© 2020. Published by The Company of Biologists Ltd | Development (2020) 147, dev179051. doi:10.1242/dev.179051

REVIEW

Understanding generation and regeneration of pancreatic β cells from a single-cell perspective

Xin-Xin Yu and Cheng-Ran Xu*

ABSTRACT

Understanding the mechanisms that underlie the generation and regeneration of β cells is crucial for developing treatments for diabetes. However, traditional research methods, which are based on populations of cells, have limitations for defining the precise processes of β-cell differentiation and trans-differentiation, and the associated regulatory mechanisms. The recent development of single-cell technologies has enabled re-examination of these processes at a single-cell resolution to uncover intermediate cell states, cellular heterogeneity and molecular trajectories of cell fate specification. Here, we review recent advances in understanding β-cell generation and regeneration, *in vivo* and *in vitro*, from single-cell technologies, which could provide insights for optimization of diabetes therapy strategies.

KEY WORDS: Pancreas, β Cell, Single-cell RNA-seq, Cell lineage differentiation, Trans-differentiation, Cellular plasticity

Introduction

The pancreas is an important internal organ for the digestion of nutrients and maintenance of blood glucose homeostasis, the functions of which are executed by the exocrine and endocrine compartments, respectively. The exocrine compartment consists of acinar cells that secrete various digestive enzymes, and ductal cells that transport these enzymes into the duodenum. The endocrine compartment senses changes in blood glucose and executes regulatory functions via the islets of Langerhans, stereotypically organized structures that are composed of five hormone-secreting cell types: insulin (Ins)-secreting β cells, glucagon (Gcg)-secreting α cells, somatostatin (Sst)-secreting δ cells, pancreatic polypeptide (Ppy)-secreting PP cells, and ghrelin (Ghrl)-secreting ε cells (reviewed by Pan and Wright, 2011; Bastidas-Ponce et al., 2017; Larsen and Grapin-Botton, 2017). It is the dysfunction of insulin-producing β cells that leads to diabetes (DeFronzo et al., 2015; Katsarou et al., 2017).

Affecting approximately 451 million people, diabetes is a worldwide health problem and imposes a heavy burden on patients and societies (Cho et al., 2018). Type 1 diabetes (T1D), which accounts for 5-10% of cases, is caused by insufficient secretion of insulin due to attack of β cells by the immune system. Type 2 diabetes (T2D) is much more common and is caused by insulin resistance of surrounding tissues, such as the liver, muscle and fat. Currently, the major approaches used to alleviate the symptoms of diabetes are the transplantation of cadaveric islets and exogenous insulin injection, but these have the disadvantages of donor shortage and inaccurate glucose control, respectively (Aguayo-Mazzucato and Bonner-Weir, 2018; Tan et al., 2019). Sustainable sources of functional β cells or islets represent a promising method for improved diabetes treatment. The sources for β cells include: endogenous regeneration of β cells; plasticity and trans-differentiation of non-β cells into insulin-secreting cells; and exogenous induction of β cells from human embryonic stem cells (hESCs) (Aguayo-Mazzucato and Bonner-Weir, 2018; Zhou and Melton, 2018). To successfully generate mature β cells, or construct whole islets *in vitro*, we must understand the pathways and regulatory mechanisms that guide the development and maturation of pancreatic endocrine lineages. To induce endogenous regeneration of β cells through promoting β-cell proliferation, or trans-differentiation of non-β cells into insulin-secreting cells, we must understand the level of cellular heterogeneity in these populations, the mechanisms that regulate cell plasticity and the molecular relationships between endocrine lineages. All these efforts will facilitate development of cell therapies for diabetes.

In the past few decades, research in both animal models and human specimens has furthered our understanding of β-cell development and regeneration to drive exciting advances in diabetes therapy. However, we still do not fully understand the molecular characteristics, cell lineage differentiation pathways and cell fate plasticity of pancreatic endocrine cells. Fortunately, owing to the recent development of single-cell technologies, researchers have been able to re-examine the regulation of development and regeneration of endocrine cells at the single-cell level. Single-cell technology is well suited for the study of complex organs such as the pancreas and facilitates the mapping of the developmental trajectory of each cell lineage, which will further our understanding of the regulation of cell fate determination.

Many single-cell approaches have been developed in recent years (summarized in Table 1). Among these, single-cell RNA sequencing (scRNA-seq) has been commonly used in developmental and regenerative biology. Different scRNA-seq methods (Box 1) have been used to: describe cell subpopulations or cell heterogeneity; identify novel marker genes in cell populations; define the pathways of lineage development and transition; identify regulatory cues for cell fate determination; and trace an organism's cell lineage tree (Kumar et al., 2017; Griffiths et al., 2018; Pijuan-Sala et al., 2018; Potter, 2018; Chan et al., 2019; Chen et al., 2019; Morris, 2019).

In the pancreas field, many studies have used single-cell technology to resolve key issues that have been unresolved by cell population-based studies, and to provide insights into β-cell neogenesis, maturation, regeneration and heterogeneity (summarized in Tables 2-4). This Review summarizes recent pancreatic studies that have increased our understanding of the generation and regeneration of β cells from a single-cell perspective. We largely focus on scRNA-seq studies, but do mention other single-cell approaches where appropriate.

Ministry of Education Key Laboratory of Cell Proliferation and Differentiation, College of Life Sciences, Peking-Tsinghua Center for Life Sciences, Peking University, Beijing, 100871, China.

*Author for correspondence (cxu@pku.edu.cn)

C.-R.X., 0000-0002-0583-4464

Received 7 October 2019; Accepted 20 February 2020

Development (2020) 147, dev179051. doi:10.1242/dev.179051

Table 1. Examples of single-cell techniques

Single-cell techniques	Type of information	Reference
Single-cell RNA sequencing (scRNA-seq)	Global transcriptome	Reviewed by Chen et al., 2019
Single-cell CyTOF (scCyTOF)	Expression of selected proteins	Reviewed by Spitzer and Nolan, 2016
Single-cell assay for transposase-accessible chromatin using sequencing (scATAC-seq)	Epigenome: chromatin accessibility	Cusanovich et al., 2015; Chen et al., 2018; Clark et al., 2018; Satpathy et al., 2019
Single-cell chromatin immunoprecipitation sequencing (scChIP-seq)	Epigenome: DNA-associated protein binding sites	Ai et al., 2019; Carter et al., 2019; Grosselin et al., 2019; Kaya-Okur et al., 2019; Wang et al., 2019
Single-cell DNA methylation (scDNA methylation)	Epigenome: transcription activity	Reviewed by Karemaker and Vermeulen, 2018
Single-cell Hi-C (scHi-C)	Epigenome: 3D chromatin organization	Nagano et al., 2013; Ramani et al., 2017; Zhou et al., 2019
Single-molecule fluorescence *in situ* hybridization (smFISH)	Spatial transcriptome	Reviewed by Mayr et al., 2019

Pancreas development

Development of the rodent pancreas

In rodents, the pancreas is derived from two origins (the dorsal and ventral endoderm) that respond to different sets of signals from surrounding tissues to form dorsal and ventral pancreatic buds at embryonic day (E) 9.0 and E9.5, respectively (Pan and Wright, 2011; Bastidas-Ponce et al., 2017) (Fig. 1). These buds are composed of multipotent progenitor (MP) cells expressing *Pdx1* and the buds fuse at completion of intestinal rotation at ∼E12.5 (Pan and Wright, 2011; Shih et al., 2013). Subsequently, MP cells differentiate into tip and bipotent trunk cells. Tip cells further specify into acinar cells, whereas trunk cells differentiate into ductal or endocrine cells. Cell fate determination of trunk cells is regulated by Neurog3 (Ngn3), a key transcription factor (TF) for the formation of endocrine progenitors (EPs). It is generally accepted that the endocrine cells originate from *Ngn3*$^{+}$ EPs and that the specification of each endocrine lineage is temporally distinct (Gradwohl et al., 2000; Gu et al., 2002; Heller et al., 2005; Johansson et al., 2007). Although, as a population, *Ngn3*$^{+}$ can produce all specialized endocrine cells, individual *Ngn3*$^{+}$ cells are unipotent precursors to differentiate into one of the endocrine cell types (Desgraz and Herrera, 2009). Pancreas development undergoes two transitionary periods: a first wave transition (from E9.5-E12.5) during which the endocrine cells that are formed are primarily *Gcg*-expressing cells or multi-hormonal cells (Pan and Wright, 2011; Shih et al., 2013; Bastidas-Ponce et al., 2017), and a second wave transition (from E12.5 to birth), which is the main period of endocrine cell formation. Previous reviews have summarized the developmental process, and related regulatory TFs and signals for each pancreatic lineage (Pan and Wright, 2011; Shih et al., 2013; Bastidas-Ponce et al., 2017; Larsen and Grapin-Botton, 2017).

Genetic studies in rodent models have uncovered the main framework of pancreatic lineage differentiation and have identified many important factors involved in regulating key steps of pancreatic lineage differentiation (Pan and Wright, 2011; Shih et al., 2013; Bastidas-Ponce et al., 2017; Larsen and Grapin-Botton, 2017). However, traditional studies that are based on populations of cells and limited marker genes may mask cell heterogeneity and obscure intermediate progenitor cells that are involved in cell lineage differentiation. These limitations thus affect analyses of cellular differentiation pathways and their underlying regulatory mechanisms. Therefore, decoding developmental process at single-cell resolution can guide us to a more comprehensive understanding of pancreatic organogenesis.

Development of the human pancreas

Compared with our understanding of rodent pancreatic development, we know little regarding the development of human fetal pancreas. The human pancreas is also derived from a dorsal and a ventral endoderm domain; the dorsal bud is detected at 26 dpc (day post conception) and the ventral bud at ∼30 dpc. The buds are fused upon gut rotation between 6-8 weeks of gestation (Piper et al., 2004; Jennings et al., 2013). Generally, the lineage hierarchy and molecular features of human pancreas are considered to be very similar to mouse, and the details of human pancreas development have been reviewed elsewhere (Pan and Brissova, 2014; Jennings et al., 2015; Baeyens et al., 2018; Petersen et al., 2018). In brief, *PDX1*$^{+}$ MP cells segregate into tip and trunk compartments, and the endocrine cells are generated from bipotent trunk cells through a transient *NGN3*$^{+}$ state. Deficiency of *NGN3* leads to the loss or reduction of endocrine cells in humans (Pinney et al., 2011; Rubio-Cabezas et al., 2011; McGrath et al., 2015; Zhu et al., 2016). Importantly, the functions of some key TFs are conserved in regulating cell identity between humans and mice: α-cell formation requires *ARX* (Itoh et al., 2010), and β cells specifically express *NKX6-1* in adult islets (Riedel et al., 2012). However, species differences do exist between human and mouse pancreatic

Box 1. scRNA-seq methods: advantages and disadvantages

Many scRNA-seq methods have been developed in recent years, including well-based methods, such as Smart-seq2 (Picelli et al., 2014), STRT-seq (Islam et al., 2012), STRT-seq-2i (Hochgerner et al., 2017) and CEL-seq2 (Hashimshony et al., 2016), as well as droplet-based methods, such as Drop-seq (Macosko et al., 2015), inDrop (Klein et al., 2015a) and the commercial 10x Genomics platform (Zheng et al., 2017). Generally, droplet-based methods provide greater cell throughput than well-based methods. Each method has its own advantages and disadvantages, and some of them have been systematically compared (Haque et al., 2017; Picelli, 2017; Ziegenhain et al., 2017; Zhang et al., 2019). In short, the well-based methods, such as Smart-seq2, have higher sensitivity for gene detection, but they also cost more per cell. Smart-seq2 generates full-length transcripts and better detects low-redundant transcripts. It is suitable for distinguishing subtle differences in transcripts that may be important in defining intermediate cell populations and developmental trajectory. The droplet-based methods are based on a microfluidic device and have significant advantages of generating a large number of cells from tissue at a lower cost, but at the expense of sensitivity. Therefore, the droplet-based methods are suitable for capturing rare cells and identifying cell composition in tissues, but might have limitations in defining intermediate progenitor cells and precise developmental pathways.

Development (2020) 147, dev179051. doi:10.1242/dev.179051

Table 2. Summary of the insights into differentiation and maturation of pancreatic lineages from single-cell studies of the mouse and human pancreas

Species	Tissue	Stage	Method	Number of cells	Conclusion	Reference
Mouse	Dorsal and ventral pancreas	E9.5, E10.5	Smart-seq2 scRNA-seq	447	Defining the developmental pathways of two populations of *Pdx1*-expressing cells ($Pdx1^{low}$ and $Pdx1^{high}$) in the ventral and dorsal pancreas. $Pdx1^{low}$ cells are intermediate progenitors to become hepatoblasts, extrahepatic bile ducts and $Pdx1^{high}$ pancreatic progenitors in ventral region, whereas $Pdx1^{low}$ cells are precocious endocrine cells in the dorsal region.	Li et al., 2018
Mouse	Dorsal pancreas	E9.5-E17.5	Smart-seq2 scRNA-seq	2702	Identification of developmental trajectory with four branch nodes and several intermediate cell states during pancreatic development, including the generation pathway toward first wave of α cells. Identification of four developmental stages of EPs (EP1-EP4). Identification of repressed role of ERK pathway for induction of α and β lineages.	Yu et al., 2019
Mouse	Pancreas	E12.5-E15.5	10x Genomics scRNA-seq	36,351	Identification of pancreatic endocrinogenesis roadmap. Identification of signature genes showing the same transient expression dynamics as *Ngn3*.	Bastidas-Ponce et al., 2019
Mouse	Pancreas	E13.25, E15.25	Smart-seq2 scRNA-seq	303	Combining lineage tracing strategy, quantitative biophysical modeling and scRNA-seq to define developmental process of pancreatic precursors.	Sznurkowska et al., 2018
Mouse	Pancreas	E13.5	Fluidigm C1 platform scRNA-seq	77	Identification of SLC38A5 as a differentiation marker of potential α cell precursors.	Stanescu et al., 2017
Mouse	Pancreas	E12.5, E14.5, E17.5	10x Genomics scRNA-seq	18,294	Identification of heterogeneity in the developing mesenchyme of the pancreas. Mapping developmental trajectory from Fev^{+} population toward α and β cells.	Byrnes et al., 2018
Mouse	Pancreas	E12.5-E15.5, E18.5	SORT-seq scRNA-seq	4620	Identification of roadmap and dynamic gene signatures toward α and β cells.	van Gurp et al., 2019
Mouse	Pancreas	E13.5-E15.5	Smart-seq scRNA-seq	440	Combining single cell transcriptomic analysis and 3D microscopic imaging, the authors propose a new model of islet formation during pancreas development.	Sharon et al., 2019a
Mouse	Pancreas	E14.5	InDrop platform scRNA-seq	1635	$Myt1^{+}Ngn3^{+}$ cells are biased towards β cells because of higher methylation at the enhancer region of *Arx*.	Liu et al., 2019
Mouse	Pancreas	E14.5, E16.5	Drop-seq scRNA-seq	17,234	Identification of four EP subtypes; compared with E14.5 EPs, E16.5 EPs have a higher tendency to generate β cells and exhibit a temporary shift in chromatin-accessible regions to enrich β cell-associated motifs.	Scavuzzo et al., 2018
Mouse	Pancreas	E15.5, E18.5	10x Genomics scRNA-seq	13,531	Characterizing the single-cell transcriptomes of mouse and hESC-derived endocrine progenitors. hESC-derived endocrine cells are similar to β cells.	Krentz et al., 2018
Mouse	β and α cells	E17.5, P0, P3, P9, P15, P18, P60	Smart-seq2 scRNA-seq	866	Defining maturation pathway and heterogeneity of β and α cells. Identification of proliferative β and α cells which show synchronously mature state with quiescent cells.	Qiu et al., 2017
Mouse	β Cells	P1, P7, P14, P21, P28	Fluidigm C1 platform scRNA-seq	387	Defining the maturation pathway and heterogeneity of β cells. Amino acid uptake and ROS levels promote β-cell proliferation.	Zeng et al., 2017
Mouse	β Cells	Adult	Fluidigm C1 platform scRNA-seq	207	Age-related alterations in gene expression in young and old mouse cells are similar.	Xin et al., 2016b
Human	Pancreas	9 WD	sc-qPCR	683	Addressing the developmental path and heterogeneity of the sorted human fetal pancreatic populations. Identification of three branches – β-track, α and γ-track, and δ-track – originated from the EP cells.	Ramond et al., 2018
Human	Islets	Juvenile, young adult, adult/ middle aged	Smart-seq2 scRNA-seq	2544	The aging of endocrine cells is associated with increased transcriptional noise and cell identity drift.	Enge et al., 2017

