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Current Opinion in Genetics & Development

Genetic and epigenetic control of early mouse development

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A decade after cloning the sheep Dolly, the induction of pluripotency by transcription factors has further revolutionized the possibilities of reprogramming a cell's identity, with exciting prospects for personalized medicine. Establishing totipotency during natural reproduction remains, however, exceedingly more efficient than in reproductive cloning or in transcription factor-based reprogramming. Understanding the molecular mechanisms directing acquisition of totipotency during early embryogenesis may enable optimization of protocols for induced reprogramming. Recent studies in mouse embryonic stem cells (ESCs) show that self-renewal and pluripotency are efficiently maintained by a core set of transcription factors when intrinsic differentiation inducing signals are blocked. In early embryos, the specification of the pluripotent epiblast and two differentiating lineages (trophectoderm and primitive endoderm) is controlled by transcription factors that are regulated by autoactivating and reciprocal repressive mechanisms as well as by ERK-mediated signaling. Chromatin-based regulatory mechanisms also contribute to the identity of ESCs and early embryos. During gametogenesis, genomes undergo extensive epigenetic reprogramming. This may underlie the efficient acquisition of totipotency during subsequent preimplantation development.

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Current Opinion in Genetics & Development 2009, **19**:113–121

This review comes from a themed issue on
Chromosomes and expression mechanisms
Edited by Marie-Laure Caparros, Amanda Fisher and Matthias Merkenschlager

Available online 7th April 2009

0959-437X/$ – see front matter

DOI 10.1016/j.gde.2009.03.004

Introduction

Mammalian development starts by the fusion of two highly differentiated, transcriptionally silent germ cells, leading to the formation of the totipotent embryo. Embryonic stem cells (ESCs) are pluripotent cells that are derived from preimplantation embryos. In ESCs, cell identity is primarily regulated by transcription factors while chromatin-based repressive and activating modifiers serve transcriptional corepressor and coactivating functions. Transcription factors also drive lineage specification in early embryos. Genomes are, however, also subjected to major changes in chromatin states during the early phase of preimplantation development. It is widely thought that these changes enable acquisition of totipotency. The potential of such epigenetic reprogramming was illustrated by nuclear transfer experiments, where the epigenome of a fully differentiated somatic cell could be reprogrammed by the cytoplasm of an enucleated oocyte. The success rate of reproductive cloning is, however, very low compared to natural reproduction [1]. We therefore propose that resetting of epigenetic modifications on chromatin during germ cell development provides parental genomes the competence to effectively support changes in cell identity during early embryogenesis.

This review discusses the recent advances in our understanding of the molecular mechanisms underlying self-renewal and pluripotency in ESCs. We subsequently report on the specification of the first three cell lineages in the early embryo. We finally describe the dynamic changes in chromatin states occurring during early embryogenesis and discuss the possible contribution of transgenerational inheritance of epigenetic information from germ cells to the early stages of development.

Ground state for ESC self-renewal

The three transcription factors Oct3/4, Sox2, and Nanog are considered master regulators of pluripotency *in vivo* and *in vitro* [2–6] and share many target genes [7,8]. Nanog is required *in vivo* for the formation of the epiblast [3] and later in development for the maturation of primordial germ cells (PGCs) [9••]. It is, however, not necessary for self-renewal of ESCs, though low levels predispose toward differentiation [9••]. Since genomes are subjected to extensive epigenetic reprogramming during epiblast and PGC development, Nanog may be involved in such reprogramming processes [9••]. Stella and Pecam1 also show variable expression in ESCs that correlate with developmental potential [10•]. This cell-to-cell heterogeneity reflects a characteristic of mouse ESCs: the balance between pluripotency and being primed for differentiation.

Mouse ESCs are commonly cultured in media containing the cytokine leukemia inhibitory factor (LIF) and either serum or bone morphogenetic protein (BMP). LIF activates STAT3 which promotes self-renewal and serum/BMP induces proteins that inhibit differentiation. LIF/

gp130 signaling, however, is not required for inner cell mass (ICM) formation in developing blastocysts. Smith and colleagues showed that the propensity of ESCs for differentiation is a cell-intrinsic feature [11••]. It involves activation of the mitogen-activated protein kinases (Erk1/2) in response to autocrine signaling via the fibroblast growth factor-4 (Fgf4) pathway [12]. Accordingly, in absence of LIF and BMP, inhibition of ERK signaling is sufficient to suppress innate differentiation stimuli. Additional inhibition of glycogen synthase kinase-3 (GSK3) further stabilizes self-renewal [11••]. These data indicate that ESCs have a 'ground-state' program for self-renewal that does not require extrinsic signals if effectively protected from intrinsic differentiation stimuli [11••].

