Cdx2 expression regulates trophectoderm lineage specification in mouse embryos

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Evaluation of: **Deb K, Sivaguru M, Yong HY and Roberts RM: Cdx2 gene expression and trophectoderm lineage specification in mouse embryos. *Science* 311, 992-996 (2006) [1].**

In a variety of animals such as *Drosophila* and *Xenopus*, the earliest stages of development are coordinated by the asymmetrical distribution of maternal transcripts and protein in the egg. In these animals, the position of blastomeres formed by embryo division and the expression products they inherit from egg cytoplasm determines their developmental fates. Thus far, attempts to determine whether such a specification system exists within mammalian embryos have yielded conflicting results. A recent report by Deb and colleagues [1] provides some strong evidence that the distribution of Cdx2 products may predetermine the fate of mouse blastomeres at the two-cell embryo stage. Their elegant data suggest that the asymmetrical localization of Cdx2 mRNA and protein in the oocyte and embryo controls the designation of trophectoderm and inner cell mass lineages, and that Cdx2 expression is necessary for proper blastocyst formation.

Introduction

The first differentiation event in mammalian development is the commitment of cells (blastomeres) of the dividing pre-implantation embryo to one of the two lineages within a future blastocyst: trophectoderm or inner cell mass (ICM). While in a large number of organisms it is clear that the destiny of the blastomeres is predetermined in the egg, there is still debate over when blastomere commitment occurs during mammalian development. Multiple studies have addressed this issue using a variety of approaches, including labeling individual blastomeres with membrane soluble dye [2] or β -galactosidase reporter under CAG-control in a Cre-LoxP system [3], labeling zona pellucida of the embryo with oil drops [4], time-lapse and confocal microscopy [5,6]. However, such efforts have produced conflicting results.

A recent report by Deb and colleagues [1] provides some strong evidence that the distribution of Cdx2 products may predetermine the fate of mouse blastomeres at the 2-cell embryo stage. The caudal-type homeodomain transcription factor Cdx2 is required for trophectoderm formation and axial elongation during mouse development [7,8]. It has been shown to be essential for directing blastomeres to the trophectoderm lineage by repressing their Oct3/4 and Nanog expression [9]. Consequently, its absence stalls embryogenesis prior to implantation.

Results from the paper

In their elegant and comprehensive study, Roberts' group [1] first compared expression of the transcription factor Cdx2 in cells descending from each of the two-cell-stage blastomeres. Their data showed that Cdx2 was only expressed in the nuclei of embryonic cells derived from the late-dividing but not the first-dividing blastomere. Next, they utilized lineage tracing and RNA interference knock-down experiments to demonstrate that the late-dividing blastomere is the precursor of the trophectoderm. Finally, using *in situ* hybridization and PCR techniques, Roberts and colleagues detected asymmetry in Cdx2 mRNA distribution from the oocyte stage all the way to the blastocyst.

To trace individual blastomeres from the two-cell embryo, the authors injected high-molecular-weight (40,000 d) neutral Texas red-conjugated dextran into one of the blastomeres and traced its lineage to the blastocyst stage. Unlike a lighter dextran-conjugated dye that quickly diffused into the other blastomere, the high-molecular-weight tracer did not spread from the injected blastomere, and at the blastocyst stage, staining was confined more or less exclusively to either trophectoderm 49% (25/51) or to the ICM 41% (21/51). All of the embryos showing red fluorescence in the lagging blastomere at the three-cell stage and progressing through the four-, eight-cell, and

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morula stages to the blastocyst showed red fluorescence in the trophectoderm. In these cases the ICM was generally unlabeled, except for the region adjoining the mural trophectoderm. By contrast, when the fluorescent dye-labeled blastomere had divided first, in nine out of ten blastocysts red fluorescence localized to the ICM, suggesting that the leading blastomere at the two-cell stage contributes mostly to ICM, while the lagging blastomere contributes to trophectoderm. To confirm that the tracer was confined to the trophectoderm, the blastocysts with tracer-labeled trophectoderm were immunostained with Cdx2, and examined through optical planes under the confocal microscope. In all the embryos tested ($n = 10$), the Cdx2-positive cells were also labeled with the tracer dye. The number of dye-positive and Cdx2-positive trophectoderm cells in individual blastocysts were identical, indicating that the lagging blastomere gives rise entirely to trophectoderm in CF-1 outbred mice. By injecting one of the two-cell-stage blastomeres with conjugated dye along with Cdx2 small interfering RNA (siRNA), the investigators determined that silencing of Cdx2 in the lagging, but not the leading, blastomere disrupts trophectoderm formation, resulting in an abnormal blastocyst lacking a cavity.

