, non-canonical domains of histone PTMs do not necessarily occur at the same stage in all mammalian species, they are a common feature of mammalian epigenetic reprogramming around the time of fertilization.

Histone PTMs typical of facultative heterochromatin, such as H3K27me3 and H2AK119 ubiquitylation (H2AK119ub), are also found in broad domains covering around one-third of the genome in mouse oocytes[71–74]. ncH3K27me3 is enriched at intergenic regions, non-canonical imprinted genes and non-transcribed promoters lacking DNA methylation and is largely non-overlapping with ncH3K4me3 (refs. 60,63,71–75). This landscape of histone PTMs in oocytes results in the resetting of chromatin to a bivalent state that persists until the post-implantation blastocyst. Pig and bovine oocytes also contain broad domains of ncH3K27me3, but in these species, there is greater overlap between ncH3K27me3 and ncH3K4me3 domains[57,65,70], although H3K27me3 is largely depleted outside CpG continents in pig oocytes[57]. Human oocytes, in keeping with the presence of canonical H3K4me3, also have a canonical H3K27me3 pattern, lacking ncH3K27me3 broad domains[67].

H3K36 methylation has a crucial role in regulating the epigenome in mouse oocytes. H3K36me3, catalysed by the methyltransferase SETD2, is found at transcribing gene bodies in growing oocytes and persists after oocytes undergo transcriptional silencing[76]. H3K36me3 accumulation negatively correlates with both ncH3K4me3 and ncH3K27me3 domains[76]. H3K36me2 and H3K36me3 recruit DNMT3A to direct DNA methylation at those transcribed gene bodies[77]. Remarkably, loss of H3K36 methylation leads to a marked alteration in the DNA methylation landscape in mouse oocytes, which indicates that H3K36me3 has a role in setting the epigenome for the next generation[76,77]. In keeping with this, loss of H3K36me2 and H3K36me3 leads to the global redistribution of H3K4me3 and H3K27me3 in mouse oocytes[76], and expression of the dominant-negative H3.3K36M mutant in mouse oocytes, which cannot be methylated, leads to developmental defects upon fertilization[77,78]. H3K36me3 strongly correlates with DNA methylation also in pig, bovine and rat oocytes, which suggests that there may be a conserved mechanism for establishing DNA methylation in mammalian oocytes[57].

Chromatin changes at fertilization

The distinct chromatin configurations of the two parental genomes unite in the zygote. With the exception of imprinted regions and some classes of retrotransposons, a conserved genome-wide demethylation of methyl-CpG occurs — by both active enzymatic processes and passive dilution across cleavage cycles — but with different dynamics in the two genomes[79–85]. One of the earliest events after fertilization is nucleosome assembly following protamine eviction on the paternal genome, which is apparent within 1 h after fertilization in mice. In particular, HIRA-mediated incorporation of H3.3 is essential for de novo nucleosome assembly[2,86–88]. In addition, DAXX-dependent incorporation of H3.3 at paternal pericentric heterochromatin is essential for chromosome stability[89]. H3.3 has a non-canonical, genome-wide distribution pattern in zygotes, characterized by an even distribution in both parental genomes, probably because H3.3 is the dominant form of H3 at this stage of development[90]. The redistribution of H3.3 into a more canonical distribution pattern — for example, enrichment at TSSs — occurs at ZGA but, intriguingly, depends on DNA replication but not on transcription[90]. Notably, the genomic redistribution of H3.3 at ZGA depends on the replication-dependent chaperone CAF1 (encoded by *Chaf1*), which incorporates the H3.1 and H3.2 variants[90]. CAF1 downregulation, which induces mouse embryonic stem cells to adopt a two-cell stage-like phenotype[91], also partially recapitulates the 'fuzzy' (less well-defined) nucleosome positioning that is typical of zygotes and two-cell-stage embryos and leads to a partial phenotype of non-canonical, broad H3.3 distribution[92], which emphasizes the biological relevance of chromatin assembly and nucleosome positioning for control of totipotency. In fact, mouse embryos without CAF1 activity do not develop beyond the morula stage (approximately 16 cells) and exhibit upregulation of transposable elements, highlighting a potential role for CAF1 in promoting the deposition of repressive histone PTMs at the earliest stages of embryogenesis[93–95].

The histones incorporated in the paternal genome after protamine eviction are largely hyperacetylated and hypomethylated,

Box 1 | In vitro models of early mammalian embryonic development

Rare populations of cells that molecularly and functionally resemble totipotent early embryonic stages at the time of zygotic genome activation (ZGA) have been discovered in both mouse and human embryonic stem cell cultures[32,175,176]. In mice, these cells are typically marked by expression of the mouse endogenous retrovirus MERV-L, which is specific to two-cell-stage embryos, whereas in humans, various markers specific to ZGA have been used[177,178]. Several factors, including transcription factors, epigenetic regulators, small molecules, microRNAs, metabolic cues and osmotic stress, have been shown to regulate the emergence of these cells in culture[179–181]. Improving the protocols used to generate cells that more closely resemble totipotent cells both molecularly and functionally will greatly assist research in this area in the future[182]. In particular, it will be important to determine which of the large number of artificial inducers that have been identified in culture are involved in cellular reprogramming to totipotency in vivo. The standardization of cell culture protocols and the creation of robust, realistic guidelines to test cellular potency will also be of great use.

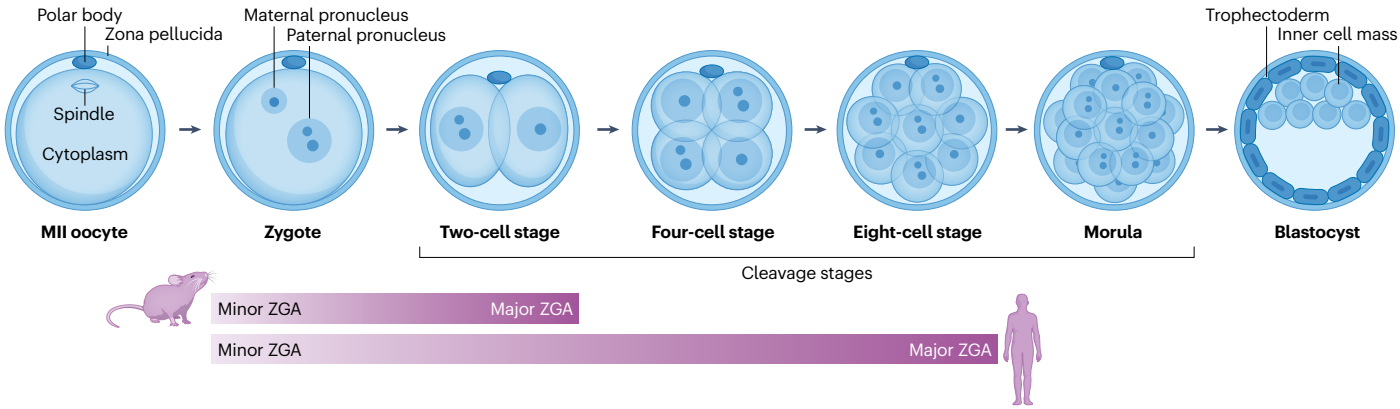


Fig. 1 | Overview of pre-implantation embryonic development in mice and humans. The relative timings of minor and major zygotic genome activation (ZGA) are indicated for mouse and human embryos. Fertilization of the oocyte by the sperm generates a totipotent zygote, which undergoes several cleavage divisions during pre-implantation development to generate the blastocyst, which is composed of an outer layer of trophectoderm and the inner cell mass. The morula stage refers to embryos that have undergone the process of compaction, when cellular adhesion and polarization take place at around the eight-cell stage and before the formation of the blastocyst. MII, metaphase II.

