Feature Article

Chromatin role in early programming of embryos

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Introduction

Normal embryo development is a critical component for normal fertility and is essential for the propagation and survival of species, the success of animal breeding and production programs, and the efficient use of assisted reproductive technologies (ARTs). The embryo life begins at fertilization and its progress depends on a series of interplaying events between the parental gametes. These events comprise the make-up and preservation of the normal embryo ploidy, the remodeling of the chromatin inherited from each gamete into a functional bi-paternal genome, and the coordination of the cell cycle progression, cell function, and cell differentiation. There are many molecular events participating in these processes, not only those involved in turning on and off the genes controlling development, cell function, and differentiation, but also for the regulation of cell homeostasis and the balance between cell survival and cell integrity. While cell survival is essential for embryo development, cell integrity is essential for the health of individuals and their progeny. It is known that a significant proportion of embryos fail to develop and become arrested at early stages of development, before the first cell lineage specification and blastocoel formation, particularly in embryos that are cultured in vitro as part of ART protocols. The goal of this review paper is to highlight some of the known mechanisms that participate in the regulation of the embryo developmental program and its reprogramming.

Building and Preserving the Normal Embryo Program

Changes in the chromatin structure

Chromatin remodeling to support normal embryo development starts immediately after fertilization. The sperm chromatin undergoes a vast rearrangement, which comprises the replacement of protamines by histones. This is followed by DNA replication and the merging of the paternal and maternal chromosomes, thus rebuilding a diploid bi-paternal genome in the first mitotic division (Figure 1). The exchange of protamines by histones involves the phosphorylation of protamines with the participation of the serine/arginine protein kinase 1 (SRPK1), which enables the recruitment of the histone chaperones Nucleoplasmin 2 (NPM2), involved in the extraction of protamines, and the histone cell cycle regulator (HIRA), involved in H3.3 deposition (Gou et al., 2020). Replacement of core and linker histone variants is also necessary for totipotency acquisition, cell differentiation, and normal embryo development. For example, the three variants of the core histone H2A, H2A.X, H2A.Z, and macroH2A, are present in the chromatin of oocytes, but only the H2A.X variant is abundant in the pronuclei of zygotes after fertilization (Nashun et al., 2010). The deposition of the H3.3 variant is important for normal embryo development and chromatin stability (Gou et al., 2020).

Implications

- Normal embryo development depends on many molecular events that include changes in the chromatin structure, epigenetic modifications, DNA damage repair, expression of transcription factors, and the coordination of cell cycle progression and cell differentiation.
- Failures in the proper regulation of one or more events of early development may result in embryo arrest, which often occurs around the oocyte to embryo transition when the embryo genome is activated, or in embryos that may have lower potential for full development and production of healthy offspring.
- The complete elucidation of the early embryo programming regulation has effective implications on fertility, animal conservation, animal production, animal breeding programs, the success of in vitro embryo technologies, and the reprogramming of cells to a totipotent state.
Replacement of variants of the linker histone H1, which comprises 11 variants divided into somatic and germline-specific subtypes, also alter the chromatin structure and function during embryo programming. For example, the somatic variants are associated with chromatin compaction and silencing of endogenous retroviruses via the establishment of the repressive epigenetic marks H3K9me3 and H3K27me3, and the inhibition of the permissive mark H3K36me2 (Willcockson et al., 2021). On the other hand, the oocyte-specific variant, H1foo, is associated with chromatin relaxation and maintenance of cell pluripotency. The H1foo is quickly assembled into the sperm chromatin after fertilization and remains the most abundant H1 variant until the activation of the embryo genome when it is replaced by the somatic variants.

In addition to the exchanges of nuclear proteins, the establishment of the embryo developmental program depends on the activity of ATP-dependent nucleosome-remodeling complexes, which modulate nucleosome spacing and access to transcription factors. Among the different families of nucleosome-remodeling enzymes, the switch/sucrose non-fermentable (SWI/SNF or BAF) complex plays important roles in the regulation of early embryo development. Depletion or inactivation of components of the SWI/SNF complex, including Brg1 (or SMARCA2), ARID1A, and SNF2H (or SMARCA5), hampered embryo genome activation (EGA) and development. The expression of Brg1 around the EGA stage is correlated with the developmental potential of embryos (Glanzner et al., 2017).

