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Genes controlling pancreas ontogeny

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ABSTRACT The pancreas develops from two separate and independent endodermal primordia. The molecular events supporting the early morphological changes that give rise to the formation of the dorsal and ventral pancreatic buds result from coordinated responses to extrinsic and intrinsic signals. The extrinsic signals are involved in processes dictating whether progenitor cells remain as immature or as committed precursors. After specification, the sequential activation of transcription factors determines cell autonomously the commitment and differentiation of these progenitors. During pancreas development, the roles of extrinsic and intrinsic signals are variable, depending on the particular competence of each progenitor cell. We summarize in this review the main events, at the level of gene expression, which are involved in the early stages of pancreas development.

KEY WORDS: *islet, pancreas, progenitor, endocrine, development, beta cell*

The pancreas is an exocrine and endocrine gland of the digestive system (Fig. 1A). The exocrine part represents 95-99% of the total pancreatic mass. It consists of serous acini of highly polarized cells that produce digestive enzymes (amylase, lipase, phospholipase) as well as pro-enzymes (elastase, procarboxypeptidase, trypsinogen, pepsinogen, deoxyribonuclease, ribonuclease), which are stored in zymogen granules located in their apical pole (Fig. 1CD). Once secreted with water and bicarbonates into the lumen of the acinus, they become activated and forwarded through the ductal network to the duodenum, for the intestinal digestion of nutrients. The ductal tree begins within the acini with very small ducts lined by centroacinar cells, followed by intercalated, intralobular and finally interlobular ducts (Fig. 1CD).

The endocrine pancreas is composed of islets of Langerhans scattered within the exocrine tissue, representing 1-5% of the pancreatic mass (Fig. 1BC). Adult islets are composed of different cell types characterized by the production of specific hormones: glucagon by α-cells, insulin by β-cells, somatostatin by δ-cells and pancreatic polypeptide by PP-cells. A rare fifth endocrine cell type, the ε-cell, secreting ghrelin, represents about 1% of the embryonic endocrine pancreas, but disappears after birth. In rodents, islets are composed of a central core of β-cells, which represent about 80% of all islet cells, surrounded by a mantle composed of the three other cell types (Fig. 1E). Insulin and glucagon control blood glucose levels, whereas PP and ghrelin are orexigenic hormones and somatostatin regulates the secretion of insulin, glucagon and PP.

Specification of pancreatic fate

In Mammals, the pancreatic differentiation program is induced in the foregut/midgut junction of the endoderm by factors released from the mesoderm at the 6-10 somites stage (6-10s) (Fig. 2). Initially, the dorsal endoderm is adjacent to the notochord, before the fusion of the two dorsal aortae at 12-20s (which corresponds to E8.75-9.0 in mice). At this stage, the dorsal pancreatic endoderm is near the dorsal aorta and the dorsal mesenchyme, and the ventral-lateral pancreatic endoderm is adjacent to the septum transversum (i.e. the primordium of the diaphragm) and the cardiogenic mesoderm. The

Abbreviations used in this paper: Apc, Adenomatous polyposis coli ; Arx, Aristaless-related homeobox; bHLH, basic helix-loop-helix; Bmp, Bone morphogenic protein; bZIP, basic leucine zipper; CCK, Cholecystokinin; CPA1, Carboxypeptidase A1; Cx, Connexin; Dhh, Desert hedgehog; E, Embryonic day; EGF, Epidermal growth factor; FGF, Fibroblast growth factor; Fox, Forkhead homeobox; Glut2, Glucose transporter 2; Hes1, Hairy enhancer of split; Hex, Haematopoietically expressed homeobox; Hh, Hedgehog; Hnf, Hepatocyte nuclear factor; Isl1, Islet1; Maf, Musculoaponeurotic fibrosarcoma; Ngn3, Neurogenin 3; P, Postnatal day; Pax, Paired homeobox; Pdx1, Pancreatic and duodenal growth factor 1; Prox1, Prospero-related homeobox; Ptch1, Patch1; Ptf1a, Pancreas specific transcription factor 1a; s, somites; Shh, Sonic hedgehog; Sox, Sry related hydroxymethylglutaryl homeobox ; Srf, Serum response factor; TGFβ, Transforming growth factor β; VEGF, Vascular Endothelial Growth Factor.

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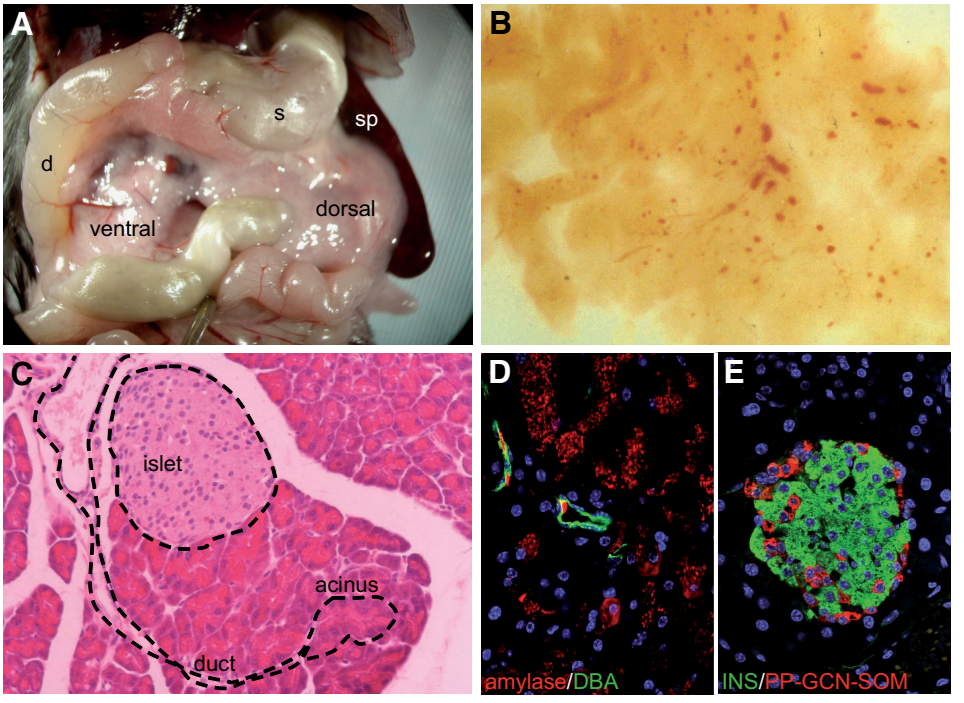

Fig. 1. Macroscopic and microscopic views of the adult mouse pancreas. (A) *The duodenal portion of the pancreas ("ventral"), derived from the ventral primordium, is attached to the duodenum (d) and stomach (s), whereas the splenic ("dorsal", derived from the dorsal primordium) pancreas extends towards the spleen (sp).* **(B)** *Wholemount adult mouse pancreas perfused with dithizone, which specifically labels β-cells. The endocrine pancreas is composed of islets of Langerhans (stained in red) scattered among the exocrine tissue.* **(C)** *Paraffin section stained with hematoxylin-eosin. Endocrine cells are small and pale, and concentrate in islets. The exocrine gland is composed of secretory units, the serous acini, and excretory ducts: intercalated (small), intralobular and interlobular (large) ducts. Magnification, x100.* **(D)** *Immunofluorescence of pancreatic cells. Exocrine cells are acinar cells containing amylase-positive zymogen granules (red immunofluorescence) and ductal cells, stained with the DBA lectin (green immunofluorescence).* **(E)** *Islet cells are distributed so as to form a central core of* insulin *(INS)-expressing cells (green immunofluorescence) surrounded by a mantle of cells expressing either,* PP, glucagon *(GCN) or* somatostatin *(SOM) (red immunofluorescence). (D-E) Magnification, x400.*

first signs of pancreas organogenesis occur at 22-25s (E9.5). The dorsal mesenchyme condenses and the adjacent endodermal region evaginates to form the dorsal bud. The ventral bud, adjacent to the liver diverticulum appears later, at 30s. Through continuous signals originating from the adjacent mesoderm, pancreatic epithelial cells proliferate and migrate to generate an evagination that branches and invades the surrounding mesenchyme (Fig. 3AB). When the gut rotates clockwise (56-60s; E13.5 in mice), the ventral bud is brought beside the dorsal bud.

