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The Endocrine Pancreas: insights into development, differentiation and diabetes

Teresa L. Mastracci,

Department of Genetics and Development, Columbia University

Lori Sussel

Department of Genetics and Development, Columbia University

Teresa L. Mastracci: tm2377@columbia.edu; Lori Sussel: lgs2@columbia.edu

Abstract

In the developing embryo, appropriate patterning of the endoderm fated to become pancreas requires the spatial and temporal coordination of soluble factors secreted by the surrounding tissues. Once pancreatic progenitor cells are specified in the developing gut tube epithelium, epithelial-mesenchymal interactions, as well as a cascade of transcription factors, subsequently delineate three distinct lineages, including endocrine, exocrine and ductal cells. Simultaneous morphological changes, including branching, vascularization, and proximal organ development, also influence the process of specification and differentiation. Decades of research using mouse genetics have uncovered many of the key factors involved in pancreatic cell fate decisions. When pancreas development or islet cell functions go awry, due to mutation in genes important for proper organogenesis and development, the result can lead to a common pancreatic affliction, diabetes mellitus. Current treatments for diabetes are adequate but not curative. Therefore researchers are utilizing the current understanding of normal embryonic pancreas development *in vivo*, to direct embryonic stem cells toward a pancreatic fate with the goal of transplanting these *in vitro* generated “islets” into patients. Mimicking development *in vitro* has proven difficult; however, significant progress has been made and the current differentiation protocols are becoming more efficient. The continued partnership between developmental biologists and stem cell researchers will guarantee that the *in vitro* generation of insulin-producing beta cells is a possible therapeutic option for the treatment of diabetes.

Correspondence to: Teresa L. Mastracci, tm2377@columbia.edu; Lori Sussel, lgs2@columbia.edu.

Further Reading/Resources

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Introduction

The pancreas is made up of exocrine and endocrine compartments. Acinar cells, which form the exocrine tissue, produce digestive enzymes that are secreted through a ductal network and aid in digestion. The endocrine compartment is organized into structures known as the islets of Langerhans, which are composed of five types of hormone-producing cells. In mice, islet organization appears as a central core of insulin-producing beta cells surrounded by alpha (which express glucagon), epsilon (ghrelin), delta (somatostatin), and PP (pancreatic polypeptide) cells.

Disorders that afflict the pancreas can occur in both the exocrine and endocrine glands and research into the development of the pancreas has provided much insight into the aetiology of many of these diseases. The most common form of pancreatic cancer is adenocarcinoma, which is believed to arise from ductal and non-ductal origins (reviewed in [1, 2]); cancers affecting islet cells are far more rare.[3] Pancreatitis is an inflammation in the pancreas caused by the activation of digestive enzymes produced in the acini, which can secondarily affect endocrine pancreas survival and function. Diabetes mellitus is the disease most commonly associated with the endocrine pancreas, with onset attributable to genetic, metabolic, and/or physiological dysfunction. This review synthesizes our current understanding of pancreas development from studies in the embryo, with emphasis on those using mouse models, and discusses how this knowledge is assisting in the pursuit of therapeutics for the treatment of diabetes.

I. PANCREAS DEVELOPMENT

I.1 Patterning the endoderm

Between embryonic day (E) 8.5 and E9, the developing embryo rotates from a lordotic to a fetal position, which internalizes the endoderm layer to form a primitive gut tube. The gut tube epithelium is influenced by secreted factors and signalling pathways that induce regional transcriptional responses to create anterior-posterior patterning along the length of the tube. All endoderm-derived organs, including the oesophagus, lungs, thyroid, thymus, stomach, pancreas, liver and intestine, will eventually develop from this simple epithelial tube.[4] Specifically, the dorsal pancreatic bud evaginates from the region of the dorsal foregut endoderm that lies between the putative stomach and intestine domains. Similarly, a region of the ventral foregut endoderm is patterned to become the ventral pancreatic bud, in proximity to the liver and biliary domain. Inductive and permissive cues from neighbouring regions influence ensuing organ specification. Figure 1 depicts stages during early embryonic development where signalling from adjacent tissues and cells influences the patterning and specification of the pancreatic endoderm. Many of the specific signals that influence early pancreatic patterning will be discussed.

Retinoic acid (RA) signalling from the paraxial mesoderm is required to define the endodermal anterior-posterior position of the pancreas as demonstrated by studies in Zebrafish and Xenopus.[5, 6] In the mouse, RA has been found to have specific effects on the dorsal pancreatic domain. *Aldh1a2* (formerly known as RALDH2), the gene that encodes the enzyme required to synthesize RA, is expressed in the dorsal pancreatic mesenchyme,

and deletion of *Aldh1a2* leads to dorsal pancreatic agenesis.[7] Additionally, *Pdx1* expression is lost in the dorsal, but not ventral, pancreatic domain[7], which demonstrates the importance of RA to the development of the dorsal pancreatic bud. In addition to RA, FGF (fibroblast growth factor) signalling is important for early patterning of the endoderm.[8] In particular, FGF4-mediated signalling directly patterns the gut tube endoderm to promote posterior fates and inhibit anterior fates.[9]

The notochord has been implicated as the source of signals that permit differentiation of the dorsal foregut endoderm fated to become pancreas; removal of the notochord was found to abolish the expression of pancreatic genes in the adjacent endodermal tissue.[10] Further studies revealed the absence of *Shh* (sonic hedgehog) expression in the pancreatic endoderm, whereas adjacent regions of the gut tube rostral or caudal to the pancreatic domain express *Shh*.[11] Moreover, *in vitro* studies determined that FGF2 (fibroblast growth factor 2) and activin, signalling factors expressed in the notochord, repress endodermal *Shh* and induce the pancreatic gene *Pdx1* and pancreatic fates.[12] Therefore the current model synthesized from studies in the mouse and chick, holds that in the dorsal foregut endoderm induction of the pancreatic program specifically requires FGF and activin to inhibit endodermal *Shh*, which permits mesenchymal PTCH1 (patched homolog 1) to initiate pancreatic specification.[12]

The above studies clearly identify factors originating from the notochord (FGF, activin) and paraxial mesoderm (RA, FGF) as necessary to affect dorsal pancreatic patterning. The factors influencing ventral foregut endoderm emanate from the cardiac mesoderm (source of FGFs) and the septum transversum mesenchyme (source of BMPs), which are positioned in proximity to the liver and ventral pancreatic domains. This was determined, in part, using *ex vivo* recombination studies from late gastrulation-stage embryos; the mesoderm and ectoderm were identified to differentially induce endodermal genes, providing evidence that the endoderm receives instructions from adjacent germ layers.[8] In addition, soluble factors expressed in the mesoderm and primitive streak, create regional identity in areas of the endoderm, thus establishing organ domains.[8] Without these initial instructions, ventral patterning does not proceed.

Subsequent work from Zaret and colleagues[13] determined the importance of BMP and the timing of ventral specification. Using three to four somite (3-4S) stage embryos and half-embryo cultures it was determined that BMP signalling specifically influenced the expression of hepatic-specific genes. BMP signalling had the opposite effect on the pancreatic program, whereby *Pdx1* expression was induced by noggin (NOG), an inhibitor of BMP signalling, and inhibited by BMP4; an effect that was reversed in the 5-6S stage embryos.[13] In addition, conditional ablation of *Smad4* at the 5-6S stage (using *Foxa3-Cre*) showed defective ventral pancreatic development[13], whereas ablation at the 7-8S stage (using *Pdx1-Cre*) produced no pancreatic phenotype.[14] Ultimately, it was determined that BMP signalling in the ventral foregut is necessary to induce *Pdx1* expression and the pancreatic program. These studies demonstrate that ventral foregut patterning signals are progressive and dynamic, with opposing effects on the hepatic and pancreatic programs.

I.2 Pancreatic Patterning and Specification

Induction of the ventral pancreatic endoderm occurs one day after that of the dorsal pancreatic endoderm; however in both cases, signals from the mesoderm lead to patterning of the epithelium and evagination of the pancreatic buds. The subsequent activation of a complex network of transcription factors (TFs) in the pancreatic progenitor cells is critical for downstream developmental events. Using mouse models, many TFs have been identified as both necessary and sufficient for pancreas development.

First and foremost, *Pdx1* (pancreatic and duodenal homeobox 1; formerly known as *Ipf1*, *Stf1*) marks the pancreatic progenitor cells. Loss of *Pdx1* results in pancreatic agenesis [15–17], and mutation leads to maturity onset diabetes of the young (MODY4)[18] or permanent neonatal diabetes [19]. Expression of *Pdx1* is induced in foregut endoderm cells in a broad domain that encompasses the dorsal and ventral pancreas, the duodenum, and a portion of the stomach.[20] Subsequent coexpression of *Pdx1* and the basic helix-loop-helix TF *Ptf1a* (pancreas-specific transcription factor 1a; formerly known as *p48*) defines pancreatic progenitor cells and allows for the pancreatic program to proceed.[21] As development progresses, *Pdx1* expression becomes down-regulated in the cells that form the endocrine progenitor population, and by late gestation is selectively maintained at high levels in the beta cells and lower levels in the acinar cells.[22, 23] Recent studies have demonstrated that these dynamic changes in *Pdx1* expression levels are necessary for proper endocrine differentiation.[24] Moreover, Wright and colleagues have recently demonstrated that altering the level of *Pdx1* expression in the NEUROG3+ (neurogenin3; formerly known as *Ngn3*) progenitor cells can alter alpha and beta cell ratios both in the embryo and the adult pancreas.[25] As with many TFs, timing, location and level of expression can result in different effects on differentiation and disease.