Development (2020) 147, dev179051. doi:10.1242/dev.179051

Table 3. Summary of the molecular features and heterogeneity of adult pancreatic cells

Species	Tissue	Age	Method	Number of cells	Conclusion	Reference
Mouse	Islets	Adult	Fluidigm C1 platform scRNA-seq	341	Identification of cell-type-specific TFs and pathways in islet cells.	Xin et al., 2016c
Mouse	Pancreas	Adult	smFISH	–	Identification of β-cell heterogeneity: a subpopulation of β cells express higher mRNA levels of insulin and other secretory genes, and contain higher ribosomal RNA and proinsulin, but lower insulin proteins, suggesting that they may be basal secretors.	Farack et al., 2019
Human	Islets	18 days to 65 years	scCyTOF	–	Quantification of human islet composition. Proliferation capacities of β, α and δ cells decline with age. β and α cells exhibit heterogeneous proliferative capacity.	Wang et al., 2016a
Mouse	Acinar cells	Adult	Smart-seq2 scRNA-seq	108	Identification of acinar heterogeneity: a proliferative subpopulation is capable of long-term self-renewal and displays higher *STMN1* expression.	Wollny et al., 2016
Human	Islets	Adult	Smart-seq2 scRNA-seq	70	Identification of marker genes for islet cell types and diabetes risk genes.	Li et al., 2016
Human	Islets	Adult (healthy and T2D)	Smart-seq2 scRNA-seq	2209	Identification of endocrine lineage-specific genes and T2D-associated genes. Identification of heterogeneity of acinar, α and β cells.	Segerstolpe et al., 2016
Human	Islets	Children, adult (healthy, T1D and T2D)	Smart-seq scRNA-seq	635	T2D donor α and β cells exhibit similar expression profiles to children. Identification of sonic hedgehog signals that may be involved in regulating α-cell proliferation.	Wang et al., 2016b
Human	Islets	Adult	CEL-seq scRNA-seq	1728	Description of putative subpopulations of ductal cells with distinct potential to differentiate into endocrine and acinar cells using the StemID algorithm.	Grün et al., 2016
Human	Islets	Adult	CEL-seq2 scRNA-seq	3005	Identification of cell-type-specific genes. Identification of heterogeneity of acinar and β cells. Enrichment of α and β cells by surface markers CD24 and TM4SF4.	Muraro et al., 2016
Human	Islets	Adult (healthy and T2D)	Fluidigm C1 platform scRNA-seq	3709	Identification of islet cell-type-specific genes and disease-related genes. Identification of transcriptomic differences between mice and humans.	Xin et al., 2016a
Human	Islets	Adult (healthy and T2D)	Fluidigm C1 platform scRNA-seq	638	Identification of cell-type-specific genes and T2D-related genes, and several interesting receptors in δ cells.	Lawlor et al., 2017
Human	Islets	Adult	10x Genomics scRNA-seq	19,174	Identification of five subpopulations of healthy human β cells with variable insulin gene expression and UPR activation.	Xin et al., 2018
Human and mouse	Islets	Adult	InDrop platform scRNA-seq	8629 1886	Identification of cell-type-specific genes. Identification of heterogeneity of ductal and β cells.	Baron et al., 2016

development (Pan and Brissova, 2014; Jennings et al., 2015; Baeyens et al., 2018; Petersen et al., 2018). For example, *NKX2-2* is downstream of *NGN3* in humans but upstream of *Ngn3* in mice (Jennings et al., 2013). Moreover, the order of emergence of the endocrine lineages is different: the insulin-expressing cells appear first in humans, whereas the glucagon-expressing cells appear first in mice (Piper et al., 2004; Jeon et al., 2009). In addition, there has been no clear evidence of first wave transition during human pancreatic development (Villasenor et al., 2008; Jennings et al., 2013).

Notably, researchers should be cautious when applying conclusions drawn from rodent models to human developmental processes, because the differences in cell lineage differentiation pathways and the regulatory mechanisms between the species have not been comprehensively addressed. Therefore, defining a blueprint for the development of the human pancreatic lineage is crucial for our understanding of the developmental process and regulatory mechanisms of the human pancreas. However, because of the scarcity of human tissues, large animal models with similar physiology to humans, such as pigs and non-human primates, can be used as alternative models of human pancreatic research (Zhu et al., 2014; Bakhti et al., 2019; Kim et al., 2019 preprint). Next, we review the single-cell transcriptomic studies of pancreas development in rodent models and humans.

Defining pancreatic lineage differentiation pathways using single-cell studies

Recent research using well-based scRNA-seq approaches (Box 1), has produced a more comprehensive understanding of pancreatic development, including the differentiation trajectories of lineages and intermediate cell states (Table 2), as well as the key genes of each cell population (Tables 2 and 3).

Differentiation and heterogeneity of MP cells

Although the generation of MP cells occurs at the earliest stage of pancreatogenesis and MP cells are not the direct progenitors of endocrine lineages; the generation of high-quality MP cells is a crucial step for obtaining functional endocrine β cells (Russ et al., 2015). To understand MP cell differentiation in the dorsal and ventral pancreatic regions in mice, Smart-seq2 scRNA-seq has been performed on *Pdx1*$^+$ cells from both regions at E9.5 and E10.5 (Li et al., 2018). These analyses have revealed that *Pdx1*$^+$

Development (2020) 147, dev179051. doi:10.1242/dev.179051

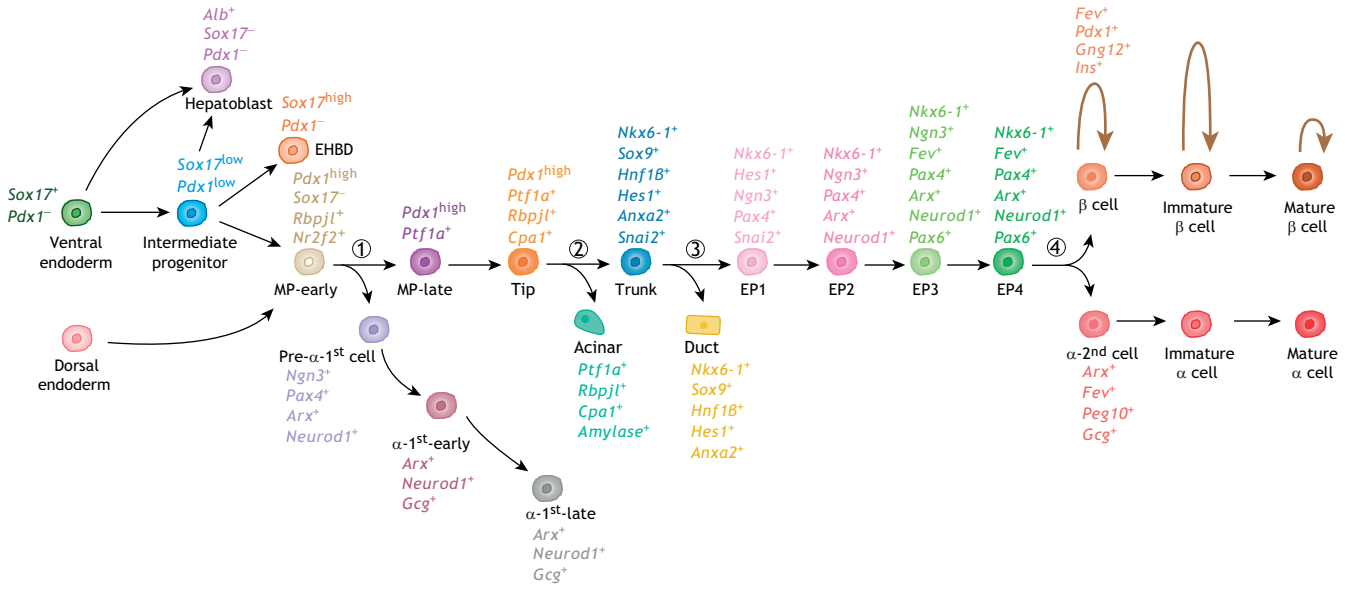

Fig. 1. Developmental model of mouse pancreatic exocrine lineages and α/β lineages. The pancreas is derived from the ventral and dorsal endoderm, which stepwise differentiate into exocrine and endocrine lineages. In the ventral region, Pdx1^{low} intermediate progenitors are multipotent, with the potential to become hepatoblasts, extrahepatic bile ducts (EHBDs) and Pdx1^{high} multipotent progenitor (MP) cells. Starting from MP-early cells, there are four branched nodes (1-4) along the pancreatic development pathway. Endocrine progenitor (EP) cells can be divided into four stages (EP1-EP4). Marker genes of each cell population are listed. The height of brown arrows represents the proliferative ability of β cells, and immature cells have a higher proliferative rate.

cells are heterogeneous, and can be divided into Pdx1^{low} and Pdx1^{high} populations in both the dorsal and ventral regions (Fig. 1). In the ventral region, Pdx1^{low} cells represent intermediate MPs that will become hepatoblasts, extrahepatic bile ducts (EHBDs) and Pdx1^{high} MP cells. However, in the dorsal region, Pdx1^{low} cells are the first-wave of endocrine cells and Pdx1^{high} cells are MP cells that, presumably, directly differentiate from endoderm cells. Therefore, this work has furthered our understanding of the differences between dorsal and ventral pancreatic programs (Rodriguez-Seguel et al., 2013; Li et al., 2018) (Fig. 1).

Subsequently, MP cells initiate stepwise differentiation toward the exocrine and endocrine lineages. Our recent work has comprehensively mapped the multistep developmental trajectory of exocrine and endocrine (α- and β-cell) lineages by Smart-seq2 scRNA-seq from the E9.5-E17.5 dorsal pancreas using various mouse lines (Yu et al., 2019). Along this pancreatic lineage differentiation trajectory, we have identified several intermediate cell states and branch points (nodes) at which progenitor cells undergo fate changes (Fig. 1). From our data, MP cells can be divided into MP-early (E9.5) and MP-late (E10.5) cells according to the developmental time of their appearance. MP-early cells serve as the first node and differentiate into tip cells or the first wave of α cells (α-1st cells) through intermediate states (MP-late and pre-α-1st cells, respectively) (Fig. 1, 1). At the second node, tip cells further specify into trunk and acinar cells (Fig. 1, 2), and trunk cells represent the third node for differentiation into ductal cells and EPs (Fig. 1, 3).

Differentiation and heterogeneity of EPs

As the endocrine pancreatic lineages, especially α- and β-cell lineages, are responsible for homeostasis of blood glucose and are therefore relevant to translational medicine for therapy, the majority of single-cell studies have focused on endocrinogenesis (Table 2). Several groups have surveyed the process of pancreatic endocrine generation and have revealed that mouse EPs are heterogeneous, with dynamic changes in featured gene expression and a temporal propensity towards differentiation into distinct islet lineages (Byrnes et al., 2018; Scavuzzo et al., 2018; Bastidas-Ponce et al., 2019; van Gurp et al., 2019; Yu et al., 2019). However, the sub-

Table 4. Cell conversion from non-β cells to β cells in adults

Species	Tissue	Method	Number of cells	Conclusion	Reference
Mouse	α Cells	Smart-seq2 scRNA-seq	182	scRNA-seq transcriptomic analysis provides evidence for α-cell conversion to β cells following *Arx* and *Dnmt1* ablation in mice.	Chakravarthy et al., 2017
Human	Islets	Smart-seq2 scRNA-seq	106	scRNA-seq analysis show that in artemether-treated α cells from primary human islets, the downregulation of α cell-specific genes and upregulation of key β cell-specific genes resulted in a conversion of α to β-like cells.	Li et al., 2017
Human	*In vitro* modified α cells	10x Genomics scRNA-seq	532	scRNA-seq analysis resolved the transcriptomic profiles and reprogramming status of converted α cells with ectopic expression of *PDX1* and *MAFA* to β cells.	Furuyama et al., 2019

Development (2020) 147, dev179051. doi:10.1242/dev.179051

classification of EP cells defined by these different groups are not completely consistent.

