CURRENT OPINION

Transcriptional and epigenetic control of early life cell fate decisions

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Purpose of review
Global epigenetic reprogramming of the parental genomes after fertilization ensures the establishment of genome organization permissive for cell specialization and differentiation during development. In this review, we highlight selected, well-characterized relationships between epigenetic factors and transcriptional cell fate regulators during the initial stages of mouse development.

Recent findings
Blastomeres of the mouse embryo are characterized by atypical and dynamic histone modification arrangements, noncoding RNAs and DNA methylation profiles. Moreover, asymmetries in epigenomic patterning between embryonic cells arise as early as the first cleavage, with potentially instructive roles during the first lineage allocations in the mouse embryo. Although it is widely appreciated that transcription factors and developmental signaling pathways play a crucial role in cell fate specification at the onset of development, it is increasingly clear that their function is tightly connected to the underlying epigenetic status of the embryonic cells in which they act.

Summary
Findings on the interplay between genetic, epigenetic and environmental factors during reprogramming and differentiation in the embryo are crucial for understanding the molecular underpinnings of disease processes, particularly tumorigenesis, which is characterized by global epigenetic rewiring and progressive loss of cellular identity.

Keywords
cell fate, development, embryo, epigenetics, reprogramming, transcription

INTRODUCTION

Development starts at fertilization, when the sperm and egg fuse to create the zygote, which will, through subsequent cleavages and differentiation, give rise to all cells in the new organism. Following fertilization, the specialized and asymmetric epigenomic patterns of the maternal and paternal genomes are largely reset to provide a clean slate supporting the development of the new animal. Embryo-specific organization of the genome is then established with patterning gradually becoming more restricted and specialized, supporting lineage specification during embryogenesis. The first cell differentiation event during mouse development is the distinction of extraembryonic trophectoderm from the pluripotent inner cell mass (ICM) during the morula/blastocyst stage, an event primarily driven by developmental signaling pathways and transcriptional master regulators of the two cell fates.

Generally considered as equipotent, the cells of the early mouse embryo preceding lineage allocation nevertheless harbor some functional differences. In certain cases, these arise as early as two-cell stage of development, when blastomeres are considered totipotent (meaning that they can contribute to both embryonic and extraembryonic tissues). For instance, only a subset of mouse embryos contain two totipotent cells at the two-cell stage, while the majority constitute blastomere pairs in which only one of the blastomeres has the ability to singularly maintain development of a healthy blastocyst [1].

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KEY POINTS

- In the early mammalian embryo, global epigenetic reprogramming followed by establishment of epigenetic patterns influences the emergence of distinct cell lineages from undifferentiated blastomeres.
- Specification of cell identity during early development is guided by the interaction of transcriptional master regulators with epigenetic factors and chromatin organization.
- Noncanonical distribution of histone and DNA modifications, and asymmetries in epigenetic factor localization are a hallmark of mouse preimplantation blastomeres, with potential to instruct lineage allocation.
- The extent to which parentally inherited epigenomic differences contribute to early embryonic prepatterning and blastomere plasticity, and influence downstream development and differentiation remains to be elucidated.

In this review, we focus on the different gene-regulatory mechanisms influencing chromatin and genome function preceding the first cell differentiation events and discuss how their dynamics and asymmetries influence lineage decisions in the mouse embryo (Fig. 1).

TRANSCRIPTION FACTORS

Transcription factors (TFs) can bind DNA cis-regulatory elements in a sequence-specific manner and modulate transcriptional output of their target genes (reviewed in [2,3]). Recruitment and binding of transcription factors to their cognate sites can be facilitated by cooperative interactions among different transcription factors and by interactions with chromatin components (reviewed in [4]).

The first lineage segregation between the pluripotent ICM and the trophectoderm, which will give rise to the placenta, is guided by lineage-specific transcription factors, resulting from a polarization of the outer cells of the morula and a subsequent activation of the Hippo signaling pathway (reviewed in [5]). Mechanistically, this pathway results in the dephosphorylation of YAP1, allowing for its nuclear translocation where it acts as a co-activator for TEAD4, forming a complex that induces expression of Cdx2 and Gata3, transcriptional master regulators of the trophectoderm lineage [6,7]. The activation of the Hippo pathway leads to downregulation of the pluripotency factor SOX2 in trophectoderm precursors, a mechanism dependent on TEAD4 but not CDX2 [8]. CDX2 itself is dispensable for establishment of the trophectoderm but necessary for the maintenance of its function [9]. CDX2 can be co-expressed with OCT4, a core pluripotency transcription factor, in a cross-antagonistic manner with the transcription factors inhibiting each other's activity [10,11]. Despite Cdx2 expression, morula blastomeres retain a high level of plasticity until the 32-cell stage during which they can interconvert lineages [12]. However, shortly thereafter, cells expressing high CDX2 levels lose their ability to convert to the ICM [13].