The expression domain of *Ptf1a* early in pancreas development is restricted to multipotent progenitor cells in the developing pancreatic epithelium [20, 26], which gives rise to the endocrine, exocrine and ductal lineages. *Ptf1a* was originally thought to be an exocrine-specific gene[27]; however, null mutations of *Ptf1a* results in pancreatic agenesis, with only a small remaining dorsal pancreatic rudiment.[26, 28] Interestingly, descendants of *Ptf1a*-expressing cells were observed in the duodenum of the null and found to express pancreatic markers. Moreover, when *Pdx1* is expressed under the control of *Ptf1a* regulatory sequences in *Pdx1* null embryos, the pancreatic agenesis phenotype is rescued.[26] Therefore both *Pdx1* and *Ptf1a* are important for early pancreas development; however, specification of the pancreatic domain proceeds in the absence of either or both of these factors.[26, 28] This is depicted in Figure 2, which shows glucagon-expressing cells in the dorsal pancreatic primordium that forms in the absence of either *Pdx1* or *Ptf1a*.

The FOXA genes are expressed in the dorsal and ventral endoderm. Similar to *Pdx1* and *Ptf1a*, the pancreas-specific simultaneous deletion of *Foxa1* (forkhead box A1) and *Foxa2* (forkhead box A2) (in $Foxa1^{flox/flox};Foxa2^{flox/flox};Pdx1\text{-}cre$ embryos) leads to pancreatic hypoplasia.[29] Additionally, FOXA1 and FOXA2 regulate *Pdx1* expression through occupancy of a distal *Pdx1* enhancer, and this regulation of *Pdx1* controls pancreatic anlagen expansion and differentiation.

In addition to its broader role in general endoderm specification[30], another gene identified as important in pancreatic specification is the SRY-related HMG-box TF, *Sox17*. The Wells group[31] showed that the ventral pancreas and extrahepatobiliary system are derived from a common progenitor cell that coexpresses *Pdx1* and *Sox17* at E8.5. By E10.5 the *Sox17*+ biliary cells and the *Pdx1*+ ventral pancreatic cells occupy separate domains. When *Sox17* is deleted from the ventral foregut at E8.5 (using *Foxa3-cre*), biliary structures are lost and ectopic pancreas tissue is found in the liver and common duct. Overexpression of *Sox17* in the *Pdx1*+ domain resulted in the opposite phenotype, with loss of the ventral pancreas and ectopic biliary tissue in the pancreatic domain. As *Hes1* is also expressed in the biliary domain [32], further analysis of *Hes1* null embryos revealed an expanded *Sox17* expression domain with scattered *Sox17*+ cells in the dorsal pancreas. The previously reported phenotype of gall bladder agenesis and ectopic pancreatic tissue in the common duct of *Hes1* null [32] embryos supports observations by the Wells group that functionally links HES1 and SOX17 in controlling biliary development. Figure 3 depicts sagittal sections from an E10.5 wild-type embryo where the dorsal and ventral pancreatic domains marked by PDX1 are separate from the SOX17+ biliary domain.

Sox9 (SRY-box containing gene 9) expression overlaps with *Pdx1* in the early pancreatic buds and is also a marker of pancreatic progenitor cells. By E15.5, *Sox9* becomes restricted to a subset of *Pdx1*+ cells in the epithelial cords that are also *Hes1*+ and mitotically active.[33] Depletion of the progenitor cell pool in the pancreas-specific deletion of *Sox9* (in $Sox9^{flox/flox}$;*Pdx1-cre* embryos) leads to pancreatic hypoplasia.[33] Subsequent lineage tracing revealed that *Sox9*+ cells located in the epithelial cords give rise to both endocrine and exocrine cells.[34] Furthermore, mice hypomorphic for *Sox9* show reduced endocrine cell mass, but overall organ size remains unchanged, which suggests that SOX9 is involved in endocrine cell fate determination.[34] In two studies that lineage trace *Sox9*-expressing cells in the wild-type and injured states, it was determined that, while *Sox9* was expressed continuously throughout the pancreatic and biliary ductal epithelium and *Sox9*+ cells have the capacity to give rise to all cell types in the embryonic pancreas[35], *Sox9*+ cells do not give rise to endocrine cells in normal or injured postnatal/adult pancreas.[36]

As evidenced by the studies outlined in this section, signals originating from adjacent tissues must intersect on the primitive gut tube in order for pancreatic organ formation to begin. Subsequently, a large number of TFs are activated to influence the patterning and specification of this early pancreatic domain, as well as the later decision of cells to maintain the pancreatic program (studies summarized in Table 1). Importantly, it has become increasingly clear that a unique combination of endoderm-specific and pancreatic-specific TFs must interact to specify the different pancreatic cell fates and to direct islet cell lineage decisions.

I.3 Morphological development and cellular interactions in pancreas development

Morphological changes in the pancreas and the presence of neighbouring non-pancreatic cells have a profound affect on development and differentiation. In particular, many genes expressed in the pancreatic mesenchyme have been identified to influence proper pancreas formation. *Fgf10* (fibroblast growth factor 10) is expressed in the mesenchyme surrounding

the early pancreatic buds. Pancreatic specification is normal in *Fgf10* null mice; however, subsequent branching morphogenesis, pancreatic growth, and differentiation do not occur resulting in severe pancreatic hypoplasia.[37] While the pancreatic buds form and *Pdx1*+ progenitor cells were identified in the epithelium at E9.5, maintenance of *Pdx1* expression was lost by E10.5 in the dorsal pancreas and reduced in the ventral pancreas; without FGF10 signalling in the mesenchyme *Pdx1*+ progenitor cells were not maintained in the epithelium.

Isl1 (ISL1 transcription factor, LIM/homeodomain; formerly known as islet1) is expressed in the mesenchymal cells that surround the dorsal pancreatic bud. During development and in the adult pancreas, *Isl1* is also expressed in postmitotic islet cells. Deletion of *Isl1* leads to an arrest in embryonic development around E9.5, following the initial pancreatic budding.[38] Interestingly, in this mutant the mesenchyme surrounding the dorsal bud does not form. Using explant culture of embryos null for *Isl1*, it was demonstrated that all differentiated islet cells are lost, and exocrine differentiation is absent in the dorsal but not ventral bud.[38] Therefore ISL1 is required for two distinct stages of pancreas development including the development of the mesenchyme, which influences pancreas specification, as well as differentiation of both endocrine and exocrine cells.

Signals from the vasculature can also affect pancreas organogenesis. Yoshitomi and Zaret [39] describe how emergence of the dorsal bud and expression of *Pdx1* require endothelial cell interactions or the presence of the aorta. *Ptf1a* expression is also induced in the dorsal pancreatic endoderm by aortic endothelial cells; however later in development, endogenous pancreatic endothelial cells localize to the trunk epithelium where they function to limit acinar cell differentiation.[40] Endothelial signalling does not appear to be sufficient for inducing endocrine differentiation but can enhance insulin expression and beta cell proliferation in response to external stimuli (reviewed in [41]).

A study by Villasenor et al. [42] highlights branching morphogenesis as another determinant in pancreatic development and differentiation. The authors performed an extensive anatomical characterization of branching and epithelial cell dynamics in the embryonic pancreas. Subsequently, it was demonstrated that EPH (eph-related receptor tyrosine kinase) signalling is required to support proper branching morphogenesis. *Ephb2*/*Ephb3* compound mutants display reduced pancreas size and thickness, and many wildtype branching features were not observed; later stage embryos have reduced exocrine mass, and defects in branch length and pancreas size were observed in the adult. Concomitantly, mesenchymal gene expression was altered; however, most striking was the change in epithelial dynamics. Defective EPH signalling lead to disorganized rosette structures, early lumen collapse, and an overall delay in tubulogenesis and remodelling.[42] Given that the ductal epithelium houses the endocrine progenitor cells, the effect of altered branching may indirectly affect the differentiation of other pancreatic cell lineages.

Late in development, islet cell aggregation is another morphological feature that, when defective, can influence proper pancreatic function. The overexpression of dominant negative E-cadherin (cadherin1, *Cdh1*) in beta cells resulted in perturbed cell clustering at E13.5, and abrogation of the coalescing of endocrine cells into islets at E17.5.[43] Further studies identified that RAC1 (RAS-related C3 botulinum substrate 1) signalling

can modulate CDH1 mediated cell adhesion thereby affecting islet cell migration.[44] Clearly, the expression of factors within and surrounding the progenitor cells has a critical affect on pancreatic patterning as evidenced by the multiple models of pancreatic agenesis and hypoplasia; however, changes to the anatomical morphology of the organ and the influence of intercalated endothelial cells also impacts organ development. Additional reviews of signalling pathways as well as cellular interactions that affect pancreas development can be found in the "Further Reading/Resources" section.