Based on our Smart-seq2 scRNA-seq study, EPs were defined as the cells that differentiate from bipotent trunk cells and express a cluster of marker genes including *Ngn3* and *Neurod1*, but do not yet express hormonal genes (Yu et al., 2019). Our study has shown that the rapid fate shift of EPs can be divided into four developmental stages (EP1-EP4) and involves a cascade of gene expression for a series of TFs (Fig. 1). Compared with a fraction of *Ngn3* lower-expressing trunk cells, *Ngn3* expression increases in EP1, peaks in EP2, decreases in EP3 and returns to background level in EP4, and *Neurod1* starts to become expressed in EP2 cells. The EP4 population serves as a branch node to generate α and β cells (Yu et al., 2019) (Fig. 1, 4). Scavuzzo and colleagues have classified *Ngn3*-expressing cells into four subpopulations at E14.5, namely N14_1-N14_4 (Scavuzzo et al., 2018). However, the N14_2 population expresses markers of mesenchymal cells and deviates from the developmental trajectory formed by other three subpopulations. Considering that droplet-based scRNA-seq inevitably causes doublet contamination, the existence of the N14_2 population needs to be verified. Based on the expression patterns of marker genes, N14_1 roughly corresponds to the *Ngn3*-expressing trunk cells and the EP1 population; N14_3 is similar to the EP2 population, and N14_4 covers EP3 and a fraction of EP4 cells. In another study, Fev^{+} cells are defined as an intermediate EP population because Fev^{+} cells connect *Ngn3*-expressing cells and hormone-expressing cells on a pseudotime-ordered pathway. It has been confirmed by lineage tracing that Fev^{+} cells produce the majority of endocrine cells (Byrnes et al., 2018). This finding is consistent with previous studies that have shown that *Fev* is induced by *Ngn3*, and is expressed in EP cells and islet cells (Miyatsuka et al., 2009; Ohta et al., 2011). Notably, *Fev* is highly expressed in EP3 and EP4 cells (Yu et al., 2019), and N14_4 cells (Scavuzzo et al., 2018), and these *Fev*-expressing EP cells have also been confirmed by other scRNA-seq studies (Bastidas-Ponce et al., 2019; van Gurp et al., 2019). Later, Fev^{+} cells that co-express paternally expressed 10 (*Peg10*) tend to differentiate into α cells, whereas those that co-express G protein subunit gamma 12 (*Gng12*) are biased to become β cells (Byrnes et al., 2018) (Fig. 1, 4).

Several studies have provided evidence to support that the development and heterogeneity of EP cells are regulated at the chromatin level (Scavuzzo et al., 2018; Yu et al., 2018; Liu et al., 2019). Our work has uncovered a regulatory role of the histone demethylase Jmjd3 (also known as Kdm6b) during the EP cell transition (Yu et al., 2018). We used an Ngn3-GFP knock-in mouse strain to purify Ngn3-GFP^{low} (containing *Ngn3*-expressing trunk cells and EP1 cells) and Ngn3-GFP^{high} cells (mainly including EP2-EP4 cells). Genetic knockout of *Jmjd3* in the pancreas impairs the transition of EP cells from Ngn3-GFP^{low} to Ngn3-GFP^{high} states and subsequent islet formation. Using scRNA-seq, we found that Jmjd3 only affects the efficiency of the cell fate transition from Ngn3-GFP^{low} to Ngn3-GFP^{high} but does not alter the transcriptomes and developmental pathways of $Ngn3^{high}$ cells (Yu et al., 2018). Liu and colleagues have proposed that the EPs are transcriptionally uniform but epigenetically distinct (Liu et al., 2019). Using scRNA-seq, the authors have shown that mouse $Ngn3^{+}$ cells heterogeneously express *Myt1*, which encodes a TF involved in regulating endocrine islet cell differentiation and function (Wang et al., 2007, 2008). $Myt1^{+}Ngn3^{+}$ and $Myt1^{-}Ngn3^{+}$ cells are biased toward β- and α-cell fates, respectively. $Myt1^{+}Ngn3^{+}$ cells highly express DNA methyltransferase 1 (*Dnmt1*), resulting in a higher level of DNA methylation at the enhancer region of *Arx*, a key TF for α-cell differentiation. Thus, the expression of *Arx* is repressed in $Myt1^{+}Ngn3^{+}$ cells (Liu et al., 2019). In addition, mouse *Ngn3*-expressing cells at E14.5 and E16.5 show differences in transcriptome and chromatin accessibility (Scavuzzo et al., 2018). E16.5 cells exhibit lineage bias towards β cells, because motifs of TFs that positively regulate β-cell generation are enriched at the open chromatin regions (Scavuzzo et al., 2018). Supporting this temporal lineage bias model, Sharon and colleagues combined scRNA-seq and 3D microscopic imaging of whole-mount immunostaining to propose a growing peninsula model to describe islet formation in mouse embryonic pancreas (Sharon et al., 2019a). In this model, EP cells are considered to leave the epithelial cord but remain attached and form a growing bud, which depends on the continuous recruitment of the differentiated endocrine cells. Notably, the earlier appearing α cells are pushed outward by the β cells generated later, and eventually the α cells reside in the peripheral region of the mouse islets (Sharon et al., 2019a).

Taken together, these studies have revealed the heterogeneity of EP cells during endocrinogenesis; however, the classifications of EP cells generated by different scRNA-seq platforms are not uniform. Integrated analysis of these datasets might determine the correspondence between these classifications. The heterogeneity of EP cells may reflect their differentiation ability or tendency for a specific endocrine lineage, which might be epigenetically regulated at a distinct developmental time. In addition, combining scRNA-seq analysis with traditional genetic, epigenetic and imaging methods has become a new trend in pancreatic developmental biology, and provides useful tools to address these questions.

Differentiation and heterogeneity of other cell types

Although the developmental trajectories of α- and β-lineage generation have been identified using scRNA-seq (as described above), we know very little about the developmental pathways of ε-, δ- and PP-cell lineages, except that all these endocrine lineages were derived from $Ngn3^{+}$ EPs (Gradwohl et al., 2000; Gu et al., 2002; Heller et al., 2005). Recent scRNA-seq studies have captured a 'branch' of ε cells, marked by *Ghrl* and *Irs4*, which are generated from $Ngn3^{+}$ EP cells in the mouse embryonic pancreas (Liu et al., 2019; Sharon et al., 2019a). Droplet-based scRNA-seq methods are inefficient in capturing sufficient numbers of δ and PP cells that are required to define developmental pathways on the trajectory map, presumably because of the low abundance of δ and PP cells during the early stage of endocrinogenesis. In addition, owing to high noise and low sensitivity for low-level transcripts, droplet-based scRNA-seq methods may have limited potential in defining cell types with only subtle differences at the transcriptomic level and therefore may have a reduced capacity to define intermediate progenitor cells and precise developmental pathways. Therefore, well-based deep-sequenced scRNA-seq of the enriched ε, δ and PP cells might be a promising approach to uncover the precise pathways of these rare endocrine populations.

scRNA-seq analysis has also uncovered the heterogeneity and developmental dynamics of mesenchymal cells (Byrnes et al., 2018); however, their cell-cell interaction with pancreatic cells requires more extensive investigation.

Differentiation and heterogeneity in the human pancreas

Because of the scarcity of human fetal samples, research on human fetal pancreatic development has been limited and has led to the overestimation of conservation of lineage hierarchy and transcriptome between mice and humans. Using single-cell qPCR, Ramond and colleagues have profiled the transcription profiles of

Development (2020) 147, dev179051. doi:10.1242/dev.179051

91 genes in four populations (population A-D) enriched by cell-surface markers, from 9 weeks of development (WD) in the human fetal pancreas. Through this approach, they revealed the cell composition of each population and branched differentiation pathway of endocrine cells (Ramond et al., 2018). Populations A and B mainly include pancreatic progenitors, population C contains EP cells and population D consists of endocrine cells. Moreover, they identify three lineage trajectories that originate from EP cells: β-track, α and γ-track, and δ-track. Importantly, this work depicted a continuous developmental process from pancreatic progenitors to endocrine cells in 9 WD human pancreas and provides a paradigm to explore the developmental trajectories and regulatory mechanisms of the human fetal pancreatic lineages through a single-cell approach, which allows the effective use of scarce samples. Future efforts should focus on comprehensive and unbiased assessment of human fetal pancreatic development using scRNA-seq in combination with other methods.

Single-cell studies define endocrine lineage maturation pathways

Once specified during embryonic development, immature endocrine cells mature into functional endocrine cells (Salinno et al., 2019). Most studies have focused on the maturation of β cells, because of translational medicine potential for the therapy of diabetes. Immature β cells are highly proliferative and respond poorly to glucose stimulation (Blum et al., 2012; Henquin and Nenquin, 2018). Postnatal development is a crucial period for β-cell maturation, during which they acquire physiological functions, such as glucose stimulated insulin secretion (GSIS) in response to extracellular glucose. In both mice and humans, the maturation process of β cells coincides with cell cycle exit (Piccand et al., 2014; Stewart et al., 2015) and is marked by gene expression changes, such as increased expression of urocortin 3 (*Ucn3*) (Blum et al., 2012) and decreased neuropeptide Y (*Npy*) (Rodnoi et al., 2017). Moreover, the TFs *Mafb* and *Mafa* are expressed in mouse immature and mature β cells, respectively, and this conversion is an important feature of mouse β-cell development (Nishimura et al., 2006). However, unlike in mice, *MAFB* is constantly expressed in adult human β cells (Dai et al., 2012). During human pancreas development, the ratio of β cells that express glucose-sensing proteins increases, mainly after 15 WD (Richardson et al., 2007). In rodents, the postnatal maturation of β cells is driven by weaning (dietary change from high-fat milk to high-carbohydrate chow) and studies have suggested that miRNAs have a central role in regulating postnatal β-cell maturation (Jacovetti et al., 2015; Stolovich-Rain et al., 2015). Furthermore, signaling pathways such as mammalian target of rapamycin (mTOR) (Ni et al., 2017; Sinagoga et al., 2017), AMP-activated protein kinase (AMPK) (Jaafar et al., 2019) and WNT (Dorrell et al., 2016) have been proposed to regulate β-cell maturation, but need further investigation.

Recent studies at the single-cell level have broadened our understanding of cellular heterogeneity, proliferation and maturation of the β and α lineages (Qiu et al., 2017; Zeng et al., 2017). Single-cell transcriptomic analyses have revealed the genes that are specifically expressed in proliferative versus quiescent cells or immature versus mature cells during endocrine lineage maturation. Excluding cell cycle-related genes, the proliferative β cells exhibit similar transcriptomic profiles to the quiescent β cells, and remain synchronized with the pseudotemporal process of maturation (Qiu et al., 2017). In addition, prenatal and juvenile – but not adult – β cells exhibit heterogeneity in maturation state (Qiu et al., 2017). Moreover, investigation of the maturation pathway and concomitant gene expression dynamics of α cells has shown that β cells and α cells use different regulatory strategies for maturation: α cells tend to downregulate gene expression during maturity, whereas β-cell maturation is accompanied by upregulation of many genes (Qiu et al., 2017). Zeng and colleagues have observed that, during the process of β-cell maturation, the genes related to mitochondrial activity (such as *Ndufv1*) and amino acid metabolism (such as *Slc7a2* and *Lamtor5*) are downregulated, and the data indicated that Srf is a regulator for maturation-associated genes (Zeng et al., 2017). The gene expression profiles of cells recovered from young and old mice are similar at the transcriptomic level, suggesting that aging is not associated with β-cell dysfunction in mice (Xin et al., 2016b). However, in humans, aging is associated with increased transcriptional noise and cell identity drift (Enge et al., 2017).

In summary, scRNA-seq has been used to systematically investigate the development of pancreatic lineages at various stages in mice, and these findings may provide insights to improve and evaluate the efficiency of hESC induction. Nonetheless, we must be cautious when extrapolating these conclusions to human pancreatic development. Thus, it is important to resolve the developmental processes of human pancreatic lineages, as has been done in mouse.

Regeneration of β cells

Proliferation of endogenous β cells

Stimulating proliferation of β cells is a straightforward approach for the endogenous regeneration of β cells for diabetes therapy. β Cells undergo a burst in proliferation during the early postnatal period, followed by a decrease in proliferation and functional maturation (Finegood et al., 1995; Meier et al., 2008; Gregg et al., 2012). Proliferation of adult β cells is relatively slow (Teta et al., 2005), but it is believed that a burst of proliferation can occur under certain physiological conditions, such as obesity and pregnancy, to increase insulin secretion and meet high metabolic demand (Klöppel et al., 1985; Parsons et al., 1992; Sorenson and Brelje, 1997; Rahier et al., 2008). These findings indicate that at least a portion of β cells possess the flexibility to adapt to the changing microenvironment to maintain homeostasis. Therefore, exploiting the heterogeneity in proliferation and function of β cells may inform the development of therapeutic approaches that specifically and controllably stimulate β-cell proliferation. Research based on single-cell technologies has extended our knowledge on proliferation and heterogeneity of endocrine cells, especially β cells.

β-Cell proliferation primarily occurs during the perinatal period, when β cells are in an immature state. Hence, comparison of immature and mature β cells is expected to provide clues regarding the molecular mechanism of proliferation, including the key regulatory genes and signaling pathways. At the single-cell transcriptomic (Qiu et al., 2017; Zeng et al., 2017) and proteomic levels (Wang et al., 2016a), β-cell proliferation rate declines with age, both in mice or humans. In mice, immature β cells display high expression of the cell cycle inhibitor *p57* (*Cdkn1c*), distinct from the *p18* (*Cdkn2c*) expression observed in mature β cells. This finding suggests distinct regulatory strategies of cell cycle inhibition (Qiu et al., 2017). In addition, amino acid metabolism and mitochondrial reactive oxygen species (ROS) play important roles in promoting postnatal β-cell proliferation in mice (Zeng et al., 2017). The supplementation of amino acids to cultured islets from postnatal day (P)28 mice enhances β-cell proliferation, and inducible overexpression of radical scavenger catalase to clear ROS in the mitochondria of β cells reduces the proliferation rate and the mass of β cells (Zeng et al., 2017). In addition, single-cell mass cytometry analysis, profiling 24 proteins

Development (2020) 147, dev179051. doi:10.1242/dev.179051

which are associated with β-cell proliferation, function and heterogeneity, has identified three subgroups of human β cells, two of which contain proliferating β cells (Wang et al., 2016a).

β-Cell heterogeneity has been reported by different groups; however, the classification of 'heterogeneity' is inconsistent between various studies (reviewed by Nasteska and Hodson, 2018; Mawla and Huising, 2019; Wang and Kaestner, 2019). Antibodies against ST8SIA1 and CD9 can classify human β cells into four antigenic subpopulations with different gene expression profiles and GSIS function (Dorrell et al., 2016). Several scRNA-seq analyses have revealed heterogeneity of human β cells in maturation and in response to metabolic stress (Baron et al., 2016; Muraro et al., 2016; Segerstolpe et al., 2016; Xin et al., 2018). Healthy human β cells exhibit heterogeneity reflecting distinct cell states with variable insulin gene expression and unfolded protein response (UPR) activation. One of the cell states (with low insulin but high UPR-related gene expression) is enriched in proliferative cells, and represents cells that have recovered from stress (Xin et al., 2018). This result is consistent with previous studies that reveal the role of UPR in response to metabolic stress to compensate β-cell failure and trigger proliferation to adapt to cellular environment changes (Rabhi et al., 2014; Sharma et al., 2015). Notably, some studies have not detected heterogeneity in human β cells at the transcriptomic level (Li et al., 2016; Wang et al., 2016b; Xin et al., 2016a; Lawlor et al., 2017), which may be partly because of the sensitivity and throughput of scRNA-seq technology or sample variation between individuals (Table 3). Using single-molecule fluorescence *in situ* hybridization (smFISH) in intact pancreatic tissues of mouse, Farack and colleagues have identified a small subset of 'extreme' β cells that express a higher mRNA level of insulin and other secretory genes. These cells contain higher ribosomal RNA and proinsulin, but lower insulin protein, suggesting they may be basal secretors (Farack et al., 2019). In future investigations, stable and high-throughput approaches and larger sample sizes will be needed to examine β-cell heterogeneity in greater detail. Taken together, these studies show that β cells display heterogeneity in their ability to proliferate and enact functional responses to stress and metabolic demand.