The presumptive region of the pancreas and duodenum is the only part of the primitive gut devoid of *Shh* expression. This inhibition, mediated by adjacent mesodermic structures, is required for pancreas organogenesis. Pancreatic and duodenal progenitor cells are characterized by the expression of the transcription factor *Pancreatic and duodenal homeobox 1 (Pdx1)*. The initial induction of *Pdx1* is triggered by the transcription factor Hnf6. Other factors, such as Hb9 and Isl1 in the dorsal bud, and Hex1 in the ventral bud, are required for *Pdx1* expression. Afterwards, the maintenance of Πδξ1 activity depends on mesodermal signals, such as FGFs, which contribute to the induction of *Pancreas transcription factor (Ptf1a)* in pancreatic progenitors.

Role of the notochord and the cardiac mesoderm

The repression of *Shh* in the dorsal pancreatic endoderm is mediated by the adjacent notochord (Kim *et al.*, 1997). This inhibition is permissive for the expression of *Pdx1*. Hebrok and others determined that TGFβ ligand (activin βB) and FGF2 are sufficient to inhibit *Shh* expression in the dorsal endoderm, thus allowing normal pancreas development (Kim *et al.*, 1997). *Shh* repression and the concomitant pancreatic differentiation were observed in isolated chick endoderm (stage 12) cultured in the presence of high concentrations of activin βB. A similar effect was obtained when FGF2 concentration was low. On the contrary, disruption of activin βB-dependent TGFβ signaling in mice lacking activin receptors (*ActRIIA -/-; ActRIIB -/-*), or under extreme FGF2 concentrations (too low and too high), permitted the expression of *Shh* in the dorsal endoderm, leading to pancreas development disruption (Hebrok *et al.*, 1998). Accordingly, the disruption of Hh signaling, by treating mice with cyclopamine (an inhibitor of Smoothened) or in *Shh-/-* and *Shh-/-; Ihh+/-* mutants, promoted the pancreatic differentiation program in non-pancreatic endoderm (rostral stomach and duodenum) (Kim and Melton, 1998).

Similarly, in the anterior leading edge of the embryonic endoderm, *Shh* repression defines the presumptive region from which the ventral pancreatic primordium starts to grow at the 2-6s stage (Deutsch *et al.*, 2001). The cardiac mesoderm releases FGFs, which induce *Shh* expression in the endoderm adjacent to the heart, thus triggering the hepatic development program. The more distal endoderm, on the contrary, is exposed to low FGF levels and does not express *Shh,* therefore adopting the pancreatic program.

Role of vascular structures and the dorsal mesenchyme

The close vicinity of blood vessels and pancreatic endoderm is essential for pancreatic development. At E8.75-9.0 (12-20s), the two dorsal aortae fuse, separating the notochord from the pancreatic endoderm. The removal of the aorta inhibits pancreas development in *Xenopus* endoderm; however the pancreas is rescued when the endoderm is cocultured with other non-aortic endothelial cells (Lammert *et al.*, 2001).

The involvement of endothelial cells, whether directly or as vessels containing circulating factors, was also demonstrated using *Ilk-/-* mice, in which the deletion of VEGF receptor inhibits blood vessel formation and thus the dorsal mesenchyme fails to develop (Shalaby *et al.*, 1995, Yoshitomi, 2004). In *Flk-/-* em-

bryos, *Pdx1* is normally expressed in the pancreatic endoderm at 20-25s, yet the dorsal bud does not form and only few Ptf1a$^-$/Pdx1$^+$ cells remain, whereas the pancreatic development of the ventral endoderm is not affected. Interestingly, the E8.5-E9.5 *Flk-/-* dorsal endoderm gives rise to a normal pancreas if cocultured with wild type aorta, thus showing that endothelial cells are supporting cells necessary for dorsal pancreas development.

The dorsal mesenchyme, which is placed between the dorsal aorta and the endoderm, is also involved in promoting the development of the dorsal pancreas, as shown in mice deficient for *islet1 (Isl1)* or the adhesion molecule *N-Cadherin (Cdh2)*. In these mutants there is no dorsal mesenchyme, and dorsal pancreas development is impaired. Indeed, dorsal pancreas development is modulated by the vascular structures of the dorsal mesenchyme. This was shown after restoration of cardiac development through the cardiac-specific expression of *N-Cadherin* in the *Cdh2-/-* background: this is sufficient to reestablish dorsal pancreas development. Similarly, the coculture of aortic cells and dorsal mesenchyme ensures the survival of Isl1$^+$ cells in the latter (Jacquemin *et al.*, 2006).

The survival signals from the aorta allow the dorsal mesenchyme to secrete FGF10, which in turn promotes dorsal pancreas development (Jacquemin *et al.*, 2006). FGF10 is known to promote the accumulation of *Pdx1*+ pancreatic progenitor cells. The disruption of FGF signaling leads to pancreatic hypoplasia. Inversely, induction of FGF signaling in animals overexpressing *Fgf10*, or *in vitro* with FGF10 treatment, leads to excessive proliferation of progenitor cells and the formation of an oversized pancreas.

Altogether, these results highlight the critical role of the pancreatic mesoderm as source of permissive factors for the development of the pancreas.

Hepatocyte nuclear factor6 (Hnf6)

The Onecut homeodomain transcription factor *Hepatocyte nuclear factor 6 (Hnf6)* is expressed from 8s, shortly prior *Pdx1* induction, in the foregut/midgut region of the endoderm, which gives rise to the pancreatic diverticuli. Later, *Hnf6* is expressed in Pdx1 + progenitors, and becomes restricted to ductal and acinar cells from E18.

Hnf6 is required for adequate *Pdx1* expression, downstream of Hb9 and HNF3β. Therefore, in *Hnf6-/-* embryos the induction of *Pdx1* is delayed, affecting the size of the pancreas from E10.5 to adulthood, without modified proliferation or apoptosis (Jacquemin *et al.*, 2003).

Pancreatic and duodenal growth factor1 (Pdx1)

The expression of *Pdx1* starts in the ventral pancreas at E8.5, and about 12 hours later in the dorsal pancreas, as well as in the caudal stomach and proximal duodenum, after *Hnf6* induction. By E16.5, *Pdx1* expression diminishes in exocrine lineages and, from E19.0, it is restricted to β-cells and to 20% of δ-cells (Fig. 3C).

Pdx1 expression is necessary for pancreas development. Pdx1 is expressed in all epithelial cells of the developing pancreas and represents the earliest marker of progenitors generating endocrine and exocrine pancreatic cells. The absolute requirement of Pdx1 during pancreas development was confirmed by genetic loss-of-function experiments. In humans, heterozygous mutations in *Pdx1* highly predispose to MODY4 diabetes and adult-onset Type II diabetes, and complete absence of *Pdx1* results in pancreatic aplasia (Stoffers *et al.*, 1997). Similarly, in mice, *Pdx1* disruption inhibits pancreas development, allowing only a limited growth of the dorsal bud (Jonsson *et al.*, 1994). In addition, *Pdx1* mutations are frequently associated with pyloric atresia and replacement of duodenal epithelium by Glut2$^+$ bile duct-like epithelium. Nevertheless, ectopic *Pdx1* expression is not sufficient to promote the pancreatic program in non-pancreatic chick endoderm (Grapin-Botton *et al.*, 2001), whereas concomitant expression of *Pdx1* and *Ptf1a* is sufficient to convert non-pancreatic endoderm into pancreatic precursors in *Xenopus* embryos (Afelik *et al.*, 2006).

