# Sorting Out Fate Determination

Mostafa Bakhti,[1,2,3] Aimée Bastidas-Ponce,[1,2,3,4] and Heiko Lickert[1,2,3,4,*]
[1]Institute of Diabetes and Regeneration Research, Helmholtz Zentrum München, Neuherberg, Germany
[2]Institute of Stem Cell Research, Helmholtz Zentrum München, Neuherberg, Germany
[3]German Center for Diabetes Research (DZD), Neuherberg, Germany
[4]Technical University of Munich, Medical Faculty, Munich, Germany
*Correspondence: heiko.lickert@helmholtz-muenchen.de
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**How organ morphogenesis specifies cell fate and whether organ progenitors are predetermined or specified via niche signals are critical developmental biology questions. In this issue of *Developmental Cell*, Nyeng et al. (2019) modulate cell-cell adhesion in the pancreas and provide evidence that progenitors are plastic and instructed by niche signals.**

The developmental mechanisms linking morphogenesis and cell fate decisions are not well understood. One example is pancreas organogenesis, which starts with formation of an epithelial primordium that progressively gets patterned and segregates into central trunk and peripheral tip domains. Further development of these domains results in the differentiation of acinar cells in the tip and ductal and endocrine cells from the trunk. During the establishment of the tree-like tubular network, endocrine progenitors (EPs) arise from the bipotent trunk epithelial cells. Increased levels of Neurogenin3 (Neurog3) expression specify endocrine cells that delaminate to form hormone-producing cells in the islets of Langerhans (Bakhti et al., 2019). One of the key questions that remained to be addressed in this organ system is how lineage segregation occurs during pancreas development. Are the three main pancreatic lineages (acinar, ductal, and endocrine) derived from intrinsic predetermined progenitors? Or does progenitor differentiation depend on extrinsic signals from the surrounding niche? Although the common notion is that pancreas architecture defines lineage decision, this idea is challenged by recent studies reporting that predetermined unipotent progenitors exist before organ patterning (Larsen et al., 2017; Sznurkowska et al., 2018). Now, in this issue of *Developmental Cell*, Nyeng et al. (2019) reconcile apparently opposing models of progenitor predetermination and niche instruction by showing that differential cell-cell surface tension (dictated by a combination of adhesion and cortical tension) in pancreatic progenitors is important for cell sorting and pattern formation before environmental signals in the trunk and tip niche further lead to cell-fate determination.

For their study, the authors established several mosaic mutant and/or reporter mouse lines and performed long-term live imaging on organotypic pancreatic cultures over 2 days. They found that during early stages of pancreas development, the cells in the center actively move toward the periphery over a remarkably long distance. This raised the question of whether the progenitors were predetermined before their movement or instructed by the niche that they moved into. A first hint came from the careful analysis of immune localization studies of the protein p120-catenin. Before tip-trunk patterning, pancreatic progenitors express heterogeneous levels of p120-catenin (p120ctn$^{low}$ and p120ctn$^{high}$) and are randomly intermingled. However, the authors observed that during pattern formation, p120ctn$^{low}$ cells move toward the periphery and p120ctn$^{high}$ cells remain in the center (Figure 1A). This suggested that differential cell-cell surface tension of progenitors determines cell sorting and pattern formation and, potentially through niche signals, defines trunk (duct/endocrine) and tip (acinar) fates. To test this idea directly, the authors generated mosaic p120ctn or E-cadherin mutant mice to change cell-cell surface tension in a mosaic fashion. This led to the movement and enrichment of p120ctn-KO cells in the peripheral tips. Notably, the p120ctn-low or -KO (knockout) cells induced an acinar cell program after relocating toward the peripheral tip, which implies that the cells were not predetermined before movement but instead acquired the acinar fate in the tip domain. Thus, increased acinar differentiation is not a direct consequence of p120ctn reduction or loss but is a secondary result of cell repositioning, indicating that differential cell-cell surface tension eventually predetermines cellular fate through cell sorting.

The authors further found a remarkable reduction in p120ctn protein levels in insulin-expressing cells, as compared to bipotent ductal and Neurog3$^{+}$ EPs in mouse and human (Figure 1A). This finding indicates that after tip-trunk patterning, transient downregulation of cell-cell adhesion further segregates the endocrine lineage from the ductal epithelium. In addition, mosaic loss of p120ctn increased delamination of Neurog3$^{+}$ KO cells, which resulted in decreased numbers of β cells and increased numbers of α cells. This suggests that the duration of EP residency within the epithelium impacts endocrine lineage allocation. Accordingly, α cells are formed at early stages during development when cell polarity and cell-cell adhesion are not yet well established in the trunk epithelium, and these cells leave the niche faster. On the other hand, β cells are determined at later stages within the well-polarized and p120ctn$^{high}$ trunk epithelium. They stay longer in the niche due to higher cell-cell adhesion, which is downregulated upon delamination. Along the same line, the Semb laboratory has previously shown the requirement of established apical cell polarity for β-cell, but not α-cell, formation (Löf-Öhlin et al., 2017). Thus, the current study further supports the notion that cell polarity and adhesion-mediated

