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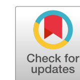
Review

The first cell fate decision in pre-implantation mouse embryos



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ABSTRACT

Fertilization happens when sperm and oocytes meet, which is a complicated process involving many important types of biological activation. Beginning in the 2-cell stage, an important event referred to as zygotic genome activation (ZGA) occurs, which governs the subsequent development of the embryo. In ZGA, multiple epigenetic modifications are involved and critical for pre-implantation development. These changes occur after ZGA, resulting in blastomeres segregate into two different lineages. Some blastomeres develop into the inner cell mass (ICM), and others develop into the trophectoderm (TE), which is considered the first cell fate decision. How this process is initiated and the exact molecular mechanisms involved are fascinating questions that remain to be answered. In this review, we introduce some possible developmental models of the first cell fate decision and discuss the signalling pathways and transcriptional networks regulating this process.
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1. Introduction

Fertilization is initiated when oocytes interact with sperm in the female oviduct [1]. This process is accompanied by various genetic and epigenetic modifications. After fertilization, the maternal mRNA and proteins are gradually degraded (Fig. 1A), and the zygotic genome begins to undergo activation in the 2-cell stage, which is termed zygotic genome activation (ZGA) [2]. During the process of ZGA, there are many important events, including chromatin remodelling, DNA demethylation, etc [3]. The embryo is totipotent at the 2-cell stage and exhibits cell plasticity from the 4-cell stage onward. After several cell divisions, the embryos begin to undergo compaction (Fig. 1B), and the cell fates of blastomeres begin to segregate to inner cell mass (ICM) or trophectoderm (TE) fate, which is referred to as the first cell fate decision. The ICM subsequently develops into a fetus, whereas the TE develops into the supporting placenta. It is very important to study the mechanism of the first cell fate decision to guarantee correct embryogenesis. Many elements, such as cell polarity [4], cell position [5,6], mechanical forces [7–9], and metabolism [10], affect the first cell fate decision.

In this review, we introduce developmental models of mouse early embryos and the most recent advances in the understanding of the first cell fate decision.

2. Epigenetic modification in pre-implantation development

Mammalian chromatin organization undergoes severe reprogramming after fertilization. Oocytes in the MII phase show homogeneous chromatin lacking topologically associating domains (TADs) and compartments, whereas the higher-order structure of chromatin decreases after fertilization [11]. Parental chromosomes are spatially separated and display distinct compartmentalization until the 8-cell stage. A non-canonical H3K4me3 mark (ncH3K4me3) is found in mature oocytes, restricted to Cp-G-rich regions of promoters. ncH3K4me3 is erased, and canonical H3K4me3 is established in the late 2-cell stage when ZGA begins [12]. Despite extensive parental asymmetry in DNA methylomes, chromatin accessibility between the parental genomes is globally comparable after ZGA [13]. ZGA is a critical process for new life in which epigenetic modifications were poorly understood until recently. H3 lysine methylation (H3K4me3) and acetylation (H3K27ac) in mouse immature and MII oocytes and 2-cell and 8-cell embryos were profiled by a μChip-seq method [14]. In another breakthrough, H3K4me3 and H3K27me3 in mouse pre-

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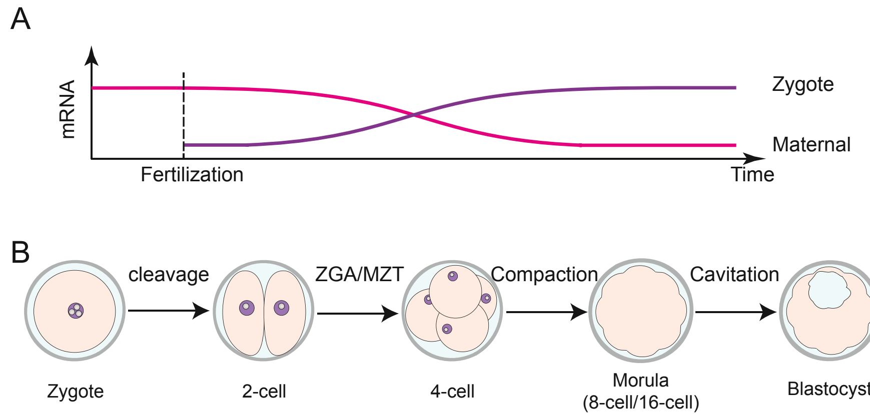