Recent evidences suggest that the number of Pdx1$^+$ progenitors formed between E8.5 and E12.5 determines the final size of the pancreas (Stanger *et al.*, 2007). The size of the pool of Pdx1$^+$ progenitors depends on the proper commitment and proliferation of early endodermal cells into Pdx1$^+$ cells. The partial ablation of Pdx1$^+$ progenitors or the disruption of Notch, FGF or Wnt/β-catenin signaling leads to a very similar phenotype, i.e. a reduced number of Pdx1$^+$ progenitors. This reduction is associated with pancreatic hypoplasia. This suggests that pancreas growth is fixed by the amount of Pdx1+ progenitor cells, and that it follows an intrinsic program (Stanger *et al.*, 2007).

The tight regulation of *Pdx1* expression allows Pdx1 to have multiple roles, depending on its temporal and spatial expression pattern. *Pdx1* is expressed in the different domains of the caudal

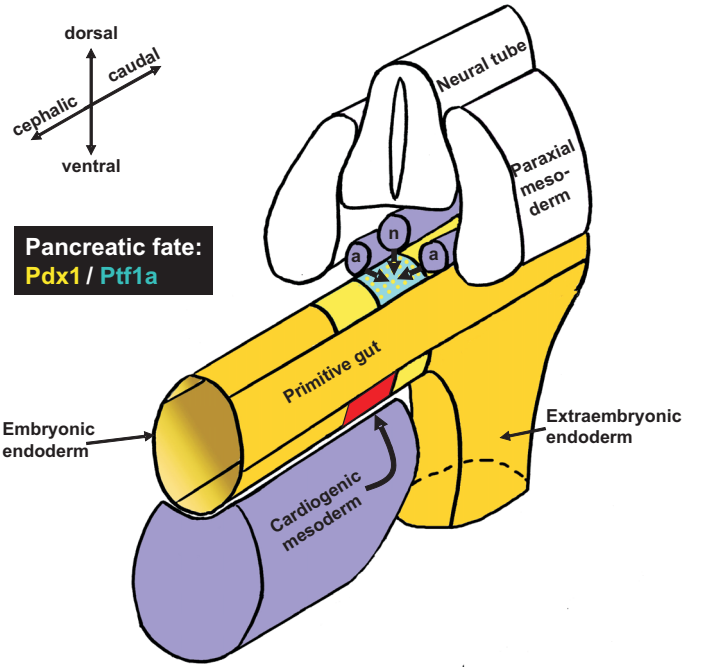

Fig. 2. Schematic view of the foregut/midgut endodermal junction of an E8.5 mouse embryo, oriented along the cephalo-caudal axis. *The dorsal endoderm is programmed towards the pancreatic fate in response to mesodermal factors (secreted from the notochord and aortae, in purple); this dorsal endoderm is characterized by the coexpression of Pdx1 (in yellow) and Ptf1a (in blue). Ventrally, the cardiogenic mesoderm defines the presumptive liver (red) in the ventral endoderm. Adjacent to it, at the leading edge of the embryonic endoderm, the ventral pancreatic primordium, which only expresses Pdx1, is specified by unknown mechanisms. n, notochord; a, aortae.*

stomach, the ventral and dorsal pancreas and the rostral duodenum. The spatial specificity of *Pdx1* transcription was revealed by deleting the enhancer region I-II-III of *Pdx1* promoter (Fujitani *et al.*, 2006). The homozygous loss of region I-II-III aborted the ventral budding, while the dorsal bud became hypoplastic, leaving the stomach and duodenum unaffected. The hemizygous deletion of the same region only affected the maturation of endocrine progenitors, without any other alteration. Intriguingly, these phenotypes were not complementary.

Until E16.5, the whole pancreatic epithelium expresses *Pdx1*, but its requirement is different throughout development. Using an inducible and reversible transgenic model of *Pdx1* repression, Holland *et al.* showed that *Pdx1* expression is required for early pancreas morphogenesis between E10.5 and E12.5 (Holland *et al.*, 2002). In addition, Pdx1 is necessary from E13.5 for the genesis of exocrine tissue. Indeed, in absence of *Pdx1*, *Ptf1a* induction is suppressed, which alters the normal expression of acinar markers (Hale *et al.*, 2005). These results demonstrate that Pdx1 induces *Ptf1a* activity during early pancreas morphogenesis, and this is critical for the maturation of the exocrine compartment.

