Historical Perspective: Beginnings of the β-Cell

Current Perspectives in β-Cell Development

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Over the course of the last half-century, we have gleaned much about the developmental biology of the pancreas and in particular the insulin-producing β-cell, the autoimmune destruction of which results in type 1 diabetes. Deciphering the mechanisms driving β-cell neogenesis in vivo holds great allure, in large part because of the potential therapeutic applications that stand to be gained.

The field of pancreas development was arguably established by such scientists as Rutter, Grobstein, Wessells, and Cohen in the 1960s. Their seminal studies were the first to demonstrate the significance of the mesenchyme in supporting development of the pancreas or indeed any organ. Their work was further extended by Teitelman and colleagues who did much to characterize pancreatic differentiation via immunohistochemistry. Over the last two decades, the emergence of increasingly elaborate knockout and transgenic mouse technologies has exponentially expanded our insight into the signaling pathways and transcriptional nexus governing pancreas development. Thus, it is fitting that this review will mainly focus on the development of the pancreas and β-cells in the mouse.

Through recapitulating endogenous signaling pathways governing β-cell neogenesis in the embryo, it has recently proven possible to generate insulin-producing cells in vitro from human embryonic stem cells (hESCs) (1). Although this milestone accentuates the great therapeutic potential of studying β-cell neogenesis in vivo, the currently insufficient functionality of hESC-derived insulin cells argues the case for further examination of β-cell development in order to understand how and why such engineered cells differ from their endogenous counterparts. It is likely that resolving these differences will lie in better characterizing the relationships between the many signaling pathways and key factors already known to govern the pancreatic program in regards to spatial and temporal pancreatic expression and their impact on pancreatic differentiation. It is envisaged that such incremental knowledge gains will be applied to the optimization of current stepwise hESC differentiation protocols to generate transplantable insulin cells more reminiscent of endogenous β-cells. Although the direction of the field in broad terms is described above, in the following review, which will focus on β-cell development from pancreatic specification onward, attention will be drawn to more specific, outstanding questions in the current field as they arise.

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SPECIFYING THE PANCREAS

Pancreas development in the mouse is first morphologically discernable at embryonic day (e)8.75–9.0 with the outgrowth of dorsal and ventral pancreatic "buds" (Fig. 1*A-D*) on opposing sides of the foregut endoderm, marked by expression of the transcription factor Pdx1 (refer to Table 1 for an overview of transcription factors regulating pancreas morphogenesis and β-cell neogenesis and summarized phenotypes of mouse mutants). Pictet et al. (2) were the first, however, to propose that such budding is preceded by the acquisition of a so-called pancreatic state by prepancreatic endoderm cells, a process they coined the primary transition. Subsequent work has shown this endodermal state to be conferred by a combination of cell intrinsic regulators whose expression in the gut endoderm is governed by extrinsic, mesodermally derived signals. These include members of the hedgehog, transforming growth factor (TGF)-β, fibroblast growth factor (FGF), bone morphogenetic protein (BMP), and retinoic acid (RA) families of secreted factors. Much remains unknown about how these pathways communicate with one another to specify pancreatic endoderm, and it is likely that future work will do much to dissect the interactions between them.

MESODERMAL CUES

FGF, BMP, and hedgehog signaling. While expressed throughout early foregut endoderm, Sonic hedgehog (Shh) and Indian hedgehog (Ihh) are specifically excluded from the prepancreatic domain, a phenomenon crucial for pancreas specification because hedgehog signaling has been shown to inhibit both pancreas formation and Pdx1 expression ([3] and references therein). Experiments in the chick have suggested that notochord-derived signals, such as the TGF-β superfamily member activin-βB and FGF2 repress *Shh* in the adjacent dorsal prepancreatic endoderm (4–6). However, hedgehog exclusion from the ventral prepancreatic endoderm is achieved via a distinct notochord-independent mechanism. Intricate work by Zaret and colleagues ([7] and references therein) predominantly using embryo culture models has shown that this ventral foregut endoderm is bipotential, giving rise to both liver and ventral pancreas with the latter being formed by default. FGF signaling from the adjacent cardiac mesoderm instead diverts ventral endoderm toward a liver fate by inducing local Shh expression while the ventral pancreatic program is initiated by those cells not exposed to cardiac FGF. Concordantly, BMP signaling from the septum transversum mesoderm similarly induces hepatic

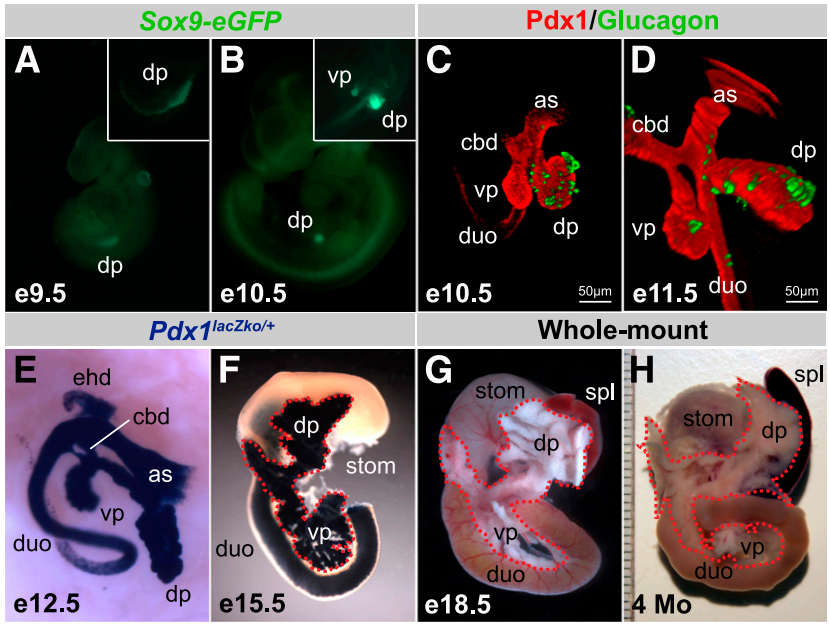

FIG. 1. Gross morphology of the developing pancreas. Sox9-eGFP demarcates the dorsal pancreatic endoderm (dp) at e9.5 (*A*) and both dorsal (dp) and ventral (vp) pancreatic buds at e10.5 (*B*) (embryos shown at same scale). Staining for Pdx1 highlights the epithelium of the pancreatic buds, antral stomach (as), common bile duct (cbd), and duodenum (duo) at e10.5 (*C*) and e11.5 (*D*); early glucagon$^+$ endocrine cells differentiate at the periphery of the dorsal pancreatic bud and, following a temporal delay, in the ventral pancreatic bud (*z*-stack projections shown at same scale). X-Gal staining for *lacZ* expressed from the *Pdx1* locus highlights the dorsal bud-derived (dp) and ventral bud-derived (vp) pancreas, antral stomach (as), common bile duct (cbd), extrahepatic bile ducts (ehd), and duodenum (duo) at e12.5 (*E*) and e15.5 (*F*). Gross morphology of the perinatal (e18.5) (*G*) and adult (four-month-old) (*H*) pancreas: stom, stomach; spl, spleen. *F–H*: Pancreas tissue is demarcated by red dashed lines. (A high-quality digital representation of this figure is available in the online issue.)

fate in ventral endoderm cells and, reciprocally, suppresses adoption of pancreatic fate ([7] and references therein). Recent studies by the Zaret laboratory using a novel half-embryo culture system have however revealed that just a few hours later in development, BMP signaling contrarily promotes pancreatic fate in ventral endoderm (7), exemplifying how dynamically cells change their competence to respond to the inductive effects of programming signals in development. Although these findings illustrate the great utility of half-embryo and organ culture models, the experimental system is not without caveats. These include developmental delay in comparison with in situ organs, high explant-to-explant variability, incomplete tissue exposure to exogenously applied agonists, and failure to fully recapitulate in vivo developmental processes. Thus, explant studies are of most benefit when complementing in vivo genetic analyses. It currently remains unknown whether the indicated liver-pancreas bipotentiality of foregut endoderm truly exists at the level of a single endoderm cell, or whether the foregut endoderm population is heterogeneous in terms of organ lineage commitment.