I.4 Endocrine Differentiation

The endocrine versus exocrine lineage decision can only occur early in development when the progenitors are multipotent.[45] Notch signalling participates in this lineage decision, although it also plays complex roles in other aspects of pancreas development and function (see additional review [46]). Notch signalling in pancreatic progenitor cells activates the Notch target, HES1, which directly represses *Neurog3* expression to promote the exocrine cell lineage.[47] Consistently, deletion of *Dll1* (delta-like gene 1) or *Rbpj* (recombination signal binding protein for immunoglobulin kappa J region), or overexpression of *Neurog3*, results in accelerated endocrine differentiation.[48] However, the misexpression of activated Notch in pancreatic progenitor (*Pdx1*+) cells blocks the differentiation of both the exocrine and endocrine lineages, and instead promotes the maintenance of progenitor cells.[49] This suggests there are additional complexities to Notch-regulated lineage decisions. Clearly the development and differentiation of the exocrine lineage is intimately related to that of the endocrine lineage; however, the process of exocrine differentiation is a complex area of study, in its own right, which has been recently documented in a comprehensive review and will not be addressed further here.[50]

The specification and differentiation of the five endocrine cell types begins with the onset of pancreas development. A subset of hormone-expressing cells can be found as early as E9.5; however, these early "first wave" endocrine cells have not been well characterized due to the lack of definitive markers. Most endocrine cells are specified during the *secondary transition*, the stage of pancreas development between E12.5 and E15.5.[51] During this stage, key TFs necessary for endocrine specification and maturation are upregulated, resulting in the appearance of hormone-expressing cells that differentiate from the endocrine progenitors and delaminate from the ductal epithelial cords.[52]

Cells expressing NEUROG3 represent the endocrine progenitor population. Gradwohl et al.[53] established this fact with the generation of the *Neurog3* null mouse, which lacks all hormone-expressing endocrine cells; further confirmation was provided by lineage tracing experiments.[54, 55] Interestingly, using a transgenic mouse model, temporal induction of *Neurog3* influenced the proportional distribution of differentiated endocrine cell populations.[56] Additionally, the level of expression of *Neurog3* was demonstrated to be important for the endocrine versus exocrine decision in pancreatic progenitor cells[57], as well as for the delamination of endocrine cells from the epithelium.[58] These studies demonstrate that modulating temporal, spatial, and level of expression of a TF can affect specification and differentiation. In this section, we will highlight additional TFs important in the fate determination of each endocrine cell lineage.

I.4a The insulin-expressing beta cell—The most highly studied endocrine cell is the insulin-producing beta cell largely because of the implications for the treatment of diabetes. Interestingly, a number of TFs expressed in early pancreatic progenitor cells are also found to function in the beta cell. In particular, mice deficient for *Pdx1* (heterozygous deletion) are glucose intolerant.[59] Moreover, deletion of *Pdx1* specifically in the beta cell results in beta cell dysfunction.[60] While expression studies clearly identify PDX1 as an early pancreas progenitor factor, these studies discern additional functions for PDX1 in the proper functioning of mature beta cells. Furthermore, transcriptional activation of several beta cell genes, including insulin (*Ins1*) [61] and islet amyloid polypeptide (*Iapp*) [62], occurs as a result of direct regulation by PDX1.

Similar to *Pdx1*, the homeobox TF gene *Nkx6-1* (NK6 homeobox 1) is expressed throughout the epithelium early in pancreas development[63]; however, after the secondary transition it becomes restricted to beta cells. Deletion of *Nkx6-1* results in the loss of beta cell precursors.[64] Beta cells were restored to the *Nkx6-1* null pancreas by transgenic expression of either *Nkx6-1* or *Nkx6-2* from the *Pdx1* promoter; however, beta cells were not rescued with transgenic expression of *Nkx6-1* from the *Neurog3* promoter, which places *Nkx6-1* upstream of *Neurog3* in beta cell differentiation.[65] Interestingly, although the related family member, *Nkx6-2* (NK6 homeobox 2), is also expressed in a similar domain to *Pdx1* in early pancreatic development[63], the phenotype of the *Nkx6-2* null mouse showed that this TF is dispensable for normal endocrine development.[66] However, simultaneous deletion of *Nkx6-1* and *Nkx6-2* demonstrated functional redundancy between the two family members in both alpha and beta cell development, as the number of alpha and beta cells was reduced compared with the single knockout of *Nkx6-1*.[66]

Just as the function of a TF can change over the course of pancreatic development, many TFs have been identified to be necessary for the development and differentiation of the beta cell lineage while not expressed exclusively therein. Mice with a deletion of *Nkx2-2* (NK2 transcription factor related, locus 2) die from severe hyperglycemia due to the complete absence of beta cells.[67] The pancreas of this null mouse is also deficient in alpha cells and a subset of PP cells, with a concomitant increase in ghrelin-expressing cells.[68]

Another factor expressed in, but not exclusive to, the beta cell is the basic helix-loop-helix (bHLH) TF neurogenic differentiation 1 (NEUROD1, formerly known as BETA2). During embryonic development, *Neurod1* expression can be detected in all endocrine cell types, except for delta cells.[69] Mice homozygous for the deletion of *Neurod1* also die perinatally from diabetes, due to beta cell apoptosis.[70] More recently, Lee and colleagues discovered an additional function for *Neurod1* in the postnatal maturation of beta cells.[71] The deletion of *Neurod1* in all insulin-expressing cells (using both *RIP-cre* and *Pdx1-cre^{ER}*) resulted in the arrest of postnatal beta cell maturation, characterized by the expression of increased glycolytic genes and *Ldha* (lactate dehydrogenase A), and lead to severe glucose intolerance and impaired insulin secretion.[71]

Additional TFs important in beta cell development include the PAX and MAF families of genes. The absence of *Pax4* (paired box gene 4) leads to loss of beta cells [72], whereas deletion of the related family member, *Pax6* (paired box gene 6), affects predominately the

alpha and beta cell lineage, in addition to other endocrine cell types.[73, 74] Interestingly, the *Pax4/Pax6* double mutant lacks all mature endocrine cells.[75] The MAF family of basic leucine zipper TFs behaves similarly in their individual and overlapping affects on alpha and beta cells. *Mafb* (v-maf musculoaponeurotic fibrosarcoma oncogene family, protein B) null mice die at birth, and the pancreatic phenotype shows reduced alpha and beta cells despite an unchanged endocrine cell number.[76] *Mafa* (v-maf musculoaponeurotic fibrosarcoma oncogene family, protein A) null mice are viable but develop glucose intolerance and diabetes due to beta cell dysfunction.[77] Moreover, the deletion of *Mafa* lead to reduced expression of the beta cell genes *Ins1*, *Ins2*, *Neurod1*, and *Glut2*. Zhao and colleagues [78] also demonstrated that MAFA controls the level of insulin gene expression, together with *Pdx1* and *Neurod1*. Interestingly, in the developing and postnatal mouse pancreas *Mafa* expression is exclusive to the beta cell and can be used as a marker of the mature insulin-producing cells.[79, 80]

Finally, it is interesting to note that when the *Ins1* and *Ins2* genes were directly disrupted, beta cell differentiation was not affected, rather these double mutant mice showed insulin deficiency and subsequent perinatal death from diabetes [81]. Upon further investigation, enlarged islets were observed in the *Ins1/Ins2* mutant pancreata, pointing to insulin as a negative regulator of growth.[82]

I.4b The glucagon-expressing alpha cell—Similar to TFs important for beta cell development, a number of TFs have been described as markers of the glucagon-expressing cells because of their involvement in alpha cell development, despite their lack of exclusive expression.

Mice carrying a deletion of *Arx* (aristaless-related homeobox) display perinatal hypoglycemia and die shortly after birth.[83] The pancreas of the *Arx* null mouse is deficient for alpha cells after E12.5, thereby defining *Arx* as necessary for alpha cell development. However, the *Arx* mutant also shows an increase in insulin- and somatostatin-expressing cells.[83] Further study of the function of *Arx* in the pancreas uncovered that when misexpressed in *Pdx1*- or *Pax6*-expressing cells dramatic postnatal hyperglycemia and death resulted, due to a loss of beta and delta cells, and an increase in alpha and PP cells.[84] The misexpression of *Arx* in the beta cell also induced an increase in alpha and PP cell numbers, which when combined with lineage tracing determined that beta cells were being converted to alpha or PP cells.[84] Compound mutants have also identified the importance of *Arx* function in the developing pancreas. Whereas the loss of both *Arx* and *Pax4* resulted in loss of alpha and beta cells, with a concomitant increase in somatostatin-expressing cells [85], the combined loss of *Arx* and *Nkx2-2* most significantly restored the PP cell population lost in the *Nkx2-2* single mutant mouse [86].

