

A tale of division and polarization in the mammalian embryo

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Summary

The first cell fate choice to occur in mouse development generates the embryonic inner cell mass and the extra-embryonic trophectoderm. Here Korotkevich and colleagues show that the interplay between cell polarization and cell-cell contact drives the segregation of these lineages and provides a framework for how self-organization emerges in development.

One of the most perplexing and fascinating problems in developmental biology is how differences arise within a uniform field of cells. A prime example of such self-organization occurs during the very early stages of the mammalian embryo development, where an apparently homogenous ball of cells segregates into two distinct lineages: the inner cell mass (ICM) and the trophectoderm (TE). This is the first cell fate decision to take place in the embryo and demarcates embryonic and extra-embryonic cell types, as the pluripotent ICM will give rise to the fetus (as well as some extraembryonic tissues), while the TE only gives rise to the placenta. The ICM and TE start to form at the late 8-cell stage, when the mouse embryo is a compacted ball composed of cells that are polarised along the apical (surface-facing)-basal axis. When these cells divide, it results in two spatially distinct groups of cells – those situated on the surface of the embryo and those embedded within. The outer, polar cells are fated to become the TE and the inner, apolar cells the ICM. There has been much debate over how this early lineage segregation occurs, with experimental evidence providing support for two different models. The “inside-outside” model argues that position determines fate, with the inner cells becoming ICM due to their increased number of cell-cell contacts (Tarkowski and Wroblewska, 1967). In contrast, the “polarity” model suggests that the polar nature of the outside cells is what determines their fate as TE (Johnson and Ziomek, 1981). In this issue of *Developmental Cell*, Korotkevich et al. (2017) use an intricate and elegant series of experiments to provide evidence that reconciles these two models and provides insight into the basis for the first lineage segregation event.

To separate the relative importance of position versus polarization, Korotkevich and colleagues (2017) first simplify the problem by using mini-blastocysts (Tarkowski and Wroblewska, 1967; Ziomek and Johnson, 1980), where the minimal requirements for the ICM/TE fate decision could be analysed. Mini-blastocysts are isolated blastomeres from an 8-cell stage embryo that divide asymmetrically to give rise to two daughter cells that inherit different amounts of the apical domain (Figure 1a). Korotkevich and colleagues (2017) put a modern twist on this classical approach. Using fluorescent reporters of apical polarity and the cell membrane they followed by time-lapse microscopy the behaviour of the mini-blastocysts as they develop. Importantly, they observed that shortly after division, the cell with the larger apical domain envelopes the less polar cell and assumes a TE fate. Furthermore, there is a correlation between the proportion of apical domain inherited and the

levels of TE gene expression. Together, these findings point to a key role for the apical domain in determining cell fate. To test if this is the case, the authors first analysed embryos maternally and zygotically mutant for *Cdc42*, an important polarity regulator. They found that the mutant embryos not only lacked an apical domain, but they also showed reduced TE formation and expanded ICM, indicating the apical domain is required for TE formation. To test sufficiency, the authors developed a method to transplant the apical domain from an 8-cell stage blastomere onto an early, apolar blastomere. Transplantation was sufficient to drive asymmetric division and TE fate, demonstrating the fundamental role that the apical domain plays in directing TE cell identity.

But how does the apical domain direct TE fate? A key step in TE specification is the polarity-dependent localization of Angiomotin (Amot) to the apical membrane. This allows the Hippo pathway effector YAP to localise to the nucleus, which is both required and sufficient for TE differentiation (Hirate et al., 2013). Korotkevic and colleagues (2017) therefore looked at the localization of Amot and YAP in mini-blastocysts derived from an asymmetric doublet of cells (one polar and the other apolar) from 16-cell embryos, which represent the product of division at the 8-cell stage. They found that Amot was apical and Yap nuclear in the polar cell, similar to the intact embryo. Because in these doublets both cells experience adhesion from one cell contact, these results suggest that cell polarity, rather than adhesion, is primarily responsible for Hippo pathway activity in this context. To further test this hypothesis, the authors transplanted the apical domain of a blastomere onto the apolar cell of 16-cell stage-derived mini-blastocysts and showed that this was sufficient to induce apical localization of Amot and the nuclear translocation of YAP. Therefore, the apical domain is sufficient to promote YAP activity in the nucleus and thus the specification of TE fate.