Conversion of endogenous non-β cells into insulin-secreting cells

Non-β pancreatic cells, including exocrine and endocrine cells, possess the plasticity to convert into insulin-secreting β-like cells under certain injury or pathological conditions (Xu et al., 2008; Solar et al., 2009; Thorel et al., 2010; Chera et al., 2014). In addition, non-β pancreatic cells can be reprogrammed into β-like cells with the intervention of key cell type-specific TFs (Zhou et al., 2008; Yang et al., 2011; Al-Hasani et al., 2013; Courtney et al., 2013; Li et al., 2014; Miyazaki et al., 2016; Chakravarthy et al., 2017; Druelle et al., 2017; Xiao et al., 2018) or signaling pathways (Klein et al., 2015b; Lemper et al., 2015; Zhang et al., 2016; Ben-Othman et al., 2017; Li et al., 2017) (Fig. 2, Table 4).

Although advancements of *in vivo* and *in vitro* cell fate conversion have been well summarized (reviewed by Aguayo-Mazzucato and Bonner-Weir, 2018; Zhou and Melton, 2018), the extent to which the obtained β-like cells resemble primary β cells and how the dynamic conversion process occurs remain unclear. It is unclear whether the endogenous conversion occurs through a process of dedifferentiation to a progenitor state followed by re-differentiation or through a direct switch between lineages. By leveraging single-cell technologies to describe the developmental and transitionary trajectory, researchers can comprehensively reveal the progression of rapid cell fate shifts and the resulting molecular features. Indeed, an increasing number of studies have utilized scRNA-seq to understand the reprogramming of non-β to β cells (Chakravarthy et al., 2017; Li et al., 2017; Furuyama et al., 2019).

Conversion of endocrine cells

Mouse α and δ cells are plastic and are able to convert into β-like cells under extreme β-cell ablation. This plasticity depends on age: δ cells and α cells tend to adopt a β-cell fate and thus contribute to the pool of insulin-producing cells in juvenile and adult mice, respectively (Thorel et al., 2010; Chera et al., 2014). Moreover, *in vitro* re-aggregated human α or PP cells are able to become glucose-responsive, insulin-producing cells following ectopic expression of *PDX1* and *MAFA* (key TFs for β-cell development and maturation), and this plasticity is maintained in T2D α cells (Furuyama et al., 2019). Using scRNA-seq, the transcriptomic profiles and reprogramming status of converted α cells on the pseudotemporal transitionary trajectory to β cells have been resolved. These converted cells are INS and GCG bi-hormonal and are divided into three stages, in which the late state of converted cells exhibits higher expression of β cell-related genes and lower expression of α cell-related genes (Furuyama et al., 2019).

Chakravarthy and colleagues have delineated the mechanism of α-cell identity maintenance by DNMT1 and ARX, and provided evidence via lineage tracing and scRNA-seq in mice of α-cell conversion to β cells after *Arx* and *Dnmt1* ablation (Chakravarthy et al., 2017). Notably, the converted α cells acquire functional features of native β cells (Chakravarthy et al., 2017). The conversion of α to β-like cells has also been reported to be regulated by anti-

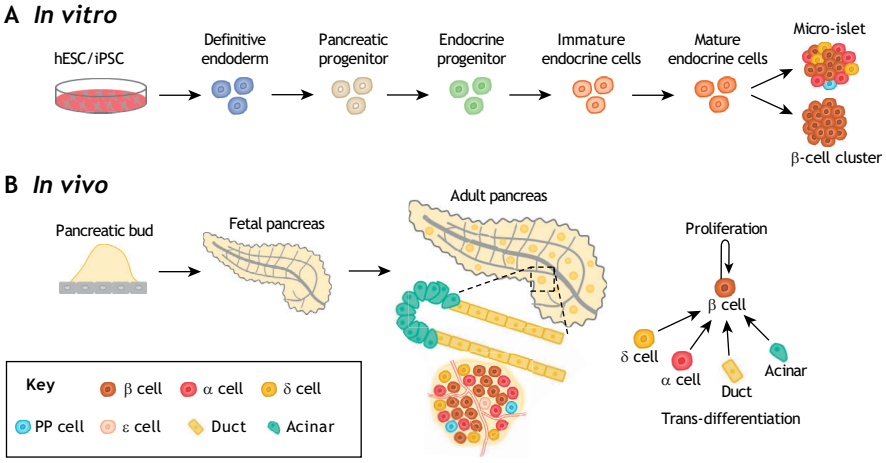

Fig. 2. Regeneration sources of β cells. (A) Stepwise induction of human embryonic stem cells (hESCs)/induced pluripotent stem cells (iPSCs) to obtain functional endocrine cells for β-cell clusters or micro-islet aggregation *in vitro*. (B) Endogenous cell fate conversion of non-β cells or self-proliferation of β cells *in vivo*. Adult α, δ, acinar and ductal cells show plasticity under certain injury or pathological conditions in mice and can be converted into β-like cells. Proliferation of β cells occurs under certain physiological conditions, such as obesity and pregnancy in mice and humans.

Development (2020) 147, dev179051. doi:10.1242/dev.179051

Box 2. Insights into characterizations of non-β islet cells from single-cell technologies

- α Cells are heterogeneous in proliferation, and exhibit the highest proliferative rate in endocrine cell types from birth to adulthood (Segerstolpe et al., 2016; Wang et al., 2016a,b).
- δ Cells express several vital receptor genes, such as *LEPR* and *GHSR*, which bind leptin and ghrelin, respectively, indicating the role of δ cells in integrating paracrine and metabolic signals (Baron et al., 2016; Muraro et al., 2016; Segerstolpe et al., 2016; Lawlor et al., 2017).
- Compared with other endocrine cell types, ε cells express a specific group of genes, including cell-surface receptors for neuropeptides, metabolites, hormones and others (Segerstolpe et al., 2016; Dominguez Gutierrez et al., 2018).
- PP cells display a gene expression profile that resembles neuronal cells, which supports previous observations (van Arensbergen et al., 2010) and suggests either similarity in cell function or developmental proximity (Muraro et al., 2016).

malaria drug artemether through activation of gamma-aminobutyric acid (GABA) signaling in zebrafish and rodent models, as well as in human islets (Li et al., 2017). Indeed, scRNA-seq showed downregulation of α cell-specific genes and upregulation of β cell-featured genes in artemether-treated α cells from primary human islets (Li et al., 2017). However, the effect of GABA signaling in stimulating α- to β-cell trans-differentiation is under debate, because although the expression of *Arx* was indeed decreased after artemether treatment, the conversion from α to β cells was not observed in the Gcg-CreER lineage tracing system or *in vitro* culture systems of mouse and human intact islets (Ackermann et al., 2018; van der Meulen et al., 2018).

Many studies have described the scRNA-seq profiles of human islets and identified the constituent cell types and their corresponding transcriptional signatures, including the less abundant δ, ε and PP cells (Baron et al., 2016; Li et al., 2016; Muraro et al., 2016; Segerstolpe et al., 2016; Wang et al., 2016b; Xin et al., 2016a; Lawlor et al., 2017; Dominguez Gutierrez et al., 2018). These datasets validated the previously known features of each cell type but have also discovered novel signatures that may play roles in cell identity and function (Box 2).

Conversion of ductal and acinar cells

The heterogeneity and corresponding plasticity of ductal and acinar cells are worthy of further investigation because these cells are considered abundant sources for *in vivo* cell fate conversion to β cells. Two subpopulations of ductal cells have been identified that display inverse expression patterns of mucin 1 (*MUC1*, a ductal cell marker), and cystic fibrosis transmembrane conductance regulator (*CFTR*, a marker for secretory cells; Baron et al., 2016), which is consistent with the finding that *CFTR* is heterogeneously expressed in ducts (Burghardt et al., 2003). Notably, the *MUC1*^{high}- and *CFTR*^{low}-expressing population localizes at the ductal terminal, whereas the inverse population is localized at the region connecting acinar and ductal cells. Using the StemID algorithm, Grün and colleagues have described putative subpopulations of ductal cells with distinct potential to differentiate into endocrine and acinar cells. However, the conclusion in this study needs to be carefully verified via other approaches such as lineage tracing (Grün et al., 2016).

Using a well-based Smart-seq2 method, Segerstolpe and colleagues have identified two clusters of acinar cells from humans: cluster 1, with elevated expression of inflammation-related genes, and cluster 2, which highly express secretory, digestive enzyme-encoding genes (Segerstolpe et al., 2016). However, Muraro and colleagues have identified four clusters of human acinar cells using a CEL-Seq2 method (Muraro et al., 2016). One cluster expresses regenerating family member 3 alpha (*REG3A*), which is involved in proliferation and differentiation of various cell types (and in tumorigenesis of pancreatic cells; Parikh et al., 2012; Xu et al., 2016), but displays lower expression of acinar marker genes, suggesting that this acinar subpopulation is in a less-mature state, with increased proliferative potential. Consistent with this finding, a subpopulation of acinar cells with proliferative activity has been identified in rodents; these cells are capable of long-term self-renewal and display high expression of stathmin 1 (*STMN1*), a cell division-related gene (Wollny et al., 2016). The distinction between subpopulations of human acinar cells in different studies might be caused by different scRNA-seq platforms, variation of human samples or the limited cell number. It should be noted that this proliferative, progenitor-like acinar population needs detailed investigation because they may contribute to the β-cell population under pathological conditions through trans-differentiation. Taken together, the findings from these studies suggest that understanding the heterogeneity of each pancreatic cell type will support selective and efficient cell fate conversion, in which the subpopulation with greater plasticity is the preferred target for therapy.

The use of scRNA-seq in future studies will aid efforts to re-examine the cell fate conversion process and resolve controversial conclusions from previous studies. scRNA-seq will capture rare converted intermediate cell states, describe their comprehensive dynamic gene expression profiles, enable mapping of the trajectory along cell state transitions, and determine vital regulatory drivers, either *in vivo* or *in vitro*. A deeper understanding will enable us to exploit cell heterogeneity and plasticity to optimize strategies for regeneration. In addition, to precisely model the conversion to insulin-secreting β-like cells, we must first understand the molecular features of each cell type in the pancreas and the corresponding dynamic changes that occur under various conditions at the single-cell level. Finally, decoding the transcriptional profiles and heterogeneity of non-β pancreatic cell types will aid establishment of a systematic framework to recapitulate the comprehensive relationship between cell lineages.

Exogenous production of β cells from hESCs

Regeneration of β cells from hESCs is a promising and sustainable approach for diabetes treatment and can provide an easily manipulated platform for exploring the mechanism of diabetes pathogenesis and for screening treatable targets. In order for findings to be clinically applicable, this approach should precisely mimic the *in vivo* process. In past decades, based on knowledge gained from animal models, significant breakthroughs have been made to create an *in vitro* inductive model that mirrors the *in vivo* developmental process to induce functional β cells. Although current protocols are able to produce glucose-responsive β-like cells with improved functions *in vitro*, such as increased GSIS capacity and relief of diabetes after transplantation, these cells are still not equivalent to mature β cells *in vivo* and require further maturation (D'Amour et al., 2006; Pagliuca et al., 2014; Rezania et al., 2014; Russ et al., 2015; Korytnikov and Nostro, 2016; Petersen et al., 2018; Sneddon et al., 2018; Nair et al., 2019; Sharon et al., 2019b; Velazco-Cruz et al., 2019). A direct comparison between primary and induced β cells is necessary to address the gap between the *in vivo* process and what we can currently recapitulate *in vitro*.

Development (2020) 147, dev179051. doi:10.1242/dev.179051

Researchers have explored the induced cell-type composition and modeled the dynamic differentiation process at the level of gene expression using single-cell qPCR (Petersen et al., 2017; Ramond et al., 2018) or scRNA-seq (Sharon et al., 2019b; Veres et al., 2019). Researchers have also compared the gene expression and GSIS ability of final induced cells with that of adult human islet cells (Petersen et al., 2017; Veres et al., 2019). Single-cell transcriptional profiling has identified distinct subpopulations and dynamic gene expression changes concomitant with the differentiation process and has shown that the majority of hESC-derived endocrine cells recapitulate the developmental trajectory toward human β cells but do not reach full maturity (Sharon et al., 2019b; Veres et al., 2019).

WNT signaling regulates endocrine cell development (Dessimoz et al., 2005; Pedersen and Heller, 2005; Heiser et al., 2006; Murtaugh, 2008). A recent study shows the inhibitory role of the WNT pathway during endocrine differentiation, verified by deletion of the WNT inhibitor APC in mice as well as by small molecule treatment *in vitro* (Sharon et al., 2019b). Moreover, in the hESC induction system, Sharon and colleagues have revealed two waves of endocrine differentiation: one tends to produce α cells at stage 4, and the other tends to generate β cells at stage 5 (Sharon et al., 2019b). Veres and colleagues have characterized the composition of cell populations and mapped the lineage trajectory during *in vitro* β-cell differentiation using high-throughput inDrops scRNA-seq (Veres et al., 2019). The stem-cell-derived islets (SC-islets) mainly contain four cell types: SC-β cells, α-like cells with *INS* expression, exocrine cells and a population that resembles enterochromaffin cells (SC-EC), which synthesize and secrete serotonin in the gut. The induced β cells express a fraction of maturity-related genes, such as *IAPP* and *SIX2*, but not others, such as *UCN3*, *MAFA* and *SIX3*. Pseudotemporal analysis shows that SC-β and SC-EC cells originate from a common *NGN3*$^{+}$ state. The polyhormonal α-like cells are considered a transient cell state to generate monohormonal α cells. In addition, the induced β cells can be enriched by cell-surface marker CD49a, for re-aggregation to improve functions (Veres et al., 2019).

