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Review

The role of heterochromatin in 3D genome organization during preimplantation development

Franka J. Rang,[1,2] Jop Kind,[1,2,3,*] and Isabel Guerreiro[1,2,*]

[1]Hubrecht Institute, Royal Netherlands Academy of Arts and Sciences (KNAW) and University Medical Center Utrecht, Uppsalalaan 8, 3584 CT Utrecht, the Netherlands

[2]Oncode Institute, the Netherlands

[3]Department of Molecular Biology, Faculty of Science, Radboud Institute for Molecular Life Sciences, Radboud University Nijmegen, Houtlaan 4, 6525 XZ Nijmegen, the Netherlands

*Correspondence: j.kind@hubrecht.eu (J.K.), i.guerreiro@hubrecht.eu (I.G.)

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SUMMARY

During the early stages of mammalian development, the epigenetic state of the parental genome is completely reprogrammed to give rise to the totipotent embryo. An important aspect of this remodeling concerns the heterochromatin and the spatial organization of the genome. While heterochromatin and genome organization are intricately linked in pluripotent and somatic systems, little is known about their relationship in the totipotent embryo. In this review, we summarize the current knowledge on the reprogramming of both regulatory layers. In addition, we discuss available evidence on their relationship and put this in the context of findings in other systems.

INTRODUCTION

At the beginning of mammalian development, the sperm and oocyte fuse to give rise to the totipotent zygote. Since the oocyte and sperm are both mature cell types with preexisting and vastly different epigenomes, all layers of epigenetic regulation undergo extensive remodeling to allow for the development of all embryonic and extra-embryonic cell types.[1] Moreover, very soon after fertilization, the embryo undergoes zygotic genome activation (ZGA), in which transcription from the zygotic genome initiates and maternally provided mRNA starts to be degraded. In mouse development, a minor wave of ZGA takes place as early as the zygotic stage, while major ZGA occurs one cleavage later at the 2-cell stage.[2] By this stage, mechanisms of transcriptional regulation have to be in place to ensure the timely activation of the correct subset of genes. The complete remodeling of the epigenome in preimplantation development offers a unique system to study the interactions between different epigenetic layers as they emerge and change. Moreover, the presence of the distinct maternal and paternal epigenetic states within the same cell provides a unique side-by-side comparison of how the initial chromatin state influences its subsequent dynamics. Studying these processes will thus not only provide insight into the foundational events of early development and epigenetic reprogramming but may also reveal fundamental principles of epigenetic interactions that hold true in any system.

Epigenetic regulation works by making DNA accessible or inaccessible to proteins such as transcription factors and the transcriptional machinery. The inaccessible and inactive fraction of the genome, referred to as heterochromatin, is physically segregated from the accessible and active fraction, or euchromatin: while euchromatin resides in the nuclear interior, the densely compacted heterochromatin tends to locate at the nuclear periphery and around nucleoli.[3,4] This inherent link between heterochromatin and spatial organization was already established decades ago in microscopy studies, and research since has further solidified this finding.[3,4] Classically, heterochromatin has been divided into two types: constitutive and facultative. The two types are associated with different types of proteins and are also spatially organized in different ways. Constitutive heterochromatin is mostly consistent across cell types and covers regions rich in repetitive elements, such as the major and minor satellite repeats at centromeres. Facultative heterochromatin, on the other hand, is cell-type specific and is associated with the repression of developmental genes.[3] Both types of heterochromatin play an important role in maintaining cell identity and genome stability through repression of genes and repeats[5–9] but have distinct structural organizations with major differences in spatial distribution, compaction level, and long-range contacts.[3] Despite the many links between heterochromatin and genome organization, relatively little is known about their interactions during the extensive reprogramming that takes place in preimplantation development. Insight into this relationship could further our understanding of epigenetic remodeling during the establishment of totipotency, as well as the complex interactions between different modes of genome regulation in general.

In this review, we summarize and interconnect the current knowledge on the remodeling of the heterochromatin and 3D organization during mammalian preimplantation development.

Since most of the research to date has been performed in mouse, we mainly focus on this model organism. In addition, we describe the links between these two modes of genome regulation that have been established in early development and how they relate to observations in other systems. Finally, we suggest avenues of further research to advance our understanding of the relationship between heterochromatin and 3D organization during preimplantation development.

REPROGRAMMING OF HETEROCHROMATIN AND 3D ORGANIZATION IN MOUSE PREIMPLANTATION DEVELOPMENT

Facultative heterochromatin

The most characteristic facultative heterochromatin marks are the mono-ubiquitination of H2A lysine-119 (H2AK119ub1) and the tri-methylation of H3 lysine-27 (H3K27me3), catalyzed by Polycomb repressive complex 1 (PRC1) and PRC2, respectively. Both complexes exist in multiple forms, and they have been shown to work both upstream and downstream of one another.[10–13] In pluripotent stages of development, starting in the blastocyst, H2AK119ub1 and H3K27me3 marks largely overlap at the promoters of developmental genes and serve to repress them. A large fraction of these promoters are also marked by the active histone post-translational modification (PTM) H3K4me3, creating a so-called bivalent domain that represents a reversible repressive state to prevent premature gene activation.[14,15] Once cells commit to a certain lineage, genes specific to the lineage lose H3K27me3/H2AK119ub1 and become active. Conversely, at many genes specific to other cell types, H3K4me3 is lost, H3K27me3 domains broaden, and a permanent repressive state is achieved.[16,17]