Retinoic acid signaling. RA, synthesized by the enzyme encoded by *Raldh2* (or *Aldh1a2*), regulates global anteroposterior patterning of gut endoderm far upstream of Pdx1. While zebrafish embryos lacking RA fail to correctly specify both pancreas and liver because of endoderm anteriorization (8), in *Xenopus* and mouse, only dorsal pancreas specification is RA-dependent (9–11). Although some evidence in *Xenopus* suggests that RA may promote pancreas formation by downregulating *Shh* (9), RA induction of the dorsal pancreatic program is probably hedgehog-independent in mammals (10).

Endothelial signals. Tissue recombination experiments and studies of endothelium-deficient *Kdr1*$^{-/-}$ (*Flk1*) mice have revealed that endothelium of the fusing dorsal aortae is required for emergence of the adjacent dorsal pancreatic endoderm and its maintenance of Pdx1 expression as well as induction of a second crucial pancreatic transcription factor, Ptf1a (12,13). The dorsal aorta endothelium has more recently been shown to also play a role in promoting the survival of dorsal pancreatic mesenchyme (14). In contrast, ventral pancreas emergence and Ptf1a expression occur independently of the underlying vitelline vein endothelium (13), consistent with the existence of distinct programs for specifying dorsal versus ventral pancreas.

PANCREAS POSITIONING

Establishing the proper anteroposterior location of the prepancreatic domain within the gut endoderm requires its interaction with the mesoderm. The transcription factors Cdx4, Bapx1 (Nkx3.2), and Hhex all serve to correctly position the emerging pancreatic buds within the gut endoderm. The posteriorly shifted pancreas, liver, and small intestine in *Cdx4*-deficient zebrafish show that endodermally expressed Cdx4 confers posterior identity to gut endoderm and may suppress adoption of pancreatic fate by preventing RA signal transduction in posterior endoderm (15). Both Bapx1 and Hhex regulate the adoption of pancreatic fate by controlling morphogenetic movements, thereby governing proximity of prepancreatic endoderm to signaling centers. Bapx1 acts in the splenic mesenchyme to correctly position and partition the developing dorsal pancreas and spleen (16). Hhex instead directs specification of the ventral pancreas by regulating proliferation and, therefore, positioning of the leading edge of ventral definitive endoderm cells that extend beyond the cardiac mesoderm during closure of the gut tube (17) (see "FGF, BMP, and hedgehog signaling" above). In *Hhex*$^{-/-}$ mice,

the bipotential ventral foregut endoderm cells do not become positioned beyond the liver-inducing influence of the cardiac mesoderm and, consequently, ventral pancreas is not specified (17).

INTRINSIC FACTORS

Assigning pancreatic fate: deciphering the pancreatic code. Pdx1 has long been regarded as the marker for identifying presumptive pancreatic progenitors because it demarcates the prepancreatic endoderm from e8.5, prior to pancreatic bud emergence. Subsequently, however, Pdx1 additionally marks the presumptive extrahepatobiliary system, antral (posterior) stomach, and duodenum (18–20) (Fig. 1*C-F*). In contrast, expression of Ptf1a in the gut endoderm is exclusively pancreas-specific (21,22). Interestingly, despite their early expression in prepancreatic endoderm, neither *Pdx1* nor *Ptf1*a is required for dorsal pancreatic specification: mouse embryos lacking either gene do form a dorsal pancreatic rudiment though it subsequently growth-arrests at around e10.5 (19–21,23). Lineage-tracing experiments have revealed that in the absence of *Ptf1*a, endodermal cells fated to form ventral pancreas switch fate and become incorporated into duodenal and common bile duct (CBD) endoderm (21,24). These studies prompted speculation that Ptf1a expression within the broader Pdx1$^+$ domain defines a so-called pancreas code instructing adoption of pancreatic identity. However, contrary to this idea, concerted loss of both *Pdx1* and *Ptf1*a is insufficient to prevent initiation of a pancreatic program in either *Xenopus* (25) or mouse (24), strongly pointing to the existence of parallel genetic programs driving specification of dorsal pancreas. Most recently, a role has been demonstrated for the transcription factor Sox17 in assigning ventral pancreatic versus extrahepatobiliary fate in ventral foregut progenitor cells (26). In the absence of *Sox17*, ventral pancreatic fate is promoted at the expense of extrahepatobiliary fate while the reverse results from *Sox17* misexpression in presumptive pancreatic progenitor cells (26). In contrast to Ptf1a and Sox17, which are functionally required for proper ventral pancreas development, the transcription factor Hb9 (encoded by *Hlxb9*) plays a crucial role in early morphogenesis of the dorsal pancreas. *Hlxb9*$^{-/-}$ mice display complete dorsal pancreas agenesis, while the early ventral pancreatic program is unperturbed (27,28).

On account of the key roles played by Pdx1 and Ptf1a in early pancreas development, one intense focus of interest is the search for transcription factors regulating their expression (reviewed in [29]). Both Foxa2 (formerly Hnf3β) and Hnf6 (Onecut1) have been identified as potential activators of *Pdx1* expression (30,31) but, in apparent contradiction to this function, are both dispensable for *Pdx1* activation and early pancreas morphogenesis (31–33). Similarly, some evidence points to the transcription factor Hnf1β (Tcf2, vHnf1) regulating *Ptf1*a (34). Phenocopying the *Ptf1*a mutant, in *Hnf1β*-nullizygous mice, the ventral pancreas is absent while the dorsal bud, which fails to express Ptf1a, growth-arrests (34). Lineage-tracing will be required to definitively test whether *Hnf1β*-deficient presumptive ventral pancreatic endoderm cells contribute to adjacent endodermally derived organs as in *Ptf1a*$^{-/-}$ mice. Based on the discordance between dorsal and ventral pancreatic phenotypes in mice deficient for the endodermally expressed factors *Ptf1*a, *Hnf1β*, *Pdx1*, or, most notably, *Hlxb9*, it has been suggested that dorsal and ventral anlagen are specified by distinct cell-intrinsic programs (35). In those mutants in which ventral pancreas morphogenesis is more severely perturbed than dorsal, it should, however, be considered that the divergent phenotypes could also be explained by differences in the morphogenetic events leading to the emergence of the two rudiments. While the larger dorsal bud arises in relative isolation, the contiguity of the emerging, smaller ventral pancreas and CBD (Figs. 1*C-D* and 2) render the two nearly undistinguishable in the event of ventral pancreatic hypoplasia. As such, early growth-arrest of an initially specified ventral pancreatic bud can result in its assimilation into the adjacent endoderm of the CBD and duodenum, manifesting in apparent ventral pancreas agenesis. For this reason, the molecular programs governing dorsal versus ventral pancreas specification are most likely more similar than currently perceived.