Epigenetic modifications and early embryo development

Early embryo programming depends on epigenetic modifications, including DNA methylation and post-translational modifications of histones. Epigenetic changes are necessary for pronuclei formation, totipotency acquisition, oocyte-to-embryo transition, embryo genome activation, and cell lineage specification and differentiation. Although the paternal and maternal genomes are exposed to the same ooplasm environment, their epigenetic status is asymmetrically regulated, and this epigenetic asymmetry persists for several cell cycles. For example, while sperm DNA is rapidly and actively demethylated via the recruitment of Tet enzymes, the oocyte DNA is passively demethylated during subsequent cell cycles (Figure 1). DNA demethylation of both paternal and maternal genomes is necessary to ensure the naïve state of the bi-parental genome and acquisition of cell totipotency. The methyltransferases Ehmt2 and SETDB1, which regulate H3K9me2 and H3K9me3, respectively, participate in protecting the maternal pronucleus from active DNA demethylation (Zeng et al., 2019). In addition to DNA methylation, the paternal pronucleus has lower levels of the repressive epigenetic marks H3K9me2/3 and H3K27me2/3 than the maternal pronucleus. On the other hand, the paternal pronucleus has higher levels of the permissive mark H3K4me3 compared to the maternal pronucleus (Figure 1). H3K4me3 levels fluctuate during early embryo development and both the capacity to methylate and demethylate H3K4 is necessary for EGA and normal embryo development. In pig embryos, attenuation of the H3K4 demethylases, KDM5B and KDM5C, which are transiently expressed around the EGA stage (Glanzner et al., 2018), affected EGA and DNA damage repair, and decreased embryo development (Glanzner et al., 2020) (Figure 2).

Normal embryo development also requires proper regulation of H3K36me3, which is a permissive epigenetic mark that is commonly co-localized with RNA polymerase II in gene bodies and regulate transcriptional elongation. Depletion of Setd2, a methyltransferase of H3K36me3, led to aberrant DNA methylation, replacement of H3K36me3 by H3K4me3 and H3K27me3 in gene sequences, abnormal H3K4me3 acquisition in imprinting sequences, and abnormal oocyte maturation and embryo development (Xu et al., 2019).

Embryo programming is also regulated by the repressive epigenetic mark H3K27me. Although H3K27me3 and H3K4me3 have opposite roles in transcriptional regulation, they co-exist in some genes that require tight regulation, such as quick activation for cellular differentiation. Following fertilization, H3K27me3 decreases in the promoter regions of developmental genes, in both paternal and maternal alleles, however, maternal alleles inherit distal H3K27me3, a difference that persists until the blastocyst stage (Zheng et al., 2016). The decrease in H3K27me3 around the EGA stage may be necessary for derepressing pluripotency genes, which are inactive during gametogenesis. Impaired EGA and embryo development was observed in response to the attenuation of the H3K27me3 demethylase KDM6B (Chung et al., 2017). Attenuation of KDM7A, another H3K27me3 demethylase, decreased embryo development and perturbed cell differentiation (Rissi et al., 2019) (Figure 2).

Another repressive epigenetic mark having important impact on the regulation of the early embryo program is H3K9me3. This epigenetic mark participates in the regulation of long terminal repeats (LTR) and DNA damage repair, through the formation of a transitional repressive heterochromatin, and it is important for the preservation of genome stability in embryos. The function of Suv39h1/b2, which are methyltransferases of H3K9me3, is necessary to maintain embryo genome stability (Peters et al., 2001). Attenuation of KDM4C, a demethylase of H3K9me3, resulted in embryo developmental arrest at the morula stage (Wang et al., 2010) (Figure 2).

