

A single-cell snapshot of cell-fate decisions

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Critical steps in the specification of embryonic cell lineages occur after implantation, but gaining insight into the molecular details of these cellular processes *in vivo* has been challenging. Jin and co-workers now report the transcriptomic signatures and molecular heterogeneity of more than 600 single cells from mouse embryos at days 5.5 and 6.5, advancing our understanding of how early embryonic cells make cell-fate decisions into mesoderm and endoderm lineages.

In mammals, a precisely controlled series of lineage specification and axis-patterning events occur during gastrulation (1). For example, the anterior-posterior polarity of the mouse embryo is established at around embryonic day 6.0, marked by the formation of two localized tissues, the primitive streak (PS)² and the anterior visceral endoderm (AVE), that establish symmetry and specify subsequent development. Epiblast (EPI) cells undergo an epithelial-mesenchymal transition, changing from closely connected to more mobile and separate cells, and ingress through the PS, followed by differentiation into mesoderm (ME) and definitive endoderm (DE). These cells further migrate, either surrounding the prospective ectoderm region to form the embryo proper or moving into the extra-embryonic region and contributing to the yolk sac, placenta, and umbilical cord (Fig. 1). So far, various strategies have been applied to identify developmentally regulated transcription factors and key signaling pathways in post-implantation mouse embryos at the tissue level (2). However, to unravel the molecular basis for the cell-fate plasticity and determination, it is essential to study the characteristics of individual cells. Unfortunately, the numbers of gastrulating cells are very limited, and so the molecular mechanisms underlying this process remain unclear.

The recently developed techniques, single-cell RNA sequencing (scRNA-Seq) and single-cell high-throughput quantitative RT-PCR (qRT-PCR), have provided new opportunities to interrogate these limited pools of developing cells at high

resolution. So far, these techniques have been applied to reveal distinct cell types in pre-implantation embryos as well as lineage segregation and pluripotency state transitions of embryonic stem cells (3–5). More recently, the technology was also applied to compare the pluripotency state between pre-implantation and early post-implantation embryos (6) and to decipher the mesodermal lineage diversification toward the hematopoietic system in the early post-implantation embryo (7). However, these studies did not explore whether these early cells had characteristics of perspective mesoendoderm (pre-MEN) cells, the precursors of ME and DE cells, leaving open the question of how these cells segregate into ME and DE cells in the PS *in vivo* and what the expression profiles of embryonic cells are that specify earliest in the EPI.

The work of Jin and colleagues (8) addresses these questions in two parts. First, they report the expression profiles of embryonic cells at the early post-implantation stage. Second, they use these data to investigate the molecular features of segregation of the DE and ME in post-implantation embryos. The authors begin by performing scRNA-Seq in combination with single-cell qRT-PCR in nearly 600 cells to obtain a global picture of transcriptomic signatures of EPI, visceral endoderm (VE), and extra-embryonic ectoderm (EXE) individual cells at the early post-implantation stage E5.5 and E6.5. The resulting single-cell gene expression data provide a rich resource for these authors and others to visualize different states of cells at the early post-implantation stages. They found many genes, both expected and new, that provide signatures of cells of both embryonic and extra-embryonic origins. For example, *Sox2* was expressed in EXE and EPI but rarely in VE, and *Otx2* was expressed in VE and EPI but rarely in EXE. Furthermore, many ligands and receptors of the Fgf signaling pathways showed cell type-specific expression patterns, with some *Fgf* genes specific to only EPI or only EXE, and the known EPI marker *Fgf5* appearing in VE cells as well. Further exploration of the functional roles of these cell type-specific genes will surely advance our understanding of how different cell lineages are specified and established during gastrulation.

Next, the authors mined their single-cell gene expression dataset looking for clues as to how EPI cells segregate into ME and DE cells in the PS *in vivo*. They were able to distinguish pre-MEN cells from EPI cells and to identify the divergence of ME and DE lineages from cells that were double-positive for the *Oct4* and *Gata6* transcription factor genes, hinting at a PS location. It is well known that mesoderm cells, both ME and extra-embryonic mesoderm (EXEM), are migrated from the EPI, which means they are similar. However, the authors found that

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² The abbreviations used are: PS, primitive streak; EPI, epiblast; DE, definitive endoderm; VE, visceral endoderm; E, embryonic day; EXE, extra-embryonic ectoderm; AVE, anterior visceral endoderm; ME, mesoderm; qRT, quantitative RT; MEN, mesendoderm; scRNA-Seq, single-cell RNA sequencing; PSC, pluripotent stem cell.