POU3F4 (POU domain, class 3, transcription factor 4; formally known as *Brn4*) is a TF expressed in glucagon-expressing cells as early as E9.5.[20] BRN4 facilitates the transcriptional activation of the glucagon gene; however, deletion of *Brn4* does not disrupt alpha cell development.[87, 88] While not exclusive, *Irx1* (iroquois related homeobox 1) and *Irx2* (iroquois related homeobox 2) are also expressed in alpha cells.[89] Interestingly, mutant mice with a deletion of *Irx2* are phenotypically normal[90]; *Irx1* null pancreata have not

yet been investigated. In contrast, *Pax6* is expressed in the early pancreatic epithelium and in differentiated endocrine cells not exclusively alpha cells [20]; however, deletion of *Pax6* results in a loss of predominantly alpha cells, in addition to other endocrine cell types.[74]

While under appreciated given its importance to beta cell differentiation, *Neurod1* is also expressed in the glucagon-expressing cells.[69, 70] In addition to the beta cell phenotype described in the *Neurod1* null, alpha cell deficiency was also observed late in development.[70] Moreover, an epistatic relationship was identified between *Neurod1* and *Nkx2-2*, whereby simultaneous loss of both TFs restores the alpha cells lost in the *Nkx2-2* null.[91] Clearly, these studies highlight the complexity of the TF network in endocrine cell fate decisions.

Similar to the deletion of the insulin genes, Hayashi et al.,[92] deleted the glucagon (*Gcg*) gene to determine the effect on alpha cells and general pancreatic function. These mice display alpha cell hyperplasia, but are viable and normoglycemic, demonstrating that glucagon is dispensable for survival.

Interestingly, in nearly all models where pancreatic development was severely compromised, a population of glucagon-expressing alpha cells remained. In addition, the mutant mouse models for specific genes identified as important in alpha cell development, including *Nkx2-2* and *Arx*, also do not display a complete loss of this endocrine cell population. These “early” endocrine cells have also been termed “first wave” endocrine cells, and in many cases exclusively express the hormone glucagon. The origin of these cells is as of yet unknown; however, as depicted by the images in Figure 4, first wave glucagon-expressing cells exist despite the deletion of factors necessary for pancreas and/or endocrine cell development, including *Pdx1*, *Ptf1a* and *Neurog3*. While research has uncovered many of the genes and signals necessary and sufficient for pancreatic specification and endocrine cell differentiation, unexplained observations like the “early” glucagon-expressing cell population remind us that there is still much to uncover.

I.4c The ghrelin-expressing epsilon cell—The ghrelin-expressing endocrine cell lineage was first identified in human pancreatic islets by Sundler and colleagues.[93] While rare compared with other endocrine populations, ghrelin+ cells were described as most abundant in embryonic islets, present in the neonate, but greatly reduced in the adult pancreas.[93] Moreover, ghrelin+ cells were identified in islets, exocrine tissue, and ductal epithelium.[93] Further analysis in the rat identified that ghrelin+ cells occasionally coexpressed glucagon or pancreatic polypeptide; these double positive cells were detectable from late gestation through early postnatal time points, and from this it was suggested that ghrelin+, glucagon+ and pancreatic polypeptide+ cells are of the same lineage.[94]

In the mouse, Sussel and colleagues [68] observed an increase in ghrelin+ cells (subsequently named “epsilon” cells) in place of the deficient beta, alpha and PP cells in the pancreas of the *Nkx2-2* null mouse. Similar to previous reports in the rat and human, epsilon cells were described as the fifth endocrine lineage and identified in small number in the wildtype islet. Additional TF mutants, including *Pax6* null embryos, were also discovered to have an increase in ghrelin+ cells.[68,95] Subsequent studies identified that, similar to the rat, ghrelin-

expressing cells and glucagon/ghrelin coexpressing cells can be found in the embryonic pancreas, and these cells derive from a Neurog3+ endocrine progenitor.[95] Interestingly, the glucagon/ghrelin coexpressing cells are lost with deletion of either *Nkx2-2*[68] or *Arx*[95], but increased with deletion of *Pax4*.[95]

In light of these studies, there is ongoing investigation to understand the epsilon cell lineage, as well as the significance of the presence of ghrelin in the pancreas. Not surprisingly the hormone ghrelin, like insulin and glucagon, was deleted (*Ghrl* null mouse) and found to be dispensable for endocrine development and differentiation.[96]

I.4d The pancreatic polypeptide-expressing PP cell—In recent years, pancreatic polypeptide (PPY)-expressing PP cells have not been studied to the same extent as alpha and beta cells. However, a number of early studies investigating the connection between all endocrine lineages uncovered a controversial relationship between PPY-expressing cells and the endocrine progenitor population. Vassalli and colleagues[52] examined the pattern of hormone expression throughout pancreas development and identified glucagon- and PPY-expressing cells at E10.5, insulin cells at E11.5 and somatostatin cells at E13.5; using RT-PCR the early appearance of *Ppy* expression was confirmed and these early PPY+ cells were believed to coexpress other hormones.[52] In the following years, hormone expression in the pancreas was re-examined by Hanahan and colleagues[97] and insulin and glucagon were found to coexpress in the E9.5 pancreas, and the presence of PPY expression was not identified until postnatal day 1. These opposing results were explained with the observation that antibodies against PPY cross reacts with a related family member, neuropeptide Y (NPY), which is expressed in early hormone+ cells.[97] Subsequent studies using *in vivo* endocrine cell ablation, determined that PP cells are indispensable for beta and delta cell differentiation because the ablation of PPY-expressing cells resulted in embryonic pancreata devoid of insulin- and somatostatin-expressing cells.[98] However, further studies by Herrera[99] utilized a lineage tracing technique and demonstrated definitively that islet beta cells do not produce PPY.

TFs exclusive to PP cells have yet to be identified; however, a number of mouse models previously discussed also describe alteration to the PP cell population. Interestingly, the deletion of *Pyy* (peptide YY), another NPY family member, results in the loss of PP cells.[100] More recently, in a *Neurog3* null background, *Neurog3* expression was temporally induced to determine if progenitor cells change their competence throughout development, and it was demonstrated that pancreatic progenitors acquire the competence to make PP cells between E10.5 and E12.5; however induction of *Neurog3* after E14.5 induced a greater number of PP cells late in development.[56]

I.4e The somatostatin-expressing delta cell—Although somatostatin-producing cells play an important role in the paracrine regulation of islet function[101], delta cell markers remain scarce, with few somatostatin cell-specific TFs identified. Inactivation of *Pax4* causes a loss of delta cells, in addition to beta cells, leading to the hypothesis that delta cells and beta cells arise from a common lineage.[72] However, a common beta/delta progenitor has yet to be identified. Alternatively, a handful of studies have described phenotypes that potentially link the alpha and delta cell lineages. Gannon et al.,[60] deleted *Pdx1* specifically

in the beta cell and found that the absence of *Pdx1* results in beta cell dysfunction and an increase in alpha and delta cell numbers. In addition, the *Arx* null pancreas displays an increase in delta cells, concomitant with a decrease in alpha cells.[85] In general, the development of the delta cell is quite under-studied and, similar to other hormone mutants, the deletion of somatostatin (*Sst*) has no reported pancreatic phenotype.[102]

Table 2 summarizes some of the key TFs noted to have defects in endocrine differentiation; Figure 5 illustrates the genesis of the hormone-producing cell lineages from the delaminating endocrine progenitors, which differentiate from the multipotent pancreatic progenitor cells that reside in the ductal epithelium of the developing pancreas.

II. DIABETES

II.1 Diabetes

Diabetes mellitus ("diabetes") represents a family of metabolic disease characterized primarily by pancreatic dysfunction. Under the umbrella of diabetes, there are multiple forms of the disease with different etiology but the same resultant endpoint. Type 1 diabetes (T1D) is caused by the autoimmune destruction of beta cells and can affect normal weight children and adults; however, childhood onset is most prevalent (reviewed in [103]). This form of diabetes is fatal if not treated with exogenous insulin to compensate for the lack of production of this hormone by the body. Although autoimmunity is the primary effecter of T1D, it is still not known which combinations of genetic and environmental stimuli trigger the immune response and why the pancreatic beta cells are specifically targeted for destruction. These are ongoing areas of research.

Type 2 diabetes (T2D) is characterized by insulin resistance, whereby there is a reduced ability to respond to insulin in the pancreatic beta cells and peripheral tissues. Ultimately, the disease is associated with the disruption of pancreatic beta cell function and the loss of beta cell mass. This form of diabetes is most prevalent in adults; however, more recently T2D has been described in an increasing number of younger individuals.[104] T2D is a polygenic disease influenced by many environmental factors, which has complicated the identification of a general underlying genetic cause as well as universal therapeutic treatments.

A third form of diabetes that is often mistaken for T1D due to its early onset, is maturity onset diabetes of the young (MODY). MODY is characterized by beta cell dysfunction due to single gene mutations. This monogenic disease has autosomal-dominant inheritance and early onset, usually in childhood or adolescence before 25 years of age.[104] The genes implicated in the pathogenesis of MODY are expressed in the beta cell, and a heterozygous mutant state has a range of resultant defects including abnormal insulin secretion, glucose sensing, and beta cell development and function. While MODY genes encode TFs important in the pancreas, a number of these genes are also expressed in other tissues, namely the liver (*HNF4A*, *GCK*, *HNF1A*, *HNF1B*, *KLF11*, *BLK*) and kidney (*HNF1B*) (Table 3). Dysfunction in these organs can also accompany the disease in individuals that harbour the associated mutation.