The experiments above provide evidence for the importance of the apical domain, but do not address the role of cell-cell contact during TE specification. This question is especially relevant given that live-cell imaging of normal embryos by the authors and others (Anani et al., 2014; Watanabe et al., 2014), has revealed that as development progresses, a number of inside cells emerge outside, subsequently become polarised and eventually assume TE fate. This raises the question of what induces the apical domain in these cells. One possible cue is changes in the degree of cell-cell contact (Ziomek and Johnson, 1980). To address this idea in a molecularly defined manner, Korotkevic et al. (2017) juxtaposed apolar single blastomeres of 8-cell stage embryos with beads either uncoated or coated with Cadherin 1 (Cdh1), a molecule previously found to be important for cell-cell adhesion during early embryogenesis (Johnson et al., 1986; Stephenson et al., 2010). They observed that an apical domain formed opposite the point of contact, regardless of whether the bead was coated with Cdh1. Thus there is a role for contact in inducing the apical domain, and this can occur independently of Cdh1-mediated adhesion.

Together, these findings from the Hiiragi lab (Korotkevic et al. 2017) provide a unified model for how cells in the mammalian embryo might acquire their first lineage identity. They suggest that those cells on the surface of the embryo develop an apical domain at their contact free surface through some as yet unknown mechanism. This apical domain performs at least three roles (Figure 1b). First, it induces TE fate by recruiting Amot that, as Hirate et al. (2013) have shown, allows the nuclear translocation of YAP and the specification of TE identity. The apical domain also directs the asymmetric division that generates the outside and inside cells that give rise to TE and ICM, respectively. This asymmetric division, together with the reduced contractility of the apical domain, has been shown to generate daughter blastomeres with distinct contractilities, which further promotes the sorting of inside and outside cells (Maitre et al., 2016). Cell relocation can re-equilibrate the proportion of cells allocated to the TE and ICM lineages to ensure that the right cell numbers will be

specified for proper developmental progression. In this manner, the relationship and feedback between cell contact and cell polarity can integrate mechanical and molecular cues to drive self-organization in the early mouse embryo, and these principles may be key underlying forces for the organization of multicellular systems.