Taken together, these works provide a blueprint and reference for hESC differentiation at single-cell resolution, which is essential for the improvement of therapeutic strategies. However, comparison of the *in vivo* and *in vitro* developmental and maturation processes is limited by the scarcity and availability of primary human tissues at continuous developmental stages.

Conclusions and perspectives

With the development of high-resolution techniques, we have entered a new era of understanding organogenesis at the single-cell level. In this Review, we have summarized the use of single-cell technologies, primarily scRNA-seq, to inform research in the field of β-cell generation and regeneration (Tables 2-4). These findings provide insights into strategies for β-cell regeneration from hESCs *in vitro*. scRNA-seq has been widely applied to pancreatic development to comprehensively describe the lineage hierarchy landscape in mice. However, it is necessary to determine whether the inferred developmental trajectory based on pseudotime analysis reflects the real developmental trajectories. These findings, based on single-cell analyses, provide insights into the strategies of β-cell regeneration from hESCs *in vitro*. In addition, scRNA-seq allows for the identification of pancreatic cell state and heterogeneity in adults, to better understand the proliferative ability and functional potential of certain cell types. Moreover, scRNA-seq studies are expected to extend our understanding of cell plasticity, as well as defining cell fate conversion pathways. However, the different conclusions drawn by different scRNA-seq platforms should be further validated through synergy with other approaches, such as microscopy and CRISPR editing. Overall, studies based on single-cell technologies have reconstituted the framework of pancreatic development and have laid the foundation for future mechanistic studies.

The combined application of new single-cell technologies (Table 1) will provide a clearer understanding of the pancreas from a multi-omics perspective. Although pancreatic cell identities have been defined by scRNA-seq, gene expression does not always indicate cellular function. Hence, protein expression patterns in individual cells can inform cell heterogeneity in function and plasticity. More importantly, during the processes of pancreas development and regeneration, the regulatory mechanisms of multistep cell fate choices and transitions need to be delineated from an epigenetic perspective. Therefore, single-cell proteomic and epigenetic studies will complement the framework of pancreatic lineage differentiation established by scRNA-seq, and inform regenerative medicine.

Decoding tissue architecture by characterizing the spatial transcriptome paired with microscopy and morphology is also important for understanding generation and regeneration of pancreatic endocrine cells. The location of distinct cell types will shed light on the function and phenotype of the cells, as neighboring cells may release differing signals to affect cell behaviors. Currently, several high-throughput spatially resolving approaches have been developed (summarized by Mayr et al., 2019); these approaches can deepen our understanding of stepwise regulation of cell fate determination in pancreas development.

In order to assemble functional islets *in vitro*, the developmental pathways and regulatory mechanisms of all endocrine lineages must be reconstructed. To characterize the features of rare δ, ε and PP cells, these cells must be enriched using gene reporter mouse strains or fluorescence-labeled antibodies at various time points. Findings from human samples will undoubtedly provide more valuable information for islet production. However, direct observations from human pancreas are limited because of sample scarcity. To maximize use of precious primary human tissues, unbiased and high-throughput methods should be applied to uncover the molecular features and regulatory mechanisms of human endocrine lineages.

Although not the focus of this Review, the implications of single-cell technologies to better understand the pathogenesis of diabetes have been summarized elsewhere (Carrano et al., 2017; Tritschler et al., 2017). However, many of the diabetes-associated genes identified in the scRNA-seq studies have not overlapped (Wang and Kaestner, 2019), which may be because of the pathogenic complexity of diabetes in individuals, the limited number of donor samples or the use of different technical platforms. To best use the datasets generated from single-cell technology, future studies need to rely on the development of more sensitive and higher-throughput experimental methodologies, and novel bioinformatics analytical tools, as well as larger sample sizes.

In conclusion, single-cell technology has extended our understanding of the complex processes of pancreas development and regeneration, and has provided novel insights for drug screening and diabetes treatment. In the near future, with the continuous development of single-cell technologies, we will have a more comprehensive and in-depth understanding of the processes and mechanisms of pancreatic endocrine development and regeneration, thus providing information for the production of human islet tissue *in vitro*.

Acknowledgements
We thank the members of the Xu laboratory for their advice and comments. We apologize that we were unable to cite many studies owing to space limitations.

Competing interests
The authors declare no competing or financial interests.

Funding
This work was supported by the National Key Research and Development Program of China (2019YFA0801500), the Ministry of Science and Technology of the People's Republic of China (973 Program 2015CB942800) and the National Natural Science Foundation of China (31521004, 31471358, 31522036, 91753138) to C.-R.X. and the China Postdoctoral Science Foundation (BX20190009) to X.-X.Y.

References

Ackermann, A. M., Moss, N. G. and Kaestner, K. H. (2018). GABA and artesunate do not induce pancreatic α-to-β cell transdifferentiation in vivo. *Cell Metab.* **28**, 787-792.e3. doi:10.1016/j.cmet.2018.07.002

Aguayo-Mazzucato, C. and Bonner-Weir, S. (2018). Pancreatic β cell regeneration as a possible therapy for diabetes. *Cell Metab.* **27**, 57-67. doi:10.1016/j.cmet.2017.08.007

Ai, S., Xiong, H., Li, C. C., Luo, Y., Shi, Q., Liu, Y., Yu, X., Li, C. and He, A. (2019). Profiling chromatin states using single-cell itChIP-seq. *Nat. Cell Biol.* **21**, 1164-1172. doi:10.1038/s41556-019-0383-5

Al-Hasani, K., Pfeifer, A., Courtney, M., Ben-Othman, N., Gjernes, E., Vieira, A., Druelle, N., Avolio, F., Ravassard, P., Leuckx, G. et al. (2013). Adult duct-lining cells can reprogram into β-like cells able to counter repeated cycles of toxin-induced diabetes. *Dev. Cell* **26**, 86-100. doi:10.1016/j.devcel.2013.05.018

Baeyens, L., Lemper, M., Staels, W., De Groef, S., De Leu, N., Heremans, Y., German, M. S. and Heimberg, H. (2018). (Re)generating human beta cells: status, pitfalls, and perspectives. *Physiol. Rev.* **98**, 1143-1167. doi:10.1152/physrev.00034.2016

Bakhti, M., Böttcher, A. and Lickert, H. (2019). Modelling the endocrine pancreas in health and disease. *Nat. Rev. Endocrinol.* **15**, 155-171. doi:10.1038/s41574-018-0132-z

Baron, M., Veres, A., Wolock, S. L., Faust, A. L., Gaujoux, R., Vetere, A., Ryu, J. H., Wagner, B. K., Shen-Orr, S. S., Klein, A. M. et al. (2016). A single-cell transcriptomic map of the human and mouse pancreas reveals inter- and intra-cell population structure. *Cell Syst.* **3**, 346-360.e4. doi:10.1016/j.cels.2016.08.011

Bastidas-Ponce, A., Scheibner, K., Lickert, H. and Bakhti, M. (2017). Cellular and molecular mechanisms coordinating pancreas development. *Development* **144**, 2873-2888. doi:10.1242/dev.140756

Bastidas-Ponce, A., Tritschler, S., Dony, L., Scheibner, K., Tarquis-Medina, M., Salinno, C., Schirge, S., Burtscher, I., Böttcher, A., Theis, F. J. et al. (2019). Comprehensive single cell mRNA profiling reveals a detailed roadmap for pancreatic endocrinogenesis. *Development* **146**, dev173849. doi:10.1242/dev.173849

Ben-Othman, N., Vieira, A., Courtney, M., Record, F., Gjernes, E., Avolio, F., Hadzic, B., Druelle, N., Napolitano, T., Navarro-Sanz, S. et al. (2017). Long-term GABA administration induces alpha cell-mediated beta-like cell neogenesis. *Cell* **168**, 73-85.e11. doi:10.1016/j.cell.2016.11.002

Blum, B., Hrvatin, S., Schuetz, C., Bonal, C., Rezania, A. and Melton, D. A. (2012). Functional beta-cell maturation is marked by an increased glucose threshold and by expression of urocortin 3. *Nat. Biotechnol.* **30**, 261-264. doi:10.1038/nbt.2141

Burghardt, B., Elkaer, M. L., Kwon, T. H., Racz, G. Z., Varga, G., Steward, M. C. and Nielsen, S. (2003). Distribution of aquaporin water channels AQP1 and AQP5 in the ductal system of the human pancreas. *Gut* **52**, 1008-1016. doi:10.1136/gut.52.7.1008

Byrnes, L. E., Wong, D. M., Subramaniam, M., Meyer, N. P., Gilchrist, C. L., Knox, S. M., Tward, A. D., Ye, C. J. and Sneddon, J. B. (2018). Lineage dynamics of murine pancreatic development at single-cell resolution. *Nat. Commun.* **9**, 3922. doi:10.1038/s41467-018-06176-3

Carrano, A. C., Mulas, F., Zeng, C. and Sander, M. (2017). Interrogating islets in health and disease with single-cell technologies. *Mol. Metab.* **6**, 991-1001. doi:10.1016/j.molmet.2017.04.012

Carter, B., Ku, W. L., Kang, J. Y., Hu, G., Perrie, J., Tang, Q. and Zhao, K. (2019). Mapping histone modifications in low cell number and single cells using antibody-guided chromatin tagmentation (ACT-seq). *Nat. Commun.* **10**, 3747. doi:10.1038/s41467-019-11559-1

Chakravarthy, H., Gu, X., Enge, M., Dai, X., Wang, Y., Damond, N., Downie, C., Liu, K., Wang, J., Xing, Y. et al. (2017). Converting adult pancreatic islet α cells into β cells by targeting both Dnmt1 and Arx. *Cell Metab.* **25**, 622-634. doi:10.1016/j.cmet.2017.01.009

Chan, M. M., Smith, Z. D., Grosswendt, S., Kretzmer, H., Norman, T. M., Adamson, B., Jost, M., Quinn, J. J., Yang, D., Jones, M. G. et al. (2019). Molecular recording of mammalian embryogenesis. *Nature* **570**, 77-82. doi:10.1038/s41586-019-1184-5

Chen, X., Miragaia, R. J., Natarajan, K. N. and Teichmann, S. A. (2018). A rapid and robust method for single cell chromatin accessibility profiling. *Nat. Commun.* **9**, 5345. doi:10.1038/s41467-018-07771-0

Chen, G., Ning, B. and Shi, T. (2019). Single-cell RNA-Seq technologies and related computational data analysis. *Front. Genet.* **10**, 317. doi:10.3389/fgene.2019.00317

Chera, S., Baronnier, D., Ghila, L., Cigliola, V., Jensen, J. N., Gu, G., Furuyama, K., Thorel, F., Gribble, F. M., Reimann, F. et al. (2014). Diabetes recovery by age-dependent conversion of pancreatic δ-cells into insulin producers. *Nature* **514**, 503-507. doi:10.1038/nature13633

Cho, N. H., Shaw, J. E., Karuranga, S., Huang, Y., da Rocha Fernandes, J. D., Ohlrogge, A. W. and Malanda, B. (2018). IDF Diabetes Atlas: global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Res. Clin. Pract.* **138**, 271-281. doi:10.1016/j.diabres.2018.02.023

Clark, S. J., Argelaguet, R., Kapourani, C.-A., Stubbs, T. M., Lee, H. J., Alda-Catalinas, C., Krueger, F., Sanguinetti, G., Kelsey, G., Marioni, J. C. et al. (2018). scNMT-seq enables joint profiling of chromatin accessibility DNA methylation and transcription in single cells. *Nat. Commun.* **9**, 781. doi:10.1038/s41467-018-03149-4

Courtney, M., Gjernes, E., Druelle, N., Ravaud, C., Vieira, A., Ben-Othman, N., Pfeifer, A., Avolio, F., Leuckx, G., Lacas-Gervais, S. et al. (2013). The inactivation of Arx in pancreatic α-cells triggers their neogenesis and conversion into functional β-like cells. *PLoS Genet.* **9**, e1003934. doi:10.1371/journal.pgen.1003934

Cusanovich, D. A., Daza, R., Adey, A., Pliner, H. A., Christiansen, L., Gunderson, K. L., Steemers, F. J., Trapnell, C. and Shendure, J. (2015). Multiplex single-cell profiling of chromatin accessibility by combinatorial cellular indexing. *Science* **348**, 910-914. doi:10.1126/science.aab1601

Dai, C., Brissova, M., Hang, Y., Thompson, C., Poffenberger, G., Shostak, A., Chen, Z., Stein, R. and Powers, A. C. (2012). Islet-enriched gene expression and glucose-induced insulin secretion in human and mouse islets. *Diabetologia* **55**, 707-718. doi:10.1007/s00125-011-2369-0

D'Amour, K. A., Bang, A. G., Eliazer, S., Kelly, O. G., Agulnick, A. D., Smart, N. G., Moorman, M. A., Kroon, E., Carpenter, M. K. and Baetge, E. E. (2006). Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells. *Nat. Biotechnol.* **24**, 1392-1401. doi:10.1038/nbt1259

DeFronzo, R. A., Ferrannini, E., Groop, L., Henry, R. R., Herman, W. H., Holst, J. J., Hu, F. B., Kahn, C. R., Raz, I., Shulman, G. I. et al. (2015). Type 2 diabetes mellitus. *Nat. Rev. Dis. Primers* **1**, 15019. doi:10.1038/nrdp.2015.19

Desgraz, R. and Herrera, P. L. (2009). Pancreatic neurogenin 3-expressing cells are unipotent islet precursors. *Development* **136**, 3567-3574. doi:10.1242/dev.039214

Dessimoz, J., Bonnard, C., Huelsken, J. and Grapin-Botton, A. (2005). Pancreas-specific deletion of β-catenin reveals Wnt-dependent and Wnt-independent functions during development. *Curr. Biol.* **15**, 1677-1683. doi:10.1016/j.cub.2005.08.037

Dominguez Gutierrez, G., Kim, J., Lee, A.-H., Tong, J., Niu, J. J., Gray, S. M., Wei, Y., Ding, Y., Ni, M., Adler, C. et al. (2018). Gene signature of the human pancreatic ε cell. *Endocrinology* **159**, 4023-4032. doi:10.1210/en.2018-00833

Dorrell, C., Schug, J., Canaday, P. S., Russ, H. A., Tarlow, B. D., Grompe, M. T., Horton, T., Hebrok, M., Streeter, P. R., Kaestner, K. H. et al. (2016). Human islets contain four distinct subtypes of β cells. *Nat. Commun.* **7**, 11756. doi:10.1038/ncomms11756

Druelle, N., Vieira, A., Shabro, A., Courtney, M., Mondin, M., Rekima, S., Napolitano, T., Silvano, S., Navarro-Sanz, S., Hadzic, B. et al. (2017). Ectopic expression of Pax4 in pancreatic δ cells results in β-like cell neogenesis. *J. Cell Biol.* **216**, 4299-4311. doi:10.1083/jcb.201704044