Finally, rare forms of neonatal diabetes manifest with other syndromes, and are the result of single gene alterations. One example is a rare syndrome characterized by neonatal diabetes and congenital hypothyroidism (NDH), which has been linked to a genetic alteration in *GLIS3*.[105] In addition, mutation in the gene *RFX6* leads to Mitchell-Riley syndrome, typified by hypoplastic pancreas, neonatal diabetes, intestinal atresia and small/absent gall bladder.[106] Additional genes implicated in rare forms of neonatal diabetes include *PTF1A*[107], *EIF2AK3* (Wolcott-Rallison syndrome[108]), *INSULIN*[109], *FOXP3* (Immunodysregulation, Polyendocrinopathy, and Enteropathy, X-linked syndrome)[110], and *KCNJ11*[111].

II.2 Current therapies

The use of exogenous insulin for the treatment of diabetes stemmed from the seminal work of Frederick Banting, Charles Best, James Collip and J.J.R. MacLeod, researchers at the University of Toronto (Canada) in 1921.[112] While the first successful extraction of insulin (at the time termed pancrein) can be traced to Nicolae Paulescu in 1921 (published in 1922)[113], the work by Banting and colleagues, who injected purified extract from fetal calf pancreas into patients affected with diabetes, revolutionized the treatment of this fatal disease. At present, the insulin prescribed to diabetic patients is biosynthetic recombinant human insulin and provides effective treatment. The creation of continuous subcutaneous insulin infusion therapy, i.e. the insulin pump, further assists patients in that the device provides consistent insulin injections.[114] Additionally, in patients where the disease has not progressed, many drugs have been designed to assist the beta cell in its proper function.

Exogenous insulin and pharmaceuticals, while gold standards of care, burden the patient with constant treatment and monitoring of their disease. Alternate therapies are being pursued with the goal of alleviating the requirement for exogenous therapeutics. The question has become, is it possible to identify a therapeutic strategy that restores islet cells, thereby returning normal pancreatic function to patients with diabetes, effectively “curing” the disease?

One such breakthrough came in 1999 at the University of Alberta (Canada), with the first human pancreatic islet transplantation.[115] The Edmonton Protocol, as it has been termed, isolates pancreatic islets from up to three donated human cadaveric pancreata and transplants these islets into the portal vein of the recipient.[115] As with any organ transplant, recipients are also administered immunosuppressive drug therapy to prevent rejection. One-year follow-up data describes that more than half of the transplant recipients do not require exogenous insulin; however, these numbers decrease with increasing post-operative time.[116] While the Edmonton protocol provides a window of curative hope, this revolutionary therapeutic option has limitations, first and foremost, the availability of donor islets. This limitation could be resolved with the *in vitro* generation of large quantities of functional “islets”.

II.3 The future

Many of the developmental studies outlined in Section I have been instrumental in guiding the creation of protocols to engineer functional insulin-producing beta cells *in vitro* for

therapeutic use in the treatment of diabetes. Ultimately, it has been demonstrated that to successfully produce pancreatic islet cells *in vitro*, the *in vivo* developmental stages must be recapitulated. These stages include the induction of definitive endoderm, the subsequent patterning and specification of the pancreatic progenitor cells, followed by the differentiation into hormone-producing endocrine cells, specifically insulin-responsive beta cells. Mimicking development is not trivial, but work in the mouse is assisting in translating the known signals required for specification and differentiation into the *in vitro* setting.

The relative ease of procurement and manipulation of mouse embryonic stem (mES) cells, as well as mouse embryonic or adult pancreatic tissue, makes these platforms ideal for the creation and testing of *in vitro* differentiation protocols. Various techniques have been used to manipulate these cells with the goal of understanding their capacity to produce insulin-expressing cells. The direct differentiation of mES cells toward endoderm using soluble factors was found to be successful, as this recapitulated the known developmental signals that induce the specification of pancreatic endoderm.[117] Building on this work, small molecules were identified that mimicked these soluble factors and produced endoderm more efficiently.[118] However, in both cases the starting cellular platform was the mES cell. From a therapeutic standpoint, the production of insulin-producing cells from adult tissue would mean that cells from a patient could be used to create customized beta cells for transplantation, circumventing the need for immunosuppressive drugs.

To that end, the creation of induced pluripotent stem (iPS) cells [119], which are adult somatic cells reprogrammed to an ES cell state using *Oct4*, *Klf4*, *cMyc*, *Sox2*, has created a new platform for testing *in vitro* protocols and the differentiation of many cell lineages (reviewed in [120]). Ultimately, the use of human iPS or approved human embryonic stem (hES) cell lines to produce differentiation protocols is more translatable to the clinical realm. To date, research in many organ systems has been successful in the *in vitro* differentiation of hES cells into cardiac (reviewed in [121]), haematopoetic[122], and neural[123] cells, thus providing great therapeutic promise for the differentiation of hES cells into pancreatic islet cells.

The manipulation of mES and iPS cells has provided adequate proof of principle that *in vitro* differentiation can be successful, in general. However, understanding the organ-specific requirements, and specifically the plasticity of cells in the pancreas, has provided further insight into the ability to direct or redirect cells toward a pancreatic fate. The capacity of adult mouse pancreatic tissue to transdifferentiate into insulin-producing cells was demonstrated; Zhou and colleagues infected adult mouse exocrine tissue with virus expressing the TF genes *Pdx1*, *Neurog3* and *MafA*, and determined that insulin-expressing cells could be produced.[124] More recently, using either the overexpression of certain TF genes or by extreme pancreatic injury, transdifferentiation between alpha and beta cells has been demonstrated in both the adult and embryonic pancreas.[84, 125]

The coalescing of decades of pancreatic development research with years of *in vitro* technology pursuits produced a successful template for the *in vitro* differentiation of hES cells toward the pancreatic lineage, first reported by ViaCyte (formerly Novocell).[126] In this study, the authors outline the manipulation of hES cells toward a hormone-producing fate *in vitro*, using soluble factors. While successful in the differentiation of definitive

endoderm[127], and pancreatic and endocrine progenitors[126], the ability to complete the differentiation process *in vitro* was not demonstrated; only after transplantation of endocrine precursors into the mouse were insulin-responsive cells produced.[128]

Building on these studies, the Keller group recently outlined a protocol with an increased differentiation potential.[129] Similar to the *in vivo* developmental program, this protocol first induces hES cells toward endoderm using FGF, followed by the addition of RA, noggin (BMP inhibitor) and cyclopamine (Hedgehog inhibitor) to generate *Pdx1*-expressing cells. Ultimately the resulting differentiated endocrine cells were in greater proportion to previous protocols, with an increased number of insulin+ cells produced. While much work still remains to be done, this *in vitro* differentiation protocol as well as those discussed throughout this section, provides great promise for the creation of insulin-producing cells for therapeutic use. The continued application of developmental biology research to *in vitro* differentiation technology may ultimately produce the long awaited therapeutic cure for diabetes.

Conclusion

Developmental biology research has, and will continue to, decipher the mystery of organogenesis. With respect to endoderm patterning, pancreatic specification and endocrine differentiation, the utilization of mouse models has been successful in deciphering many of the key factors involved in this process. Factors identified in tissue surrounding the prospective pancreatic endoderm, including SHH, activin, FGF, BMP, and RA, are necessary for the proper induction of patterning in the epithelial gut tube. Following patterning, transcription factors, most importantly PDX1, PTF1A, FOXA and SOX9, specify the pancreatic progenitor cells from which the endocrine, exocrine and ductal lineages are derived. The subsequent development and differentiation of all hormone-producing endocrine cells relies on the influence of mesenchyme and vasculature, as well as branching morphogenesis, ductal delamination and islet cell coalescing. Taken together, understanding the specific cues that allow for proper development has aided in determining the plasticity of cells in the pancreas. Researchers are now translating these key findings into *in vitro* differentiation protocols, whereby adult or embryonic stem cells can be directed toward a pancreatic fate with the goal of generating insulin-responsive beta cells for the purpose of transplantation into patients affected with diabetes. These *in vitro* protocols outline the sequential addition of soluble factors, small molecules, or drugs to mimic the *in vivo* developmental process, thereby creating the developmental milieu in a dish to direct cells to become insulin-producing beta cells. With continued support from developmental biology research, the promise of a curative therapeutic option for the treatment of diabetes is on the horizon.

