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Review

Dynamics of Histone Modifications during Mammalian Zygotic Genome Activation

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Abstract: Mammalian fertilization initiates the reprogramming of oocytes and sperm, leading to the formation of a totipotent zygote. The zygotic genome remains transcriptionally silent throughout this intricate process, undergoing the maternal-to-zygotic transition (MZT) and subsequent zygotic genome activation (ZGA). Histone modifications are pivotal in shaping cellular identity and gene expression in many mammals. Recent advances in chromatin analysis enable a detailed exploration of histone modifications during ZGA. This review delves into conserved and unique regulatory strategies, providing essential insights into the dynamic changes in histone modifications and their variants during ZGA in mammals. The objective is to explore recent advancements in leading mechanisms related to histone modifications governing this embryonic development phase in depth. These considerations will be useful for informing future therapeutic approaches that target epigenetic regulation in diverse biological contexts, ranging from regenerative medicine to cancer research. It will also contribute to the extensive areas of evolutionary and developmental biology and possibly lay the foundation for future research and discussion on this seminal topic.

Keywords: histone modifications; chromatin landscape; mammals; embryonic development; embryonic stem cells

1. Introduction

Mammalian fertilization commences with the fusion of an oocyte and a single sperm, a critical event in which these two terminally differentiated germ cells must undergo reprogramming to establish a totipotent zygote[1–3]. Notably, the zygotic genome remains transcriptionally silent during this reprogramming process[4]. As this intricate transformation unfolds, the reins of

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developmental control are handed over to the RNAs and proteins that have previously accumulated within the oocyte. This transition, during which maternal products are cleared, is commonly called the maternal-to-zygotic transition (MZT)[5,6]. This transition is meticulously coordinated with zygotic genome activation (ZGA), which signifies the initiation of transcriptional control and gene expression post-fertilization[7,8]. It becomes evident that epigenetic modifications are pivotal in orchestrating this fundamental transformation[9]. Subsequently, ZGA is succeeded by emerging distinct cell identities within embryonic cells, leading to their differentiation into the inner cell mass (ICM) and trophectoderm (TE) stages at the blastocyst stage[10].

Epigenetic modifications occurring in terminally differentiated gametes, including DNA methylation[11–13], histone modifications[14–16], chromatin accessibility[17–19], and 3D chromatin structure[20,21], can be reset to a foundational state following fertilization. This reset process is crucial for achieving totipotency and supporting the subsequent development of a new individual. The precise regulation of zygotic gene transcription is intricately linked to chromatin accessibility. It underscores epigenetic information's pivotal role in upholding cellular identity and governing gene expression. The nucleosome, serving as the fundamental unit of chromatin, consists of octamers comprising two copies of the core histone proteins H2A, H2B, H3, and H4, collectively contributing to the formation of tightly packed heterochromatin[22,23]. The modulation of chromatin accessibility is mediated through the positioning and configuration of nucleosomes, factors influenced by histone variants, and the post-translational modification of histone N-terminal tails. Several studies have increasingly suggested that histone modifications and variants are pivotal in ensuring precise control over ZGA[24–26].

Recent advancements in low-input chromatin analysis technologies have introduced innovative methods to address the challenges associated with the inaccessibility of early-stage embryos[3]. These breakthrough approaches have enabled a comprehensive investigation of the epigenetic remodeling mechanisms at the whole-genome level. In this review, our primary goal is to provide an in-depth exploration of the recent advancements in our understanding of the dynamic changes in histone modifications during the activation of the zygotic genome in mammals. These considerations will be useful for informing future therapeutic approaches that target epigenetic regulation in diverse biological contexts, ranging from regenerative medicine to cancer research.

2. Methods

To compile this review, we conducted a comprehensive search of the Google Scholar and PubMed databases for relevant references from October up to December 2023 using the following terms: "epigenetics", "histone modifications," "chromatin landscape," "mammals embryonic developments," and "embryonic stem cells". Also, the cited bibliography of each selected paper was revised to identify any further relevant publications that aligned with our search objectives. The full text of each article included in this review was examined. We finally selected only the articles written in English and Spanish.

3. Comprehensive Overview

ZGA is not a singular event but rather a period during which transcription gradually becomes activated, marked by two distinct transcriptional waves. The first, smaller wave occurs during the early cleavage divisions, while the second, more significant wave coincides with the pause in the first division cycle across diverse species[4,27]. Although the precise timing of these waves and the number of division cycles vary among species[28], the process within a given species is meticulously controlled, exhibiting highly reproducible temporal patterns. Species with rapid development, such as worms[29], frogs[30], fish[31,32], and flies[33,34], complete the MZT and enter gastrulation only hours after fertilization. In contrast, in mammals with a more prolonged development, such as mice[35,36] and humans[37,38], MZT takes one or more days. This disparity is believed to stem from the egg's nature, suggesting that each egg's unique requirements dictate different embryogenesis modes[39]. Despite these variations, fundamental processes are conserved, and in all animals, the precise onset of ZGA relies on intricately coordinated mechanisms[4]. In this context, it becomes

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evident that diverse regulatory mechanisms orchestrate gene expression to establish and define cellular identity and fate.

Among these regulatory mechanisms, the dynamic processes of promoting or removing methylation[24], acetylation[40], phosphorylation[41], SUMOylation[42], and ubiquitination[43,44] marks on histones actively participate in chromatin modification during ZGA. Histone modifications, particularly methylation and acetylation, are crucial in regulating transcription by altering chromatin structure and providing binding platforms for transcription factors and other regulators[45,46].

Histone methylation occurs on specific lysine and arginine residues of these proteins without altering their electrical charges[47]. Depending on the methylated residue, methylation can have diverse effects on gene transcription, either activating or repressing it[48]. In contrast, histone acetylation is closely associated with active gene transcription. This process, highly enriched at the transcription start site (TSS), involves changing the charge of lysine residues from basic to neutral. Histone acetylation has the effect of unpacking chromatin structure, increasing its accessibility for transcription processes[49].

The composition of a unique set of histones and their variants in the nucleosome is instrumental in loosening chromatin structure during ZGA. The subsequent sections delve into the specific roles of some of the most studied methylation and acetylation modifications in histone proteins, shedding light on their contributions during this critical developmental period.

4. H3K4me3 in Zygotic Genome Activation and Gene Expression Regulation

Histone H3 Lysine 4 trimethylation (H3K4me3) is an epigenetic modification associated with packaging DNA in eukaryotic cells, including human cells. It involves adding three methyl groups (trimethylation) to the fourth lysine residue on the histone H3 protein. This modification plays a crucial role in regulating gene expression by altering the accessibility of genes for transcription[50,51]. H3K4me3 is commonly linked to the activation of nearby gene transcription. It achieves this by facilitating chromatin remodeling through interactions with the Nucleosome remodeling factor (NURF) complex[52]. This process enhances the accessibility of DNA for transcription factors, enabling genes to be transcribed and expressed within the cell. Beyond its role in gene expression, H3K4me3 is a key player in genetic regulation related to stem cell potency and lineage determination[53]. It is predominantly found in DNA regions associated with development and the establishment of cell identity. This epigenetic modification contributes to the intricate control of gene expression, ultimately influencing cellular functions and fate.

4.1. H3K4me3 at Promoters and Across Species

H3K4me3 is a canonical activation mark typically present at gene TSSs[54], established at the 2-cell stage, and exhibiting variations among species[4]. In zebrafish, H3K4me3 is detected at many promoters before ZGA, priming genes for activation[31,54,55]. Similarly, H3K4me3 is observed in frogs before genome activation, increasing during gastrulation[56]. In contrast, in *Drosophila*, only a few promoters exhibit H3K4me3 before the major ZGA wave, implying that early transcription during the minor wave can proceed without this chromatin signature[57]. Nonetheless, a substantial upsurge in H3K4me3 accompanies the major wave of transcription in zebrafish, frogs, and *Drosophila*. In mice, early embryos exhibit broad domains of H3K4me3, which later become predominantly associated with conventional TSSs. Notably, depletion of the demethylases responsible for this shift leads to developmental arrest and downregulation of numerous ZGA genes[58].

