Published in final edited form as:

Semin Cell Dev Biol. 2012 August ; 23(6): 673–684. doi:10.1016/j.semcdb.2012.06.005.

Lineage determinants in early endocrine development

Sebastian Rieck¹, Eric D. Bankaitis¹, and Christopher V.E. Wright^{1,2}

¹Vanderbilt University Program in Developmental Biology, Department of Cell and Developmental Biology, Vanderbilt University Medical Center, Nashville, Tennessee 37232, USA

Abstract

Pancreatic endocrine cells are produced from a dynamic epithelium in a process that, as in any developing organ, is driven by interacting programs of spatiotemporally regulated intercellular signals and autonomous gene regulatory networks. These algorithms work to push progenitors and their transitional intermediates through a series of railroad-station-like switching decisions to regulate flux along specific differentiation tracks. Extensive research on pancreas organogenesis over the last 20 years, greatly spurred by the potential to restore functional β -cell mass in diabetic patients by transplantation therapy, is advancing our knowledge of how endocrine lineage bias is established and allocation is promoted. The field is working towards the goal of generating a detailed blueprint of how heterogeneous cell populations interact and respond to each other, and other influences such as the extracellular matrix, to move into progressively refined and mature cell states. Here, we highlight how signaling codes and transcriptional networks might determine endocrine lineage within a complex and dynamic architecture, based largely on studies in the mouse. The process begins with the designation of multipotent progenitor cells (MPC) to pancreatic buds that subsequently move through a newly proposed period involving epithelial plexus formation-remodeling, and ends with formation of clustered endocrine islets connected to the vascular and peripheral nervous systems. Developing this knowledge base, and increasing the emphasis on direct comparisons between mouse and human, will yield a more complete and focused picture of pancreas development, and thereby inform β -cell-directed differentiation from human embryonic stem or induced pluripotent stem cells (hESC, iPSC). Additionally, a deeper understanding may provide surprising therapeutic angles by defining conditions that allow the controllable reprogramming of endodermal or pancreatic cell populations.

Keywords

pancreas organogenesis; multipotent progenitor cell; transcription factor; tubulogenesis; tip-trunk compartmentalization; plexus remodeling; branching morphogenesis; endocrine subtype specification and maturation; islet; human pancreas; reprogramming; human embryonic stem cells; transcription factor; gene regulatory network; primary transition; secondary transition

1. Introduction

Since the successful (albeit temporary) removal of brittle type 1 diabetic patients from insulin-dependence by transplantation of cadaver-derived islets, there has been a great push

© 2012 Elsevier Ltd. All rights reserved

²Corresponding author. chris.wright@vanderbilt.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

to understand the methods that could be applied *in vitro* to obtain functioning β cells (1). Added incentive comes because the demand for β cells (or islets, as discussed later) far outstrips any conceivable cadaver-based source. To date, *in vitro* protocols have attempted to entrain hESC along a still poorly defined sequence of events, initially biasing them towards definitive endoderm, then channeling them to primitive gut tube epithelium, posterior foregut, pancreatic-endoderm progenitors and endocrine precursors, *en route* to insulin (Ins)-expressing cells. However, mature endocrine cell formation is inefficient and incomplete; the number of β -like cells formed is low, and unlike their endogenous counterparts they are poorly glucose-responsive (1, 2). And yet, intermediate-stage pro-pancreatic cells derived from such protocols that are transplanted into mice can, after several months, apparently yield mature, functional β cells (3). This *in vivo* maturation is a real ‘black box’ phenomenon: what happens post-implantation remains cryptic. It could be useful to determine the local or systemic signals and/or other factors that are driving the transplanted cells along a proper endocrine/ β -cell differentiation program. The corollary is that all current *in vitro* differentiation protocols induce a slightly incorrect, off-track program, leading to pseudo- β -cells (4). Which begs the question: What are the missing *in vivo* components that induce complete β -cell maturation *in vitro*? Is it a lack of ‘community effect’ involving planar cell polarity and apical-basal polarization present in the normal 3D epithelial structure, or is the *in vitro* differentiation process being forced along too quickly? Or, could there be just one or two missing critical factor(s), which might be identified or mimicked soon by appropriate screening methods?

hESC generally grow and differentiate in two-dimensional (2D) monolayer conditions. In contrast, the pipeline for endocrine development *in vivo* involves potentially complex 3D niches within the developing pancreatic epithelium, and these may be highly dynamic as the plexus intermediate moves towards an organized, tubular network (5). It is possible, therefore, that the current applications of inducer cocktails, although designed to mimic the normal developmental process, fail to recapitulate the finely timed triggers and signaling thresholds that exist in real tissue. We have gained some broad knowledge of how regionalized transcription factor (TF) expression distinguishes the evolving epithelial compartments (6). But, more information is needed regarding the timing, context, and expression thresholds of specific transcription factors (TFs), and how they interact with epigenetic control factors in individual genes, and in entire gene regulatory networks (GRN), to control endocrine-biased differentiation (7). The development of additional prospective cell markers and a detailed appreciation of local tissue landmarks should aid in obtaining a fundamental, sequential map of cellular transitional phases. We therefore predict, over the next few years, an almost certain substantial overhaul of our understanding of endocrine ontogeny. The new realization that the pancreatic epithelium develops through a plexus intermediate, whose resolution (to an epithelial tree) and differentiation is temporally asynchronous across the organ (6, 8), means that understanding lineage allocation could involve defining the epithelium using classical developmental biology terms such as equivalence groups (which could be relatively small, reiterated groups of cells, having a specific interaction structure), and how these groups are tiled through the plexus and resolving epithelial tree. This concept is important because cells expressing the endocrine commitment factor Neurogenin 3 (Neurog3 or Ngn3) are already delaminating from the epithelium in large numbers at the plexus stage (Figure 1C). How regionally distributed cells or groups advance developmentally with respect to each other, and how they give birth to various numbers of each hormone-positive endocrine cell, could tell us how the pancreas grows to its final size with the appropriate endocrine cell ratio within the islets of Langerhans (9). Most importantly, characterizing transient niches within the developing plexus, along with any facilitating (permissive) signals from the adjacent mesenchyme (10–12), could identify factors missing from hESC differentiation protocols. As we shall address below, small molecule or miRNA treatments, together with synthetic

scaffolds and embryoid/spheroid cultures could effectively substitute for the signaling pathways operative *in vivo*.

One critical unknown is whether it is strictly necessary to reproduce all aspects of endocrine ontogeny operating *in vivo* to achieve large-scale differentiation of functional β -cells *in vitro*. Instead of triggering a multitude of steps each via manual intervention, it is possible that a systemic factor or small set of local signals initiate relatively autonomous ('autopilot') gene regulatory 'modules' that push cells relatively automatically along the endocrine pipeline (Figure 2). Here, we highlight some of the most important instructive steps determined to date, and address the biggest current deficiencies. When appropriate, we discuss similarities and differences between mouse and other model organisms, and try to make direct comparisons to what is known on human pancreas development. At the end of the review, a perspective section describes ideas on how genetic and other studies, done with cellular resolution, would allow a reductionist deconstruction of lineage commitment and differentiation. Our goal is to convince the reader that fundamental investigations into the mechanisms regulating the spatiotemporal and cell-type specificity of endocrine development should continue as a high-energy avenue towards a deeper knowledge base for translational application involving cellular transplantation-reconstitution or reprogramming (reviewed by Nostro and Keller; this issue).

2. The primary (1°) transition: MPC proliferation and priming phase (E9.5 – E12.5)

2.1 Multipotent progenitors of the pancreatic buds

Pancreas fate is specified from dorsal and ventral regions of 'pre-differentiated' epithelial cells arising within the posterior foregut endoderm – although, interestingly, the ventral pancreas emerges from common bile duct, while the dorsal bud is attached to duodenum (suggesting different specification strategies; as [editor please insert appropriate citation here] reviewed by Dr. J. Wells). The first signs of pancreas organogenesis are noticed at approximately embryonic day (E)9.5 to E10 when epithelial cells adopt a columnar character, evaginate, and thicken into surrounding mesenchyme. These initial processes result in formation of two 'fist-like,' complex stratified epithelia surrounding a primary central lumen (5), and comprising a pool of non-polarized multipotent progenitor cells (MPC) committed to pancreas-fate and defined as 'proto-differentiated' (13). Pulse-chase lineage-tracing experiments using *Pdx1*-CreER demonstrate that all subsequent progenitors and adult pancreas cells (acinar, duct, and islet endocrine) derive from these MPC (14). Limited experiments with individual cell labeling suggest that each MPC is competent to produce descendants in all three compartments (15). The term 'primary (1°) transition' (E9.5 to ~E12.5) encompasses, in addition to the aforementioned events, a proliferative epithelial expansion of 'primary' MPC, a 'first-wave' α -cell-biased window of endocrine cell formation, a re-establishment of apicobasal cell polarity and microlumen formation, and fusion of the dorsal and ventral pancreatic buds (Figure 1A–B, 2) (16).

While distinct developmental programs drive formation of the dorsal and ventral pancreatic buds (17), the total number of primary MPC allocated to the buds during the 1° transition seems to control final pancreas size (9). This result strongly suggests that a major influence in the 1° transition is to suppress differentiation while allowing sufficient proliferation in the proto-differentiated state. Indeed, decreased MPC proliferation or unchecked endocrine differentiation, caused for example by ablating various components of the Notch signaling pathway (18–21) (reviewed in Serup, this issue), rapidly depletes the MPC pool and decreases organ size. The lack of significant growth compensation when buds are founded by smaller numbers of MPC, unlike the situation for organs such as the liver (9), suggests

that downstream progenitors of all pancreas lineages (endocrine, duct, acinar) could have a ‘preprogrammed’ number of cell cycles with which to expand the tissue, allocate cell lineages, and construct a defined architecture. Such an epithelium-intrinsic program fits well with the idea that organ size is predetermined by founder cell number, but also raises the issue of the degree of regionalized lineage bias at the early MPC stage (Figure 1A insert, 2).

Towards understanding the cell biological processes that transform the stratified epithelium of the 1° transition bud into an epithelial plexus (5, 17), some of the largest gaps cover how various lineage-biased cell populations are established to build the initial tip and trunk structures (Figure 2). A related open question is whether all primary MPCs become incorporated into the compartment that produces endocrine cells (Figure 1A insert). Primary MPC heterogeneity is supported by single cell transcript analysis (22), but are the various cell types already arranged in a particular structure, or are gene expression programs relatively imprecise at these stages, with no determined effect on lineage potential? If a mixture of lineage-biased populations within the bud is required to build the requisite early niche(s) for producing the cells that have future endocrine competence (future trunk epithelium; see below), then we might be misdirected to focus on producing completely homogeneous populations of early-pancreatic cells by *in vitro* directed differentiation (23). These issues seem important to consider with respect to future work on the epigenetic regulation of pancreas development (reviewed by Avrahami and Kaestner, this issue), which could directly control how pre-patterned primary MPC become arranged into prospective epithelial domains (24).