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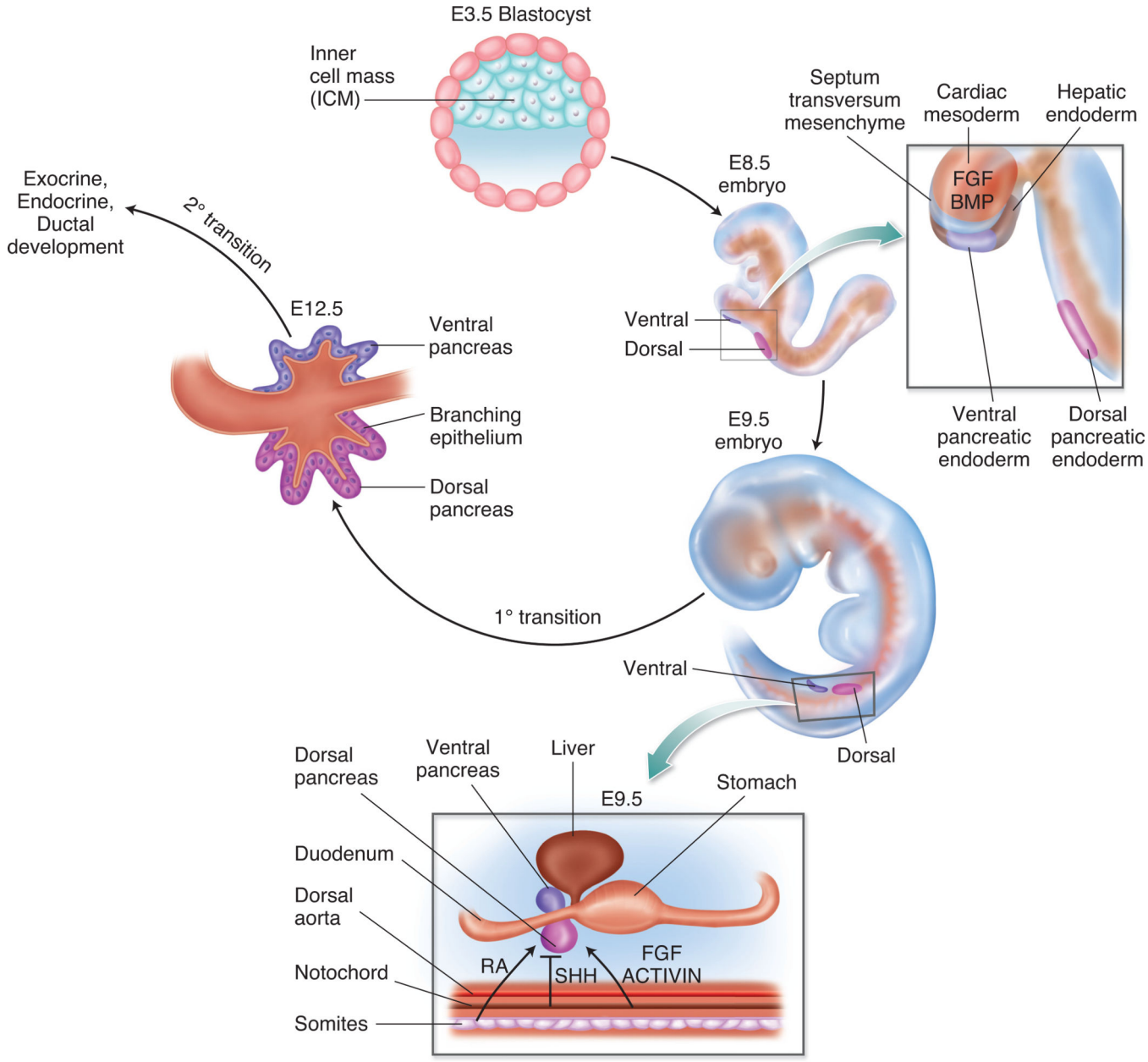

Figure 1. Pancreatic endoderm patterning and specification

The portion of the endoderm fated to become dorsal and ventral pancreas is patterned and specified due to the influence of secreted molecules and signalling pathways from adjacent tissues and cells. The influence of FGF and BMP signalling from the cardiac mesoderm and septum transversum mesenchyme, respectively, affect the patterning of ventral pancreatic endoderm. Retinoic acid (RA), SHH, FGF and ACTIVIN signalling from the paraxial mesoderm and notochord allow for patterning of the dorsal pancreatic endoderm. The stage from E9.5 through E12.5 marks the primary transition. During this period, the pancreatic progenitor cells are specified, first wave endocrine cells are present, and epithelial branching morphogenesis begins. The secondary transition occurs after E12.5 and is marked by a

major wave of endocrine cell specification and development, as well as further ductal morphogenesis and exocrine lineage differentiation.

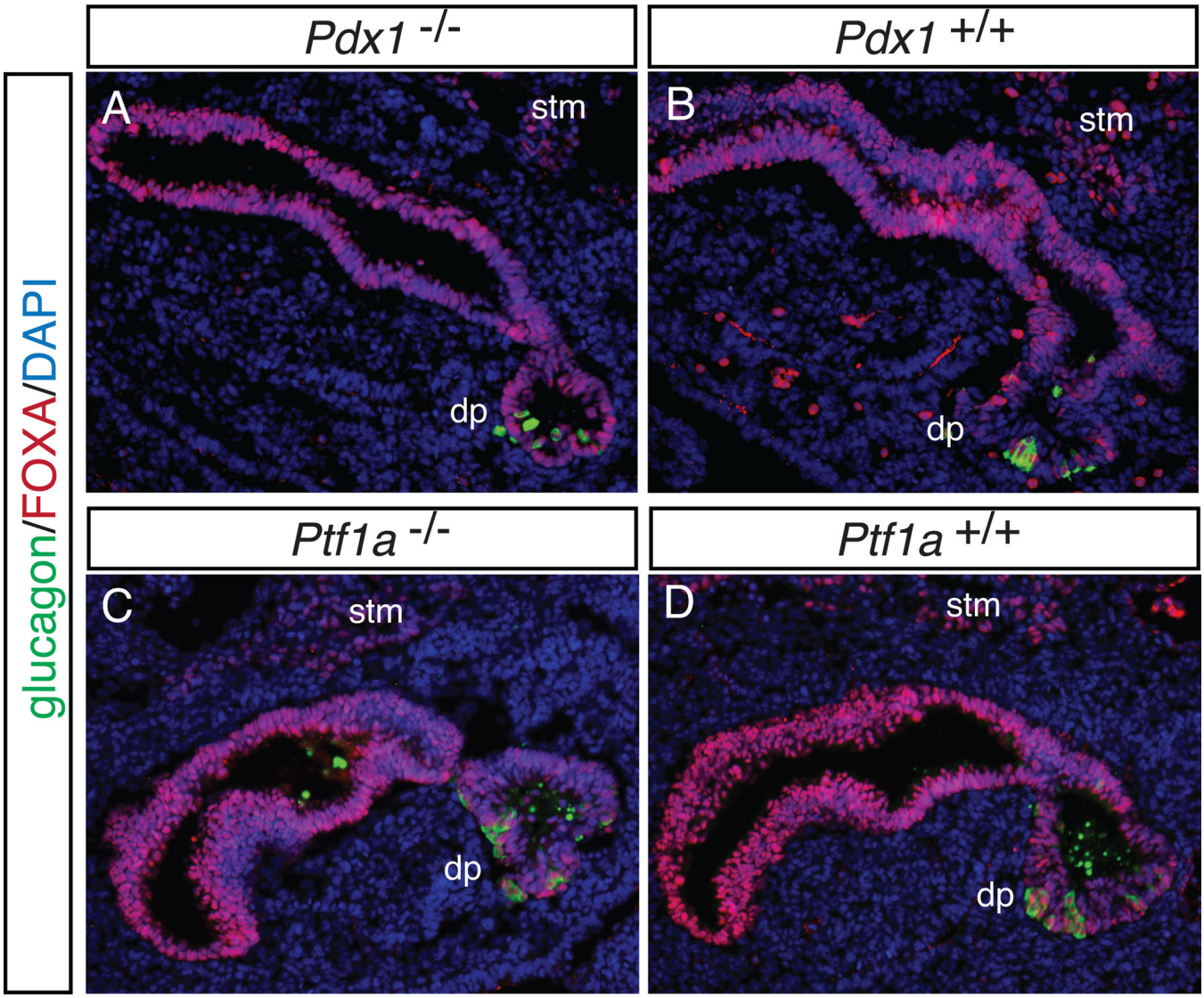

Figure 2. Pancreatic specification is unaltered in embryos lacking either *Pdx1* or *Ptf1a*

Sagittal sections from E10.5 *Pdx1* null (A) and wildtype littermate (B), as well as *Ptf1a* null (C) and wildtype littermate (D) embryos were stained by immunofluorescence with antibodies against the transcription factor FOXA (demarking endoderm) and the hormone glucagon (identifying the pancreatic domain). In both null embryos, the region fated to become the dorsal pancreas (dp) is present, contains glucagon-expressing cells, and appears similar in both the null and wildtype. stm, septum transversum mesenchyme. DAPI marks all nuclei. 20X