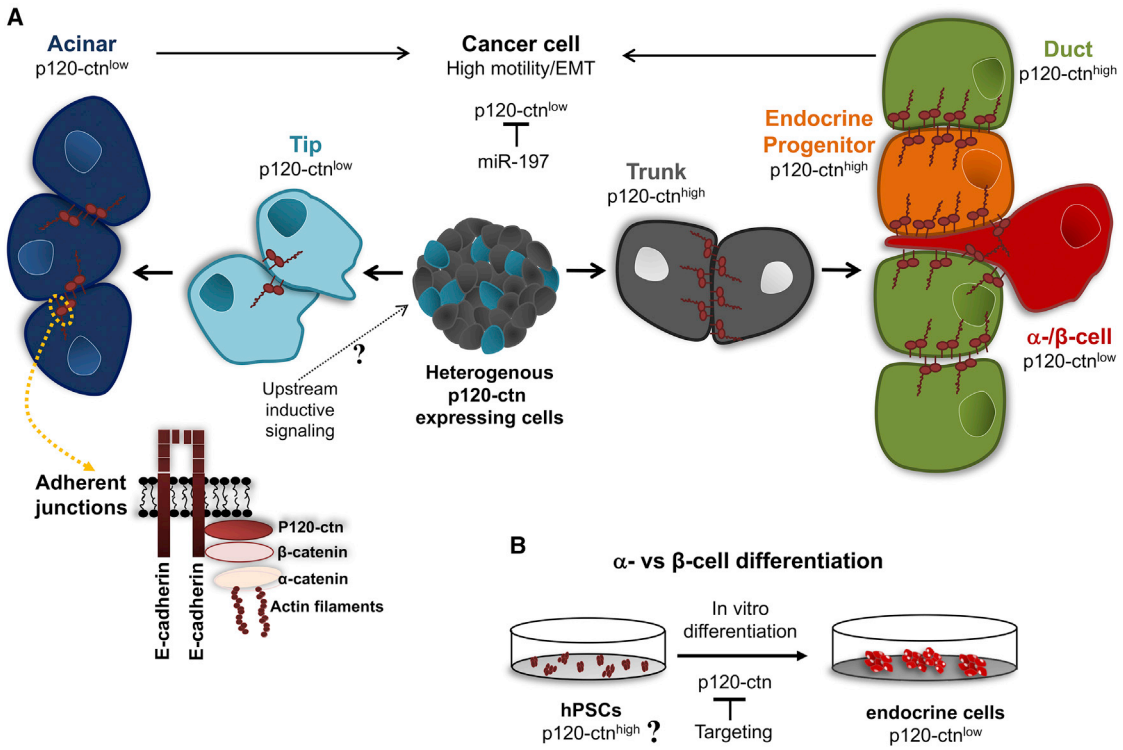


**Figure 1. Differential Expression of p120ctn Couples Pancreas Patterning and Lineage Segregation**

(A) Heterogeneous population of pancreatic progenitors segregate into tip or trunk domains based on their p120ctn protein levels where they are specified by niche signals. During endocrinogenesis, differentiating endocrine cells reduce the levels of p120ctn as compared to nearby epithelial cells. p120ctn reduction by miR-197 results in EMT in pancreatic cancer cells.

(B) Targeting of p120ctn in hPSCs may allow endocrine differentiation to be directed *in vitro*.

progenitor residency within the epithelium define endocrine subtype specification. Additionally, the majority of β cells were apically polarized and attached to the epithelial lumen upon differentiation. The authors observed that after β-cell birth, the cells lost polarity, formed membrane protrusions, and exhibited motile features before they delaminated from the epithelium and formed clusters (Figure 1A). These data challenge the current idea that β cells form after delamination, instead demonstrating that β cells are already specified and born in the trunk epithelium.

Over 60 years ago, differential cell-cell adhesion was shown to sort out the primary germ layers during gastrulation, but the molecular basis was unknown at that time (Townes and Holtfreter, 1955). The work of Nyeng et al. (2019) now further shows that differential cell-cell surface tension also directs cell sorting, tissue patterning, and cell-fate decisions in the pancreas. The authors have shown that differential expression of p120ctn first patterns pancreatic epithelium into tip and trunk and then further segregates endocrine and ductal lineages. These findings increase our understanding of pancreas patterning that drives cell differentiation. They also help to establish model systems for pancreas-related diseases (including pancreatic cancer, pancreatitis, and diabetes), develop a way to generate compact 3D pancreatic spheres for pancreatic progenitor expansion, and provide a means to induce α- and β-cell fate by modulating timing of intraepithelial progenitor residency or delamination.