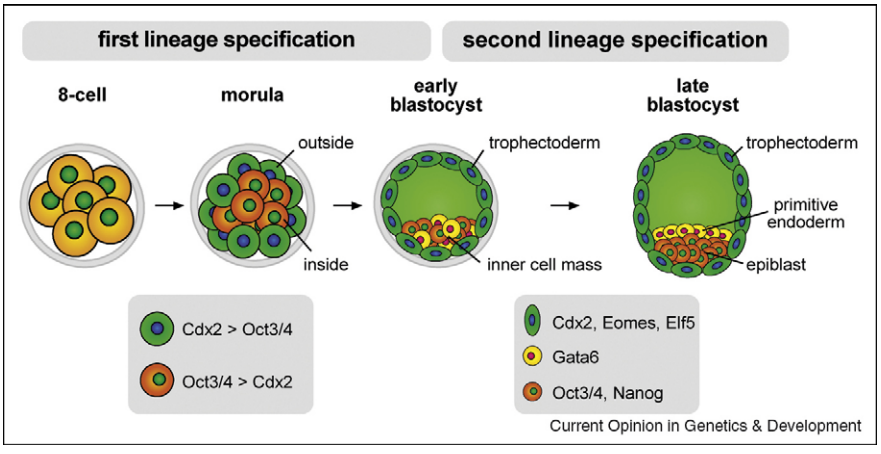
Chromatin regulators of the pluripotent ground state in ESCs

Polycomb group (PcG) proteins are evolutionarily conserved chromatin factors regulating cell identity by mediating gene repression. In mammals, they function in at least two different multiprotein complexes, called Polycomb Repressive Complex (PRC) 1 and 2. These complexes typically repress genes with key regulatory functions in development and differentiation. In ESCs, Oct3/4, Sox2, and Nanog share many targets with various PRC1 and PRC2 components [13]. Deficiency in the PRC2 components *Ezh2*, *Eed*, and *Suz12* does not, however, affect self-renewal [14–16]. In contrast, double-deficiency in the PRC1 paralogs, *Rnf2* and *Ring1a*, causes a loss of ESC identity. Repression of a substantial number of PRC1 target genes is dependent on *Oct3/4*, suggesting that PRC1 functions downstream of master ESC regulators [17••]. Importantly, regulators of DNA methylation are not required for the maintenance of ESC self-renewal [18]. The H3K9me2 and H3K9me3 demethylases, *Jmjd1a* and *Jmjd2c*, control self-renewal of ESCs. Both genes are positively regulated by Oct3/4 and are required for maintaining expression of the pluripotency genes *Tcl1* and *Nanog*, by inhibiting H3K9 methylation [5,19•].

Transcriptional networks underlying lineage specification in early embryos

The first lineage decision during mouse development leads to the establishment of the trophectoderm (TE) and ICM (see Figure 1 for details). It starts at the eight-cell stage with polarization of blastomeres and compaction of the embryo. Caudal-related homeobox 2 (Cdx2) and Oct3/4 are major transcription factors that regulate the transcriptional programs underlying TE and ICM cell identity. Expression of Cdx2 protein starts in eight-cell blastomeres and is gradually upregulated in future TE cells located toward the exterior of the embryo before blastocyst formation (Figure 1) [20•]. Interestingly, Oct3/4 and Cdx2 are coexpressed in cells until the blastocyst stage, when Oct3/4 levels decrease [20•,21••,22•]. In ESCs, forced repression of Oct3/4 or induced Cdx2 expression induces differentiation along the TE lineage

Figure 1



Lineage specification in early embryos. Lineage specification starts at embryonic day 2.5 (E2.5) in eight-cell embryos undergoing compaction and polarization [60]. Initially, the eight blastomeres show overlapping expression of the Oct3/4, Tead4, and Cdx2 transcription factors. Subsequent asymmetric and symmetric cleavage divisions result in outside-localized and inside-localized blastomeres that express, on average, higher levels of either Cdx2 or Oct3/4. At the early blastocyst stage (E3.5), three distinct cell types exist that give rise to separate lineages in E4.5 late blastocysts and later development. Trophectodermal cells, expressing Cdx2, generate all trophoblast cell types present in the fetal placenta. Within the inner cell mass, Oct3/4 and Nanog positive epiblast cells are pluripotent and give rise to the entire fetus and extra-embryonic mesoderm, whereas Gata6 positive primitive endodermal cells form the extra-embryonic endoderm layers of visceral and parietal yolk sacs. Embryo reconstruction experiments have shown that outer and inner blastomeres of the 16-cell embryo are still totipotent [60,61]. Nevertheless, depending on the cleavage direction at the four-cell stage, a bias in developmental fate toward the embryonic or extra-embryonic lineage was reported [62] that correlates with levels of histone H3 methylation at arginine 26 [63]. Thus, although specification likely starts early, developmental fate becomes only fixed late at the onset of blastocoel formation [61].