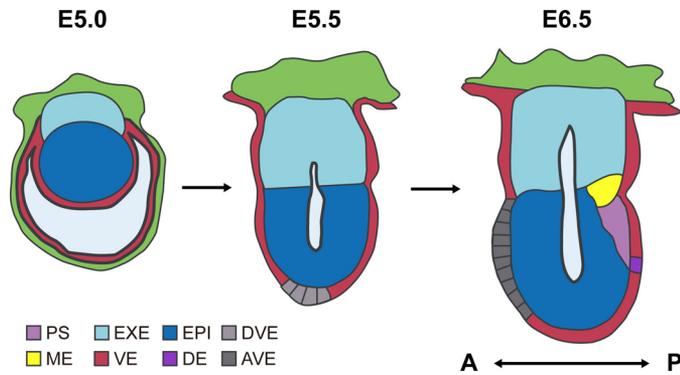


Figure 1. Early mouse post-implantation development. PS (colored light purple) formation marks the onset of gastrulation and germ-layer formation at embryonic days 5.0–6.5. EPI cells (colored blue) differentiate into the ME (colored yellow) and DE (colored purple). Surrounding the epiblast cells are the EXE and VE, a portion of which develops into the distal visceral endoderm (DVE) and then AVE; the primitive streak and anterior visceral endoderm define the anterior (A) and posterior (P) of the embryo.

a subpopulation of ME cells exhibits characteristics of the EXEM, indicating that EXEM cells are distinguished from the ME cells, even though they are migrated at the same time. For segregation of DE and ME, they found that the Wnt signaling pathway was very important: Genes involved in negatively regulating Wnt were up-regulated in DE cells, including *Cdh1*, *Cer1*, *Gsc*, *Sfrp1*, *Sfrp5*, *Shisa2*, *Six3*, and *Sox17*, whereas Wnt agonists such as *Wnt3* and *Wnt5a* were up-regulated in ME cells. These data provide new systematic insights into how the segregation of ME and DE cells might be regulated in terms of both transcriptional networks and signaling cross-talk. It will be exciting to test the function of these specific genes during lineage segregation and stem-cell differentiation.

Finally, Jin and colleagues (8) consider data drawn from other recent studies reporting single-cell transcriptomic analyses of mouse early post-implantation embryos (6, 9). In the original articles, the divergence between ME and DE cells and characteristics of the pre-MEN cells were not included; the analysis of Jin and colleagues (8) indicates those data did not report on cells similar to the DE cells in this new work. Thus, their new study uniquely improves our understanding of how early embryonic cells make cell-fate decisions toward the ME and DE lineages. This study also demonstrates that anterior-posterior regionalization in the PS occurs at an earlier stage (E6.5) than previously understood. In combination, the new data allow the authors to put forward a concise model of their findings including new relationships between cellular populations. The authors further hypothesize that pre-MEN cells are more prone to differentiate into the mesendoderm (MEN) lineage than the rest of early EPI cells. However, further experimental evidence is needed to test whether signal enforcement is required for further differentiation of pre-MEN cells and verify this proposal.

This study provides fundamental insights into the molecular basis of lineage segregation, especially the segregation of pre-

MEN, ME, DE, and EXEM lineages, during early gastrulation of mouse embryos. The single-cell gene expression dataset allows us to visualize the differentiation state of individual cells at early post-implantation stages, improving our understanding of how early embryonic cells make cell-fate decisions toward the ME and DE lineages. In-depth analysis of these data could also help to develop efficient strategies to differentiate pluripotent stem cells into regenerative medicine-relevant cells for clinical use. Recent advances have demonstrated the possibility to derive specific somatic cell lineages as 3D organoids from pluripotent stem cells (PSCs), including liver buds and minibrains. However, most of these strategies rely on differentiation systems that do not recapitulate organogenesis, mainly owing to lack of knowledge on the subpopulation structure within PSC cultures. Therefore, in-depth analysis of datasets such as reported by Jin and colleagues (8) should be helpful for improving existing strategies and developing new approaches to differentiate PSCs into regenerative medicine-relevant cells and even 3D organoids. Although the study is not yet complete and signal enforcement involved for further differentiation of gastrulating cells remains unknown, this work provides a paradigm for understanding multiple biological processes at a single-cell resolution.

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