While in most systems, H3K27me3 and H2AK119ub1 are largely located at genic regions, these two marks present an entirely different distribution during mouse preimplantation development and are independently remodeled post-fertilization (Figure 1i–1ii). These atypical profiles arise already during oocyte maturation, where both H3K27me3 and H2AK119ub1 are progressively laid down as unusually broad domains in intergenic regions.[18,19,20,21] Consequently, a much larger fraction of the genome is covered by H3K27me3 and H2AK119ub1 in oocytes (~35%) compared with later developmental stages with canonical profiles (<5%). The broad domains of H3K27me3/H2AK119ub1 have a strong overlap with regions with intermediate levels of DNA methylation, i.e., partially methylated domains (PMDs), while being excluded from fully methylated domains (FMDs).[18] Interestingly, H3K36me3 has been shown to overlap significantly with DNA methylation in oocytes and anti-correlate with H3K27me3. Depletion of H3K36me3 via the knockout of methyltransferase Setd2 resulted in the expansion of H3K27me3 distributions, indicating a role in H3K36me3 in shaping the distribution of the oocyte Polycomb marks.[22] In addition to these atypical broad domains, H3K27me3 and H2AK119ub1 are maintained at promoters of known Polycomb targets.[18,23,20,21] In sperm, most histones have been replaced by protamines to facilitate tight packaging of the chromatin,[24,25] but the remaining histones appear to retain canonical distributions of H3K27me3 and H2AK119ub1.[18,20,21]

After fertilization, the broad H3K27me3 and H2AK119ub1 domains are inherited from the oocyte on the maternal allele, while the marks on the paternal allele are rapidly removed and *de novo* enrichment starts to appear by the late zygote stage.[18,20,21] Paternal deposition of H3K27me3 is dependent on the activation of the catalytic PRC2 subunit EZH2 via phosphorylation by CDK1 during the G2/M transition.[31] The newly formed paternal H3K27me3 and H2AK119ub1 modifications form very broad domains of low enrichment, mostly in intergenic regions.[18,19,20] On the maternal allele, H3K27me3 is lost from the promoters of canonical Polycomb targets post-fertilization and is only fully recovered after implantation.[18,23,21] However, Polycomb target genes do retain H2AK119ub1, which likely suffices for gene repression, as they become upregulated upon PRC1 catalytic component disruption but not in the absence of H3K27me3.[20,21] Although the two Polycomb marks display similar distributions in gametes and the zygote, their profiles rapidly start to diverge in subsequent cleavage stages. While H3K27me3 retains its parental asymmetry and broad domains, the two alleles have largely equalized with respect to H2AK119ub1 by the end of the 2-cell stage and start to more closely resemble canonical profiles as seen in mouse embryonic stem cells (mESCs) and the blastocyst.[19,20,21]

Some attempts have been made to elucidate the interdependence of H3K27me3 and H2AK119ub1 during early development.[19,20] Conditional knockout of Eed, a core component of PRC2, results in the loss of H3K27me3 in oocytes and in preimplantation embryos until ZGA. However, H2AK119ub1 is only affected at a subset of non-canonical imprinting loci, which temporarily lose H2AK119ub1 in wild-type (WT) embryos but fail to reestablish the mark in the absence of H3K27me3.[20] Similarly, acute depletion of H2AK119ub1 in early embryos does not lead to big changes in H3K27me3 at the 4-cell stage.[20] However, maternal knockout of PRC1 subunits and consequent loss of H2AK119ub1 did lead to a decrease of H3K27me3 at a subset of genes in oocytes and early embryos. These results suggest that PRC1 may directly or indirectly work upstream of PRC2 in this system.[19]

Constitutive heterochromatin

Several histone modifications are associated with constitutive heterochromatin, including H3K9me2/3, H4K20me2/3, and H3K64me3. H3K9me3, the most extensively studied of these marks, plays a central role in mediating typical constitutive heterochromatin features. Indeed, its interaction with heterochromatin protein 1α (HP1α) results in phase separation and chromatin compaction[32–34] and also plays a role in localization of heterochromatin to the nuclear periphery.[35] In mammals, methylation of H3K9 is mediated by six histone methyltransferases (SUV39H1, SUV39H2, SETDB1, SETDB2, G9A, and GLP) that are only partially redundant and each work in different genomic contexts, as reviewed in Padeken et al.[36] Interestingly, some of these histone methyltransferases mediate H3K9me enrichment in genic regions to ensure cell-type-specific repression,[8,9] thus functioning as a form of facultative heterochromatin.

