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Authors
Liu, Zhe
Sneddon, Julie B

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Pancreatic development: one cell at a (pseudo)time

Zhe Liu & Julie B Sneddon

Gaining a comprehensive understanding of the principles guiding lineage differentiation during organogenesis is a challenge. In this issue, Yu *et al* (2019) tackle this venture by taking advantage of single-cell transcriptomic analyses and multiple mouse genetic tools. Their work provides new insights into pancreatic endocrine and exocrine cell differentiation landscapes and identifies new pathways regulating pancreatic lineage allocation *in vivo*.

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See also: **X-X Yu *et al*** (April 2019)

Intensive work on the biology of the pancreas has focused on developing better treatments for devastating pancreatic diseases, including diabetes mellitus, pancreatitis, and pancreatic cancer. Notably, elucidation of the embryonic development of the pancreas, particularly the mechanisms underlying endocrine cell specification, has provided important guidelines for deriving pancreatic cells from human pluripotent stem cells for therapeutic purposes (Sneddon *et al*, 2018).

The pancreas is comprised of both endocrine and exocrine compartments. In mice, all pancreatic epithelial lineages arise from a pool of multipotent progenitor (MP) cells in the gut endoderm, marked by expression of the transcription factor pancreatic and duodenal homeobox 1 (*Pdx1*). From embryonic day (E) 9.5 to E10.5, rapid expansion of MP cells drives pancreatic bud growth, leading to the formation of an epithelial tube by E12.5. Between E12.5 and E15.5, bi-potential cells in the trunk of the epithelial tube are restricted to ductal cells and neurogenin3 (Ngn3)-expressing endocrine progenitor (EP) cells, whereas cells at the tip adopt an acinar fate. Thereafter, EP cells undergo allocation to differentiate into hormone-producing endocrine cells within the islets of Langerhans, including glucagon-producing α cells and insulin-producing β cells. In addition to this main lineage trajectory, a wave of initial α cells has been observed in the dorsal endoderm from E9.0 to E11.0 and is considered the earliest event-marking α-cell fate specification (Shih *et al*, 2013; Fig 1A).

Over the past several years, single-cell RNA-sequencing (scRNA-seq) has enhanced our understanding of pancreatic lineage trajectories during development, with multiple groups deciphering cell lineage dynamics during pancreas organogenesis. Taken together, these studies have revealed that seemingly equivalent embryonic pancreatic progenitors contain previously underappreciated cellular heterogeneity, including a new intermediate EP population defined by the expression of *Fev* (Byrnes *et al*, 2018) a subset of EP cells expressing *Myt1* biased toward β-cell fate (Liu *et al*, 2018), and a progenitor of α cells marked by the expression of *SLC38A5* (Stanescu *et al*, 2017).

In a new study, Yu *et al* (2019) capture 2,282 high-quality cells from various knock-in or transgenic mouse strains covering the full panoply of embryonic pancreatic epithelial developmental stages from E9.5 to E17.5 and subject them to transcriptomic analyses by Smart-seq2. The increased sequencing depth of Smart-seq2 technology compared to droplet-based microfluidics (Pijuan-Sala *et al*, 2018) provides a transcriptionally comprehensive embryonic pancreatic developmental landscape.

Using principal component analysis (PCA) and Monocle pseudotime analyses, Yu *et al* (2019) have identified fifteen cell population clusters expressing distinct gene features. Among them, they find two MP subpopulations: MP-early cells and MP-late cells (Fig 1B). MP-early cells express *Pdx1* but not *NeuroD1* and are mainly found at E9.5, whereas MP-late cells show enhanced expression of *Ptf1a* and *Sox9* and include cells from E10.5 and E11.5 stages. The existence of MP-early cells and MP-late cells is confirmed via *Nr2f2* expression, which is observed in E9.5 but not E10.5 pancreatic buds.

Consistent with previous findings, EP cells present from E13.5 to E15.5 contain multiple intermediate stages (Scavuzzo *et al*, 2018). In this paper, seven subgroups of 670 genes are found to be distinctly expressed in four transient EP stages, termed EP1 through 4, which represent progressively more mature EP states (Fig 1B). In addition, 48 transcription factors (TFs), including key fate regulators *Arx*, *Pax4*, and *Ngn3*, are variably expressed across EP1-EP4 cells; these dynamically expressed TF clusters may promote EP cells and islet lineage cell specification. Notably, *Fev*, a gene defined as a marker of a novel intermediate EP in a previous study (Byrnes *et al*, 2018), was validated as a marker of the EP3 and EP4 cell stages.