Fig. 1. Fertilization and Zygotic Genome Activation (A) Dynamics of mRNA in the fertilized zygote. After fertilization, the maternal mRNA is gradually degraded until the 2-cell stage, when the mRNA of the zygote is activated (B) The first important event after fertilization is the development pattern of the embryo during the transition from maternal to zygotic expression beginning at the 2-cell stage; this process is termed the maternal–zygotic transition (MZT) or zygotic genome activation (ZGA). Thereafter, the compaction of the embryos and formation of the blastocoel indicate the initiation of the first cell fate decision.

implantation embryos, associated with gene activation and repression, respectively, were mapped via a small-scale chromatin immunoprecipitation Chip-seq method [15]. A low-input DNase I sequencing (liDNase-seq) method was utilized to profile the chromatin regulatory landscape in mouse early development, and of DNase I-hypersensitive sites (DHSs) were generated from the 1-cell to morula stages [16].

3. Developmental models of the first cell fate decision

A fertilized zygote is totipotent and can develop into an individual offspring and its supporting ex-embryonic placenta. After several cell divisions, the blastomeres acquire different cell fates to undergo specific organogenesis [17,18]. In 1967, Tarkowski and Wroblewska hypothesized the “outside - inside model” to explain the first cell fate decision (Fig. 2A). These authors thought that outer and inner blastomeres had different destinies in subsequent development. This hypothesis highlights the importance of cell position, indicating that the inner cells of the morula tend to develop into the ICM, while the outer cells develop into the TE [5]. In 1981, Johnson and Ziomek hypothesized the “polarity model”, suggesting that asymmetric cell division causes blastomeres to acquire polarity [19]. There is a spatial pattern of blastocyst formation. In the first cleavage, the 1-cell zygote divides meridionally into two similar daughter cells. However, there are two orientations in the second cleavage (Fig. 2B): meridional (M, along the animal–vegetal axis) and equatorial (E, perpendicularly to the animal–vegetal axis) [20]. Usually, 2-cell embryos first cleave in the