During preimplantation development, constitutive heterochromatin and its associated histone PTMs are extensively remodeled. Directly after fertilization, there is a strong parental

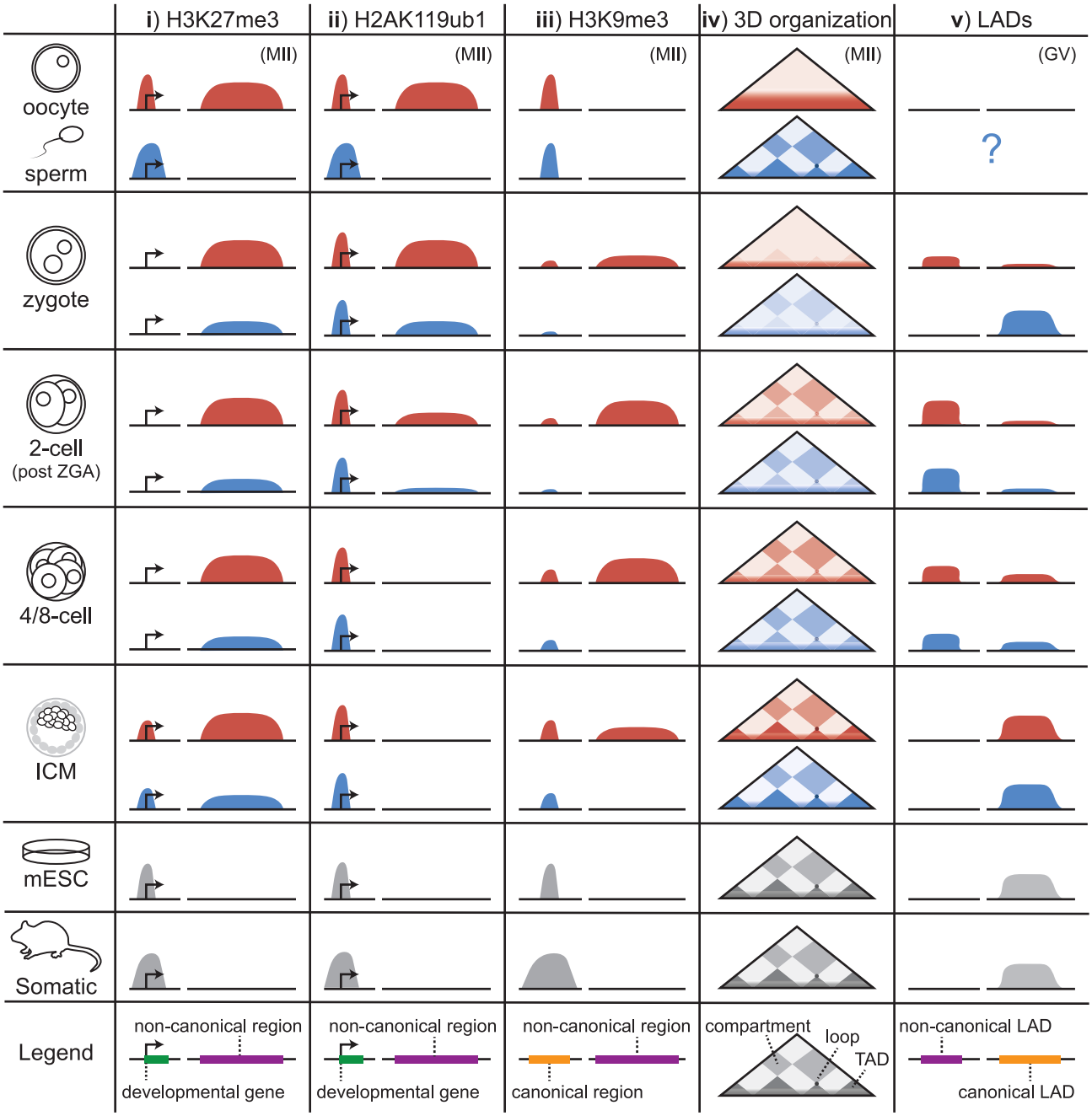

Figure 1. Schematic representation of heterochromatin and genome organization reprogramming during mouse early development

The cartoons display the reprogramming of H3K27me3 (i), H2AK119ub1 (ii), H3K9me3 (iii), 3D organization (iv), and LADs (v). The legend at the bottom provides information on the type of regions that are affected. Cartoons are based on the descriptions of ChIP-seq data for H3K27me3,[18,23] H2AK119ub1,[19,20] and H3K9me3[26]; on Hi-C data for 3D organization[27–29]; and on DamID data for LADs.[30] Since the genomics data do not cover repetitive regions, such as centromeric, pericentromeric, and telomeric regions, these are not included in the representation.

asymmetry in constitutive heterochromatin marks, which are clearly present in the maternal pronucleus while remaining largely undetected in the paternal pronucleus.[37–40] As is the case for H3K27me3 and H2AK119ub1, the maternal histone marks appear to be inherited from oocytes, while paternal H3K9me2/3 starts to be established in late zygotes by SUV39H2.[41]

Recently, the distribution of H3K9me3 in gametes and in early embryos was profiled using chromatin immunoprecipitation sequencing (ChIP-seq)[26] (Figure 1iii). Although H3K9me3 could not be detected in the early paternal pronucleus by immunofluorescence microscopy, the ChIP-seq data revealed low levels of paternal H3K9me3 at the PN3 zygote stage. This paternal signal

shows some overlap with sperm signal, suggesting that a limited amount of H3K9me3 may be inherited from the father. Interestingly, the paternal H3K9me3 that is laid down *de novo* during the zygote stage appears to lack repressive qualities, as a knockdown of the responsible histone methyltransferase results in a downregulation of affected genes at the 2-cell stage.[41] This surprising result suggests that paternal H3K9me3 at these genes may be activating rather than repressive. In the maternal pronucleus, the inherited H3K9me3 is remodeled as well, with a loss of H3K9me3 at the promoters of a set of developmental genes and a gain in intergenic regions relative to oocytes. So far, there is no evidence that H3K9me3 on the maternal allele is non-repressive at this stage, and, in fact, the genes that lose H3K9me3 in zygotes compared with oocytes are enriched for ZGA genes that are expressed at the 2-cell stage.