In humans, signals of H3K4me3 can be identified at each stage of oocyte and embryonic development. The signal remains consistently uniform in all zygotes. The levels of H3K4me3 gradually decline from the germinal vesicle to the metaphase II stage and increase from the zygote stage to the four-cell stage, reaching their lowest point at the eight-cell stage[59]. A notable surge is then observed at the blastocyst stage. Notably, it's important to highlight that H3K4me3 in human oocytes is linked to CpG-rich regions and significantly correlates with CpG density[60]. This association appears to be a species-specific feature, pivotal in marking genomic regions preferentially activated during the MZT.

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4.2. Dynamic Reprogramming of H3K4me3

Recent advancements in low-input ChIP-seq methods have allowed researchers to investigate the genome-wide landscape of H3K4me3 during ZGA and the first cell fate decisions in early embryos[61,62]. After fertilization, the H3K4me3 peaks in the paternal genome undergo swift depletion in the zygote. Recent research has unveiled that both the promoter and global H3K4me3 patterns on the paternal allele of the zygote differ significantly from those observed in sperm, indicating extensive reprogramming upon fertilization[63]. Consequently, robust paternal H3K4me3 peaks are reinstated during the major ZGA, particularly from the late two-cell stage onward[64]. Conversely, the maternal genome contains a noncanonical form of H3K4me3 (ncH3K4me3), covering broad domains in both promoters and distal regions[65]. NcH3K4me3 is already established in mature oocytes (MII), unlike in growing oocytes, where H3K4me3 maintains a conventional pattern of narrow peaks at the gene promoters. The noncanonical form of H3K4me3 is deposited during oocyte maturation and remains unaltered by canonical until the major ZGA stage. The initiation and elimination of ncH3K4me3 represent a distinctive trait observed in the genomes of oocytes and early embryos[58,66], and its removal is a crucial process for normal ZGA (Figure 1A). The deposition of the ncH3K4me3 coincides with genome silencing from fully grown oocytes to the early 2-cell stage[53,67]. The underlying maternal factors within the oocyte cytoplasm are pivotal in orchestrating the rapid change in the H3K4me3 landscape. Identifying these key maternal factors holds the potential to shed light on the mechanisms of H3K4me3 remodeling.

Broad H3K4me3 domains in both promoters and distal regions are actively removed by lysine demethylases KDM5A and KDM5B[68,69]. These enzymes allow the removal of ncH3K4me marks and resetting canonical H3K4me3 peaks until the two-cell stage[70]. The absence of Kdm5b results in the extension of the broad ncH3K4me3 domains, disrupting precise lineage differentiation[71]. On the other hand, the overexpression of Kdm5b leads to transcriptome reactivation in mature oocytes, hinting at ncH3K4me3's potential role in genome-wide silencing. Moreover, transposable elements such as B1/B2/B4 and ERVL exhibit significant overlap with ncH3K4me3 in distal regions[72], suggesting a correlation between ncH3K4me3 and repeat activities[65]. Nevertheless, the prevalence of repeats tends to be lower in MII oocytes and zygotes compared to late-stage embryos, indicating repeat activities alone cannot entirely account for the widespread distal H3K4me3 peaks observed in these cells.

During the human embryo pre-ZGA (4-cell stage), broader marks of H3K4me3 are already evident. 53% of these marks persist and become active in the 8-cell stage, while the remaining 47%, where H3K4me3 is lost, are in the promoters of development and differentiation genes[65]. These genes remain inactive during ZGA. Compared with promoter regions, weaker but widely distributed distal marks of H3K4me3 are evident in pre-ZGA embryos (Figure 1A), indicating de novo deposition of H3K4me3[17]. These distal marks decrease in the 8-cell stage and are deposited in CpG-rich and hypomethylated regions. Many of these distal marks of H3K4me3 overlap with regulatory elements and exhibit high chromatin accessibility at the 4-cell stage[73].

The interplay between H3K4me3 and DNA methylation is pivotal for understanding genome-wide regulation. Demonstrably, DNA methylation in the maternal genome exhibits an inverse correlation with ncH3K4me3. The evidence indicates that oocytes featuring ncH3K4me3 display approximately 18% CpG methylation, starkly contrasting to the 57% observed in oocytes lacking ncH3K4me3[12]. A significant correlation is also observed between H3K4me3 and CpG density in four-cell embryos, particularly for promoters with moderate CpG levels, showcasing transcriptional activity in post-ZGA stages[74]. H3K4me3 is recognized for its role in counteracting DNA methylation, along with repressive histone modifications such as Histone H3 Lysine 9 trimethylation (H3K9me3) and Histone H3 Lysine 27 trimethylation (H3K27me3)[53]. This interaction highlights the intricate regulatory mechanisms involved in gene expression. These bivalent domains play a crucial role in governing gene expression by maintaining a balance in the methylation levels of two histone proteins with opposing effects. This equilibrium enables them to remain suppressed, ready for activation in the absence of differentiation signals[75].

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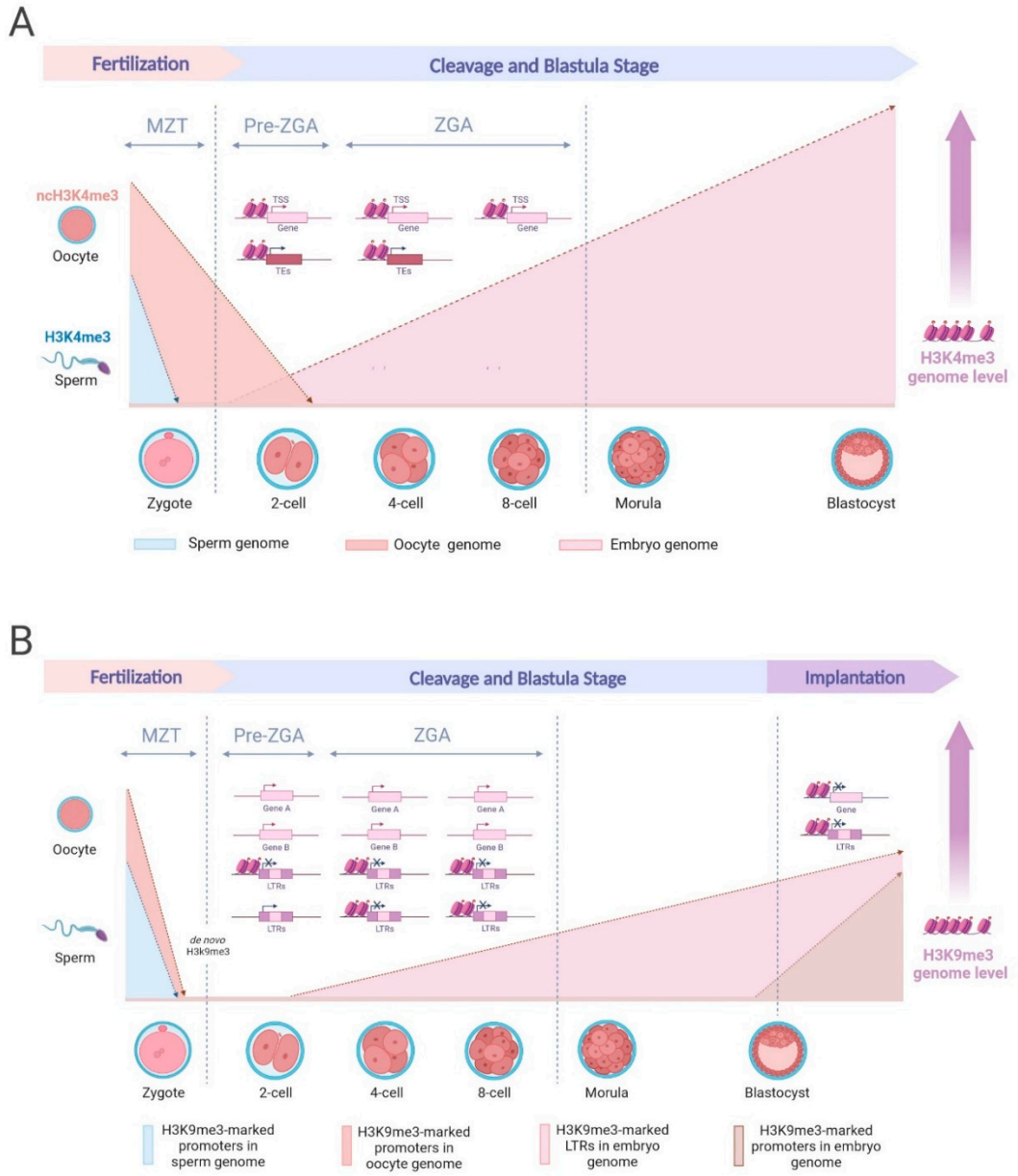