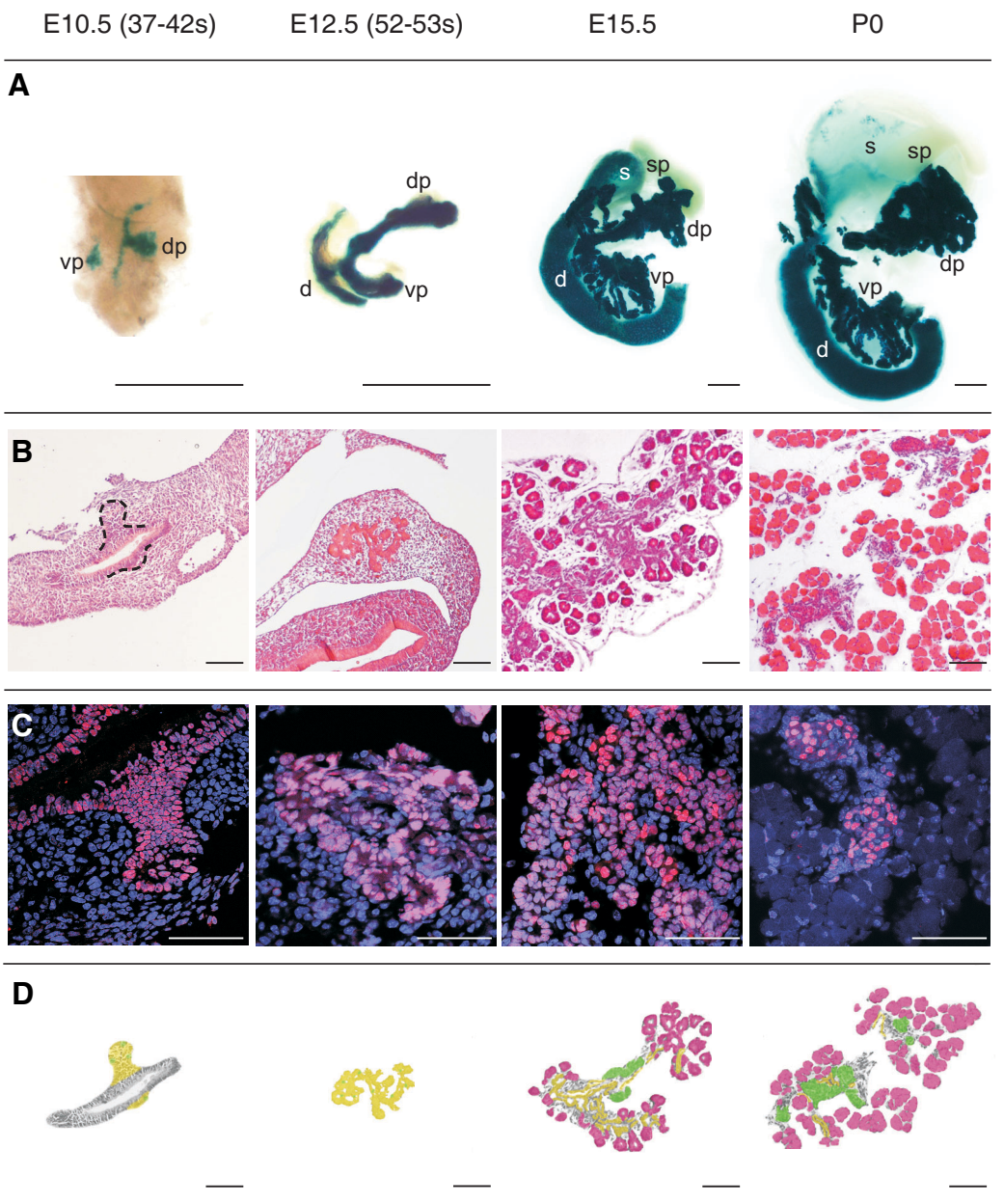

Fig. 3. Growth and expansion of the pancreatic epithelium at E10.5, E12.5, E15.5 and birth. (A) *β-galactosidase activity in* Pdx1-cre; R26R *(Herrera, 2000, Soriano, 1999) pancreas reveals the* Pdx1 *expression domain in the ventral (vp) and dorsal (dp) primordia, as well as in the caudal stomach (s) and the proximal duodenum (d). Calibration bar, 1 mm.* **(B)** *Hematoxylin-eosin stained paraffin sections. From E10.5, the pancreatic epithelium grows by branching morphogenesis, thus forming the ductal tree, from which the endocrine cells originate and delaminate to eventually form the islets within the interstitia between growing ducts. Acinar cells differentiate at the tips of the branches. Calibration bar, 100 μm.* **(C)** *Immunodetection of Pdx1. From E10.5,* Pdx1 *is widely expressed throughout the pancreatic epithelium but is progressively restricted to β-cells after birth. From E15.5, Pdx1^{High} cells are differentiating β-cells. Calibration bar, 50 μm.* **(D)** *Schematic view of differentiating cells of the endocrine (green) and acinar (pink) lineages, adjacent to the primitive or mature ductal cells (yellow). Calibration bar, 100 μm.*

Induction of Pdx1 in the dorsal endoderm

Two homeobox transcription factors, Hb9 and Isl1, are required for the initial induction of Pdx1 in dorsal pancreatic primordia. Later, persistence of *Pdx1* expression depends on FGF10 secretion by the dorsal mesoderm, which is ensured by its blood vessels.

The homeobox transcription factor Hb9 is encoded by the *Hlxb9* gene. During mouse development, *Hlxb9* expression starts at E8 in the notochord, the entire dorsal gut endoderm and the ventral endoderm. During pancreas development, *Hlxb9* expression is transient. Hb9 appears first in the ventral bud at E8, concomitant with *Pdx1* expression. Afterwards, *Hlxb9* is expressed in the dorsal bud prior to the dorsal induction of *Pdx1*. Between E10.5 and E12.5, its expression declines in both buds, but later is expressed in mature β cells.

Hb9 is an essential intrinsic signal for dorsal pancreas evagination as well as for initiation of the pancreatic program. The in-

activation of *Hlxb9* affects selectively the emergence of the dorsal bud and the dorsal activation of *Pdx1*, such that the ventral bud develops normally, although with altered islet architecture. In addition, maturation of β-cells is impaired: glucose transporter 2 (Glut2) is absent (Harrison *et al.*, 1999).

The requirement of Hb9 during pancreas development is transient. This was shown by ectopically expressing *Hlxb9* in *Pdx1*+ cells: at E15.5, the mutant pancreas was small and poorly branched because the pancreatic epithelium and adjacent mesenchyme adopted an intestinal-like differentiation program (Li and Edlund, 2001). This suggests a non-cell autonomous effect for *Hlxb9* on the surrounding mesoderm.

The second factor determinant for dorsal *Pdx1* induction is the LIM homeobox transcription factor Isl1. It was initially identified as a transcription factor binding the insulin gene enhancer region. During pancreas development, it is expressed from 15-16s (E9.0) in the dorsal pancreatic epithelium and in the gut mesenchyme (Ahlgren *et al.*, 1997). By 20-25s, *Isl1* expression becomes restricted to endocrine and mesenchymal cells of the dorsal bud.

Isl1 plays a pancreas development-promoting role, both as an extrinsic factor produced by the dorsal mesenchyme, and intrinsically expressed in pancreatic progenitors. The inactivation of *Isl1* leads to the selective depletion of the dorsal mesenchyme, associated with altered *Pdx1* expression in the dorsal epithelium (Ahlgren *et al.*, 1997). Since *Isl1-/-* embryos die at E9.5, pancreas differentiation was further analyzed in cultured explants. In absence of *Isl1*, no endocrine cells differentiate. In addition, exocrine cells appear only in the ventral bud. The depletion of acinar cells in the dorsal primordium was due to impaired development of dorsal mesenchyme in a non-cell autonomous effect, because *Isl1-/-* dorsal buds cocultured with wild type dorsal mesoderm only develop acinar cells, but not endocrine cells (Ahlgren *et al.*, 1997). This suggests that *Isl1* expression in the dorsal mesenchyme is required for its maintenance and, indirectly, for exocrine pancreas differentiation, whereas *Isl1* expressed in pancreatic progenitors is necessary for endocrine pancreas differentiation.

Induction of Pdx1 *in the ventral endoderm*

The homeobox transcription factor *Hex*, of the Antennapedia/Fushi Tarazu class, is expressed from E7.0 in the ventral-lateral foregut, which gives rise to the ventral pancreas and liver.

Hex inactivation inhibits the expansion and the anterior displacement of the ventral-lateral embryonic endoderm, due to a deficient proliferation of endodermal cells (Bort *et al.*, 2004). In absence of this morphogenetic movement, endodermal cells accumulate near the cardiac mesoderm, and thus begin the hepatic differentiation program. For this reason, the presumptive pancreatic endoderm does not form and there is no ventral induction of *Pdx1* or *Ptf1a* (Bort *et al.*, 2004). Interestingly, however, the ventral-lateral endoderm isolated from E8 *Hex-/-* embryos fully commits towards pancreatic fates *in vitro* (Bort *et al.*, 2004). These results suggest that Hex is not required for the specification of the ventral pancreatic fate, but for the proper location of pancreatic progenitors in the leading-edge of the ventral embryonic endoderm, which can then escape the influence of mesenchymal inhibitors.

Pancreas specific transcription factor1a (Ptf1a, p48)

The basic helix-loop-helix Pancreas specific transcription factor1 (Ptf1) is composed of 3 different subunits: p75, for nuclear translocation, and two heterodimeric bHLH DNA-binding subunits, p64 and Ptf1a (also called p48). *Ptf1a* mRNA is detected from E9.5 (the protein from E10) in the whole primordia. From E16, its expression becomes restricted to acinar cells.

Genetic cell tracing analyses confirmed that Ptf1a is a bona fide pancreatic marker, even better than Pdx1, which is expressed earlier but not exclusively in the pancreas. The role of Ptf1a in pancreatic specification was shown using a *Ptf1a* promoter driving the expression of *Pdx1* in a *Pdx1* knock out background: pancreas development was almost normal, with partial restoration of exocrine, ductal and endocrine cells (Kawaguchi *et al.*, 2002). *Ptf1a* expression is induced in the dorsal pancreatic bud by Pdx1. The ventral induction of *Ptf1a* remains unexplained.

Interestingly, the inactivation of *Ptf1a* selectively affects the exocrine compartment. The exocrine progenitors are reprogrammed into duodenal fates as shown by cell tracing analysis (Kawaguchi *et al.*, 2002). Directly or indirectly, *Ptf1a* inactivation also affects the endocrine pancreas, leading to the relocation of the scarce endocrine cells within the spleen. These results suggest that Ptf1a is somehow involved in both endocrine and exocrine lineages.