thus establishing an epigenetic asymmetry between the paternal and maternal pronuclei, which was initially described more than 20 years ago by immunostaining and confirmed more recently by genomics studies. The maternal epigenome is generally more stable than the paternal epigenome; the broad non-canonical domains of H3K4me3, H3K27ac and H3K27me3 that are found in mature oocytes are largely inherited in the zygote, although H3K27ac is globally depleted in oocyte meiotic chromatin[62,68,69,74]. The inheritance of histone marks between sperm DNA and paternal DNA in zygotes has been more difficult to assess, owing to technical difficulties in studying the largely compacted sperm chromatin. In mouse zygotes, the paternal genome also acquires broad ncH3K4me3 domains, albeit at lower levels than the maternal genome[62]. Interestingly, expression of H3.3K4M (a dominant-negative inhibitor of histone methylation) before fertilization, or knockdown of KMT2C and KMT2D (also known as MLL3 and MLL4) methyltransferases, which leads to reduction of H3K4me in the paternal pronucleus, causes defects in minor ZGA and developmental arrest, which suggests that de novo paternal H3K4 methylation has an important role in minor ZGA[78]. The paternal genome in mouse zygotes also acquires broad ncH3K27ac domains, and the distinct acetylation patterns of the two parental alleles become equalized by the two-cell embryonic stage[69,96]. In addition, H3K9ac has a similar distribution to H3K27ac in zygotes[69,97]. However, levels of H3K9ac are comparable between zygotes and the two-cell stage, whereas H3K27ac undergoes marked remodelling between these two stages, particularly on distal regulatory regions[69,96]. In human embryos also, H3K27ac undergoes extensive reprogramming before ZGA[68], which suggests that H3K27ac modification is highly dynamic in zygotes of both species.

Although sperm and oocyte epigenomes have different chromatin states at fertilization and there is chromatin asymmetry between parental pronuclei of the zygote, both parental genomes undergo considerable remodelling after fertilization. In addition to changes in histone and DNA methylation and histone acetylation, the chromatin accessibility and nucleosome positioning landscapes are also remodelled. In mice, chromatin accessibility profiles become largely similar at a global level between the two pronuclei as early as 6 h after fertilization[98,99]. Similarly, during reprogramming by somatic cell nuclear transfer, nucleosome remodelling occurs rapidly within 12 h and is independent of DNA replication[100]. By contrast, in human embryos, the paternal chromatin remains globally more accessible than the maternal chromatin until the four-cell stage[101]. Although nucleosome incorporation occurs early after fertilization, the global chromatin landscape before ZGA is highly atypical in mouse, human and bovine embryos. In particular, embryos before ZGA have a weak and 'noisy' ATAC-seq (assay for transposase-accessible chromatin with sequencing) signal, with fewer peaks and reduced genome coverage[102–104]. Another distinctive feature is the presence of broad accessible regions, which are particularly enriched around full-length MT2_Mm sequences, in early two-cell stage mouse embryos[104]. In accordance, a recent study using low-input MNase (micrococcal nuclease) sequencing has found that mouse zygotes have fuzzy nucleosome positioning with shorter nucleosome arrays flanking nucleosome-depleted regions[92]. Increasing regularity in nucleosome spacing and a clearly defined +1 nucleosome downstream of the TSS emerge with developmental progression in both mouse and human embryos[92,101,105].

Chromatin dynamics at *cis*-regulatory elements

cis-Regulatory elements, including promoters and enhancers, are characterized in most cell types by the presence of a nucleosome-depleted region (NDR) and the enrichment of specific chromatin marks. Active promoters are typically enriched in H3K4me3 and histone acetylation, whereas active enhancers are characterized by the presence of H3K4me1 and H3K27ac. In mouse zygotes, the promoters of minor and major ZGA genes are marked by NDRs in both parental genomes before the transcription of minor ZGA genes[75,98,105]. This has been interpreted as 'priming' for transcriptional activation. In fact, the establishment of NDRs at the zygote stage is largely independent of transcription — and of DNA replication — but is sensitive to global inhibition of histone deacetylase activity, which leads to altered NDR formation particularly in the male pronucleus[98]. Knockdown of the transcription factors MLX and RFX1, whose binding motifs are associated with NDRs in mouse zygotes, prevents NDR formation in the male chromatin and results in defective ZGA[98]. Increased promoter accessibility before transcriptional onset is also observed at later stages of mouse pre-implantation

development[99,105], and similarly, a subset of ZGA genes have accessible promoters in human embryos at the two-cell or four-cell stage, before these genes are transcribed[103,106].

Promoters are widely bound by serine-5-phosphorylated Pol II in mouse zygotes, including those promoters of genes that are not expressed at ZGA[15,16] (Fig. 2b). At the early two-cell stage of mouse embryos, Pol II becomes enriched at promoters of major ZGA genes before detectable transcription at these sites, a process termed pre-configuration[15,16]. Treatment with a Pol II elongation inhibitor during minor ZGA or knockout of *Obox* family transcription factor genes prevented Pol II pre-configuration, leading to ectopic gene activation[15,107]. Interestingly, whereas the genome-wide occupancy maps of initiating and elongating forms of Pol II are distinct in pluripotent or differentiated cells, they are practically indistinguishable in mouse embryos up to the eight-cell stage[16]. A potential interpretation of these data is that Pol II undergoes limited promoter-proximal pausing at actively transcribed genes during ZGA.

H3K4me3 is also enriched around active TSSs at the time of ZGA in all mammalian species examined so far[57,60–62,65,66]. The accumulation of H3K4me3 at TSSs results from remodelling of the ncH3K4me3 broad domains in oocytes and is dependent on transcription, at least in mice[61]. In fact, failure to remodel ncH3K4me3 into 'sharp' H3K4me3 peaks at promoters, through down-regulation of KDM5A and KDM5B demethylases, leads to ZGA failure in mouse, bovine and pig embryos[61,65,66]. In human embryos, the pattern of H3K4me3 is somewhat different: the distal ncH3K4me3 domains are remodelled into canonical H3K4me3 at the eight-cell stage — the time of ZGA in human embryos — but the accumulation of canonical H3K4me3 marks both ZGA genes and non-activated gene promoters pre-ZGA at the four-cell stage[67]. Thus, although there are some species-specific temporal dynamics, in general, gene promoters in mammalian embryos acquire the typical NDR architecture, Pol II occupancy and canonical H3K4me3 at their TSSs at the time of ZGA.