Enge, M., Arda, H. E., Mignardi, M., Beausang, J., Bottino, R., Kim, S. K. and Quake, S. R. (2017). Single-cell analysis of human pancreas reveals transcriptional signatures of aging and somatic mutation patterns. *Cell* **171**, 321-330.e14. doi:10.1016/j.cell.2017.09.004

Farack, L., Golan, M., Egozi, A., Dezorella, N., Bahar Halpern, K., Ben-Moshe, S., Garzilli, I., Tóth, B., Roitman, L., Krizhanovsky, V. et al. (2019). Transcriptional heterogeneity of beta cells in the intact pancreas. *Dev. Cell* **48**, 115-125.e4. doi:10.1016/j.devcel.2018.11.001

Finegood, D. T., Scaglia, L. and Bonner-Weir, S. (1995). Dynamics of β-cell mass in the growing rat pancreas: estimation with a simple mathematical model. *Diabetes* **44**, 249-256. doi:10.2337/diab.44.3.249

Furuyama, K., Chera, S., van Gurp, L., Oropeza, D., Ghila, L., Damond, N., Vethe, H., Paulo, J. A., Joosten, A. M., Berney, T. et al. (2019). Diabetes relief in mice by glucose-sensing insulin-secreting human α-cells. *Nature* **567**, 43-48. doi:10.1038/s41586-019-0942-8

Gradwohl, G., Dierich, A., LeMeur, M. and Guillemot, F. (2000). neurogenin3 is required for the development of the four endocrine cell lineages of the pancreas. *Proc. Natl. Acad. Sci. USA* **97**, 1607-1611. doi:10.1073/pnas.97.4.1607

Gregg, B. E., Moore, P. C., Demozay, D., Hall, B. A., Li, M., Husain, A., Wright, A. J., Atkinson, M. A. and Rhodes, C. J. (2012). Formation of a human β-cell population within pancreatic islets is set early in life. *J. Clin. Endocrinol. Metab.* **97**, 3197-3206. doi:10.1210/jc.2012-1206

Griffiths, J. A., Scialdone, A. and Marioni, J. C. (2018). Using single-cell genomics to understand developmental processes and cell fate decisions. *Mol. Syst. Biol.* **14**, e8046. doi:10.15252/msb.20178046

Grosselin, K., Durand, A., Marsolier, J., Poitou, A., Marangoni, E., Nemati, F., Dahmani, A., Lameiras, S., Reyal, F., Frenoy, O. et al. (2019). High-throughput single-cell ChIP-seq identifies heterogeneity of chromatin states in breast cancer. *Nat. Genet.* **51**, 1060-1066. doi:10.1038/s41588-019-0424-9

Grün, D., Muraro, M. J., Boisset, J.-C., Wiebrands, K., Lyubimova, A., Dharmadhikari, G., van den Born, M., van Es, J., Jansen, E., Clevers, H. et al. (2016). De novo prediction of stem cell identity using single-cell transcriptome data. *Cell Stem Cell* **19**, 266-277. doi:10.1016/j.stem.2016.05.010

Gu, G., Dubauskaite, J. and Melton, D. A. (2002). Direct evidence for the pancreatic lineage: NGN3+ cells are islet progenitors and are distinct from duct progenitors. *Development* **129**, 2447-2457.

Haque, A., Engel, J., Teichmann, S. A. and Lönnberg, T. (2017). A practical guide to single-cell RNA-sequencing for biomedical research and clinical applications. *Genome Med.* **9**, 75. doi:10.1186/s13073-017-0467-4

Hashimshony, T., Senderovich, N., Avital, G., Klochendler, A., de Leeuw, Y., Anavy, L., Gennert, D., Li, S., Livak, K. J., Rozenblatt-Rosen, O. et al. (2016). CEL-Seq2: sensitive highly-multiplexed single-cell RNA-Seq. *Genome Biol.* **17**, 77. doi:10.1186/s13059-016-0938-8

Heiser, P. W., Lau, J., Taketo, M. M., Herrera, P. L. and Hebrok, M. (2006). Stabilization of β-catenin impacts pancreas growth. *Development* **133**, 2023-2032. doi:10.1242/dev.02366

Heller, R. S., Jenny, M., Collombat, P., Mansouri, A., Tomasetto, C., Madsen, O. D., Mellitzer, G., Gradwohl, G. and Serup, P. (2005). Genetic determinants of pancreatic ε-cell development. *Dev. Biol.* **286**, 217-224. doi:10.1016/j.ydbio.2005.06.041

Henquin, J.-C. and Nenquin, M. (2018). Immaturity of insulin secretion by pancreatic islets isolated from one human neonate. *J. Diabetes Investig.* **9**, 270-273. doi:10.1111/jdi.12701

Hochgerner, H., Lönnerberg, P., Hodge, R., Mikes, J., Heskol, A., Hubschle, H., Lin, P., Picelli, S., La Manno, G., Ratz, M. et al. (2017). STRT-seq-2i: dual-index 5′ single cell and nucleus RNA-seq on an addressable microwell array. *Sci. Rep.* **7**, 16327. doi:10.1038/s41598-017-16546-4

Islam, S., Kjällquist, U., Moliner, A., Zajac, P., Fan, J.-B., Lönnerberg, P. and Linnarsson, S. (2012). Highly multiplexed and strand-specific single-cell RNA 5′ end sequencing. *Nat. Protoc.* **7**, 813-828. doi:10.1038/nprot.2012.022

Itoh, M., Takizawa, Y., Hanai, S., Okazaki, S., Miyata, R., Inoue, T., Akashi, T., Hayashi, M. and Goto, Y.-I. (2010). Partial loss of pancreas endocrine and exocrine cells of human ARX-null mutation: consideration of pancreas differentiation. *Differentiation* **80**, 118-122. doi:10.1016/j.diff.2010.05.003

Jaafar, R., Tran, S., Shah, A. N., Sun, G., Valdearcos, M., Marchetti, P., Masini, M., Swisa, A., Giacometti, S., Bernal-Mizrachi, E. et al. (2019). mTORC1-to-AMPK switching underlies beta-cell metabolic plasticity during maturation and diabetes. *J. Clin. Invest.* **130**, 4124-4137. doi:10.1172/JCI127021

Jacovetti, C., Matkovich, S. J., Rodriguez-Trejo, A., Guay, C. and Regazzi, R. (2015). Postnatal β-cell maturation is associated with islet-specific microRNA changes induced by nutrient shifts at weaning. *Nat. Commun.* **6**, 8084. doi:10.1038/ncomms9084

Jennings, R. E., Berry, A. A., Kirkwood-Wilson, R., Roberts, N. A., Hearn, T., Salisbury, R. J., Blaylock, J., Piper Hanley, K. and Hanley, N. A. (2013). Development of the human pancreas from foregut to endocrine commitment. *Diabetes* **62**, 3514-3522. doi:10.2337/db12-1479

Jennings, R. E., Berry, A. A., Strutt, J. P., Gerrard, D. T. and Hanley, N. A. (2015). Human pancreas development. *Development* **142**, 3126-3137. doi:10.1242/dev.120063

Jeon, J., Correa-Medina, M., Ricordi, C., Edlund, H. and Diez, J. A. (2009). Endocrine cell clustering during human pancreas development. *J. Histochem. Cytochem.* **57**, 811-824. doi:10.1369/jhc.2009.953307

Johansson, K. A., Dursun, U., Jordan, N., Gu, G., Beermann, F., Gradwohl, G. and Grapin-Botton, A. (2007). Temporal control of neurogenin3 activity in pancreas progenitors reveals competence windows for the generation of different endocrine cell types. *Dev. Cell* **12**, 457-465. doi:10.1016/j.devcel.2007.02.010

Karemaker, I. D. and Vermeulen, M. (2018). Single-cell DNA methylation profiling: technologies and biological applications. *Trends Biotechnol.* **36**, 952-965. doi:10.1016/j.tibtech.2018.04.002

Katsarou, A., Gudbjörnsdottir, S., Rawshani, A., Dabelea, D., Bonifacio, E., Anderson, B. J., Jacobsen, L. M., Schatz, D. A. and Lernmark, A. (2017). Type 1 diabetes mellitus. *Nat. Rev. Dis. Primers* **3**, 17016. doi:10.1038/nrdp.2017.16

Kaya-Okur, H. S., Wu, S. J., Codomo, C. A., Pledger, E. S., Bryson, T. D., Henikoff, J. G., Ahmad, K. and Henikoff, S. (2019). CUT&Tag for efficient epigenomic profiling of small samples and single cells. *Nat. Commun.* **10**, 1930. doi:10.1038/s41467-019-09982-5

Kim, S., Whitener, R. L., Peiris, H., Gu, X., Chang, C. A., Lam, J. Y., Camunas-Soler, J., Park, I., Bevacqua, R. J., Tellez, K. et al. (2020). Molecular and genetic regulation of pig pancreatic islet cell development. *Development* **147**, dev.186213. doi:10.1242/dev.186213

Klein, A. M., Mazutis, L., Akartuna, I., Tallapragada, N., Veres, A., Li, V., Peshkin, L., Weitz, D. A. and Kirschner, M. W. (2015a). Droplet barcoding for single-cell transcriptomics applied to embryonic stem cells. *Cell* **161**, 1187-1201. doi:10.1016/j.cell.2015.04.044

Klein, D., Álvarez-Cubela, S., Lanzoni, G., Vargas, N., Prabakar, K. R., Boulina, M., Ricordi, C., Inverardi, L., Pastori, R. L. and Domínguez-Bendala, J. (2015b). BMP-7 induces adult human pancreatic exocrine-to-endocrine conversion. *Diabetes* **64**, 4123-4134. doi:10.2337/db15-0688

Klöppel, G., Löhr, M., Habich, K., Oberholzer, M. and Heitz, P. U. (1985). Islet pathology and the pathogenesis of type 1 and type 2 diabetes mellitus revisited. *Surv. Synth. Pathol. Res.* **4**, 110-125. doi:10.1159/000156969

Korytnikov, R. and Nostro, M. C. (2016). Generation of polyhormonal and multipotent pancreatic progenitor lineages from human pluripotent stem cells. *Methods* **101**, 56-64. doi:10.1016/j.ymeth.2015.10.017

Krentz, N. A. J., Lee, M. Y. Y., Xu, E. E., Sproul, S. L. J., Maslova, A., Sasaki, S. and Lynn, F. C. (2018). Single-cell transcriptome profiling of mouse and hESC-derived pancreatic progenitors. *Stem Cell Rep.* **11**, 1551-1564. doi:10.1016/j.stemcr.2018.11.008

Kumar, P., Tan, Y. and Cahan, P. (2017). Understanding development and stem cells using single cell-based analyses of gene expression. *Development* **144**, 17-32. doi:10.1242/dev.133058

Larsen, H. L. and Grapin-Botton, A. (2017). The molecular and morphogenetic basis of pancreas organogenesis. *Semin. Cell Dev. Biol.* **66**, 51-68. doi:10.1016/j.semcdb.2017.01.005

Lawlor, N., George, J., Bolisetty, M., Kursawe, R., Sun, L., Sivakamasundari, V., Kycia, I., Robson, P. and Stitzel, M. L. (2017). Single-cell transcriptomes identify human islet cell signatures and reveal cell-type-specific expression changes in type 2 diabetes. *Genome Res.* **27**, 208-222. doi:10.1101/gr.212720.116

Lemper, M., Leuckx, G., Heremans, Y., German, M. S., Heimberg, H., Bouwens, L. and Baeyens, L. (2015). Reprogramming of human pancreatic exocrine cells to β-like cells. *Cell Death Differ.* **22**, 1117-1130. doi:10.1038/cdd.2014.193

Li, W., Nakanishi, M., Zumsteg, A., Shear, M., Wright, C., Melton, D. A. and Zhou, Q. (2014). In vivo reprogramming of pancreatic acinar cells to three islet endocrine subtypes. *eLife* **3**, e01846. doi:10.7554/eLife.01846

Li, J., Klughammer, J., Farlik, M., Penz, T., Spittler, A., Barbieux, C., Berishvili, E., Bock, C. and Kubicek, S. (2016). Single-cell transcriptomes reveal characteristic features of human pancreatic islet cell types. *EMBO Rep.* **17**, 178-187. doi:10.15252/embr.201540946

Li, J., Casteels, T., Frogne, T., Ingvorsen, C., Honoré, C., Courtney, M., Huber, K. V. M., Schmitner, N., Kimmel, R. A., Romanov, R. A. et al. (2017). Artemisinins target GABAA receptor signaling and impair alpha cell identity. *Cell* **168**, 86-100.e15. doi:10.1016/j.cell.2016.11.010

Li, L.-C., Qiu, W.-L., Zhang, Y.-W., Xu, Z.-R., Xiao, Y.-N., Hou, C., Lamaoqiezhong, Yu, P., Cheng, X. and Xu, C.-R. (2018). Single-cell transcriptomic analyses reveal distinct dorsal/ventral pancreatic programs. *EMBO Rep.* **19**, e46148. doi:10.15252/embr.201846148

Liu, J., Banerjee, A., Herring, C. A., Attalla, J., Hu, R., Xu, Y., Shao, Q., Simmons, A. J., Dadi, P. K., Wang, S. et al. (2019). Neurog3-independent methylation is the earliest detectable mark distinguishing pancreatic progenitor identity. *Dev. Cell* **48**, 49-63.e7. doi:10.1016/j.devcel.2018.11.048

Macosko, E. Z., Basu, A., Satija, R., Nemesh, J., Shekhar, K., Goldman, M., Tirosh, I., Bialas, A. R., Kamitaki, N., Martersteck, E. M. et al. (2015). Highly parallel genome-wide expression profiling of individual cells using nanoliter droplets. *Cell* **161**, 1202-1214. doi:10.1016/j.cell.2015.05.002

Mawla, A. M. and Huising, M. O. (2019). Navigating the depths and avoiding the shallows of pancreatic islet cell transcriptomes. *Diabetes* **68**, 1380-1393. doi:10.2337/dbi18-0019

Mayr, U., Serra, D. and Liberali, P. (2019). Exploring single cells in space and time during tissue development, homeostasis and regeneration. *Development* **146**, dev176727. doi:10.1242/dev.176727

McGrath, P. S., Watson, C. L., Ingram, C., Helmrath, M. A. and Wells, J. M. (2015). The basic helix-loop-helix transcription factor NEUROG3 is required for development of the human endocrine pancreas. *Diabetes* **64**, 2497-2505. doi:10.2337/db14-1412

Meier, J. J., Butler, A. E., Saisho, Y., Monchamp, T., Galasso, R., Bhushan, A., Rizza, R. A. and Butler, P. C. (2008). β-cell replication is the primary mechanism subserving the postnatal expansion of β-cell mass in humans. *Diabetes* **57**, 1584-1594. doi:10.2337/db07-1369