Figure 1. Epigenetic dynamics of H3K4me3 and H3K9me3 in early embryogenesis. (A) H3K4me3 experiences swift depletion in the paternal genome post-fertilization, only to be reinstated during ZGA. Conversely, the deposition of ncH3K4me3 coincides with the silencing of the genome originating from fully grown oocytes and is subsequently substituted by canonical H3K4me3 at the 2-cell stage. Throughout ZGA, chromatin accessibility becomes evident at the H3K4me3-marked TSS sites of active genes. Simultaneously, transposable elements exhibit accessibility and enrichment in H3K4me3. Progressing through development, there is a gradual escalation of H3K4me3 marks, culminating in forming a bivalent state with H3K27me3, priming the activation of developmental genes during the blastocyst stage. (B) Maternal genome-specific regions with H3K9me3 marks are more prevalent than paternal genome regions during early embryogenesis. The onset of early embryonic development triggers extensive genome-wide demethylation, resulting in the hypomethylation of actively transcribed regions, including LTRs. After fertilization, H3K9me3 marks within LTRs gradually increase during pre-implantation development. The majority of parental H3K9me3 regions are established de novo after fertilization. H3K9me3-enriched LTRs progressively emerge after the 4-cell stage and play a role in LTR silencing. Additionally, H3K9me3 marks in the promoters of developmental genes are erased after fertilization and subsequently restored after

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implantation. Abbreviations: *LTRs*, long terminal repeats; *MZT*, Maternal-to-zygotic transition; *TSS*, transcriptional start sites; *ZGA*, zygotic genome activation. Created with BioRender.com .

Another intriguing aspect of H3K4me3 dynamics involves cooperation with the histone variant H3.3, a crucial player after fertilization essential for embryo development. H3.3 turnover is associated with changes in histone modifications like H3K27me3 and H3K36me3[76]. The specific correlation between H3.3 and ncH3K4me3 remains unclear, and further investigation is required to determine if H3.3 replacement is responsible for the removal of ncH3K4me3.

While the mechanism of H3K4me3 reprogramming remains an enigma, recent studies suggest that the Histone–lysine N-methyltransferase 2 (KMT2) complex may catalyze the establishment of broad H3K4me3 domains[5,77]. Identifying the transcription factors involved in this precisely controlled process is crucial for further exploration. These major reprogramming events of both promoter and distal H3K4me3 marks offer invaluable insights into the parental-to-zygotic transition in human pre-implantation embryos, providing a deeper understanding of early embryonic development and epigenetic regulation.

5. Unveiling H3K9me3: Orchestrating Epigenetic Landscapes in Development

H3K9me3 emerges as a sentinel, staunchly guarding the integrity of heterochromatin—a densely compacted chromatin state refractory to transcriptional activities[78,79]. This histone modification, characterized by adding three methyl groups to lysine 9 of histone H3, plays a pivotal role in orchestrating cellular processes by imposing a formidable barrier to cell fate transitions.

The functional significance of H3K9me3 lies in its ability to occlude DNA, rendering it inaccessible for transcription factors—a phenomenon intricately linked to the hindrance of the transcriptional machinery[80]. This repressive chromatin environment, governed by H3K9me3, serves as a regulatory mechanism controlling gene expression patterns critical for cellular identity and function.

Recent revelations from investigations into Somatic Cell Nuclear Transfer (SCNT) embryos add a layer of complexity to our understanding of H3K9me3 dynamics[81,82]. SCNT enables animal resurrection and the treatment of human diseases by reprogramming somatic cells into pluripotent states. In this context, a method fraught with challenges in achieving efficient reprogramming, the nuanced role of H3K9me3 comes to the fore[83–85]. Unlike fertilized embryos, SCNT embryos manifest a distinctive profile marked by gradual and incomplete demethylation of H3K9me2 and H3K9me3[86,87]. This aberrant pattern significantly contributes to the failure of ZGA, highlighting the indispensable role of H3K9me3 in orchestrating the intricate balance between pluripotency and cellular differentiation.

5.1. Navigating Reprogramming Challenges and Zygotic Genome Activation

The dynamic features of H3K9me3 during early embryonic development are critical for natural reprogramming post-fertilization. It is remarkable that near-exclusive expression of the H3K9me3 demethylase named Lysine-specific demethylase 4A (KDM4D), is observed in MII oocytes and has been proven to be crucial for maintaining genomic stability and ZGA[88]. However, H3K9me3 emerges as a barrier in SCNT embryos, hindering proper ZGA and compromising development[89]. Studies indicate that H3K9me3 in donor cells obstructs somatic cell reprogramming, leading to abnormal ZGA in 2-cell SCNT embryos[90,91]. At this stage, regions resistant to reprogramming (RRRs) marked by H3K9me3 impede successful reprogramming, prompting strategies to overcome these challenges. For this reason, overexpressing Kdm4d in embryos or knocking down H3K9me3 methyltransferases (Suv39h1/h2) in donor cells proves effective in rescuing ZGA-related gene transcription and enhancing blastocyst development rates in SCNT embryos[82]. It should be noted that indiscriminate depletion of H3K9me3 can interfere with the lineage-specific deposition in SCNT blastocysts, highlighting the complexity of this process. Identifying new critical regulators involved in lineage-specific H3K9me3 deposition will add a new dimension to understanding the intricacies of epigenetic reprogramming challenges.

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The regulatory influence of H3K9me3 extends to impact the expression of repeat elements and protein-coding genes in mouse pre-implantation embryos[79]. Early embryonic development triggers extensive demethylation of the mouse genome, leading to a significant fraction of long terminal repeats (LTRs) becoming hypomethylated and actively transcribed, a process crucial for normal development. Post-fertilization, H3K9me3 marks within LTR retrotransposons gradually increase during pre-implantation development (Figure 1B), with the Chromatin assembly factor 1 subunit A (CHAF1A) complex playing a pivotal role in blastocyst formation and cell fate decisions[86]. Properly regulating these LTRs is essential in later developmental stages to maintain genome stability[92]. Studies in mouse embryonic stem cells (mESCs) have suggested that multiple H3K9me3 modifiers, such as Suv39h1/h2 and ERG-associated protein with SET domain (ESET) complexes, are responsible for retrovirus silencing through the establishment of H3K9me3 modifications[93,94].

In the postimplantation stage, a dynamic interplay of H3K9me3 marks re-establishes in promoter regions, orchestrating the repression of lineage-specific genes[86]. Transcription factors such as Pou5f1, Sox12, and Zfp105 contribute to epiblast-specific H3K9me3, while Zbed6, Elf4, and Glis2 are involved in extraembryonic-specific H3K9me3 formation[65]. Mutant studies underscore the significance of precisely regulated H3K9me3 in ensuring proper embryonic development[80]. This multifaceted role of H3K9me3 emphasizes its complex involvement in reprogramming challenges and orchestrating crucial developmental events.

