Editorial



# The apical domain defines the trophectoderm differentiation in early mammalian embryo by regulating YAP nuclear translocation

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Mammalian life initiates at the zygote, formed by the fusion of an oocyte and sperm. The first three cleavages of the mouse zygote generate eight gently connected blastomeres which show no obvious differences in developmental potencies. However, a few hours after the formation of the 8-cell stage the embryo undergoes the process of compaction, in which individual cells become tightly connected at the membranes. The first lineage specification of mouse embryos happens during the compacted 8- to 16-cell transition; these cells eventually differentiate into the extra-embryonic trophectoderm (TE) and the pluripotent inner cell mass (ICM) at blastocyst stage. In a recent study, Korotkevich and colleagues used a reduced embryo system to show that TE differentiation is driven by cell polarization and apical domain formation (1).

The early observations on pre-implantation mouse embryo cell behavior showed that cells outside of the embryo differentiate into TE, while cells inside become ICM. Tarkowski *et al.* proposed the "inside-outside model", in which a cell's fate is determined by the position of the cell within the embryo (2). However, this model does not cover the molecular mechanisms by which position-dependent differences regulate cell differentiation. In contrast, Johnson and co-workers found that polarization of cells at the apical membrane of outer cells is essential for TE differentiation. When these polarized cells asymmetrically divide along the apical-basal axis, they generate two daughter cells that show spatial differences. The outer cells remain polarized, while the inner cells become apolar (3). Johnson proposed the "polarity model", in which asymmetrically distributed cell polarity during cell division determines cell fate (4). Decades after the two models were proposed, Korotkevich and colleagues combined light-sheet live embryo imaging (5) and fluorescent reporter technologies in a series of experiments to answer the question about TE lineage differentiation.

It has been reported that the single blastomere isolated from an 8-cell stage embryo (1/8-cell) can develop into a mini-blastocyst following three cell divisions. While a normal blastocyst contains 32 cells, a mini-blastocyst contains only 4. In these experiments, when a 1/8-cell divides once to give rise to 2/16-cell doublet, it forms two daughter cells, one having polarity and the other being apolar. Thus, cells inherit apical domain asymmetrically. Interestingly, YAP and Cdx2 markers for pre-TE fate also show an asymmetric distribution between the two daughter cells (6,7). This result indicates that cells with the same position could have intrinsically different developmental potential. To further investigate the roles of position and polarization during TE fate specification, Korotkevich and colleagues took advantage of the reduced mini-blastocyst system, in which the lineage specification of TE occurs under a spatially simplified manner. The authors labelled apical domain and other developmental markers with different fluorescent reporters, then monitored the dynamic process of live blastomere development to mini-blastocyst using time-lapse light-sheet microscopy. They found that the isolated apolar 1/8-cell formed an apical domain cell-autonomously, under

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the control of an intrinsic developmental program. Majority of the 1/8-cells aligned the mitotic spindle to the apical-basal axis and underwent asymmetric division, such that the apical domain was inherited differentially by two daughter cells. Furthermore, the cell with the apical domain enveloped its apolar sister cell. Thus, there is a positive correlation between the apical domain formation and the level of Cdx2 expression, suggesting an essential role of apical domain for the TE fate.

To test the requirement of an apical domain for TE specification, the authors further analyzed Cdc42 mutant (mzCdc42$^{-/-}$) embryos, in which the apical domain is disrupted. The mzCdc42$^{-/-}$ embryos showed disorganized asymmetric division, diminished TE gene expression, and expanded ICM markers. To test the sufficiency of apical domain for TE specification, the authors developed methods to transplant the apical domain of a polarized 8-cell stage blastomere into an apolar 1/8-cell and found the transplanted apical domain was sufficient to drive asymmetric division and TE specification. In surprising observations, the authors showed that transplantation of non-apical domain into an apolar 1/8-cell delayed the formation of an apical domain from the normal 1/8-cell stage to the 2/16-cell stage. These observations indicate that additional signals function in the establishment of the apical domain within the blastomeres at this stage.

What is the connection between apical domain formation and TE specification? It was reported that polarity-dependent membrane localization of angiomotin (AMOT) regulates nuclear translocation of YAP, which is needed for TE fate specification. In the unperturbed 2/16-cell doublets, YAP shows a stronger nuclear localization in polar cells than in apolar cells, and AMOT is mostly localized to the apical domain in the polar cells, indicating that cell polarity regulates differential localization of AMOT/YAP within 2/16-cell doublets while adhesion does not. To test the possibility that the apical domain regulates the subcellular localization of AMOT and YAP, the authors transplanted the apical domain to apolar cells of 2/16-cell doublets and observed AMOT re-localization to the ectopic apical region and YAP nuclear localization in the re-polarized cell of 2/16-cell doublets. However, transplantation of non-apical domain to the apolar cells of 2/16-cell doublets showed a similar phenotype to unperturbed 2/16-cell doublets. These observations confirm that the apical domain is sufficient to promote YAP nucleus accumulation and TE fate specification.

The position of apolar cells are not fixed during developmental process. High-resolution live embryo imaging showed that a few apolar cells could move on to the surface of the embryos, and then become polarized, initiate Cdx2 expression, and eventually specify to TE fate (7,8). The question here is, what controls the apical domain formation on the surface of these cells if they cannot inherit it from the 8-cell stage? Since the polarity is always formed on the cell surface opposite to the contact region, it could be possible that cell position and cell-cell contact push the formation of the apical domain. The authors tested the mechanistic link between cell contact and apical domain in TE fate segregation. Cadherin 1 (Cdh1)-mediated cell-cell adhesion has been shown to be essential for cell fate specification (9). The authors put 1/8-cells together with Cdh1 coated or uncoated beads, and monitor the apical domain formation. Interestingly, the apical domain is formed opposite the cell-beads contact point, regardless of the Cdh1 coating. These findings indicate that cell contact directs apical domain formation, while Cdh1 does not. How cell contact directs the apical domain assembly requires future investigation.

Thus, Korotkevich and colleagues provide strong evidence of apical domain function in TE fate determination by aligning spindle orientation, directing asymmetric division, and controlling the AMOT and YAP subcellular localization. Apical domain can form on the surface membrane of the embryos in the cell-contact driven manner, which means cell contact and cell polarization work collaboratively to direct early embryos development. Meanwhile, these discoveries raise other questions. What is the intrinsic signal that temporally controls apical domain formation cell-autonomously? What is the cellular sensor that spatially directs apical domain formation at the opposite point of cell contact? How does apical domain regulate AMOT subcellular localization? In the reduced 2/16-cell doublets, Yap shows decreased nuclear signal in the apolar cells. However, it was reported that Yap is totally excluded from nucleus of the inside cells (10), which cannot be explained by the absence of apical domain. There may be an unknown mechanism controlling Yap inactivation in the inside cells. The function of cell contact in activating Hippo pathway is still elusive.

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