GROWTH OF THE PANCREAS: PROGENITOR CELL PROLIFERATION AND ORGAN MORPHOGENESIS

Following specification, the dorsal pancreatic bud arises just posterior to the presumptive stomach, while the ventral pancreas emerges opposite, ventrally adjacent to the hepatic endoderm (Figs. 1*A–D* and 2). Between e10.5 and e12.5, the two pancreatic buds grow alongside the presumptive duodenum and stomach. Rotation of the gut tube leads to their fusion on the dorsal side of the gut by e12.5 (Fig. 1*E*), forming a single organ while, simultaneously, multiple rounds of branching morphogenesis take place, initiating at e11.5 (Fig. 2). Branching morphogenesis involves the restructuring of the pancreatic epithelium into a complex and highly organized tubular network. Recent intricate studies by Semb and colleagues (36) have revealed that tube formation occurs in a multistep process: *1*) scattered microlumens arise stochastically within the pancreatic epithelium at e11.5; *2*) microlumens expand through their induction of apical cell polarity in neighboring cells; *3*) lumens coalesce with one another at e12.5 into a continuous luminal network; which *4*) remodels between e13.5 and e15.5 into a tubular network (Fig. 2). Using *Pdx1-Cre*-driven ablation in the early pancreas, Kesavan et al. (36) demonstrated that the ρ-GTPase Cdc42 plays a crucial role in tubulogenesis by concordantly establishing multicellular microlumens and apical epithelial polarity. Further extending this finding, the inducible ablation of *Cdc42* in fully polarized tubular epithelial cells results in their loss of apical cell polarity, uncovering an additional cell-autonomous role for Cdc42 in the maintenance of apical polarization (36).

PROPERTIES OF EARLY PANCREATIC EPITHELIAL CELLS

Although a few glucagon$^+$ endocrine cells differentiate (Fig. 1*C-D*) immediately following bud outgrowth (e9.5–e11.5), the majority of pancreatic cells are still undifferentiated and continue to coexpress Pdx1 (Fig. 1*C-D*) and Ptf1a. Lineage-tracing has shown such cells to give rise to all mature pancreatic cell types: the exocrine acinar and ductal cells and the five endocrine cell types (21,37). Based on these properties, Pdx1$^+$ and Ptf1a$^+$ cells of the early pancreatic epithelium have been referred to as multipotent pancreatic progenitors (MPCs) (Fig. 2). However, because in vivo lineage-tracing studies assessed the developmental potential of the entire population, it is still unclear whether progenitors of the early pancreas are a heterogeneous population with some or all being precommitted to a particular cell fate as they arise. Consistent with this model, single-cell transcript analysis has revealed the existence

TABLE 1
Overview of transcription factors governing pancreas development and β-cell neogenesis and phenotypes of respective mouse mutants

Factor	Type	Onset*	Expression/role(s)	Pancreatic phenotype of mouse mutant(s)
Bapx1 aka Nkx3.2	HD	≤e9.5	Acts in splenic mesenchyme to correctly position and partition developing d.p. and spleen (16)	*Bapx1*$^{-/-}$: d.p. undergoes metaplastic transformation into intestinal-like tissue because of prolonged interaction with splenic mesenchyme (16)
Hhex	HD	e7.0	Directs v.p. specification (17)	*Hex*$^{-/-}$: v.p. not specified: reduced proliferation of bipotential ventral foregut endoderm cells does not position them beyond prohepatic influence of the cardiac mesoderm to permit pancreatic induction (17)
Sox17	HMG box	≤e5.5	Expressed throughout endoderm postgastrulation (113); assigns ventral pancreatic vs. extrahepatobiliary fate in ventral foregut progenitor cells (26)	*Foxa3-Cre; Sox17*$^{fl/fl}$: loss of biliary primordium (gall bladder and cystic duct); ectopic pancreas in common duct (26). *Pdx1*$^{tTa/+}$; *tetO-Sox17*: d.p. and v.p. agenesis (26)
Foxa2 aka Hnf3β	winged helix	≤e5.5	Expressed throughout the early pancreatic endoderm; restricted to acinar and endocrine cells by late gestation (30;114–115); required for islet function (32;118)	*Foxa2*$^{-/-}$: embryonic lethal; absence of foregut endoderm (116;117) *Foxa3-Cre; Foxa2*$^{fl/fl}$: hypoglycemia; hypoglucagonemia; loss of α-cells (32). *Ins-Cre; Foxa2*$^{fl/fl}$: hyperinsulinemic hypoglycemia (118)
Hnf6 aka OC1	HD	≤e9.0	Expressed in MPCs and becomes restricted to acini and ducts (31;119;120); required for endocrine differentiation (33)	*Hnf6*$^{-/-}$: d.p. and v.p. hypoplastic (persists postnatally) because of delayed Pdx1 expression; loss of Ngn3^{+} endocrine progenitors; reduced endocrine cell numbers (31;33)
Hb9 (from *Hlxb9* gene)	HD	≤e8.0	Foregut and midgut endoderm, then dorsal and ventral pancreatic buds; becomes β-cell–restricted (27;28). Required for dorsal pancreas development and β-cell terminal differentiation (27;28)	*Hlxb9*$^{-/-}$: d.p. agenesis (27;28); reduced numbers of β-cells in ventral pancreas, which also showed decreased Glut2 (27;28) and Nkx6.1 expression (27); increased numbers of δ-cells (28)
Hnf1β aka Tcf2 aka vHnf1	HD	≤e8.5	Expressed in MPCs and progenitor cords (34;121); required for growth of pancreatic buds (34)	*Tcf2*$^{-/-}$: v.p. undetectable; d.p. growth-arrested ≥e10.5 and lost by e13.5 because of reduced proliferation (34)
Isl1	HD	≤e9.0	Expressed in dorsal pancreatic mesenchyme and in endocrine cells (42). Required for pancreatic epithelial growth and endocrine differentiation (42). Required for the maturation, proliferation and survival of second wave endocrine cells (110)	*Isl1*$^{-/-}$: absence of dorsal pancreatic mesenchyme and so defective exocrine differentiation; complete abrogation of endocrine development because of function in epithelial cells (42) *Pdx1-Cre*^{late}; *Isl1*$^{fl/fl}$: hyperglycemia; islet hypoplasia; reduced numbers of Pax6^{+} late endocrine progenitors; decreased proliferation and increased apoptosis of endocrine cells; impaired endocrine cell maturation (110)
Pdx1	HD	e8.5–e8.75	Expressed in MPCs (37), progenitor cords then β-cells (18–20); required for growth of pancreatic buds (19;20;24)	*Pdx1*$^{-/-}$: v.p. agenesis; d.p. growth-arrested ≥e10.5 (19;20;24) *Pdx1*$^{+/-}$: glucose intolerance; impaired GSIS; increased susceptibility to β-cell apoptosis (122)