Maintenance of uncommitted pancreatic progenitors

The initial pancreatic diverticuli are made of undifferentiated epithelial cells that proliferate and branch, invading the surrounding mesoderm. Cell specification and commitment occur through a sequential activation of genes (Fig. 4). The initial commitment into specified progenitors, whether endocrine or exocrine, is repressed by active Notch signaling and Sox9 activity, which promote progenitor expansion at the expense of their differentiation. Later, commitment towards endocrine or exocrine fates is modulated by active TGFβ signaling and by Prox1, which prevent exocrine commitment and favor the endocrine fate.

Notch signaling

The Notch pathway mediates the control of progenitor self-renewal. In the pancreas, Notch signaling controls the maintenance of progenitors in an uncommitted state, ensuring the expansion of $Pdx1^+$ progenitors up to E12.5. In this way, Notch signaling influences the final size of the pancreas (Apelqvist *et al.*, 1999). Therefore, disruption of Notch signaling triggers the premature differentiation of pancreatic progenitors (Apelqvist *et al.*, 1999).

Sox9

Members of the Sry related "high mobility group" (HMG) box (Sox) transcription factor family participate in the maintenance of undifferentiated progenitor cells in different organs, such as the central nervous system or the intestinal epithelium.

Among the Sox factors expressed in the developing pancreas, Sox9 is present in *Pdx1*-expressing progenitors from E9.5 (Seymour *et al.*, 2007). By E15.5, *Sox9* expression becomes restricted to a subset of Notch-responsive and mitotically active $Pdx1^+$ cells (Seymour *et al.*, 2007). During progenitor cell specification, *Sox9* expression mostly disappears, but persists in the adult, in centroacinar cells and in few ductal cells (Seymour *et al.*, 2007).

The conditional inactivation of *Sox9* in pancreatic Pdx1$^+$ progenitors impairs organ growth from E11.5 (Seymour *et al.*, 2007). At E18.5, the hypoplastic mutant pancreas is depleted of endocrine cells, whereas the small exocrine compartment presents defective differentiation. In absence of *Sox9*, the pool of progenitors is reduced due to increased apoptosis, decreased proliferation and premature differentiation (Seymour *et al.*, 2007). Inversely, *Fgf10* overexpression in *Pdx1*-expressing progenitors induces the expansion of undifferentiated cells, which bear Notch receptors and express *Sox9* (Seymour *et al.*, 2007).

Transforming growth factorβ (TGFβ) signaling

The developing and adult pancreas expresses TGFβ signaling ligands, like *TGFβ1, activins and bone morphogenetic proteins (Bmps)*. They are produced by epithelial cells and affect cell commitment and differentiation in paracrine ways.

TGFβ signaling activity appears to be critical for pancreas growth and differentiation. The expression of *Bmp4* in Pdx1$^+$ expressing progenitors leads to pancreas agenesis. Inversely, *Smad7* overexpression in Pdx1$^+$ progenitors impairs growth and differentiation of endocrine and acinar cells.

In addition to its role in the maintenance of unspecified pancreatic progenitors, TGFβ signaling contributes to determine the ratio of the three epithelial pancreatic cell types, favoring the endocrine lineage without affecting the ductal cell mass (Sanvito *et al.*, 1994). The disruption of TGFβ signaling in *Bmp11-/-* or *Smad2+/-* mice, results in altered proportions of acinar and endocrine cells (Harmon *et al.*, 2004).

Prospero-related homeobox transcription factor1 (Prox1)

Prox1 is first expressed at E7.5 in endodermal cells. At E8.5 (10-12s), it is expressed in the presumptive hepatic endoderm and, at 15-18s, in the dorsal pancreas. Prior to the outgrowth of the pancreatic buds, at E9.5, *Prox1* is expressed in early Pdx1$^+$ cells. By E13.5, it is expressed in Ngn3$^+$ endocrine progenitors. Finally, from E15.5, Prox1 is specifically expressed in endocrine, ductal and centroacinar cells, while it is excluded from acinar cells (Wang *et al.*, 2005).

Prox1 acts during the commitment of the different pancreatic lineages by promoting the endocrine fate. The targeted inactivation of *Prox1* hinders pancreas growth: at E11.5 the pancreatic epithelium is small and poorly branched (Wang *et al.*, 2005). In E13.5 mutant pancreas, both endocrine Ngn3$^+$ progenitors and hormone-expressing cells are reduced (Wang *et al.*, 2005).

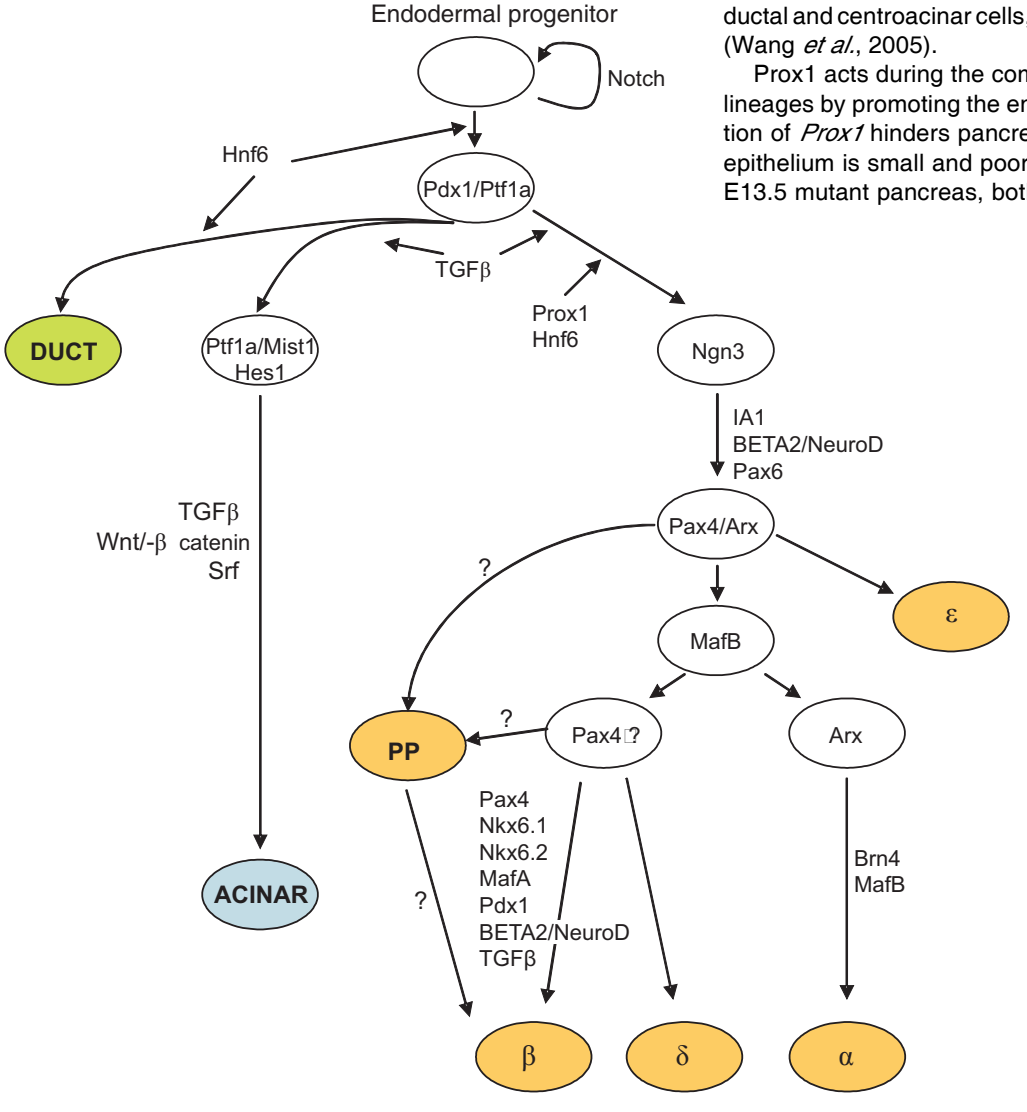

Fig. 4. Simplified model of transcription factor requirement during the specification of endocrine and exocrine pancreas.