6. H3K27me3 and H3K27ac: Dual Epigenetic Players in Zygotic Genome Activation

In the intricate landscape of epigenetic regulation governing ZGA, two histone modifications stand out as key orchestrators—Histone 3 Lysine 27 trimethylation (H3K27me3) and acetylation (H3K27ac)[4,40]. These dual epigenetic players engage in a dynamic interplay, shaping the transcriptional destiny of critical genes during the pivotal events of early embryonic development.

6.1. Individual Roles of H3K27me3

H3K27me3 is a well-known repressive histone modification associated with gene silencing, primarily within facultative heterochromatin[95]. This modification involves adding three methyl groups to lysine 27 of histone H3 and is catalyzed by Polycomb Repressive Complex 2 (PRC2)[96,97] (Figure 2A). Playing a vital role in maintaining cellular identity by repressing genes associated with alternative cell fates, the dynamics of H3K27me3 during mammalian ZGA are crucial for unraveling the transition from a transcriptionally silent state to the activation of specific gene loci[4,98]. Recent studies have unveiled the intricate interplay between H3K27me3 and other histone modifications[75,99], shedding light on regulatory networks governing early embryonic development.

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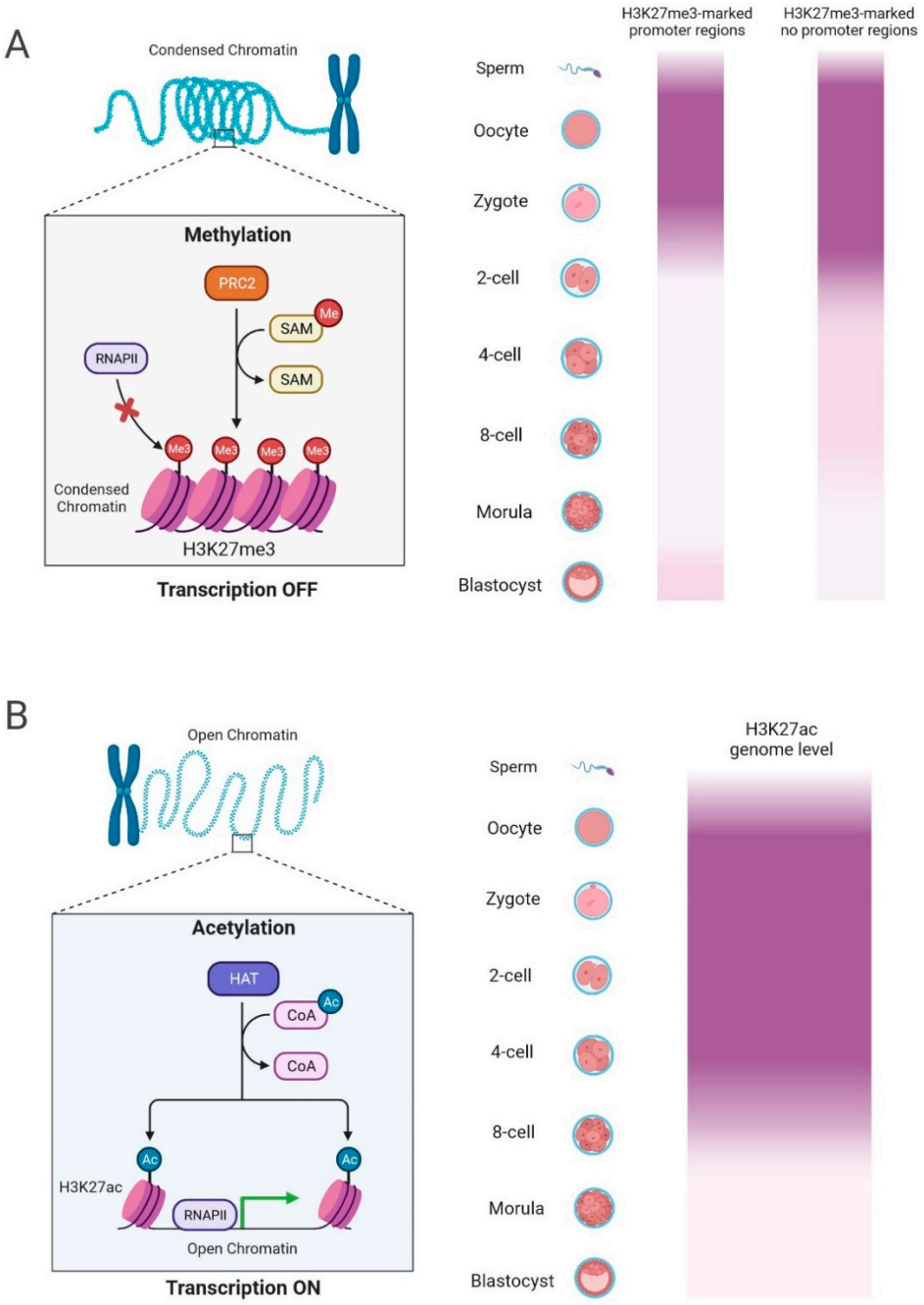

Figure 2. Antagonistic activity between H3K27me3 and H3K27ac in ZGA gene expression. (A) A polycomb repressive complex (PRC2) orchestrates the trimethylation of histone 3 on lysine 27 through histone methyltransferase activity. The SAM cofactor in Enhancer of zeste homolog 2 (EZH2), one of the PCR2 subunits, is essential to recognize the H3K27 tail selectively. The introduction of the trimethylation mark on lysine 27 results in the downregulation of nearby genes by forming heterochromatic regions. H3K27me3 in promoter regions experiences a widespread loss at the 2-cell stage. Additionally, H3K27me3 manifests in non-promoter regions in a highly pervasive and promiscuous manner. (B) A HAT facilitates the acetylation of histone 3 on lysine 27 by transferring an acetyl group from acetyl CoA. H3K27ac is an active enhancer mark, promoting a more accessible chromatin structure. It is present in both distal and proximal regions of genes, with enrichment observed at TSS. The H3K27ac marks exhibit discernible intensity in zygotes, 2-cell, and 4-cell embryos but gradually decline in 8-cell embryos. Abbreviations: *HAT*, histone acetyltransferase;

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PRC2, Polycomb Repressive Complex 2; *RNAPII*, RNA polymerase II; *SAM*, S-adenosylmethionine; *TSS*, transcriptional start sites. Created with BioRender.com.

Before ZGA onset, observed in various organism models, including zebrafish, the genome undergoes intriguing pre-patterning, defined as establishing specific epigenetic marks and chromatin configurations[7,32,100]. In this context, H3K27me3, in conjunction with H3K4me3 and H3K9me3, delineates distinctive chromatin landscapes, suggesting the existence of a primed chromatin landscape before transcription onset[75]. Throughout the MZT, the dynamics of histone modifications unfold, with H3K27me3 gradually increasing and a pronounced elevation in H3K9me3 post-ZGA[31,101,102]. This temporal sequence paints a dynamic picture of chromatin reshaping during critical developmental stages, indicating an instructive role of histone modifications in shaping the developmental transcription program even before active transcription begins.

In mouse embryos, the large-scale loss of H3K27me3 in promoter regions occurs as early as pronuclei (PN)-5 zygotes, persisting through the morula to the ICM and TE stages[103]. This loss involves global erasure in the paternal genome and selective depletion in the maternal genome's promoter regions[104,105]. Notably, a strong negative correlation between H3K27me3 enrichment and DNA methylation of promoter regions of genes in MII oocytes suggests a complex interplay between these epigenetic marks during early development[13]. On the other hand, the existence of non-canonical H3K27me3 patterns, pervasive and promiscuous in non-promoter regions[106], raises questions about their functional significance and the molecular mechanisms governing these patterns.

