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COMMENTARY

Cell fate in the early mouse embryo: sorting out the influence of developmental history on lineage choice

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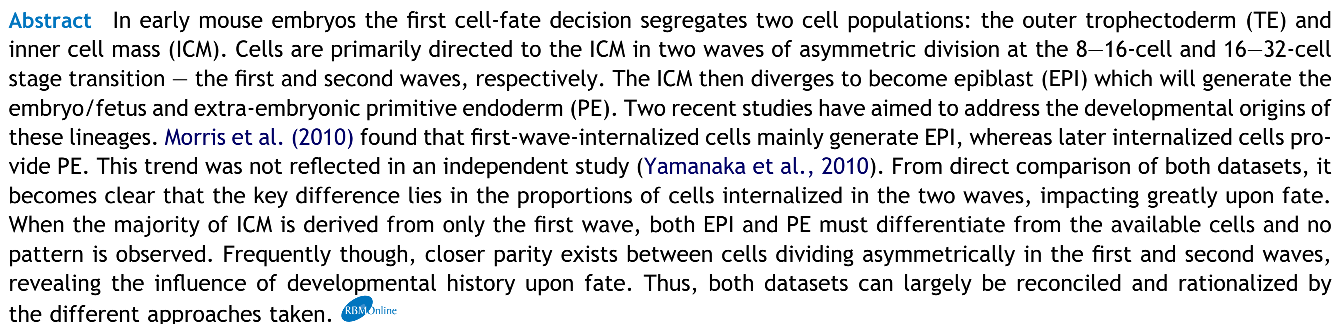
Abstract In early mouse embryos the first cell-fate decision segregates two cell populations: the outer trophectoderm (TE) and inner cell mass (ICM). Cells are primarily directed to the ICM in two waves of asymmetric division at the 8–16-cell and 16–32-cell stage transition – the first and second waves, respectively. The ICM then diverges to become epiblast (EPI) which will generate the embryo/fetus and extra-embryonic primitive endoderm (PE). Two recent studies have aimed to address the developmental origins of these lineages. Morris et al. (2010) found that first-wave-internalized cells mainly generate EPI, whereas later internalized cells provide PE. This trend was not reflected in an independent study (Yamanaka et al., 2010). From direct comparison of both datasets, it becomes clear that the key difference lies in the proportions of cells internalized in the two waves, impacting greatly upon fate. When the majority of ICM is derived from only the first wave, both EPI and PE must differentiate from the available cells and no pattern is observed. Frequently though, closer parity exists between cells dividing asymmetrically in the first and second waves, revealing the influence of developmental history upon fate. Thus, both datasets can largely be reconciled and rationalized by the different approaches taken.



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Introduction

The early development of all mammals involves a sequence of cell-fate decisions which divert cells along a pathway of restricted potential and increasing specialization. The first of these decisions is whether to become trophoectoderm (TE) or inner cell mass (ICM); cells positioned on the inside of the embryo at the 16- to 32-cell stages become ICM whereas those remaining on the outside contribute primarily to TE. The cells within the ICM then proceed to become epiblast (EPI) and give rise to the developing embryo/fetus or extra-embryonic primitive endoderm (PE, also called hypoblast), and contribute primarily to the yolk sac. An enduring and often contentious issue is how the second of these cell-fate decisions (EPI versus PE) is reached.

Recently two independent, parallel studies aimed to address the issue of PE and EPI origins: but with ostensibly contradictory findings (Morris et al., 2010; Yamanaka et al., 2010). Here, this commentary aims to address and reconcile the differing conclusions arising from these two reports.

Our own study (Morris et al., 2010) employed non-invasive lineage tracing to track all cells of the embryo from the 8-cell to late blastocyst stage, by which time PE and EPI lineages are fully sorted – as confirmed by position, morphology and protein expression of lineage markers using

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immunostaining. Utilizing this approach, we found that cells from the 8-cell stage onwards are allocated to the inside of the embryo by three successive waves of asymmetric divisions and that their time of arrival in the ICM influences the EPI/PE fate decision. Inside cells generated by the first wave (at the 8—16-cell-stage transition) give rise to the majority of EPI cells while conversely, the majority of PE cells are the progeny of inside cells generated by the second wave (16—32-cell-stage transition) (**Figure 1**A). We also found that the third wave of divisions (32—64-cell-stage transition) is able to generate inside cells, albeit very few, and that these cells exclusively form PE (Morris et al., 2010).

