Developmental Cell
Previews

Moving Inward: Establishing the Mammalian Inner Cell Mass

Bob Goldstein[1,*] and Daniel P. Kiehart[2,*]
[1]Biology Department, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA
[2]Biology Department, Duke University, Durham, NC 27708, USA
*Correspondence: bobg@unc.edu (B.G.), dkiehart@duke.edu (D.P.K.)
http://dx.doi.org/10.1016/j.devcel.2015.08.007

Early in mammalian development, a few cells move to the center of the embryo to establish the inner cell mass—the early precursor of the fetus. In this issue of *Developmental Cell*, Samarage et al. (2015) shed light on how these cells move inward.

Way back when you were little, *very* little, you made one of your first important decisions. Approximately 4 days after the egg from your mother was fertilized by the sperm from your father, you were part of a ball of about 12 cells. One of your cells took a new path, leaving the embryo's surface to move into the center of the ball of cells (Figure 1). Before this, all of the cells had been at the embryo's surface. After this initial decision by one of the cells to move inward, other cells also then internalized, joining the first cell in the middle. Those internalized cells became you, whereas the remaining outer layer of cells produced the extraembryonic tissue that attached you to your mother's uterus and developed into the placenta.

In this issue of *Developmental Cell*, Samarage, White, Álvarez et al. (2015) identify how forces are produced to move the first cell into the center of a mammalian embryo. They started by labeling cell membranes of mouse embryos and filming the embryos at high resolution, and then they used these movies to computationally segment the embryos. This method allowed the fine details of each cell's shape and position to be visualized and quantified over time. The resulting movies are truly beautiful and very informative.

Previously, cells had been shown to arrive at the center of the early mouse embryo by oriented cell division: some surface cells divide in an orientation that delivers one daughter cell to the inside. Recent imaging of early mouse embryos confirms that this indeed occurs but establishes that this division orientation is rare, particularly for the first cells to internalize (Watanabe et al., 2014). Overall, division angle is a poor predictor of which cells contribute to the inner cell mass. This left open the question of how cells moved interiorly.

Samarage et al. (2015) followed the movements of the earliest internalizing cells, which make the largest contribution to the inner cell mass (a few cells internalize later). Consistent with the earlier reports, they saw that few of the earliest inner cells were placed on the inside by oriented cell divisions. Instead, divisions appeared to be oriented randomly. Moreover, they found that in some embryos, through the 16-cell stage, no cells were internalized by oriented division. In particular, the first cell to end up in the interior was generally born on the outside, and its outer, apical domain gradually shrinks until no exterior surface remains.

Apical constriction—the shrinkage of the apical surfaces of certain cells—is known to play a central role in some key morphogenetic movements of animal embryos, including gastrulation in many animals and neural tube formation in vertebrates (Sawyer et al., 2010). Apical constriction is generally driven by the contractions of actomyosin assemblies or networks that either line apical junctions (so-called junctional belts) or crisscross the cortex just under the apical cell surface (so-called medioapical arrays) (Martin and Goldstein, 2014). Consistent with this, Samarage et al. (2015) show that a myosin II-enriched ring can be found near the edges of the apical surface of internalizing cells, and targeting myosin by small interfering RNA prevented cell internalization by apical constriction. Together, these findings imply that the forces driving cell internalization are produced from the apical side of the cell. If a cell was distinguished by having higher tension in its apical cortex, this might result in its apical surface shrinking smaller and smaller while pulling neighboring cells over the top.

To directly compare forces in different parts of the embryo, Samarage et al. (2015) made targeted cuts at junctional belts and at medioapical arrays in the embryo with a focused laser and watched the recoil. This strategy is comparable to cutting a stretched rubber band and watching it pop apart to learn something about how much tension it bears (Kiehart et al., 2000; Ma et al., 2009). Junctions of apically constricting cells recoiled more quickly and extensively when cut than did other junctions, implying as predicted that junctions of the apically constricting cells were under higher tension. Similar cuts in the medioapical array showed that it, too, was under higher tension in apically constricting cells than in other cells. Interestingly, although microscopy showed only weak distribution of actin filaments or myosin II in the apical cortex, targeting the middle of the apical surface with the laser caused recoil from the targeted site, suggesting that some forces are produced and/or transmitted across the apical surface.