Examining H3K27 methylation dynamics during minor and major ZGA in mouse embryos reveals valuable insights[24]. The di-methylation and tri-methylation of H3K27 emerge as pivotal players in transcriptional silencing. H3K27me2, exclusively and robustly expressed in the female pronucleus during PN stages 2–3 and 4–5, significantly increases from 2- to 8-cell stage embryos, reaching its highest point in early blastocysts[107]. In contrast, H3K27me3 exhibits distinct staining patterns, which are prominent in the female pronucleus during PN stages 2–3 and detectable in both male and female pronuclei during PN stages 4–5[108]. This expression weakens in 2-cell stage embryos, intensifies in 4-cell stage embryos, diminishes in 8-cell stage embryos, and becomes barely detectable in early blastocysts (Figure 2A). The dynamic fluctuations in H3K27me2 and H3K27me3 offer critical insights into their roles throughout the early stages of embryonic development[109], strongly suggesting their involvement in the intricate regulation of gene expression and chromatin accessibility.

SCNT embryos present a unique challenge regarding H3K27me3. While SCNT embryos exhibit strong H3K27me3 signals in all pseudopronuclei at the 1-cell stage, distinct from the asymmetric marks in normally fertilized embryos, the erasure of H3K27me3 from donor somatic cells doesn't necessarily improve SCNT efficiency[110]. Studies indicate that overexpression of Lysine-specific demethylase 6A (KDM6A), an H3K27me3 demethylase, enhances ZGA-related gene expression in SCNT embryos[111,112], but this enhancement doesn't translate into improved birth rates or Nuclear-transfer embryonic stem cells (ntESC) establishment efficiency[65]. Conversely, the knockdown of Lysine-specific demethylase 6B (KDM6B), another H3K27me3 demethylase, promotes both ZGA and SCNT efficiency[113]. The imprinting of H3K27me3-dependent genes is aberrant in SCNT embryos[114], emphasizing the crucial role of H3K27me3 in epigenome reprogramming. The complexity deepens with the discovery of maternal H3K27me3-mediated imprinting, transitioning to DNA methylation dependence in extra-embryonic cells after implantation[106,115].

In human embryonic development, H3K27me3 dynamics differ from those in mice. Notably, in human GV oocytes, H3K27me3 is selectively deposited in promoters of developmental genes and partially methylated domains, deviating from the pattern observed in mouse oocytes[116]. This initial divergence sets the stage for further discrepancies during human pre-implantation embryo development, where the resetting of H3K27me3 follows a unique trajectory compared to its counterpart in mice. Specifically, human embryos at ZGA (8-cell stage) manifest a striking absence of H3K27me3 signals[37], indicating a comprehensive erasure of this histone modification on both parental genomes. The correlation between the absence of core components of PRC2 in human

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embryos and the concurrent loss of H3K27me3 adds an intriguing layer to the understanding of epigenetic regulation[117]. This distinct scenario raises questions about regulatory mechanisms, such as the absence of imprinting regulation like X chromosome inactivation, which plays a pivotal role during early mouse embryogenesis.

Furthermore, the divergence continues as H3K27me3-mediated imprinted genes identified in mouse early embryos, and having human orthologs, seem to undergo de novo deposition of H3K27me3 in humans[95,115]. However, verifying the existence of H3K27me3-controlled imprinting in human early embryos necessitates further investigation. Further analysis reveals asymmetric H3K27me3 patterning between ICM- and TE-specific genes[118,119], hinting at potential preferential deposition between distinct cell types. These intricate dynamics of H3K27me3 provide a nuanced understanding of its roles in regulating gene expression, chromatin accessibility, and imprinting during the complex process of embryonic development.

6.2. Distinctive Functions of H3K27ac in ZGA

In stark contrast with H327m3, H3K27ac is generally associated with active transcription[54]. Catalyzed by histone acetyltransferases (HATs), adding acetyl groups to lysine 27 of histone H3 induces a more open chromatin structure, facilitating the binding of transcription factors and promoting gene expression (Figure 2B). As the zygotic genome undergoes activation, the presence of H3K27ac at specific genomic loci marks regions poised for transcriptional activity, a crucial indicator for the timely and precise expression of genes directing cell fate decisions and embryonic development[120].

Recent studies in mice have explored the existence of broad H3K27ac domains in embryos before the ZGA stage[40]. These investigations revealed that H3K27ac signals cover 17.6% of the mouse genome in zygotes, but this coverage drops below 10% in post-ZGA embryos[58]. Broad domains (>10 kb) of H3K27ac are widely detected in PN5 zygotes, some exceeding 20 kb. In contrast, broad domains > 20 kb become limited in mouse embryos at the 2-cell and 8-cell stages[40].

Comparing H3K27ac-enriched regions between mouse gametes and zygotes underscores a higher inheritance of regions from oocytes compared to those transmitted by spermatozoa. The data highlight the notable resemblance in the distribution of the H3K27ac signal across the entire genome of the mouse zygote to that of the mouse MII oocyte, in contrast to sperm and early post-ZGA embryos[58,63]. Additionally, a significant extension of H3K27ac domains, exceeding 20 kb, is observed in the zygote compared to oocytes and sperm, indicating a reprogramming of the H3K27ac pattern after fertilization.

In the mouse zygote, 68.3% of narrow H3K27ac peaks, with lengths less than 10 kb, originate de novo in regions without H3K27ac enrichment in mouse gametes. Most broad H3K27ac domains (> 10 kb) are established de novo by extending H3K27ac peaks in gametes post-fertilization. Of these domains, 8.0% are exclusively inherited from the oocyte, 4.8% are from the spermatozoa, and 0.5% are from shared broad domains between the oocyte and sperm. An additional comparison between H3K27ac patterns in spermatozoa and the human 2-cell embryo reveals that merely 0.4% of broad H3K27ac domains in the human 2-cell embryo are inherited from spermatozoa. Primarily, these broad H3K27ac domains in human 2-cell embryos do not originate from sperm[40].

Exploring the dynamics of histone H3K27ac in early human embryos unravels intriguing patterns. Utilizing an efficient ChIP-seq method, these studies demonstrate a genomic distribution of broad H3K27ac domains spanning over 10 kb in 2-cell and 4-cell embryos[54], with some domains exceeding 50 kb[40]. These broad domains markedly decreases in 8-cell embryos and subsequent stages, suggesting an association with zygotic genome activation in humans. Notably robust H3K27ac signal intensity is observed in zygotes, 2-cell, and 4-cell embryos before decreasing in 8-cell embryos (Figure 2B), supporting the observation of broad domains in the earliest stages of development.

Furthermore, enriched regions with H3K27ac in early embryos have been characterized, emphasizing their prevalence in intergenic and intronic regions. The H3K27ac signal spreads across the promoters of protein-coding genes at the 2- and 4-cell stages while being depleted at TSSs.

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However, after the 8-cell stage, the H3K27ac signal concentrates around the TSS[40]. Interestingly, most promoters with H3K27ac at the zygotic genome activation stage exhibit broad H3K27ac domains before this activation[8]. The association between broad H3K27ac domains and partially methylated domains (PMDs) suggests a dynamic interplay between H3K27ac marks and DNA methylation during the early stages of human embryonic development[121]. In particular, promoters covered by broad H3K27ac domains show higher CpG densities than those without a broad H3K27ac signal[122].