Miyatsuka, T., Li, Z. and German, M. S. (2009). Chronology of islet differentiation revealed by temporal cell labeling. *Diabetes* **58**, 1863-1868. doi:10.2337/db09-0390

Miyazaki, S., Tashiro, F. and Miyazaki, J.-I. (2016). Transgenic expression of a single transcription factor Pdx1 induces transdifferentiation of pancreatic acinar cells to endocrine cells in adult mice. *PLoS ONE* **11**, e0161190. doi:10.1371/journal.pone.0161190

Morris, S. A. (2019). The evolving concept of cell identity in the single cell era. *Development* **146**, dev169748. doi:10.1242/dev.169748

Muraro, M. J., Dharmadhikari, G., Grün, D., Groen, N., Dielen, T., Jansen, E., van Gurp, L., Engelse, M. A., Carlotti, F., de Koning, E. J. P. et al. (2016). A single-cell transcriptome atlas of the human pancreas. *Cell Syst.* **3**, 385-394.e3. doi:10.1016/j.cels.2016.09.002

Murtaugh, L. C. (2008). The what, where, when and how of Wnt/β-catenin signaling in pancreas development. *Organogenesis* **4**, 81-86. doi:10.4161/org.4.2.5853
Nagano, T., Lubling, Y., Stevens, T. J., Schoenfelder, S., Yaffe, E., Dean, W., Laue, E. D., Tanay, A. and Fraser, P. (2013). Single-cell Hi-C reveals cell-to-cell variability in chromosome structure. *Nature* **502**, 59-64. doi:10.1038/nature12593
Nair, G. G., Liu, J. S., Russ, H. A., Tran, S., Saxton, M. S., Chen, R., Juang, C., Li, M.-L., Nguyen, V. Q., Giacometti, S. et al. (2019). Recapitulating endocrine cell clustering in culture promotes maturation of human stem-cell-derived β cells. *Nat. Cell Biol.* **21**, 263-274. doi:10.1038/s41556-018-0271-4
Nasteska, D. and Hodson, D. J. (2018). The role of beta cell heterogeneity in islet function and insulin release. *J. Mol. Endocrinol.* **61**, R43-R60. doi:10.1530/JME-18-0011
Ni, Q., Gu, Y., Xie, Y., Yin, Q., Zhang, H., Nie, A., Li, W., Wang, Y., Ning, G., Wang, W. et al. (2017). Raptor regulates functional maturation of murine beta cells. *Nat. Commun.* **8**, 15755. doi:10.1038/ncomms15755
Nishimura, W., Kondo, T., Salameh, T., El Khattabi, I., Dodge, R., Bonner-Weir, S. and Sharma, A. (2006). A switch from MafB to MafA expression accompanies differentiation to pancreatic β-cells. *Dev. Biol.* **293**, 526-539. doi:10.1016/j.ydbio.2006.02.028
Ohta, Y., Kosaka, Y., Kishimoto, N., Wang, J., Smith, S. B., Honig, G., Kim, H., Gasa, R. M., Neubauer, N., Liou, A. et al. (2011). Convergence of the insulin and serotonin programs in the pancreatic β-cell. *Diabetes* **60**, 3208-3216. doi:10.2337/db10-1192
Pagliuca, F. W., Millman, J. R., Gürtler, M., Segel, M., Van Dervort, A., Ryu, J. H., Peterson, Q. P., Greiner, D. and Melton, D. A. (2014). Generation of functional human pancreatic β cells in vitro. *Cell* **159**, 428-439. doi:10.1016/j.cell.2014.09.040
Pan, F. C. and Brissova, M. (2014). Pancreas development in humans. *Curr. Opin Endocrinol. Diabetes Obes.* **21**, 77-82. doi:10.1097/MED.0000000000000047
Pan, F. C. and Wright, C. (2011). Pancreas organogenesis: from bud to plexus to gland. *Dev. Dyn.* **240**, 530-565. doi:10.1002/dvdy.22584
Parikh, A., Stephan, A.-F. and Tzanakakis, E. S. (2012). Regenerating proteins and their expression, regulation, and signaling. *Biomol. Concepts* **3**, 57-70. doi:10.1515/bmc.2011.055
Parsons, J. A., Brelje, T. C. and Sorenson, R. L. (1992). Adaptation of islets of Langerhans to pregnancy: increased islet cell proliferation and insulin secretion correlates with the onset of placental lactogen secretion. *Endocrinology* **130**, 1459-1466. doi:10.1210/en.130.3.1459
Pedersen, A. H. and Heller, R. S. (2005). A possible role for the canonical Wnt pathway in endocrine cell development in chicks. *Biochem. Biophys. Res. Commun.* **333**, 961-968. doi:10.1016/j.bbrc.2005.05.189
Petersen, M. B. K., Azad, A., Ingvorsen, C., Hess, K., Hansson, M., Grapin-Botton, A. and Honoré, C. (2017). Single-cell gene expression analysis of a human ESC model of pancreatic endocrine development reveals different paths to β-cell differentiation. *Stem Cell Rep.* **9**, 1246-1261. doi:10.1016/j.stemcr.2017.08.009
Petersen, M. B. K., Gonçalves, C. A. C., Kim, Y. H. and Grapin-Botton, A. (2018). Recapitulating and deciphering human pancreas development from human pluripotent stem cells in a dish. *Curr. Top. Dev. Biol.* **129**, 143-190. doi:10.1016/bs.ctdb.2018.02.009
Piccand, J., Meunier, A., Merle, C., Jia, Z., Barnier, J.-V. and Gradwohl, G. (2014). Pak3 promotes cell cycle exit and differentiation of β-cells in the embryonic pancreas and is necessary to maintain glucose homeostasis in adult mice. *Diabetes* **63**, 203-215. doi:10.2337/db13-0384
Picelli, S. (2017). Single-cell RNA-sequencing: the future of genome biology is now. *RNA Biol.* **14**, 637-650. doi:10.1080/15476286.2016.1201618
Picelli, S., Faridani, O. R., Björklund, A. K., Winberg, G., Sagasser, S. and Sandberg, R. (2014). Full-length RNA-seq from single cells using Smart-seq2. *Nat. Protoc.* **9**, 171-181. doi:10.1038/nprot.2014.006
Pijuan-Sala, B., Guibentif, C. and Göttgens, B. (2018). Single-cell transcriptional profiling: a window into embryonic cell-type specification. *Nat. Rev. Mol. Cell Biol.* **19**, 399-412. doi:10.1038/s41580-018-0002-5
Pinney, S. E., Oliver-Krasinski, J., Ernst, L., Hughes, N., Patel, P., Stoffers, D. A., Russo, P. and De León, D. D. (2011). Neonatal diabetes and congenital malabsorptive diarrhea attributable to a novel mutation in the human neurogenin-3 gene coding sequence. *J. Clin. Endocrinol. Metab.* **96**, 1960-1965. doi:10.1210/jc.2011-0029
Piper, K., Brickwood, S., Turnpenny, L. W., Cameron, I. T., Ball, S. G., Wilson, D. I. and Hanley, N. A. (2004). Beta cell differentiation during early human pancreas development. *J. Endocrinol.* **181**, 11-23. doi:10.1677/joe.0.1810011
Potter, S. S. (2018). Single-cell RNA sequencing for the study of development, physiology and disease. *Nat. Rev. Nephrol.* **14**, 479-492. doi:10.1038/s41581-018-0021-7
Qiu, W.-L., Zhang, Y.-W., Feng, Y., Li, L.-C., Yang, L. and Xu, C.-R. (2017). Deciphering pancreatic islet β cell and α cell maturation pathways and characteristic features at the single-cell level. *Cell Metab.* **25**, 1194-1205.e4. doi:10.1016/j.cmet.2017.04.003
Rabhi, N., Salas, E., Froguel, P. and Annicotte, J.-S. (2014). Role of the unfolded protein response in β cell compensation and failure during diabetes. *J. Diabetes Res.* **2014**, 795171. doi:10.1155/2014/795171
Rahier, J., Guiot, Y., Goebbels, R. M., Sempoux, C. and Henquin, J. C. (2008). Pancreatic β-cell mass in European subjects with type 2 diabetes. *Diabetes Obes. Metab.* **10** Suppl. 4, 32-42. doi:10.1111/j.1463-1326.2008.00969.x
Ramani, V., Deng, X., Qiu, R., Gunderson, K. L., Steemers, F. J., Disteche, C. M., Noble, W. S., Duan, Z. and Shendure, J. (2017). Massively multiplex single-cell Hi-C. *Nat. Methods* **14**, 263-266. doi:10.1038/nmeth.4155
Ramond, C., Beydag-Tasöz, B. S., Azad, A., van de Bunt, M., Petersen, M. B. K., Beer, N. L., Glaser, N., Berthault, C., Gloyn, A. L., Hansson, M. et al. (2018). Understanding human fetal pancreas development using subpopulation sorting, RNA sequencing and single-cell profiling. *Development* **145**, dev165480. doi:10.1242/dev.165480
Rezania, A., Bruin, J. E., Arora, P., Rubin, A., Batushansky, I., Asadi, A., O'Dwyer, S., Quiskamp, N., Mojibian, M., Albrecht, T. et al. (2014). Reversal of diabetes with insulin-producing cells derived in vitro from human pluripotent stem cells. *Nat. Biotechnol.* **32**, 1121-1133. doi:10.1038/nbt.3033
Richardson, C. C., Hussain, K., Jones, P. M., Persaud, S., Lobner, K., Boehm, A., Clark, A. and Christie, M. R. (2007). Low levels of glucose transporters and K+ATP channels in human pancreatic beta cells early in development. *Diabetologia* **50**, 1000-1005. doi:10.1007/s00125-007-0644-x
Riedel, M. J., Asadi, A., Wang, R., Ao, Z., Warnock, G. L. and Kieffer, T. J. (2012). Immunohistochemical characterisation of cells co-producing insulin and glucagon in the developing human pancreas. *Diabetologia* **55**, 372-381. doi:10.1007/s00125-011-2344-9
Rodnoi, P., Rajkumar, M., Moin, A. S. M., Georgia, S. K., Butler, A. E. and Dhawan, S. (2017). Neuropeptide Y expression marks partially differentiated β cells in mice and humans. *JCI Insight* **2**, e94005. doi:10.1172/jci.insight.94005
Rodriguez-Seguel, E., Mah, N., Naumann, H., Pongrac, I. M., Cerda-Esteban, N., Fontaine, J.-F., Wang, Y., Chen, W., Andrade-Navarro, M. A. and Spagnoli, F. M. (2013). Mutually exclusive signaling signatures define the hepatic and pancreatic progenitor cell lineage divergence. *Genes Dev.* **27**, 1932-1946. doi:10.1101/gad.220244.113
Rubio-Cabezas, O., Jensen, J. N., Hodgson, M. I., Codner, E., Ellard, S., Serup, P. and Hattersley, A. T. (2011). Permanent neonatal diabetes and enteric anendocrinosis associated with biallelic mutations in NEUROG3. *Diabetes* **60**, 1349-1353. doi:10.2337/db10-1008
Russ, H. A., Parent, A. V., Ringler, J. J., Hennings, T. G., Nair, G. G., Shveygert, M., Guo, T., Puri, S., Haataja, L., Cirulli, V. et al. (2015). Controlled induction of human pancreatic progenitors produces functional beta-like cells in vitro. *EMBO J.* **34**, 1759-1772. doi:10.15252/embj.201591058
Salinno, C., Cota, P., Bastidas-Ponce, A., Tarquis-Medina, M., Lickert, H. and Bakhti, M. (2019). β-cell maturation and identity in health and disease. *Int. J. Mol. Sci.* **20**, 5417. doi:10.3390/ijms20215417
Satpathy, A. T., Granja, J. M., Yost, K. E., Qi, Y., Meschi, F., McDermott, G. P., Olsen, B. N., Mumbach, M. R., Pierce, S. E., Corces, M. R. et al. (2019). Massively parallel single-cell chromatin landscapes of human immune cell development and intratumoral T cell exhaustion. *Nat. Biotechnol.* **37**, 925-936. doi:10.1038/s41587-019-0206-z
Scavuzzo, M. A., Hill, M. C., Chmielowiec, J., Yang, D., Teaw, J., Sheng, K., Kong, Y., Bettini, M., Zong, C., Martin, J. F. et al. (2018). Endocrine lineage biases arise in temporally distinct endocrine progenitors during pancreatic morphogenesis. *Nat. Commun.* **9**, 3356. doi:10.1038/s41467-018-05740-1
Segerstolpe, A., Palasantza, A., Eliasson, P., Andersson, E.-M., Andréasson, A.-C., Sun, X., Picelli, S., Sabirsh, A., Clausen, M., Bjursell, M. K. et al. (2016). Single-cell transcriptome profiling of human pancreatic islets in health and type 2 diabetes. *Cell Metab.* **24**, 593-607. doi:10.1016/j.cmet.2016.08.020
Sharma, R. B., O'Donnell, A. C., Stamateris, R. E., Ha, B., McCloskey, K. M., Reynolds, P. R., Arvan, P. and Alonso, L. C. (2015). Insulin demand regulates β cell number via the unfolded protein response. *J. Clin. Invest.* **125**, 3831-3846. doi:10.1172/JCI79264
Sharon, N., Chawla, R., Mueller, J., Vanderhooft, J., Whitehorn, L. J., Rosenthal, B., Gürtler, M., Estanboulieh, R. R., Shvartsman, D., Gifford, D. K. et al. (2019a). A peninsular structure coordinates asynchronous differentiation with morphogenesis to generate pancreatic islets. *Cell* **176**, 790-804.e13. doi:10.1016/j.cell.2018.12.003
Sharon, N., Vanderhooft, J., Straubhaar, J., Mueller, J., Chawla, R., Zhou, Q., Engquist, E. N., Trapnell, C., Gifford, D. K. and Melton, D. A. (2019b). Wnt signaling separates the progenitor and endocrine compartments during pancreas development. *Cell Rep.* **27**, 2281-2291.e5. doi:10.1016/j.celrep.2019.04.083
Shih, H. P., Wang, A. and Sander, M. (2013). Pancreas organogenesis: from lineage determination to morphogenesis. *Annu. Rev. Cell Dev. Biol.* **29**, 81-105. doi:10.1146/annurev-cellbio-101512-122405
Sinagoga, K. L., Stone, W. J., Schiesser, J. V., Schweitzer, J. I., Sampson, L., Zheng, Y. and Wells, J. M. (2017). Distinct roles for the mTOR pathway in postnatal morphogenesis, maturation and function of pancreatic islets. *Development* **144**, 2402-2414. doi:10.1242/dev.146316
Sneddon, J. B., Tang, Q., Stock, P., Bluestone, J. A., Roy, S., Desai, T. and Hebrok, M. (2018). Stem cell therapies for treating diabetes: progress and remaining challenges. *Cell Stem Cell* **22**, 810-823. doi:10.1016/j.stem.2018.05.016
Solar, M., Cardalda, C., Houbracken, I., Martín, M., Maestro, M. A., De Medts, N., Xu, X., Grau, V., Heimberg, H., Bouwens, L. et al. (2009). Pancreatic exocrine

duct cells give rise to insulin-producing β cells during embryogenesis but not after birth. *Dev. Cell* **17**, 849-860. doi:10.1016/j.devcel.2009.11.003

