Origin of cellular asymmetries in the pre-implantation mouse embryo: a hypothesis

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The first cell fate decision during mouse development concerns whether a blastomere will contribute to the inner cell mass (ICM; which gives rise to the embryo proper) or to trophectoderm (TE; which gives rise to the placenta). The position of a cell within an 8- to 16-cell-stage embryo correlates with its future fate, with outer cells contributing to TE and inner cells to the ICM. It remains unknown, however, whether an earlier pre-pattern exists. Here, we propose a hypothesis that could account for generation of such a pre-pattern and which is based on epigenetic asymmetry (such as in histone or DNA methylation) between maternal and paternal genomes in the zygote.

1. Introduction: the first cell fate decision

The first cell fate specification during mouse development relates to the decision whether a blastomere will contribute to the inner cell mass (ICM) or trophectoderm (TE). The precise timing of and mechanism underlying this decision are fundamental aspects of early development, but they remain unknown. Asymmetric cell division that occurs at the 8- to 16-cell stage inevitably generates two types of cell: polar cells located outside of the embryo proper and apolar cells located inside. Outer polar cells eventually become TE, whereas inner apolar cells will contribute to the ICM. Polar and apolar cells have intrinsic differences that establish the outer–inner configuration [1,2]. The position of a cell within the embryo therefore determines its future fate. An important question then arises as to whether the mouse embryo is pre-patterned or not before the position-dependent cell specification that occurs after compaction. If no earlier pre-patterning exists, then the first cell fate decision would be based solely on the differential positioning of cells on the inside or outside of the embryo at the 8- to 16-cell stage [2,3]. Theoretically, symmetry breaking can be initiated without a pre-patterning molecule by stochastic disturbance of a self-organizing system, as previously proposed [4]. Alternatively, recent studies suggest the possibility that an earlier pre-pattern may indeed exist in the mouse embryo [5]. *Drosophila* has been found to have a pre-pattern in oocytes and zygotes. Several molecules that act as maternal determinants of the future body axis (for example, *bicoid* mRNA) are thus localized asymmetrically in both oocytes and fertilized embryos of *Drosophila* [6]. No such molecule has been identified in the mouse oocyte or embryo, although certain molecular and structural asymmetries have been described in unfertilized mouse oocytes. For example, Par4 protein is asymmetrically localized in mouse oocytes [7], but whether this protein is unevenly distributed among blastomeres after fertilization remains unknown. In addition, transcriptome asymmetry in terms of mRNA regionalization within mouse oocytes and zygotes has been identified, but no such asymmetry was detected between blastomeres within 2- or 3-cell-stage embryos [8]. However, several recent studies suggest molecular differences exist between blastomeres of the mouse embryo as early as the 4-cell stage. These molecular heterogeneities include the level of H3R17me2 and H3R26me2 (dimethylation of histone H3 at arginines 17 and 26, respectively) [9] and the level of *Cdx2* expression [10]. The notion that differences between blastomeres arise as early as the 4-cell stage is supported by cell fate studies. For example, the orientation and the order of cell division from the 2- to the 4-cell stage influence the fate of a blastomere [11]. Furthermore, genetic labelling of cells at the 4-cell stage by the rainbow technique shows the presence of
individuals that will contribute either to ICM or TE, in a significant number of embryos [12].

Given that the molecular mechanism for the position-dependent cell specification in mouse embryos has been extensively studied and discussed elsewhere, we address, in this paper, the origin of pre-patterning, based on the assumption that the mouse embryo is indeed pre-patterned before the position-dependent cell specification (while a growing number of studies suggest the presence of pre-pattern in the mouse embryo, one cannot completely exclude an alternative possibility. Therefore, in this paper, we would like to maintain that the presence of pre-pattern in the mouse embryo is a strong possibility).