Future work should address how p120ctn and cell-cell surface tension heterogeneity is regulated and drives patterning and cell-fate determination. Moreover, how differential expression of p120ctn and other adhesion junction molecules in pancreatic progenitors is regulated on a transcriptional and post-transcriptional level deserves further attention. This is particularly important in case of cancer metastasis that is highly connected to changes in cell-cell adhesion. For instance, targeting of p120ctn by miR-197 was shown to increase epithelial-mesenchymal transition (EMT) in pancreatic cancer cells (Hamada et al., 2013) (Figure 1A). Modulating cell-cell adhesion in metastatic tumor cells might move them into a different niche and change the cellular phenotype, such as the initiating step of acinar-to-ductal metaplasia during pancreatic cancer initiation. Furthermore, the molecular signature of heterogeneous progenitors based on their cell-cell adhesion levels needs to be studied using tools such as single-cell transcriptomics combined with measuring cell-surface adhesion proteins. This will contribute to a better understanding of how tissue morphogenesis, pattering, and differentiation are linked. Finally, the evidence that human endocrine cells also reduce levels of p120ctn upon differentiation suggests that modulating cell-cell adhesion in a temporal controlled manner might allow for the efficient generation of endocrine cell subtypes from human pluripotent stem cells (hPSCs) (Figure 1B).

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# The Multiple Ways Nuclei Scale on a Multinucleated Muscle Cell Scale

Caitlin B. Moffatt[1] and Orna Cohen-Fix[1,*]
[1]The Laboratory of Biochemistry and Genetics, The National Institute of Diabetes and Digestive and Kidney Diseases, The National Institutes of Health, Bethesda, MD 20892, USA
*Correspondence: ornac@niddk.nih.gov
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**In mononucleated cells, nuclear size scales with cell size, but does this relationship extend to multinucleated cells? In this issue of *Developmental Cell,* Windner et al. (2019) examine scaling of nuclei in multinucleated *Drosophila* muscle fibers and identify global and local cellular inputs that contribute to nuclear size regulation.**

Nuclear size scaling is a phenomenon conserved from single-celled to multicellular eukaryotic organisms: within a given cell type, there is a relatively constant nuclear:cell volume ratio, and this ratio is disrupted in many cancers (Jevtić and Levy, 2014). The significance of this ratio and its underlying mechanism are poorly understood. In this issue of *Developmental Cell*, Windner et al. (2019) explore nuclear scaling in multinucleated *Drosophila* muscle fiber cells. Given that multinuclear muscle fibers have distinct functional domains, this experimental approach could be used to explore the relationship between nuclear size and nuclear function. Such a link might also explain the distinct nuclear:cell volume ratios observed in different cell types. Moreover, the study of multinucleated cells allows for the assessment of global (i.e., cell-wide), regional (at a functional cellular domain), and local (nuclear surroundings) factors that might affect nuclear scaling. Indeed, the findings of this study show that all three contribute to nuclear size.

As in humans, *Drosophila* muscle fibers form by the fusion of diploid myoblasts, creating one cell with multiple nuclei—the myotube or muscle fiber (Figure 1A). The number of fusing myoblasts determines the number of nuclei per cell. Those nuclei then increase in ploidy via endoreplication, leading to an increase in gene expression and resulting in an increase in cell size (Figure 1B). Windner et al. (2019) focused on the *Drosophila* VL3 and VL4 larval muscle cells. VL3 muscle fibers are larger than VL4 cells and contain more nuclei, but in both cells, nuclei are evenly spaced, with predominantly one row of nuclei in VL4 cells and two rows in VL3. Due to their flat configuration, 2D measurements accurately reflect 3D values, facilitating the quantification of different parameters that may contribute to nuclear scaling (e.g., cell size, nuclear size, internuclear distances, etc.). Moreover, being in *Drosophila* provided the advantage of genetic tractability. Previous studies in *Drosophila* have linked muscle dysfunction with defects in nuclear positioning, a phenomenon also frequently seen in human muscular disorders such as muscular dystrophies (Metzger et al., 2012 and Folker and Baylies, 2013). Thus, multinucleated model systems recapitulating human musculature, such as the one employed here, provide an opportunity to study the relationship between nuclear behavior and disease.

In an elegant set of experiments, Windner et al. (2019) observed a range of nuclear sizes within a given cell. Nonetheless, as in mononucleated cells, total nuclear size correlated strongly with cell size in both VL3 and VL4 cells. This indicates that there are one or more global factors dispersed throughout the cell that determine the collective volume of the nuclei. Interestingly, however, larger nuclei tended to be located toward the center of the cell (Figure 1C). Thus, although there is