The function of enhancers during early embryonic development is still under investigation. Earlier work in mouse embryos showed that plasmid-encoded reporters require an enhancer for robust expression at the two-cell embryonic stage but not in the oocyte or at the zygote stage[108]. More recently, however, putative enhancers identified by cap analysis gene expression with sequencing (CAGE-seq) in mouse oocytes were shown to drive reporter gene transcription from a distal location, which is a characteristic of functional enhancers[96]. These putative enhancer sites are marked by H3K27ac and, surprisingly, also by H3K4me3 in mouse oocytes and early pre-implantation embryos[96]. Mapping of putative enhancer RNA loci — which are enriched in H3K27ac within open chromatin regions across developmental stages — has revealed a highly dynamic enhancer landscape in mouse pre-implantation embryos[109]. An increase in both the number and proportion of distal NDRs occurs from the eight-cell stage in mouse embryos, which also become enriched in H3.3, which suggests that there is an expansion of the number of enhancers at this developmental time[90,99]. Distal sites at the eight-cell stage are enriched in binding sites for lineage-determining transcription factors such as OCT4, and the accessibility of a subset of these sites depends on OCT4 (refs. 99,105). The transcription factor YY1 is also enriched in distal NDRs in mouse embryos and is required for the formation of regularly spaced nucleosome arrays at thousands of enhancer-like elements at the eight-cell stage[92]. Human embryos, compared with mice, have a greater proportion of distal NDRs relative to promoters before ZGA, which often coincide with transposable elements that are expressed at ZGA, such as long interspersed nuclear elements (LINEs), HERV-K and SINE-VNTR-Alu (SVA) retrotransposons[106].

In both mouse and human embryos, the histone PTMs that are characteristic of regulatory regions emerge at and depend on ZGA, which involves the resolution of ncH3K27ac and ncH3K4me3 domains into 'sharp' enhancer and promoter peaks at this stage[61,68,69,96,110]. Enzymes that mediate H3K27ac remodelling include the histone deacetylases HDAC1, which is essential for early embryonic development[111], and NAD^+-dependent SIRT1 (ref. 110). Moreover, not surprisingly, transient inhibition of CBP and p300 histone acetyltransferase activities in mouse zygotes and early two-cell stage embryos leads to failure of both minor and major ZGA and embryonic arrest at the two-cell stage; also, knockdown of p300 in human zygotes leads to defects in ZGA[23,68,69]. Targets of CBP and p300 activity include distal regions that gain a pre-configuration of H3K27ac at the early two-cell stage, thus potentially functioning as enhancers[69,96]. In addition to the remodelling of H3K4me3 and H3K27ac at ZGA, the promoter-proximal nucleosome architecture also changes concomitantly with ZGA in mouse, bovine and human embryos, characterized by a marked increase in the number and width of promoter-proximal NDRs[99,101,105,106,112]. The widening of promoter-proximal NDRs correlates with increased transcriptional activity, and the formation of a subset of these wide NDRs (more than 300 bp) depends on transcription, which is in contrast

Box 2 | RNA modifications in early embryonic development

More than 100 chemical modifications of cellular RNAs have been identified, with emerging functions as post-transcriptional epigenetic regulators[183]. These modifications can affect the stability, processing, secondary structure, interactions and localization of various RNA species, both coding and non-coding[184]. Among them, N^6-methyladenosine (m^6A) is catalysed co-transcriptionally by METTL3–METTL14 and METTL16, is specifically read by proteins of the YTH, IGF2BP and HNRNP families, and is erased via active demethylation by FTO or ALKBH5. Depletion or inhibition of many of these factors results in early embryonic lethality[185–190]. m^6A regulates the expression of long terminal repeats in the inner cell mass of mouse blastocysts and in mouse embryonic stem cells[187,191–193]. Widespread m^6A modification across the coding and non-coding transcriptome has been reported in mouse oocytes and embryos[189,194–196]. m^6A is also enriched in transcripts from zygotic genome activation-specific genes and transposable elements such as the mouse endogenous retrovirus MERV-L, wherein it contributes to their timely decay; m^6A also contributes to the stability of maternal mRNAs and transposable elements, such as the highly abundant MTA retrotransposons[189,194–196]. Several transcripts from genes important for lineage allocation, such as *Oct4*, *Nanog*, *Cdx2* and *Yap1*, are marked by m^6A in early embryos, which suggests that RNA modifications provide an additional layer of regulation to chromatin and DNA modifications during development[189,194,196]. In the future, it will be important to dissect the interplay between RNA and chromatin modifications in embryonic development and to determine whether m^6A or other RNA modifications affect the embryonic translatome, which has been recently studied genome-wide[197–199].