While the early bud is predominantly disposed towards MPC proliferation and maintenance, some differentiated endocrine cell formation does occur during the 1° transition (Figure 2). A review by Jørgensen et al. (2007) is an excellent visual aid, providing 3D rotating views of mouse pancreas development (25). The expression of *Ngn3*, a bHLH TF gene that is required for endocrine cell differentiation, is detected within the dorsal bud (26). These ‘first wave’ endocrine cells appear as clusters of cells adjacent to the dorsal bud starting at E9.5 (20, 25). Of note, the apparent ‘aggregate delamination’ of endocrine cells observed during the 1° transition (rather than single-cell exit as currently assumed for later-stage epithelium of the secondary (2°) transition) could be similar to the delamination/budding mechanisms that may predominate in the formation of human β and other endocrine cells in human pancreas (Pan FC and Wright CV, preliminary observations, proposed in (27)). The potential for improving hESC/iPSC differentiation *in vitro* seems strong; for example, reproducing aggregate delamination in culture might aid derivation of proper β cells. During the 1° transition, glucagon⁺ (Gcg⁺) cells are the majority of these budding cells, although evidence of Ins, somatostatin, pancreatic polypeptide, and ghrelin expression in 1° transition buds exists at transcript (22) and protein level (25). Nevertheless, the fate and potential physiological functions of first-wave endocrine cells is at best controversial. Some have argued that Ins⁺ and Gcg⁺/Ins⁺ cells are short-lived, not contributing to the adult islets (28). But, pulse-chase lineage tracing and “add-back” studies with *Ngn3*-CreER concluded that some persist throughout pancreas development, becoming incorporated into the core and periphery of adult islets (8, 14). How these initial endocrine cells become integrated during the 2° transition plexus formation-resolution stages, towards the later establishment of normal islet architecture, is unknown.

While β cells seem to have a similar representation in adult dorsal and ventral-bud-derived tissues, in mice and humans (29, 30), the early discordance in dorsal/ventral developmental programs suggests that distinct cellular characteristics could exist between distinct ventrally and dorsally derived populations of the same islet cell type (31, 32). Indeed, a recent zebrafish study performed lineage-labeling of β cells specifically coming from dorsal or ventral bud and showed different gene expression profiles and mitotic indices (33). While

the ventral bud in the mouse also expresses Ngn3, immunofluorescence analysis showed delayed emergence (relative to dorsal bud) of only glucagon, pancreatic polypeptide and somatostatin-positive cells before dorsal/ventral bud fusion (25). These results suggest that even if the dorsal and ventral differentiation tracks generate equivalent β cells, it could be that one or the other program trajectory is more efficient or robust in producing high-fidelity β cells. Therefore, developing more precise lineage-tracing tools (that more finely segment early MPC populations) could help catalog key characteristic mechanisms of proliferation, specification, lineage allocation, and differentiation in the different early-stage MPC, all relevant to hESC/iPSC programming.

2.2 Transcriptional program underlining early MPC formation and maintenance

As we have alluded above, we would like to have a comprehensive atlas representative of prospective cell behaviors and fates, drawn precisely onto a map of the dynamic epithelium. The set of instructive cues that direct pancreatic MPC toward the endocrine lineage should include extrinsic factors from adjacent mesenchyme that interact with epithelium-intrinsic signals (as reviewed by Serup, this issue), and would include short-range para/juxtacrine influences, and feedback inhibition effects. These combinatorial signals would engage and affect gene activity and short/long-term epigenetic effects to comprise gene regulatory networks (GRN) within the MPC and derived populations. As part of this MPC-specific GRN, pancreatic epithelium TFs play dynamic, level-, and context-dependent roles in regulating cell fate specification. It is important to realize that specific TFs operate in multiple stages of pancreatic endocrine development. Furthermore, the presence/absence of other TFs, and cellular responsiveness, varies according to the cell's developmental history: the cumulative influences to date, blended with major effects from the epigenetic landscapes, with stage and cell-type variation. We strongly anticipate that TFs will be found to have, presumably through locus-selective coregulator recruitment and complementary epigenetic effects, activator or repressor functions, which are operative during specific phases of endocrine ontogeny, as suggested, for example, for Nkx2.2 by Sussel and colleagues (34). In our view, it is also particularly attractive to consider that, even within the same cell, one TF could activate or repress different sets of target loci. We highlight several of these principles through a discussion of Pdx1, Ptf1a, Sox9, Nkx6, and Hnf1 β . We refer the reader to more exhaustive reviews of pancreas TF action (17, 35, 36).

2.2.1 Pdx1, Ptf1a, Sox9, Nkx6, and Hnf1 β co-expression define pancreatic MPC—Stable pancreatic fate allocation is reinforced by interactions between a network of TFs. Pdx1, Ptf1a, Sox9, Nkx6 (here to represent both Nkx6.1 and Nkx6.2) and Hnf1 β are widely expressed in the MPC of the dorsal and ventral buds (14, 19, 37–39). Long-term TF-gene-based lineage-tracing (labeling during embryogenesis, analysis of adult tissue) gives similar results for all of these factors: cells in all three pancreatic compartments (endocrine, acini, ducts) derive from primary MPC expressing these TFs (14, 37, 40–42). Evidence for the Nkx6 factors comes from their spatiotemporal patterns of protein production (38). We hypothesize that regulatory interactions between these TFs ('loosely' defining a potential MPC-specific-GRN (7)) is a central process in priming MPC to become stably pancreas-committed, and capable of subsequent future segregation into endocrine vs. exocrine progenitor compartments (Figure 2A). Indeed, emerging evidence for such a TF network exists as there are Sox9 and Hnf1 β binding sites on putative *Ptf1a* promoter regions (39, 43). In addition to Ptf1a, Hnf1 β forms a transcription regulatory cascade with Hnf6, a regulator of pancreas specification (44). Ptf1a-mediated regulation of *Pdx1* through a specific *cis*-regulatory region might in part ensure the Ptf1a-Pdx1 coexpression state in early bud MPC (45), and Ptf1a might also stabilize MPC character through additional direct genetic interactions with *Hnf6*, *Nkx6.1* and *Mnx1* (46).

The temporal endurance of the MPC-specific GRN confers robustness to maintaining progenitor status while expanding the MPC pool during the 1° transition (Figure 2A). Removing single components, or altering the expression levels of *Pdx1*, *Ptf1a*, *Sox9* or *Hnf1β* can achieve GRN destabilization. While individual ablation of these TFs indicates their requirement for pancreas specification and bud outgrowth (19, 39, 47–49), in some cases cells of certain lineages still arise, including multiple types of endocrine cell. Importantly, this phenomenon raises the notion that there already exists phenotypic heterogeneity amongst the early bud cells (Figure 1A). Such heterogeneity, possibly resulting from the asynchronous evolution of differential competencies among and between resolving populations, could account for the spectrum of cellular responses caused by various genetic interventions at this stage. Furthermore, if processes such as proliferation and structure are coupled, then phenotypes perturbing proliferation could secondarily affect progression to differentiation. As global phenotypes might misrepresent such coupling, interpretations of mutant phenotypes by more focused mosaic analyses are needed. Finally, the idea that there are accessory endocrine-cell formation pathways could fit with reports that some animals, such as the mammal *Psammomys obesus* (sand rat), make a pancreas and β cells without apparently having a *Pdx1* gene, and raising the proposal that non-standard programs (independent of *Pdx1* and *Ptf1a*) could be harnessed to make endocrine cells for therapeutic application (50).

Ptf1a null mice lack a ventral pancreas, with extremely stunted growth and differentiation in the dorsal bud, leaving a *Sox9*⁺ and *Hnf1β*⁺ rudimentary epithelium (potentially indicating a limited degree of ‘trunk epithelium’ commitment) with a small number of relatively mature β and α cells strung alongside (37) (and Bankaitis ED and Wright CV, preliminary observations). On the other hand, most *Ptf1a*-deficient cells experience a different outcome, productively occupying and adopting normal cell fates within the adjacent common bile duct and duodenum (50). Similarly, in both mice and humans, total ablation of *Pdx1* causes almost complete pancreas agenesis (51, 52). In *Pdx1*^{-/-} mouse embryos, a highly retarded dorsal bud is formed with substantial *Gcg*⁺ α cells and few transient *Ins*⁺ cells. The ventral bud cells might either be re-routed to common bile duct or arrested in a non-proliferative yet still pancreas-specified state (50). *HNF1β* ablation leads to loss of *Ptf1a* expression, and results in a similar phenotype to *Ptf1a* ablation, namely absent ventral bud and transient dorsal bud (39). Ablating *Sox9* specifically in early pancreas MPC results in progenitor depletion and hypoplasia of both dorsal and ventral pancreas, through decreased proliferation, increased cell death, and precocious differentiation. Therefore, *Sox9* is essential to maintain MPC proliferation, survival and undifferentiated character (19). In addition to complete ablation, reducing the level of activity of a TF can lead to variegated phenotypes. Deleting 5' *cis*-regulatory regions (specifically ‘Areas I-II-III’ (53)) from *Pdx1* creates a hypomorphic allele (54) that does not affect pancreas specification from endoderm *per se*, but outgrowth and endocrine differentiation of the anlagen is highly abnormal. The distal region of the dorsal bud tissue differentiates as a simple dilated duct-like structure, while the gut-proximal tissue undergoes partial exocrine and endocrine formation. Implications from this particular study include there being different regional qualities within the buds, revealed as a differential gut-distal vs. gut-proximal response to reduced *Pdx1* expression (54). These studies suggest that the consequence of TF ablation or misregulation depends greatly on the cellular context in which it is ‘received’, even during the early proto-differentiated stage. Thus, the contribution that each component of the GRN makes toward determining MPC-behavior needs to be defined better, on the basis of both independent and cross-regulatory interactions, directly through chromatin immunoprecipitation (ChIP), epigenetics analysis, RNA-Sequencing based studies, and potentially aiming at gaining this knowledge within lineage-biased populations.

2.2.2 Spatial delineation between transcription factors resolves lineage in MPC

Starting at approximately E12.5, the MPC population begins to segregate into architecturally and functionally distinct tip and trunk domains (Figure 1B; as mentioned above, a transition possibly facilitated by earlier heterogeneity in the MPC). A reiterative intraepithelial and anlage-wide communication process could expand ‘tip vs. trunk segregation’ in an organized manner as the epithelium moves into and through the plexus state. During the early period of formation of the continuous plexus epithelium, MPC activity becomes progressively restricted to its many, perhaps incompletely resolved, tips (MPC having the basic marker signature $Pdx1^+$, $Ptf1a^+$, $CpaI^+$, $cMyc^{HIGH}$). The events during this phase must be considered quite different from the classical ‘branching morphogenesis’ of the lung and other organs, with clear implications as to the cellular communication niches that are possible (17). Quite dramatically, tip MPC activity becomes depleted within about a day, becoming essentially nonexistent by ~E14.5. The tip compartment as a whole becomes rapidly switched to the pro-acinar program (6) (reviewed by Cleveland, Sawyer, ... Leach, this issue). The non-tip, central ‘epithelial cord’, or trunk domain, contains a pool of cells with bipotency toward endocrine and duct fates (42).