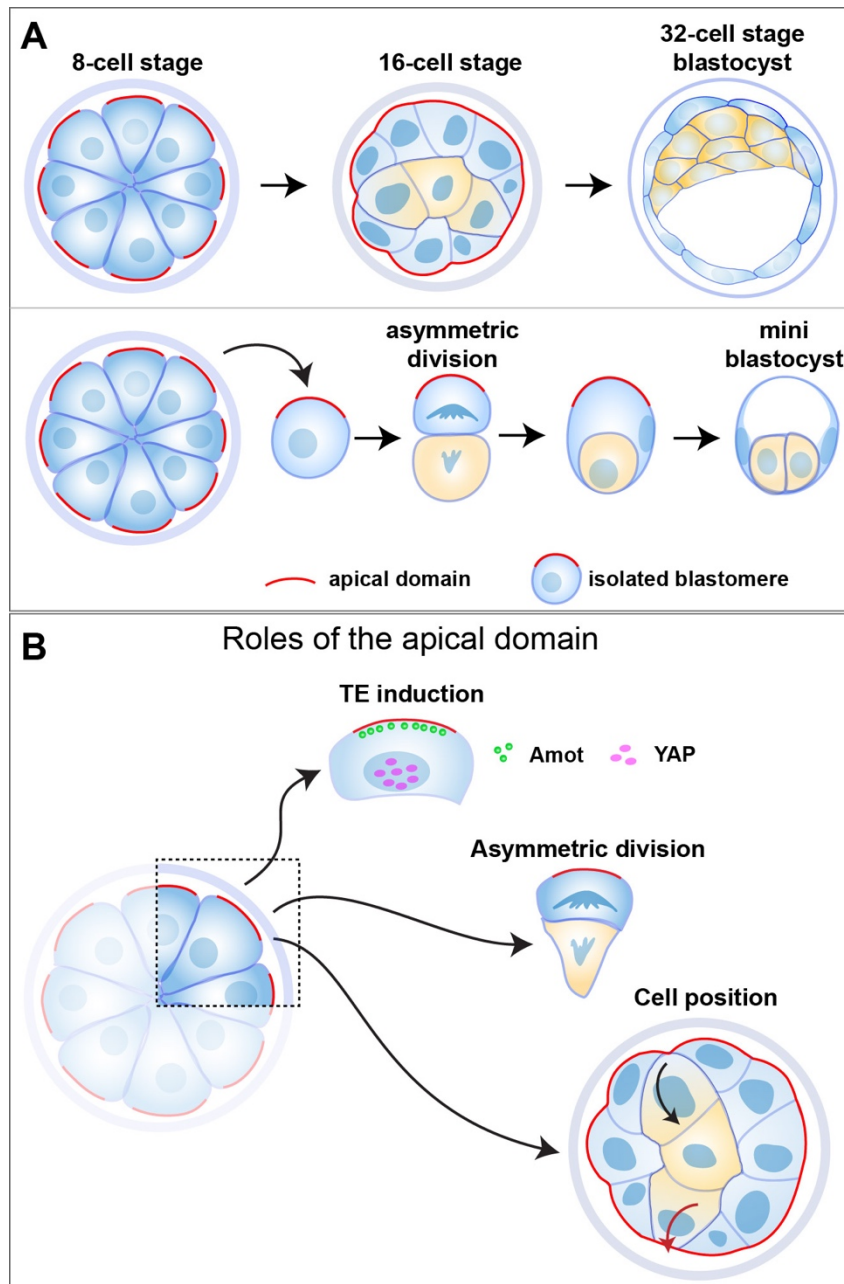


Figure 1. Importance of the apical domain for inner cell mass (ICM) versus trophectoderm (TE) lineage segregation. (a) Diagrammatic representation of the developmental stages of a mini-blastocyst. (b) Roles of the apical domain in TE specification. The apical domain is required to regulate the Hippo pathway, to drive asymmetric cell division and to ensure that polarised cells remain on the outside of the embryo.

Anani, S., Bhat, S., Honma-Yamanaka, N., Krawchuk, D., and Yamanaka, Y. (2014). *Development* 141, 2813-2824.

Hirate, Y., Hirahara, S., Inoue, K., Suzuki, A., Alarcon, V.B., Akimoto, K., Hirai, T., Hara, T., Adachi, M., Chida, K., *et al.* (2013). *Curr Biol* 23, 1181-1194.

Johnson, M.H., Maro, B., and Takeichi, M. (1986). *J Embryol Exp Morphol* 93, 239-255.

Johnson, M.H., and Ziomek, C.A. (1981). *Cell* 24, 71-80.

Korotkevich, E., Niwayama, R., Cortois, A., Friese, S., Berger, N., Buccholz, F., and Hiiragi, T. (2017). *Dev Cell* 40,

Maitre, J.L., Turlier, H., Illukkumbura, R., Eismann, B., Niwayama, R., Nedelec, F., and Hiiragi, T. (2016). *Nature* 536, 344-348.

Stephenson, R.O., Yamanaka, Y., and Rossant, J. (2010). *Development* 137, 3383-3391.

Tarkowski, A.K., and Wroblewska, J. (1967). *J Embryol Exp Morphol* 18, 155-180.

Watanabe, T., Biggins, J.S., Tannan, N.B., and Srinivas, S. (2014). *Development* 141, 2279-2288.

Ziomek, C.A., and Johnson, M.H. (1980). *Cell* 21, 935-942.