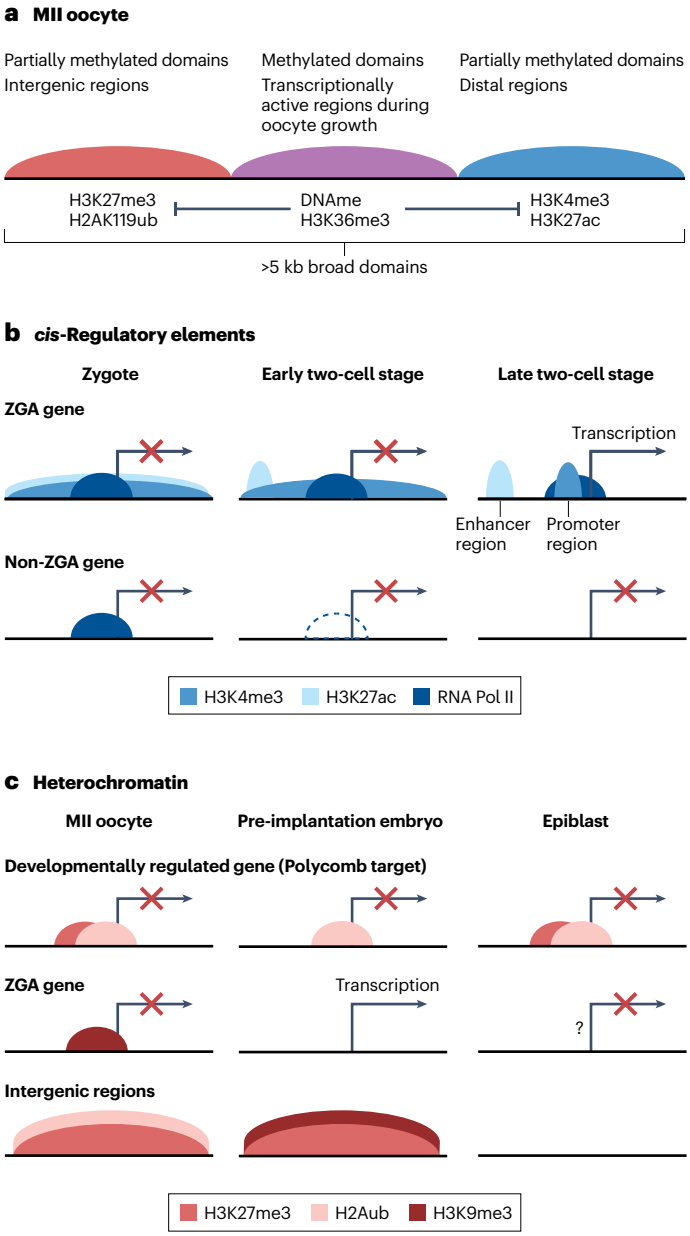


Fig. 2 | Dynamics of histone post-translational modifications during early mouse embryonic development. a, Depiction of the general pattern of broad domains of non-canonical histone post-translational modifications (PTMs) across the genome in metaphase II (MII) oocytes in mice. Oocytes contain regions of high CpG DNA methylation (DNAme) associated with histone H3 lysine 36 trimethylation (H3K36me3), which correspond to domains containing actively transcribed genes during oocyte growth. Oocytes also contain partially methylated and hypomethylated DNA regions that are enriched in broad, non-canonical domains of H3K27me3 or H3K4me3. H3K36me3 accumulation negatively correlates with both H3K4me3 and H3K27me3 non-canonical domains. K3K27me3 and H2AK119 ubiquitylation (H2AK119ub) are coupled in oocytes and enriched in intergenic regions. H3K27 acetylation (H3K27ac) colocalizes with H3K4me3 in mouse oocytes and is enriched in distal regulatory regions. **b**, Remodelling of active histone PTMs at *cis*-regulatory regions, from the zygote to late two-cell-stage embryos at the time of zygotic genome activation (ZGA). Loading of RNA polymerase II (RNA Pol II) in the zygote occurs at genes that are both transcribed and non-transcribed during ZGA. The pre-configuration (enrichment before transcription) of RNA Pol II specifically at promoters of major ZGA genes is shown at the early two-cell stage. At ZGA genes, broad non-canonical domains of H3K27ac colocalize with broad domains of H3K4me3 in zygotes. H3K27ac undergoes marked remodelling between zygotes and the two-cell stage of embryos, resolving into 'sharp' peaks particularly on distal regulatory regions such as enhancers. H3K4me3 is remodelled into sharp peaks at gene promoters by the late two-cell stage at the time of ZGA. Non-ZGA genes here refers to genes that have RNA Pol II enrichment at promoters in zygotes but are not activated at ZGA and gradually lose RNA Pol II enrichment by ZGA (as indicated by the dashed curve at the early two-cell stage). **c**, General organization of heterochromatic histone PTMs in MII oocytes, during pre-implantation development and in the epiblast layer of the inner cell mass (blastocyst stage). At Polycomb targets, such as developmentally regulated genes, H2A ubiquitylation (H2Aub) is inherited from oocytes and retained after fertilization in pre-implantation embryos. By contrast, H3K27me3 marks in oocytes are erased at fertilization, and de novo H3K27me3 marks only emerge in the epiblast of late blastocysts. ZGA genes are marked by H3K9me3 in oocytes, which is lost between fertilization and their activation at the two-cell stage. It is currently unclear which, if any, heterochromatic histone PTMs these genes acquire after they are activated (indicated by the question mark in the epiblast stage). H3K27me3 and H2AK119ub form broad domains at intergenic regions in oocytes, which lose H2AK119ub by the two-cell stage. Domains containing both H3K27me3 and H3K9me3 are found transiently at intergenic regions during pre-implantation development.

to the transcription-independent establishment of NDRs in the zygote[101,103,105]. Once established, NDRs are largely maintained during development and contain characteristic motifs for transcription factor binding. Among those, the binding motif for NFYA is the most prevalent in NDRs of mouse two-cell-stage embryos, which suggests that promoters and regulatory regions are bound by NFYA at this stage. Accordingly, NFYA knockdown results in defective ZGA and developmental arrest at the morula stage[99]. Mouse pre-implantation development is also characterized, unusually, by the presence of accessible chromatin at transcriptional end sites, which gradually decreases until the blastocyst stage[104].

Heterochromatin formation after fertilization

How heterochromatin forms at the beginning of development is a fundamental question in embryogenesis. Both facultative heterochromatin and constitutive heterochromatin have distinctive features in pre-implantation embryos that differ from pluripotent or differentiated cells (Fig. 2c). For example, whereas H3K27me3 and H2AK119ub are typically coupled and characteristic of facultative heterochromatin in differentiated cells, their localization across genomic regions and their temporal dynamics are uncoupled in early mouse embryos[71–73]. At typical Polycomb targets, such as developmentally regulated genes, in pre-implantation embryos, H2AK119ub is either inherited from oocytes and retained after fertilization or deposited by the Polycomb repressive complex 1 (PRC1) from the two-cell stage onwards. By contrast, H3K27me3 marks found in oocytes are erased from promoters at fertilization in zygotes, and de novo H3K27me3 marks, which are deposited by PRC2, only emerge in the epiblast layer at the late blastocyst stage[60,71–74,113]. This de novo deposition of H3K27me3 occurs concurrently with the re-establishment of bivalent domains. Because of the prominent presence of H2AK119ub in cleavage stage embryonic cells, a role for H2AK119ub in 'priming' or acting as a placeholder for Polycomb targets has been suggested. Indeed, acute depletion of H2AK119ub by expression of the Polycomb repressive deubiquitinase (PR-DUB)

complex in zygotes leads to ectopic expression of later developmental genes and developmental failure, which indicates that H2AK119ub has a repressive function in early mouse embryos[71]. Similarly, depletion of RING1A and RING1B (also known as RNF2) — which are catalytic components of PRC1 — in mouse oocytes results in failure to silence inappropriate gene expression in oocytes and subsequent maternal infertility[73,114]. Depletion of the deubiquitinase USP16 during oocyte growth leads to a failure to remove H2AK119ub globally, including from ZGA genes, and results in defects in ZGA and mouse embryonic development[113]. As H3K27me3 is largely erased after fertilization in human, pig and bovine embryos, similarly to mouse embryos, the predominant role of H2AK119ub in maintaining repression in mouse embryos may be conserved in other mammals[57,65,67]. A recent study in bovine embryos questions the extent to which H3K27me3 marks are erased in zygotes, although this may be owing to cross-reactivity of the antibody used in this study with H3K18me3 (refs. 70,115).

In mouse and rat embryos, maternally inherited H3K27me3, but not H2AK119ub, is maintained in broad domains that persist to the pre-implantation blastocyst stage but are lost in post-implantation embryos[57,71,74,75]. Some of these H3K27me3 domains function as a non-canonical imprinting mechanism to regulate allelic expression[75,116]. Most evidence suggests that this may be a rodent-specific mechanism, based on the global loss of H3K27me3 in other mammalian embryos, but this remains to be formally demonstrated[57,67]. On the paternal allele in mouse zygotes, intergenic regions also accumulate broad domains of ncH3K27me3 after fertilization, although H3K27me3 enrichment is much lower than for the maternal allele and occurs on different regions. This allelic H3K27me3 asymmetry persists throughout mouse pre-implantation development[74,117].

The features of constitutive heterochromatin are best exemplified by pericentric heterochromatin, which in most cell types is silenced through H3K9me3 deposition mediated by the histone methyltransferases SUV39H1 and SUV39H2. In mouse zygotes, however, paternal pericentric heterochromatin is established by Polycomb repressive complexes and its associated PTMs, whereas maternal pericentric heterochromatin is marked by SUV39-dependent H3K9me3 (refs. 88,117–119). This results in an asymmetry that persists until the eight-cell stage. By contrast, in human embryos, both paternal and maternal pericentric heterochromatin are marked by canonical H3K9me3 (ref. 120).