The authors also examined Cdx2 transcript distribution by *in situ* hybridization and RT-PCR, showing that Cdx2 mRNA is localized toward the vegetal pole of oocytes, reorients after fertilization, and becomes concentrated in the late-dividing, two-cell-stage blastomere. The asymmetry in Cdx2 mRNA distribution was persistent up to the blastocyst stage, where Cdx2 mRNA was found only in trophectoderm cells.

Significance & future perspectives

These findings have important ramifications for both basic research and clinical applications. They provide insight into the first differentiation step in mammalian development and thus contribute to our understanding of one of the fundamental aspects of developmental biology. In addition, they may have potentially significant implications for embryonic stem cell (ESC) derivations and *in vitro* fertilization techniques. If the results obtained from mice can be extended to human embryos, they suggest that the utility and consequences of harvesting single blastomeres for prenatal genetic diagnosis or generation of ESCs may be contingent on the choice of blastomere. For example, blastomeres predisposed by Cdx2

expression to form trophectoderm may be less likely to form ESC lines. On the other hand, an embryo may better survive the removal of a trophectoderm-lineage blastomere.

Interestingly, the authors do not address the reversibility of Cdx2 effects. While their experiments using siRNA clearly demonstrate that the knock-down of Cdx2 in the lagging two-cell-stage blastomere leads to aberrant blastocyst development and prevents formation of the blastocel cavity, they do not explain how this data can be reconciled with the ability of single 2-, 4- and even 8- and 16-cell-stage blastomeres to form both extraembryonic and embryonic lineages and give rise to full organisms [10]. One possible explanation is that the initial distribution of Cdx2 products promotes Cdx2 expression in certain blastomeres, predisposing them to trophectoderm lineage, but that this effect is cell contact dependent. In this scenario, cell-cell interactions between the blastomeres would promote unequal distribution of Cdx2; once a blastomere became dissociated from the others, Cdx2 distribution would be reset, and totipotency restored. This resetting of the Cdx2 distribution may be due to the changes that loss of cell-cell interactions may induce in cytoskeleton and/or receptor-mediated signal transduction. It would therefore be very interesting to see whether the expression of Cdx2 mRNA and protein is affected by the dissociation of blastomeres.

The results published by Roberts group are in direct conflict with the data recently published by Motosugi and colleagues [6] who used time-lapse video microscopy and fluorescent cell labeling to demonstrate a lack of patterning in pre-blastocyst mouse embryos. Multiple conflicting reports have been published on this subject [2-6]. While it is not clear at this stage what accounts for the differences in the results obtained by various groups, one possible explanation is variation in the mouse strains used to obtain embryos. Indeed, variability among mouse strains has been shown to account for different phenotypes observed for certain gene knockouts and differences in the position of the first cleavage plane [4]. If this proves to be the case, it would be important to establish how this discrepancy relates to Cdx2 expression, and which model corresponds most closely to the situation in human embryos.

An additional level of complexity is added to this data when we consider the report by Fujimori and colleagues [3] that incoherent clonal growth and drastic cell mixing occur after the blastocyst stage in mouse embryos. These authors suggest

Executive summary

- Asymmetric distribution of maternal transcripts and proteins in the egg allows lineage specification in multiple organisms including *Drosophila* and *Xenopus*.
- The idea that this is also the case in mammalian development remains controversial.
- Cdx2 is essential for trophectoderm specification through repressing Oct3/4 and Nanog expression.
- The distribution of Cdx2 mRNA and protein may predetermine the fate of mouse blastomeres at the two-cell embryo stage.
- These data have some potentially important implications for embryonic stem cell derivations and *in vitro* fertilization techniques.
- The authors do not explain how single 2-, 4- and even, 8- and 16-cell-stage blastomeres can form both extra-embryonic and embryonic lineages and give rise to full organisms.
- A possible explanation may be that the observed Cdx2 distribution is reversible and cell contact-dependent, at least during early embryo divisions.
- Mammalian embryos may have plasticity in determining blastomere fate.

that the first cell specification event, i.e., partitioning cell fate between the inner cell mass and trophectoderm, can occur between the two- and four-cell stage, yet the cell fate is not determined until much later in development. If these data are taken into account, Cdx2 may predispose blastomeres of the early mouse embryo to assume trophectoderm differentiation without determining their ultimate fate. This may suggest that Cdx2 in mammals performs its evolutionarily conserved function in pre-determining blastomeres to the trophectoderm lineage, but that mammalian development requires additional plasticity and thus limits the role of Cdx2, allowing for flexibility in regulating embryo development.

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