After blastocyst formation, the ICM further segregates into the epiblast (Epi), which will give rise to the embryo proper and differentiate into the three germ layers, and the extraembryonic primitive endoderm (PrE), which will contribute to the yolk sac (reviewed in [5]). Initially co-expressed in the early ICM [14,15], the classic Epi specifier NANOG, and PrE-specific transcription factor GATA6 adopt a mutually exclusive 'salt-and-pepper' expression pattern around embryonic day (E) 3.5 [16]. Lack of either factor results in the loss of the cell lineage it specifies [17–20]. During the resolution of the ICM, there is an antagonistic relationship between NANOG and GATA6 [21,23]. Nevertheless, ICM plasticity is retained beyond the bifurcation of NANOG and GATA6 expression patterns, and cells can interconvert between Epi and PrE fates until E4.5 [22,23]. The PrE/Epi divergence is guided by differential Fibroblast Growth Factor (FGF) signaling and activation of the mitogen-activated protein kinase (MAPK) pathway, the action of which causes specification towards PrE [16,24,25]. Phosphorylation of MAPK-effector ERK triggers an initially reversible priming towards PrE through a redistribution of cofactors of the transcriptional machinery, leading to the suppression of pluripotency genes and allowing for the activation of PrE genes [26,27▪]. Expression patterns of MAPK signaling components in the early ICM are heterogeneous with Epi-precursors expressing FGF4 ligand and PrE-precursors expressing FGFR2 receptor [19,28,29]. Modulating the MAPK pathway in embryos shifts the PrE-to-Epi ratio, with Fgf4-null embryos unable to maintain Gata6 expression [25,30,31]. In addition to FGFR2, FGFR1 is expressed throughout the ICM, and its activity is involved in PrE specification, as well as allowing Epi cells to exit the earlier, naive pluripotent state and progress towards a later, primed state [32,33].

CHROMATIN MOBILITY AND LONG NON-CODING RNAS PRECEDING LINEAGE ALLOCATION

As transcription factors function within the chromatin context, it is logical to hypothesize that the