Globally, levels of H3K9me3 and its genomic locations are extensively reprogrammed during mammalian pre-implantation development; H3K9me3 is both acquired and lost across many genomic regions in both parental genomes[95,121–123]. H3K9me3 is enriched on genic regions in mammalian oocytes, suggesting that it has a role in transcriptional silencing in oocytes[70,95,122]. In keeping with this, loss of the H3K9me3 demethylase KDM4A in mouse oocytes leads to the spreading of H3K9me3 into ncH3K4me3 broad domains that contain ZGA genes, resulting in defective ZGA upon fertilization[124]. After fertilization, H3K9me3 becomes enriched on intergenic regions that overlap with maternal H3K27me3 domains, and both of these histone PTMs are depleted on promoters[70,95,122,123]. In particular, the enrichment of H3K9me3 on species-specific LTRs, and to a lesser extent LINE-1 elements, is observed in mouse, bovine and human pre-implantation embryos. The presence of H3K9me3 at these elements has been linked to their repression at a time of genome-wide DNA demethylation[70,95,122,123,125]. However, depletion of H3K9me3 in early mouse embryos does not lead to the derepression of transposable elements, probably owing to the additional need for dedicated transcription factors for transposon activation[121,126]. In accordance, knockdown of several heterochromatin proteins from the SUMO or SETDB1 pathways leads to loss of H3K9me3 on some LTRs, but derepression of these LTRs occurs only upon depletion of CAF1, which suggests that nucleosome assembly or other heterochromatic PTMs, such as H4K20me3, have more important roles than H3K9me3 in LTR repression at this developmental stage[94,95]. Removal of H3K9me2 through depletion of the methyltransferase EHMT2 (also known as G9A) also has minimal effects on LTR expression in mouse embryos[127,128].

Indeed, the non-repressive nature of H3K9me3 in early mouse embryos might suggest that their heterochromatin is in an immature state. Experimentally induced gain of H3K9me3 at ZGA in mouse embryos is not sufficient to prevent the activation of transposable elements or gene expression in general, although it does lead to developmental arrest[121,129]. However, targeting H3K9me3 to SVA retrotransposons in the developing human embryo, which are enriched at putative ZGA enhancers, does lead to the repression of many ZGA genes and developmental delay[123]. Thus, although H3K9me3 may not have a primary role in repressing the expression of genes and transposable elements in early mammalian embryos, targeted ectopic acquisition of H3K9me3 may be capable of inducing transcriptional silencing. By contrast, reprogramming efficiency upon somatic cell nuclear transfer is improved markedly by the removal of H3K9me3, which clearly establishes H3K9me3 as a barrier to reprogramming in vivo, perhaps resulting from a more mature heterochromatin state in the differentiated donor cells that are used for nuclear transfer, in which H3K9me3 is fully repressive[130–132].

Spatial chromatin dynamics upon fertilization

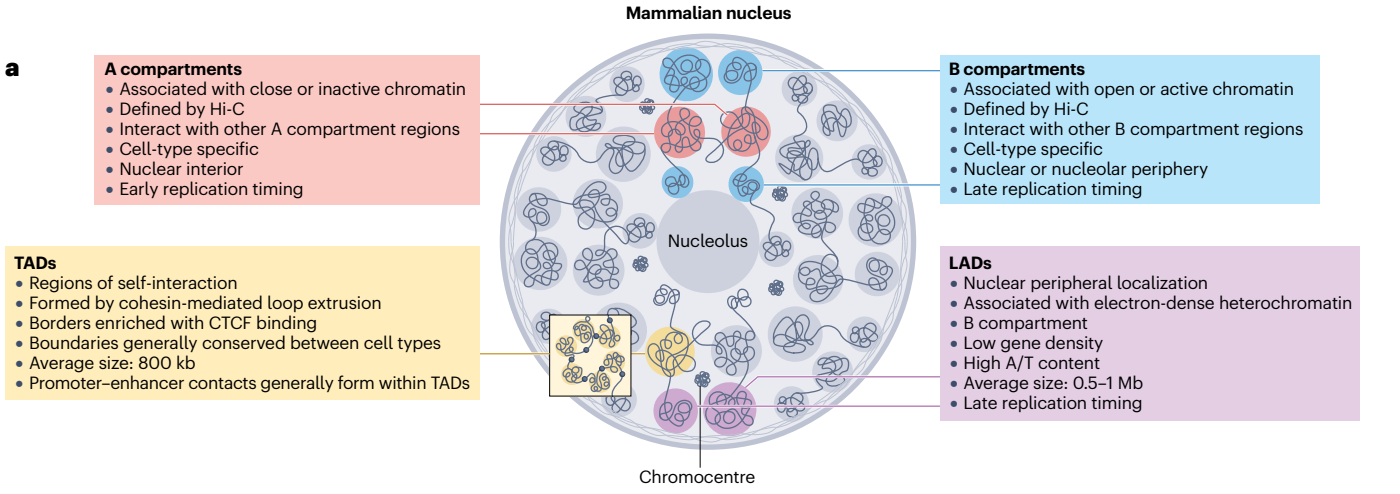
The 3D organization of chromatin inside the nucleus provides an additional layer of epigenetic regulation. The genome folds into largely conserved organizational structures that include chromosome territories, topologically associating domains (TADs), and A or B (A/B) compartments. The genome is also organized into spatially positioned domains with respect to nuclear landmarks, such as lamina-associated domains (LADs) at the nuclear lamina in the periphery of the nucleus and nucleolar-associated domains (Fig. 3a). All of these structures undergo marked changes during early embryonic development and exhibit unique features of genome organization, as detailed below[133] (Fig. 3b).

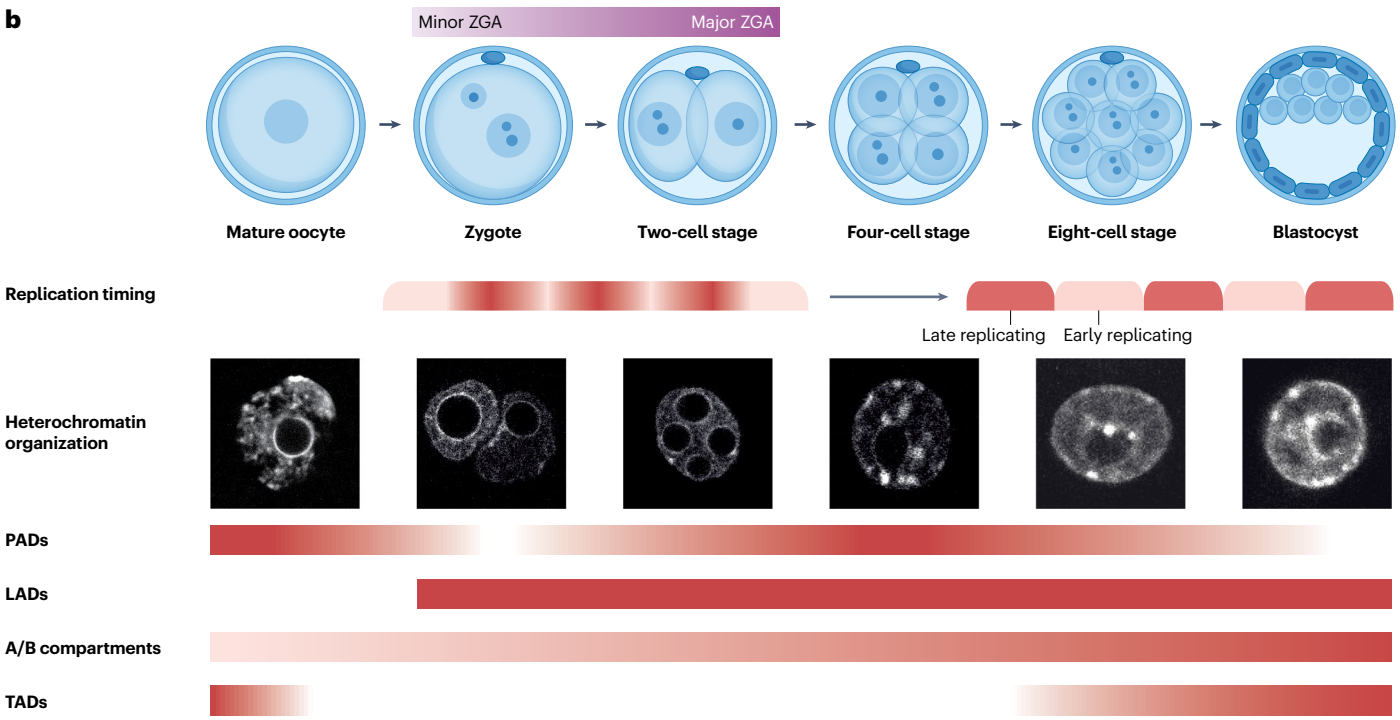
Three-dimensional organization of heterochromatin