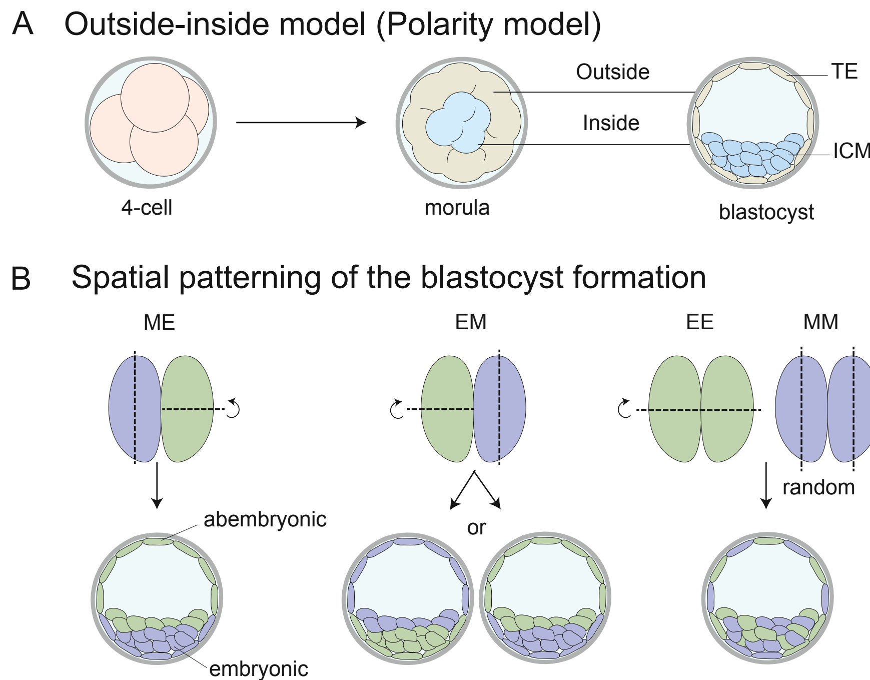


Fig. 2. Developmental Models of Cell Fate Decision and Spatial Patterning (A) “Inside-outside” model (“polarity” model): inner cells and outer cells of the morula take on different cell destinies during cell fate decisions (B) Spatial patterning in blastocyst formation: There are two cleavage orientations (meridional (M) and equatorial (E)) in 2-cell stage embryos. If the blastomeres divide in different orders, the contribution of the blastomeres is distinct.

M orientation and then in the E orientation to form 4-cell embryos. If 2-cell embryo cleavage occurs in an ME order, blastomeres in the M orientation tend to develop into embryonic tissues, and those in the E orientation tend to develop into abembryonic tissues. EM orientation may also occur, resulting in the development of E orientation blastomeres developing into either embryonic or abembryonic tissues. However, if the embryos undergo an MM or EE orientation order, the blastomeres develop randomly into embryonic or abembryonic tissues [21,22].

With breakthroughs in molecular biology technologies, two new hypotheses that could explain the mechanism of the first cell fate decision have been put forth. One hypothesis is the “equivalence hypothesis”, indicating that all blastomeres in 2-cell and 4-cell embryos are homogeneous and that differences between them do not affect the cell fate decision [23–25]. After the 8-cell stage, two cell fates (ICM and TE) are determined through symmetrical and asymmetric division. The other hypothesis is the “asymmetric hypothesis” [26]. This hypothesis indicates that the distribution of components between daughter cells is asymmetrical during cleavage [4,27]. After several cell divisions, the difference between the blastomeres is increased. Finally, the difference causes the separation of cell fates.

4. Signalling pathways regulating ICM and TE formation

Hippo signalling is one of the major pathways regulating the first cell fate decision in mammals (Fig. 3A); this pathway used to be considered a tumour suppressor signalling pathway and is conserved in mice and humans[28,29]. In mouse blastocysts, the Hippo signalling pathway is inactive in trophoblast lineages but active in the ICM. The main components of this pathway regulating cell fate decisions are *Yap* and *Tead4*.[30] In *Tead4*-null embryos, expression of *Cdx2* is absent, and all blastomeres developed into the ICM, without any TE cells, suggesting that *Tead4* is essential for *Cdx2* activation.[31,32] In addition, the activation of Hippo signalling is mediated by the phosphorylation of *Yap*, which is active in the inner cells of the morula rather than the outer cells[33]. However, the transcriptional coactivator *Yap* is not phosphorylated in the TE, and Hippo signalling is repressed [34]. The unphosphorylated *Yap* is transported into the cell nucleus. There, *Yap* acts in coordination with *Tead4* to form the *Yap-Tead4* complex, which binds to the enhancer of *Cdx2* and *Gata3* to promote their expression. The expression of *Cdx2* and *Gata3* leads to TE specification during the cell fate decision[32,35]. Conversely, under the activated Hippo signalling pathway in the ICM, *Yap* is phosphorylated by *Nf2* and its downstream target LATS1/2 kinase (large tumour suppressor kinase 1/2), thereby preventing it from entering the nucleus to activate *Cdx2* and *Gata3*[36,37]. Another experiment showed that angiomotin is a key regulator determining that the hippo pathway is active in inner cells and inactive in outer cells of early embryos (Fig. 3A), which can regulate the localization of Yap and compensate for the absence of Lats1/2 kinases[38].