The production of endocrine cells starts within the early bud (early α -cell generation) and continues as tip-trunk segregation occurs, now beginning to produce β , δ , PP, and ϵ cell types (Figure 2) (8, 13, 38, 55–57). Although specific TF combinations direct assignment to pancreas, the intertwined processes of proliferation and epithelial morphogenesis segregate cells to different locations and therefore interdependencies, which thus allow concomitant separation of TF groupings, leading to location-distinct restriction of differentiation pathways. Amongst the TFs discussed above, $Ptf1a^+/Pdx1^+$ define the tip domain, and $Sox9^+/Nkx6.1^+/Hnf1\beta^+/Pdx1^+$ the trunk domain. Here, a general principle emerges of progressive mutual exclusion of lineage potential. Mutually repressive activity between $Ptf1a$ and $Nkx6$ developed during tip-trunk compartmentalization could represent one of the bistable switch mechanisms that functionally segregate compartment competence (Figure 2B). While $Nkx6$ factors are required for trunk formation, tip (pro-acinar) formation requires $Ptf1a$, and each factor seems sufficient to repress initiation of the other lineage program (48, 58). A relevant trunk-promoting influence could be direct transcriptional repression of $Ptf1a$, because trunk TFs $Nkx6.1$, $Hnf1\beta$ and $Sox9$ can bind $Ptf1a$ regulatory regions (39, 43, 58). Other intraepithelial mechanisms including intercellular signals such as Notch signaling, and cofactor variation (the transition from MPC-defining $Ptf1a^{RBP-J}$ to pro-acinar $Ptf1a^{RBP-JL}$), also establish and reinforce selectivity of downstream targets of putative GRNs over space and time (18, 19, 59, 60). However, the lack of a comprehensive atlas of TF expression patterns with precise cross-reference to prospective, defined cell-differentiation capacity *in situ* means that knowledge is frustratingly rudimentary regarding possible TF crosstalk mechanisms. We currently have only vague definitions as to the location and nature of the tip-vs.-trunk boundary, how it develops during plexus formation/resolution (questions such as: is the boundary broad then cellularly precise?), and if cells for some period (perhaps especially those at the tip or near the boundary) vacillate between competence states; that is, exhibit tip-trunk metastability. In reality, however, a trunk epithelium competent to make endocrine cells already exists during the plexus stage; many $Ngn3^+$ cells are produced during this period (Figure 1C). What we need to know now is what specific cellular alterations occur, associated with the development of explicit tip-trunk segregation, to enable the switch from primarily an early α -cell-competence to the ability to give birth to other endocrine lineages. The reason for presenting these ideas in some depth, here and in the next section, is to advance the idea that the TF-driven GRNs that control endocrine-cell-type-specific differentiation are closely allied to the type (and maturity) of epithelial structure that cells find themselves within – and which, of course, their progenitors helped create. In the future, understanding how regionally distinct *inter*cellular signals establish *intra*cellular transcriptional specificity, operating differentially through complex motifs on

TF genes (such as the *Pdx1 cis*-regulatory region), should help define how cells are funneled into and entrained along lineage pathways.

3. Interdependence of tubulogenesis/plexus formation and endocrine differentiation (E12–E15)

A landmark study by Villaseñor et al. (2010) describes tubulogenesis within the dorsal bud in considerable detail (5). At E9.5, the anlage comprises a multi-layered, unpolarized inner cell region located between the duodenal lumen (the primary central lumen) and an outer basement membrane that is apposed to mesenchyme. An epithelialization process begins at ~E10.5 when initially single cells acquire apical polarity, with epithelial rosettes being formed when apical surface is generated within adjacent cells (61). Dispersed throughout the bud, several rosettes open central microlumens along their common apical surfaces, which continuously expand through polarized canals (Figure 1A). Independently organized luminal structures fuse with each other to form the framework of a multiluminal network within the multilayer epithelium (Figure 1B). At ~E12.5, this primary ductal plexus continuously remodels to form ultimately a ‘single layered’ polarized tip-trunk compartmentalized epithelium of the 2° transition. It is in this dynamic context that a trunk-localized production pipeline for endocrine cell birth, basal delamination and differentiation is developed.

Evidence is emerging that proper utility of apical polarity is essential for establishing the initial tubular network, and for the correct allocation of MPC towards pancreatic compartmental and cell-typespecific fates (Figure 2B). In studies by Kesavan et al. (2009), ablation in *Pdx1*⁺ MPC of *Cdc42*, a Rho-GTPase required for establishing and maintaining apical cell polarity, blocked the spreading of apical polarity between neighboring MPC, causing abnormal tubulogenesis and highly perturbed tip-trunk compartmentalization. Normal epithelial structure is an important determinant of the birth niche for endocrine cells, and the *Cdc42* mutation has strong effects on their formation. The authors of the study proposed a non-cell-autonomous mechanism: the poorly structured and fragmented epithelium abnormally exposed MPC to extracellular matrix (ECM). Improper contact with substrates such as laminin, which in the normal organ represent ‘bud-peripheral’ interactions, then promoted acinar fate and compromised endocrine cell number (61). In other words, *Cdc42* operates through a direct role in tissue architecture to generate microenvironments permissive for funneling multipotent pancreatic progenitors towards the duct/endocrine (not acinar) program. In line with this notion, *EphB2/EphB3* receptor mutations lead to epithelial disorganization through altered epithelial adhesion, causing disrupted epithelial rosettes, disturbed microlumen formation, delayed plexus remodeling, and overall reduction of endocrine and exocrine mass (5).

Interestingly, misregulation of specific endocrine lineage determinants alters epithelial structure, showing that cellular differentiation and development of epithelial architecture are linked. Indeed, in mice deficient for *Ngn3*, the pancreas displays enlarged ducts and fewer branching tips at E13.5, perhaps as a feedback effect from lack of *Ngn3*⁺ pro-endocrine cells departing the trunk epithelium. Induced overexpression of *Ngn3* by a Notch-signaling inhibitor (gamma-secretase inhibitor; GSI) results in thinner ducts and increased ‘pro-acinar’ tip cells (62). We propose that such tight integration reflects a differentiation framework, programmed within the dynamic epithelium that controls number, distribution and frequency of cell-fate choices during the 2° transition, as an essential determinant of final cell-type composition and organization of the mature organ.

While endocrine cell formation occurs throughout the 2° transition, we believe that it is naïve to assume that the spatial organization of lineage-committed domains is similar in all

regions of the pancreas. For example, the luminal plexus across one bud can have distinct regionalized behaviors: plexus resolution and tip elaboration (Figure 1C) (5). Complex, reciprocal crosstalk between ever-evolving epithelial structures and the various extrinsic tissues can set up distinct signaling environments. This might result in different intermediate types of equivalence groups representing different points along the lineage continuum (Figure 2). Integrated intrinsic and extrinsic mechanisms could affect the type of equivalence-group tiling in a pancreas-wide manner, as well as within individual mature trunk epithelial domains.

Although the developmental logic employed in epithelial maturation (including how branching patterns become distributed over time and space) remains undefined, it should be relatively easy to see that the type of branching mechanism (i.e., branch extension and/or lateral branching through tip splitting; (5, 6, 63)) predominating in one region or another, and how advanced it is, would affect intraepithelial cell-type specification. First, we predict that the processes yielding newly formed branch tips could well produce local feedback signals that affect differentiation potential within the tip-proximal trunk epithelium. Second, differential expression of mesenchymally expressed genes such as *Fgf10* throughout the expanding pancreas (these patterns need to be determined much more precisely) can transiently enforce specific transitional states within the epithelium (12). Third, and an area in dire need of more exact study, signals from vascular endothelial cells intertwined within the embryonic pancreas might significantly affect self-renewal, branching, and differentiation decisions of progenitor populations along the tip-trunk structure (64, 65) (reviewed by Villasenor, and Cleaver, this issue). Analogously, there are reports of the similarly intermingled neural-crest-derived cells exhibiting non-cell-autonomous influences on β -cell maturation (66). It remains possible that the neural crest has even more important functions (67). Based on the proposal that epithelial structure is strictly tied to the behavior of progenitors, it is but a short leap to propose that other well known pathways, including ones connected to the epithelium's 'internal sense of direction' (not just apical-basal, but for instance recognizing where tip-trunk interfaces are located) could be important contributors. We should not be surprised to see reports that planar cell polarity pathways contribute to the structural and signaling framework regulating endocrine birth. Finally, how proliferation is integrated within this differentiation framework to determine progenitor behavior and fate, through direct and/or indirect contributions, remains unclear (Figure 1D). It almost goes without saying that precise mapping of the various progenitor classes and transitional intermediates, and committed or differentiating cell populations, within this framework will provide insight on how to replicate the process by *in vitro* differentiation.

4. Secondary (2°) Transition: Endocrine specification within the bipotent trunk domain (E13.5-E16.5)

4.1 Heightened Ngn3 expression defines endocrine commitment within the trunk epithelium

Lineage labeling of trunk-localized TFs (Sox9 and Hnf1 β) during the 2° transition demonstrates that partitioning into duct and endocrine lineages occurs from trunk-resident populations (40, 42). These studies strongly indicate the existence of a bipotent progenitor population within the trunk epithelium. Importantly, single cell labeling studies do not yet exist to show if and when individual trunk cells move from true bipotency towards separated but intermingled endocrine or duct monopotencies. As one could imagine, one goal is to find markers that distinguish trunk cells prospectively, or at the earliest moment of this separation, to allow cell capture and analysis, leading to study of the inputs (intercellular and cellautonomous) and outputs that regulate how they choose duct or endocrine fate. One seminal discovery seems to be that the decision between endocrine and duct lineage involves

the regulation of Ngn3 levels (Figure 1D, 2C). Studies using a hypomorphic *Ngn3* allele demonstrated that the failure to reach a certain Ngn3 threshold results in decreased endocrine differentiation, and instead causes cells to increase their movement to alternate exocrine (duct, acinar) fates (62, 68). Studies from Gradwohl et al. (2012) suggest that while the early 1° transition MPC might be competent to express Ngn3, as MPC/acinar fate becomes segregated, the Ngn3-expressing competence shifts to the trunk domain (69). The heightened expression of Ngn3 in trunk progenitors is necessary and sufficient to trigger delamination away from the epithelium, through a process suggestive of epithelial-to-mesenchymal transition (EMT) (62, 70) (Figure 2D). The issue of endocrine cell birth, particularly of β cells, is perhaps misleadingly simple and straightforward to state: can we understand how Ngn3 is initiated (what limits it, what promotes it), and what is it within the mature trunk domain that provides β -cell competence.

4.2 The endocrine cell birth niche

The majority of delaminating Ngn3^{HIGH} cells are, by large-scale lineage-scoring studies, postmitotic and pre-committed to a single islet cell lineage (14, 26, 55, 68, 71–74). The longevity and productivity of the endocrine birth niche(s) must be tuned somehow to the number of endocrine cells that are required for proper islet assembly and function. However, the timing and mechanism of fate specification (most critically, α versus β , δ , PP, or ϵ) could be regulated by true intra-epithelial mechanisms, or to some extent be random within the epithelium yet influenced by feedback from newly Ngn3^{HIGH} cells that are able to communicate (feedback) their decision to enter one or another endocrine lineage. Either way, it seems important to derive much better knowledge of the mechanisms that impinge on the developmental history of Ngn3^{HIGH} cells. An accurate definition of the 2° transition endocrine birth niche is to date severely limited by the disappointing relative dearth of high-resolution molecular, cell biological and genetic tools, reviewed below, that could (prospectively) describe trunk epithelium heterogeneity at cellular resolution. Or, as mentioned earlier, the type of intercellular activity occurring within putative equivalence groups. The focus must now shift towards defining the niche within individual segments of trunk epithelium (Figure 1D).