Pericentric heterochromatin is organized inside the nucleus of differentiated cells into chromocentres. The nuclei of early mammalian embryos are large and maintain chromosome territories in a canonical Rabl configuration[134,135]. A distinctive feature acquired during oogenesis is the spatial arrangement of centromeres, including both pericentromeric and centromeric DNA, around nucleolar-like bodies[136–138]. The localization of pericentromeric repeats at the nucleolar-like bodies is essential for development and for heterochromatin silencing[139]. This 3D arrangement persists until ZGA in mouse and bovine embryos (but has not been investigated in other species), whereupon chromocentre formation occurs gradually. Interestingly, chromocentre formation depends on the transcription of pericentromeric repeats[140–142] and is accompanied by changes in their biophysical properties, involving a transition from a liquid-like state to a solid-like state[143].

Polycomb-associating domains

Another distinctive feature of the 3D genome organization of mouse oocytes is the formation of cohesin-independent Polycomb-associating domains (PADs)[144,145], which form from long-range self-association in the





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	Replication timing	LADs	A/B compartments	TADs
Inhibition of transcription elongation (DRB)	No global effect	Collapsed	Not determined	Not determined
Inhibition of transcription initiation and elongation (α-amanitin)	No global effect	Collapsed	No global effect	No global effect
Inhibition of replication (aphidicolin)	-	No global effect	No global effect	Collapsed

3D space of domains marked by both ncH2AK119ub and ncH3K27me3 (ref. 144). The formation of PADs in mature oocytes and the silencing of genes therein depend on PRC1-mediated H2AK119ub but not on PRC2-mediated H3K27me3 (ref. 144). PADs, similar to ncH3K27me3, transiently reappear exclusively on the maternal allele from the two-cell stage to the eight-cell stage of the embryo, in an H3K27me3-dependent manner, although the functional relevance of this is unknown. ncH3K27me3 domains are also present in the oocytes of other nonhuman mammals, but the presence of PADs has not been investigated in these species.

Fig. 3 | Three-dimensional chromatin architecture during early mouse development. a, General characteristics of 3D chromatin organization, showing A and B compartments, lamina-associated domains (LADs) and topologically associating domains (TADs). **b**, The relative dynamics of 3D chromatin organization during early embryonic development in mice, in relation to the timings of minor and major zygotic genome activation (ZGA). For replication timing, pink and red regions refer to early and late replicating domains, respectively, which become progressively more well-defined across pre-implantation development. Single confocal microscopy sections of DAPI-stained nuclei show the reorganization of DAPI-dense pericentric heterochromatin from the periphery of nucleolar-like bodies to form chromocentres from the four-cell stage onwards. Polycomb-associating domains (PADs) are detected in mature oocytes and re-emerge exclusively on the maternal allele after fertilization but are lost by the blastocyst stage. By contrast, LADs are not detectable in mature oocytes but are present from the zygote stage onwards. A and B compartments are not detected in mature oocytes, and they gradually emerge during pre-implantation embryonic development. TADs are detected in mature oocytes but not in zygotes and gradually consolidate during pre-implantation development. **c**, The dependence of features of 3D chromatin organization on transcription initiation and/or elongation and DNA replication in mouse embryos. 5,6-Dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) inhibits transcription elongation by RNA polymerase II; α-amanitin is a general inhibitor of RNA polymerase, which is used at concentrations that inhibit RNA polymerase II in these studies; and aphidicolin is an inhibitor of DNA replication.

Lamina-associated domains

LADs form immediately after fertilization in mice, being one of the earliest forms of nuclear organization to occur during development[146]. LADs are undetectable in oocytes and thus are not inherited through the maternal germline but are established de novo in the maternal pronucleus[146]. The establishment of LADs is independent of DNA replication[146] but histone PTMs seem to be involved in this process (Fig. 3c). H3K4me3 is important for the establishment of paternal LADs in the zygote as expression of the H3K4me3 demethylase KDM5B in the early zygote leads to a failure to detect LADs in the paternal pronucleus, whereas maternal LADs are largely unaffected[146]. This suggests that there are distinct, allele-specific mechanisms for the establishment and restructuring of LADs. Embryonic LADs have features typical of LADs found in somatic cells, including high AT content, correlation with the B compartment (heterochromatin) and low levels of gene expression[146]. However, LADs at the late two-cell stage of embryos are an exception, with these LADs being more fragmented, having greater cell-to-cell variability and having decreased AT content[146].

LADs undergo considerable remodelling during pre-implantation development in mice, particularly around the time of ZGA[146–148]. LADs reorganize gradually throughout the complete cell cycle of two-cell-stage mouse embryos[147]. Inhibition of transcription at ZGA markedly alters LAD organization at the two-cell stage[147] (Fig. 3c). LAD restructuring at the two-cell stage is largely correlated with and dependent on transcriptional activation at ZGA, and there is an increased number of LADs at this stage that belong to the A compartment (euchromatin)[146–148]. The relevance of this observation is unclear, but it suggests that the layers of nuclear organization (such as LADs and A/B compartments) might be uncoupled during early mammalian development. Maternal ncH3K27me3-marked regions (probably corresponding to embryonic PADs) also contribute to the repositioning of some LADs to the nuclear interior at the two-cell stage of mouse embryos[148]. LADs have not been mapped in any other species so far, and thus, whether the dynamics of their establishment and remodelling are conserved beyond mice remains to be determined.

A/B compartments and chromosome folding

Mouse oocytes gradually lose A/B compartments in the nucleus during growth, whereas sperm have a more canonical nuclear organization, with additional long-range interactions probably reflecting their increased compaction[149–151]. Likewise, in mouse zygotes, compartmentalization is greater in paternal pronuclei than in maternal pronuclei, although A/B compartments consolidate progressively over cleavage stages in both alleles[149,152,153]. The gradual establishment of nuclear compartments has also been observed in human and pig embryos[153,154]. Globally, the establishment of nuclear compartments in mouse pre-implantation embryos is independent of transcription at ZGA, as compartment consolidation occurs in the presence of the Pol II inhibitor α-amanitin, and is also largely unaffected by blocking DNA replication[146,149,152] (Fig. 3c). Nuclear A/B compartments in mouse embryos have the expected enrichments of transcriptional and chromatin features as based on somatic cells, such as H3K4me3 and accessible chromatin in A compartments, and DNA methylation and H3K27me3 in B compartments[149,152,155]. These compartments are also demarcated by a genetic signature, with SINE-B1 elements being more abundant in A compartments and LINE-1 elements being more abundant in B compartments[156].