In addition to the Hippo signalling pathway, the Notch signalling pathway (Fig. 3A) is related to the first cell fate decision by regulating the expression of *Cdx2*[39]. The Notch signalling pathway is active in trophoblast cells. In this pathway, the Notch intracellular domain (*NICD*) can bind to recombination signal binding protein for immunoglobulin kappa J region (*RBPJ*) to form the *NICD-RBPJ* complex, which targets TE-specific genes[40]. Additionally, both the *Yap-Tead4* and *NICD-RBPJ* complexes bind to the enhancer of *Cdx2* to promote its transcription, thus causing making blastomeres develop a TE fate. The mechanism by which Notch signalling coordinates with Hippo signalling remains to be elucidated.

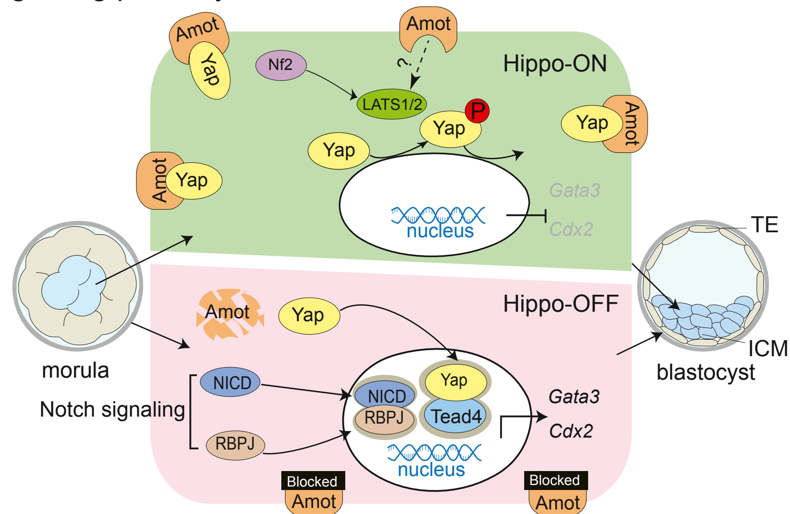
5. Transcriptional regulation in the ICM and TE

Both the ICM and TE exhibit specific transcription patterns, which are distinct from each other (Fig. 3B). The representative transcriptional factors in the ICM include *Oct4*, *Sox2*, *Sall4*, *etc*. In mice, *Sox2* can be detected throughout the oocyte maturation period. It is constantly expressed in the nucleus of each blastomere from the 2-cell stage to the 8-cell stage. Beginning in the 16-cell stage, *Sox2* is restricted to being expressed in the ICM lineage instead of the TE[41]. However, the lack of *Sox2* does not affect blastocyst formation but results in abnormalities in post-implantation embryos[42]. *Oct4* starts to be expressed in the 2-cell stage and remains uniformly distributed in each blastomere until the mid-blastula stage. In the late blastula stage, *Oct4* accumulates in the ICM. If Oct4 is deleted, the ICM cells are not pluripotent, which are restricted to differentiation trophoblast lineage[43]. Distinct from the ICM, the core transcriptional factors for the TE lineage are *Cdx2*, *Eomes*, *Elf5*, *Gata3*, *etc*. Nevertheless, there are some transcriptional factors that are expressed in both the ICM and TE. *Tead4* is expressed in both regions at the early blastula stage, but the expression level in the ICM is lower than that in the TE[33]. *Cdx2* is a specific marker gene of early development of the TE, which begins to be expressed at the 8-cell stage and is asymmetrically expressed in each blastomere of the morula[44]. In the early zygote, maternal *Cdx2* can be detected, but the exact role of *Cdx2* requires further investigation[45]. Moreover, the expression of *Cdx2* is coordinated with the expression of ICM marker genes, including *Oct4* and *Nanog*, and ultimately accumulates in the TE after the mid-blastula stage[46]. The *Cdx2*-null homozygous embryos can form blastocoel, but fails to implant[47]. The expression level of *Gata3* in 1-cell and 2-cell embryos is very low. Until the 4-cell stage, *Gata3* is consistently highly expressed in the blastocyst. At the blastocyst stage, the expression of *Gata3* is restricted to the TE lineage[48].