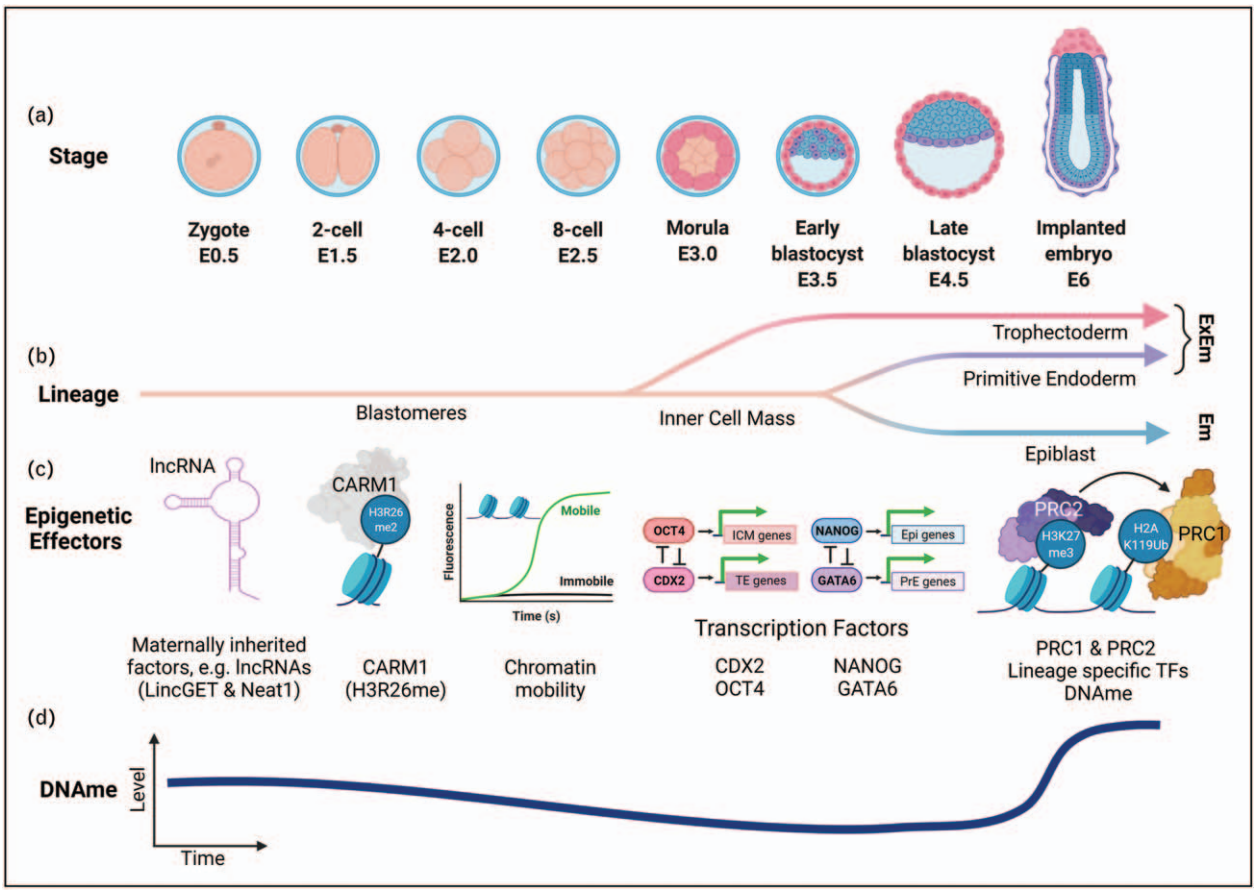

FIGURE 1. Epigenetic and transcription factors regulating cell fate allocation during mouse preimplantation development. (a) Stages of embryonic development from fertilization until implantation and (b) their respective lineage trajectories arising during early differentiation. At the morula stage, the blastomeres adopt either trophectoderm or ICM fate. The ICM subsequently differentiates into the PrE and Epi. ExEm stands for extraembryonic, Em stands for embryonic. (c) Effectors with ascribed instructive roles in the first cell fate decisions depicted below the corresponding developmental stage where they act. Initial heterogeneities are dependent on the distribution of maternally inherited factors, such as lncRNAs (zygote stage), which can impact the tethering of chromatin regulator CARM1 (two-cell stage). CARM1 is in turn associated with an increased level of pluripotency factor expression and chromatin mobility, and higher contribution of cells to the ICM (morula stage). Later, transcription factors ensure proper lineage segregation during the first (trophectoderm/ICM) and second (Epi/PrE) cell fate decisions. Around the time of implantation, DNA methylation (DNAme) and Polycomb Repressive Complexes (PRC) help guide lineage restriction. (d) Loss of DNA methylation levels during reprogramming occurs between the zygote and blastocyst stages, after which the DNA methylation levels are rapidly increased. Figure was made using Biorender.com.

interplay between genome organization and transcription factor action cumulatively contribute to cell plasticity and lineage allocation. In 2011, it was shown that the kinetics of OCT4 on chromatin in four-cell and eight-cell stage embryos differ between individual blastomeres and that differential OCT4 dynamics are predictive of lineage patterning and cell position within the embryo: cells displaying slower OCT4 kinetics are more likely to contribute to inner cells of the morula at compaction [34]. A follow-up study using photo-activatable fluorescence correlation spectroscopy in four-cell embryos found similar results for SOX2: blastomeres with long-lived SOX2 chromatin association contribute more readily towards the pluripotent lineage, in a manner regulated by H3R26 dimethylation [35]. This histone modification, deposited by arginine methyltransferase CARM1, is found to be naturally asymmetrically distributed between cells already at the four-cell stage, depending on the cleavage plane of the two-cell stage blastomeres. Lower levels of H3R26me2 in four-cell stage blastomeres are associated with a subsequent higher propensity of these cells to contribute to trophectoderm compared with ICM [36]. Conversely, increasing H3R26me2 levels through the overexpression of CARM1 in one of the

two-cell stage blastomeres leads to an upregulation of NANOG and SOX2 expression, as well as an increase in histone H3.1 mobility in its progeny [37], and results in higher contribution of these cells to the pluripotent ICM [36]. Presumably, higher accessibility of underlying DNA in ICM-destined cells, caused at least partly by faster histone exchange, facilitates longer and/or more stable association of pluripotency factors with embryonic chromatin.

Additionally, CARM1 has been reported to physically interact with PRDM14 and long non-coding (lnc) RNAs LincGET and Neat1, all of which have been proposed to anchor CARM1 to its cognate sites on chromatin [38,39▪,40▪]. LincGET itself is differentially expressed between the sister blastomeres already at the two-cell stage but only through interaction with CARM1 is it able to induce SOX2 and NANOG expression [40▪]. Similarly, it was found that depletion of Neat1 causes developmental arrest at the morula/early blastocyst stage, possibly due to increased expression of CDX2 [39▪]. Cumulatively, these data point to a dynamic interplay between different epigenetic players, transcription factor levels and underlying genomic context in guiding cell fate allocation during development.