An independent study by Yamanaka et al. (2010) used a different methodology. With the aim of testing the developmental and molecular origins of PE differentiation, a lineage-tracing approach was taken in which individual cells were permanently labelled, their time of internalization monitored and their fate assayed at postimplantation stages. Using this approach, the authors found no clear association between the developmental history of individual ICM cells and later cell fate. Rather, they propose that PE and EPI segregation is stochastic, here meaning random, and progressive as the blastocyst matures, in a fibroblast growth factor/mitogen-activated protein kinase-dependent manner (Yamanaka et al., 2010).

In an attempt to resolve the differing conclusions arising from these two studies, we compared both datasets in detail. In addition to the differing origins of EPI and PE, we found drastically different numbers of observed first-wave asymmetric cell divisions (an average 2.84 in Morris et al. versus 4.8 in Yamanaka et al.), which transforms ICM composition (first-wave-derived 50% versus 80%). This trend is inverted for second-wave asymmetric cell divisions (an average 5.1 versus 2.4).

Debate regarding cell allocation to the ICM is longstanding. The ICM was originally thought to be exclusively generated by first-wave asymmetric divisions (Johnson and Ziomek, 1981). Further work demonstrated that subsequent internalization events at the 16—32-cell stage transition are required to provide sufficient cells to establish the ICM (Fleming, 1987; Pedersen et al., 1986), although there was little consensus on the proportions of cells derived from

D Late 8-cell stage injection

Uninjected		Injected	
Polarized	Unpolarized	Polarized	Unpolarized
22 (92)	2 (8)	3 (33)	6 (67)
	n=24 embryos		n=9 embryos

Figure 1 (**A**) Model from Morris et al., 2010(a,b). Internal cells generated in the first wave of asymmetric division (blue) are biased to generate EPI, whereas cells generated in subsequent waves (red) are biased to generate PE over EPI. Black arrows indicate orientation of cell division. (**c**) EPI and PE progenitors intermingle in the ICM of the early blastocyst. (**d**) In the mature blastocyst, PE is fully sorted from EPI. (**B**) Relationship between ICM composition and the proportion of first-wave-derived cells generating EPI (blue) and PE (red) (Morris et al., 2010). Green dashed line = an 80% first-wave-derived ICM produces an equal distribution between EPI and PE; orange dashed line = a 50% first-wave-derived ICM results in 75% of the first wave generating EPI. (**C**) Disruption of polarity following microinjection of one blastomere in a late 8-cell-stage embryo. Eight-cell embryos were collected in M2 medium containing BSA from superovulated F1 (C57BL/6xCBA) females mated with F1 males and cultured in KSOM medium (Millipore). Individual blastomeres of mid- or late 8-cell embryos were injected with rhodamine dextran (Invitrogen) and fixed in 4% paraformaldehyde immediately following injection. Primary antibody used was rabbit anti-aPKC (Santa Cruz Biotechnology). Immunostaining was carried out as described previously (Plusa et al., 2005). Multichannel images were acquired on multiple sections using an Olympus FV-1000 confocal microscope. Red = rhodamine dextran; green = aPKC; blue = Hoechst staining. (**D**) Polarization levels in uninjected and injected blastomeres from **B**. Values in parentheses are percentages. aPKC = atypical protein kinase C; EPI = epiblast; ICM = inner cell mass; PE = primitive endoderm; TE = trophectoderm.(For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

each stage. More recent reports suggest a closer parity between these ICM populations (Bischoff et al., 2008; Dard et al., 2009; Jedrusik et al., 2008; Plusa et al., 2005). It is clear though that the range is wide, owing to the highly variable frequency of first-wave asymmetric divisions within an embryo (from none to six), with the majority of embryos (85%) exhibiting from one to four divisions (Dard et al., 2009; Morris et al., 2010).