Thus, apical constriction, and not oriented cell divisions, mediates internalization of these earliest cells in the mouse. The data from the Samarage et al. (2015) study reveal that in the early mouse embryo, cells become internalized very much as they do in other models, such as *C. elegans* and *Drosophila*. In these systems, similar dynamics have been seen, and laser cuts have led to similar conclusions (Sawyer et al., 2010; Martin and Goldstein, 2014).

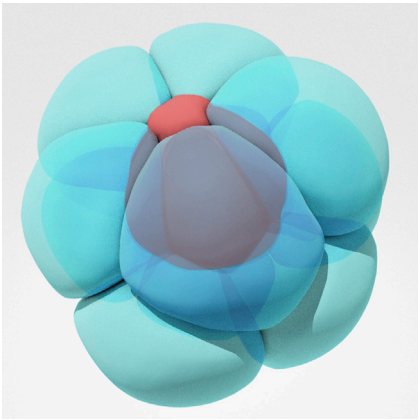

Figure 1. Cell Internalization at the 12-Cell Stage
A single cell (red) among the others (blue) at the 12-cell stage of the mouse embryo internalizes and is the first cell to contribute to the inner cell mass, which gives rise to the fetus. Illustration by Janet Iwasa.

Samarage et al. (2015) play down a role for cadherins in specifying which cells internalize, because E-cadherin, unlike myosin-II, is not differentially localized in the apically contracting cells. Nevertheless, disrupting cadherin function prevents internalization of any cells, and it remains an open question as to how rearrangement of cell junctions is coordinated with apical constriction to mediate internalization. In addition, while extracellular matrix is not a prominent feature in the early mouse embryo, how cadherin-based adhesion is integrated with adhesion mediated by other cell-surface proteins remains unknown.

There is evidence in the early mouse embryo that cell positioning plays a causal role in determining critical gene activity. Where a cell lives, whether on the inside or the outside of the embryo, contributes to its transcriptional identity (Stephenson et al., 2012; Schrode et al., 2013; Bedzhov et al., 2014; Samarage et al., 2015). This highlights the importance of positioning cells correctly.

How, then, are certain cells chosen to undergo apical constriction and take up residence in the inner cell mass? Experiments have teased out some predictors of which cells are likely to internalize, but no perfect predictor appears to exist (Bedzhov et al., 2014; Anani et al., 2014). The decision may be stochastic; perhaps any one of the 12 cells can move inward to begin production of the inner cell mass. By this model, little-understood stochastic fluctuations in the biomechanical properties of cells could lead one cell to accumulate apical tension and perhaps inhibit its sister cell and other neighbors from doing the same. Indeed, after following over 100 embryos, Samarage et al. (2015) reported that they never saw two sister cells constricting simultaneously. Thus, how this very early decision of yours was made when you were little remains a mystery, but it might have involved something as simple as one of your cells choosing to undergo apical constriction.

REFERENCES

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

Bedzhov, I., Graham, S.J.L., Leung, C.Y., and Zernicka-Goetz, M. (2014). Philos. Trans. R. Soc. Lond. B Biol. Sci. *369*, 20130538.

Kiehart, D.P., Galbraith, C.G., Edwards, K.A., Rickoll, W.L., and Montague, R.A. (2000). J. Cell Biol. *149*, 471–490.

Ma, X., Lynch, H.E., Scully, P.C., and Hutson, M.S. (2009). Phys. Biol. *6*, 036004.

Martin, A.C., and Goldstein, B. (2014). Development *141*, 1987–1998.

Samarage, C.R., White, M.D., Álvarez, Y.D., Fierro-González, J.C., Henon, Y., Jesudason, E.C., Bissiere, S., Fouras, A., and Plachta, N. (2015). Dev. Cell *34*, this issue, 435–447.

Sawyer, J.M., Harrell, J.R., Shemer, G., Sullivan-Brown, J., Roh-Johnson, M., and Goldstein, B. (2010). Dev. Biol. *341*, 5–19.

Schrode, N., Xenopoulos, P., Piliszek, A., Frankenberg, S., Plusa, B., and Hadjantonakis, A.-K. (2013). Genesis *51*, 219–233.

Stephenson, R.O., Rossant, J., and Tam, P.P.L. (2012). Cold Spring Harb. Perspect. Biol. *4*, a008235.

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