Interestingly, the H3K9me3 domains that are gained post-fertilization strongly overlap with the maternally inherited H3K27me3,[26] while in pluripotent and differentiated cells, these marks only show a limited overlap at gene promoters.[8,17,42] The H3K9me3 enrichment in H3K27me3 domains starts to decrease around the morula stage and is completely lost after implantation, thus slightly preceding the loss of non-canonical H3K27me3.[26] Meanwhile, starting at the 4-cell stage, H3K9me3 gains enrichment at canonical sites, such as long terminal repeat (LTR) retro-transposons. For a number of LTRs, the gain of H3K9me3 at the 4-cell stage coincides with their downregulation, implying that H3K9me3 may play a role in the timely repression of repeats during development.[26]

Together, these results show that H3K9me3 is extensively remodeled in the early embryo and displays several unusual characteristics, such as a lack of repression by newly gained paternal domains and an extensive overlap with H3K27me3 at non-canonical sites. While the relevance of these features is still unclear, H3K9me3 does seem to play an important role in the temporal regulation of repeat expression.

3D genome organization

Heterochromatin is intimately linked to the spatial organization of the genome. One important aspect of this organization is the 3D positioning of genomic regions relative to one another in the nucleus. The genome is organized in a multi-layered manner. At the highest level of organization, chromosomes form distinct territories within the nucleus, while interactions between different chromosomes remain limited.[43,44] The chromatin further partitions into two compartments: the active compartment A is associated with higher levels of gene expression, active histone marks, and GC content, while the opposite holds true for the inactive compartment B.[44] Within these compartments, domains of preferential interactions occur, referred to as topologically associating domains (TADs).[45,46] Mechanistically, TADs are formed by the continuous process of loop extrusion whereby the ring-shaped protein cohesin continually extrudes a loop of DNA until it is halted at a boundary element or dissociates from the DNA.[47–51] The process of loop extrusion within domain boundaries likely plays a role in bringing together regulatory elements with their target genes while excluding interaction with off-target genes outside the boundaries.[52] The most prominent boundary element in vertebrates is the insulator protein CCCTC-binding factor (CTCF). The binding sites of CTCF determine regions across which loops cannot be extruded, and, consequently, pairwise interactions across boundaries are rarer. In bulk methods such as Hi-C, this phenomenon thus gives rise to contact domains that often have focal points of increased interactions at boundary elements where loops frequently stall.[53]

As is the case for heterochromatin, the 3D organization of the genome is extensively remodeled during preimplantation development (Figure 1iv). The chromatin from the maternal and paternal gametes exists in entirely different organizational states at the moment of fertilization. Since the oocyte is stalled at metaphase of meiosis II prior to fertilization, the maternal chromosomes are strongly condensed and structurally similar to mitotic chromosomes, lacking loops, TADs, and compartments, while being enriched for interactions at 1–7 Mb.[27] Meanwhile, the paternal genome is packaged tightly in the sperm nucleus, which has a volume over ten times smaller than that of somatic cells.[24,25,54] Despite this extreme compaction, the overall 3D organization of sperm is similar to mESCs and somatic cells, albeit somewhat enriched for long-range interactions.[28,55,56] Following fertilization, both parental genomes are rapidly remodeled to a state with little consistent 3D architecture in the zygote embryo. At this stage, TADs and loops are barely visible in the Hi-C interaction matrices[27,28] and only become evident when averaging across multiple sites.[29,57] While the paternal genome shows weak compartmentalization, the maternal genome lacks compartments.[27–29] Nevertheless, the paternal chromatin has fewer distal (>2 Mb) interactions, suggesting that it may be in a more relaxed state than the maternal genome during the zygote stage.[27]

The allelic differences present in zygote are largely resolved in the 2-cell embryo. In addition, starting at the end of the 2-cell stage (post-ZGA), all levels of chromatin organization get progressively stronger and have been completely established by the time of implantation.[27,28] Despite the conspicuous concurrence with transcriptional activation, transcription itself does not appear to be necessary to consolidate the 3D genome architecture.[27,28] Rather, blocking the process of DNA replication appears to prevent further establishment of chromatin organization.[28] Despite these insights, it is still largely unclear what factors cause the lack of organization in the early embryo and which factors subsequently are responsible for its reestablishment.

Lamina-associated domains

Another important aspect of genome organization involves the spatial positioning of DNA within the nucleus. Most exemplary of this is the segregation of heterochromatic chromatin at the nuclear lamina (NL), a filamentous network at the inner nuclear membrane. Regions of the genome associated with the NL are referred to as lamina-associated domains (LADs). These broad regions have a median size of ~500 kb and are characterized by a high density of long interspaced nuclear element (LINE) repeats, low gene density, and low levels of gene expression.[58,59] LADs are frequently enriched for H3K9me2/3[42,58,60,61] and to a limited extent for H3K27me3.[42,58,62] In line with their heterochromatic nature, LADs show a strong correspondence with the inactive B compartment identified by Hi-C.[53,63]

As is the case of the 3D interactions, NL contacts are extensively reorganized during preimplantation development[30] (Figure 1v). In zygotes, the paternal genome forms well-defined LADs, while the maternal genome shows weaker and more inconsistent NL interactions in regions with features atypical for LADs. Interestingly, LADs seem to be largely absent in the developing oocyte (germline vesicle [GV]), implying that LADs are established *de novo* for the maternal genome.[30] At the zygote stage, the genome contacts the NL, but electron spectroscopic imaging shows an absence of compacted chromatin in these regions,[64] suggesting that these LADs exist in a relatively decondensed state unique to the zygote stage.