The events comprising cell fate determination of α cells have been relatively understudied compared to those of β cells. By generating a new α-cell reporter mouse strain (Gcg-P2A-GFP), the authors demonstrate several new steps along the trajectory of α-cell differentiation. At E9.5-E10.5, an early "first-wave" of α cells (pre-α-1st cells) expressing *Arx*, but not *Gcg*, derives from

Diabetes Center, University of California, San Francisco, San Francisco, CA, USA. E-mail: julie.sneddon@ucsf.edu
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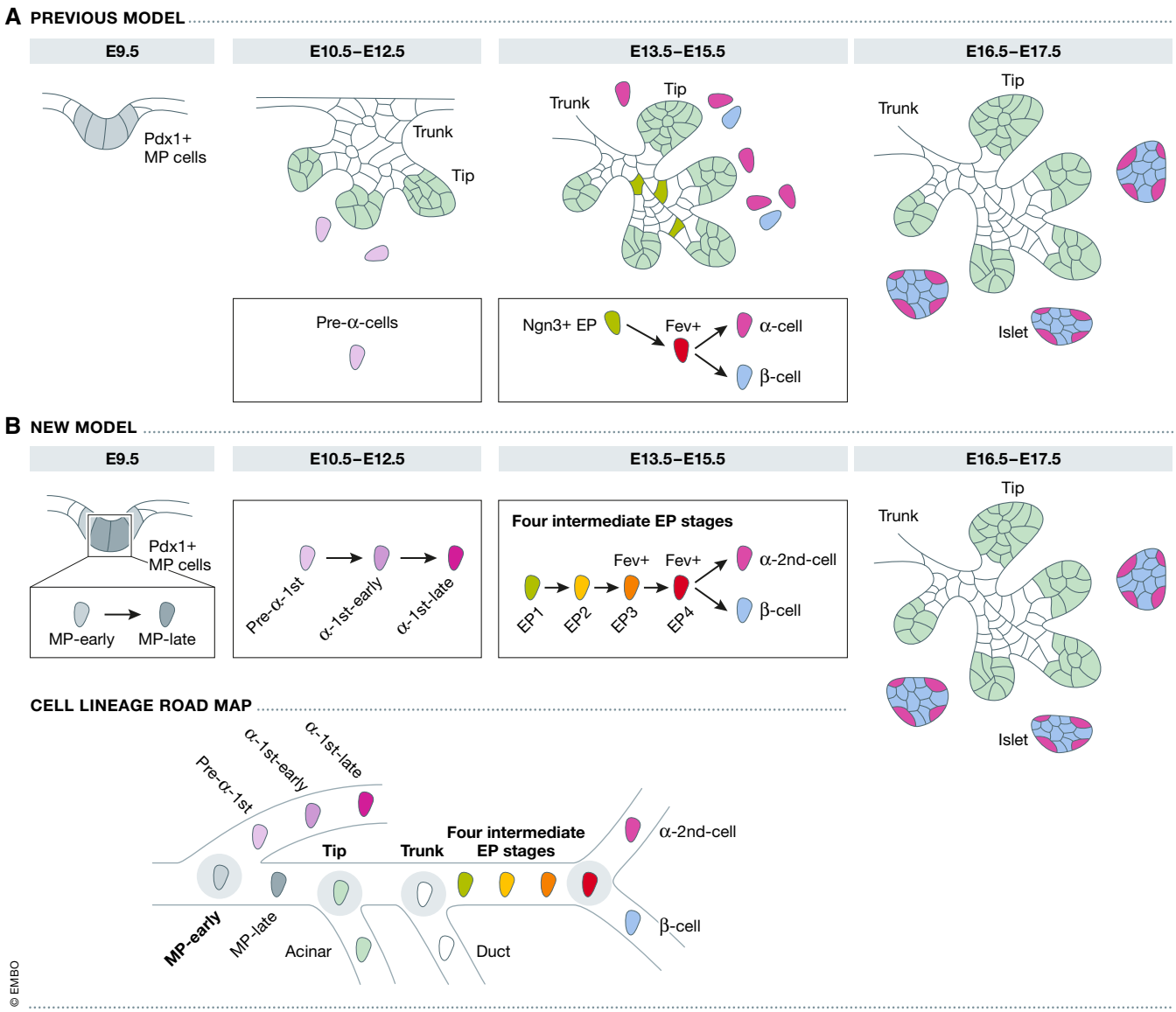

Figure 1. Single-cell transcriptomic analyses of pancreatic organogenesis in the mouse embryo.
Schematic illustration of cellular transition and branching models of pancreatic endocrine and exocrine cell differentiation based on previous (A) versus current (B) data.

MP-early cells; at E10.5-E13.5, pre-α-1st cells further give rise to α-1st-early and α-1st-late cells, which both express *Gcg* and *Arx*, while a well-differentiated "second-wave" of α-cell (α-2nd cells) highly expresses *Gcg* at E15.5 (Fig 1B). Collectively, these data indicate that α cells follow a previously unexplored step-wise path toward maturation. Furthermore, Yu *et al* (2019) also provide new insights into key transcriptional regulators controlling α cell maturation, which now await further investigation.