We propose several questions whose answers should move us towards a better definition of the endocrine birth niche and how hormone-type is selected. Does the mature trunk epithelium behave as a collection of independent equivalence groups? How does their activity vary spatiotemporally according to epithelial maturation as a whole? How are signals recognized and integrated from adjacent mesenchyme, vascular, and neural crest cells, including just-delaminated Ngn3^{HIGH} cells, or ECM? Intrinsic and non-cell-autonomous processes prime the TF code and epigenome as cells move from the bipotent progenitor condition towards endocrine specification and commitment. This interplay is critical: the epithelial endocrine cell production pipeline needs to work just long enough and at the right speed to provide the numerous endocrine cells, yet leave enough duct cells to produce the final duct network. Without the appropriate cellular resolution, we should be careful to use the ‘salt and pepper’ distribution of Ngn3 expression throughout the trunk to invoke the classical lateral inhibition model for Notch signaling, in which ‘pioneer endocrine cells’ would prevent acquisition by adjacent cells of the ‘default’ endocrine fate (75). Some parts of the model are probably correct: under the *Ngn3*-null condition, *Ngn3* promoter activity expands within the trunk domain, suggesting Ngn3 protein restricts its own expression territory, possibly via non-cell-autonomous induction of bHLH repressors (62, 68). However, the function of Notch signaling within the 2° transition trunk epithelium remains to be tested at cellular resolution (reviewed in Serup, this issue). It remains possible that more complex versions allow adoption of endocrine commitment in clusters (not as individual cells), and ‘aggregate delamination’. More work is required at the cell biological

level, and improvements in deep-tissue high-resolution microscopy and real-time imaging will likely help. The developmental history of the cells (potentially reflecting recent secondary transition influences laid over those from even much earlier stages) that is recorded in the epigenome through successive cell divisions, and intrinsic to the bipotent progenitors, might affect the type of endocrine lineage adopted under the Notch lateral inhibition process. The idea of a residual ‘chromotypic’ imprint (an imprint at the chromatin level) that predisposes cells toward one cell fate or another is important to consider. A relevant discovery here is the pre patterning of chromatin states identified during early foregut endoderm patterning (24) (Figure 2).

5. Islet subtype lineage diversification and maturation

In the mouse, pro-endocrine cells appear to aggregate into interconnected cell clusters quite soon after delamination, so that they appear still associated with the trunk epithelium (their ability to maintain endocrine-endocrine contact likely connected to the rapid switch from E-cadherin (epithelial) to N-cadherin), and from these aggregates become organized into the future islets of Langerhans (76). The *Ngn3*^{HIGH} state leads to the expression of *Snail2*, *NeuroD1*, *Insm1*, and *Rfx6* important for endocrine precursors to delaminate, survive, and move into mono-hormone-expressing pro-endocrine cells (70, 77–80). With current limitations in the methods to lineage-trace multiple, transient cell populations within the trunk, we cannot accurately define so far when and where commitment is made towards a specific islet subtype (Figure 1D). Is the choice already determined while intraepithelial, just before/after acquiring the *Ngn3*^{HIGH} state, or only solidified after delamination in the pro-endocrine cells? Do these decisions reflect prior inputs that are imprinted and conserved through successive cell divisions from former progenitor cells? Some of the TFs and their interactions that work to allocate and drive maturation of endocrine subtypes have been uncovered (Figure 2E–F). Furthermore, a relatively unbiased screen for TFs that can together elicit *de novo* conversion to β -like cells from adult acinar cells – essentially a conversion between ‘developmental cousins’ – led to the identification of *Pdx1*, *MafA*, and *Ngn3* carried in an adenovirus cocktail (81). There remain gaps in our knowledge of what cell states can ‘interpret properly’ instructive cues derived from such TF expression (for instance, if all acini are not equivalently responsive to the aforementioned TF cocktail, what determines their conversion competence), and if this effect can be reproduced in human tissue, or *in vitro*. Related issues cover the nature of the competence state at an epigenetic level, and if and how such states could be used to better identify points of endocrine cell type diversification (Figure 2).

Perturbing cell-type-specific TF networks through genetic studies of *Arx*, *Pax4*, *Nkx2.2*, and *Pdx1* all lead to alterations in the overall allocation of endocrine subtypes within the pancreas, although the total endocrine number is seemingly maintained (Figure 2E). *Nkx2.2* ablation causes an extreme lineage switch from β , α , and PP-cells towards ghrelin-producing ϵ cells (56, 82). Potential strong genetic interactions between *Nkx2.2* and *Pax4* are indicated because *Pax4*-null mice exhibit an increase in both α and ϵ cells at the expense of β and δ cells (56). *Pax4* and *Arx* are required for β and α -cell fates, respectively, a lineage decision in part determined by their direct mutually repressive relationship (83, 84). For example, *Arx* global mutants show loss of α -cells and increased β and δ cells, complementary to the allocation defects detected in *Pax4* mutants. Also, misexpression of *Pax4* or *Arx* in terminally differentiated mature endocrine cell types induces α -to- β or β -to- α conversion, respectively (85, 86). Interestingly, co-ablation of *Arx/Pax4* causes a massive increase in δ cells at the expense of α and β cells, suggesting that δ -cell differentiation requires the absence of *Arx* and *Pax4* (83). *Pdx1* expression becomes restricted to delaminated pro- β cells (cells at the stage of initiating insulin expression), and is heightened (in the mouse at least) relative to the developing acini, trunk epithelial cells, and other pro-endocrine

subtypes (87). Strikingly, preventing the *Pdx1* up-regulation by deleting *cis*-regulatory regions results in increased α , δ and PP cells and reduced β -cell number and maturity (54, 88). Lineage-tracing analysis provided some evidence that the increased α and δ cells result from increased proliferation, suggesting that endocrine cell intercommunication (that is, inhibitory signals from β cells to adjacent cell types), in addition to specification regulation, is involved in generating the normal ratios of islet subtypes (88). Yang et al. (2011) asked whether raising expression of *Pdx1* throughout the *Ngn3*⁺ population during the 2° transition biases specification towards the β fate (89). Indeed, *Ngn3*-based *Pdx1*-overexpression imposed a delayed (until the postnatal stage), but then rapid, α -to- β cell conversion, leading to reprogrammed β cells that were indistinguishable from normal β cells. It was proposed that *Pdx1* elicited a temporally cryptic ‘chromotypic’ effect (priming at the chromatin and/or epigenetic level), without causing an immediate phenotypic change. Potentially important connections were made between *Pdx1* and the cellular localization of the histone methyltransferase Set7/9 (nuclear in β cells, cytoplasmic in α cells), which is proposed to enhance chromatin accessibility and transcription of β -cell target genes (90). Thus, the chromotypic imprint imparted on *Ngn3*⁺ endocrine progenitors could predispose their robust conversion towards the β -cell fate, in response to (currently undetermined) physiological triggers or regulatory check points that are normally encountered during the final, postnatal, maturation phase of islet cell development (Figure 2). *Nkx2.2* has been linked to the stabilization of β -cell identity starting from the endocrine progenitor stage by cooperating with *de novo* DNA methyltransferase Dnmt3a, the repressor cofactor Grg3 and histone deacetylase Hdac1, in the repression of transcription of the α -cell determinant *Arx* (34).

The maturation of delaminated endocrine cells, initially into immature mono-hormone cells and then towards physiological readiness, is thought to occur from the 2° transition and into postnatal growth (91) (Figure 2). In addition to the possible feedback and communication mechanisms alluded to previously, there might be influences from gap-junction or electrical coupling between endocrine cells, and contributions from the vasculature and nervous system (64, 66, 67, 92). Incorrect spatiotemporal deployment or absence of these components, for example ephrinA-EphA bidirectional signaling, Ncadherin expression, the correct levels of Hnf6- and NeuroD1-expression, and connexin-mediated gap junctions, can lead to impaired islet function (76, 93–99). The correct expression sequence of the basic leucine-zipper (bZIP) TFs, MafA and MafB, seems vital for α and β -cell maturation. Both MafA/MafB are expressed in immature β cells, but resolve over time to an exclusive α -cell-specific MafB versus β -cell-specific MafA expression profile (Figure 2F) (3, 100–102). How yet-undefined postnatal physiological triggers cue the final fixing of endocrine cell type determination and maturation in association with eliciting MafA vs. MafB resolution, and if they might be connected to major physiological events such as weaning, is unknown (Figure 2). It is interesting to note here that the period of conversion of the *Pdx1*-overexpressing α cells proposed by Yang et al. (2011) seems to overlap quite well with the Maf/MafB resolution period. A more extensive list and discussion of additional TFs can be found in other excellent reviews (17, 35).

6. Future horizons

6.1 Pancreas development: are all principles translatable from mouse to human?

For human therapy, current *in vitro* differentiation protocols inevitably focus on human ESC or iPSC (reviewed in Nostro and Keller, this issue). While study of murine endocrine development led to these protocols and continues to provide clues for improving the programming of such cells towards defined endocrine fates, one should be mindful of how to detect (and apply) any major variations in the rules that mouse and human pancreas development follow. Today, there is not a clear answer to this question, because we have such a primitive understanding of human pancreas development. One interesting area

deserves note: the discovery, by genetic analysis in humans, of potential differences in the genetics of pancreas bud formation and proliferation. Until recently, the cause of most cases of human pancreas agenesis was not known (103), but *Gata6* heterozygous null mutations have now been linked to a majority, in contrast to the lack of a drastic phenotype in *Gata6*-heterozygous mice (104). It remains to be seen if the mouse has a species-specific backup built in that arises from activity of a sister Gata gene, or exhibits a different threshold requirement for Gata6 function with respect to early pancreas organogenesis (105). Thus, while the core series of lineage-steps and developmental themes could be highly analogous in mouse and human endocrine development, significant functionally important differences might exist, which could be valuable in modifying differentiation *in vitro* or undertaking reprogramming *in vivo*. Diverse mechanisms could attain the same goal of creating endocrine cells that have similar function. For example, dorsal and ventral budding occurs in human essentially as it does in mouse (31, 106), and TFs such as Pdx1 and Ngn3 are expressed during human fetal pancreas development (106–108). However, the specific islet architecture and cell-type proportions vary between the species. For example, the relative ‘intermixing’ of endocrine subtypes in human islets is often called out as distinct from the canonical core-mantle structure of mice (29). Importantly, whether the contrast in islet composition and architecture reflects only slight differences in the endocrine pipeline between species remains to be addressed (108) (Figure 2). Under the premise that a homogenous population of transplanted β cells, as opposed to heterogeneous islet clusters, may be a poor surrogate (although a sufficient stop-gap) for restoring euglycemia to patients, such information could help decide what type of functional islet organoids might be aimed for in hESC/iPSC differentiations.

6.2 Potential novel endocrine lineage determinants: miRNAs, small molecules, and epigenetics

The direct reprogramming of somatic cells into pluripotent stem cells by the *Oct3/4*, *Sox2*, *Klf4*, and *cMyc* TFs demonstrates the principle that core GRNs can be manipulated to effect massive alterations in even long-term ‘fixed’ cell fates (109). Therefore, additional related methods should be developed that achieve similar results with respect to endocrine differentiation. Candidate entry points should include ways to establish and coordinate the correct interface between intrinsic signals and extrinsic interactions in various tissues, compartments within tissues, and individual cells within compartments. ‘Intrinsic manipulation’ would include affecting TF expression, including with miRNAs or other non-coding RNA regulators, using small molecules to mimic signaling pathways or stimulate intracellular signal transduction, and altering epigenetic landscapes to perhaps decrease the resistance to induced plasticity and specific reprogramming. ‘Extrinsic manipulation’ would include building the correct architecture for appropriate spatiotemporal distribution of intercellular signaling pathways through the use of nano-engineered scaffolds, use of natural and artificial biopolymers, and trying to achieve large-scale 3D culture conditions. These newer approaches, which are currently in a kind of ‘fringe use’, could first be used to dissect more precisely the physiologically relevant cell fate transitions, but then to provide potential shortcuts through or even around them. Proof of principle studies have been reported for some of these potential tools. For example, miRNAs can reprogram human somatic cells, or mouse embryonic fibroblasts to induced pluripotent stem cells (110, 111), and can cause directed transitions to specific cell types such as cardiomyocytes, osteoblasts, and various neural- and hematopoietic cell-types (112–118). For *in vitro* hESC differentiation, the small molecule indolactam V affects the organ choice of progenitors of the definitive endoderm; its administration increases flux of these progenitors toward Pdx1⁺ MPC, potentially providing increased throughput (Figure 2A) (119). Furthermore, small molecules that affect chromatin-modifying enzymes, such as histone deacetylase (HDAC) inhibitors, greatly improve reprogramming of the aforementioned TFs in inducing pluripotency in somatic

cells (120). Indeed, it is now becoming clear that HDACs and other chromatin modifiers are involved at either major decision points, or for fidelity-tuning of specific steps in specification and lineage allocation during pancreas development (121–123). DNA methyltransferases (DNMTs) are involved in maintaining the identity of adult β cells (124). Epigenetic signatures along the various stages of endocrine development are likely very dynamic, suggesting the need to identify the most significant hallmarks properly (most likely, in well-resolved cell populations identified by specific marker sets, flow cytometry and small-scale analytical methods), before learning how to establish, manipulate and/or remove them as appropriate in the *in vitro* context (125). In a complementary sense, certain pathophysiological insults, such as injury and inflammation, might destabilize even ‘fixed’ adult fates, making cells more amenable to reprogramming; again with possible application to generating specific cell types therapeutically (126–128). With these principles in mind, the identification of novel protocols that use multiple avenues to modify the TF networks and push cells along and between the different endocrine differentiation routes will likely be highly fruitful.