All the transcriptional factors function as a network, influencing and interacting with each other. Niwa et al found that overexpression of *Cdx2* in mESCs can inhibit the expression of *Oct4*, resulting in the conversion of mESCs to a TE cell fate. Likewise, the expression of *Oct4* can inhibit the expression of *Cdx2*. *Cdx2* can bind to the promoters of *Oct4* and *Nanog*, inhibiting the transcription of pluripotent genes and inducing the TE fate[49]. Zhang et al showed that the expression of *Cdx2* was upregulated via the deletion of *Sall4*. *Sall4* can also guarantee the expression of *Oct4*, maintaining the pluripotency of the ICM[50]. Recently, *Sox21*, a target gene of *Sox2* that is regulated by *Oct4*, is important in maintaining the cell fate of the ICM[41]. Overexpression of *Arid3a* can also induce TE cell fate[51].

6. Heterogeneous expression in the first cell fate decision

Although many studies have attempted to explain the mechanism regulating the first cell fate decision, it is still unknown whether this process supports the “equivalence hypothesis” or the “asymmetric hypothesis”. Beginning in the 8-cell and 16-cell morula stages, there are transcriptional and protein-level differences between blastomeres, including differences in *Oct4*[52], *Sox2* [53] and other specific critical factors (Fig. 4A). However, it is not clear precisely when the heterogeneity of blastomeres originates. In a previous lineage tracing study, after two daughter cells from the 2-cell stage divided out of sync, the descendants of them to divide exhibited distinct cell cycles, which demonstrated that the blastomeres in the 2-cell stage were not the same[54]. Another challenging study showed that each 4-cell blastomere has full developmental potentials, according to its spatial origin and fate of their progeny[55].



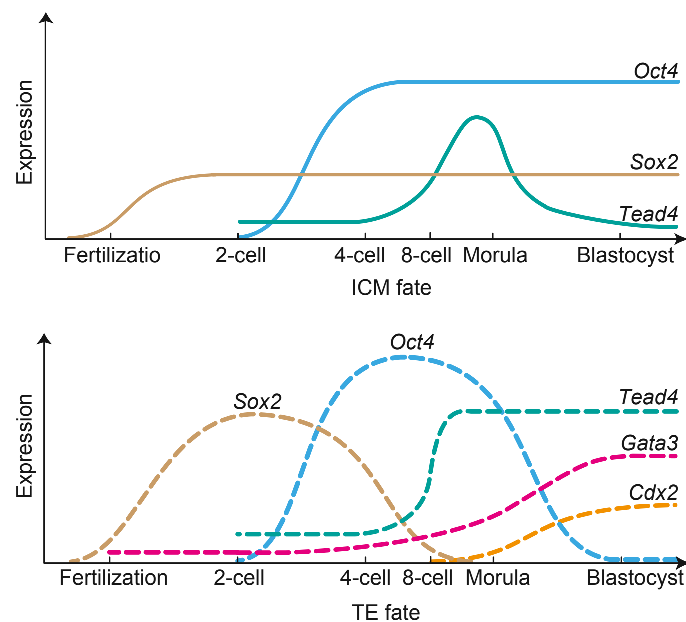


Fig. 3. Signalling and Transcriptional Regulation in ICM and TE Fates (A) Hippo pathway in the ICM and TE: Unphosphorylated *Yap* can be transported into the cell nucleus to bind with *TEAD4* to activate the expression of *Cdx2*. Phosphorylated *Yap* in the ICM remains outside of the cell nucleus, resulting in the expression of *Oct4*. The Notch pathway in the TE: *NICD* and *RBPJ* form a complex that activates the expression of *Cdx2*. Angiomotin (Amot) is a key factor regulating the activation of *Yap* (B) Expression patterns of specific genes in ICM and TE fate.