2. Epigenetic differences among early blastomeres: a sign of a pre-pattern?

Two recent studies have revealed epigenetic differences among blastomeres as early as the 4-cell stage, suggesting that the onset of the first cell fate decision may take place earlier than the 8- to 16-cell stage. First, global levels of H3R17me2 and H3R26me2 were found to differ among blastomeres of 4-cell-stage embryos that have undergone tetrahedral division patterns [9]. Those cells showing higher levels of H3R17me2 and H3R26me2 preferentially contributed to the ICM, whereas those with lower levels were biased to become TE. When an expression vector for Carm1 (the enzyme that mediates the transfer of methyl groups to arginine residues, including H3R17 and H3R26) was injected into one blastomere of a 2-cell embryo, the injected cell exhibited upregulation of Nanog and Sox2 expression and preferentially contributed to the ICM [13].

Another study reporting an epigenetic difference among blastomeres was concerned with the behaviour of Oct3/4, a key transcription factor required for pluripotency [14]. The intracellular behaviour of Oct3/4 was examined by monitoring the kinetics of nuclear export, import, and retention of a green fluorescent protein (GFP):Oct3/4 fusion protein in embryos at the 4- and 8-cell stages. Two types of Oct3/4 behaviour were observed, probably reflecting differences in the access of this transcription factor to its binding sites in the genome. One type of behaviour was characterized by low rates of nuclear import and export as well as by immobility of Oct3/4 (presumably as a result of Oct3/4 being tightly bound to its target sites). Cells that manifested such behaviour underwent asymmetric cell division, generating inside cells and outside cells, and they preferentially contributed to the ICM and TE. The other type of behaviour was characterized by high Oct3/4 mobility (Oct3/4 is presumably not bound to its target sites in these cells). Cells manifesting this pattern of behaviour underwent symmetric cell division, generating only outside cells, and they showed a biased contribution to TE.

Several important questions remain to be addressed. For example, are these two epigenetic differences—in the levels of H3R17me2 and H3R26me2 at the 4-cell stage and in Oct3/4 behaviour at the 4- and 8-cell stages—related to each other? How might low Oct3/4 mobility lead to asymmetric division? When and how is differential histone modification (H3R17me2 and H3R26me2) generated among blastomeres? If Carm1 is the only enzyme that methylates arginine residues, we need to know its expression pattern and distribution in oocytes, zygotes and cleavage-stage embryos. Zygotic Carm1 mutants develop normally until late embryonic stages [15]. However, Carm1 may function as a maternal factor, so it will be interesting to characterize embryos of maternal Carm1 knockout mice. Some of these questions can be addressed with current technology.

3. Epigenetic asymmetries between maternal and paternal genomes

During gametogenesis and preimplantation development, epigenetic asymmetry is apparent between maternal and paternal genomes. Sperm DNA is tightly associated with protamines, but on fertilization, these are replaced by histones that are acetylated, but not methylated, at H3K9 or H3K27. By contrast, DNA and associated histones (such as H3) in the oocyte are highly methylated. Histone methyltransferase activity present in oocytes is responsible for H3 methylation in these cells. On fertilization, the maternal genome exists in a nucleosomal configuration with histone methylation inherited from the oocyte.

Differential histone modification between parental genomes in the preimplantation embryo includes mono-methylation of histone H3 at lysine 9 (H3K9me) and H3 trimethylation at lysine 27 (H3K27me3). Given that the maternal genome of the oocyte contains histone H3 methylated at K9, whereas the paternal genome of the sperm does not [16,17], fertilization gives rise to an asymmetry in H3K9me between parental genomes, and this asymmetry persists at the 2-cell stage. De novo methylation of H3K9 occurs in both parental genomes at the 4-cell stage, however, which erases the asymmetry in H3K9me by the 8-cell stage. As described above, the level of H3R26me2 also exhibits asymmetry among blastomeres of the 4-cell-stage embryo, being high in those blastomeres fated to contribute to the ICM but low in those fated to become mural TE [9]. The origin of this asymmetry in H3R26me2 is unknown, however. It may be generated by differential de novo methylation or demethylation after fertilization, or it may hark back to differential methylation between parental genomes.