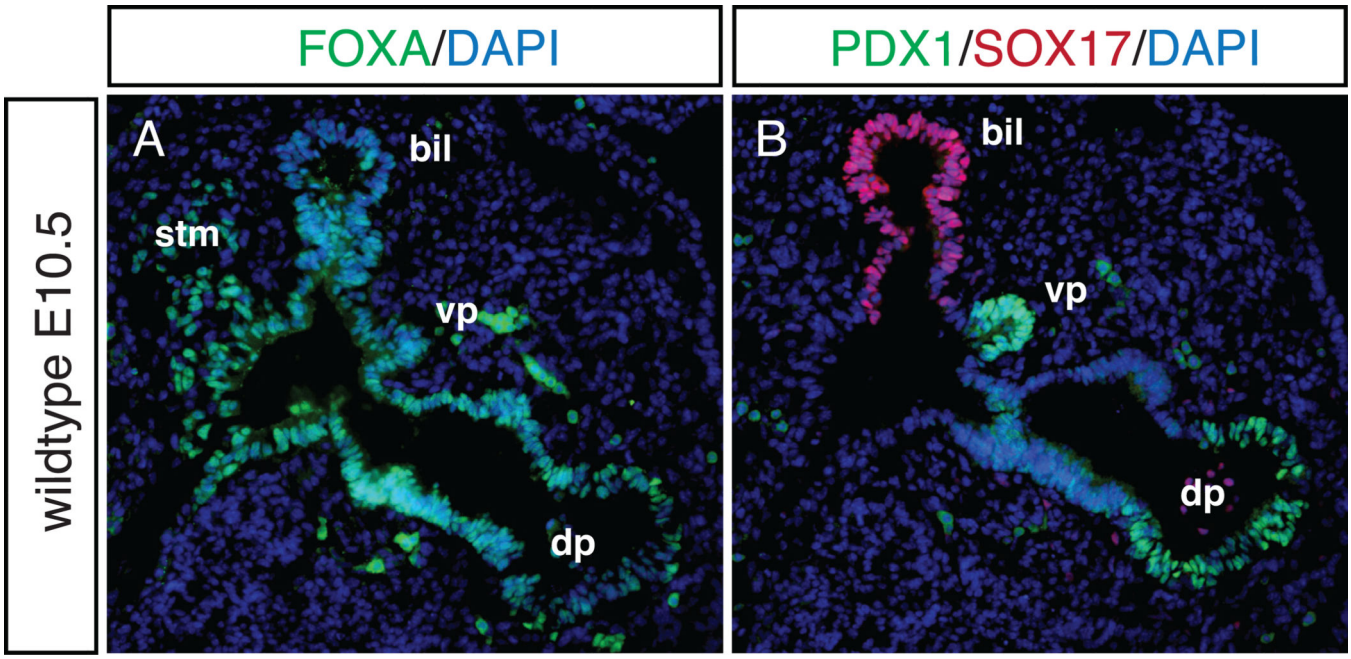

Figure 3. Pancreatic and biliary domains in the wildtype E10.5 embryo

Adjacent sagittal sections from E10.5 wildtype embryo, stained by immunofluorescence with antibodies against FOXA (A), and PDX1, SOX17 (B). The domains of the dorsal pancreas (dp), ventral pancreas (vp), biliary domain (bil) and septum transversum mesenchyme (stm) are noted. While all domains express FOXA (A), PDX1+ cells are found only in the pancreatic domains, and are distinct from the SOX17+ cells in the biliary domain (B). DAPI marks all nuclei. 20X

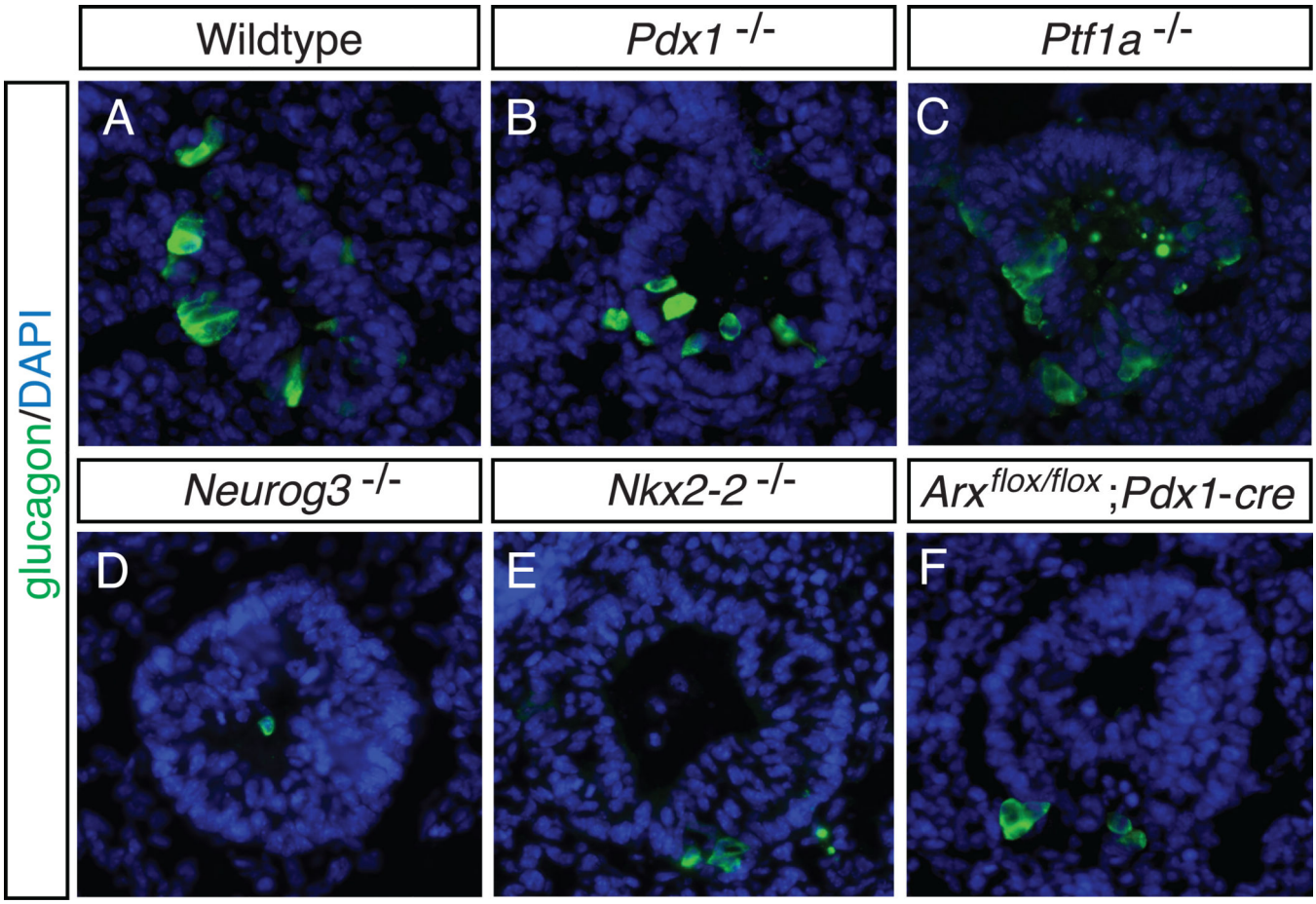

Figure 4. Early "first wave" glucagon-expressing alpha cells

Early "first wave" alpha cells are present in mouse models despite the deletion of factors necessary for pancreas development and endocrine specification. Sagittal sections of E10.5 embryos from a representative wildtype littermate (A), *Pdx1* null (B), *Ptf1a* null (C), *Neurog3* null (D), *Nkx2-2* null (E), and pancreas-specific deletion of *Arx* (F), were stained by immunofluorescence for the hormone glucagon (A–F). In all images glucagon-expressing cells are present. All images are of the dorsal pancreas. DAPI marks all nuclei. 40X