Single-cell RNA sequencing analysis revealed that heterogeneity of gene expression in early mouse embryos appears from the 2-cell stage to the 8-cell stage[56]. The heterogeneity of early mouse embryos induces many biological processes, such as cell division, gene expression and epigenetic modification. All of these types of heterogeneity affect the first cell fate decision in mouse preimplantation development. One important type of modification is the heterogeneity of histone H3 methylation. The heterogeneity of H3R17me and H3R26me in the 4-cell stage leads to the plasticity of early blastomeres. The hypermethylation of H3R17 and H3R26 facilitates the development of blastomeres into the ICM lineage[57]. Importantly, the heterogeneous expression of *Carm1* (a histone H3 modified regulatory enzyme) in the 4-cell stage also affects histone H3 methylation, regulating the first cell fate decision[57]. Additionally, some non-coding genes, such as *Neat1* and its partner *p54nrb*, can combine with *Carm1* to form a paraspeckle (Fig. 4B), specific nuclear architecture impacting proper lineage allocation and pre-implantation development[58]. Goolam et al proved that *Sox21*, a target gene of *Sox2*, showed a significantly heterogeneous pattern in the 4-cell stage. Interestingly, *Sox21* can also respond to the methylation of *Carm1*, which could lead the cell fate to the ICM lineage[41]. In summary, all of these studies have proven that the heterogeneity of histone methylation modifications and gene expression in the 4-cell stage plays an important role in the bias of the first cell fate decision.

Regarding the exploration of whether there is heterogeneity of expression before the 4-cell stage, Wang et al found that the long noncoding RNA *LincGET* played an important role in pre-implantation development[59]. When *LincGET* was deleted, the development of early mouse embryos arrested at the 2-cell stage. They also proved that *LincGET* was expressed heterogeneously between the two daughter cells at the 2-cell stage. *LincGET* was