Parental genomes also show different levels of DNA methylation [17,18]. The paternal genome in the male pronucleus undergoes rapid demethylation after fertilization, with DNA methylation being largely lost before the first mitosis. This active demethylation is mediated by Tet3, a dioxygenase that converts 5-methylcytosine to 5-hydroxymethylcytosine and which is present specifically in the male pronucleus [19]. By contrast, the maternal genome in the female pronucleus remains highly methylated until cleavage stages, when methylation is passively lost over the entire genome. This loss of DNA methylation is followed by active methylation throughout the genome during the blastocyst stage. The pattern of DNA methylation thus changes rapidly between fertilization and the blastocyst stage, but asymmetry in this pattern exists between the male and female pronuclei.

How is the paternal genome specifically demethylated by Tet3, whereas the maternal genome is protected from Tet3-mediated demethylation? Whereas Tet3 mRNA is abundant in oocytes and zygotes, Tet3 protein is specifically enriched in the male pronucleus (it is localized to the cytoplasm at later preimplantation stages). How then is Tet3 protein excluded from the female pronucleus? PGC7 is required for
this exclusion of Tet3 protein given that the latter is present in both pronuclei of PGC7-deficient zygotes [20,21]. PGC7 is thought to prevent recruitment of Tet3 to the female pronucleus by binding to H3K9me2, a type of histone modification present in the maternal genome, but absent from the paternal genome [20]. If this is the case, then DNA methylation and histone methylation are interconnected and may regulate each other. Does parental epigenetic asymmetry influence cell fate specification, in particular the decision to contribute to the ICM or TE? There is currently no direct evidence to support such an influence. PGC7 acts as a maternal factor essential for preimplantation development [22,23]. Embryos lacking maternal PGC7 rarely reach the blastocyst stage, with most remaining at the 1-cell, 2-cell or morula stage [23], making it difficult to examine whether the ICM–TE decision is impaired in such mutant embryos. Many genes responsible for the regulation of H3 methylation have been identified, but their roles in preimplantation development and cell fate specification have not been fully investigated. The histone methyltransferases Suv39h1 and Suv39h2 are responsible for parental asymmetry in H3K9me3 [24]. Interestingly, female zygotic null mutants for Suv39h1 or Suv39h2 are infertile [25]. The methyltransferases Prdm3 and Prdm16 are implicated in parental asymmetry of H3K9me [26]. Zygotic null mutants for Prdm3 or Prdm16 do not survive beyond the late gestation stage or manifest hematopoietic and immune defects at the adult stage, respectively. The maternal role of these proteins remains unknown, however.

4. A hypothesis: epigenetic asymmetry between parental genomes can account for pre-patterning

Epigenetic asymmetry between maternal and paternal genomes could theoretically generate an early pre-pattern. We here propose a hypothetical model for generation of epigenetic and transcriptional differences among blastomeres as early as the 2-cell stage (figure 1). In this model, the two parental genomes exhibit asymmetric modification (either DNA or histone methylation), with this modification existing in the maternal genome at the 1-cell stage. This modification is maintained on the original chromosome but is not inherited by a daughter chromosome after cell division. Because zygotic transcription begins at the 2-cell stage, only the blastomere that has inherited the modified maternal genome will express at this stage an unknown regulatory gene (or genes) that will influence the later ICM–TE fate decision. Given a role for the plane of cell division at the 2-cell stage [11], blastomeres derived from the vegetal or animal pole may preferentially inherit genomes in the defined epigenetic states. At the 4-cell stage, de novo modification occurs equally on maternally and paternally derived genomes, which may or may not erase the epigenetic asymmetry. Validation of this model will require investigation of the maternal role of genes involved in asymmetric epigenetic modification as well as identification of specific genes that are
regulated by asymmetric epigenetic modification and influence the first cell fate specification.

5. Concluding remarks
The position of a cell within the mouse embryo at the 8- to 16-cell stage determines its fate. Although it is unknown whether an earlier pre-pattern contributes to the first cell fate decision, epigenetic differences among blastomeres probably exist as early as the 2- or 4-cell stage. Such epigenetic differences among blastomeres could be generated by asymmetry in histone or DNA modification between maternal and paternal genomes. Verification of a role for such asymmetry in embryonic pre-patterning awaits further study.

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