REVIEW

On the origin of the β cell

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The major forms of diabetes are characterized by pancreatic islet β-cell dysfunction and decreased β-cell numbers, raising hope for cell replacement therapy. Although human islet transplantation is a cell-based therapy under clinical investigation for the treatment of type 1 diabetes, the limited availability of human cadaveric islets for transplantation will preclude its widespread therapeutic application. The result has been an intense focus on the development of alternate sources of β cells, such as through the guided differentiation of stem or precursor cell populations or the transdifferentiation of more plentiful mature cell populations. Realizing the potential for cell-based therapies, however, requires a thorough understanding of pancreas development and β-cell formation. Pancreas development is coordinated by a complex interplay of signaling pathways and transcription factors that determine early pancreatic specification as well as the later differentiation of exocrine and endocrine lineages. This review describes the current knowledge of these factors as they relate specifically to the emergence of endocrine β cells from pancreatic endoderm. Current therapeutic efforts to generate insulin-producing β-like cells from embryonic stem cells have already capitalized on recent advances in our understanding of the embryonic signals and transcription factors that dictate lineage specification and will most certainly be further enhanced by a continuing emphasis on the identification of novel factors and regulatory relationships.

Diabetes is rapidly becoming a global epidemic, with a staggering health, societal, and economic impact. Recent estimates by the American Diabetes Association suggest that the lifetime risk of developing diabetes for Americans born in the year 2000 is one in three. Diabetes results when insulin production by the pancreatic islet β cell is unable to meet the metabolic demand of peripheral tissues such as liver, fat, and muscle.

A reduction in β-cell function and mass leads to hyperglycemia (elevated blood sugar) in both type 1 and type 2 diabetes. In type 1 diabetes, autoimmune destruction of the β cell itself severely reduces β-cell mass, resulting in marked hypoinsulinemia and potentially life-threatening ketoacidosis. In contrast, during the progression to type 2 diabetes, impaired β-cell compensation in the setting of insulin resistance (impaired insulin action) eventually leads to β-cell failure and a modest but significant reduction in β-cell mass (Maclean and Ogilvie 1955; Butler et al. 2003; Yoon et al. 2003). More recently, autoimmunity has been detected in a subset of patients with type 2 diabetes, which has led to a revision of the classification to include LADA, latent autoimmune diabetes of adulthood, underscoring the continuum between type 1 and type 2 diabetes, and raising questions as to the role of immunity and inflammation in β-cell dysfunction and death in type 2 diabetes (Syed et al. 2002; Pozzilli and Buzzetti 2007). Conversely, forms of ketosis prone diabetes due to severe β-cell dysfunction but without evidence of autoimmunity are now recognized (Balasubramanyam et al. 2008). Decreased β-cell function also underlies early-onset monogenic forms of diabetes termed MODY (maturity-onset diabetes of the young) resulting from mutations in transcription factors that regulate β-cell development and differentiation. Mutations in MODY genes are also found in patients with common late-onset type 2 diabetes (Fajans et al. 2001). Thus, reduced β-cell number and function underlie the progression of the full spectrum of diabetes, prompting intense effort to develop new sources of insulin-producing β cells for replacement therapies.

Islet transplantation is a cell replacement approach whose widespread applicability as a treatment for diabetes has been curtailed by limited donor islet availability and the toxicity of immunosuppressive drug regimens. The success of islet transplantation was markedly improved by the introduction of the Edmonton protocol, which involved the transplantation of greater numbers of islets as well as a glucocorticoid-free immunosuppressant regimen that reduces but does not eliminate β-cell toxicity (Shapiro et al. 2000; Shapiro et al. 2006). However, the long-term success of this approach is still limited by a gradual reduction in β-cell function, resulting in a return to insulin dependence in a majority of subjects, as well as by the ongoing severe mismatch between the numbers of cadaveric donor islets available and those needed to treat patients with type 1 diabetes.

As a result, basic research has continued to focus on methods of generating β cells from alternative sources, such as transdifferentiation from related cell types or

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differentiation from stem and progenitor cells (Hogan et al. 2008). Although success in achieving stem cell differentiation into insulin expressing cells has been highly variable and even controversial, recent reports indicate highly promising results in the derivation of endoderm-like cells from embryonic stem (ES) cells and subsequent in vitro or in vivo differentiation into insulin-expressing β-like cells (D'Amour et al. 2005, 2006; J. Jiang et al. 2007; W. Jiang et al. 2007; Phillips et al. 2007; Shim et al. 2007; Kroon et al. 2008). This research was directly guided by fundamental advances in our understanding of the extracellular signals and transcription factors that dictate the embryonic development of the pancreas. This review emphasizes recent advances in our knowledge of both extracellular signals/signaling pathways and transcription factors that govern the formation of β cells during pancreas development. Although tremendous progress in delineating the molecular underpinnings of β development has been made, there is still much to be learned regarding the regulatory relationships among signaling pathways and transcription factors and how these processes guide pancreatic specification, endocrine differentiation, and β-cell maturation.

Overview of mouse pancreas development

The definitive endoderm, from which the pancreas arises, begins as a flat sheet of cells that is specified during gastrulation (Fig. 1). Genes required for definitive endoderm formation include *Wnt/β-catenin*, *Nodal*, *GATA4/6*, *FoxA2*, *Sox17*, and *Mix* (Grapin-Botton and Constam 2007; Zorn and Wells 2007). At the beginning of somitogenesis, closure of the gut tube starts as the anterior region of this sheet folds posteriorly, and eventually joins the anteriorly moving posterior fold. Specification of the pancreatic domain within the gut endoderm is mediated by a combination of mesodermally derived signals, including but not limited to TGFβ (transforming growth factor) superfamily members, retinoic acid (RA), and fibroblast growth factors (Fgf) (Grapin-Botton and Constam 2007; Spence and Wells 2007). Pancreatic specification becomes evident around embryonic day 8.5 (E8.5, eight to 10 somites) with the expression of pancreatic duodenal homeobox 1 (Pdx1) in two ventral domains, preceding expression of Pdx1 in the single dorsal domain at E8.5–E8.75. At this time, mesenchyme accumulates around the prospective dorsal and ventral pancreatic anlagen, with one of the two ventral domains eventually disappearing.

Around E9.5, epithelial budding into the surrounding mesenchyme occurs with subsequent branching morphogenesis. Recent evidence indicates that multipotent progenitors that give rise to hormone-expressing endocrine cells, ductal cells, and digestive enzyme-producing acinar cells of the exocrine compartment are located at the tips of the branching network and are marked by Pdx1, Ptf1a, cMyc, and carboxypeptidase A (CPA) (Zhou et al. 2007). Although some endocrine cells, mostly glucagon$^+$, are evident at E9.5 during the primary transition, most of the hormone-expressing cells that will comprise

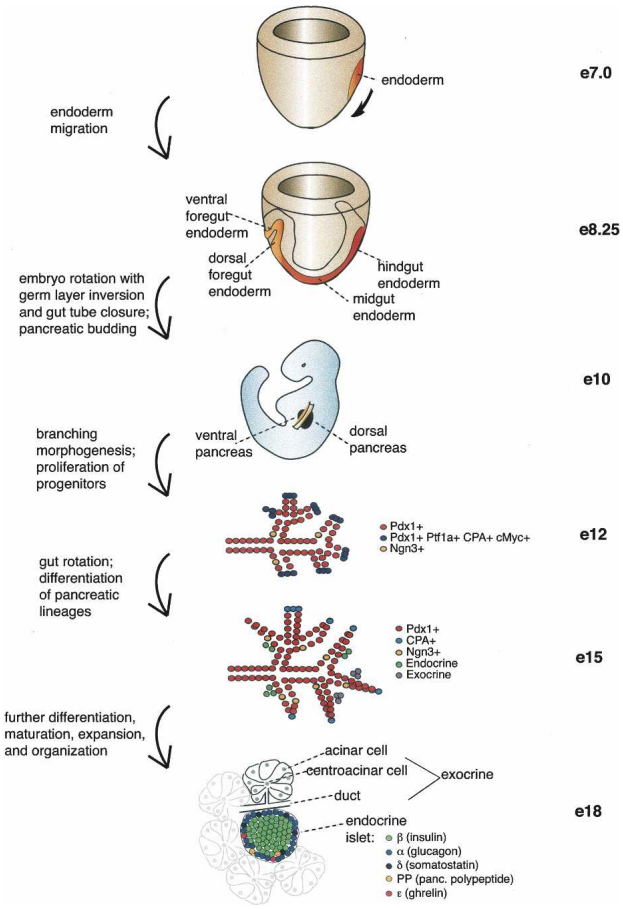

Figure 1. Overview of mouse pancreatic organogenesis. Drawing depicts mouse embryonic development from early primitive streak stage (E7) through endoderm migration and specification, pancreatic budding, branching morphogenesis, and differentiation of pancreatic lineages. Multipotent progenitors that give rise to all pancreatic lineages express *Pdx1*, *Ptf1a*, *CPA*, and *cMyc* in early pancreas development, while exocrine progenitors express *CPA* during mid-pancreatic development. At all stages, endocrine progenitors express *Ngn3*.

the endocrine islets begin to rapidly emerge around E13.5, a period called the secondary transition. Reports on the precise appearance of the different lineages vary, but by E13.5–E14.5 all five hormone-expressing endocrine lineages (α, β, δ, ε, and PP) are detectable. Emergence of amylase-expressing acinar cells commences around this time as well. The development of the ventral bud is generally delayed compared with the dorsal bud, except with regard to the differentiation of the acinar lineage. By this time, the gut tube has rotated, bringing the two buds into proximity. As embryogenesis proceeds, organ growth and differentiation continue by forming digestive enzyme-producing acinar cell clusters that empty into the ductal network leading into the duodenum, interspersed with endocrine cells that organize by delaminating from the epithelium and forming islet clusters (for comprehensive reviews, see Pictet et al. 1972; Slack 1995; Jorgensen et al. 2007).

Signaling pathways

Extracellular factors that modulate signaling pathways are required for distinct aspects of pancreas formation. The early specification of the pancreatic domain within the gut endoderm requires escape from Hedgehog signaling as well as regulation by endothelially derived signals and Fgf. The Fgf, Notch, Wnt, TGFβ superfamily and EGF pathways influence the growth of the early pancreas. TGFβ family members and EGF are also important in lineage specification and differentiation. Little is known about how these pathways cross-talk and how they regulate transcription factor cascades during pancreatic development (Kim and Hebrok 2001). Recent advances in signaling pathway cross-talk and their impingement on the transcriptional hierarchy will be particularly highlighted throughout this review.