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TABLE 1
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Factor	Type	Onset*	Expression/role(s)	Pancreatic phenotype of mouse mutant(s)
Ptf1a	bHLH	e8.5–e8.75	Expressed in MPCs (21), then acinar cells (22); promotes ventral pancreatic fate (21); governs acinar program (21;23;62)	*Ptf1a*$^{-/-}$: v.p. agenesis (cells diverted to intestinal and CBD fate); d.p. growth-arrested ≥e10.5; in d.p., endocrine cells present but acinar cells lost (21;23;24)
Hes1	bHLH		Maintains MPCs by preventing cell cycle exit and differentiation (49–51) by repressing *Ngn3* (53) and *p57* (49), respectively	*Hes1*$^{-/-}$: d.p. and v.p. hypoplasia because of MPC pool depletion through precocious differentiation (49–51); acinar dysgenesis (50); ectopic pancreas in distal stomach, duodenum, and CBD (123)
Sox9	HMG box	≤e9.0	Expressed in MPCs and progenitor cords (55;63;124); required for growth of pancreatic buds (55) and generation of Ngn3^{+} endocrine progenitors (63)	*Pdx1-Cre*^{early}*; Sox9*$^{fl/fl}$: d.p. and v.p. hypoplasia because of MPC pool depletion; increased apoptosis, reduced proliferation, and precocious differentiation (55) *Pdx1-Cre*^{early}*; Sox9*$^{fl/+}$: islet hypoplasia because of reduced numbers of Ngn3^{+} endocrine progenitor cells (63)
Nkx6.1	HD	≤e9.0	Expressed in MPCs, Ngn3^{+} endocrine progenitors, then β-cells (62;93). Specifies MPCs toward an endocrine/ductal fate (62). Required for β-cell differentiation (93)	*Nkx6.1*$^{-/-}$: 85% reduction in β-cell numbers (93); additional loss of α-cells in *Nkx6.1*$^{-/-}$; *Nkx6.2*$^{-/-}$ mutants (125). *Nkx6.1*$^{-/-}$: ectopic trunk Ptf1a expression and loss of Ngn3^{+} cells; both more acute in *Nkx6.1*$^{-/-}$; *Nkx6.2*$^{-/-}$ mice (62)
Nkx6.2	HD	≤e10.5	Expressed in MPCs, then by e15.5, only in some glucagon^{+} cells and acinar cells (125). Functionally equivalent to Nkx6.1 (126)	*Nkx6.2*$^{-/-}$: no obvious pancreatic phenotype (125)
Ngn3	bHLH	≤e9.5	Endocrine progenitor cell marker then later expressed in islet cells (51;74;75); cell-autonomously induces commitment to endocrine lineage (51); required for formation of all endocrine cells (74). Maintains endocrine function (75)	*Ngn3*$^{-/-}$: absence of all pancreatic endocrine and intestinal enteroendocrine cells (74;78;79); acinar dysmorphogenesis (74) *Ins2-Cre; Ngn3*$^{fl/-}$: hyperglycemia; impaired β-cell maturation (75)
Ia1 aka Insm1	Zinc finger	≤e9.5	Expressed in endocrine progenitors and some differentiated endocrine cells in *Ngn3*-dependent manner (83;84)	*Ia1*$^{-/-}$: impaired differentiation of pancreatic α- and β-cells and gut enteroendocrine cells (84)
Neuro D1 aka Beta2	bHLH	≤e9.5	Expressed in differentiating and mature endocrine cells (83;86). Required for normal islet formation (86) and for the acquisition and maintenance of the fully functional, glucose-responsive β-cell phenotype (111)	*NeuroD1*$^{-/-}$: islet hypoplasia and dysgenesis, endocrine apoptosis and aberrant differentiation; disrupted enteroendocrine differentiation (86). *RIP-Cre* (or *Pdx1-Cre*^{ERT})*; NeuroD1*$^{fl/-}$: hyperglycemia; reduced pancreatic insulin (*ins1* mRNA reduced); β-cell immaturity (111)
Nkx2.2	HD	≤e9.5	Expressed in MPCs, then α-, β-, and PP-cells (87). Required for β- and α-cell development (87;89)	*Nkx2.2*$^{-/-}$: β-cell loss and α- and PP-cell reduction; ε-cell numbers expanded (87;89)
Pax4	HD	≤e9.5	Expressed in endocrine progenitors then becomes restricted to β- and δ-cells (88); *Ngn3*-dependent expression. Promotes allocation to β-cell fate (88–90)	*Pax4*$^{-/-}$: β- and δ-cell loss; ε- and α-cell numbers expanded (88–90)

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TABLE 1
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Factor	Type	Onset*	Expression/role(s)	Pancreatic phenotype of mouse mutant(s)
Arx	HD	≤e9.5	Expressed in α- and PP-cells in *Ngn3*-dependent manner (94;95); promotes acquisition of α- and PP-cell fate; represses β- and δ-cell fate (94)	*Arx*$^{-/-}$: α-cell loss; β- and δ-cell numbers expanded (94)
Rfx6	Winged helix	≤e9.0	Expressed in endocrine progenitors and all islet cell types in *Ngn3*-dependent manner (97;98). Restricts expression of Nkx6.1 (97)	*Rfx6*$^{-/-}$: small bowel obstruction; incompletely penetrant pancreatic hypoplasia; loss of all hormone-expressing (except PP-) cells; partial activation of endocrine program (97)
MafA	Basic leucine zipper	≤e13.5	β-Cell–specific activator of insulin gene transcription (104 [review]); exclusive expression in insulin^{+} cells from e13.5 onwards (105;107)	*MafA*$^{-/-}$: no obvious pancreatic developmental phenotype; development of glucose intolerance postnatally (108)
MafB	Basic leucine zipper	≤e10.5	Expressed in insulin^{+} and glucagon^{+} cells by e12.5; restricted to α-cells postnatally (106;107). Regulates transcription of key factors required for α- and β-cell maturation (109)	*MafB*$^{-/-}$: α- and β-cell numbers reduced throughout pancreas development while total endocrine cell numbers unaffected; insulin^{+} cell neogenesis delayed (109)

*Onset of expression in or adjacent to the prepancreatic or pancreatic domain. aka, also known as; d.p., dorsal pancreas; GSIS, glucose-stimulated insulin secretion; v.p., ventral pancreas.

of six distinct cell types in e10.5 pancreas based upon expression profile (38). Whether a single cell in the early embryonic pancreas is truly multipotent has yet to be demonstrated: only through single-cell colony-forming assays can unequivocal proof of multipotency be acquired.

Maintenance of early progenitor pool size is critical for normal pancreas morphogenesis. A recent study (39) using temporally controlled ablation has shown that while progenitor depletion prior to e9.5 has negligible impact on final pancreas size, their diminution up until e11.5 causes pancreatic hypoplasia which persists into adulthood. Thus, the bulk of the progenitor pool is generated between e8.5 and e12.5 and if a proportion is lost, compensatory growth is unable to occur. As such, the size of the initial progenitor pool predetermines final pancreas size.

ROLE OF MESENCHYMAL SIGNALS

The seminal tissue recombination experiments of Golosow, Grobstein, Wessells, and Cohen in the 1960s revealed