Transcription factors and early embryo development

Changes in chromatin structure and epigenetic marks can either facilitate or restrict the access of transcription factors (TFs) to regulatory elements of genes involved in the coordination of early embryo development. There is evidence from studies with bovine embryos indicating that open chromatin regions are enriched for maternal TFs binding elements before EGA, while binding sites for homeobox TFs that regulate
embryo transcription become more common at the EGA stage (Halstead et al., 2020). Several TFs involved in the EGA have been identified, which include the transcription intermediary factor 1-α (TIF1α), the nuclear transcription factor Y (NFY), the DUX family (e.g., the mouse Dux and the human DUX4), the maternal factors developmental pluripotency-associated 2, 3 and 4 (Dppa2-3-4), which bind to the Dux promoter, and the Zinc finger and SCAN domain-containing protein 4 (ZSCAN4). Depletion of TIF1α led to aberrant localization of RNA polymerase II and embryo development arrest (Torres-Padilla and Zernicka-Goetz, 2006). Inactivation of either Dppa2 or Dppa3 reduced EGA transcripts and decreased embryo development and quality (Eckersley-Maslin et al., 2019). In addition, Dppa2 and Dppa4 were shown to promote a permissive epigenetic pattern by preventing DNA methylation and facilitating H3K4me3. ZSCAN4 is implicated in EGA through its interactions with Dppa2, Dppa4, and Dux (Eckersley-Maslin et al., 2019). ZSCAN4 is also involved in the regulation of DNA damage repair in embryos and its attenuation decreased blastocyst formation (Takahashi et al., 2019; Srinivasan et al., 2020).

Ubiquitination and early embryo development

Normal embryo programming is also regulated by protein modifications induced by the ubiquitin system. Ubiquitin is a small protein that binds to other proteins, which is referred to as ubiquitination, and it modulates target proteins degradation, function, or localization. The enzymes that drive the ubiquitination process are classified as E1 or ubiquitin-activating enzymes, E2 or ubiquitin-conjugating enzymes, and E3 or ubiquitin ligases. Another family of proteins with similar functions are the small ubiquitin-like modifiers (SUMO). Ubiquitination participates in the regulation of several processes during early development such as fertilization, degradation of maternally derived proteins during EGA transition, DNA damage repair, histone methylation, and genomic imprinting. For example, the ubiquitin ligase NEDL2 is important for sperm decondensation after fertilization (Mao et al., 2021). In addition, SUMO proteins (e.g., SUMO-1, SUMO2) are involved in the regulation of development, epigenetic modifications, expression of pluripotency-related genes, and genome stability in embryos (Liu et al., 2020).
Transposable elements and early embryo development

Transposable elements (TEs) are also important regulators of cell functions during early embryo development. TEs are mobile DNA sequences that can replicate and insert in different sites of the genome and are grouped according to their mechanism of action as retrotransposons or class I, and DNA transposons or class II. The class I TEs are further classified, based on the presence or not of long terminal repeats (LTR) in both extremities, as LTR or non-LTR. Retrotransposons play more relevant roles than transposons on transcriptional regulation during embryo development. This includes endogenous retrovirus (ERVs), which belong to the LTR group, and the long (LINEs) and short (SINEs) interspersed nuclear elements, which belong to the non-LTR group. ERVs are the most important TEs and are regulated by epigenetic changes (e.g., DNA methylation, histone modifications), with the participation of DNA methyltransferases, TET proteins, lysine methyltransferases, and lysine demethylases. ERVs affect gene regulation by inserting into the genome near gene sequences and by using their LTRs promoters to regulate transcription and alternative splicing. LINEs and SINEs also participate in transcription regulation during embryo development and both account for approximately 30% of the human genome (Elbarbary et al., 2016). TEs affect embryo development by interacting with TFs and regulating gene expression and EGA (Fu et al., 2019). Examples of TFs involved in EGA that are known to interact with TEs include Dux, Dppa2, Dppa4, and Zscan4 (Hendrickson et al., 2017; Eckersley-Maslin et al., 2019).