The parental asymmetry in NL association is strongest in the zygote and starts to diminish in subsequent stages, but is only fully resolved by the time of implantation.[30] At the 2-cell stage, the maternal LADs strengthen, while the paternal LADs are reorganized to resemble the maternal LADs more closely. At this stage, many LADs considered to be constitutive in somatic cells have dissociated from the NL. These regions only partially relocate to the NL at the 8-cell stage, but are fully recovered in the inner cell mass (ICM) of the blastocyst.[30] To date, such extensive reprogramming and loss of constitutive LADs has not been observed in any other system. The extreme remodeling and less condensed state of LADs are unique to the preimplantation embryo, suggesting that NL association may be regulated differently during these early stages of development. However, the exact mechanisms behind these events remain unclear at the moment.

THE INTERACTIONS OF HETEROCHROMATIN AND 3D ORGANIZATION IN PLURIPOTENT AND SOMATIC SYSTEMS

Constitutive heterochromatin and 3D organization

In many biological systems, indirect and direct links have been established between heterochromatin and 3D-genome architecture. Early Hi-C studies revealed that H3K9me3 and H3K27me3 are enriched in separate subcompartments of compartment B.[42,53] Moreover, polymer models of chromatin organization suggest that compartmentalization can be largely explained by homotypic interactions between heterochromatic regions.[65,66] These results suggest that heterochromatin may play an important role in the establishment of compartments. In the case of H3K9me3, phase separation of HP1α is a likely candidate for driving heterochromatin compartmentalization.[32–34] HP1α directly binds H3K9me3 via its chromodomains. In addition, it possesses a shadow domain, enabling dimerization and binding of other heterochromatin proteins, as well as unstructured regions that facilitate phase separation.[32,34,67] Direct evidence that H3K9me3 has the potential to impact compartment status has been provided by experiments showing that the ectopic enrichment of H3K9me3 results in the switch of some compartment A regions to compartment B.[68] In addition, H3K9me3 seems to be influenced by and have an influence on the formation of loops. Studies have shown that the process of loop extrusion can disrupt H3K9me3 heterochromatin domains[69] and weaken compartment interactions.[48,51] Heterochromatin marked by H3K9me3/HP1α/HP1β in turn impacts the formation of stable loops by preventing CTCF from binding in these regions.[70]

In addition to its role in 3D topology, H3K9me is important for the recruitment of chromatin to the NL via linker proteins.[35,71] In *C. elegans*, the protein CEC-4 has been shown to be directly responsible for tethering H3K9me-marked DNA to the nuclear periphery during embryonic development.[72] While the mechanisms of tethering chromatin to the NL are less clear in mammalian systems, the NL-associated proteins LBR, LAMIN A, and LAP2β seem to play important and partially redundant roles.[73–75]

Facultative heterochromatin and 3D organization

The Polycomb marks H3K27me3 and H2AK119ub1 have been associated with specific contacts between distal genomic regions in multiple systems.[76–80] In microscopy experiments, such Polycomb-associated interactions are visible as distinct foci, referred to as Polycomb bodies.[81] The Polycomb interactions may be partially mediated by phase separation of the protein CBX2, a subunit present in some forms of PRC1.[82] However, other mechanisms could also play a role since the PRC1 protein PHC2 cannot phase separate, but its mutation does lead to the ablation of Polycomb bodies.[76] Based on these results, it seems like PRC1 is more involved in the establishment of 3D interactions than PRC2. The formation of Polycomb interactions is independent from cohesin or CTCF, as their depletion does not lead to the disappearance of such interactions and even appears to strengthen them.[78,79,83] Since chromatin marked by H3K27me3/H2AK119ub1 is enriched in a separate subcompartment,[42,53] these interactions may contribute to their compartmentalization away from other chromatin types.

While H3K27me3 is enriched in a subset of LADs,[42,58,62] no evidence for H3K27me3-mediated tethering has been found to date. On the contrary, recent work in mESCs and neural progenitor cells (NPCs) suggests that H3K27me3 could serve as a repellant for NL association.[84]

A LACK OF 3D ORGANIZATION IN ZYGOTE IN THE PRESENCE OF HETEROCHROMATIC MARKS

Given the known relationship of Polycomb and H3K9me3 with 3D organization, the observed lack of strong loops, TADs, and compartments in the mouse zygote is quite striking. As mentioned earlier, the paternal genome initially has undetectable levels of all heterochromatin modifications and only accumulates low levels of H3K9me3, H3K27me3, and H2AK119ub1 by the end of the zygotic stage.[31,37–40,85,86] Nevertheless, compartments are already clearly present in the paternal pronucleus, albeit weaker than at later stages.[27–29] Meanwhile, the maternal genome is enriched for multiple heterochromatin marks but shows little to no compartmentalization. Based on these observations, it would seem that the presence of heterochromatic histone modifications is neither necessary nor sufficient for compartmentalization, as demonstrated by the paternal and maternal states, respectively. However, a role for the very low levels of H3K9me2/3 that accumulate in the paternal pronucleus cannot be excluded.