The dynamics of typical H3K27ac peaks during early embryogenesis reveal significant changes at the 8-cell stage. Broad H3K27ac domains transition to typical H3K27ac peaks in human embryos during this phase. A detailed analysis unveils that approximately 80% of H3K27ac peaks are in distal regions, i.e., non-promoter regions, at the 8-cell stage. Nevertheless, over 70% of human promoters exhibit H3K27ac enrichment at this stage[40]. Studies also confirm high expression in genes whose promoters are marked with H3K27ac and/or combined with the H3K4me3 modification[54,123]. Throughout early development, from the 8-cell stage to the 6-week stage, H3K27ac peaks in promoter regions exhibit greater stability than distal H3K27ac peaks, and the number of promoters marked with H3K4me3 undergoes minimal changes. Despite this stability, many stage-specific genes are expressed during the morula, blastocyst, and 6-week embryos, and this expression is correlated with stage-specific modifications of H3K27ac in promoters[40]. These findings reinforce the notion that establishing H3K27ac modification in promoters plays a crucial role in the temporal regulation of gene expression during human embryogenesis.

7. Dynamics of Other Histone Modifications in Early Embryonic Development

7.1. H3K36me3 Dynamics Unveiled: Allelic Reprogramming in Early Mouse Embryos

The methylation of H3K36 (H3K36me), a highly conserved process from yeast to humans, is intricately associated with transcribed regions, playing pivotal roles in transcription fidelity[124,125], RNA splicing[126,127], and DNA repair[128,129]. In mammals, SET domain containing 2 (SETD2) emerges as the primary methyltransferase responsible for catalyzing H3K36 trimethylation (H3K36me3) *in vivo*[130–132]. SETD2 facilitates the interaction with RNA polymerase II, orchestrating the coupling of H3K36me3 with transcription elongation[133]. Unlike H3K4me3, H3K36me3 exhibits a positive correlation with DNA methylation, recruiting DNA methyltransferase 3A and 3B (DNMT3A/B) and maintaining this association in various mammalian cells[134,135].

Several studies underscore the critical roles of SETD2 levels and H3K36me3 in establishing and safeguarding the maternal DNA methylome during oogenesis and early embryo development. In mice, SETD2-depleted oocytes experience a significant loss of H3K36me3, leading to invasions of H3K4me3 and H3K27me3 into regions formerly marked by H3K36me3[65]. Additionally, SETD2-deficient oocytes result in an aberrant DNA methylome characterized by the loss of maternal imprints and anomalous deposition of H3K4me3 instead of DNA methylation, particularly at imprinted control regions (ICRs)[136].

Furthermore, the scarcity of SETD2 has been demonstrated to induce defects in oocyte maturation and embryonic lethality. Mice deficient in SETD2 do not survive beyond embryonic day (E) 10.5 - E11.5[137]. Notably, the overexpression of H3.3K36M (lysine to methionine mutant) in mouse metaphase II (MII) oocytes results in reduced H3K36me3 levels and compromised embryo viability[138].

The study of allelic reprogramming of H3K36me3 post-fertilization in early mouse embryos has provided valuable insights. Given the transient inheritance of maternal marks H3K4me3 and H3K27me3, influencing processes like ZGA[53], imprinted X chromosome inactivation[139], and gene expression[140], the inquiry arises regarding the inheritance of H3K36me3 in early embryos and its potential interaction with other epigenetic marks. Recent immunofluorescence studies have uncovered the presence of H3K36me3 at all stages except in paternal pronuclei shortly after fertilization[136]. Analyses of H3K36me3 through ChIP-seq in sperm and discrimination of parental strains via single-nucleotide polymorphisms (SNPs) revealed a significant allelic imbalance in 1-cell

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embryos, with a notably higher number of maternal reads than paternal reads[141,142]. Surprisingly, H3K36me3 inherited from oocytes appears present in 1-cell embryos but diminishes considerably by the late 2-cell stage and is lost by the 8-cell stage[143]. Conversely, most, if not all, H3K36me3 peaks in sperm are lost in zygotes[73]. The temporal transition of H3K36me3 from parental to zygotic patterns aligns closely with ZGA, suggesting allelic reprogramming during early embryo development[144]. Notably, maternal H3K27me3 persists beyond ZGA in the blastocyst, potentially influencing the deposition of zygotic H3K36me3. Genes with paternal-specific H3K36me3, as opposed to maternal-specific H3K36me3, exhibit a preference for reciprocal allelic H3K27me3. This group includes a significant number of H3K27me3-controlled imprinted genes, indicating the occurrence of H3K36me3 in early embryos and its role in marking allele-specific gene expression[65].

7.2. Histone H3R26me2: Pivotal in Cell Fate Determination in Embryos

After ZGA, embryos undergo multiple cell divisions before the first segregation into cell lineages, giving rise to TE and ICM cells. TE lineage, marked by Cdx2 and Gata3 expression, plays a role in placental development[145–147]. In contrast, cells in the ICM, recognized by pluripotent factors, undergo differentiation into epiblast and primitive endoderm, giving rise to all embryonic tissues and certain extraembryonic membranes[148–150]. During differentiation, the HIPPO pathway regulates the TE lineage[151,152], and epigenetic modifications consolidate the ICM lineage[153].

Previously explored studies have highlighted the role of Histone 3 Arginine 26 dimethylation (H3R26me2) as a recently identified epigenetic mechanism[154]. This process, predominantly governed by Coactivator-associated arginine methyltransferase 1 (CARM1), has been reported to influence pluripotency in both mouse embryos and mESCs[155]. The overexpression of CARM1 in embryonic stem cells (ESC) and early mouse embryos' blastomeres drives an increase in H3R26me2 at pluripotent gene promoters[156–158], such as Oct4/Pou5f1[159], Nanog[160], and Sox2[161,162]. This epigenetic mark linked to gene activation determines the elevated expression of these genes, closely related to cell fate determination and the pluripotent capacity of these cells.

The asymmetrical distribution of H3R26me2 in blastomeres is detected in embryos as early as the four-cell stage[157]. Blastomeres with higher levels of CARM1 and H3R26me2 contribute more significantly to the formation of the ICM[163]. Furthermore, higher levels of H3R26me2 enhance the expression of pluripotent genes and facilitate Sox2 binding to its targets, contributing to ICM specification. Additionally, CARM1 overexpression can increase the frequency of asymmetric cell divisions, leading cells to adopt a more internal position in the embryo[163]. Alongside CARM1, another chromatin regulator named PR domain-containing 14 (PRDM14) is also asymmetrically expressed in four-cell embryos, thus modulating the level of H3R26me2 to favor contribution to the ICM[164–166].

Early cell fate determination in mouse embryos has recently been suggested to commence at the late 2-cell stage[167]. At this point, a long non-coding RNA (lncRNA) known as LincGET is transiently and asymmetrically expressed in the nucleus, extending from the 2-cell to the 4-cell stage[159]. LincGET interacts with CARM1, accumulating it in nuclear granules that require the presence of the Nuclear Paraspeckle Assembly Transcript 1 (NEAT1) and its partner Nuclear RNA-binding protein 54 kDa (P54NRB)[167,168]. It results in a significant increase in the H3R26me2 level, activation of ICM-specific gene expression, positive regulation of transposons, and an increase in global chromatin accessibility. It is important to note that introducing LincGET into one of the blastomeres of 2-cell embryos can potentially redirect their differentiation toward the ICM[159].

The lncRNA named Neat1 is also required to mark H3R36me2, which is CARM1-dependent and crucial for ICM specification [169]. NEAT1 shows an asymmetrical expression among blastomeres in 4-cell embryos and recruits CARM1 in paraspeckle nuclear foci[144]. Disruption of NEAT1 results in a decrease in H3R26me2, an increase in Cdx2 expression, and a biased specification toward the TE lineage[157,170].

In summary, the asymmetry in the distribution of H3R26me2 emerges as one of the early signals guiding lineage specification. The variability in H3R26me2 is carefully regulated by the asymmetrical expression of CARM1, PRDM14, LincGET, and NEAT1 in the early blastomeres. Although the cause

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of the initial skewed expression of CARM1/PRDM14/LincGET/NEAT1 in 2-cell and 4-cell embryos is not fully understood, these findings provide a clearer understanding of the molecular events orchestrating cell fate determination in the early stages of embryonic development.