Genome damage/stability and early embryo development

Genome damage, repair, and stability are critical for normal embryo development. Genome damage can be caused by endogenous and exogenous genotoxic factors, and may involve DNA strand breaks, collapsed DNA replication forks, and damages to histones and other DNA-binding proteins. To maintain genome stability, cells use complementary mechanisms involving DNA damage sensors, DNA damage repair, DNA replication, and cell cycle checkpoints, which may lead to cell recovery, cell adaption, or cell arrest/death. DNA damage
repair also involves replacement of histone variants, ubiquitination, and epigenetic modifications. DNA double-strand breaks (DSBs) are the most biologically significant genotoxic lesions with potential severe effects on genomic stability and cell survival. Possible consequences of DSBs include chromosome breakage and rearrangement, and mutagenesis, which may have severe consequences for cell physiology and development. In this regard, DNA mutations occurring at the beginning of embryonic life can be transmitted to tissues and germs cells, and consequently passed on to the offspring. Early developing embryos seem to repair DSBs by using mainly the homologous recombination (HR) pathway, which is error-free, as opposed to the nonhomologous end-joining (NHEJ) pathway, which is error-prone (Bohrer et al., 2018) (Figure 3). However, at earlier stages of development embryos seems less capable of regulating cell cycle checkpoints, which may facilitate the propagation of genetic errors. The incidence of DSBs was shown to alter embryo cell cleave kinetics and reduce development to the blastocyst stage (Bohrer et al., 2013). Oxidative stress and endoplasmic reticulum stress are associated with an increased incidence of DSBs during early embryo development (Dicks et al., 2020) (Figure 3).

**Understanding the Early Embryo Program Through Its Reprogramming**

Somatic cell nuclear transfer (SCNT) into enucleated oocytes was the pioneering method developed to investigate cell reprogramming. Lessons from SCNT studies pave the way for the creation of induced pluripotent stem cells (iPS). More recently, a sub-population of mouse totipotent cells resembling 2-cell stage embryos (2-cell like cells or 2-CLCs) was identified, and along with SCNT are helping with deciphering the mechanisms regulating early embryo development, cell totipotency, and differentiation. In SCNT, the embryo genome originates from a differentiated cell, and is reprogrammed back to a totipotent state, which enables examining embryo development mechanisms in a backward perspective.

Regarding the reprogramming of the chromatin structure, SCNT studies revealed that extensive reprogramming of core (e.g., H2A, H2A.Z, H3.3) and linker (somatic and oocyte-specific H1) histone variants are involved in the reacquisition of cell totipotency. However, the specific roles of each variant on chromatin remodeling, EGA, genome repair and stability, and cell redifferentiation have not been fully characterized. There is evidence indicating that the expression of sperm procamines in somatic cells prior to SCNT may improve cell reprogramming (Loi et al., 2021).

Studies using SCNT embryos have helped identifying several epigenetic marks involved in the regulation of embryo development and cell differentiation and reprogramming. There is evidence from different studies suggesting that SCNT embryos partially retain the transcriptional memory inherited from the donor cells, which may be associated with high levels of H3K4me2/3. Injection of mRNA for KDM5B, a demethylase of H3K4, into bovine SCNT embryos helped re-setting the transcription program in the developing embryos and improved blastocyst rates and quality (Zhou et al., 2020). Abnormal regulation of development in SCNT embryos was also correlated with impaired H3K27me3 reprogramming, and failure in H3K27me3 reestablishment was associated with placental defects (Matoba et al., 2018). Insufficient H3K27me3 reprogramming may also be associated with abnormal Xist expression and X chromosome inactivation in SCNT embryos. Lower expression of the H3K27me3 demethylase KDM6A was observed in SCNT embryos, and embryo development was improved by inducing its expression after SCNT. Persistent H3K9me3 was identified as a major epigenetic barrier for cell reprogramming and SCNT embryo development (Matoba et al., 2014). Both methyltransferases and demethylases of H3K9me3 participate in the regulation of cell reprogramming. It was observed that the demethylase of H3K9me3, KDM4B, was expressed at the 2-cell stage of mouse SCNT embryos that developed to the blastocyst stage, but it was not expressed in those that had an impaired development (Liu et al., 2016). Moreover, injection of mRNA for KDM4D, a demethylase of H3K9me3, into mouse SCNT embryos increased reprogramming efficiency and full-term development eight-fold compared to control SCNT embryos (Matoba et al., 2014). The effect of H3K9me3 as an epigenetic barrier for cell reprogramming seems to be conserved among species, since its attenuation also increased development of bovine, porcine, and non-human pri-mates SCNT embryos.

Genome damage repair and stability are also critical elements for successful cell reprogramming and development of SCNT embryos. Higher incidence of DSBs was observed in SCNT embryos, which had a negative correlation with embryo quality (Bohrer et al., 2013). Alleviation of oxidative stress and endoplasmic reticulum stress, which are known to increase DNA damage, improved cell viability, and development of SCNT embryos (Liang et al., 2017). In addition, treatment with inhibitors of histone deacetylases, which are commonly
used to improve cell reprogramming, enhanced DSBs repair in SCNT embryos (Bohrer et al., 2014), which further supports a link between epigenetic regulators, cell reprogramming, and genome stability in SCNT embryos.