On the horizon, we imagine that much more spatiotemporally defined, cellular level, studies must be carried out not just in the mouse, but in parallel on human ‘organogenesis in a dish’ (129), or on fragments of human tissue transplanted for their continued development in a mouse host. We need to move much further than the current primitive blueprint for pancreas organogenesis and endocrine ontogeny, which seldom takes into account the asynchrony of the developmental process (local and across the developing organ), and try harder to identify local interactions and single cell effects. What do we need? A non-exhaustive list would include: a more precise catalog of architectural landmarks in the plexus and tip-trunk structure, meticulously specific sets of prospective markers for intermediate cell states, methods for following living cells in 3D and real-time for substantial periods along the endocrine ontogenetic process, and ways of recognizing even fleeting and highly localized deployment of intercellular signals.

Current publications in the field continue to demonstrate too much acceptance of ‘global characterization’ of developmental phenotypes. Achieving high cellular resolution with the improved tools in future studies will, in some ways, force an intellectual return to reductionist analysis, for example, breaking down asynchronous epithelial development into manageable units (the ‘tiling’ referred to above). Exploring the use of cell surface markers is expected to improve the accurate classification of competencies in defined cell populations (23, 130). For example, CD142 seems relatively specific for Pdx1⁺ MPC, allowing flow-cytometric sorting and analysis of gene expression and the epigenetic landscape on cell-fate-instructive genes (131). We also need better progress in understanding the process of endocrine cell maturation, because postnatal development might include incredibly important fixation steps in endocrine ontogeny, serving to deposit the final resistance against interconversion and stabilize lineage fidelity. Against the backdrop of a dynamic, asynchronous trunk epithelial niche for the birth of the various endocrine cells, attention should be prioritized on communication methods (gap junctions, lateral inhibition). Further help would come from developing dual-lineage-tracing methods to follow progenitor heterogeneity through two or more sequential steps in the program. With obvious appreciation of the technical hurdles and time investment, we restate here the attractiveness of pushing toward real-time characterization of endocrine cell birth and development, at single-cell resolution. One imagines, for example, video renderings of mosaic or non-mosaic genetic analysis of cells (normal and gene-perturbed), identified via coexpression of different fluorescent proteins, flashing one or another color at critical transition points. Such visualization methods would not just be aesthetically pleasing, and academically informative on the cell biology and genetics of pancreas development as another example of vertebrate organogenesis, but also extend our understanding of the physiological connections between

endocrine lineages, and with the vasculature and peripheral nervous system. The translational application would be to discover how artificial manipulations, briefly discussed above, could result in an effective pipeline for endocrine cells and fully functional islets of Langerhans (Figure 2).

Acknowledgments

We acknowledge helpful discussions with members of the Wright laboratory, and input from anonymous peer reviewers. We regret the omission of multiple important contributions that we could not discuss because of space constraints. This work was funded by NIH 1U01DK089570 to C.V.E.W. and NIH NRSA 2T32DK7061-37 to S.R.

References

1. D'Amour KA, Bang AG, Eliazar S, Kelly OG, Agulnick AD, Smart NG, Moorman MA, Kroon E, Carpenter MK, Baetge EE. Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells. *Nat Biotechnol.* 2006; 24:1392–1401. [PubMed: 17053790]
2. Zhang D, Jiang W, Liu M, Sui X, Yin X, Chen S, Shi Y, Deng H. Highly efficient differentiation of human ES cells and iPS cells into mature pancreatic insulin-producing cells. *Cell Res.* 2009; 19:429–438. [PubMed: 19255591]
3. Kroon E, Martinson LA, Kadoya K, Bang AG, Kelly OG, Eliazar S, Young H, Richardson M, Smart NG, Cunningham J, Agulnick AD, D'Amour KA, Carpenter MK, Baetge EE. Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. *Nat Biotechnol.* 2008; 26:443–452. [PubMed: 18288110]
4. Yang YP, Wright C. Chemicals turn human embryonic stem cells towards beta cells. *Nat Chem Biol.* 2009; 5:195–196. [PubMed: 19295520]
5. Villasenor A, Chong DC, Henkemeyer M, Cleaver O. Epithelial dynamics of pancreatic branching morphogenesis. *Development.* 2010; 137:4295–4305. [PubMed: 21098570]
6. Zhou Q, Law AC, Rajagopal J, Anderson WJ, Gray PA, Melton DA. A multipotent progenitor domain guides pancreatic organogenesis. *Dev Cell.* 2007; 13:103–114. [PubMed: 17609113]
7. Servitja JM, Ferrer J. Transcriptional networks controlling pancreatic development and beta cell function. *Diabetologia.* 2004; 47:597–613. [PubMed: 15298336]
8. Johansson KA, Dursun U, Jordan N, Gu G, Beermann F, Gradwohl G, Grapin-Botton A. Temporal control of neurogenin3 activity in pancreas progenitors reveals competence windows for the generation of different endocrine cell types. *Dev Cell.* 2007; 12:457–465. [PubMed: 17336910]
9. Stanger BZ, Tanaka AJ, Melton DA. Organ size is limited by the number of embryonic progenitor cells in the pancreas but not the liver. *Nature.* 2007; 445:886–891. [PubMed: 17259975]
10. Bhushan A, Itoh N, Kato S, Thiery JP, Czernichow P, Bellusci S, Scharfmann R. Fgf10 is essential for maintaining the proliferative capacity of epithelial progenitor cells during early pancreatic organogenesis. *Development.* 2001; 128:5109–5117. [PubMed: 11748146]
11. Landsman L, Nijagal A, Whitchurch TJ, Vanderlaan RL, Zimmer WE, Mackenzie TC, Hebrok M. Pancreatic mesenchyme regulates epithelial organogenesis throughout development. *PLoS Biol.* 2011; 9:e1001143. [PubMed: 21909240]
12. Kobberup S, Schmerr M, Dang ML, Nyeng P, Jensen JN, MacDonald RJ, Jensen J. Conditional control of the differentiation competence of pancreatic endocrine and ductal cells by Fgf10. *Mech Dev.* 2009; 127:220–234. [PubMed: 19969077]
13. Pictet RL, Clark WR, Williams RH, Rutter WJ. An ultrastructural analysis of the developing embryonic pancreas. *Dev Biol.* 1972; 29:436–467. [PubMed: 4570759]
14. Gu G, Dubauskaite J, Melton DA. Direct evidence for the pancreatic lineage: NGN3+ cells are islet progenitors and are distinct from duct progenitors. *Development.* 2002; 129:2447–2457. [PubMed: 11973276]
15. Fishman MP, Melton DA. Pancreatic lineage analysis using a retroviral vector in embryonic mice demonstrates a common progenitor for endocrine and exocrine cells. *Int J Dev Biol.* 2002; 46:201–207. [PubMed: 11934148]

16. Neoptolemos, J. Pancreatic cancer. Vol. 2 v. New York: London: Springer; 2010. SpringerLink (Online service); p. xxixp. 1390
17. Pan FC, Wright C. Pancreas organogenesis: from bud to plexus to gland. *Dev Dyn*. 2011; 240:530–565. [PubMed: 21337462]
18. Jensen J, Pedersen EE, Galante P, Hald J, Heller RS, Ishibashi M, Kageyama R, Guillemot F, Serup P, Madsen OD. Control of endodermal endocrine development by Hes-1. *Nat Genet*. 2000; 24:36–44. [PubMed: 10615124]
19. Seymour PA, Freude KK, Tran MN, Mayes EE, Jensen J, Kist R, Scherer G, Sander M. SOX9 is required for maintenance of the pancreatic progenitor cell pool. *Proc Natl Acad Sci U S A*. 2007; 104:1865–1870. [PubMed: 17267606]
20. Ahnfelt-Ronne J, Jorgensen MC, Klinck R, Jensen JN, Fuchtbauer EM, Deering T, MacDonald RJ, Wright CV, Madsen OD, Serup P. Ptf1a-mediated control of Dll1 reveals an alternative to the lateral inhibition mechanism. *Development*. 2011; 139:33–45. [PubMed: 22096075]
21. Fujikura J, Hosoda K, Iwakura H, Tomita T, Noguchi M, Masuzaki H, Tanigaki K, Yabe D, Honjo T, Nakao K. Notch/Rbp-j signaling prevents premature endocrine and ductal cell differentiation in the pancreas. *Cell Metab*. 2006; 3:59–65. [PubMed: 16399505]
22. Chiang MK, Melton DA. Single-cell transcript analysis of pancreas development. *Dev Cell*. 2003; 4:383–393. [PubMed: 12636919]
23. McKnight KD, Wang P, Kim SK. Deconstructing pancreas development to reconstruct human islets from pluripotent stem cells. *Cell Stem Cell*. 2010; 6:300–308. [PubMed: 20362535]
24. Xu CR, Cole PA, Meyers DJ, Kormish J, Dent S, Zaret KS. Chromatin "prepattern" and histone modifiers in a fate choice for liver and pancreas. *Science*. 2011; 332:963–966. [PubMed: 21596989]
25. Jorgensen MC, Ahnfelt-Ronne J, Hald J, Madsen OD, Serup P, Hecksher-Sorensen J. An illustrated review of early pancreas development in the mouse. *Endocr Rev*. 2007; 28:685–705. [PubMed: 17881611]
26. Gradwohl G, Dierich A, LeMeur M, Guillemot F. neurogenin3 is required for the development of the four endocrine cell lineages of the pancreas. *Proc Natl Acad Sci U S A*. 2000; 97:1607–1611. [PubMed: 10677506]
27. Hara A, Kadoya Y, Kojima I, Yamashina S. Rat pancreatic islet is formed by unification of multiple endocrine cell clusters. *Dev Dyn*. 2007; 236:3451–3458. [PubMed: 17973333]
28. Herrera PL. Adult insulin- and glucagon-producing cells differentiate from two independent cell lineages. *Development*. 2000; 127:2317–2322. [PubMed: 10804174]
29. Cabrera O, Berman DM, Kenyon NS, Ricordi C, Berggren PO, Caicedo A. The unique cytoarchitecture of human pancreatic islets has implications for islet cell function. *Proc Natl Acad Sci U S A*. 2006; 103:2334–2339. [PubMed: 16461897]
30. Baetens D, Malaisse-Lagae F, Perrelet A, Orci L. Endocrine pancreas: three-dimensional reconstruction shows two types of islets of langerhans. *Science*. 1979; 206:1323–1325. [PubMed: 390711]
31. Slack JM. Developmental biology of the pancreas. *Development*. 1995; 121:1569–1580. [PubMed: 7600975]
32. Matsuura K, Katsumoto K, Fukuda K, Kume K, Kume S. Conserved origin of the ventral pancreas in chicken. *Mech Dev*. 2009; 126:817–827. [PubMed: 19651207]
33. Hesselton D, Anderson RM, Beinat M, Stainier DY. Distinct populations of quiescent and proliferative pancreatic beta-cells identified by HOTcre mediated labeling. *Proc Natl Acad Sci U S A*. 2009; 106:14896–14901. [PubMed: 19706417]
34. Papizan JB, Singer RA, Tschen SI, Dhawan S, Friel JM, Hipkens SB, Magnuson MA, Bhushan A, Sussel L. Nkx2.2 repressor complex regulates islet beta-cell specification and prevents beta-to-alpha-cell reprogramming. *Genes Dev*. 2011; 25:2291–2305. [PubMed: 22056672]
35. Oliver-Krasinski JM, Stoffers DA. On the origin of the beta cell. *Genes Dev*. 2008; 22:1998–2021. [PubMed: 18676806]
36. Gittes GK. Developmental biology of the pancreas: a comprehensive review. *Dev Biol*. 2009; 326:4–35. [PubMed: 19013144]