Sorenson, R. L. and Brelje, T. C. (1997). Adaptation of islets of Langerhans to pregnancy: β-cell growth, enhanced insulin secretion and the role of lactogenic hormones. *Horm. Metab. Res.* **29**, 301-307. doi:10.1055/s-2007-979040

Spitzer, M. H. and Nolan, G. P. (2016). Mass cytometry: single cells, many features. *Cell* **165**, 780-791. doi:10.1016/j.cell.2016.04.019

Stanescu, D. E., Yu, R., Won, K.-J. and Stoffers, D. A. (2017). Single cell transcriptomic profiling of mouse pancreatic progenitors. *Physiol. Genomics* **49**, 105-114. doi:10.1152/physiolgenomics.00114.2016

Stewart, A. F., Hussain, M. A., García-Ocaña, A., Vasavada, R. C., Bhushan, A., Bernal-Mizrachi, E. and Kulkarni, R. N. (2015). Human β-cell proliferation and intracellular signaling: part 3. *Diabetes* **64**, 1872-1885. doi:10.2337/db14-1843

Stolovich-Rain, M., Enk, J., Vikesa, J., Nielsen, F. C., Saada, A., Glaser, B. and Dor, Y. (2015). Weaning triggers a maturation step of pancreatic β cells. *Dev. Cell* **32**, 535-545. doi:10.1016/j.devcel.2015.01.002

Sznurkowska, M. K., Hannezo, E., Azzarelli, R., Rulands, S., Nestorowa, S., Hindley, C. J., Nichols, J., Göttgens, B., Huch, M., Philpott, A. et al. (2018). Defining lineage potential and fate behavior of precursors during pancreas development. *Dev. Cell* **46**, 360-375.e5. doi:10.1016/j.devcel.2018.06.028

Tan, S. Y., Mei Wong, J. L., Sim, Y. J., Wong, S. S., Mohamed Elhassan, S. A., Tan, S. H., Ling Lim, G. P., Rong Tay, N. W., Annan, N. C., Bhattamisra, S. K. et al. (2019). Type 1 and 2 diabetes mellitus: a review on current treatment approach and gene therapy as potential intervention. *Diabetes Metab. Syndr.* **13**, 364-372. doi:10.1016/j.dsx.2018.10.008

Teta, M., Long, S. Y., Wartschow, L. M., Rankin, M. M. and Kushner, J. A. (2005). Very slow turnover of β-cells in aged adult mice. *Diabetes* **54**, 2557-2567. doi:10.2337/diabetes.54.9.2557

Thorel, F., Népote, V., Avril, I., Kohno, K., Desgraz, R., Chera, S. and Herrera, P. L. (2010). Conversion of adult pancreatic α-cells to β-cells after extreme β-cell loss. *Nature* **464**, 1149-1154. doi:10.1038/nature08894

Tritschler, S., Theis, F. J., Lickert, H. and Böttcher, A. (2017). Systematic single-cell analysis provides new insights into heterogeneity and plasticity of the pancreas. *Mol. Metab.* **6**, 974-990. doi:10.1016/j.molmet.2017.06.021

van Arensbergen, J., Garcia-Hurtado, J., Moran, I., Maestro, M. A., Xu, X., Van de Casteele, M., Skoudy, A. L., Palassini, M., Heimberg, H. and Ferrer, J. (2010). Derepression of Polycomb targets during pancreatic organogenesis allows insulin-producing beta-cells to adopt a neural gene activity program. *Genome Res.* **20**, 722-732. doi:10.1101/gr.101709.109

van der Meulen, T., Lee, S., Noordeloos, E., Donaldson, C. J., Adams, M. W., Noguchi, G. M., Mawla, A. M. and Huising, M. O. (2018). Artemether does not turn α cells into β cells. *Cell Metab.* **27**, 218-225.e4. doi:10.1016/j.cmet.2017.10.002

van Gurp, L., Muraro, M. J., Dielen, T., Seneby, L., Dharmadhikari, G., Gradwohl, G., van Oudenaarden, A. and de Koning, E. J. P. (2019). A transcriptomic roadmap to α- and β-cell differentiation in the embryonic pancreas. *Development* **146**, dev173716. doi:10.1242/dev.173716

Velazco-Cruz, L., Song, J., Maxwell, K. G., Goedegebuure, M. M., Augsornworawat, P., Hogrebe, N. J. and Millman, J. R. (2019). Acquisition of dynamic function in human stem cell-derived β cells. *Stem Cell Rep.* **12**, 351-365. doi:10.1016/j.stemcr.2018.12.012

Veres, A., Faust, A. L., Bushnell, H. L., Engquist, E. N., Kenty, J. H.-R., Harb, G., Poh, Y.-C., Sintov, E., Gürtler, M., Pagliuca, F. W. et al. (2019). Charting cellular identity during human in vitro β-cell differentiation. *Nature* **569**, 368-373. doi:10.1038/s41586-019-1168-5

Villasenor, A., Chong, D. C. and Cleaver, O. (2008). Biphasic Ngn3 expression in the developing pancreas. *Dev. Dyn.* **237**, 3270-3279. doi:10.1002/dvdy.21740

Wang, Y. J. and Kaestner, K. H. (2019). Single-cell RNA-seq of the pancreatic islets–a promise not yet fulfilled? *Cell Metab.* **29**, 539-544. doi:10.1016/j.cmet.2018.11.016

Wang, S., Zhang, J., Zhao, A., Hipkens, S., Magnuson, M. A. and Gu, G. (2007). Loss of Myt1 function partially compromises endocrine islet cell differentiation and pancreatic physiological function in the mouse. *Mech. Dev.* **124**, 898-910. doi:10.1016/j.mod.2007.08.004

Wang, S., Hecksher-Sorensen, J., Xu, Y., Zhao, A., Dor, Y., Rosenberg, L., Serup, P. and Gu, G. (2008). Myt1 and Ngn3 form a feed-forward expression loop to promote endocrine islet cell differentiation. *Dev. Biol.* **317**, 531-540. doi:10.1016/j.ydbio.2008.02.052

Wang, Y. J., Golson, M. L., Schug, J., Traum, D., Liu, C., Vivek, K., Dorrell, C., Naji, A., Powers, A. C., Chang, K.-M. et al. (2016a). Single-cell mass cytometry analysis of the human endocrine pancreas. *Cell Metab.* **24**, 616-626. doi:10.1016/j.cmet.2016.09.007

Wang, Y. J., Schug, J., Won, K.-J., Liu, C., Naji, A., Avrahami, D., Golson, M. L. and Kaestner, K. H. (2016b). Single-cell transcriptomics of the human endocrine pancreas. *Diabetes* **65**, 3028-3038. doi:10.2337/db16-0405

Wang, Q., Xiong, H., Ai, S., Yu, X., Liu, Y., Zhang, J. and He, A. (2019). CoBATCH for high-throughput single-cell epigenomic profiling. *Mol. Cell* **76**, 206-216.e7. doi:10.1016/j.molcel.2019.07.015

Wollny, D., Zhao, S., Everlien, I., Lun, X., Brunken, J., Brüne, D., Ziebell, F., Tabansky, I., Weichert, W., Marciniak-Czochra, A. et al. (2016). Single-cell analysis uncovers clonal acinar cell heterogeneity in the adult pancreas. *Dev. Cell* **39**, 289-301. doi:10.1016/j.devcel.2016.10.002

Xiao, X., Guo, P., Shiota, C., Zhang, T., Coudriet, G. M., Fischbach, S., Prasadan, K., Fusco, J., Ramachandran, S., Witkowski, P. et al. (2018). Endogenous reprogramming of alpha cells into beta cells, induced by viral gene therapy, reverses autoimmune diabetes. *Cell Stem Cell* **22**, 78-90.e4. doi:10.1016/j.stem.2017.11.020

Xin, Y., Kim, J., Okamoto, H., Ni, M., Wei, Y., Adler, C., Murphy, A. J., Yancopoulos, G. D., Lin, C. and Gromada, J. (2016a). RNA sequencing of single human islet cells reveals type 2 diabetes genes. *Cell Metab.* **24**, 608-615. doi:10.1016/j.cmet.2016.08.018

Xin, Y., Okamoto, H., Kim, J., Ni, M., Adler, C., Cavino, K., Na, E., Murphy, A. J., Yancopoulos, G. D., Lin, C. et al. (2016b). Single-cell RNAseq reveals that pancreatic β-cells from very old male mice have a young gene signature. *Endocrinology* **157**, 3431-3438. doi:10.1210/en.2016-1235

Xin, Y., Kim, J., Ni, M., Wei, Y., Okamoto, H., Lee, J., Adler, C., Cavino, K., Murphy, A. J., Yancopoulos, G. D. et al. (2016c). Use of the Fluidigm C1 platform for RNA sequencing of single mouse pancreatic islet cells. *Proc. Natl Acad. Sci. USA* **113**, 201602306. doi:10.1073/pnas.1602306113

Xin, Y., Dominguez Gutierrez, G., Okamoto, H., Kim, J., Lee, A.-H., Adler, C., Ni, M., Yancopoulos, G. D., Murphy, A. J. and Gromada, J. (2018). Pseudotime ordering of single human β-cells reveals states of insulin production and unfolded protein response. *Diabetes* **67**, 1783-1794. doi:10.2337/db18-0365

Xu, X., D'Hoker, J., Stangé, G., Bonné, S., De Leu, N., Xiao, X., Van de Casteele, M., Mellitzer, G., Ling, Z., Pipeleers, D. et al. (2008). β cells can be generated from endogenous progenitors in injured adult mouse pancreas. *Cell* **132**, 197-207. doi:10.1016/j.cell.2007.12.015

Xu, Q., Fu, R., Yin, G., Liu, X., Liu, Y. and Xiang, M. (2016). Microarray-based gene expression profiling reveals genes and pathways involved in the oncogenic function of REG3A on pancreatic cancer cells. *Gene* **578**, 263-273. doi:10.1016/j.gene.2015.12.039

Yang, Y.-P., Thorel, F., Boyer, D. F., Herrera, P. L. and Wright, C. V. E. (2011). Context-specific α- to-β-cell reprogramming by forced Pdx1 expression. *Genes Dev.* **25**, 1680-1685. doi:10.1101/gad.16875711

Yu, X.-X., Qiu, W.-L., Yang, L., Li, L.-C., Zhang, Y.-W. and Xu, C.-R. (2018). Dynamics of chromatin marks and the role of JMJD3 during pancreatic endocrine cell fate commitment. *Development* **145**, dev163162. doi:10.1242/dev.163162

Yu, X.-X., Qiu, W.-L., Yang, L., Zhang, Y., He, M.-Y., Li, L.-C. and Xu, C.-R. (2019). Defining multistep cell fate decision pathways during pancreatic development at single-cell resolution. *EMBO J.* **38**, e100164. doi:10.15252/embj.2018100164

Zeng, C., Mulas, F., Sui, Y., Guan, T., Miller, N., Tan, Y., Liu, F., Jin, W., Carrano, A. C., Huising, M. O. et al. (2017). Pseudotemporal ordering of single cells reveals metabolic control of postnatal β cell proliferation. *Cell Metab.* **25**, 1160-1175.e11. doi:10.1016/j.cmet.2017.04.014

Zhang, M., Lin, Q., Qi, T., Wang, T., Chen, C.-C., Riggs, A. D. and Zeng, D. (2016). Growth factors and medium hyperglycemia induce Sox9+ ductal cell differentiation into β cells in mice with reversal of diabetes. *Proc. Natl. Acad. Sci. USA* **113**, 650-655. doi:10.1073/pnas.1524200113

Zhang, X., Li, T., Liu, F., Chen, Y., Yao, J., Li, Z., Huang, Y. and Wang, J. (2019). Comparative analysis of droplet-based ultra-high-throughput single-cell RNA-seq systems. *Mol. Cell* **73**, 130-142.e5. doi:10.1016/j.molcel.2018.10.020

Zheng, G. X. Y., Terry, J. M., Belgrader, P., Ryvkin, P., Bent, Z. W., Wilson, R., Ziraldo, S. B., Wheeler, T. D., McDermott, G. P., Zhu, J. et al. (2017). Massively parallel digital transcriptional profiling of single cells. *Nat. Commun.* **8**, 14049. doi:10.1038/ncomms14049

Zhou, Q. and Melton, D. A. (2018). Pancreas regeneration. *Nature* **557**, 351-358. doi:10.1038/s41586-018-0088-0

Zhou, Q., Brown, J., Kanarek, A., Rajagopal, J. and Melton, D. A. (2008). In vivo reprogramming of adult pancreatic exocrine cells to β-cells. *Nature* **455**, 627-632. doi:10.1038/nature07314

Zhou, J., Ma, J., Chen, Y., Cheng, C., Bao, B., Peng, J., Sejnowski, T. J., Dixon, J. R. and Ecker, J. R. (2019). Robust single-cell Hi-C clustering by convolution- and random-walk-based imputation. *Proc. Natl. Acad. Sci. USA* **116**, 14011-14018. doi:10.1073/pnas.1901423116

Zhu, H., Yu, L., He, Y. and Wang, B. (2014). Nonhuman primate models of type 1 diabetes mellitus for islet transplantation. *J. Diabetes Res.* **2014**, 785948. doi:10.1155/2014/785948

Zhu, Z., Li, Q. V., Lee, K., Rosen, B. P., González, F., Soh, C.-L. and Huangfu, D. (2016). Genome editing of lineage determinants in human pluripotent stem cells reveals mechanisms of pancreatic development and diabetes. *Cell Stem Cell* **18**, 755-768. doi:10.1016/j.stem.2016.03.015

Ziegenhain, C., Vieth, B., Parekh, S., Reinius, B., Guillaumet-Adkins, A., Smets, M., Leonhardt, H., Heyn, H., Hellmann, I. and Enard, W. (2017). Comparative analysis of single-cell RNA sequencing methods. *Mol. Cell* **65**, 631-643.e4. doi:10.1016/j.molcel.2017.01.023