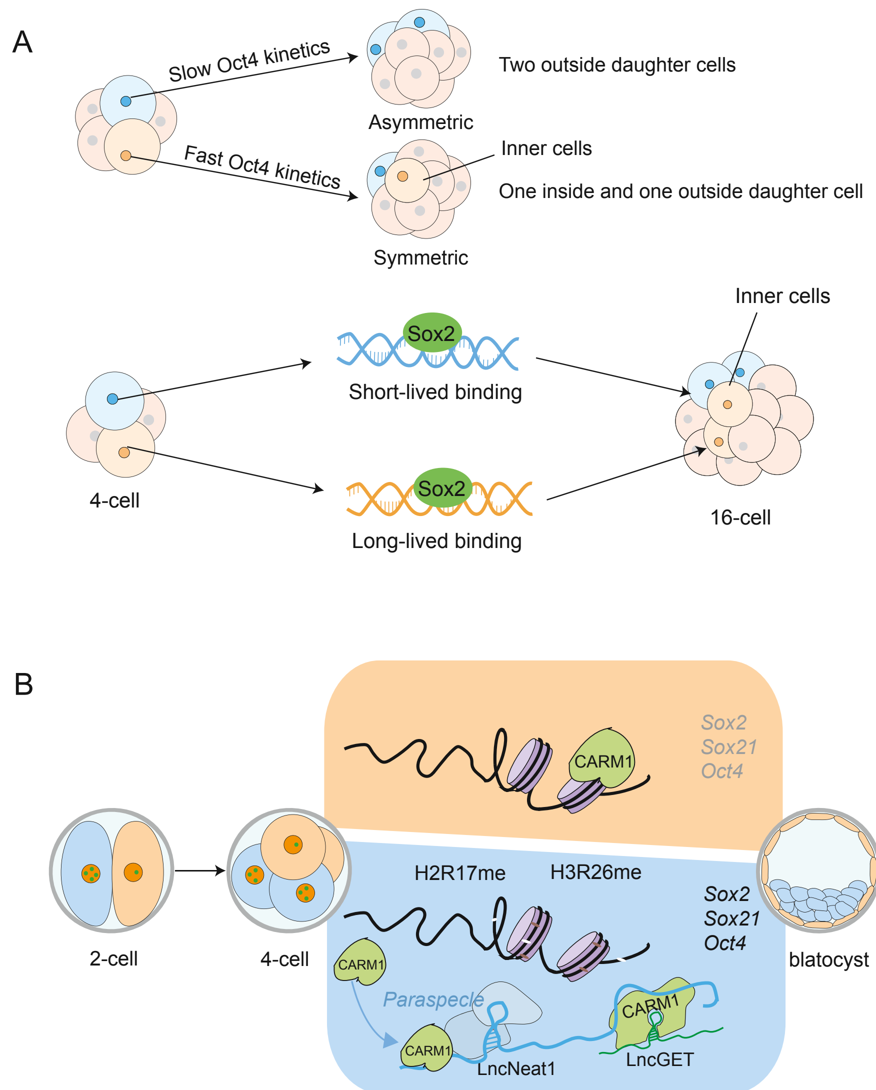


Fig. 4. Heterogeneous Expression in Early Blastomeres (A) *Oct4* shows distinct kinetics in blastomeres at the 4-cell stage, resulting in cell fate segregation. Additionally, Sox2 shows different binding windows and also affects cell fate decision (B) Heterogeneous expression in the 4-cell stage: CARM1 is heterogeneously expressed in the 4-cell stage, resulting in the upregulation of Oct4 and Sox21 (target gene of Sox2) and decision of the ICM. *LincGET* is heterogeneously expressed in the blastomeres of 2-cell embryos and binds to CARM1 and affects ICM formation. In addition, Neat1 combines with Carm1 to form a paraspeckle to regulate ICM/TE cell fates.

transiently expressed (2-cell and 4-cell stages) and asymmetrically distributed to every blastomere. *LincGET* and CARM1 formed a complex to increase chromatin accessibility, promoting H3R26me modifications and activating ICM-specific genes (Fig. 4B). As a result, *LincGET* guaranteed ICM gene expression, and bias toward the ICM lineage fate was observed when *LincGET* was overexpressed[60]. This is the first evidence that the first cell fate decision is related to the 2-cell stage. It also confirms that the heterogeneity of histone H3 methylation is critical for the first cell fate decision.

7. Perspectives

Spatial and temporal accuracy of early embryo development is essential for subsequent pregnancy and foetal development; thus, the first cell fate decision is a matter of widespread interest. The early “outside - inside” hypothesis suggested that the cell fate of blastomeres is segregated from the 8-cell and 16-cell morula stages because the different positions of blastomeres decide their distinct destinies. Later, the “polarity” hypothesis suggested that cell fate decision begins in the 4-cell stage because different cleavage orientations and orders cause blastomeres to contribute differently to embryonic and abembryonic tissues. A recent study showed that the critical noncoding RNA-*LincGET* is distributed asymmetrically to the two blastomeres at the 2-cell stage, which plays an important role in ICM fate specification[60]. Therefore, the first cell fate decision might be initiated in ZGA, an earlier stage after fertilization. Whether there are other important regulators that are differentially expressed in 2-cell blastomeres and related to the first cell fate segregation requires further investigation. In addition, these mechanisms that regulate the first cell decision may also occur in other species, and additional evidence will be required to address this possibility. In summary, exploring the exact mechanism underlying the first cell fate decision is beneficial for achieving a better understanding of embryogenesis patterns and early development.

Conflicts of interest

The authors declare that there is no conflicts of interest.

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