Specification of endocrine progenitors

Very early in pancreatic primordia, the first wave of endocrine cell generation, so-called "first transition", is characterized by the appearance of glucagon- (E9.5), pancreatic polypeptide family- (E10.5), insulin- (E10.5) and ghrelin-producing cells (E10.5) within the primitive ductal epithelium (Fig. 3D). These early hormone-expressing cells are different from other endocrine cells appearing during the "secondary transition" (E13.5-E15.5), as they probably do not contribute to the endocrine cell pool of mature pancreas. Originating from specific progenitors expressing *Ngn3*, the endocrine cells at the "secondary transition" differentiate, while migrating and grouping to form islet-like clusters at E16, and finally islets from E18-19, during the "third transition". The specification into the mature endocrine cell types relies on the activity of early factors involved in the segregation of the endocrine lineages (IA1, Arx, Pax4, Nkx2.2,

Nkx6.1, MafB), and of late factors involved in the maturation of committed endocrine cells (Pax6, Isl1, Pdx1, Brn4, Hb9, MafA).

Hepatocyte nuclear factor6 (Hnf6)

Hnf6 activates *Pdx1* expression and is involved in endocrine commitment/differentiation, but is absent in mature endocrine cells. In *Hnf6-/-* animals there is a drastic downregulation of *Ngn3* expression, which therefore affects the differentiation of all endocrine cell types (Jacquemin *et al.*, 2000). Mice are viable, but intolerant to glucose. Endocrine cells appear scattered and become arranged in islets only 2-3 weeks after birth, with altered architecture and impaired β-cell differentiation (Jacquemin *et al.*, 2000).

Neurogenin3 (Ngn3)

The bHLH transcription factor *Ngn3* is expressed from E8.5 in the pancreatic endoderm, peaking at E15.5 and diminishing at birth (Apelqvist *et al.*, 1999). It is transiently co-expressed with *Nkx6.1* and *BETA2/NeuroD1* prior to hormone production. Cell tracing studies demonstrated that while all pancreatic endocrine cell derive from progenitors having expressed *Ngn3* during development, the early glucagon- and insulin-expressing cells formed before the "secondary transition" originate independently of *Ngn3*-expression. Whether *Ngn3* expression persists after birth is controversial. Two reports, using *in situ* hybridization and immunohistochemistry, failed to detect *Ngn3* expression in the adult pancreas (Jensen *et al.*, 2000, Schwitzgebel *et al.*, 2000). On another hand, cell tracing analyses designed to tag *Ngn3*+ cells only in the adult, revealed the presence of few *Ngn3*-expressing cells, devoid of hormone expression, in some islets. Their maintenance in the adult suggests that they might contribute to islet cell renewal.

Inactivation of *Ngn3* induces islet agenesis (Gradwohl *et al.*, 2000). Expression of islet-specific transcription factors (*Isl1, Pax4, Pax6 and BETA2/NeuroD1)* is suppressed in *Ngn3*-null pancreas, suggesting that *Ngn3* acts upstream of these factors. The ectopic expression of *Ngn3* in the presumptive endodermal regions of stomach and duodenum is sufficient to initiate the endocrine program, with the differentiation of almost only α-cells (Apelqvist *et al.*, 1999).

Ngn3 expression is repressed by active Notch signaling (Apelqvist *et al.*, 1999). Thus, Notch disruption leads to premature expression of *Ngn3* and accelerated endocrine differentiation (Apelqvist *et al.*, 1999). In addition, *Ngn3* expression is also modulated by Hnf6 (Jacquemin *et al.*, 2000).

The mechanisms involved in endocrine specification downstream of *Ngn3* are still to be defined. Recently, Johansson *et al.* studied whether all Ngn3$^+$ cells are alike at different developmental stages, i.e. whether their competence over time is maintained or not (Johansson *et al.*, 2007). Using a system to precisely activate Ngn3 in *Pdx1*-expressing progenitors at different timepoints, they showed that from E11.5, the induced *Ngn3* activity favored the differentiation towards β- and PP- cell phenotypes, while *Ngn3* induction from E14.5 promoted δ-cell differentiation (Johansson *et al.*, 2007).

IA1

The *Insulinoma-associated 1 (Insm1)* gene, which encodes the zinc-finger transcription factor IA1, is a direct target of *Ngn3* : IA1 is absent in the *Ngn3*-null pancreas, but is unaffected in mutants for *BETA2/NeuroD1, Arx , Pax4* and *Pax6* (Mellitzer *et al.*, 2006). Ectopic *Ngn3* expression in pancreatic ductal cells induces *Insm1* prior to *Pax4, BETA2/NeuroD1* and *Nkx2.2* (Mellitzer *et al.*, 2006). During pancreatic development, IA1 appears in scattered cells from E10.5 and is expressed in most of Ngn3$^+$ cells at E15.5 (Mellitzer *et al.*, 2006). In developing and mature pancreas, *Insm1* expression is excluded from the exocrine lineage (Mellitzer *et al.*, 2006, Gierl, 2006 #2439). Whether *Insm1* expression persists in postnatal islet cells is controversial (Mellitzer *et al.*, 2006, Gierl, 2006 #2439).

Insm1 expression by it-self is not sufficient to drive the endocrine program, but it promotes the effect of Ngn3 (Mellitzer *et al.*, 2006). The few endocrine cells formed in *insm-/-* embryos have a deficient maturation at E15.5. In β-cells, the expression of *Nkx6.1* and *Proconvertase1/3* is downregulated, while MafA is absent. In α-cells, the activity of *MafB*, *Arx* and *Pax6* is also downregulated (Gierl *et al.*, 2006).

NKX transcription factors

The Nkx protein family consists of a large group of homeodomain transcription factors. In the developing pancreas, three Nkxs, 2.2, 6.1 and 6.2 are regulators of endocrine pancreas development.

Nkx2.2

From E9.5, *Nkx2.2* expression is wide in the pancreatic region. After E12.5, its expression becomes restricted to a subset of Ngn3$^+$ cells (Sussel *et al.*, 1998) and persists in the adult, in differentiated α-, β- and PP-expressing cells.

Nkx2.2 is required for β-, α- and PP-cell commitment and differentiation. In *Nkx2.2-/-* newborns, β-cells are absent and α- and PP-cells are reduced, while ε-cell numbers increase (Sussel *et al.*, 1998). In mutant islets, the majority of endocrine cells have impaired differentiation (Sussel *et al.*, 1998).

Nkx2.2 and *Nkx6.1* (see below) co-express in Ngn3$^+$ progenitors and in mature β-cells, which suggests that they may cooperate together during β-cell differentiation. Indeed, the epistatic activity of *Nkx2.2* on *Nkx6.1* was demonstrated by comparing single and double knock-out phenotypes (Sander *et al.*, 2000).

Nkx6.1 and Nkx6.2

Their high degree of homology suggests that there is functional redundancy between 6.1 and 6.2. However, *Nkx6.1* and *Nkx6.2* expression domains are distinct. Nkx6.1 is first detected in E10.5 pancreatic epithelium, only in Ngn3$^+$ progenitors and then in adult β-cells (Sander *et al.*, 2000). *Nkx6.1* expression is suppressed in *Pdx1*-/- pancreata and, inversely, it is ectopically induced after *Pdx1* overexpression. Early insulin- and glucagon-positive cells do not express *Nkx6.1*.