37. Kawaguchi Y, Cooper B, Gannon M, Ray M, MacDonald RJ, Wright CV. The role of the transcriptional regulator Ptf1a in converting intestinal to pancreatic progenitors. *Nat Genet.* 2002; 32:128–134. [PubMed: 12185368]
38. Henseleit KD, Nelson SB, Kuhlbrodt K, Hennings JC, Ericson J, Sander M. NKX6 transcription factor activity is required for alpha- and beta-cell development in the pancreas. *Development.* 2005; 132:3139–3149. [PubMed: 15944193]
39. Haumaitre C, Barbacci E, Jenny M, Ott MO, Gradwohl G, Cereghini S. Lack of TCF2/vHNF1 in mice leads to pancreas agenesis. *Proc Natl Acad Sci U S A.* 2005; 102:1490–1495. [PubMed: 15668393]
40. Kopp JL, Dubois CL, Schaffer AE, Hao E, Shih HP, Seymour PA, Ma J, Sander M. Sox9+ ductal cells are multipotent progenitors throughout development but do not produce new endocrine cells in the normal or injured adult pancreas. *Development.* 2011; 138:653–665. [PubMed: 21266405]
41. Furuyama K, Kawaguchi Y, Akiyama H, Horiguchi M, Kodama S, Kuhara T, Hosokawa S, Elbahrawy A, Soeda T, Koizumi M, Masui T, Kawaguchi M, Takaori K, Doi R, Nishi E, Kakinoki R, Deng JM, Behringer RR, Nakamura T, Uemoto S. Continuous cell supply from a Sox9-expressing progenitor zone in adult liver, exocrine pancreas and intestine. *Nat Genet.* 2010; 43:34–41. [PubMed: 21113154]
42. Solar M, Cardalda C, Houbracken I, Martin M, Maestro MA, De Medts N, Xu X, Grau V, Heimberg H, Bouwens L, Ferrer J. Pancreatic exocrine duct cells give rise to insulin-producing beta cells during embryogenesis but not after birth. *Dev Cell.* 2009; 17:849–860. [PubMed: 20059954]
43. Lynn FC, Smith SB, Wilson ME, Yang KY, Nekrep N, German MS. Sox9 coordinates a transcriptional network in pancreatic progenitor cells. *Proc Natl Acad Sci U S A.* 2007; 104:10500–10505. [PubMed: 17563382]
44. Poll AV, Pierreux CE, Lokmane L, Haumaitre C, Achouri Y, Jacquemin P, Rousseau GG, Cereghini S, Lemaigre FP. A vHNF1/TCF2-HNF6 cascade regulates the transcription factor network that controls generation of pancreatic precursor cells. *Diabetes.* 2006; 55:61–69. [PubMed: 16380477]
45. Wiebe PO, Kormish JD, Roper VT, Fujitani Y, Alston NI, Zaret KS, Wright CV, Stein RW, Gannon M. Ptf1a binds to and activates area III, a highly conserved region of the Pdx1 promoter that mediates early pancreas-wide Pdx1 expression. *Mol Cell Biol.* 2007; 27:4093–4104. [PubMed: 17403901]
46. Thompson N, Gesina E, Scheinert P, Bucher P, Grapin-Botton A. RNA Profiling and Chromatin Immunoprecipitation-Sequencing Reveal that PTF1a Stabilizes Pancreas Progenitor Identity via the Control of MNX1/HLXB9 and a Network of Other Transcription Factors. *Mol Cell Biol.* 2012; 32:1189–1199. [PubMed: 22232429]
47. Jonsson J, Carlsson L, Edlund T, Edlund H. Insulin-promoter-factor 1 is required for pancreas development in mice. *Nature.* 1994; 371:606–609. [PubMed: 7935793]
48. Krapp A, Knofler M, Ledermann B, Burki K, Berney C, Zoerkler N, Hagenbuchle O, Wellauer PK. The bHLH protein PTF1-p48 is essential for the formation of the exocrine and the correct spatial organization of the endocrine pancreas. *Genes Dev.* 1998; 12:3752–3763. [PubMed: 9851981]
49. Sellick GS, Barker KT, Stolte-Dijkstra I, Fleischmann C, Coleman RJ, Garrett C, Gloyn AL, Edghill EL, Hattersley AT, Wellauer PK, Goodwin G, Houlston RS. Mutations in PTF1A cause pancreatic and cerebellar agenesis. *Nat Genet.* 2004; 36:1301–1305. [PubMed: 15543146]
50. Burlison JS, Long Q, Fujitani Y, Wright CV, Magnuson MA. Pdx-1 and Ptf1a concurrently determine fate specification of pancreatic multipotent progenitor cells. *Dev Biol.* 2008; 316:74–86. [PubMed: 18294628]
51. Stoffers DA, Zinkin NT, Stanojevic V, Clarke WL, Habener JF. Pancreatic agenesis attributable to a single nucleotide deletion in the human IPF1 gene coding sequence. *Nat Genet.* 1997; 15:106–110. [PubMed: 8988180]
52. Offield MF, Jetton TL, Labosky PA, Ray M, Stein RW, Magnuson MA, Hogan BL, Wright CV. PDX-1 is required for pancreatic outgrowth and differentiation of the rostral duodenum. *Development.* 1996; 122:983–995. [PubMed: 8631275]

53. Gerrish K, Gannon M, Shih D, Henderson E, Stoffel M, Wright CV, Stein R. Pancreatic beta cell-specific transcription of the *pdx-1* gene. The role of conserved upstream control regions and their hepatic nuclear factor 3beta sites. *J Biol Chem*. 2000; 275:3485–3492. [PubMed: 10652343]
54. Fujitani Y, Fujitani S, Boyer DF, Gannon M, Kawaguchi Y, Ray M, Shiota M, Stein RW, Magnuson MA, Wright CV. Targeted deletion of a cis-regulatory region reveals differential gene dosage requirements for *Pdx1* in foregut organ differentiation and pancreas formation. *Genes Dev*. 2006; 20:253–266. [PubMed: 16418487]
55. Schwitzgebel VM, Scheel DW, Connors JR, Kalamaras J, Lee JE, Anderson DJ, Sussel L, Johnson JD, German MS. Expression of neurogenin3 reveals an islet cell precursor population in the pancreas. *Development*. 2000; 127:3533–3542. [PubMed: 10903178]
56. Prado CL, Pugh-Bernard AE, Elghazi L, Sosa-Pineda B, Sussel L. Ghrelin cells replace insulin-producing beta cells in two mouse models of pancreas development. *Proc Natl Acad Sci U S A*. 2004; 101:2924–2929. [PubMed: 14970313]
57. Herrera PL, Huarte J, Sanvito F, Meda P, Orci L, Vassalli JD. Embryogenesis of the murine endocrine pancreas; early expression of pancreatic polypeptide gene. *Development*. 1991; 113:1257–1265. [PubMed: 1811941]
58. Schaffer AE, Freude KK, Nelson SB, Sander M. *Nkx6* transcription factors and *Ptf1a* function as antagonistic lineage determinants in multipotent pancreatic progenitors. *Dev Cell*. 2010; 18:1022–1029. [PubMed: 20627083]
59. Fukuda A, Kawaguchi Y, Furuyama K, Kodama S, Horiguchi M, Kuhara T, Koizumi M, Boyer DF, Fujimoto K, Doi R, Kageyama R, Wright CV, Chiba T. Ectopic pancreas formation in *Hes1* - knockout mice reveals plasticity of endodermal progenitors of the gut, bile duct, and pancreas. *J Clin Invest*. 2006; 116:1484–1493. [PubMed: 16710472]
60. Masui T, Swift GH, Deering T, Shen C, Coats WS, Long Q, Elsasser HP, Magnuson MA, MacDonald RJ. Replacement of *Rbpj* with *Rbpjl* in the PTF1 complex controls the final maturation of pancreatic acinar cells. *Gastroenterology*. 2010; 139:270–280. [PubMed: 20398665]
61. Kesavan G, Sand FW, Greiner TU, Johansson JK, Kobberup S, Wu X, Brakebusch C, Semb H. *Cdc42*-mediated tubulogenesis controls cell specification. *Cell*. 2009; 139:791–801. [PubMed: 19914171]
62. Magenheimer J, Klein AM, Stanger BZ, Ashery-Padan R, Sosa-Pineda B, Gu G, Dor Y. *Ngn3*(+) endocrine progenitor cells control the fate and morphogenesis of pancreatic ductal epithelium. *Dev Biol*. 2011; 359:26–36. [PubMed: 21888903]
63. Puri S, Hebrok M. Dynamics of embryonic pancreas development using real-time imaging. *Dev Biol*. 2007; 306:82–93. [PubMed: 17448459]
64. Magenheimer J, Ilovich O, Lazarus A, Klochendler A, Ziv O, Werman R, Hija A, Cleaver O, Mishani E, Keshet E, Dor Y. Blood vessels restrain pancreas branching, differentiation and growth. *Development*. 2011; 138:4743–4752. [PubMed: 21965615]
65. Lammert E, Cleaver O, Melton D. Induction of pancreatic differentiation by signals from blood vessels. *Science*. 2001; 294:564–567. [PubMed: 11577200]
66. Plank JL, Mundell NA, Frist AY, LeGrone AW, Kim T, Musser MA, Walter TJ, Labosky PA. Influence and timing of arrival of murine neural crest on pancreatic beta cell development and maturation. *Dev Biol*. 2010; 349:321–330. [PubMed: 21081123]
67. Nekrep N, Wang J, Miyatsuka T, German MS. Signals from the neural crest regulate beta-cell mass in the pancreas. *Development*. 2008; 135:2151–2160. [PubMed: 18506029]
68. Wang S, Yan J, Anderson DA, Xu Y, Kanal MC, Cao Z, Wright CV, Gu G. *Neurog3* gene dosage regulates allocation of endocrine and exocrine cell fates in the developing mouse pancreas. *Dev Biol*. 2010; 339:26–37. [PubMed: 20025861]
69. Beucher A, Martin M, Spenle C, Poulet M, Collin C, Gradwohl G. Competence of failed endocrine progenitors to give rise to acinar but not ductal cells is restricted to early pancreas development. *Dev Biol*. 2011; 361:277–285. [PubMed: 22056785]
70. Gouzi M, Kim YH, Katsumoto K, Johansson K, Grapin-Botton A. *Neurogenin3* initiates stepwise delamination of differentiating endocrine cells during pancreas development. *Dev Dyn*. 2011; 240:589–604. [PubMed: 21287656]