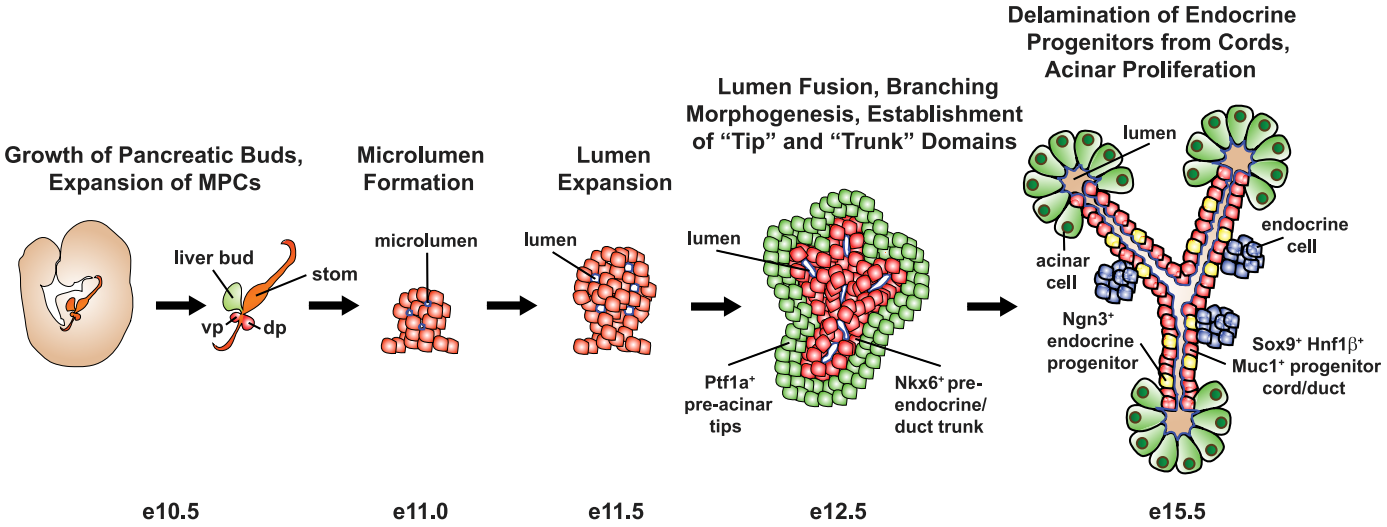

FIG. 2. Overview of major events in pancreatic morphogenesis. By e10.5, the anlagen of the dorsal (dp) and ventral (vp) pancreas, liver, stomach (stom), and duodenum are established; rapid growth of the pancreatic buds occurs through expansion of the MPC population. From around e11.0 on, neighboring cells undergo cell polarization, generating microlumens with a common apical surface. Over the next few days, microlumen expansion and fusion forms a ductal luminal network; branching morphogenesis of the pancreatic epithelium occurs concordantly with segregation of MPCs by e12.5 into an acinar-committed Ptf1a^{+} "tip" domain and an Nkx6^{+} central "trunk" field fated to give rise to the ductal network and endocrine cells. By e15.5, the midpoint of the secondary transition, much acinar cell neogenesis has occurred and the acinar compartment expands predominantly through mitosis. Ngn3^{+} endocrine progenitor cells delaminate from the Sox9^{+} Hnf1β^{+} Muc1^{+} ductal epithelium and give rise to endocrine cells, which coalesce to form nascent islets.

that growth and differentiation of pancreatic epithelium is crucially dependent upon diffusible, permissive signals from the adjacent pancreatic mesenchyme (40,41). It is only relatively recently, through the use of transgenic and gene knockout mice that we have begun to characterize these mesenchymal cues that comprise not only diffusible signaling molecules but transcription factors also.

Isl1. RA promotes the expression of the transcription factor Isl1 in the dorsal pancreatic mesenchyme (10). Without Isl1, the dorsal pancreatic mesenchyme is almost entirely absent at e9.5, and the dorsal pancreas growth-arrests, revealing a crucial role for Isl1 in promoting pancreatic growth and differentiation through a requirement in the maintenance of the dorsal pancreatic mesenchyme (42).

FGF signaling. Of the broad spectrum of FGF ligands and their receptors expressed in the developing rodent pancreas, the role of the FGF10-FGFR2IIIb axis is arguably the best characterized. *FGF10* is expressed in dorsal and ventral pancreatic mesenchyme from e9.5–e11.5, coincident with both pancreatic epithelial expression of it's receptor FGFR2IIIb and rapid pancreatic epithelial growth (43). Without either FGF10 or FGFR2b, dorsal and ventral pancreatic hypoplasia occurs because of reduced proliferation of the early progenitor pool, showing mesenchymal FGF10 signaling to be required for MPC maintenance (43,44). Reinforcing this conclusion, studies by Norgaard et al. (45) have demonstrated that ectopic *Pdx1* promoter-driven expression of *FGF10* in pancreatic epithelium both maintains cells in an undifferentiated state and promotes their proliferation.

BMP and Wnt signaling. Although studied in substantially less depth than the FGF signaling pathway, recent studies have suggested that Wnt and BMP signaling in the mesenchyme also provide important cues for expansion of the early pancreatic epithelium (46,47). The PHD-finger protein pygopus was initially identified in *Drosophila* as a nuclear coactivator of Wg (fly homolog of mammalian Wnt) signaling (reviewed in [48]). Deficiency for the *pygopus* homolog *mPygo2* in mice results in pancreas hypoplasia because of decreased progenitor cell proliferation after e12.5 when canonical Wnt signaling is both active in the pancreatic mesenchyme and mPygo2-dependent (46). The absence of pancreatic hypoplasia after conditional ablation of *mPygo2* in the pancreatic epithelium suggests that decreased mesenchymal Wnt signaling accounts for the observed pancreas hypoplasia in *mPygo2* null mutant mice (46). While Wnt signaling activity is also detected in pancreatic epithelial cells (46), BMP signaling as revealed by phospho-Smad1,5,8 immunoreactivity is restricted to the early pancreatic mesenchyme in both mouse and chick (47). The observation that abrogation of BMP signaling with the BMP antagonist Noggin results in pancreatic hypoplasia and diminished epithelial branching (47) suggests a role for mesenchymal BMP signaling in promoting growth of the early pancreatic epithelium. One hindrance in genetically dissecting the roles of Wnt and BMP signaling in pancreatic morphogenesis is redundancy between the large number of ligands and receptors that can mask important gene functions in loss-of-function models.

EPITHELIAL CELL INTRINSIC CUES

In addition to extrinsic signals from the mesenchyme, pancreatic growth is also governed by signaling pathways and transcription factors that operate within the epithelium. While a few studies have begun to address the relationship between signaling events and transcriptional responses in progenitors, the mechanisms through which specific signaling pathways affect gene transcription remain largely unexplored.

The Notch pathway. While the aforementioned study by Norgaard et al. (45) has hinted that the effects of mesenchymal FGF10 signaling are transduced through Notch pathway activation in the pancreatic epithelium, overwhelming evidence supports the involvement of Notch in the maintenance of undifferentiated progenitors. Inactivation of the Notch pathway constituents *Hes1* (the Notch downstream effector in the pancreas), *RBP-Jκ*, or *Δ-like 1* (*Dll1*) in mice leads to cell cycle arrest, precocious endocrine differentiation, and MPC pool depletion, resulting in pancreatic hypoplasia (49–52). As well as repressing the cyclin-dependent kinase inhibitor *p57* in MPCs (49), Hes1 also represses the pancreatic proendocrine transcription factor *Ngn3* (53). Furthermore, Hes1 and the related protein Hey1 inhibit *Ptf1*a (54), which in addition to being required for pancreas specification, subsequently promotes acquisition of acinar fate (21,23). Thus, Notch signaling within the pancreatic epithelium maintains progenitors in an undifferentiated state.

Sox9. Similar to Pdx1 and Ptf1a (19–21,23), the transcription factor Sox9 is crucial for proper expansion of the MPC pool to generate a normal pancreas. Conditional ablation of *Sox9* in MPCs results in almost complete pancreas agenesis because of MPC pool depletion through reduced proliferation, apoptosis, and premature differentiation (55). Loss of Hes1 in Sox9-deficient MPCs indicates that Sox9-mediated progenitor cell maintenance may be at least partially Notch-dependent (55). Future work will ascertain at what level of the Notch pathway interaction with Sox9 occurs.

The Wnt pathway. Attenuation of the β-catenin–dependent canonical Wnt pathway in MPCs has been shown by some (though not by all) groups to result in decreased progenitor proliferation, manifesting in pancreatic hypoplasia at birth (56,57). Intriguingly, hyperactivation of canonical Wnt signaling in MPCs also produces hypoplasia, due apparently to loss of mesenchymal FGF10 signaling, concomitant with increased hedgehog signaling (58,59). However, analysis of temporally inducible Wnt ablation and hyperactivation mouse models will be needed to determine whether the observed pancreatic hypoplasia truly reflects a role for Wnt signaling in pancreatic progenitor cell expansion or whether it instead results from earlier endodermal patterning defects.