Hedgehog signaling

Three members of the Hedgehog family, Sonic (Shh), Indian (Ihh), and Desert (Dhh), are expressed in the developing gut (for a comprehensive review, see Lau et al., 2006). All three proteins have similar binding affinities and function by interacting with the Patched receptor, relieving its repression of the G-protein-coupled receptor Smoothened, thereby initiating a signaling cascade that results in nuclear translocation of Gli transcription factors. Gli1 and Gli3 function as transcriptional activators and repressors, respectively, while both functions have been attributed to Gli2. Shh and Ihh are expressed throughout the early foregut endoderm but are excluded from the pancreatic domain.

Hedgehog signaling inhibits pancreas formation and early Pdx1 expression (Apelqvist et al. 1997; Kim and Melton 1998; Hebrok et al. 2000). Conversely, inhibition of Shh signaling is a critical step for the early specification of both pancreatic buds and for permitting Pdx1 expression. In the chick embryo, evagination of the dorsal bud occurs in response to mesodermal notochord-derived signals, including activin-βB, a TFGβ family ligand, which represses Shh, thereby enabling Pdx1 expression and inducing a pancreatic fate (Kim et al. 1997; Hebrok et al. 1998; Kim and Melton 1998). Consistent with the idea that activin signaling is necessary for inhibition of Shh and pancreatic specification, activin receptor II loss of function mouse mutants have decreased pancreatic size with a concomitant misexpression of Shh and reduced Isl1 expression (Kim et al. 2000). In contrast, ventral pancreas development is initiated when presumptive pancreatic endoderm migrates away from the high Fgf signaling environment of the cardiac mesoderm that up-regulates Shh and evades septum transversum mesoderm-derived bone morphogenetic protein (BMP) signaling, both of which normally specify a liver fate (Deutsch et al. 2001; Rossi et al. 2001).

RA-mediated inhibition of Shh has also been suggested, although its role in mammals is unclear. In *Xenopus* and zebrafish, RA signaling inhibits Shh and induces both pancreas gene expression in dorsal endoderm and endocrine differentiation (Stafford and Prince 2002; Chen et al. 2004; Stafford et al. 2004; Pan et al. 2007). In contrast, the phenotype of mouse embryos deficient for the RA-synthesizing enzyme Raldh2 suggests a Shh-independent pathway mediating RA effects on pancreas development. Raldh2 is expressed in mouse dorsal mesenchyme adjacent to pancreatic progenitors until E12.5, and RA signaling is detected in both mesenchyme and pancreatic epithelium. *Raldh2*-deficient mice do not develop the dorsal pancreatic bud and have fewer mesenchymal cells and decreased expression of the transcription factors, Hb9, Isl1, Pdx1, and Prox1 (Martin et al. 2005; Molotkov et al. 2005). However, this was not associated with ectopic expression of Ihh or Shh in the pancreas. These studies suggest that RA signaling in the mouse might influence early dorsal pancreatic development in a hedgehog-independent manner, by regulating expression of Isl1, Pdx1, or other factors, directly or indirectly.

Hedgehog signaling may also regulate pancreatic size and endocrine mass by its activity later in pancreas development. Expression of Ihh and Patched1 can be detected in the pancreas by E13.5 (Hebrok et al. 2000). Transgenic overexpression of Shh or Ihh under the control of the *Pax4* promoter at mid-development dramatically inhibits formation of epithelial cells, acinar cells, and Neurogenin3 (Ngn3)-expressing endocrine precursors and decreases β-cell proliferation (Kawahira et al. 2005), suggesting that Hh signaling could regulate the number of pancreatic progenitors by modulating survival, proliferation, or differentiation state. Similarly, increased Hh activity in hedgehog inhibitor *Hhip*-null mice leads to defects in pancreas morphogenesis, reductions in pancreatic size, and endocrine cell formation likely in part due to delayed expression of Fgf10 in the mesenchyme, which normally promotes proliferation of $Pdx1^+$ progenitors (Bhushan et al. 2001; Hart et al. 2003; Kawahira et al. 2003; Norgaard et al. 2003). This study provides the first indication that hedgehog signaling influences mesenchymal function.

Endothelial signals

Mesodermally derived aortic endothelial cells are important in dorsal pancreatic specification following initiation of Pdx1 expression. Aortic endothelial cells in close proximity to the dorsal $Pdx1^+$ endoderm induce this region to express the pancreatic transcription factor Ptf1a and to maintain Pdx1 expression (Lammert et al. 2001; Yoshitomi and Zaret 2004). Moreover, the dorsal bud fails to emerge in *Flk-1*$^{-/-}$ embryos, which lack these endothelial cells likely due to the apoptotic loss of the overlying dorsal mesenchyme, thereby revealing a second role for the endothelium (Jacquemin et al. 2006). Even following specification of the dorsal pancreatic bud, interaction with overlying dorsal mesenchyme is necessary for further development, at least in part due to its expression of Fgf10 beginning at 15–20 somites (E9.5), which together with the transcription factor Hepatocyte Nuclear Factor 6 (HNF6), contributes to initial induction

of Pdx1 expression and enhances aorta-induced Ptf1a expression.

Intraislet endothelial cells also play an essential role in endocrine development. Both the developing endoderm and islets express vascular endothelial growth factor (VEGF) and its receptor Flk-1 (Christofori et al. 1995; Dumont et al. 1995; Kuroda et al. 1995; Vasir et al. 2001). In mature islets, β cells synthesize VEGF-A, which may attract endothelial cells, evidenced by the marked reduction in intraislet endothelial cell numbers in mice with β-cell-specific deletion of *VEGF-A* (Nikolova et al. 2006). Conversely, transgenic overexpression of VEGF-A driven by the *Pdx1* promoter induces formation of hypervascular hyperplastic islets (Lammert et al. 2001). Endothelial cells in turn synthesize the basement membrane laminin chains α4 and α5, which in culture studies up-regulate insulin gene expression and β-cell proliferation by interacting with β1-integrin containing laminin receptors on the β cell (Nikolova et al. 2006). The role of the endothelium in β-cell proliferation is further supported by the observation that vascular proliferation is closely associated temporally with the peak of β-cell proliferation in rat pancreatic development (Johansson et al. 2006). Altogether, these studies demonstrate the critical role of the endothelium in both pancreas specification and β-cell differentiation and proliferation.

Fgf signaling

In addition to the role of Fgf2 in initiating Pdx1 expression in the pancreatic primordium, multiple members of the Fgf family have also been described to regulate expansion and differentiation of the pancreatic epithelium. Expression of multiple Fgf ligands and receptors, including alternatively spliced isoforms, has been detected in the early rodent pancreas (Le Bras et al. 1998; Cras-Meneur and Scharfmann 2002; Elghazi et al. 2002; Dichmann et al. 2003; Hart et al. 2003). FgfR1c is detected in both the epithelium and mesenchyme, while FgfR1b, FgfR2b, and FgfR4 are detected exclusively in the epithelium and FgfR2c is present in the mesenchyme. Expression of the ligands Fgf1, Fgf7, Fgf9, Fgf10, Fgf11, and Fgf18 is also detected early in the developing pancreas (Dichmann et al. 2003). The role for Fgf9 is inferred from genetic mouse models with targeted disruption of its receptor, FgfR3, that reveal FgfR3 as a negative regulator of pancreatic epithelial cell expansion (Arnaud-Dabernat et al. 2007). In contrast, the Fgfr2b ligands Fgf1, Fgf7, and Fgf10, have all been shown to induce epithelial cell proliferation and switch the default fate of pancreatic epithelial explants from endocrine differentiation toward an exocrine fate (Miralles et al. 1999; Bhushan et al. 2001; Elghazi et al. 2002; Dichmann et al. 2003; Pulkkinen et al. 2003).

The most well-characterized of the Fgf ligands involved in pancreas development is Fgf10. Fgf10 is expressed by the dorsal and ventral mesenchyme from E9.5 to E12.5. Transgenic overexpression or targeted deletion of *Fgf10* in mice indicates that Fgf10 regulates bud growth and morphogenesis by promoting proliferation of $Pdx1^+$ epithelial pancreatic progenitors after its initial role to induce Pdx1 expression and maintain Ptf1a expression (Bhushan et al. 2001; Hart et al. 2003; Norgaard et al. 2003; Jacquemin et al. 2006). Fgf10 also maintains Notch signaling and Hes1 expression, thus preventing differentiation of progenitors (Hart et al. 2003; Norgaard et al. 2003; Miralles et al. 2006). These studies have begun to clarify the mechanism behind the well-observed phenomenon that mesenchyme is required for pancreatic bud growth and morphogenesis as well appropriate lineage differentiation (Golosow and Grobstein 1962; Wessels and Cohen 1967; Gittes et al. 1996; Miralles et al. 1998).

Notch signaling

In the classic Notch signaling pathway, interaction of the membrane-bound ligands Delta or Serrate with the Notch receptor on the adjacent cell leads to cleavage of the Notch intracellular domain (ICD) (for a comprehensive review of this pathway, see Louvi and Artavanis-Tsakonas 2006). The cleaved ICD translocates to the nucleus and interacts with the transcription factor RPB-Jκ (recombining binding protein suppressor of hairless), thereby activating expression of basic helix–loop–helix (bHLH) proteins of the hairy and enhancer of split family (Hes). Hes transcription factors, in turn, actively repress differentiation factors. Multiple Notch ligands and receptors are expressed in the early rodent pancreas, including Notch1-4, Delta-like 1 (Dll1), Dll3, Jagged 1 (Jag1), Jag2, Serrate 1, Serrate 2, and the Notch signaling target Hes1 (Apelqvist et al. 1999; Jensen et al. 2000b; Lammert et al. 2000). Promoter reporter assays indicate that Hes1 represses expression of both the bHLH factor *Ngn3*, a marker of endocrine precursors, and the cyclin-dependent kinase inhibitor *p57*, thus regulating cell cycle exit (Lee et al. 2001; Georgia et al. 2006). Mice deficient for Notch signaling pathway components such as RBP-Jκ, Dll1, or Hes1, display increased numbers of $Ngn3^+$ cells, premature endocrine differentiation and a hypoplastic pancreas that is likely the result of premature depletion of pancreatic precursor cells (Apelqvist et al. 1999; Jensen et al. 2000b; Fujikura et al. 2006). Similarly, transgenic overexpression of Notch3 ICD, a Notch signaling inhibitor, leads to decreased epithelial branching and decreased bud size, low Ptf1a and Hes1 expression, increased $Ngn3^+$ and $hormone^+$ cells, and decreased numbers of mature acinar cells. Conversely, mice expressing a constitutively active Notch1 (Notch1-ICD) in the Pdx1 domain display reduced $Ngn3^+$ and endocrine cell number as well as impaired acinar differentiation (Hald et al. 2003; Murtaugh et al. 2003). Expressing Notch1-ICD in the $Ngn3^+$ population also prevents differentiation (Murtaugh et al. 2003). HES family members Hes1, Hey1, and Hey2 directly bind Ptf1 and inhibit its activity in promoter reporter assays, suggesting that Notch signaling also regulates exocrine differentiation by regulating Ptf1 activity (Esni et al. 2004; Ghosh and Leach 2006). All together, these studies indicate that Notch signaling in the early developing pancreas is responsible for maintaining the undifferentiated state of

pancreatic progenitors, enabling proper growth and morphogenesis, and suppressing exocrine and endocrine differentiation by inhibiting Ptf1 activity and Ngn3 expression, respectively. Further investigation will be required to assess the specific roles of individual Notch signaling components in pancreas development.