8. Functional Diversity of Histone Variants in the Activation of the Zygotic Genome

The histone variants exhibit distinct positioning and dynamics within cells, assembling into nucleosomes through different molecular chaperones. They interact with various chromatin remodeling complexes, replacing canonical histones, or undergoing substitution with other variants during cellular development and differentiation[171–173]. Structural differences introduced by a central histone variant can impact histone interactions, transforming nucleosome stability and chromatin opening or compaction[174]. Among these, histone H2A variants are recognized for coordinating early embryonic genome chromatin remodeling by replacing conventional H2A in a subset of nucleosomes[175,176].

The macroH2A histone variant is a central histone related to canonical H2A, possessing a long non-histone domain (NHD) at the C-terminal[177]. Previous studies have implicated macroH2A in epigenetic gene silencing events, including X chromosome inactivation[178,179]. However, macroH2A1 is expressed at similar levels in both male and female cells[180], suggesting its function extends beyond X chromosome inactivation. Further analyses have revealed that macroH2A can inhibit transcription by negatively regulating the binding of the NF-kappaB transcription factor and preventing SWI/SNF chromatin remodeling)[181–183]. MacroH2A is localized in the chromatin of germ vesicles in oocytes, associated with mature oocyte chromosomes, and abundant in the first polar body. After fertilization, a transient asymmetry is observed, with macroH2A preferentially associating with the female pronucleus. This maternal reserve of macroH2A is lost in late-stage 2 pronuclei (2PN), resulting in normal embryos at 2, 4, and 8-cell stages lacking macroH2A, except in residual polar bodies[181,184]. As macroH2A is a repressive H2A variant and should be progressively lost as the embryo becomes transcriptionally active, it is not detected in major ZGA[7,102]. MacroH2A protein expression reappears in embryos after the 8-cell stage and persists in morula and blastocysts, where nuclear macroH2A is present in both trophectodermal cells and the inner cell mass[181]. This finding suggests that embryos complete their initial three or four-cell cycles without macroH2A. These findings imply significant modifications in macroH2A variant content in the chromatin of developing embryos before implantation.

Another identified H2A variant is H2A.X, which plays a role in DNA repair[185,186]. In mammals, H2A.X shares up to 95% sequence similarities with canonical H2A and is highly conserved across species[187]. H2A.X contains a unique SQ motif at its C-terminus and is invariant in sequence and position relative to its C-terminus across species[188,189]. Recent studies have demonstrated that H2A.X regulates Cdx2 and its specific extraembryonic genes, determining the developmental potential of stem cells[190,191], and indicating regulatory functions of H2A.X in the transcriptional network related to cellular fate control.

H2A.X is the main H2A variant deposited on chromatin in cleavage-stage embryos in mice and humans with ZGA activity. This histone variant is specifically expressed in 1-2 cell stage mouse embryos[192] and shows an enrichment trend in human embryos at the 4-8 cell cleavage stage[174]. The proper amount of H2A.X ensures that genes involved in ZGA are at relatively normal expression levels. A recent study in ESCs identified that H2A.X inhibits the expression of genes mediated by Dux[193,194], a factor directly involved in ZGA stimulation, by binding to its locus, confirming that the dynamic incorporation of this histone variant finely modulates developmental progression.

The H2A.Z histone variant from yeast to mammals constitutes approximately 4 to 10% of total H2A histones [195]. Its multifaceted role includes crucial functions in transcriptional control[196], DNA repair[197], heterochromatin formation[198,199], and genetic stability[200,201]. Genomically, H2A.Z integrates into chromatin, playing an essential regulatory role in transcription. Despite its functional relevance, studies on H2A.Z in early developmental stages have been constrained by the lethality accompanying its mutation in various organisms[202–204]. However, multiple studies

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concur that it plays a crucial role as a regulator in the activation and transcription of genes during ZGA[195,205].

Two isoforms of H2A.Z have been identified, differing in only three amino acids. These variants, H2A.Z.1 and H2A.Z.2, are encoded by separate genes, H2AFZ and H2AFV, respectively[201]. Despite the subtle difference of three amino acids between these isoforms, they perform specialized functions related to their interactions. While H2A.Z.2 preferentially associates with H3K4me3, it has been confirmed that H2A.Z.1 interacts more efficiently with Bromodomain-containing protein 2 (BRD2)[196,206,207].

The deposition of H2A.Z on the TSS of the zygotic genome is facilitated by an ATPase chaperone known as Domino in *Drosophila*[208]. This deposition precedes ZGA and RNA polymerase II (Pol II) binding to chromatin, indicating its contribution to preparing genes for transcriptional activation[207]. Although the mammalian orthologs of Domino, Snf2 Related CREBBP Activator Protein (SRCAP) and E1A Binding Protein P400 (EP400)[209], have not been fully explored during early embryogenesis, previous studies have shown that EP400 is essential for the identity of ESCs[210], and EP400 mutant mice are lethal when homozygous[211]. This observation underscores the need for further research to better understand the dynamics of SRCAP and EP400 in the context of early developmental regulation.

During minor ZGA, H2A.Z is symmetrically expressed in male and female pronuclei in embryos at PN 2–3 and embryos at PN 4–5. However, variant expression slightly decreases in embryos at the 2-cell stage, reaching a higher level in embryos at the 4-cell stage. In embryos at the 8-cell stage and early blastocyst, the expression level of H2A.Z decreases, suggesting a temporal regulation of its function during later stages of embryonic development[212]. It has been verified that H2A.Z deposited by Domino in *Drosophila* and its mammalian orthologs, provided by the mother, are necessary for the transcriptional activation of thousands of genes at the onset of ZGA; the lack of expression of these, including regulators of this process, leads to embryonic death[195]. In mESCs, H2A.Z in chromatin is linked to H3K4me3, present in both active and bivalent promoters, but not in repressed genes[205]. This correlation pattern is maintained in human embryonic stem cells (hESCs)[213]. These findings expand our current understanding of ZGA regulation, emphasizing the importance of chromatin in this process. Given the evolutionary conservation of H2A.Z and the fundamental principles of ZGA, it is speculated that histone variants could play similar roles during mammalian embryogenesis. Future research in this direction will illuminate the complex process by which chromatin states and transcription factors jointly orchestrate zygotic genome activation.

Finally, we encounter the H3.3 variant, which has sparked considerable interest due to its distinctive role in remodeling the male and female genomes during fertilization and the early stages of embryonic development. This histone plays a vital function in maintaining genomic integrity in mammals[214]. Encoded by two different genes, H3f3a and H3f3b, H3.3 generates an identical protein product[215]. Its constitutive expression in cells and its incorporation into chromatin independently of DNA synthesis underscore its relevance in the biological context. In the case of mice, ZGA is associated with the extensive incorporation of the H3.3 variant into parental genomes[216]. Both in sperm and oocytes, H3.3 is enriched, with mature oocytes being particularly rich in H3 mRNA, leading to the formation of maternal H3.3 after activation. This histone also plays a crucial role in forming the male pronucleus during fertilization[217].

A detailed study using a mouse model marked with H3.3B-HA reveals the asynchronous activation of paternal and maternal genomes[76]. The early deposition of paternal H3.3 in the zygotic genome contrasts with the delay in maternal deposition until the four-cell stage. Maternally stored H3.3 in oocytes is essential for cleavage and the lesser ZGA[218]. Its global deposition in the paternal genome during the transition from protamine to histone[219], with a preferential enrichment in CpG-rich TSS, highlights its critical role. Depletion of maternal H3.3 can result in the loss of H3K27ac, leading to failure in the lesser ZGA and early embryo arrest[220]. Mechanically, the deposition of maternal H3.3 in the sperm genome removes repressive histone modifications, promotes the establishment of active modifications, and, in turn, enables the initiation of the lesser ZGA of the paternal genome. In summary, current findings emphasize that paternal chromatin remodeling

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mediated by H3.3 is essential for developing pre-implantation embryos and activating the paternal genome during embryogenesis, providing valuable insights into fundamental biological processes.