CHROMATIN MODIFICATIONS IN THE EARLY EMBRYO

The first of two genome-wide waves of epigenetic reprogramming in the animal's life cycle takes place immediately after fertilization, with the presumptive aim of 'resetting' the chromatin landscape inherited from the highly specialized gametes. This establishes a clean slate of the embryonic epigenome preceding (and allowing for) cell differentiation. Below, we outline the best characterized chromatin modifications associated with regulation of embryogenesis and differentiation.

DNA methylation

DNA methylation occurs directly on the DNA molecule in a CpG dinucleotide context and is traditionally associated with transcriptional silencing (reviewed in [41]). Although overall stable in somatic tissues, DNA methylation patterns are globally reprogrammed following fertilization and during the specification of the germline.

In the early embryo, progressive loss of DNA methylation takes place, ultimately resulting in a hypomethylated genome at the blastocyst stage (Figure 1d) [42,43]. This occurs as a consequence of the absence of DNA methylation maintenance normally carried out by DNMT1 [42,43], as well as active removal through the action of Ten-eleven Translocation (TET) enzymes. In the zygote, the paternal genome is demethylated more rapidly than the maternal one, through the action of TET3 [44–48]. Maternal chromosomes are protected from this mechanism by STELLA/Dppa3, which recognizes H3K9me2, deposited during oogenesis [49]. This distinction is not clear-cut: TET3 has been reported to demethylate parts of the maternal genome, blurring the segregation of demethylation mechanisms between the parental genomes [50–52]. Although pervasive, it is important to note that DNA demethylation in preimplantation embryos is not absolute, with imprinting control regions and some transposable elements (in particular IAPs) escaping the reprogramming process [53]. From the blastocyst stage, DNA methylation levels increase through the action of de novo DNA methyltransferases DNMT3A and DNMT3B [54]. DNA methylation is dispensable for the formation of extra-embryonic lineages [55], consistent with the reported hypomethylated states in extraembryonic tissues and the higher expression levels of DNMT3A/B in the post-implantation epiblast [56]. Despite the differential requirements and levels of DNA methylation between cell types of the blastocyst, DNA methylation asymmetries in cleavage stage blastomeres have thus far not been implicated as early regulators of the first lineage decision event as they chiefly arise following cell fate allocation.

H3K27me3 and H2AK119Ub1

Polycomb repressive complexes 1 and 2 (PRC1&2) deposit histone modifications H2A monoubiquitylation (H2AUb1) and H3K27 trimethylation (H3K27me3), respectively, which correlate with repression of gene activity and the restriction of cell fate during development in various animal model organisms [57–61]. PRC1 can be recruited to chromatin by its interaction with H3K27me3, suggesting a temporal order of PRC function on chromatin (PRC2 precedes PRC1) [62,63]. However, during preimplantation development, an asymmetric distribution exists between H3K27me3 and H2AK119Ub1 across the genome [64▪▪,65▪▪,66]. After fertilization, global erasure of H3K27me3 and targeted depletion at promoter regions occur at the paternal and maternal genomes, respectively [66–68]. A gradual gain of H3K27me3 follows between the two-cell and morula-to-blastocyst transition and in the postimplantation epiblast [71], concomitantly with the initial cell fate specifications in the embryo. Genetic studies have revealed PRC2 to be dispensable during preimplantation development but essential at the onset of

gastrulation, when cells set a course towards distinct developmental trajectories [69,70]. Interestingly, PRC2 KO has almost no effect on H2AK119Ub1 distribution in the embryo, which is expected in a somatic context [64▪▪,65▪▪,71] after a near-complete loss of H3K27me3. Conversely, in embryos, PRC1 loss-of-function phenotypes are embryonic lethal, causing developmental arrest at the two-cell stage [75]. Recently, variants of PRC1 have been implicated in mediating the noncanonical pattern of H3K27me3. PRC1 variants can mediate the recruitment of PRC2 independently of preexisting H3K27me3. PRC2 can bind H2AK119Ub1, which in turn stimulates its catalytic activity and deposition of H3K27me3 (PRC1 precedes PRC2) [64▪▪,65▪▪,72]. Thus, contrary to the dogma, preimplantation embryos are characterized by a PRC1-mediated regulation of PRC2.