Topologically associating domains

The consolidation of TADs also occurs progressively during mouse pre-implantation development; TADs are barely detectable before the eight-cell stage and their emergence depends on CTCF[149,152,157]. Human and pig pre-implantation embryos also undergo progressive TAD formation, and in human embryos, CTCF has been shown to be necessary but not sufficient for TAD formation[153,154]. CTCF is bound to chromatin as early as the zygote stage and depletion of both maternal and zygotic CTCF leads to developmental failure at the time of implantation in mice[157,158]. Surprisingly, structure-related transcriptional changes are not observed in CTCF-depleted embryos and the first cell fate specification is not affected, which suggests that TADs are not necessary for the establishment of gene expression programmes, at least until implantation[157]. The establishment of TADs in mice is largely independent of transcription during ZGA, whereas in humans, TAD establishment is prevented by inhibiting ZGA[149,152,153] (Fig. 3c). Thus, in general, TADs are progressively established during mammalian pre-implantation development, but the molecular dependencies on developmental processes such as ZGA for the formation of TADs differ between species.

Replication timing

Replication timing, which refers to the defined order in which the genome is replicated during S phase, is a major epigenetic feature[159]. The coordinated order in which specific genomic regions undergo replication leads to their segregation into early and late replicating domains, which are highly associated with other chromatin features and aspects of nuclear organization in differentiated cells[160]. LADs and B compartments (heterochromatin) typically correspond with late replicating domains, whereas inter-LADs correspond mainly with A compartments (euchromatin) and replicate early during S phase. In human embryonic stem cells, a near-complete disruption of the

Glossary

A or B (A/B) compartments
The organization of chromosomes into two general compartments in the nucleus, broadly corresponding to active (A) euchromatin and inactive (B) heterochromatin.

Bivalent
The existence of both activating and repressive chromatin modifications at specific loci, particularly observed at developmental genes in embryonic stem cells.

Blastocyst
A conserved, early embryonic stage in mammalian development consisting of an outer layer of cells (trophectoderm) surrounding a fluid-filled cavity known as the blastocoel and the inner cell mass.

Cap analysis gene expression with sequencing
(CAGE-seq). A method for the identification of the 5′ end of capped RNA by biotinylation of the cap and subsequent purification followed by sequencing.

Chromocentres
Highly compacted regions of the nucleus consisting of repetitive centromeric and pericentromeric DNA derived from one or more chromosomes.

Chromosome territories
Regions of the nucleus that are preferentially occupied by particular chromosomes.

Cleavage stage
The stage of early development in which the fertilized egg undergoes cellular divisions without significant growth, typically until the formation of the blastocyst in mammals.

Constitutive heterochromatin
Regions of the genome that remain condensed and inactive throughout the cell cycle and across tissues.

CpG continents
Megabase-scale domains enriched in cytosine and guanine that are detected in non-rodent mammals and that are hypomethylated in non-transcribed regions in oocytes.

Epigenome
A description of the chromatin composition across the genome, including DNA and histone modifications, in a given cell type.

Facultative heterochromatin
Regions of the genome that are variably condensed and inactive across development and tissues.

Histone variants
Histone proteins that substitute for canonical core histones in nucleosomes, which can modify the structure and function of the nucleosome. In contrast to canonical histones, histone variants can be incorporated in nucleosomes at any stage of the cell cycle, not only at S phase.

Inner cell mass
The group of cells in the inner part of the mammalian blastocyst that give rise to the embryo and from which embryonic stem cells are derived.

Long terminal repeat
(LTR). A pair of identical DNA sequences flanking retroviral genomes, containing *cis*-regulatory sequences involved in regulating the transcription of the viral genome.

Maternal-to-zygotic transition
(MZT). The process by which the control of development switches from maternal products (RNAs and proteins) to exclusively embryonic products derived from the embryonic genome.

Nucleolar-like bodies
Also known as nucleolus precursor bodies. Spherical structures within early embryonic nuclei, thought to be precursors of nucleoli, that become functional from the late two-cell stage of embryonic development.

Pericentric heterochromatin
Constitutive heterochromatin located adjacent to centromeric regions, marked by repetitive satellite DNA.

Pluripotency
The ability of a cell to give rise to the three germ layers, including germline, but lacking the ability to give rise to a full organism by itself without contribution from extra-embryonic tissues.

Polycomb-associating domains
(PADs). Self-interacting, cohesin-independent compartmental domains marked by H3K27me3.

Polycomb targets
Genes that are typically repressed by the Polycomb repressive complexes PRC1 and PRC2, comprising mainly developmentally regulated genes.

Rabl configuration
The distribution of chromosomes in the nucleus in which telomeres and centromeres are located at opposite poles of the nucleus, which is found throughout eukaryotes.

Somatic cell nuclear transfer
The process by which the nucleus of a somatic donor cell is transferred into an enucleated egg, which enables the generation of a clonal embryo of the original donor cell.

Topologically associating domains
Regions of the genome containing preferentially self-interacting DNA sequences on a scale of hundreds of kilobases that are thought to be formed by cohesin-mediated loop extrusion and boundary formation by CTCF.

Totipotent
The ability of a cell to give rise, by itself, to a complete organism, including extra-embryonic tissues.

Transcriptional silencing
A highly conserved transcriptionally quiescent state observed in mature oocytes.

Trim-Away
A technique originally developed in mouse oocytes to target specific proteins for degradation using specific antibodies.

Trophectoderm
The outer layer of the mammalian blastocyst, which forms extra-embryonic tissues that are responsible for implantation and support of the developing embryo.

Zygotic genome activation
(ZGA). Also known as embryonic genome activation. The first transcription of the embryonic genome after fertilization, which does not necessarily take place at the zygote stage.

replication timing programme results in changes in histone PTMs and inter-compartment and intra-compartment interactions[161], which suggests that replication timing acts upstream of such chromatin features, thereby potentially having a central role in establishing the epigenome.

In mouse embryos, the replication timing programme is initially not well defined and is only gradually consolidated after the two-cell stage[162,163] (Fig. 3b). Zygotes and two-cell-stage embryos have a poorly defined, fuzzy replication timing programme, such that early and late replication domains are not well separated. The early and late domains then gradually segregate to span the complete S phase during subsequent embryonic stages up to the blastocyst stage[162]. Thus, although the replication timing programme in zygotes and two-cell-stage embryos may seem to be less well defined within S phase compared with later-stage embryos[164], genomic regions do replicate earlier

(or later) than others in zygotes and two-cell-stage embryos[162,163,165], which is consistent with the existence of a partial replication timing programme, albeit 'fuzzier'. These interpretations are supported by studies from the 1990s that documented the typical microscopy patterns of early, mid and late S phase using analogue incorporation in zygotes[166,167]. In addition, maternal pericentromeric regions replicate later than the paternal counterparts[163], which highlights that there are differences in replication timing at these specific heterochromatic loci between the parental genomes. This is also in line with earlier cytological work tracking the incorporation of bromodeoxyuridine around the nucleolar-like bodies in mouse zygotes[142,166]. Differences in replication timing may reflect the differences in heterochromatin constitution that are characteristic of the pericentromeric repeats of maternal and paternal genomes at these early stages, as described above.