Wnt signaling

Wnts are secreted glycoproteins that bind to Frizzled receptors and LRP5–LRP6 coreceptors in the plasma membrane, thereby recruiting Dishevelled and inhibiting the Axin/APC (Adenomatous Polyposis coli)/GSK-3β (glycogen synthase kinase 3β)/CK1α (casein kinase 1α) complex, leading to stabilization of β-catenin. Upon nuclear translocation, β-catenin interacts with transcription factors such as TCF/LEF to activate gene transcription. In the absence of active Wnt signaling, β-catenin is targeted for proteosomal degradation by CK1α- and GSK-3β-mediated phosphorylation and subsequent ubiquitination. In addition to its role in the canonical Wnt signaling pathway, β-catenin also binds E-cadherin in adherens junctions (for a comprehensive review of this pathway, see Klaus and Birchmeier 2008). Expression of multiple Wnt ligands (1, 2b, 5a,7b, 11, and 16), Frizzled receptors (2, 3, 4, 5, 6, 7, 8, and 9), Wnt coreceptors LRP5 and LRP6, and Wnt inhibitors Fsrp (1–4) has been detected in the developing pancreas, in the mesenchyme, and in the epithelium (Heller et al. 2002).

Multiple genetic mouse models have been derived to elucidate the role of Wnt/β-catenin signaling in vivo, including transgenic mouse models of Wnt or Wnt inhibitor Frz8CRD-igG overexpression, as well as Cre-Lox-mediated deletion of *APC* and stabilization or deletion of *β-catenin* using various *Pdx1-Cre* lines (Heller et al. 2002; Dessimoz et al. 2005; Murtaugh et al. 2005; Papadopoulou and Edlund 2005; Heiser et al. 2006; Strom et al. 2007; Wells et al. 2007). Taken as a whole, these studies suggest that β-catenin mediates overall pancreas growth, by regulating both early proliferation of pancreatic epithelial progenitor cells as well as acinar cell differentiation and proliferation.

Recently, single-nucleotide polymorphisms in the *TCF7L2* locus have been linked to type 2 diabetes in humans and associated with decreased insulin secretion, implying an important role for Wnt/β-catenin signaling in β cells (Cauchi et al. 2007; Salonen et al. 2007; Sladek et al. 2007; Loder et al. 2008). In mice, Wnt3a treatment of cultured β cells or islets stimulates proliferation, and in vivo expression of active β-catenin in β cells postnatally leads to β-cell expansion (Rulifson et al. 2007). Genetic studies manipulating this pathway through conditional ablation or overexpression of a stabilized form of β-catenin, however, have given conflicting phenotypes with regard to the developing endocrine compartment. In agreement with the adult role of Wnt signaling, expression of the Wnt inhibitor Axin in the Pdx1 domain during development decreases β-cell mass (Rulifson et al. 2007). Expression of stabilized β-catenin also increases pancreatic proliferation (Heiser et al. 2006; Rulifson et al. 2007). By contrast, slightly earlier expression of stabilized β-catenin in the epithelium, driven by a different *Pdx1-Cre* recombinase strain, modulates mesenchymal Fgf10 and Hh signaling, reducing Pdx1 expression in pancreatic progenitors and decreasing the number of insulin$^+$ cells (Heiser et al. 2006). Similarly, conditional ablation of *β-catenin* in the Pdx1 domain gave divergent results in three studies, two of which showed no effect on the developing endocrine compartment while the third showed a decrease in endocrine islet cell number (Dessimoz et al. 2005; Murtaugh et al. 2005; Wells et al. 2007). While these discrepancies may be due in part to methodological differences in the quantitative assessment of the endocrine compartment, the precise timing of perturbation of the β-catenin signal also has a profound impact on the phenotypic result, likely reflecting highly dynamic, temporally, and spatially distinct roles for β-catenin in the developing endocrine compartment.

TGF-β signaling

The secreted growth factors in the TGFβ superfamily include TGFβ, Inhibin, Activin, BMP, and growth differentiation factors (GDF). Expression of the TGFβ signaling pathway components has been detected in the pancreatic epithelium and mesenchyme and in mature and developing islets. These include the ligand subunits of TGF-β1-3, activinβB, inhibin-α, BMP2, BMP4, BMP5, BMP6, BMP7, GDF11, ligand antagonists such as follistatin, the receptors TβR-II, ActR-IA, ActR-IB, ActR-IIA, ActR-IIB, BMPR-IA, BMPR-IB, BMPR-II, and intracellular mediators such as Smad2 (Ogawa et al. 1993; Feijen et al. 1994; Furukawa et al. 1995; Verschueren et al. 1995; Crisera et al. 1999; Tremblay et al. 2000; Dichmann et al. 2003).

Activin receptors (ActRII-A and ActRII-B), which may be bound by ligands in multiple TGFβ subclasses, are expressed early in pancreatic epithelium and later in islets (Yamaoka et al. 1998; Shiozaki et al. 1999; Kim et al. 2000; Goto et al. 2007). Mice harboring null or dominant-negative mutations in these receptors as well as the downstream mediator *Smad2,* display decreased endocrine–hormone expression and islet hypoplasia, supporting a role for activin/TGFβ signaling in islet development and differentiation. Mice overexpressing Smad7, a potent inhibitor of TFGβ superfamily signaling, in the Pdx1 domain have β-cell hypoplasia (Smart et al. 2006). Together, these studies underscore the role of TGFβ family signaling in endocrine development, but whether signaling influences formation, survival, or expansion of endocrine progenitors or of differentiated endocrine cells has not been established.

In addition to its previously mentioned role in notochord-mediated specification of the early pancreatic bud and in regulating early branching morphogenesis, activin signaling has other important functions in lineage specification (Ritvos et al. 1995). For example, the ligand Activin B is detected at E12.5 in glucagon$^+$ cells and by E18.5 it is localized to islets (Maldonado et al. 2000). The

inhibitor follistatin is present in the mesenchyme prior to E12.5 and reappears in islets at E18.5. Cultured explant data indicate that follistatin mimics mesenchyme by inhibiting endocrine differentiation and promoting exocrine development (Miralles et al. 1998). Consistent with this, adult mice with a targeted disruption of Follistatin like 3 (FSTL3) exhibit increased islet size (Mukherjee et al. 2007). Thus, activin may be permissive for endocrine differentiation, such that inhibition by follistatin may inhibit endocrine differentiation and redirect it toward an exocrine lineage.

Another ligand member of the TGFβ superfamily, GDF11, regulates development of the exocrine and endocrine compartments (Harmon et al. 2004; Dichmann et al. 2006). *GDF11*$^{-/-}$ mice display increased numbers of Ngn3^{+} endocrine precursors, suggesting an inhibitory role for GDF11 in endocrine development (Harmon et al. 2004). The increase in islet precursor number does not result in a corresponding increase in mature islet cells, implying a distinct role for GDF11 in terminal islet cell differentiation. GDF11 can bind type II activin receptors (ActRII-A, ActRII-B), thereby eliciting Smad2 phosphorylation (Oh et al. 2002). Not surprisingly, *Smad2*$^{+/-}$ mice display similar pancreatic defects to *GDF11*$^{-/-}$ mice, thereby identifying an additional TGFβ family pathway that influences endocrine development. Additionally, the acinar compartment is reduced in *GDF11*$^{-/-}$ mice, attributing an additional function to GDF11 in exocrine development, possibly by influencing progenitor cell fate decision between endocrine and exocrine lineages (Harmon et al. 2004; Dichmann et al. 2006).

Other than their role in the early inductive events leading to pancreas specification, the function of BMP ligands within the developing pancreas is unclear. Culture studies suggest that BMPs 4–6 may promote proliferation of pancreatic epithelial cells and endocrine differentiation (Jiang et al. 2002; Jiang and Harrison 2005; Hua et al. 2006). In contrast, mouse embryos expressing BMP6 under the control of the *Pdx1* promoter display pancreatic agenesis (Dichmann et al. 2003). The source of the BMPs within the pancreas that could mediate these effects in vivo has not been established. In adult animals, studies of β-cell deletion of *BMPR1A* as well as overexpression of its ligand BMP4 indicate that this pathway positively regulates insulin expression and processing as well as glucose-stimulated insulin secretion (Goulley et al. 2007).

In summary, the TGFβ family of ligands regulates multiple processes during pancreas development after pancreatic specification, including both exocrine and endocrine development. Elucidation of the mechanisms by which each of the individual ligands regulates these processes will require further investigation of the signaling pathways and genetic targets affected by specific ligand and receptor isoforms.

Transcription factors

Over the past 15 years, targeted gene disruption in mice has provided enormous insight into the role that transcription factors play during embryonic development of the mouse pancreas. In particular, conditional gene ablation has revealed that many transcription factors have early roles in pancreas development as well as later distinct roles in specific lineage formation, differentiation, and function (Table 1). Most of the known pancreatic transcription factors, with few exceptions, are expressed prior to or near the onset of pancreas development. Further, many of these factors regulate one another through positive feedback loops; as a result, earlier models of the transcriptional hierarchy have evolved into less linear schemes (Fig. 2). As development proceeds, networks regulating specific lineages segregate from one another and are stabilized. In addition to suggesting pathways that can be manipulated to differentiate progenitor cells into β cells, understanding these cross-regulatory networks may also provide insights into how fully differentiated cells might transdifferentiate; e.g., how a mature acinar cell might be reprogrammed to become a fully differentiated insulin-producing β cell.