71. Desgraz R, Herrera PL. Pancreatic neurogenin 3-expressing cells are unipotent islet precursors. *Development*. 2009; 136:3567–3574. [PubMed: 19793886]
72. Miyatsuka T, Kosaka Y, Kim H, German MS. Neurogenin3 inhibits proliferation in endocrine progenitors by inducing Cdkn1a. *Proc Natl Acad Sci U S A*. 2010; 108:185–190. [PubMed: 21173230]
73. Jensen J, Heller RS, Funder-Nielsen T, Pedersen EE, Lindsell C, Weinmaster G, Madsen OD, Serup P. Independent development of pancreatic alpha- and beta-cells from neurogenin3-expressing precursors: a role for the notch pathway in repression of premature differentiation. *Diabetes*. 2000; 49:163–176. [PubMed: 10868931]
74. Villaseñor A, Chong DC, Cleaver O. Biphasic Ngn3 expression in the developing pancreas. *Dev Dyn*. 2008; 237:3270–3279. [PubMed: 18924236]
75. Lee JC, Smith SB, Watada H, Lin J, Scheel D, Wang J, Mirmira RG, German MS. Regulation of the pancreatic pro-endocrine gene neurogenin3. *Diabetes*. 2001; 50:928–936. [PubMed: 11334435]
76. Miller K, Kim A, Kilimnik G, Jo J, Moka U, Periwai V, Hara M. Islet formation during the neonatal development in mice. *PLoS One*. 2009; 4:e7739. [PubMed: 19893748]
77. Gierl MS, Karoulias N, Wende H, Strehle M, Birchmeier C. The zinc-finger factor Insm1 (IA-1) is essential for the development of pancreatic beta cells and intestinal endocrine cells. *Genes Dev*. 2006; 20:2465–2478. [PubMed: 16951258]
78. Smith SB, Qu HQ, Taleb N, Kishimoto NY, Scheel DW, Lu Y, Patch AM, Grabs R, Wang J, Lynn FC, Miyatsuka T, Mitchell J, Seerke R, Desir J, Eijnden SV, Abramowicz M, Kacet N, Weill J, Renard ME, Gentile M, Hansen I, Dewar K, Hattersley AT, Wang R, Wilson ME, Johnson JD, Polychronakos C, German MS. Rfx6 directs islet formation and insulin production in mice and humans. *Nature*. 2010; 463:775–780. [PubMed: 20148032]
79. Naya FJ, Huang HP, Qiu Y, Mutoh H, DeMayo FJ, Leiter AB, Tsai MJ. Diabetes, defective pancreatic morphogenesis, and abnormal enteroendocrine differentiation in BETA2/neuroD-deficient mice. *Genes Dev*. 1997; 11:2323–2334. [PubMed: 9308961]
80. Rukstalis JM, Habener JF. Snail2, a mediator of epithelial-mesenchymal transitions, expressed in progenitor cells of the developing endocrine pancreas. *Gene Expr Patterns*. 2007; 7:471–479. [PubMed: 17185046]
81. Zhou Q, Brown J, Kanarek A, Rajagopal J, Melton DA. In vivo reprogramming of adult pancreatic exocrine cells to beta-cells. *Nature*. 2008; 455:627–632. [PubMed: 18754011]
82. Sussel L, Kalamaras J, Hartigan-O'Connor DJ, Meneses JJ, Pedersen RA, Rubenstein JL, German MS. Mice lacking the homeodomain transcription factor Nkx2.2 have diabetes due to arrested differentiation of pancreatic beta cells. *Development*. 1998; 125:2213–2221. [PubMed: 9584121]
83. Collombat P, Hecksher-Sorensen J, Broccoli V, Krull J, Ponte I, Mundiger T, Smith J, Gruss P, Serup P, Mansouri A. The simultaneous loss of Arx and Pax4 genes promotes a somatostatin-producing cell fate specification at the expense of the alpha- and beta-cell lineages in the mouse endocrine pancreas. *Development*. 2005; 132:2969–2980. [PubMed: 15930104]
84. Collombat P, Mansouri A, Hecksher-Sorensen J, Serup P, Krull J, Gradwohl G, Gruss P. Opposing actions of Arx and Pax4 in endocrine pancreas development. *Genes Dev*. 2003; 17:2591–2603. [PubMed: 14561778]
85. Collombat P, Hecksher-Sorensen J, Krull J, Berger J, Riedel D, Herrera PL, Serup P, Mansouri A. Embryonic endocrine pancreas and mature beta cells acquire alpha and PP cell phenotypes upon Arx misexpression. *J Clin Invest*. 2007; 117:961–970. [PubMed: 17404619]
86. Collombat P, Xu X, Ravassard P, Sosa-Pineda B, Dussaud S, Billestrup N, Madsen OD, Serup P, Heimberg H, Mansouri A. The ectopic expression of Pax4 in the mouse pancreas converts progenitor cells into alpha and subsequently beta cells. *Cell*. 2009; 138:449–462. [PubMed: 19665969]
87. Guz Y, Montminy MR, Stein R, Leonard J, Gamer LW, Wright CV, Teitelman G. Expression of murine STF-1, a putative insulin gene transcription factor, in beta cells of pancreas, duodenal epithelium and pancreatic exocrine and endocrine progenitors during ontogeny. *Development*. 1995; 121:11–18. [PubMed: 7867492]

88. Gannon M, Ables ET, Crawford L, Lowe D, Offield MF, Magnuson MA, Wright CV. *pdx-1* function is specifically required in embryonic beta cells to generate appropriate numbers of endocrine cell types and maintain glucose homeostasis. *Dev Biol.* 2008; 314:406–417. [PubMed: 18155690]
89. Yang YP, Thorel F, Boyer DF, Herrera PL, Wright CV. Context-specific alpha- to-beta-cell reprogramming by forced *Pdx1* expression. *Genes Dev.* 2011; 25:1680–1685. [PubMed: 21852533]
90. Ogiwara T, Vanderford NL, Maier B, Stein RW, Mirmira RG. Expression and function of *Set7/9* in pancreatic islets. *Islets.* 2009; 1:269–272. [PubMed: 21099283]
91. Hang Y, Stein R. *MafA* and *MafB* activity in pancreatic beta cells. *Trends Endocrinol Metab.* 2011; 22:364–373. [PubMed: 21719305]
92. Brissova M, Shostak A, Shiota M, Wiebe PO, Poffenberger G, Kantz J, Chen Z, Carr C, Jerome WG, Chen J, Baldwin HS, Nicholson W, Bader DM, Jetton T, Gannon M, Powers AC. Pancreatic islet production of vascular endothelial growth factor--a is essential for islet vascularization, revascularization, and function. *Diabetes.* 2006; 55:2974–2985. [PubMed: 17065333]
93. Johansson JK, Voss U, Kesavan G, Kostetskii I, Wierup N, Radice GL, Semb H. N-cadherin is dispensable for pancreas development but required for beta-cell granule turnover. *Genesis.* 2010; 48:374–381. [PubMed: 20533404]
94. Konstantinova I, Nikolova G, Ohara-Imaizumi M, Meda P, Kucera T, Zarbalis K, Wurst W, Nagamatsu S, Lammert E. EphA-Ephrin-A-mediated beta cell communication regulates insulin secretion from pancreatic islets. *Cell.* 2007; 129:359–370. [PubMed: 17448994]
95. Greiner TU, Kesavan G, Stahlberg A, Semb H. *Rac1* regulates pancreatic islet morphogenesis. *BMC Dev Biol.* 2009; 9:2. [PubMed: 19126201]
96. Gannon M, Ray MK, Van Zee K, Rausa F, Costa RH, Wright CV. Persistent expression of *HNF6* in islet endocrine cells causes disrupted islet architecture and loss of beta cell function. *Development.* 2000; 127:2883–2895. [PubMed: 10851133]
97. Bosco D, Haefliger JA, Meda P. Connexins: key mediators of endocrine function. *Physiol Rev.* 2011; 91:1393–1445. [PubMed: 22013215]
98. Gu C, Stein GH, Pan N, Goebbels S, Hornberg H, Nave KA, Herrera P, White P, Kaestner KH, Sussel L, Lee JE. Pancreatic beta cells require *NeuroD* to achieve and maintain functional maturity. *Cell Metab.* 2010; 11:298–310. [PubMed: 20374962]
99. Caton D, Calabrese A, Mas C, Serre-Beinier V, Wonkam A, Meda P. Beta-cell crosstalk: a further dimension in the stimulus-secretion coupling of glucose-induced insulin release. *Diabetes Metab.* 2002; 28:3S45–3S53. discussion 43S108–112. [PubMed: 12688633]
100. Artner I, Hang Y, Mazur M, Yamamoto T, Guo M, Lindner J, Magnuson MA, Stein R. *MafA* and *MafB* regulate genes critical to beta-cells in a unique temporal manner. *Diabetes.* 2010; 59:2530–2539. [PubMed: 20627934]
101. Nishimura W, Kondo T, Salameh T, El Khattabi I, Dodge R, Bonner-Weir S, Sharma A. A switch from *MafB* to *MafA* expression accompanies differentiation to pancreatic beta-cells. *Dev Biol.* 2006; 293:526–539. [PubMed: 16580660]
102. Artner I, Le Lay J, Hang Y, Elghazi L, Schisler JC, Henderson E, Sosa-Pineda B, Stein R. *MafB*: an activator of the glucagon gene expressed in developing islet alpha- and beta-cells. *Diabetes.* 2006; 55:297–304. [PubMed: 16443760]
103. Allen HL, Flanagan SE, Shaw-Smith C, De Franco E, Akerman I, Caswell R, Ferrer J, Hattersley AT, Ellard S. *GATA6* haploinsufficiency causes pancreatic agenesis in humans. *Nat Genet.* 2011; 44:20–22. [PubMed: 22158542]
104. Morrissey EE, Tang Z, Sigrist K, Lu MM, Jiang F, Ip HS, Parmacek MS. *GATA6* regulates *HNF4* and is required for differentiation of visceral endoderm in the mouse embryo. *Genes Dev.* 1998; 12:3579–3590. [PubMed: 9832509]
105. Watt AJ, Zhao R, Li J, Duncan SA. Development of the mammalian liver and ventral pancreas is dependent on *GATA4*. *BMC Dev Biol.* 2007; 7:37. [PubMed: 17451603]
106. Piper K, Brickwood S, Turnpenny LW, Cameron IT, Ball SG, Wilson DI, Hanley NA. Beta cell differentiation during early human pancreas development. *J Endocrinol.* 2004; 181:11–23. [PubMed: 15072563]