ESTABLISHING ENDOCRINE- AND EXOCRINE-COMMITTED COMPARTMENTS

Between e12 and e14 (the branching phase of pancreas development), the apparently homogeneous field of MPCs in the early pancreatic buds resolves into an acinar-committed distal "tip" domain (marked by *Cpa*, *c-Myc*, and *Ptf1*a) (60) and an endocrine- or ductal-committed central core or "trunk" region (61) (Fig. 2). Only recently has insight been gained into the molecular mechanism governing this early lineage allocation. Through genetic loss- and gain-of-function studies, our laboratory showed that Nkx6 (Nkx6.1 and Nkx6.2) and Ptf1a transcription factors are required and sufficient to repress the alternative lineage program and to specify MPCs toward an endocrine/ductal or acinar fate, respectively (62). Reciprocal repression between Nkx6 factors and Ptf1a constitutes a bistable switch in MPCs that governs the equilibrium between endocrine and acinar cell neogenesis and is thus crucial for normal cell differentiation.

THE SECONDARY TRANSITION: DIFFERENTIATION FROM SECONDARY PROGENITORS

At e13.5, during the midpoint of the branching phase of pancreas development, a window of major cell differentiation begins, known as the secondary transition (2). Over the next three days, all five endocrine cell types arise: the β, α, δ, ε, and pancreatic polypeptide (PP) cells, which, respectively, synthesize and secrete the endocrine hormones insulin, glucagon, somatostatin, ghrelin, and pancreatic polypeptide.

While the existence of MPCs during early pancreas organogenesis is beyond contention, it is only now becoming established whether such a cell population persists later in development and if so, where they reside. During the secondary transition, the early MPC marker Sox9 becomes restricted to the embryonic ductal tree within which $Ngn3^+$ endocrine progenitor cells are intercalated (55) (Fig. 2). Short-term *Sox9-eGFP*-based lineage-tracing revealed that from e15.5 until birth, endocrine cells arise from $Sox9^+$ cells via transient hormone$^-$ $Ngn3^+$ endocrine progenitor cells (63). eGFP labeling similarly showed a proportion of acinar cells to arise from $Sox9^+$ cells at the onset of the secondary transition although, unlike endocrine cells, the acinar compartment appears to expand predominantly through proliferation in late embryogenesis (63). Subsequent recent genetic lineage-labeling studies in mice expressing Cre-ER^{T2} driven by the promoters of either ductally expressed *Hnf1β* (61) or *Mucin1* (*Muc1*) (64) have largely corroborated these findings, showing secondary transition ductal cells to give rise to endocrine cells via $Ngn3^+$ endocrine progenitors, as well as differentiated ductal cells. Taken together, these studies support the existence of a $Sox9^+$ $Hnf1\beta^+$ $Muc1^+$ secondary progenitor cell population comprising the embryonic ductal tree (thus, recently coined progenitor cords) (Fig. 2). Future work is required to determine *1*) whether individual secondary progenitors are multipotent and *2*) if lineage-restricted, whether they are committed to distinct fates dependent upon their location (distal vs. proximal) within the ductal tree.

SIGNALS REGULATING ENDOCRINE VERSUS ACINAR DIFFERENTIATION

Findings from early tissue recombination experiments (65) that the pancreatic mesenchyme promotes acinar differentiation in the pancreatic epithelium, led to the notion that the pancreatic epithelium forms endocrine tissue by "default" (66). Subsequent work has shown that at e12.5, while the mesenchyme is necessary for acinar differentiation, it represses the endocrine program (67). Both effects are reproduced by the antagonist of the TGF-β ligand activin B, *follistatin*, which is expressed in e12.5 pancreatic mesenchyme (68). Examination of temporally inducible mouse models for both abrogation or hyperactivation of the TGF-β signaling pathway in the pancreatic mesenchyme and/or epithelium will be needed to dissect how this pathway influences the endocrine program.

Recent work has reinforced the notion that mesenchyme-derived extracellular matrix components, such as laminin-1, promote acinar cell development (36). Inhibition of *laminin-1* translation has been shown to abrogate acinar differentiation of pancreatic epithelial cells while exerting no influence on endocrine differentiation (69,70). Concordantly, the disintegration of the pancreatic epithelium in the absence of *Cdc42* results in enhanced epithelial exposure to pancreatic mesenchyme and laminin-1, manifesting in increased acinar differentiation at the expense of endocrine and ductal differentiation (36). Moreover, antibody-mediated inhibition of laminin-1 function in *Cdc42*-deficient pancreatic explants significantly reduces acinar cell differentiation, confirming the proacinar activity of mesenchymal laminin-1 (36).

A similar role has been suggested for FGF signals from the mesenchyme: the FGFR2b ligands FGFs 1, 7, and 10 all favor acinar cell development (71). While it is still unclear whether the mesenchyme is a relevant source of Wnt proteins, evidence suggests that canonical Wnt signaling in the pancreatic epithelium also promotes acinar growth (56,72). A role for Wnt signaling in endocrine differentiation is, however, contentious: *Pdx1-Cre*-mediated *β-catenin* deletion has been shown by some (73) to decrease β-cell numbers, but not by others (56,72). This discrepancy may be contingent on the *Pdx1-Cre* line used. Reporter-monitored, temporally inducible, and efficient ablation of *β-catenin* in pancreatic epithelium will be required to comprehensively establish the precise role of Wnt signaling in endocrine differentiation.

SPECIFYING THE FIVE ENDOCRINE LINEAGES

Ngn3 induces endocrine commitment. Expression of Ngn3 in pancreatic progenitors has been shown to be crucial for inducing commitment to the endocrine lineage in a cell-autonomous manner (51). While Ngn3 is expressed in pancreatic buds by e9.5, expression does not peak until the secondary transition after which it is progressively downregulated to almost undetectable levels in differentiated endocrine cells (51,74,75). Lineage-tracing studies have shown that $Ngn3^+$ cells are pan-endocrine progenitors giving rise to all five endocrine cell types in the pancreas (37) (Fig. 3). Recent studies have further revealed that a high *Ngn3* expression level is critical for this endocrine commitment. While lowered *Ngn3* gene dosage in mice heterozygous or hypomorphic for *Ngn3* results in elevated numbers of $Ngn3^+$ pancreatic progenitors, some of these (likely low) *Ngn3*-expressing cells are misallocated to ductal or acinar fates (76). When extended to a single-cell level, however, results of a recent in vivo clonal analysis suggest individual $Ngn3^+$ cells to be unipotent, with each $Ngn3^+$ cell being the precursor of a single endocrine cell (77). This finding is, however, inconsistent with the currently prevailing model of endocrine cell subtype specification (Fig. 3 Model A) to be discussed later. Concordant with $Ngn3^+$ cells being panendocrine progenitors, *Ngn3*-nullizygous mice exhibit a complete loss of all endocrine cells in both pancreas (74) and intestine (78,79) while in contrast, gastric endocrine cell development in the glandular stomach is only partly abrogated (79,80). Surprisingly, however, presumptive *Ngn3* null mutations in humans manifest only in loss of intestinal endocrine cells (81), hinting at divergent mechanisms for pancreatic endocrine cell specification between mouse and human.