This review will emphasize transcription factors with recently discovered or newly expanded roles in pancreatic progenitors (Ptf1, Pdx1, Sox9), islet progenitors (HNF1β, Ngn3, and Pax4), lineage specification (Nkx2.2, Nkx6.1) and β-cell differentiation (Maf A/B, HNF4α, Foxa2). Studies elucidating the regulation of these factors by signaling pathways will be highlighted. Due to space constraints and the growing list of transcription factors expressed during pancreas development, this review is necessarily incomplete; however, a concise summary of the transcription factors with roles in pancreas development and human disease is provided in Table 1. For more comprehensive reviews of the entire hierarchy, of the distinct aspects of the hierarchy governing dorsal versus ventral pancreas development and of the embryonic pancreas development of other model organisms, the reader is referred to several excellent recent reviews (Jensen 2004; Servitja and Ferrer 2004; Jorgensen et al. 2007).

Pancreatic progenitors

Ptf1 Ptf1 is a heterooligomeric transcription factor composed of bHLH p48, p64, and p75 subunits that together control exocrine pancreas gene expression, including *elastase 1*(*Ela1*) (Cockell et al. 1989; Roux et al. 1989; Sommer et al. 1991; Krapp et al. 1996; Liu et al. 2001). Ptf1a (p48) is detected throughout the early pancreatic epithelium and later becomes restricted to acinar cells. The complete absence of exocrine cells in *Ptf1a*-null mice led to the initial interpretation that Ptf1a was exclusively required for the formation of the exocrine pancreas (Krapp et al. 1998). This concept was revised after elegant lineage tracing experiments revealed that Ptf1a is expressed in the pancreatic progenitors that give rise to endocrine, ductal, as well as acinar lineages (Kawaguchi et al. 2002). In the absence of Ptf1a, these progenitors adopt an intestinal fate. Thus, Ptf1a is important for both pancreatic specification and the development of all pancreatic lineages. More recent studies in

Table 1. *Transcription factors that regulate pancreatic β-cell development and function*

Factor	Role(s)	Genetic mouse models	Human genetics
Isl1	Required for dorsal mesenchyme; contributes to exocrine and endocrine lineage formation	*Isl1*$^{-/-}$: no dorsal mesenchyme or dorsal bud endocrine cells	Heterozygous: nonsense mutation (Q310X) in Japanese patient with type 2 diabetes
Hlxb9	Role in early dorsal pancreatic epithelial specification and later β-cell development and differentiation	*Hlxb9*$^{-/-}$: dorsal pancreatic agenesis; normal dorsal mesenchyme; reduced islet size and ventral pancreas β-cell number	Curarrino syndrome; sacral agenesis; no reported diabetes or glucose intolerance
Hex	Required for ventral pancreas formation	*Hex*$^{-/-}$: no ventral pancreatic budding and morphogenesis; reduced proliferation leads to failure of presumptive pancreatic endoderm displacement away from hepatic inductive signals of cardiogenic mesoderm	Association of type 2 diabetes susceptibility near *HHEX*
Prox1	Influences exocrine vs. endocrine fate decisions during the secondary transition	*Prox1*$^{-/-}$: pancreatic hypoplasia and dysmorphogenesis; premature cell cycle exit	
HNF1β	Required for specification and differentiation of visceral endoderm; HNF6^{+}/HNF1β^{+} cells possible precursors to Ngn3^{+} endocrine progenitors	*HNF1β*$^{-/-}$: small pancreatic dorsal bud rudiment at E9.5 that regresses by E13.5.	Heterozygous: monogenic diabetes (MODY5); cystic kidney disease
HNF6	Regulates early inductive events, endocrine differentiation and ductal development	*HNF6*$^{-/-}$: hypoplastic pancreas at E10.5; reduction in endocrine cell number and maturation	
Ptf1a	Expressed in pancreatic progenitors; regulates exocrine pancreas gene expression	*Ptf1a*$^{-/-}$: markedly hypoplastic pancreas; endocrine cells present but exocrine cells absent in dorsal pancreatic rudiment; key regulator of cerebellar neurogenesis.	Homozygous: pancreatic and cerebellar agenesis
Pdx1	Expressed in pancreatic progenitors; required for pancreatic growth and development of all pancreatic lineages; imparts epithelial competence to respond to mesenchymally derived growth signals	*Pdx1*$^{-/-}$: pancreatic agenesis; small rudimentary buds with few glucagon^{+} cells. *Pdx1*$^{+/-}$: glucose intolerance, impaired glucose stimulated insulin secretion, age-dependent decrease in β-cell mass, decreased in β-cell survival; impaired islet compensation in response to genetic models of insulin resistance.	Homozygous: congenital pancreatic agenesis heterozygous: monogenic diabetes (MODY4) and type 2 diabetes
Pbx1	Pdx1–Pbx complexes required for pancreatic growth, cell migration, and expansion of all lineages during development	*Pbx1*$^{-/-}$: disordered mesenchyme at E10.5; hypoplastic dorsal and ventral pancreas at E14; poorly branched epithelium; reduced cellular proliferation rates; cell-autonomous epithelial defect; role in mesenchyme; *Pbx1*$^{+/-}$: impaired glucose tolerance, hypoinsulinemia; *Pdx1*$^{+/-}$ *Pbx1*$^{+/-}$: overt diabetes, impaired insulin secretion	
Sox9	Expressed in Pdx1^{+} pancreatic progenitors; maintains Notch signaling in the progenitor pool	*Pdx1-Cre;Sox9*$^{loxP/loxP}$: small dorsal and ventral pancreatic rudiments populated by Sox9^{+} cells that escaped deletion; reduced acinar cell amylase staining; increased apoptosis and decreased proliferation of Pdx1^{+} progenitors at E11.5.	Campomelic dysplasia
Sox4	At E18.5, expressed in endocrine islets and subset of acinar cells.	*Sox4*$^{-/-}$: grossly normal morphology at E12.5; pancreatic explants develop fewer endocrine cells.	
GATA-4	Expressed at E9.5; by E15.5, restricted to acinar cells; persists postnatally	Tetraploid complementation of *GATA-4*$^{-/-}$ ES cells: required in early ventral pancreas formation	Congenital heart defects
GATA-6	Expressed at E9.5; expressed in developing ducts, endocrine precursors and endocrine cells E14.5–E15.5; later restricted to endocrine islets	Tetraploid complementation of *GATA-6*$^{-/-}$ ES cells: required in early ventral pancreas formation	

continued on next page

Table 1. *(continued)*

Factor	Role(s)	Genetic mouse models	Human genetics
Ngn3	Ngn3$^+$ cells are endocrine progenitors	*Ngn3*$^{-/-}$: devoid of intestinal and pancreatic endocrine lineages; abnormal pancreatic acinar morphogenesis	Homozygous: congenital malabsorptive diarrhea, no reported diabetes
NeuroD1	Expressed in all pancreatic endocrine cells including earliest glucagon$^+$ cells at E9.5.	*NeuroD1*$^{-/-}$: hyperglycemic at birth and die perinatally; small disorganized islets; secretion-defective acinar cells	Heterozygous: monogenic diabetes (MODY6)
Insm1	Direct target of Ngn3; may function as a transcriptional repressor	*IA-1*$^{-/-}$: reduced endocrine cell number; accumulate endocrine precursors without hormone expression; impaired differentiation of intestinal endocrine precursors	
Myt1	Expressed in endocrine progenitors cells; appears to be downstream from Nkx6.1	*Ngn3-DN Myt1*: reduction of insulin$^+$ and glucagon$^+$ cells. *Myt1*$^{-/-}$ and pancreas *Cre; Myt*$^{loxP/loxP}$: multihormone expressing cells	
Pax6	Roles in α-cell differentiation and β-cell development	*Pax6*$^{-/-}$: islet disorganization; absence of glucagon$^+$ cells, lack eyes *Pax6*$^{smalleye/smalleye}$: decreased numbers of all endocrine lineages; disorganized islets; reduced hormone gene expression	Homozygous: aniridia
Pax4	Expressed in endocrine progenitors; may function as a transcriptional repressor	*Pax4*$^{-/-}$: marked reduction in β- and ∂-cell number; increased α-cell and ε–cell numbers; *Pax4-Notch1-ICD:* Pax4$^+$ cells switch their fate to ductal cells	
Nkx2.2	First detected at E9.5; by E15.5 restricted to α, β, and PP cells; functions as both transcriptional activator and repressor	*Nkx2.2*$^{-/-}$: lack β-cells and decreased numbers of α and PP cells, hyperglycemic at birth, die perinatally; increased numbers of PC1/3 expressing cells that are negative for other hormones; later identified as ghrelin$^+$ ε endocrine cells	
Nkx6.1	Expressed in pancreatic progenitors and endocrine precursors; progressively restricted to β cells; functions as both transcriptional activator and repressor	*Nkx6.1*$^{-/-}$: reduced islet size; markedly reduced β-cell number after secondary transition.	
MafA	Identified as the β-cell-specific RIPE3b1 factor that activates the insulin promoter. Expression in insulin$^+$ cells begins at E13.5 and remains through adulthood.	*MafA*$^{-/-}$: no apparent defects in pancreatic or β-cell development; become glucose intolerant postnatally, impaired secretogogue-stimulated insulin secretion	
MafB	Expressed in β and α cells at E12.5 and restricted to α cells postnatally	*MafB*$^{-/-}$: reduced numbers of insulin$^+$ and glucagon$^+$ cells during development; delay in the emergence of insulin$^+$ cells until after the onset of MafA expression at E13.5	
Foxa1	First detected at E7.0 in midline	*Foxa1*$^{-/-}$: normal pancreas organogenesis; impaired insulin secretion and decreased proglucagon expression	
Foxa2	Expressed at E6.5 in the anterior primitive streak and node, then in foregut endoderm, notochord, and floor plate, prior to pancreas formation	*Foxa2*$^{-/-}$: die at E11 from multiple defects, *Foxa3-Cre;Foxa2*$^{loxP/loxP}$: impaired α-cell differentiation. β-cell-*Cre; Foxa2*$^{loxP/loxP}$: abnormal fuel stimulated insulin secretion, calcium responses and exocytosis.	
HNF1α	Expressed at E10.5, in endocrine and acinar cells by E15.5, and in adult β cells	*HNF1*α$^{-/-}$: develop hyperglycemia at 2 wk postnatally; defective insulin secretory responses	Heterozygous: monogenic diabetes (MODY3)
HNF4α	Expressed in primitive endoderm, gut endoderm, pancreatic epithelium, and postnatally in endocrine and exocrine cells	*HNF4*α$^{-/-}$: embryonic lethal by E6.5. *RIP-Cre; HNF4*α$^{loxP/loxP}$: impaired glucose tolerance, glucose-stimulated insulin secretion, and calcium responses; impaired β-cell mass expansion during pregnancy	Heterozygous: monogenic diabetes (MODY1)
TCF7L2	Key factor in Wnt signaling; possible role in GLP-1 regulated insulin secretion and β-cell replication; maintenance of small intestine stem cells.	*Tcf7l2*$^{-/-}$: absence of proliferative compartments in crypt regions between intestinal villi; pancreatic phenotype not reported.	First gene consistently involved in type 2 diabetes susceptibility in all major ethnic groups

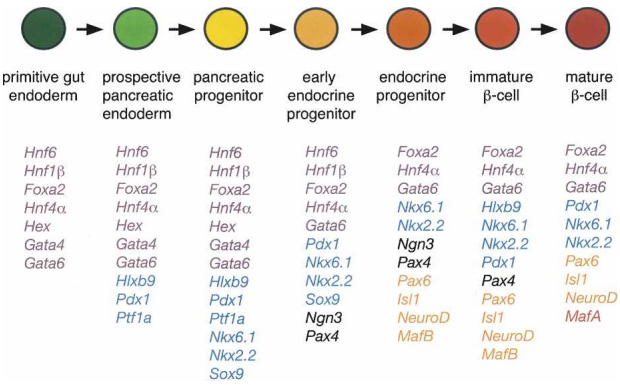

Figure 2. Transcription factor profile during stages of β cell formation from endodermal derivatives. Diagram indicates transcription factors expressed at each stage of differentiation. Factors initially expressed at a particular stage are color-coded as follows: gut endoderm (purple), pancreatic endoderm progenitor (blue), early endocrine progenitor (black), endocrine progenitor (orange), β cell (red).