Nkx6.2 is expressed like *Nkx6.1* through the pancreatic epithelium from E10.5. By E15.5, it is expressed in α- and acinar cells, and is excluded from *Ngn3*+ cells, but around birth its expression disappears (Sander *et al.*, 2000).

The disruption of *Nkx6.1* reveals its involvement in β-cell proliferation, specifically after E12.5, leading to a decreased β-cell mass (Sander *et al.*, 2000). In absence of Nkx6.1, *Nkx6.2*-expressing cells are more numerous, while keeping a normal spatial and temporal expression pattern, which suggests a potential functional compensation (Henseleit *et al.*, 2005). In absence

of *Nkx6.2*, the pancreas develops normally; however, the co-inactivation of *Nkx6.2* and *Nkx6.1* reveals its requirement for the normal proliferation of β- and α-cells. In *Nkx6.1-/-; Nkx6.2-/-* pancreata, the number of Ngn3$^+$ progenitors is conserved, as well as that of δ- and PP-cells, but β- and α-cell numbers are reduced (Henseleit *et al.*, 2005).

Pax4 and Arx

In the developing mouse pancreas, both *Pax4* and *Arx* are expressed from E9.5 (Collombat *et al.*, 2003, Sosa-Pineda *et al.*, 1997). Even if *Arx* and *Pax4* overlap at E13.5 in *Ngn3*-expressing progenitors, they become mutually exclusive from E18.5. Pax4 is restricted to progenitors differentiating into β- and δ- cells, whereas *Arx* is expressed in progenitors differentiating into α- and ε-cells (Collombat *et al.*, 2003). Postnatally, *Pax4* is not expressed, or at very low levels in β-cells and *Arx* expression persists in mature α-cells.

Pax4 and *Arx* act downstream of *Ngn3* within a regulatory network that determines the final proportions of the different endocrine cell types. Pax4 and Arx have opposite roles. According to their expression pattern, Pax4 promotes the commitment of Ngn3$^+$ progenitors into β- and δ-lineages, whereas Arx promotes α- and ε-lineages. *Pax4-/-* islets are composed of α- and ε-cells, and lack β- and δ-cells (Sosa-Pineda *et al.*, 1997). Similarly, *Arx -/-* islets are composed of β- and δ-cells exclusively (Collombat *et al.*, 2003).

The expression of *Pax4* is directly promoted by Ngn3 and Hnf1α, and is repressed by Arx (Collombat *et al.*, 2003). Pax4 and Arx inhibit each other directly (Collombat *et al.*, 2003). In *Pax4-/-* pups, *Arx* expression is upregulated, while in *Arx -/-* newborns *Pax4* is upregulated. Interestingly, the double inactivation of *Pax4* and *Arx* results in a normal number of endocrine cells, which produce somatostatin, and when pups start suckling, these cells begin to co-express PP (Collombat *et al.*, 2005).

Musculo-aponeurotic fibrosarcomaB (MafB)

MafA and MafB are members of Musculo-aponeurotic fibrosarcoma family of leucine zipper (ZIP) transcription factors. They activate *Insulin* and *glucagon* transcription. Both Mafs are involved in the maturation of committed endocrine cells: MafA in β-cell differentiation and MafB in the lineages of both α- and β-cells (Nishimura *et al.*, 2006).

MafB is selectively expressed in mature α-cells, independently of Pax4 and Pax6 (Artner *et al.*, 2006), but its expression pattern during development reveals a role for MafB in the lineages of both α- and β-cells (Nishimura *et al.*, 2006). During pancreas development, the first insulin-cells express *MafB* and then switch to *MafA* instead, after *Nkx6.1* and *Pdx1* induction, like mature β-cells.

Maintenance of islet cell identity

Differentiated endocrine cells maintain their characteristics thanks to the permanent expression of maturation genes. The transcription factor Pax6 is necessary for the expansion and maturation of all endocrine cells, and for islet organization. TGFβ signaling controls the number and differentiation of β-cells, by promoting their differentiation. Three transcription factors, MafA, Pdx1 and BETA2/NeuroD, participate in the transcription of *Insulin* and together they represent a "molecular signature" of β-cell identity. Concerning α-cell identity, the transcription factor Brn4 is determinant for *glucagon* expression.

Paired homeobox transcription factor6 (Pax6)

Pax6 is expressed in scattered cells from E9.0 and becomes restricted to islet cells after birth. With a genetic cell tracing analysis it was shown that Pax6 is expressed in the progenitors of all islet cell types.

Pax6 is involved in the expansion of all islet endocrine cell types. Its inactivation reduces the number of endocrine cells, in particular that of α-cells. The few endocrine cells that differentiate, are clustered into disorganized islets, and produce quantitatively less hormones. The late requirement of Pax6 in endocrine cell maturation was demonstrated by its conditional inactivation in *Pax6*-expressing cells only (Ashery-Padan *et al.*, 2004). In *Pax4-/-; Pax6-/-* double mutants, the adult pancreas is completely devoid of endocrine cells, a phenotype which mimics the combined phenotypes of each simple mutant. Thus, cells expressing only *Pax6* differentiate into α-cells and cells co-expressing *Pax4* and *Pax6* differentiate into β-, δ-, ε- and PP-cells (Collombat *et al.*, 2003).

Maintenance of β-cell identity

TGFβ signaling

TGFβ modulates the proportions of acinar and endocrine cells, and promotes the differentiation of β-cells (Harmon *et al.*, 2004).

Disruption of TGFβ signaling results in the accelerated formation and accumulation of Ngn3$^+$ progenitors, with impaired β-cell differentiation (Harmon *et al.*, 2004). In these conditions, the islet mass is slightly increased, and composed of defective β-cells. In *Gdf11-/-* pancreata, β-cells are immature: they express *Nkx6.1* but not *Insulin* (Harmon *et al.*, 2004). Similarly, the conditional expression of the inhibitory *Smad7* in adult β-cells impairs the expression of the β-cell markers, *Insulin, MafA, Menin* and its target gene, *p27^{Kip1}*, associated with diabetes onset (Smart *et al.*, 2006).

Downstream of TGFβ signaling, the transcription factor Klf11 activates the glucose-induced transcription of *Insulin*. Mutations of this factor impair its transcriptional activity, and are associated with human early-onset type II diabetes (Neve *et al.*, 2005).

MafA

MafA is restricted to β-cells in adult pancreas. MafA is an activator of *Insulin*, binding to the enhancer elements RIPE3b/C1-A2 of the insulin promoter, in response to glucose. The other transcription factors interacting with the conserved insulin enhancer elements are Pdx1, which binds the A3 box, and BETA2/NeuroD1, which binds the E1 element.

MafA is involved in the maintenance of β-cell identity. *MafA* inactivation does not alter β-cell development, but in adult knock-out mice, the β-cell mass becomes dysfunctional, such that there is diabetes onset 12 weeks after birth. The expression of β-cell markers such as *Insulin1, Insulin2, Pdx1, BETA2/NeuroD1* and *Glut2* is downregulated and the β-cell mass decreases due to β-cell apoptosis (Zhang *et al.*, 2005). This late phenotype suggests that MafA is not necessary for embryonic

pancreas development but is required for the maintenance of a functional β-cell mass.

In the program of endocrine differentiation, *MafA* is located downstream of *Pax4* and *Pax6* (Sosa-Pineda, 2004). In turn, MafA contributes to activate *Pdx1* expression and thus to maintain the mature β-cells.

BETA2/NeuroD

The bHLHL transcription factor BETA2/NeuroD is a potent transcriptional activator of *Insulin*, by binding to the E1 box of the RIP3b/C1-A2 enhancer of the insulin promoter. *BETA2/NeuroD* is expressed from E9.5 in scattered cells of the pancreatic epithelium and from E14.5, in *Ngn3*-expressing cells. After birth, its expression is restricted to β-cells.