H3K4me3

H3K4me3 is deposited by MLL1 and MLL2 methyltransferases (reviewed in [57]), and generally associated with promoters of actively transcribed genes. In oocytes, H3K4me3 exhibits a noncanonical pattern, which is established gradually during oogenesis through the action of MLL2 [73–75]. These noncanonical domains are broad and abundant (covering promoters, intergenic regions, distal regions and transposable elements), and found on a subset of CpG islands, regardless of their transcriptional status [73,74,76▪]. After fertilization, the pattern of H3K4me3 inherited from the oocyte is reprogrammed through the action of histone demethylases KDM5A and KDM5B [73]. Disruptions of KDM5A/B cause defects in preimplantation development and aberrant resolution of noncanonical H3K4me3 patterning in a transcription-dependent manner [74]. The paternal genome acquires broad, weak regions of H3K4me3, which are replaced by a canonical H3K4me3 pattern at the two-cell stage [74]. Interestingly, H3K4me3 is found over transposable elements at the two-cell stage, which in turn correlates with their transient developmental expression [77]. Both H3K27me3 and H3K4me3 display noncanonical patterning in the oocyte, which is rapidly erased after fertilization. What role could these unique chromatin markings play during oogenesis and are they necessary for proper progression through the earliest developmental stages? The broad distribution of these histone post-translational modifications over large genomic regions argues against their role in fine-tuned regulation of specific genes they decorate and rather points to a more general function prior to transcriptional activation of the genome.

Interestingly, a subset of developmental promoters in the embryonic epiblast harbor both H3K4me3 and the seemingly antagonistic H3K27me3 histone mark. These genomic regions are termed bivalent. Bivalency has been proposed to function as a 'poising' mechanism, pausing genes in an inactive or lowly expressed state, while maintaining the potential for rapid activation upon developmental cues [78–80]. The embryo contains low levels of bivalent chromatin around implantation, which increases in the Epi at peri-implantation. Whether the acquisition and/or resolution of dually marked chromatin domains can play an instructive role in the first cell fate decisions or reflects the transcriptional status of different cell types in the blastocyst remains to be elucidated.

CONCLUSION AND OUTSTANDING QUESTIONS

Despite rapid and pervasive changes in genome organization and function, cell morphology and signaling pathways, early embryogenesis is an incredibly robust and concerted process resulting in the emergence of specialized cell lineages from the same DNA content. Following the principles of regulative development [81], the fate of the cleavage-stage mouse blastomeres is not predetermined by a gradient of maternally provided factors. Nevertheless, differences in chromatin markings, transcription factor dynamics and noncoding RNA species can be detected between cells as early as the two-cell stage. Here, we discussed some of the most-understood gene-regulatory factors influencing early cell fate decisions, and while many more are being continuously uncovered and characterized (such as RNA-binding proteins and metabolites), open questions remain. How are functional asymmetries established and propagated in the near-identical cells of early embryos, and do they play a role in lineage allocation? Are distinct epigenomic patterns between blastomeres a result of differences in local concentrations of epigenetic factors found already in the zygote? How prominent is the role of stochasticity and transcriptional noise in the eventual establishment of regulatory feedback loops and downstream signal amplification? When and how do heterogeneities at the transcription factor level become sufficiently stable to induce lineage allocation, and is chromatin organization instructive during this process? Does the simultaneous expression of different lineage-specifying transcription factors prolong the developmental time window before final lineage commitment? Finally, the extent to which internal and external signals (such as environmental stress or nutrient composition) have the ability to influence the embryonic epigenome and 'nudge' lineage allocation at the onset

of development remains poorly understood. With our increasing ability to molecularly probe early developmental events at unprecedented spatial and temporal resolution, these exciting biological questions will undoubtedly keep developmental biology aficionados busy in the coming years.

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Conflicts of interest

There are no conflicts of interest.

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- ■■ of outstanding interest

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This article together with reference [65■■] dissects the temporal dynamics and between PRC1 and PRC2 during oocyte growth and after fertilization. Although in oocytes, depletion of PRC1 subunits causes loss of H2AK119Ub1 and leaves H3K27me3 largely unaffected, in the preimplantation embryo there is a dependence of H3K27me3 on the preceding H2AK119Ub1. These results show that PRC1 functions in regulating PRC2 activity after fertilization.

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The authors deplete the catalytic subunit of PRC2, and show that consequent loss of H3K27me3 in the early embryo leaves H2AK119Ub1 mostly unaffected. Additionally, the acute loss of H2AK119Ub1 in the zygote leaves H3K27me3 unaffected until the four-cell stage where the embryos arrest, suggesting together with reference [64■■] that the PRC1-mediated patterning occurs in the oocyte before fertilization.

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