Ptf1a-null mice indicate that Ptf1a is not absolutely required for formation of Ngn3$^+$ endocrine progenitor cells or endocrine β cells, which are present in the remaining dorsal pancreatic rudiment (Burlison et al. 2008).

A substantial emerging literature indicates significant interactions between Ptf1 and the Notch signaling pathway. Notch signaling components modulate Ptf1 activity and therefore exocrine differentiation, in Notch signaling-dependent and -independent manners. Hes1, Hey1, and Hey2 directly interact with p48/Ptf1a, inhibiting its activity (Esni et al. 2004; Ghosh and Leach 2006).The Notch mediator RBP-J can bind Ptf1a in the Ptf1 complex and enhance Ptf1 transactivation of the Area III enhancer of the *Pdx1* promoter, possibly a Notch signaling-independent effect (Obata et al. 2001; Beres et al. 2006; Miyatsuka et al. 2007; Wiebe et al. 2007). Chromatin immunoprecipitation (ChIP) experiments demonstrate in vivo binding of Ptf1 to this *Pdx1* promoter region at E11.5. Consistent with this observation, deletion of RBP-J in the Ptf1 domain starting at E10.5 leads to decreased Pdx1 expression, epithelial growth and acinar cell differentiation (Fujikura et al. 2007). Masui et al. (2007) suggest that the RBP-J/Ptf1a complex during the secondary transition activates transcription of its paralog RBP-JL, which can replace RBP-J in this complex and mediate activation of acinar digestive enzymes. Taken together, these studies suggest that Ptf1 is important in the specification of early pancreatic progenitors, in the regulation of *Pdx1* expression and in acinar cell differentiation and that these functions occur in part through interaction with RBP proteins and Hes family members.

Pdx1 Pdx1 is a homedomain transcription factor whose gene is mutated in early onset monogenic human diabetes (MODY4) (Stoffers et al. 1997a). Pdx1 is expressed as early as E8.5 in the dorsal and ventral endoderm regions that give rise to pancreatic buds, as well as in the common bile duct, distal stomach, Brunner's glands, and duodenal epithelium (Ohlsson et al. 1993; Guz et al. 1995; Stoffers et al. 1999). Pancreatic expression continues in the pancreatic epithelium and high level expression progressively becomes restricted to pancreatic β cells and a small subpopulation of δ and PP cells (Miller et al. 1994; Guz et al. 1995). Low levels are detected in the nuclei of acinar cells.

Lineage tracing studies reveal that Pdx1 expressing cells, like those expressing Ptf1a, are pancreatic progenitor cells, since all pancreatic lineages develop from these cells (Herrera 2000; Gu et al. 2002). *Pdx1*-null mice display pancreatic agenesis, as well as an abnormal duodenal epithelium and decreased numbers of enteroendocrine cells (Jonsson et al. 1994; Offield et al. 1996). Similarly, human congenital pancreatic agenesis is caused by homozygous deletion of IPF1, the human ortholog of Pdx1 (Stoffers et al. 1997b). Close examination of the pancreatic endoderm in *Pdx1*$^{-/-}$ mice reveals the presence of small rudimentary buds with some glucagon$^+$ cells, indicating that Pdx1 is not essential for initial pancreatic induction but is required for pancreatic growth and development of the full spectrum of pancreatic lineages. In pancreatic explants, growth of *Pdx1*$^{-/-}$ epithelium does not occur in the presence of wild-type mesenchyme, indicating that Pdx1 imparts competence to the epithelium to respond to mesenchymally derived growth signals (Ahlgren et al. 1996). Furthermore, *Pdx1*-null mice do not contain Ngn3$^+$ pancreatic endocrine progenitors after E9.5 (Burlison et al. 2008). The specific requirement for Pdx1 in the formation, survival, or proliferation of endocrine progenitor cells remains to be determined.

A variety of genetic models that permit spatially and temporally regulated expression of Pdx1 reveal additional roles for Pdx1 in endocrine and exocrine development. In vivo transgenic reporter mice reveal specific conserved regions in the *Pdx1* promoter that confer endocrine and β-cell-specific expression (Gannon et al. 2001). Areas I and II impart endocrine expression, while Area III confers β-cell specificity. Mice homozygous for deletion of the Area I, II, and III containing enhancer region recapitulate the pancreatic phenotype of *Pdx1*-null mice, including the absence of a ventral bud, a hypoplastic dorsal bud, and impaired endocrine differentiation (Fujitani et al. 2006). In vivo Tet-off-mediated down-regulation of *Pdx1* throughout embryonic development also recapitulates the *Pdx1*-null phenotye (Holland et al. 2002). In contrast, *Pdx1* down-regulation starting at E12.5–E13.5 leads to a selective defect in acinar cell differentiation and markedly reduced levels of Ptf1a, indicating a later role for Pdx1 in the exocrine pancreas (Hale et al. 2005). Cre-lox-mediated deletion of *Pdx1* in the β cell in late development leads to a defect in β-cell proliferation (Gannon et al. 2008). All together, these studies indicate that Pdx1 plays crucial roles in pancreatic growth and in the differentiation of both exocrine and endocrine lineages.

Postnatally, high level Pdx1 expression becomes restricted to islet β cells and β-cell growth and function become exquisitely sensitive to Pdx1 protein level. *Pdx1* heterozygosity, cre-lox-mediated deletion of *Pdx1* in the β cell, as well as Tet-off-mediated down-regulation of

Pdx1 in adult mice all lead to overt hyperglycemia, highlighting the importance of Pdx1 in mature β-cell differentiation (Ahlgren et al. 1998; Brissova et al. 2002; Holland et al. 2005). In addition to its role in glucose-stimulated insulin secretion, Pdx1 is also important in β-cell survival and in the functional and morphological compensatory response in genetic models of insulin resistance (Brissova et al. 2002, 2005; Johnson et al. 2003; Kulkarni et al. 2004).

The specificity whereby Pdx1 regulates gene transcription is dictated in part by its interaction with other transcription factors and cofactors. One set of partners are the atypical TALE (three-amino-acid loop extension) homeodomain containing factors, including Pbx and Meis. In a heterotrimeric complex with Pdx1 and MEIS, Pbx regulates expression of *Elastase I* in acinar cells and *CK19* in ductal cells (Swift et al. 1998; Liu et al. 2001; Deramaudt et al. 2006). In partnership with Prep1 and Pdx1, Pbx regulates expression of *somatostatin* and with Prep1 alone Pbx silences *glucagon* expression in non-α cells (Goudet et al. 1999; Herzig et al. 2000). Heterodimer formation with Pdx1 requires the FPWMK pentapeptide motif conserved in Pdx 1. To determine the role of Pdx1–Pbx complexes in vivo, Dutta et al. (2001) performed rescue experiments of null *Pdx1* embryos with a wild-type *Pdx1* transgene or a Pbx interaction motif-deficient *Pdx1* mutant. As compared with wild-type, animals with the mutant transgene have a hypoplastic pancreas with small disorganized islets and hormone$^+$ ductal cells. Decreased proliferation of all cell lineages is observed at birth, suggesting that Pdx1–Pbx complexes are required for pancreatic growth, cell migration, and expansion of all lineages during development. The development of diabetes in transheterozygous *Pdx1*$^{+/-}$ *Pbx1*$^{+/-}$ mice provides genetic evidence for the importance of this interaction in β cells (Kim et al. 2002). Remarkably, no β-cell transcriptional targets of the Pdx1–Pbx heterodimer have been reported.

Sox Twelve members of the *Sox* gene family of high-mobility group (HMG) transcription factors are expressed in the pancreas during development (Lioubinski et al. 2003). *Sox4* transcript is detectable in the pancreas by E10.5 and peaks at E12.5 (Wilson et al. 2005). By E18.5, Sox4 is present in endocrine islets and subset of acinar cells (Lioubinski et al. 2003). *Sox4*$^{-/-}$ embryos show grossly normal morphology at E12.5 (Wilson et al. 2005). Pancreatic explants from these mice develop fewer endocrine cells, indicating a specific role for Sox4 in islet development. More recently, Sox4 has been implicated in the regulation of insulin secretion in adult β cells (Goldsworthy et al. 2008).

An exciting recent discovery is the critical role of Sox9 in the maintenance of the pancreatic progenitor pool. Sox9 is first detected in the pancreatic epithelium in Pdx1$^+$ progenitors at E9.0–E9.5, and is later coexpressed with Hes1 cells (Seymour et al. 2007). Consistent with the idea that Sox9 expression marks progenitor cells, the numbers of Sox9$^+$ cells are unchanged in *Ngn3* or *Nkx6.1*-null animals, whereas in transgenic *Pdx1* promoter–*Fgf10* embryos, where ectopic expression of Fgf10 maintains Pdx1$^+$ cells in their progenitor state, Sox9 expression is abnormally maintained at E18.5. Deletion of *Sox9* in the Pdx1 domain results in increased apoptosis and decreased proliferation of Pdx1$^+$ progenitors. An increase in glucagon$^+$ and Isl1$^+$ cells indicates premature differentiation, and decreased percentages of Pdx1$^+$ cells that coexpress Hes1 in these mice suggests that Sox9 is required to maintain Notch signaling in the progenitor pool.