A plausible reason for the lack of H3K9me3-driven compartmentalization in the maternal pronucleus may be that the HP1α

protein is not present in the zygote.[40,87,88] Microscopy studies present conflicting results on the timing of the first appearance of HP1α: one study observed HP1α starting in late S phase of the 2-cell embryo,[88] while another study only detected the protein post-implantation.[87] If indeed HP1α is expressed starting from the late 2-cell stage, this would coincide with the increase in compartment strength post-ZGA[27,28] and the start of chromocenter formation.[89] In line with this hypothesis, a recent study in *Drosophila* embryos showed that HP1a (the fly homolog of HP1α) plays an important role in the establishment of strong B compartments at ZGA.[90] The lack of compartmentalization in the mouse maternal pronucleus could thus potentially be attributed to a lack of heterochromatin phase separation driven by, e.g., HP1.

The lack of heterochromatin-driven interactions just after fertilization is in line with the idea that heterochromatin initially exists in an immature state.[41] After ZGA, the expression of additional heterochromatic proteins may contribute to the maturation of heterochromatin[41,91] and in turn promote the consolidation of the B compartment.

H3K27me3-enriched interaction domains at the 2-cell stage

Although heterochromatin-mediated interactions seem absent in zygotes, Polycomb-driven interactions do make a unique appearance in the 2-cell embryo (Figures 2A–2D), albeit with different characteristics from Polycomb interactions as observed in mESCs. Two studies independently identified interaction domains that arise specifically on the maternal allele at the 2-cell stage and are strongly enriched for H3K27me3,[79,92] which were coined Polycomb-associated domains (PADs) by Du et al. PADs display increased interactions both within and between domains, establishing compartment-like interactions at a smaller scale. The existence of these interactions is rather brief, as the domains are largely lost by the 8-cell stage and completely absent in the 64-cell stage. PADs are initially established during oocyte development between the growing oocyte II (GO II) and fully grown oocyte (FGO) stages. In metaphase II (MII) oocytes, the interaction domains have disappeared and are only reestablished after fertilization.[79]

Conditional maternal knockout of the core PRC2 subunit *Eed* resulted in a substantial loss of H3K27me3, but, surprisingly, PADs were largely unaffected (Figure 2E). However, embryos derived from *Eed* knockout (KO) oocytes and WT sperm were incapable of reforming PADs at the late 2-cell stage (Figure 2F). This implies that functional PRC2 may play a role in their reestablishment, either by PRC2-mediated interactions or by bookmarking via H3K27me3. Conditional KO of the catalytic subunits of PRC1 (*Ring1*/*Rnf2*) resulted in a loss of H2AK119ub1 and a weakening of long-range (2–5 Mb) inter-PAD interactions (Figure 2G), while intra-PAD and short-range inter-PAD interactions were largely unaffected.[79] The effect of this loss on PAD reestablishment in the late 2-cell embryo could not be determined, as mutant embryos arrest before this stage. Interestingly, H2AK119ub1 is largely lost at regions of maternally biased H3K27me3 by the end of the 2-cell stage,[20] calling into question its role in PAD reestablishment. Together, these results suggest that PRC1 and PRC2 both play important roles in PAD formation but exert their effect at different developmental stages.

After the 2-cell stage, PADs gradually weaken and are lost, potentially due to the loss of PAD regulators.[79] Alternatively, the presence of H3K4me3, which is transiently enriched in PADs at the 4-cell stage,[92] could lead to the dissociation of PAD proteins from the chromatin. Another, non-mutually exclusive, explanation is that the regular chromatin architecture mediated by loop extrusion starts to take shape after the 2-cell stage[27,28] and may disrupt PAD interactions. In support of this, loss of loop extrusion via KO of *cohesin* resulted in an increase in PAD interaction strength in oocytes.[79]

The biological relevance of Polycomb interactions is unclear. Loss of maternal EED, and consequently H3K27me3 and PADs, results in minor changes in gene expression during preimplantation stages, problems with non-canonical imprinting by the blastocyst stage, and post-natal overgrowth.[93,94] However, it is unknown whether the loss of EED, H3K27me3, and/or PADs is responsible for the observed phenotype. Finding a way to perturb the interactions without affecting H3K27me3 may give some insight into which extent the Polycomb-mediated 3D architecture is instructive at this stage of development.