In vivo, the competence of progenitors to generate the distinct endocrine cell types changes throughout the course of pancreatic development and is autonomous to the pancreatic epithelium (82). Temporally inducible expression of *Ngn3* in $Pdx1^+$ progenitors of $Ngn3^{-/-}$ mice has revealed that progressively later *Ngn3* activation induces first $glucagon^+$ cells, then $insulin^+$ cells, PP^+ cells, and finally $somatostatin^+$ cells (82). Attempts to dissect the mechanism(s) governing adoption of a given endocrine lineage by $Ngn3^+$ progenitors have largely focused upon

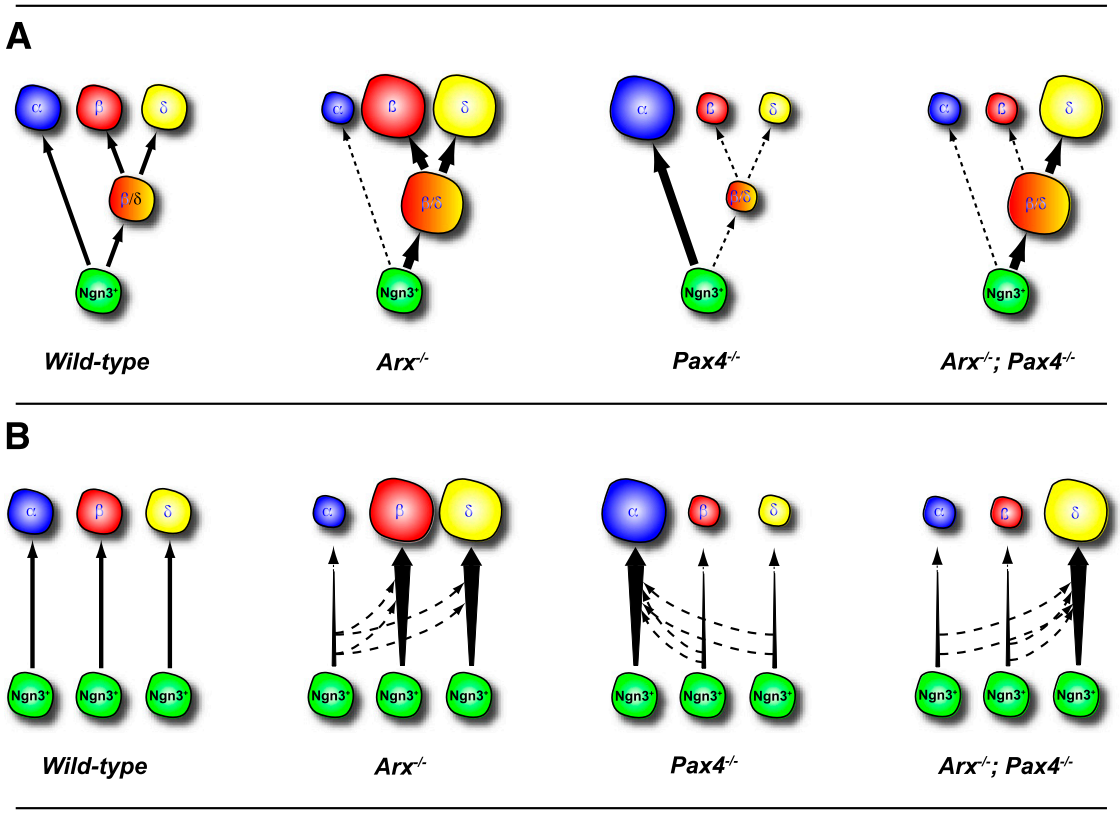

FIG. 3. Alternative models of endocrine subtype specification from endocrine progenitor cells. Model *A*: Prevailing model of endocrine subtype specification in *wild-type*, *Arx*$^{-/-}$, *Pax4*$^{-/-}$, and compound *Arx*$^{-/-}$; *Pax4*$^{-/-}$ mutants, based on the assumption that cells make binary fate decisions involving an intermediary hypothetical β-/δ-cell precursor cell downstream of Ngn3. Model *B*: Alternative model in which Arx and Pax4 function to stabilize lineage decisions downstream of Ngn3. Note that PP- and ε-cell populations are excluded for the sake of clarity.

the identification of Ngn3 downstream targets. However, it is important to consider that the timing of endocrine subtype specification relative to *Ngn3* activation remains unknown (77), thus illustrating the need for studies focusing on the specific timing of cell fate specification as well as the identification of temporally and spatially constrained cues (both cell-intrinsic and -extrinsic) that affect endocrine subtype choices.

DOWNSTREAM TARGETS OF NGN3

While it is still unclear whether islet cell type specification occurs prior to *Ngn3* induction, manipulation of transcriptional programs downstream of Ngn3 has provided insight into the molecular control of islet cell terminal differentiation. Moreover, these studies have revealed that considerable plasticity between the five endocrine cell types still exists after *Ngn3* expression has been initiated.

The zinc-finger transcription factor Ia1 (Insm1) was identified as a direct transcriptional target gene of Ngn3 (83). Mice deficient for Ia1, which is coexpressed with Ngn3 in both endocrine progenitor cells and some differentiated endocrine cells (83,84), exhibit impaired terminal differentiation of α- and β-cells as well as gut enteroendocrine cells associated with downregulation of transcription factors that promote β-cell differentiation (84). How Ngn3-induced *Ia1* activation initiates the endocrine program is currently unknown. Further work will be necessary to determine whether *Ia1* functions in the endocrine pancreas as a transcriptional repressor as biochemical studies indicate (85).

During pancreas development, NeuroD1 (Beta2) is expressed from e9.5 in a partially overlapping manner with Ia1 in differentiating and mature endocrine cells (83,86); concordantly, *NeuroD1* deficiency in mice results in generation of hypoplastic, disorganized islets, probably because of perturbed endocrine survival and differentiation (86). Intriguingly, inactivation of *Ia1* (84) or *NeuroD1* (86) phenocopies the enteroendocrine dysgenesis of *Ngn3*$^{-/-}$ mice, alluding to parallels between transcriptional programs governing endocrine differentiation in pancreas and gut.

Both Nkx2.2 and Pax4 are expressed in pancreatic epithelium by e9.5; while Nkx2.2 thereafter becomes restricted to α-, β-, and PP-cells (87), Pax4 becomes confined to β- and δ-cells (88). Intriguingly, without *Nkx2.2*, β-cells are lost and numbers of α- and PP-cells reduced while the normally scarce ghrelin^{+} ε-cell population is greatly expanded (87,89). Similarly, inactivation of *Pax4* also manifests in a loss of β-cells and expansion of the ghrelin^{+} population (88,89), but unlike *Nkx2.2* mutants, ghrelin^{+} cells in *Pax4*-deficient mice coexpress glucagon (90). These studies demonstrate that Nkx2.2 and Pax4 are both crucially required for development of the β-cell lineage. Consistent with this notion, recent studies have revealed that the ectopic expression of *Pax4* in the early pancreas, endocrine compartment, or even α-cells, forces the adoption of a β-cell fate, showing Pax4 to be both necessary and sufficient to promote β-cell lineage commitment (91).

In vitro studies have suggested Nkx2.2 to directly regulate *Nkx6.1* (92); concordantly, *Nkx2.2; Nkx6.1* compound nullizygous mutants exhibit similar changes in endocrine

subtype ratios as seen in *Nkx2.2*$^{-/-}$ mice, suggesting a function for *Nkx6.1* downstream of *Nkx2.2* (93). Nkx6.1 is broadly expressed in the pancreatic epithelium from e9.0, is later expressed in endocrine progenitors, then subsequently becomes β-cell–restricted (93). Unlike *Nkx2.2* mutants, *Nkx6.1*-null mice specifically lose only the β-cell lineage (93). The β-cell loss in *Nkx6.1*-deficient mice could, however, also be secondary to the early endocrine to acinar cell fate switch caused by *Nkx6.1* abrogation in MPCs (62), thus illustrating the general limitation of studying specific gene functions in null mutant animals. The specific role of Nkx6.1 in β-cell specification/differentiation remains to be defined and will require conditional gene inactivation in a temporally and spatially regulated manner.