107. Piper Hanley K, Hearn T, Berry A, Carvell MJ, Patch AM, Williams LJ, Sugden SA, Wilson DI, Ellard S, Hanley NA. In vitro expression of NGN3 identifies RAB3B as the predominant Ras-associated GTP-binding protein 3 family member in human islets. *J Endocrinol.* 2010; 207:151–161. [PubMed: 20807725]
108. Jeon J, Correa-Medina M, Ricordi C, Edlund H, Diez JA. Endocrine cell clustering during human pancreas development. *J Histochem Cytochem.* 2009; 57:811–824. [PubMed: 19365093]
109. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell.* 2006; 126:663–676. [PubMed: 16904174]
110. Subramanyam D, Lamouille S, Judson RL, Liu JY, Bucay N, Derynck R, Bluelloch R. Multiple targets of miR-302 and miR-372 promote reprogramming of human fibroblasts to induced pluripotent stem cells. *Nat Biotechnol.* 2011; 29:443–448. [PubMed: 21490602]
111. Judson RL, Babiarz JE, Venere M, Bluelloch R. Embryonic stem cell-specific microRNAs promote induced pluripotency. *Nat Biotechnol.* 2009; 27:459–461. [PubMed: 19363475]
112. Fu JD, Rushing SN, Lieu DK, Chan CW, Kong CW, Geng L, Wilson KD, Chiamvimonvat N, Boheler KR, Wu JC, Keller G, Hajjar RJ, Li RA. Distinct roles of microRNA-1 and -499 in ventricular specification and functional maturation of human embryonic stem cell-derived cardiomyocytes. *PLoS One.* 2011; 6:e27417. [PubMed: 22110643]
113. Cheng LC, Pastrana E, Tavazoie M, Doetsch F. miR-124 regulates adult neurogenesis in the subventricular zone stem cell niche. *Nat Neurosci.* 2009; 12:399–408. [PubMed: 19287386]
114. Liu K, Liu Y, Mo W, Qiu R, Wang X, Wu JY, He R. MiR-124 regulates early neurogenesis in the optic vesicle and forebrain, targeting NeuroD1. *Nucleic Acids Res.* 2010; 39:2869–2879. [PubMed: 21131276]
115. Delaloy C, Liu L, Lee JA, Su H, Shen F, Yang GY, Young WL, Ivey KN, Gao FB. MicroRNA-9 coordinates proliferation and migration of human embryonic stem cell-derived neural progenitors. *Cell Stem Cell.* 2010; 6:323–335. [PubMed: 20362537]
116. Ivey KN, Muth A, Arnold J, King FW, Yeh RF, Fish JE, Hsiao EC, Schwartz RJ, Conklin BR, Bernstein HS, Srivastava D. MicroRNA regulation of cell lineages in mouse and human embryonic stem cells. *Cell Stem Cell.* 2008; 2:219–229. [PubMed: 18371447]
117. Chen CZ, Li L, Lodish HF, Bartel DP. MicroRNAs modulate hematopoietic lineage differentiation. *Science.* 2004; 303:83–86. [PubMed: 14657504]
118. Li H, Xie H, Liu W, Hu R, Huang B, Tan YF, Xu K, Sheng ZF, Zhou HD, Wu XP, Luo XH. A novel microRNA targeting HDAC5 regulates osteoblast differentiation in mice and contributes to primary osteoporosis in humans. *J Clin Invest.* 2009; 119:3666–3677. [PubMed: 19920351]
119. Chen S, Borowiak M, Fox JL, Maehr R, Osafune K, Davidow L, Lam K, Peng LF, Schreiber SL, Rubin LL, Melton D. A small molecule that directs differentiation of human ESCs into the pancreatic lineage. *Nat Chem Biol.* 2009; 5:258–265. [PubMed: 19287398]
120. Huangfu D, Maehr R, Guo W, Eijkelenboom A, Snitow M, Chen AE, Melton DA. Induction of pluripotent stem cells by defined factors is greatly improved by small-molecule compounds. *Nat Biotechnol.* 2008; 26:795–797. [PubMed: 18568017]
121. Noel ES, Casal-Sueiro A, Busch-Nentwich E, Verkade H, Dong PD, Stemple DL, Ober EA. Organ-specific requirements for Hdac1 in liver and pancreas formation. *Dev Biol.* 2008; 322:237–250. [PubMed: 18687323]
122. Haumaitre C, Lenoir O, Scharfmann R. Histone deacetylase inhibitors modify pancreatic cell fate determination and amplify endocrine progenitors. *Mol Cell Biol.* 2008; 28:6373–6383. [PubMed: 18710955]
123. Lenoir O, Flosseau K, Ma FX, Blondeau B, Mai A, Bassel-Duby R, Ravassard P, Olson EN, Haumaitre C, Scharfmann R. Specific control of pancreatic endocrine beta- and delta-cell mass by class IIa histone deacetylases HDAC4, HDAC5, and HDAC9. *Diabetes.* 2011; 60:2861–2871. [PubMed: 21953612]
124. Dhawan S, Georgia S, Tschen SI, Fan G, Bhushan A. Pancreatic beta cell identity is maintained by DNA methylation-mediated repression of Arx. *Dev Cell.* 2011; 20:419–429. [PubMed: 21497756]
125. van Arensbergen J, Garcia-Hurtado J, Moran I, Maestro MA, Xu X, Van de Casteele M, Skoudy AL, Palassini M, Heimberg H, Ferrer J. Derepression of Polycomb targets during pancreatic

- organogenesis allows insulin-producing beta-cells to adopt a neural gene activity program. *Genome Res.* 2010; 20:722–732. [PubMed: 20395405]
126. Thorel F, Nepote V, Avril I, Kohno K, Desgraz R, Chera S, Herrera PL. Conversion of adult pancreatic alpha-cells to beta-cells after extreme beta-cell loss. *Nature.* 2010; 464:1149–1154. [PubMed: 20364121]
127. Xu X, D'Hoker J, Stange G, Bonne S, De Leu N, Xiao X, Van de Casteele M, Mellitzer G, Ling Z, Pipeleers D, Bouwens L, Scharfmann R, Gradwohl G, Heimberg H. Beta cells can be generated from endogenous progenitors in injured adult mouse pancreas. *Cell.* 2008; 132:197–207. [PubMed: 18243096]
128. Wang AY, Ehrhardt A, Xu H, Kay MA. Adenovirus transduction is required for the correction of diabetes using Pdx-1 or Neurogenin-3 in the liver. *Mol Ther.* 2007; 15:255–263. [PubMed: 17235302]
129. Spence JR, Mayhew CN, Rankin SA, Kuhar MF, Vallance JE, Tolle K, Hoskins EE, Kalinichenko VV, Wells SI, Zorn AM, Shroyer NF, Wells JM. Directed differentiation of human pluripotent stem cells into intestinal tissue in vitro. *Nature.* 2010; 470:105–109. [PubMed: 21151107]
130. Dorrell C, Grompe MT, Pan FC, Zhong Y, Canaday PS, Shultz LD, Greiner DL, Wright CV, Streeter PR, Grompe M. Isolation of mouse pancreatic alpha, beta, duct and acinar populations with cell surface markers. *Mol Cell Endocrinol.* 2011; 339:144–150. [PubMed: 21539888]
131. Kelly OG, Chan MY, Martinson LA, Kadota K, Ostertag TM, Ross KG, Richardson M, Carpenter MK, D'Amour KA, Kroon E, Moorman M, Baetge EE, Bang AG. Cell-surface markers for the isolation of pancreatic cell types derived from human embryonic stem cells. *Nat Biotechnol.* 2011; 29:750–756. [PubMed: 21804561]

Highlights

- Transcription factor networks underlie primary MPC maintenance and lineage choice.
- Behavior of endocrine progenitors is closely tied to the 3D epithelial structure of the developing pancreas.
- Heightened Ngn3 expression defines endocrine commitment within the trunk epithelium.
- Islet subtype diversification and maturation involves specific transcription factor interactions and potential epigenetic inputs.
- Deeper understanding and successful mimicking of extrinsic and intrinsic interactions will improve endocrine subtype differentiation attempts *in vitro*.

A. E10.75 Microlumen onset **B.** E12.5 Primary ductal plexus

(C) E14.5-E15.5 plexus remodeling

D. E14.5-E15.5 endocrine birth niche

Figure 1. Overview model, mouse pancreas bud and endocrine cell development

Schematics were much influenced by Kesavan et al. 2009 and Villasenor et al. 2010 (5, 61).

A: At ~E10.75, single epithelial cells throughout the stratified body of the dorsal bud (unpolarized body cells are pink) acquire apical polarity (red dot) and begin to recruit adjacent cells to form multiple rosettes (top three light green ‘bottle’ shaped cells). Microlumens open up (white circle in the center of light green cells) and come together along polarized canals (yellow dotted lines opening up to solid yellow patent lumens) eventually amassing other MPC towards a common apical surface (individual red dots). The spatial location might bias MPC towards either a pro-acinar fate (cap cell; turquoise) or an endocrine/duct fate (green cell). The primary central lumen continuous with the duodenal lumen is orange. **A insert:** MPC within a single rosette might exhibit segregating biases towards different subtypes, especially endocrine or duct (different shades of green). The asterisk designates the first cell to acquire apical polarity. **B:** By E12.5, the primary ductal plexus has formed. Explicit compartmentalization of apically localized cells into tip (blue nucleated cells; note some are not in ‘tip’ locations) or trunk (green nucleated cells) epithelium occurs intermixed with tip-disposed MPC (mixed green-blue nucleated cells) with selfreplicative potential (red arrows). Remaining cap cells (turquoise cells without nuclei) and body cells (pink cells without nuclei) seem to eventually join the epithelium (17). **C:** At E14.5–15.5, the whole dorsal pancreas epithelium (overview whole-mount on the right) shows asynchronous, regionalized architecture: a distinct, complex plexus in central regions of the anlagen (left bottom), but a more resolved tree-like structure is seen in ‘exterior’ regions, with multiple tip elaborations (left top). Large numbers of Ngn3⁺ cells (red nuclei) are delaminating (apical surface designated by Muc1 in white). These asynchronous compartments might have variable endocrine differentiation competence. **D:**

The basal delamination of post-mitotic Ngn3^{HIGH} endocrine precursors (solid-shaded green) already assigned to a final hormone⁺ fate (light green, β ; dark green, α) occurs from the trunk epithelium. In adult islets, small clusters of β cells (light green) or α cells (dark green) may arise by delayed replication of individual hormone⁺ cells (71). The possibility that (future identifiable) specific mitotic intermediates precede each Ngn3^{HIGH} state is indicated (gradient-shaded cells in niche 1 and 2). The general bipotent trunk epithelial population is indicated in pink. In the adult islet, light green cells are β cells with dark green α , δ , PP and ϵ cells, forming the canonical mouse islet core-mantle structure.

Figure 2. The endocrine ontogeny pipeline with β cell bias

The context-dependent interplay between extrinsic and intrinsic signals determine a series of railroad-switching-type events (**exclamation mark signs** and **square boxes A–F**) that lead ‘predifferentiated’ progenitors down the pancreatic endocrine lineage and ultimately the β cell conduit (emphasized in a black color), instead of alternate fates (shown in grey). Some degree of epigenetic priming might bias different progenitor states toward endocrine differentiation as early as uncommitted-pancreatic progenitors (solid red tab indicates stages where known epigenetic influences occur, “hazy” red tabs indicate no direct experimental data). Defined expression levels and genetic interactions of specific TFs determine self-replicating (circular arrow) MPC-behavior (**Box A–B**; equilibrium arrows), and movement towards either ‘first wave’ hormone-positive endocrine cells or eventual segregation of acinar-fated tip cells. Microlumen formation provides the groundwork for the integration of extrinsic cues that push MPC toward a bipotent endocrine/duct state (**Box B**). Specific interfaces defined by the remodeling tubular epithelial arbor (‘3D epithelial architecture’ green tab) determine the sites of endocrine competence (‘endocrine-bias’ purple arrow) and delamination of endocrine precursors characterized by heightened Ngn3-expression (**Box C–D**). Establishment of context-specific TF networks (**Box E–F**), and priming at chromatin-level might predispose endocrine precursors towards a β cell fate, among the other endocrine subtype possibilities (in grey). These intrinsic factors might also ensure robustness of β cell identity in subsequent transitional states (pro- β cell and immature β cell) in the face of any final endocrine maturation checkpoints (‘physiological trigger’ blue tab; ‘endocrine maturation’ purple arrow right).