HETEROCHROMATIN ASSOCIATION WITH THE NL IN EARLY DEVELOPMENT

Constitutive heterochromatin and NL association

As discussed previously, heterochromatin marks and NL association are mechanistically linked in several systems. While both aspects of chromatin state have been individually studied in mouse preimplantation development, almost no direct comparison has been made between them.[30] Remarkably, there are clearly defined LADs in the paternal pronucleus in the zygote,[30] while H3K9me2/3 has been shown to be strongly depleted from the paternal genome at this stage.[26,38–40] These paternal LADs are similar to those observed in mESCs and largely overlap a set of LADs that are constitutively present across cell types.[30] Therefore, zygotic and mESC LADs have been theorized to represent the default interactions with the NL that can be further adapted by cell-type-specific programs.[59,95,96] If this is the case, the interactions of the paternal genome with the NL may be driven by sequence rather than chromatin state. Indeed, there is some evidence for NL association driven by the presence of a (GA)n or GA-rich motif in other systems,[73,75] although constitutive LADs are rather enriched for AT isochores.[95] In the maternal pronucleus, on the other hand, H3K9me2/3 is present,[26,38–40] along with a more unconventional and variable LAD profile.[30] While no direct comparison has been made between available H3K9me3 and NL association profiles, several clues indicate that here also NL tethering may be independent of this histone mark. Firstly, neither H3K9me2 nor H3K9me3 appears to be localized at the nuclear periphery of the maternal pronucleus.[38–41] This is especially striking for H3K9me2, which almost exclusively localizes to the nuclear periphery in both pluripotent and somatic cells across species.[71] Moreover, overexpression of the lysine-9-specific demethylase KDM4B in the zygote results in a dramatic decrease in H3K9me3, but no subsequent effect is seen on the NL association profile in either the paternal or maternal pronucleus.[30] In addition, electron spectroscopic imaging of the zygote revealed that no condensed chromatin is

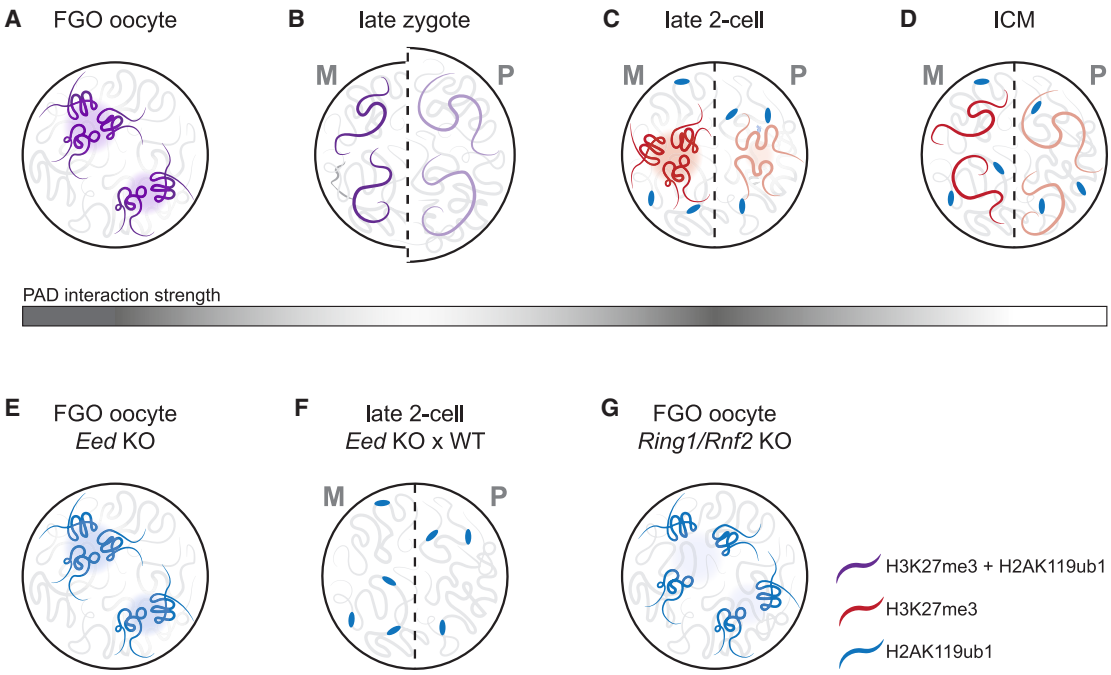

Figure 2. Putative model of changes in PADs during early mouse development

(A) In FGO oocytes, broad domains of overlapping H3K27me3/H2AK119ub1 form intra- and inter-PAD interactions.

(B) In the zygote, H3K27me3/H2AK119ub1 is more strongly enriched on the maternal allele, but broad domains with moderate enrichment of both marks also exist on the paternal allele. On both alleles, inter-PAD interactions are lost, and intra-PAD interactions are very weak.

(C) By the late 2-cell stage, H2AK119ub1 has been largely lost from broad H3K27me3 domains and is enriched at canonical Polycomb sites. Intra- and inter-PAD interactions are strong on the maternal allele and weak on the paternal allele.

(D) In blastocyst, all PAD interactions have been lost. Broad domains of H3K27me3 remain.

(E) In *Eed* KO oocytes, H3K27me3 is lost, but PAD interactions are unaffected.

(F) 2-cell stage embryos derived from *Eed* KO oocytes cannot reform PAD interactions.

(G) In *Ring1/Rnf2* KO oocytes, H2AK119ub1 is lost, and long-range PAD interactions are weakened.

Model based on Du et al.[79] and Collombet et al.[92]

present at the nuclear periphery,[64] unlike at other developmental stages, indicating that zygotic LADs exist in a decondensed state and may be targeted to the NL in a chromatin-independent manner. The zygote thus seems to represent a unique system in which interactions between the DNA and NL are entirely independent of H3K9me2/3. This could indicate that NL tethering would be a sequence-, rather than chromatin-, driven process at this stage. Alternatively, localization at the periphery of the zygotic nucleus could be a passive process rather than an active recruitment, in which genomic regions would locate to other nuclear compartments and LADs would arise by exclusion.