In addition to identifying new pancreatic progenitor subtypes along the path toward differentiation, the authors also highlight the concept of pancreatic cell branching nodes, whereby progenitor cells undergo fate restriction and slowly become definitive, differentiated endocrine or exocrine cell types. Taking advantage of PCA and pseudotemporal analyses, Yu and colleagues describe four key lineage branching nodes in the trajectory taken by differentiating epithelial cells. In this pancreatic development landscape, the first node is composed of MP-early cells, which then enter the MP-late cell stage and develop into tip cells. The second branch node is made up of the tip cells, which generate acinar and trunk cells. Trunk-early cells are considered the third node, where the bi-potential trunk-progenitor cells differentiate into ductal cells and EP cells, and the EP4 cells are the fourth and major branching node, from which islet lineage allocation begins (Fig 1B). This branching information shapes the skeleton of the overall pancreatic epithelial lineage trajectories.

Precise transcriptional regulation is required during α-cell and β-cell fate allocation. In this study, Yu *et al* (2019) find that the extracellular signal-regulated kinase (ERK) pathway is down-regulated during the

cell fate restriction of trunk cells to EP cells, which will further generate definitive islet cells. Administration of ERK signaling inhibitor U0126 to *in vitro* cultured *Ins1-RFP* or G*cg-GFP* pancreatic explants at E13.5 resulted in an increased percentage of RFP- or GFP-expressing cells after 48 h, suggesting that the repression of ERK signaling may be important for α-cell and β-cell fate specification. This finding reveals the power of scRNA–seq in examining key fate regulation pathways, but awaits further confirmation via orthogonal methods, such as mouse genetics.

Taken together, Yu *et al* (2019) provide a comprehensive roadmap of murine embryonic pancreatic epithelial cell lineage trajectories and transcriptional landscapes, which hold the potential to guide the application of developmental principles to therapeutic use. To that end, defining the developmental similarities between mouse and human pancreas will be an important next step. While scRNA–seq has broadened our understanding of pancreas development at the transcriptional level, future work can further flesh out the roles of epigenetic and post-transcriptional regulation to achieve an even more thorough understanding of pancreas organogenesis. As a complex organ, the pancreas contains diverse non-epithelial cell populations (e.g., nerves, smooth muscle cells, endothelium, and mesenchyme) that provide important cues for epithelial development (Golosow & Grobstein, 1962; Lammert *et al*, 2001). As described previously, the pancreatic mesenchyme harbors rich cellular heterogeneity that is yet to be functionally characterized (Byrnes *et al*, 2018). Integrating mesenchymal cell heterogeneity and lineage trajectories when investigating pancreas organogenesis will assist in modeling pancreas development as a whole.

References

Byrnes LE, Wong DM, Subramaniam M, Meyer NP, Gilchrist CL, Knox SM, Tward AD, Ye CJ, Sneddon JB (2018) Lineage dynamics of murine pancreatic development at single-cell resolution. *Nat Commun* 9: 1 – 17

Golosow N, Grobstein C (1962) Epitheliomesenchymal interaction in pancreatic morphogenesis. *Dev Biol* 4: 242 – 255

Lammert E, Cleaver O, Melton D (2001) Induction of pancreatic differentiation by signals from blood vessels. *Science* 294: 564 – 567

Liu J, Banerjee A, Herring CA, Attalla J, Hu R, Xu Y, Shao Q, Simmons AJ, Dadi PK, Wang S, Jacobson DA, Liu B, Hodges E, Lau KS, Gu G (2018) Neurog3-independent methylation is the earliest detectable mark distinguishing pancreatic progenitor identity. *Dev Cell* 48: 49 – 63 e7

Pijuan-Sala B, Guibentif C, Göttgens B (2018) Single-cell transcriptional profiling: a window into embryonic cell-type specification. *Nat Rev Mol Cell Biol* 19: 399 – 412

Scavuzzo MA, Hill MC, Chmielowiec J, Yang D, Teaw J, Sheng K, Kong Y, Bettini M, Zong C, Martin JF, Borowiak M (2018) Endocrine lineage biases arise in temporally distinct endocrine progenitors during pancreatic morphogenesis. *Nat Commun* 9: 1 – 21

Shih HP, Wang A, Sander M (2013) Pancreas organogenesis: from lineage determination to morphogenesis. *Annu Rev Cell Dev Biol* 29: 81 – 105

Sneddon JB, Tang Q, Stock P, Bluestone JA, Roy S, Desai T, Hebrok M (2018) Stem cell therapies for treating diabetes: progress and remaining challenges. *Cell Stem Cell* 22: 810 – 823

Stanescu DE, Yu R, Won K-J, Stoffers DA (2017) Single cell transcriptomic profiling of mouse pancreatic progenitors. *Physiol Genomics* 49: 105 – 114

Yu X-X, Qiu W-L, Yang L, Zhang Y, He M-Y, Li L-C, Xu C-R (2019) Defining multistep cell fate decision pathways during pancreatic development at single-cell resolution. *EMBO J* 38: e100164