9. Future Perspectives and Applications of ZGA Histone Modifications in Stem Cell Research

In this review, we delved into the dynamics of histone modifications during the ZGA in mammals, unveiling an intricate network of molecular events that govern this pivotal process. The findings suggest potential therapeutic approaches for epigenetic regulation in biological contexts, particularly in regenerative medicine and cancer research.

The versatility of ESCs and their unique ability to regenerate any differentiated cell place them at the forefront as a pluripotent cell type of significant interest in research and medicine. This interest extends beyond conventional ethical and cultural considerations. The prospect of applying specific epigenetic marks to these cells generates considerable expectations for their use as revolutionary therapeutic alternatives, opening the possibility of addressing diverse diseases, including neurodegenerative conditions, osteoarticular disorders, and cancer.

In the field of neuroscience, disruptions in histone methylation have been implicated in various processes, including inflammation[221,222], quiescence of neural stem cells[223,224], and neurodegenerative and psychiatric disorders[225,226]. Recent research has established connections between epigenetic regulation and brain aging[227,228]. An increase in H3K4me3 has been associated with the re-entry of mature brain cells into the cell cycle [229], emphasizing the importance of maintaining a proper balance of this histone for neuronal function. The plasticity of histone methylation patterns provides a unique window for interventions with specific cellular therapies. While these findings are promising, they are not without ethical considerations, demanding a comprehensive understanding of the molecular complexities of the involved organ. This scenario continues to pave the way for deeper studies and critical reflections at the intersection of molecular biology and ethics.

Understanding the significance of histone modifications in modulating cellular changes has significantly advanced through reprogramming somatic cells into induced pluripotent stem cells (iPSCs)[230]. Adequate reprogramming of a somatic cell into an iPSC requires not only the introduction of stemness genes but also the reorganization of its chromatin, modifying specific epigenetic marks that enable pluripotency[231]. Histone modifications in neurodifferentiation with iPSCs have become a focus of various studies to improve therapy effectiveness [232,233]. In these pluripotent stem cells, genes associated with differentiation exhibit H3K27me3 as a repressive mark, while genes related to cell renewal display H3K4me3 as an activating mark. Recent developments in epigenetics have enabled the exploration of drugs that target these therapeutic pathways[234,235], facilitating the effective reprogramming of somatic cells into iPSCs.

The scientific community underscores the relevance of epigenetic regulations in bone development and repair within the realm of regenerative medicine[236]. A crucial regulatory sequence in the epigenetic control of the osteogenic commitment of Wharton's jelly-derived mesenchymal stem cells (MSCs) has been identified [237]. These findings reveal that those repressive marks on the SP7 gene promoter result from a weak transcriptional response during osteoblast differentiation. Additionally, the enrichment of the H3K4me1 mark on the P1 promoter of the RUNX2 gene is associated with the repression of key regulatory genes. Conversely, the presence of activating histone marks in these regions is linked to the induction of osteogenic differentiation of Wharton's jelly-derived MSCs. In this context, the epigenome emerges as a biomarker to assess the efficacy and safety of stem cell differentiation. The natural stimulation of the adult stem cell niche after bone tissue injury implies significant potential for clinical translation without requiring cell transplants. Pluripotency and cell differentiation can benefit from manipulating activating marks such as H3K27ac and H3K36me3, which could be essential for bone repair by stimulating osteogenic activity.

Histone modifications also provide an opportunity to address cancer through therapeutic strategies regulating the genetics of cancer cells[238,239]. Various studies have highlighted the central role of H3K9me3 in gene regulation, especially in hematopoietic disorders such as acute myeloid leukemia (AML)[240,241]. Other epigenetic changes, such as H3K27me3 and H3K4me3, are

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recognized as important markers in tumor progression[242,243] and cell differentiation[244], respectively, and are extensively studied in oncology. Additional research on post-translational histone modifications in cancer cell lines has identified specific patterns of modifications in histones H3 and H4 and their variants[245]. These studies describe how inhibiting Enhancer of zeste homolog 2 (EZH2), a key enzyme in H3K27 methylation, significantly reduces tumor burden in breast cancer cell lines in mice[246,247]. Similarly, the presence of H3K9me3 in double-strand breaks activates the acetyltransferase activity of Tip60[248], which is essential for efficient DNA repair, suggesting that abnormal histone methylation patterns could influence DNA repair efficiency and contribute to cancer.

Our review provides a detailed insight into the dynamics of histone modifications during ZGA in mammals, highlighting various promising avenues as targets for application in the biological context of specific diseases, as outlined in this section. This knowledge could be crucial for developing protocols to differentiate ESCs and iPSCs with specific histone modifications, thus opening the possibility of applying these cellular therapies in humans to address medical conditions. Additionally, the mentioned perspectives have therapeutic implications and suggest intriguing paths for future research in the field of reproductive medicine. Another promising research direction is exploring specific transcription factors and cofactors orchestrating the reprogramming of other histones during ZGA. This approach would shed light on the molecular actors driving this critical process and could have applications in both fundamental understanding of biology and future therapeutic developments. Furthermore, conducting a comparative analysis of the dynamics of studied histones across different species could provide valuable evolutionary insights into the role of these modifications in early embryonic development, offering a broader understanding of comparative biology. Finally, understanding the specific mechanisms of histone reprogramming during early embryonic development in embryos produced by SCNT not only presents itself as a fundamental model for basic research but also paves the way for innovative approaches in animal cloning, regenerative medicine, and treating human diseases.

10. Conclusions

The in-depth analysis of ZGA accentuates the intricacies of this essential process in embryonic development. This review emphasizes that, regardless of variations in embryonic development, ZGA shares highly conserved control mechanisms in mammals. These finely orchestrated mechanisms establish gene expression, shaping cellular identity and fate. The pivotal role of histone modifications, including the methylation and acetylation of specific lysine and arginine residues on H3 and their variants, significantly impacts the activation or repression of gene transcription during ZGA. A comprehensive understanding of the dynamics of histone modifications during this period unveils novel perspectives for therapeutic applications. The potential to manipulate ESC and iPSC with specific histone modifications emerges as a promising approach for developing targeted cellular therapies.

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Abbreviations

AML	Acute myeloid leukemia
BRD2	Bromodomain-containing protein 2
CARM1	Coactivator-associated arginine methyltransferase 1
CHAF1A	Chromatin assembly factor 1 subunit A
ESCs	Embryonic stem cells
ESET	ERG-associated protein with SET domain
EZH2	Enhancer of zeste homolog 2
HATs	Histone acetyltransferases
hESCs	Human embryonic stem cells
ICM	Inner cell mass
ICRs	Imprinted control regions
iPSCs	Induced pluripotent stem cells
KDM4D	Lysine-specific demethylase 4A
KDM6A	Lysine-specific demethylase 6A
KDM6B	Lysine-specific demethylase 6B
KMT2	Histone–lysine N-methyltransferase 2
lncRNA	Non-coding RNA
LTRs	Long terminal repeats
mESCs	Mouse embryonic stem cells
MSCs	Mesenchymal stem cells
MZT	Maternal-to-zygotic transition
NEAT1	Nuclear Paraspeckle Assembly Transcript 1
NHD	Non-histone domain
ntESC	Nuclear-transfer embryonic stem cells
NURF	Nucleosome remodeling factor
P54NRB	Nuclear RNA-binding protein 54 kDa
PMDs	Partially methylated domains
PN	Pronuclei
PRC2	Polycomb Repressive Complex 2
PRDM14	PR domain-containing 14
RRRs	Regions resistant to reprogramming
SCNT	Somatic Cell Nuclear Transfer
SETD2	SET domain containing 2
SNPs	Single-nucleotide polymorphisms
TE	Trophectoderm
TSS	Transcription start site
ZGA	Zygotic genome activation

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