The recent dissection of the role played by Arx in endocrine cell development has yielded insight into the mechanisms regulating the segregation of the α- and β-cell lineages. Arx is expressed in a Ngn3-dependent fashion from e9.5 in the pancreas whereafter its expression becomes confined to α- and PP-cells (94,95). Abrogation of *Arx* activity causes α-cell deficiency while numbers of β- and δ-cells are increased (94), revealing Arx to be both required for α-cell fate acquisition and repression of β- and δ-cell fate, the mirror opposite of the role of Pax4 with which Arx is coexpressed during early endocrine differentiation. Concordantly, ectopic *Arx* expression in the endocrine precursors or mature β-cells expands α- and PP-cell numbers at the expense of β-cells (95), showing Arx to be both necessary and sufficient to promote α- and PP-lineage commitment. These findings are consistent with studies showing that Pax4 and Arx mutually and directly inhibit one another's transcription at the promoter level (96). The observation that both α- and β-cells in *Pax4; Arx* compound mutant mice are replaced by δ-cells, which later initiate ectopic PP expression (96), has led to a refinement of the model by which Pax4/Arx govern islet cell subtype choices downstream of Ngn3. Namely, it has been proposed that when one of the two factors is activated in preference to the other in endocrine progenitors, Arx specifies α-cell fate while Pax4 first permits commitment toward a β-/δ-cell fate by repressing *Arx* then subsequently driving a hypothetical bipotential β-/δ-cell precursor cell toward a β-cell fate (96) (Fig. 3 Model A). This prevailing model is based on the assumption that two cells with different endocrine subtype identity arise from an intermediary progenitor downstream of Ngn3, which is in apparent contradiction to the recent observation that each Ngn3^{+} cell only gives rise to one endocrine cell and is therefore unipotent (77). Reconciling this apparent contradiction, an alternative explanation for the observed phenotypes is that Arx and Pax4 function to stabilize lineage decisions downstream of Ngn3 rather than reallocating cells at the level of a hypothetical intermediate precursor cell (Fig. 3 Model B). Additional studies are clearly required to determine when and how endocrine subtype identity is established and stabilized during development.

Similar to Arx and Pax4, most recently, loss-of-function studies in both mouse (97) and zebrafish (98) have intriguingly hinted at a role for the transcription factor Rfx6 in controlling endocrine cell subtype choice. Rfx6, which is activated by Ngn3, is expressed broadly throughout the epithelium of the mouse gut by e9.0 and within a subset of endocrine progenitor cells during the secondary transition, becoming restricted to islet cells by adulthood (97,98). *Rfx6* exhibits a parallel expression pattern in zebrafish pancreas (98). Consistent with its endocrine expression, mice lacking *Rfx6* exhibit a loss of all mature endocrine cell types with the notable exception of the PP-cells, which unusually express the β-cell marker Nkx6.1 (97). In zebrafish, *Rfx6* abrogation results in failure of the non–β-cell endocrine lineages to differentiate from endocrine progenitors while the β-cells fail to coalesce into an islet (98). Future studies will be needed to determine the specific roles played by Rfx6 in the transcription factor network orchestrating endocrine differentiation.

ISLET FORMATION AND β-CELL MATURATION

As birth nears, the exocrine pancreas grows rapidly, primarily through mitotic expansion of differentiated acinar cells. Meanwhile, from e16.5 onwards, the endocrine cells coalesce into polyclonal clusters to form functional islets that, in the mouse, comprise a central core of β-cells and an outer mantle composed of the other four endocrine cell types. Disrupted islet cytoarchitecture following β-cell–specific deletion of cadherins or neural cell adhesion molecule has revealed a requirement for cell adhesion molecules in β-cell–β-cell adhesion during islet formation (99,100). In addition, in vitro evidence (101) supports a role for matrix metalloproteinase enzymes in facilitating endocrine cell migration via extracellular matrix degradation. However, this hypothesis has not been borne out in vivo (102).

During the early postnatal period, the β-cells acquire the ability to regulate insulin secretion in response to glucose (103), requiring the expression of the glucose transporter Glut2 and prohormone convertase PC1/3 to cleave proinsulin to active insulin. The recent characterization of the roles played by the transcription factors MafA and MafB in pancreas development has offered insight into the mechanisms governing β-cell terminal differentiation. MafA, which was initially identified as a β-cell–specific activator of insulin transcription (reviewed in [104]), is exclusively expressed in insulin^{+} cells from e13.5 onward (105). In contrast, MafB is expressed in both insulin^{+} and glucagon^{+} cells by e12.5 but becomes restricted postnatally to α-cells (106). Thus, maturing β-cells undergo a developmental MafB→MafA switch (107). Apparently conflicting with its expression in immature insulin^{+} cells, MafA function is dispensable for β-cell development, most likely because of functional compensation by MafB (108). However, deletion of *MafB* reduces numbers of insulin^{+} and glucagon^{+} cells and delays the development of insulin^{+} cells until the onset of *MafA* expression (109). Loss of *MafB* is associated with downregulation of factors required for β-cell maturation and function such as Pdx1, MafA, Nkx6.1, and Glut2. MafB is thus crucially required for the terminal differentiation of both α- and β-cells by acting as a master activator of hormone gene transcription and key regulators of β-cell differentiation and function.

Serving as a potent example of individual transcription factors exhibiting multiple roles during pancreatic development, conditional deletion of *Isl1* has recently unmasked a crucial requirement for this transcription factor in endocrine cell maturation (110). Ablation of *Isl1* immediately prior to the secondary transition results in a severe reduction in the number of mature endocrine cells prior to the eventual loss of endocrine cell mass contributed by the changes in cell proliferation and survival (110). Paralleling the multiple roles played by Isl1 in pancreatic organogenesis, recent studies have also uncovered

additional, later roles for Ngn3 and its downstream target, NeuroD1, in the acquisition and maintenance of the terminally differentiated, fully functional β-cell phenotype (75,111). Future work will undoubtedly focus on further elucidating the roles of Isl1 and other endocrine differentiation factors in β-cell maintenance and function as the requisite genetic tools become available.

FUTURE PERSPECTIVES

Despite the wealth of knowledge we have amassed to date, many questions in the field of pancreas and β-cell development remain unanswered, as this review has sought to illustrate. It is only relatively recently, for example, that evolving transgenic mouse technology has provided insight into the roles of transcription factors such as Ptf1a and Sox17 in allocating pancreatic fate at the expense of other endodermally derived organs. The inability of any single gene deletion to prevent formation of the early pancreatic anlage hints at the complexity of the transcriptional network governing this process and the involvement of as yet unidentified players, which further work will undoubtedly unmask. How known transcriptional regulators interact with one another and with extraneous signaling pathways is also ripe for future examination, whether in the control of pancreatic specification, growth, or cytodifferentiation.

Outstanding among current questions in the field is the issue of multipotency of individual progenitors at single-cell resolution. This area of investigation would greatly benefit from the development of a culture system in which organ development can be initiated from single cells in vitro, as recently established for cells in the intestinal crypts (112). Another still understudied area is the question of whether or how the physical location of cells in the developing pancreas bestows lineage-restriction upon progenitors. Open questions include whether all ductal cells in the secondary transition progenitor cords function as progenitor cells; whether such progenitors are homogeneously or heterogeneously lineage-restricted; and if the latter, the factor(s) governing progenitor commitment or differentiation into ductal, endocrine, or acinar lineages. Finally, as illustrated in this review, we still know very little about when and how the five different endocrine cell subtypes are specified during development. It is anticipated that much effort will be expended in the future with the goal of answering some of these questions. It is hoped that acquiring more comprehensive insight into the processes governing β-cell neogenesis in vivo will enable the in vitro generation of unlimited quantities of functional insulin-producing cells for the successful management and eventual cure of diabetes.

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