Several transcription factors, including Dux, Dppa2, and Dppa4, have been identified as enhancers of EGA and development in mouse SCNT embryos. For example, induction of Dux expression enhanced EGA and development of SCNT embryos and improved derivation of iPSC cells (Yang et al., 2020a). In addition, injection of Dux miRNA prior to SCNT corrected abnormal levels of H3K9 acetylation in genome regions essential for EGA, thus improving gene activation (Yang et al., 2020b).

Abnormal activation of TEs involved in the regulation of EGA has been associated with abnormal development of SCNT embryos. For example, it has been found that LTRs and LINEs were among the major components of reprogramming resistant regions with high levels of H3K9me3, which were identified to affect EGA and development of mouse SCNT embryos (Matoba et al., 2014). In addition, overexpression of KDM6A, a demethylase of H3K27me3, induced the reactivation of a subset of ERVs in SCNT embryos and improved cell reprogramming efficiency (Yang et al., 2018). These findings provided solid evidence that TEs play critical roles in the reacquisition of cell totipotence and normal development in SCNT embryos.

Conditions Affecting Normal Embryo Programming

Given the complex molecular interactions involved in the proper coordination of early embryo development, it is not surprising that normal embryo programming is highly sensitive to its developmental milieu, including nutrients, metabolites, and environmental factors. This is particularly relevant for the use of ARTs requiring long in vitro culture and/or gamete/embryo manipulations (e.g., in vitro fertilization, sex-sorted sperm, intracytoplasmic sperm injection, gametes/embryo cryopreservation). For example, in vitro embryo culture conditions were associated with molecular and cellular alterations in preimplantation bovine embryos and fetal growth compared with embryos that developed in vivo (Lazzari et al., 2002). Moreover, culture with nutrients excess (e.g., glucose, lipids), or high oxygen tension, has important detrimental consequences on embryo metabolism, development, and viability (Sciorio and Smith, 2019; de Lima et al., 2020; Desmet et al., 2020).

Defective regulation of embryo programming may also emanate from deficiencies inherited in the gametes (e.g., immaturity, altered epigenetic marks, genome and organelle damages), which may result in embryos with decreased capacity to regulate cell functions and development, or having excessive metabolic demands required for the activation of stress coping mechanisms. In this regard, embryo viability has been associated with a less active metabolism, which is observed in embryos having less damages or superior repair capacity (Leese et al., 2007). In addition, embryo cleavage kinetics is affected by DNA damage (Bohrer et al., 2015), and both embryo metabolism and cleavage kinetics have been used to select embryos with superior capacity for postimplantation development (Sugimura et al., 2012). Therefore, providing an ideal development milieu is critical for the normal regulation of the embryo program.

Figure 4. Embryo regulation and outcomes. Proper embryo programming and normal development depend on several events affecting chromatin structure, integrity and function. Altered regulation end/or excessive stress or damages may cause embryo death or result in embryos having lower potential for development or carrying alterations having potential detrimental consequences for health and production. This figure was assembled using Biorender.
The establishment and proper regulation of the embryo development program depends on several conditions that include changes in the chromatin structure, epigenetic modifications, protein modifications, activation of transcription factors and retrotransposons, and genome repair and stability (Figure 4). Failures in the proper regulation of these events can result in either embryo development arrest and death or eventually embryo survival, but resulting in embryos having lower potential to continue developing, implanting, and producing a normal offspring, or even contribute to the creation and segregation of cell anomalies (Figure 4). Although many molecular mechanisms participating in the regulation of embryo development have been identified and partially characterized, particularly in mouse embryos, much remains to be accomplished for the complete elucidation of the embryo programming mechanisms in the different species. In this regard, studies investigating cell reprogramming from SCNT embryos and 2CLCs have been providing solid contributions. Better understanding the embryo programming will help to mitigate fertility issues, increase the efficiency of animal breeding program, animal production, and improve the success rate of embryo-based technologies.

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