From our observations, this highly regulative cell allocation has a distinct influence upon patterning. In the 32-cell blastocyst, the ICM accommodates 11 cells on average, although the number of first-wave asymmetric divisions can vary, from one to five. ICM occupancy following the first wave directly impacts on the number of second-wave asymmetric divisions, such that in the case where a high number of cells have divided asymmetrically in the first wave, there are few second-wave asymmetric divisions and vice versa, in agreement with earlier findings (Bischoff et al., 2008; Fleming, 1987). From lineage tracing, we see that cells internalized by asymmetric division in the first wave are more likely to contribute to EPI (75%), whereas 85% of cells internalized in later cleavages form PE. This relationship is highly dependent on the number of cells internalized in the first wave: when few inside cells arise from the first wave there is a very high correlation between EPI fate and first-wave internalization. Whereas when the ICM consists mainly of first-wave cells (up to 90%), the PE must be derived from the available cell population and this trend disappears (**Figure 1B**).

In the Yamanaka et al. study, if we exclude apoptotic cells, the first wave generates 50% PE, 18% EPI and 32% both lineages. In the few cases of second-wave asymmetric divisions, these generated 50% PE, 12.5% EPI and 17.5% both lineages. As mentioned above, these embryos have 4.8 cells internalized on average at the 8—16-cell stage transition resulting in 80% of the ICM derived from the first wave. If we examine our data considering only embryos with high numbers of first-wave asymmetric cell divisions, we see remarkably similar results (**Figure 1B**, green dashed line). However, we observed an overall average of 2.84 asymmetric divisions in the 8—16-cell stage transition. This results in an equal balance between first- and second-wave-derived ICM, and it is in these cases that the link between cell fate and developmental history becomes clear (**Figure 1B**, orange dashed line). Furthermore, in the case where few second-wave-derived cells contributed to the ICM, the majority form PE. This is in agreement with the Yamanka et al. dataset where, considering exclusive contributions, the majority of such cells (80%, four cells) form PE rather than EPI (20%, one cell). Excluding cells that contribute to both lineages is appropriate here, as it is important to note these mixed populations may obscure any preimplantation biases as a result of the differential proliferation of derivative tissues in postimplantation stages.

Our study reported mostly asymmetric division as the chief mechanism of cell internalization, where one daughter is deposited inside the embryo upon cell division. In contrast to this, the study by Yamanaka and colleagues identified cell engulfment as the major cause. Intriguingly, it has been reported that down-regulation of polarity can lead to cell internalization by cell engulfment (Plusa et al., 2005). This led us to investigate whether microinjection of blastomeres at the 8-cell stage, the time when they are developing their apical—basal polarity, as employed in the Yamanka study to label cells, could itself result in disruption of cell polarity. We found that this is indeed the case: **Figure 1C** shows disruption of apical localization of the polarity marker molecule atypical protein kinase C following microinjection of rhodamine dextran into one blastomere of the 8-cell-stage embryo (in 67% of cases, **Figure 1D**). It should be stressed that this effect is only observed when cells are injected late in this cell cycle so as to prevent the lineage marker from spreading into sister cells of the previous division. This leads us to the possibility that the Yamanaka et al. microinjection approach encourages more cells of the ICM to be generated at the 8—16-cell stage transition. An alternative and likely scenario centres on the stain of mice studied. In previous work with outbred ICR embryos (Pedersen et al., 1986), fewer cells (three to four) are internalized in the first wave, although this is also the case in the second wave, resulting in the ICM possessing a higher proportion of first-wave-derived cells (70%). In all cases, this would obscure any relationship between time of internalization and cell fate (**Figure 1B**).

The differences in the reports of Morris et al. and Yamanaka et al. can, by in large, be reconciled. The key difference lies in the proportions of cells internalized in each wave of asymmetric division, which impacts greatly upon cell fate. This outcome can be rationalized by the different approaches taken or possibly due to differences in mouse strains. Using microinjection to label cells may disrupt cell polarity, leading to a disproportionate number of cells to be internalized in the first wave. Embryos with such a high proportion of inside cells coming from the first wave are rare (∼15%) (Dard et al., 2009; Morris et al., 2010) and, unlike the majority of embryos, a correlation between internalization time and cell fate is not present in these cases. Together, these studies paint embryogenesis as a highly dynamic and regulative process with subtle trends that influence cell fate. Hopefully these trends will help us to uncover the molecular mechanisms that enable such dynamic behaviour within a highly organized system.

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