Interestingly, the maturation of replication timing occurs progressively through embryonic cleavage stages, with different features of replication timing evolving at different developmental stages towards a configuration more similar to that of somatic cells[162,164,165]. For example, zygote, two-cell-stage and four-cell-stage embryos have a similar degree of variability of the replication programme across cells, but the heterogeneity of the replication timing programme across genomic regions is greatest in zygotes, and two-cell-stage embryos then starts to decrease from the four-cell stage onwards[162]. Thus, several molecular steps contribute to the gradual consolidation of the replication timing programme in development. This behaviour is highly reminiscent of the gradual establishment of nucleosome organization and the maturation of A/B compartments during cleavage stages, which suggests that there might be a functional link between these features. However, this contrasts with LAD formation, which occurs rapidly after fertilization[146]. As expected, B compartments and LADs are late replicating already by the zygote stage, but a clear distinction between the early and late replication of A compartments and B compartments, respectively, only emerges at later embryonic stages as development proceeds[162,165]. The replication timing of ZGA genes depends on the presence of Pol II but not on their transcriptional elongation[162], which suggests that Pol II might have a role in establishing the early epigenome independently of its transcriptional activity. Interestingly, although preventing ZGA through transcriptional inhibition equalizes the replication timing of A compartments and B compartments[162], the formation of these compartments and the strength of their distinction are unaffected[149,162] (Fig. 3c).

Thus, these recent observations indicate that at the beginning of mammalian development, the embryonic genome has not only distinctive molecular features of chromatin but also distinctive organization of other epigenetic features such as replication timing. The process of DNA replication itself also seems to have distinctive features in embryos. For example, the speed of the replication fork is slow at the beginning of development and increases as development proceeds in mouse embryos[164,168], which suggests that a reduction in cellular plasticity is accompanied by an increase in DNA replication fork speed, coupled with a more well-defined replication timing programme. Whether the slow replication fork speed is functionally linked to the less well-defined replication timing programme in mouse zygotes and two-cell-stage embryos remains to be addressed experimentally, but the changes in fork speed are conserved in human embryos[169]. In human zygotes, the slow replication fork speed is accompanied by replication fork stalling and the accumulation of unreplicated sites in G2 of the cell cycle with the consequent accumulation of DNA damage[169]. It is tempting to speculate that such observations are related to the well-known high incidence of aneuploidy in human embryos[169].

Although replication timing has a key role in chromatin and cellular identity, our knowledge of replication timing is so far limited to mouse embryos; furthermore, the regulators of replication timing and its consolidation in vivo are not yet known. Whether LADs, A/B compartments and replication timing have similar regulatory pathways remains to be studied. Recently, the telomere-associated protein RIF1 has been shown to regulate the emergence of replication timing in mouse embryos. Interestingly, depletion of RIF1 does not majorly alter LADs[170], which supports the notion that the layers of genome organization may be molecularly independent, at least to some extent, in early embryos. Disentangling the molecular, functional and temporal relationships between these aspects of genome organization will shed additional light on the mechanisms that drive the establishment of the epigenome at the beginning of embryonic development.

Conclusions and perspectives

Studies in mammalian embryos have expanded our knowledge of how the cellular machinery mediates the inheritance, establishment and regulation of chromatin features and the epigenome. Low-input genomics studies on embryonic chromatin over the past decade have reinforced and greatly expanded the view that chromatin and nuclear organization in early mammalian embryos are highly atypical at multiple levels. These epigenomic features are gradually transformed to a more canonical organization upon ZGA and loss of totipotency, which suggests that they have important roles in the rapid and marked cellular reprogramming that occurs upon fertilization.

Several transcription factors have now been identified that have largely redundant roles in the regulation of ZGA[41,171]. How the distinct and dynamic features of embryonic chromatin influence the binding and function of such transcription factors in the developing embryo remains an important outstanding question. The relationship between chromatin signatures and gene expression in the embryo has been addressed to some extent through genetic manipulations of epigenetic modifiers, although the direct and indirect effects of chromatin features on transcription need to be resolved by more mechanistic studies, such as by profiling the histone PTM landscape upon such manipulations. In addition, a more in-depth integration of existing epigenomic datasets is needed, and this, combined with the inclusion of additional epigenetic datasets in the coming years, will provide further insights into the unusual epigenetic landscape in early mammalian embryos. Moreover, functional insights into the effects of epigenetic reprogramming will require manipulations of epigenetic modifiers combined with the analysis of multiple aspects of epigenetic information to provide insights into the crosstalk between epigenetic features. It will also be important to investigate the interplay with other aspects of genome function in the embryo that are known to affect chromatin organization, such as DNA replication and repair. Finally, the interplay between the distinct metabolic properties of the embryo[172,173] and chromatin regulation through the shared use of various metabolites that contribute to regulation of the epigenome (epi-metabolites) will also be important to explore further.

In terms of genomic interactions, early embryonic chromatin is enriched in short-range contacts and lacks clearly demarcated higher-order organizational features such as TADs and A/B compartments. This correlates with the relatively open and accessible chromatin that is characteristic of such early developmental stages, although whether these features are linked is currently unclear. It is also in accord with prior observations that histones have high mobility in the chromatin of early embryos[174]. However, certain chromosome regions

consistently interact with the nuclear lamina and nucleolar-like bodies in early embryos, which indicates that a spatial structure with respect to nuclear landmarks exists shortly after fertilization; this may provide the initial framework for the establishment of nuclear organization in the developing mammalian embryo. It will be important in the future to uncover how, mechanistically, LADs are established and their importance in the formation of other layers of nuclear organization, such as A/B compartments and heterochromatin domains. In addition, the relatively disordered replication timing programme in early embryos is reminiscent of the fuzzy distribution of nucleosomes and poorly defined nuclear organization, and thus, a potential functional link between these features warrants further investigation.

In conclusion, mammalian pre-implantation embryos use remarkably well-conserved mechanisms of nuclear remodelling during reprogramming at fertilization, although there are some important species-specific distinctions, particularly between human and mouse embryos, such as the lack of broad histone PTM domains in human oocytes. Future work in this area will advance our understanding of how early mammalian embryonic development is regulated, of how reprogramming is achieved in vivo, and of the molecular control of totipotency and pluripotency. Moreover, studies of embryonic chromatin will provide new insights into the regulation of nuclear processes and the de novo establishment of epigenetic features such as A/B compartments, heterochromatin and active regulatory regions.

Published online: 03 April 2025

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Acknowledgements
The authors thank T. Schauer for the discussions and comments on the manuscript. Research in the Torres-Padilla laboratory is funded by the Helmholtz Association, the German Research Council (SFB1064, SPP2202), the Marie Skłodowska-Curie Actions (RepliFate, ChromDesign) and the National Institutes of Health 4DN programme (grant number 5U01DK127391-03).

Author contributions
Both authors contributed to the discussion of the content, writing the article, and review and/or editing of the manuscript before submission.

Competing interests
M.-E.T.-P. is a member of the Ethics Advisory Panel of MERCK.

Additional information
Peer review information *Nature Reviews Genetics* thanks Zhiyuan Chen and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.