Facultative heterochromatin and NL association

Compared with H3K9me2/3, even less research has been done on the relationship between Polycomb and NL association during preimplantation development. However, some features of the newly gained H3K27me3/H2AK119ub1 domains in oocytes and zygotes are reminiscent of regions that constitutively associate with the NL in other systems. For example, the atypical Polycomb domains on both the maternal and paternal allele are broad, located in intergenic regions, and are enriched for genes families such as olfactory receptors,[18] all of which are characteristic features of constitutive LADs. The Polycomb domains show an extensive overlap with PMDs in oocytes,[18] while LADs also show a strong overlap with the PMDs in somatic tissues.[97] Moreover, constitutive LADs are AT rich,[95] and PRC1 seems to be preferentially targeted to AT-rich regions in the late paternal pronucleus.[85,86,98]

Interestingly, both the non-canonical maternal and paternal H3K27me3/H2AK119ub1 domains seem to appear at moments when conventional NL association is lost: for the maternal allele, the Polycomb marks are established in the oocyte,[18,19] while LADs are known to be largely absent in GV oocytes.[30] Conversely, paternal Polycomb domains are laid down by the end of the zygote stage,[18,19,20] while paternal LADs are reprogrammed between the zygote and late 2-cell stages.[30] In light of the recent work suggesting that H3K27me3 may be inhibitory to NL contacts,[84] it would be interesting to investigate whether Polycomb has a role in reprogramming LADs in early development.

NL ASSOCIATION AND 3D ORGANIZATION

NL association and 3D genome organization are both aspects of the spatial chromatin architecture. However, the exact ways in which 3D folding and NL localization of the chromatin influence

one another are not yet fully understood. In early Hi-C experiments, it was established that the B compartment shows a very strong overlap with LADs,[53,63] which is in concordance with the heterochromatic nature of both. In addition, LAD boundaries frequently coincide with TAD boundaries,[45] suggesting that regions within a TAD have a shared affinity for NL association and may be targeted to the NL periphery as a unit. This idea is supported by single-cell maps of NL association in a human cell line that show that larger genomic regions usually associate with the NL as a whole rather than having focal and independent points of attachment.[63] So, while the exact relationship between genome folding and NL association is still not entirely clear, these results show that the two modes of spatial organization clearly intersect and influence one another.

To investigate the connection between these two modes of chromatin organization in early development, NL-association profiles obtained from early embryos have been compared with the available Hi-C data of the same stages.[30] This comparison showed that LADs are present in zygotes prior to the establishment of clear TADs. Moreover, TAD boundaries gain in strength at zygotic LAD boundaries during the early embryonic stages, suggesting that LADs precede TADs as a form of genome organization and may even serve as a starting point for further maturation of the 3D structure. In line with a structuring role for the NL, a genomic tiling imaging study showed that interaction domains form at the nuclear periphery in single paternal pronuclei, despite an absence of a clear genome structure in aggregate profiles.[99] Based on these observations and the results from human single-cell LAD profiles,[63] it would be interesting to determine whether LADs still associate with the NL in a coordinated manner in the absence of a strong TAD structure or whether each locus now independently contacts and dissociates from the NL.

Another interesting observation came from the comparison of LADs with compartments in the preimplantation embryo. While LADs that are constant during early development show consistent overlap with the B compartment, a large part (39%) of LADs at the 2-cell stage belong to compartment A.[30] Interestingly, LADs that are established *de novo* in the 2-cell embryo and persist throughout development (11%) typically fall in the A compartment at this stage but switch to the B compartment by the 8-cell stage. This suggests that, at least in some cases, NL association may prime regions for a switch to the B compartment. Together, these results show that although LADs correlate highly with the B compartment in most systems, this is not necessarily the case during the first stages of embryogenesis. Moreover, in the preimplantation embryo, changes in LAD structure may direct, or at least indicate, future changes in compartmentalization. The stronger role of NL association in compartmentalization during early development could potentially be due to the absence of conventional heterochromatin. Early development can thus provide new insight into the mechanisms by which TADs get established, as well as the possible influence of NL association in shaping nuclear structure.

Conclusion

During the early stages of embryonic development, all layers of epigenetic regulation are extensively reprogrammed, including the heterochromatin and genome organization. While the intricate relationship between these two modalities is starting to be unraveled in pluripotent and somatic systems, little is known about these interactions during preimplantation development. Here, we have reviewed the current knowledge on constitutive chromatin, facultative heterochromatin, and nuclear organization from the moment of fertilization until implantation in mouse embryogenesis. In addition, we have discussed the available data on their interconnectedness. From this, it seems like both heterochromatin and nuclear organization exist in immature states in the early embryo. Both start to mature by the end of the 2-cell stage, after ZGA, with conventional features such as TADs, compartments, and chromatin compaction emerging. Before this moment, particularly in the zygote stage, the immature chromatin state appears to result in a weaker relationship between heterochromatin marks, 3D organization, and NL association. The immature state and atypical relationship between the different modes of genome regulation could be the result of the absence of important effector proteins involved in processes such as phase separation and chromatin compaction. Further research into the epigenetics of early development will be necessary to fully understand these processes, as well as their relevance to the establishment of totipotency and subsequent development.

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AUTHOR CONTRIBUTIONS

F.R., I.G., and J.K. conceived the topic. F.R. wrote the manuscript and made figures. All authors reviewed and edited the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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