Sox9 also participates in a cross-regulatory network during early pancreas development. Sox9 is coexpressed with Ngn3 at E14.5, and in vitro and cell based reporter studies suggest that Sox9 positively regulates *Ngn3* (Lynn et al. 2007). To determine whether Sox9 regulates Ngn3 via *HNF1*β, *HNF6*, and *Foxa2*, factors that have been implicated in *Ngn3* regulation (Fig. 3), EMSA, ChIP, and siRNA-mediated gene silencing studies were performed, which indicated that Sox9 directly regulates expression of these factors (Lynn et al. 2007). Conversely, knockdown of Foxa2 also decreases *Sox9* levels in ductal cells, suggesting a cross-regulatory relationship between these factors. Moreover, Sox9 binds a site in its own promoter, suggesting autoregulation. The relevance of these coregulatory relationships during the transition from Sox9-mediated maintenance of a progenitor pool to endocrine differentiation warrant further exploration.

Islet progenitors

HNF1β HNF1β plays early roles in visceral endoderm differentiation and overall pancreas development, as well as a specific role in the precursor population for Ngn3-expressing islet progenitor cells. HNF1β is expressed in the primitive endoderm and later in the epithelium of both pancreatic buds, eventually becoming relatively restricted to ductal cells by E14, although expression in endocrine cells continues at very low but detectable levels (Barbacci et al. 1999; Coffinier et al. 1999a,b; Maestro et al. 2003). In *HNF1*β-null embryoid

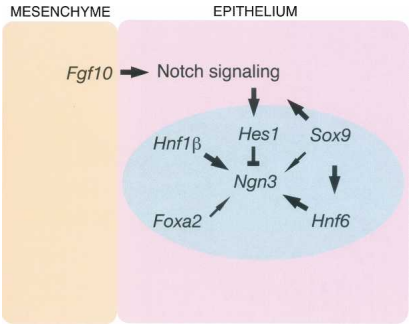

Figure 3. Regulation of endocrine progenitor marker *Ngn3*. Schematic depicts the factors and pathways within the epithelium and the mesenchyme that regulate *Ngn3* expression. Large arrows indicate regulation that has been demonstrated by in vivo mouse models, while small arrows denote regulation supported by cell culture and in vitro evidence.

bodies, activation of *HNF4α*, *HNF1α*, and *Foxa3* is reduced, suggesting direct or indirect regulatory relationships among these factors. Further studies have confirmed that HNF1β likely directly activates *Foxa3* (Hiemisch et al. 1997). Rescue of the visceral endoderm defect using tetraploid aggregation chimeric embryos reveals that HNF1β is also required for pancreatic organogenesis (Haumaitre et al. 2005). Mice lacking *HNF1*β have a small pancreatic dorsal bud rudiment at E9.5 that expresses Pdx1 and Hb9 and reduced HNF6, but no detectable Ptf1a and Ngn3. Altogether these data suggest that HNF1β may be important in directly or indirectly activating pancreatic (*Ptf1a*, *HNF6*) and endocrine (*Ngn3*) gene expression. HNF1β activates *HNF6* in the embryo (Poll et al. 2006). In turn, *HNF6*$^{-/-}$ animals have reduced HNF1β, suggesting that *HNF6* and *HNF1*β regulate one another during early pancreas development; this could be an underlying mechanism that contributes to diabetes development in humans with MODY5, which results from mutations in the *HNF1*β locus (Horikawa et al. 1997; Maestro et al. 2003). Moreover, morphologic studies imply that HNF6$^+$/HNF1β$^+$ cells may be precursors to the Ngn3$^+$ endocrine progenitor population (Maestro et al. 2003).

Ngn3 The bHLH transcription factor Ngn3 is first detected in the embryonic pancreatic epithelium at E9.5, peaks by E15.5, and declines after birth, such that little if any expression is present in the adult (Gradwohl et al. 2000; Gu et al. 2003). Unlike humans where presumptive null mutations result in endocrine cell loss in the gut but not in the pancreas, *Ngn3*-null mice are completely devoid of both intestinal and pancreatic endocrine lineages and pancreatic acinar morphogenesis is abnormal (Gradwohl et al. 2000; Jenny et al. 2002; Lee et al. 2002a; Schonhoff et al. 2004; Bjerknes and Cheng 2006; Wang et al. 2006; Jensen et al. 2007). Conversely, overexpression of Ngn3 in the Pdx1 domain leads to premature endocrine differentiation toward the glucagon lineage at the expense of the exocrine lineage (Apelqvist et al. 1999). These studies, together with lineage tracing and morphological studies, indicate that Ngn3$^+$ cells are endocrine progenitors (Jensen et al. 2000a; Schwitzgebel et al. 2000; Gu et al. 2002).

The mechanisms by which Ngn3$^+$ cells give rise to the different endocrine lineages are poorly understood. However, a recent study using inducible reexpression of *Ngn3* in *Ngn3*-null pancreas at various times throughout development suggests that the context of different developmental time windows dictates lineage allocation. Early Ngn3 induction (E8.7) produces glucagon$^+$ cells, whereas later induction additionally leads to insulin$^+$ and PP$^+$ cells, and even later induction (E14.5) contributes somatostatin$^+$ cells while decreasing glucagon$^+$ cells (Johansson et al. 2007). The specific signals that influence competence of Ngn3$^+$ progenitors to generate specific endocrine lineages at each developmental stage remain to be elucidated.

Various Ngn3 targets have been identified by in vitro, cell-based, and in vivo *Xenopus* studies, including *NeuroD/BETA2*, *IA-1*, *Pax4*, *Nxk2.2*, and *Ngn3* itself (Huang et al. 2000; Smith et al. 2003; Watada et al. 2003; Smith et al. 2004; Mellitzer et al. 2006). More recently, microarrays using E13 and E15 pancreata of wild-type and *Ngn3*-null mice were performed and a new list of potential Ngn3 targets was generated (Petri et al. 2006). Furthermore, gene expression profiles of isolated Ngn3$^+$ cells and their descendants from E13.5–E17.5 embryos will aid in identifying developmentally determined gene regulatory relationships of high potential importance to in vitro differentiation of mature β cells (Gu et al. 2004; White et al. 2008).

Insulinoma-associated antigen 1 (*IA-1*), a zinc finger transcription factor that may function as a transcriptional repressor, has been identified as a direct target of Ngn3 (Goto et al. 1992; Breslin et al. 2002). *IA-1*-null mice have reduced numbers of endocrine cells and they accumulate endocrine precursors without hormone expression (Gierl et al. 2006; Mellitzer et al. 2006).

The zinc finger transcription factor *Myt1*, which encodes for two isoforms, is expressed in Ngn3 expressing endocrine progenitors cells during development and appears to be downstream from Nkx6.1 (Henseleit et al. 2005). In chick, ectopic expression of the Notch inhibitor, Manic Fringe, promotes expression of Ngn3 and cMyt1/3 as well as endocrine differentiation (Xu et al. 2006). Expression of a dominant-negative form of Myt1 in the Ngn3 domain leads to a reduction in the number of insulin$^+$ and glucagon$^+$ cells (Gu et al. 2004). Surprisingly, pancreas-specific and global *Myt1*-null mice have a mild endocrine defect consisting of the presence of multihormone expressing cells (Wang et al. 2007). Compensation by Myt1 paralogs may be masking a critical role for this gene family; targeted disruption of the other paralogs, individually and in combination, may be required to fully appreciate the importance of this gene family in endocrine differentiation.

Pax4 The paired domain transcription factor Pax4 is expressed by E9.5 in the pancreas, becomes restricted to insulin$^+$ cells, and is absent in the adult (Sosa-Pineda et al. 1997; Wang et al. 2004). Homozygous disruption of *Pax4* leads to a marked reduction in the number of β and δ cells, along with increased numbers of α cells. Interestingly, these mice also contain increased number of ghrelin$^+$ ε cells, as does the *Nkx2.2* knockout, suggesting a genetic interaction between these two factors (Prado et al. 2004). Lineage tracing reveals that Pax4$^+$ cells contribute to all endocrine lineages, indicating that, similar to Ngn3, Pax4 may mark endocrine precursors (Greenwood et al. 2007; Wang et al. 2008). Interestingly, expression of the Notch1-ICD in this population switches the fate of Pax4-expressing cells to ductal cells, consistent with Notch signaling preventing commitment to an endocrine fate (Greenwood et al. 2007).

Lineage specification and β*-cell differentiation*

Nkx2.2 Nkx2.2 is an NK type of homeodomain transcription factor whose expression is first detected at E9.5

in the pancreatic epithelium, and by E15.5 becomes restricted to endocrine cells, specifically α, β, and PP cells (Sussel et al. 1998). *Nkx2.2*-null mice lack β cells and are hyperglycemic at birth. Decreased numbers of α and PP cells are observed as well. Interestingly, a group of PC1/3-expressing cells that are negative for other hormones is expanded within the islets. These cells were later identified as ghrelin$^+$ ε endocrine cells, a fifth endocrine cell type that derives from Ngn3$^+$ progenitor cells and normally disappears postnatally (Prado et al. 2004). Whether these cells represent an alternative lineage to β cells derived from a common intermediate progenitor or are themselves β-cell precursors remains to be determined.

Nkx2.2 may function as both a transcriptional activator and transcriptional repressor (Doyle et al. 2007; Doyle and Sussel 2007). The phenotypes of transgenic mice expressing fusion proteins containing the Nkx2.2 homeodomain (HD) fused to either the VP16 activation domain or the Engrailed repressor domain (Engr) suggest that Nkx2.2 functions in part as a repressor for α- and β-cell formation during development and as an activator for β-cell maturation and postnatal function. The *Nkx2.2HD-Engr* transgene completely rescues the reduction in α-cells and partially rescues β-cell number in *Nkx2.2*-null embryos, while *Nkx2.2HD-VP16* has no effect, implying at least in part a repressive role for Nkx2.2 in development. However, expression of β-cell maturation factors MafA and Glut2 is not recovered. Postnatally, the presence of the *Nkx2.2HD-Engr* transgene on a wild-type background decreases endogenous Nkx2.2 levels as well as expression of MafA, Glut-2, and insulin, suggesting a requirement for Nkx2.2 activator function in this context. This example demonstrates the markedly different roles that transcription factors may play at different times during development and highlights the need to confirm transcriptional targets in vivo at the appropriate time point in order to elucidate relevant regulatory relationships.