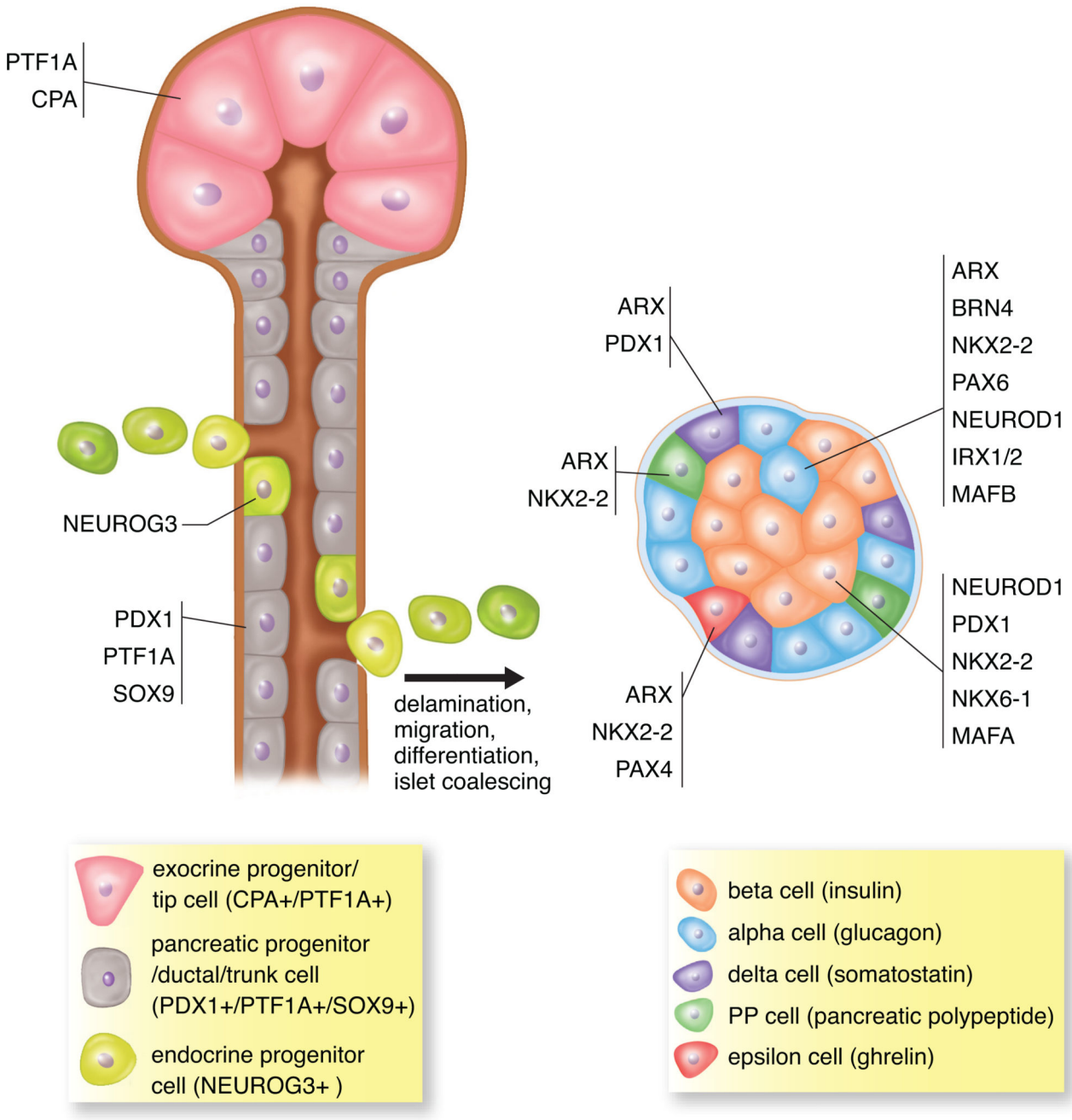

Figure 5. Islet cell specification in the developing pancreas

Multipotent pancreatic progenitor cells (PDX1+/PTF1A+/SOX9+) are present in the ductal epithelium in the developing pancreas. In addition, multipotent CPA+/PTF1A+ cells at the tips in early development are multipotent progenitor cells but later in development are restricted to differentiating into only the exocrine lineage. All hormone-producing endocrine cell lineages are derived from the endocrine progenitor cells (NEUROG3+), which delaminate from the ductal epithelium. Endocrine progenitors can differentiate into all five hormone-expressing cell types including insulin-producing beta cells, glucagon-

producing alpha cells, somatostatin-producing delta cells, pancreatic polypeptide-producing PP cells and ghrelin-producing epsilon cells. As development proceeds, these differentiated hormone+ cells will coalesce and form the islets of Langerhans. The influence of various transcription factors determines the specific endocrine cell produced.

Table 1

Summary of knockout mouse models with severe pancreatic phenotypes

Gene(s)	Genotype studied	Gross Pancreatic Phenotype	Reference
Pdx1	*Pdx1−/−*	pancreatic agenesis	Jonsson J et al., Nature 1994
Ptf1a	*Ptf1a−/−*	pancreatic agenesis	Krapp A et al., Genes Dev 1998; Kawaguchi Y et al., Nat Genet 2002
Foxa1, Foxa2	*FoxA1$^{flox/flox}$;FoxA2 $^{flox/flox}$;Pdx1-cre*	pancreatic hypoplasia	Gao N et al., Genes Dev 2008
Sox9	*Sox9 $^{flox/flox}$;Pdx1-cre*	pancreatic hypoplasia	Seymour PA et al., PNAS 2007
Fgf10	*Fgf10−/−*	pancreatic hypoplasia	Bhushan A et al., Dev 2001
Cdh2	*Cdh2−/−*	dorsal pancreatic agenesis	Esni F et al., Dev Biol 2001
Hlxb9	*Hlxb9−/−*	dorsal pancreatic agenesis	Harrison KA et al., Nat Genet 1999; Li H et al., Nat Genet 1999
Raldh2	*Raldh2−/−*	dorsal pancreatic agenesis	Martin M et al., Dev Biol 2005
Isl1	*Isl1−/−*	Lack of dorsal mesenchyme formation and exocrine differentiation; islet cells absent	Ahlgren U et al., Nature 1997
Sox17	*Sox17$^{flox/flox}$;FoxA3-cre*	Absent biliary structures; ectopic pancreas tissue in the liver and common duct	Spence JR et al., Dev Cell 2009
Hes1	*Hes1−/−*	Gall bladder agenesis; ectopic pancreas in the common duct	Sumazaki R et al., Nat Genet 2004; Fukuda A et al., J Clin Invest 2006

Table 2

Summary of knockout mouse models that affect endocrine differentiation

Gene	Genotype studied	Viability	Pancreatic Phenotype (expression changes vs. to WT)					Reference
			alpha	beta	delta	epsilon	PP	
Neurog3	*Neurog3−/−*	perinatal death	absent*	absent	absent	absent	absent	Gradwohl G et al., PNAS 2000
Pdx1	*Pdx1$^{flox/flox}$;RIP-cre*	viable/diabetic	increase	decrease	increase			Gannon M et al., Dev Biol 2008
Nkx6-1	*Nkx6-1−/−*	perinatal death		decrease				Sander M et al., Dev 2000
Nkx6-2	*Nkx6-2−/−*	viable						Henseleit KD et al., Dev 2005
Nkx6-1/Nkx6-2	*Nkx6-1−/−; Nkx6-2−/−*	perinatal death	decrease	decrease				Henseleit KD et al., Dev 2005
Nkx2-2	*Nkx2-2−/−*	perinatal death	decrease*	absent		increase	decrease	Sussel et al., Dev 1998
Neurod1	*Neurod1−/−*	perinatal death	decrease	absent				Naya FJ et al., Genes Dev 1997
Nkx2-2/Neurod1	*Nkx2.2−/−; Neurod1−/−*	perinatal death		absent		increase		Chao CS et al., Dev Biol 2007
Pax4	*Pax4−/−*	perinatal death	increase	absent	absent			Sosa-Pineda B et al., Nature 1997
Pax6	*Pax6−/−*	perinatal death	decrease	decrease	decrease	increase	decrease	Sander M et al., Genes Dev 1997
Pax4/Pax6	*Pax4−/−; Pax6−/−*	perinatal death	absent	absent	absent		absent	St-Onge L et al., Nature 1997
MafA	*MafA−/−*	viable/diabetic		dysfunction				Zhang C et al., MCB 2005
MafB	*MafB−/−*	perinatal death	decrease	decrease				Artner I et al., PNAS 2007
Arx	*Arx−/−*	perinatal death	absent*	increase	increase			Collombat P et al., Genes Dev 2003
Foxa2	*Foxa2$^{flox/flox}$;Foxa3-cre*	perinatal death	decrease					Lee CS et al., Dev Biol 2005

* first wave alpha cells present

Table 3

Genes implicated in Maturity Onset Diabetes of the Young (MODY)

MODY	Gene	Gene Name	Former gene names	Resulting Defect	Reference
MODY 1	*HNF4A*	hepatocyte nuclear factor-4-alpha	*HNF4, TCF14*	insulin secretion and beta cell mass	Bell GI et al., PNAS 1991; Stoffel M et al., PNAS 1996
MODY 2	*GCK*	glucokinase	*GK, GLK, HK4, LGLK*	glucose sensing by beta cells; hepatic glucose storage	Froguel P et al., Nature 1992
MODY 3	*HNF1A*	hepatocyte nuclear factor-1 alpha	*TCF1, HNF1*	insulin secretion and beta cell mass	Vaxillaire M et al Nat Gen 1995; Menzel S. et al., Diabetes 1995
MODY 4	*PDX1*	pancreas/duodenum homeobox protein-1	*IPF1, STF1, IDX1*	transcriptional regulation of beta cell development and function	Stoffers DA et al., Nat Gen 1997
MODY 5	*HNF1B*	hepatic nuclear factor-1 beta	*TCF2, HNF2*	insulin secretion and beta cell mass; 'renal cysts and diabetes syndrome'	Horikawa Y et al., Nat Gen 1997
MODY 6	*NEUROD1*	neurogenic differentiation 1	*BETA2*	transcription/regulation of beta cell development and function	Malecki MT et al., Nat Gen 1999
MODY 7	*KLF11*	kruppel-like factor-11	*TIEG2*	impaired insulin promoter activation leading to decreased insulin expression	Neve B et al., PNAS 2005
MODY 8	*CEL*	carboxyl-ester lipase	*BSDL, BSSL*	lipase function in pancreatic acinar cells; 'exocrine pancreatic dysfunction'	Raeder H et al., Nat Gen 2006
MODY 9	*PAX4*	paired box gene 4		repressed activity of the insulin and glucagon promoters	Plengvidhya N et al., J Clin Endocr Metab. 2007
MODY 10	*INS*	insulin	proinsulin	insulin gene processing	Edghill EL et al., Diabetes 2008; Molven A et al., Diabetes 2008
MODY 11	*BLK*	tyrosine kinase, B-lymphocyte specific		beta cell function (insulin synthesis and secretion)	Kim SH et al., Diabetes 2004; Borowiec M et al., PNAS 2009