Nkx6.1 Nkx6.1 is another NK-type homeodomain transcription factor that is first detected in the pancreatic epithelium at E10.5. As development progresses, Nkx6.1 is expressed in Ngn3$^+$ endocrine precursors and becomes restricted to the pancreatic β cell, where it is maintained postnatally (Rudnick et al. 1994; Sander et al. 2000). *Nkx6.1*$^{-/-}$ embryos have normal pancreatic size at E18.5, with reduced islet size due to a marked reduction of the β-cell lineage. The early population of insulin$^+$ cells at E12.5 is unaffected, suggesting that Nkx6.1 is only necessary for the second wave of β-cell differentiation starting at the secondary transition. A role for Nkx6.1 in α-cell development has also been suggested (Henseleit et al. 2005; Nelson et al. 2007).

Similar to Nkx2.2, Nkx6.1 may function as a transcriptional repressor (Mirmira et al. 2000). Cell line-based evidence suggests that Nkx6.1 represses *glucagon* expression and regulates insulin secretion in the mature β cell (Schisler et al. 2005; Gauthier et al. 2007). Nkx6.1 also appears to function as a transcriptional activator by positively regulating its own transcription (Iype et al. 2004). Moreover, there is some evidence suggesting postranscriptional regulation of Nkx6.1 (Watada et al. 2000).

A highly related gene, *Nkx6.2*, is also expressed in the pancreatic epithelium beginning at E10.5 (Nelson et al. 2005). Transgenic expression of Nkx6.2 rescues β-cell differentiation in an *Nkx6.1*-null as effectively as an *Nkx6.1* transgene, suggesting a high degree of functional redundancy between these family members (Nelson et al. 2007). Although *Nkx6.2*-null mice have no obvious pancreatic phenotype, double *Nkx6.1/Nkx6.2*-null mice have a further reduction in the number of insulin$^+$ cells compared with *Nkx6.1*-null animals, and an additional reduction in glucagon$^+$ cells, which is not observed in either *Nkx6.1*- or *Nkx6.2*-null mice (Henseleit et al. 2005; Alanentalo et al. 2006). This result suggests an additional role for Nkx6 factors in α cell development.

Mafs The basic leucine zipper proteins of the large Maf family, MafA, MafB, and c-Maf, are expressed in pancreatic islets. MafA was independently identified by three groups as the β-cell-specific RIPE3b1 factor that binds the well characterized *RIPE3b1* element of the *insulin* promoter (Kataoka et al. 2002; Olbrot et al. 2002; Matsuoka et al. 2003). MafA directly interacts with Pdx1 and BETA2 to synergistically activate *insulin* gene transcription (Aramata et al. 2005; Zhao et al. 2005). Interestingly, all three Mafs are capable of activating the *insulin* gene, although direct regulation of *glucagon* by MafB and c-Maf has been observed as well (Matsuoka et al. 2003; Kataoka et al. 2004; Artner et al. 2006).

Onset of MafA expression occurs at E13.5 in the first insulin producing cells and remains in this population until adulthood (Matsuoka et al. 2004). Expression is markedly reduced in *Nkx6.1*$^{-/-}$ mice positioning MafA downstream from Nkx6.1. Surprisingly, *MafA*-null mice do not exhibit defects in pancreatic or β-cell development but become glucose intolerant postnatally, revealing a role for MafA in glucose-stimulated insulin secretion (Zhang et al. 2005).

In contrast, MafB is expressed in both β and α cells starting at E12.5 and becomes restricted to α cells postnatally (Artner et al. 2006). *MafB*-null mice have reduced numbers of both insulin$^+$ and glucagon$^+$ cells during development, with a delay in the emergence of insulin$^+$ cells until after the onset of MafA expression at E13.5, suggesting a role for MafB in both α- and β-cell differentiation and/or maturation (Artner et al. 2007). Decreased expression of Pdx1, Nkx6.1, and Glut-2 is observed in insulin$^+$ cells after E15.5, suggesting al regulatory role for MafB in β-cell differentiation. Consistent with this, MafB occupies the *MafA*, *Glut2*, and *Nkx6.1* promoters in vivo. Developmental studies suggest that insulin$^+$ cells switch from a MafB$^+$ to a MafA$^+$ state during development, and that this process is associated with up-regulation of Pdx1 expression (Nishimura et al. 2006). This idea is consistent with the recent identification of *MafA* as a direct transcriptional target of Pdx1 (Raum et al. 2006). Another study suggests that *Pdx1* is itself a target of MafA (Samaras et al. 2003), emphasizing the cross-

regulatory nature of the maturing β-cell transcription factor network.

Forkheads The Forkhead family of transcription factors includes Foxa1 (HNF3α), Foxa2 (HNF3β), and Foxa3 (HNF3γ). Foxa2 is the first of these to be expressed in the embryo at E6.5 in the anterior primitive streak and node and becomes expressed in the foregut endoderm, notochord, and floor plate, prior to pancreas formation (Ang et al. 1993; Kaestner et al. 1993, 1994; Monaghan et al. 1993; Sasaki and Hogan 1993, 1994). *Foxa2*-null mice die at E11 from multiple defects (Ang and Rossant 1994; Weinstein et al. 1994); thus, the role of Foxa2 in pancreas development was determined by conditional gene ablation. Interestingly, pancreatic specification and growth are unaltered in *Foxa3-Cre;Foxa2* $^{loxP/loxP}$ mice, but α-cell differentiation is impaired, resulting in a reduced number of α cells and reduced glucagon gene expression (Lee et al. 2005b). In the mature β cell, Foxa2 regulates insulin secretion (Sund et al. 2001; Lantz et al. 2004; Gao et al. 2007). Mice with *Foxa2* deletion in β cells using *RIP-Cre* transgene display increased insulin secretion in response to amino acids consistent with the observed reduction the Foxa2 targets *Sur1*, *Kir6.2*, and *Hadhsc*, which are mutated in various forms of human congenital hyperinsulinism (Sund et al. 2001; Lantz et al. 2004). More recently, derivation of adult Tam-inducible *Pdx1-Cre ERT2; Foxa2* $^{loxP/loxP}$ mice reveals additional novel roles for Foxa2 in β-cell calcium responses and in the formation and docking of insulin containing secretory granules (Gao et al. 2007).

In vitro and cell line studies identify *Pdx1* and *Ngn3* as direct Foxa2 targets (Wu et al. 1997; Gerrish et al. 2000; Marshak et al. 2000; Lee et al. 2001). Interestingly, dysregulation of *Ngn3* has not been observed in the absence of *Foxa2* in vivo, and dysregulation of *Pdx1* by *Foxa2* deletion has only been observed in the differentiated β cell and not during early pancreas development, suggesting that this regulatory relationship depends on developmental context (Lee et al. 2002b,b; Gao et al. 2007).

Foxa1 is first detected at E7.0 in midline endoderm cells and Foxa3 expression begins at E8.5 in the midgut to hindgut region (Ang et al. 1993; Monaghan et al. 1993). Both *Foxa1*- and *Foxa3*-null mice appear to have normal pancreas organogenesis (Kaestner et al. 1998, 1999; Shih et al. 1999). *Foxa1*$^{-/-}$ mice have impaired insulin secretion and decreased *proglucagon* expression, revealing *glucagon* as a novel target of Foxa1 (Kaestner et al. 1999; Shih et al. 1999; Vatamaniuk et al. 2006).

Surprisingly, despite the early expression and important role for forkhead factors in early endoderm, Foxa1, Foxa2 and Foxa3 are not individually essential for overall pancreas development. Similarly, these factors are not individually required for liver development, but simultaneous deletion of *Foxa1* and *Foxa2* in the endoderm leads to absence of hepatic induction, indicating redundancy of these factors during liver development (Lee et al. 2005a) and leaving open the possibility of a critical early role for these factors in pancreas development as well.

HNF4α HNF4α is nuclear hormone receptor that is mutated in MODY1 (Yamagata et al. 1996). It is expressed in the primitive endoderm at E4.5, in the gut endoderm at E8.5, the pancreatic epithelium at E9.5, and postnatally in both endocrine and exocrine cells (Duncan et al. 1994; Nammo et al. 2008). Isoforms result from both alternative splicing and expression driven by alternative promoters, P1 and P2. *HNF4α*-null mice are embryonic lethal by E6.5 (Chen et al. 1994), requiring conditional gene ablation to define the role of this factor during β-cell development. β-cell-specific deletion of *HNF4α* in *RIP-Cre; HNF4α* $^{loxP/loxP}$ adult mice leads to impaired glucose tolerance, glucose-stimulated insulin secretion, and calcium responses (Gupta et al. 2005; Miura et al. 2006). Moreover, β-cell mass expansion during pregnancy is impaired in these animals due to reduced HNF4α dependent-transcription of *ST5* (*suppression of tumorigenicity 5*), a novel regulator of Ras/ERK signaling (Gupta et al. 2007). This animal model has proven useful in the identification of multiple HNF4α targets, including the K-channel subunit *Kir6.2* (Gupta et al. 2005). Interestingly, no change in *HNF1α* mRNA is observed, in contradiction to the previously proposed model of HNF4α-mediated regulation of *HNF1α*. Other studies using islet ChIP/promoter arrays, expression of dominant-negative or wild-type HNF4α in cell lines or ES cell-derived visceral endoderm have identified a multitude of putative transcriptional targets, although the in vivo regulation of many of these are still to be determined (Stoffel and Duncan 1997; Wang et al. 2000; Bartoov-Shifman et al. 2002; Odom et al. 2004; Thomas et al. 2004; Gupta et al. 2005).

Recently, the regulatory relationships among HNF4α and other factors including HNF1α, HNF1β, Foxa2, and HNF6 were examined throughout liver development (Kyrmizi et al. 2006). ChIP assays for HNF1α, HNF1β, Foxa2, HNF4α, HNF6, LRH, C/EBPα, COUP-TFII, GATA6, and RNA pol-II, were performed using liver tissue from E14.5, E18.5, P2 (postnatal day 2), and P45. The results revealed a dynamic recruitment of transcription factors to individual promoters that varies depending on the developmental time point. For instance, occupancy of the *HNF1α* promoter by HNF1β is only detected at E18.5. Moreover, six of these factors appear to regulate one another and downsteam targets as part of a cross-regulatory network. Furthermore, hepatocyte-specific deletion of *HNF4α* in adult liver had little effect on expression of these other hepatic factors, while deletion in the embryo had a marked effect on expression *HNF1α*, *HNF1β*, *Foxa2*, and *HNF6*, among others, indicating a developmental stage-specific requirement of these factors for cross-regulation. This study and others highlight the importance of identifying promoter occupancy and regulatory relationships in the appropriate developmental context.

Directed differentiation of ES cells

These advances in our understanding of the signaling pathways and transcription factors that regulate the em-

bryonic development of the pancreas have informed efforts to derive glucose-responsive β-like cells from human and mouse ES cells (Lumelsky et al. 2001; Hori et al. 2002; Kim et al. 2003; Blyszczuk et al. 2004; Vaca et al. 2008). For a detailed review of directed differentiation of ES cells to pancreatic endocrine cells, see Spence and Wells (2007). The use of the Nodal mimic Activin A to generate an endodermal intermediate has significantly enhanced the efficiency of generating β-like cells in a stepwise fashion by sequential treatment with a variety of factors (Kubo et al. 2004; D'Amour et al. 2005, 2006; J. Jiang et al. 2007; W. Jiang et al. 2007; Phillips et al. 2007; Shim et al. 2007; Kroon et al. 2008). One notable example was published by D'Amour et al. (2006) (Fig. 4). Using an in vitro differentiation protocol, treatment of human ES cells with Activin A and Wnt followed by Activin A alone resulted in the generation of definitive endoderm, marked by expression of Sox17, FoxA2, the mouse Cerberus homolog, CER, and the chemokine receptor, CXCR4. Then, addition of Fgf10 and the hedgehog signaling inhibitor cyclopamine and the subsequent addition of RA led to the generation of primitive gut tube-like cells marked by HNF1β and HNF4α, followed by posterior foregut-like cells that expressed Pdx1, HNF6, and Hb9. Finally, treatment with the Notch signaling inhibitor DAPT and the glucagon-like peptide 1 (GLP-1) receptor agonist Exendin-4 with the subsequent addition of insulin-like growth factor 1 (IGF-1) and hepatocyte growth factor (HGF), sequentially generated pancreatic/endocrine precursors marked by expression of Nkx6.1, Ngn3, Pax4, Nkx2.2, and finally cells expressing endocrine hormones, including insulin. GLP-1 promotes fetal β-cell maturation in culture, and HGF is a β-cell mitogen (Otonkoski and Hayek 1995; Otonkoski et al. 1996; Garcia-Ocana et al. 2000; Movassat et al. 2002), whereas IGF-1 plays postnatal roles in β-cell differentiation and survival (Ueki et al. 2006). Thus, by attempting to recapitulate the signaling cascades governing embryonic development and β-cell differentiation, cells expressing a transcription factor signature resembling that of β cells were generated from ES cells. Although the importance of this contribution cannot be overstressed, the efficiency of this protocol in generating insulin-expressing cells was still low (7%), and the cells themselves were poorly glucose-responsive, indicating incomplete differentiation, further evident by the absence of MafA expression. These cells may more closely resemble "primary transition" endocrine cells.

In a more recent study, the same group modified their protocol to generate pancreatic endoderm-like cells from hES cells (Kroon et al. 2008). The resulting cell mixture, which included Pdx1, Foxa2, HNF6, and Nkx6.1 express-

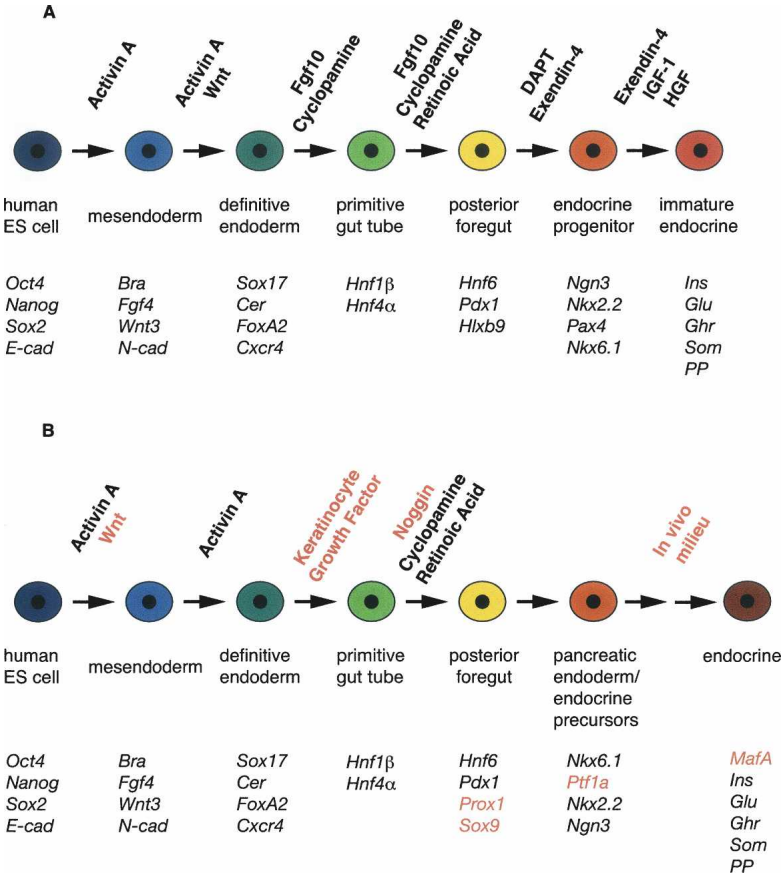

Figure 4. Differentiation of β-like cells from human ES cells. Diagrams depicts protocols used by D'Amour et al. (2006) (*A*) and Kroon et al. (2008) (*B*). Exogenous factors added to induce differentiation into particular lineages are indicated above, and transcription factors expressed at each stage are denoted in italics *below*. Differences between the protocols are indicated in red. Adapted by permission from Macmillan Publishers Ltd.: (*Nature Biotechnology*, http://www.nature.com/nbt) from D'Amour et al. (2006) (© 2006) (*A*) and Kroon et al. (2008) (© 2008) (*B*).

ing pancreatic endoderm-like cells as well as nonpancreatic endoderm, mesoderm, and ectoderm-like cells, was implanted into epididymal fat pads of immunocompromised SCID-Bg mice. Within a 3-mo period, endocrine cells were generated within the implants in vivo, a proportion of which were insulin/Pdx1/Nkx6.1/MafA expressing β-like cells. These cells were glucose-responsive, as measured by serum human insulin and C-peptide levels that approached the levels achieved by a curative dose of human islets transplanted into a parallel set of recipients. The presence of hES-derived pancreatic endoderm implants prevented development of overt hyperglycemia upon streptozotocin treatment, which selectively targets mouse pancreatic β cells. This study clearly demonstrated the potential to generate glucose-responsive β-like cells from hES cells in vivo. However, the occurrence of a teratoma in one graft could impact the clinical utility of this approach. Since the endogenous β cells of the host were not destroyed until after the implants were engrafted, it is also unclear whether the generation of fully functional β-like cells from pancreatic endoderm-like cells would be impaired under the hyperglycemic conditions that might be present in diabetic human recipients.

Concluding remarks

The last decade has been marked by tremendous progress in the elucidation of the basic transcriptional hierarchy governing β-cell development, the identification of critical signaling pathways that impinge on this hierarchy and the application of this fundamental knowledge toward the development of β-cell replacements to complement the inadequate supply of human islets available for transplantation into patients with diabetes. Parallel progress in human islet transplantation to achieve at least short-term insulin independence raises considerable hope for cell-based therapy for diabetes. Significant challenges, however, remain to be overcome before ES- or other progenitor-derived β-cell replacements can be used in a clinical setting. The efficiency of differentiation into a stable mature β-cell phenotype, whether from ES cells, adult progenitor cells, or even differentiated cells of other lineages, must be considerably improved, and the generation of unwanted cell types (including potentially cancerous cells) suppressed. Complementary progress in addressing immunological issues of rejection and modulation of recipient autoimmunity must also continue.

Improvements in both efficiency and fidelity of guided ES cell differentiation will rely on new advances in our understanding of the extracellular signals governing each lineage choice along the path of β-cell development. One notable gap is the identity of the extracellular signals that promoted ES cell differentiation toward a mature β-cell fate in vivo (Kroon et al. 2008). The inability to achieve the same outcome in the culture dish reflects the paucity of knowledge of the factors that normally regulate β-cell maturation during late embryonic and neonatal development in vivo. Similarly, our knowledge of the bidirectional signaling between pancreatic mesenchyme and epithelium during early pancreas development remains limited. Other emerging areas include the critical role of cell–cell contact in limiting cellular plasticity (Minami et al. 2008) and the ability of severe injury and inflammation, perhaps through the destruction of cell–cell interactions and the production of cytokines, to unleash previously unappreciated regenerative pathways in vivo (Xu et al. 2008).

Knowledge of the transcription signature characteristic of each stage of β-cell development has been critical to the advances thus far, but a refinement of the in vivo transitions between pancreatic progenitor and endocrine progenitor and between endocrine progenitor and mature β cell will further extend the efficiency and fidelity of β-cell development ex vivo. Already a distinct transcription factor profile has begun to emerge for immature β cells and similarly for early endocrine progenitors (Fig. 2). Recent advances in high-throughput technology will greatly assist in this endeavor. A thorough comparative study of the gene expression profiles of cells isolated from definitive and visceral endoderm, pancreatic progenitors including single-cell preparations, $Ngn3^{+}$ endocrine progenitors at different stages, and islets, has already enabled the determination of a "genetic identity" of different cell types at different stages of development and the identification of novel regulatory elements (Chiang and Melton 2003; Gu et al. 2004; Sherwood et al. 2007; White et al. 2008).

Many of the regulatory relationships that have been studied in the mature β cell have not been directly examined during embryonic development of the pancreas. Further, until recently, the regulation of targets by specific transcription factors has been determined primarily using in vitro and cell culture systems in the form of electrophoretic mobility shift assays (EMSA) and promoter/reporter studies. With the advent of ChIP to examine transcription factor occupancy in vivo, siRNA gene-silencing techniques and more widespread application of conditional gene ablation, the relevance of specific target gene regulation can now be more easily established in vivo.

Combining high-throughput ChIP and promoter arrays (Odom et al. 2004; Keller et al. 2007) and more recently high-throughput sequencing (Johnson et al. 2007), using material from developing pancreas along with gene expression profiles of specific pancreatic cell types from mice with targeted genetic mutations (Petri et al. 2006; Svensson et al. 2007) will advance our knowledge of the regulatory relationships among transcription factors.

Ultimately, the integration of these new advances in the extracellular signals and a refined transcription factor network governing each stage of β-cell development will redefine our understanding of how insulin-producing β cells are derived from primitive endoderm in vivo. There is considerable hope that the application of this knowledge will optimize efforts to generate β cells from precursor or stem cells ex vivo, thereby providing a potentially limitless source of β cells for transplantation into patients with diabetes as well as providing the first

tractable cell-based model of islet β-cell development, which will find wide application among basic scientists who endeavor to understand this process.

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