BETA2/NeuroD is a downstream target of Ngn3, involved in the formation and maturation of the β-cell mass. *BETA2/NeuroD-/-* pancreata have impaired islet morphogenesis, with a reduction in the number of endocrine cells, especially β-cells, as a consequence of increased apoptosis from E17 (Naya *et al.*, 1997).

Pdx1

The bHLH transcription factor Pdx1 is also one of the activators of *Insulin* expression, binding to the A3 box of the RIP3b/C1-A2 enhancer of the insulin promoter. *Pdx1* expression is almost restricted to β-cells from E19.0, and is necessary for the transcriptional regulation of many β-cell markers, such as *Glut2* and *Glucokinase* (Serup *et al.*, 1995).

The conditional repression of *Pdx1* inhibits insulin expression and induces diabetes 14 days later (Holland *et al.*, 2005). Following derepression of *Pdx1*, normoglycemia is restored in 28 days. During this period, a regenerative program is induced, with compensatory neogenesis of β-cells and induction of genes activated during regeneration such as *Regenerating islet-derived (Reg)* genes (Holland *et al.*, 2005).

Hedgehog signaling

Hh signaling is necessary to maintain β-cell identity *in vitro*, whereas during development, it inhibits the pancreatic specification of the endoderm (Thomas *et al.*, 2000). Primary isolated β-cells and INS1 cells both express *Hedgehog* ligands and the receptor *Ptc1* (Thomas *et al.*, 2000). Hedgehog signals activate *Pdx1* transcription, which in turn stimulates *Insulin* expression (Thomas *et al.*, 2001). Inversely, cyclopamine treatment (Smoothen inhibitor) inhibits *Pdx1* and *Insulin* transcription (Thomas *et al.*, 2001).

Maintenance of α-cell identity

Brn4

The *POU-homeobox transcription factor 4 brain4/POU3F4 (Brn4)* is expressed from E10.5 in early glucagon$^+$ cells. Some of *Brn4*+ cells coexpress *Pax6* and *Isl1* (Heller *et al.*, 2004). At E14.5, *Brn4* expression is restricted to α-cells, but not all glucagon-expressing cells coexpress *Brn4*, suggesting that Brn4 may be a marker of α-cell progenitors (Heller *et al.*, 2004). Perinatally, *Brn4* expression is maintained in all α-cells and sometimes in PP-cells (Heller *et al.*, 2004).

Brn4 is dispensable for α-cell identity, but sufficient for *glucagon* expression. In absence of Brn4, commitment and differentiation of α-cells during development are not affected (Heller, 2004). However, *Brn4* overexpression in β-cell lines is sufficient to induce glucagon expression.

Specification of the exocrine lineage

During pancreas development, by E14.5, cells budding from the tips of ductal branches commit into the acinar fate (Fig. 3D). From E16.5, these cells become polarized and begin storing zymogen granules, which contain digestive enzymes. Acinar cells become arranged into acini and are fully mature shortly after birth.

Terminally differentiated acinar cells have a highly developed rough endoplasmic reticulum producing a large amount of digestive enzymes. These are packed within zymogen granules, which are stored in the apical pole of the cells. Upon stimulation, exocytosis of zymogen granules is initiated, releasing their content into the lumen of the acinus. Exocytosis is regulated by acetylcholine, which is secreted by the autonomous nervous system, and during food ingestion and transit, by enteric hormones from the stomach (gastrin) and the duodenum (secretin and cholecystokinin). Exocrine secretion is coordinated between acinar cells through the activity of gap junctions, which are formed by hexameric complexes of acinar-specific connexins (connexin 26 and connexin 32).

The genetic program directing the specification and differentiation of exocrine progenitors remains elusive (Fig. 4). The establishment of the exocrine mass depends on the number of *Pdx1*+ progenitor cells, and on Pdx1 levels from E13.5 (Stanger *et al.*, 2007). Two transcription factors, Ptf1a, Mist1, are required to determine the exocrine fate, but are not sufficient. Wnt/β-catenin and Notch signaling pathways participate in the expansion and differentiation of exocrine progenitors. In the adult, maintenance of the acinar cell mass relies on the activity of protective genes such as *Srf*. More specifically, Hnf6 is required for the ductal lineage.

Pdx1

Pdx1 activity is specifically required for the exocrine lineage from E13.5 (Holland *et al.*, 2002). Upon *Pdx1* repression from E13.5, the exocrine tissue is reduced and composed of immature cells. The defective ductal cells maintain *Glut2* expression, and immature acinar cells fail to express *Ptf1a*, altering the normal expression of acinar markers, such as *Cpa1* and *Amylase*. *Pdx1* expression is thus required for *Ptf1a* induction, for early pancreas morphogenesis and, from E13.5, for the commitment and maturation of the exocrine compartment.

Ptf1a (p48)

Ptf1a / p48 is required for the transcription of acinar specific genes, such as *elastase1, α-Amylase2* and *Chymotrypsinogen B* (Cockell *et al.*, 1989). In absence of Ptf1a, these genes are still expressed but less efficiently, demonstrating that Ptf1a is necessary, but not sufficient, to drive exocrine specific gene expression (Cockell *et al.*, 1995).

Mist1

The bHLHL transcription factor *Mist1* is specifically expressed in the lineage of serous secretory cells of the pancreas, the parotid

and submandibular salivary. During pancreas development, *Mist1* expression begins at E10.5 in a subset of primitive foregut cells. From E13 and throughout life, *Mist1* is expressed in developing and mature acinar cells (Pin *et al.*, 2001).

The role of Mist1 was investigated in *Mist1-/-* mice or by overexpressing a dominant negative form of *Mist1* (*Mist* ^{MB}) in acinar cells (Pin *et al.*, 2001, Zhu, 2004). The disruption of *Mist1* does not alter the prenatal development of the pancreas. However, adult *Mist1-/-* and *Mist* ^{MB} pancreata present a disorganization of acini, with a normal endocrine compartment. The acini are composed of poorly differentiated acinar cells, with few abnormal and misplaced zymogen granules, and displaying signs of stress such as cytoplasmic vacuolization and nuclear dysplasia (Pin *et al.*, 2001). In *Mist1-/-* acinar cells, the exocytosis machinery is defective (Pin *et al.*, 2001). The cholecystokinin (CKK) signaling pathway, which mediates the regulated secretion of zymogen granules, is altered. In addition, the expression of *Connexin 32* is lost, thus hampering acinar gap junction formation (Rukstalis *et al.*, 2003). The impaired maturation of acinar cells is associated with intracellular activation of the proenzyme carboxypeptidase A1 (Pin *et al.*, 2001). Indeed, with aging, mutant pancreata have features of chronic pancreatitis, namely fibrosis, necrosis, metaplastic ducts and bleeding. These results indicate that Mist1 is required for the terminal differentiation of acinar cells, ensuring their functional stability and their maintenance in the adult.

Wnt/β-catenin signaling

Wnt/β-catenin signaling is required for the expansion and differentiation of the acinar lineage. Wnt/β-catenin players are expressed in the developing pancreas from E12.5. Cytoplasmic β-catenin is present in all cells of E11.5-E13.5 pancreatic epithelia, and declines between E15.5-E17.5 to finally disappear at birth.

Since *β-catenin*-deficient mice die around E6.5, conditional genetic approaches were used to define the spatial and temporal requirement of this signaling pathway in pancreas development. The disruption of Wnt/β-catenin obtained by the inactivation of *β-catenin*, or by overexpressing a dominant negative form of *Frz8* in Pdx1^{+} progenitors, leads to the selective hypoplasia of the postnatal exocrine pancreas (Murtaugh *et al.*, 2005, Papadopoulou and Edlund, 2005). This reduction is due to reduced proliferation, concomitant with *c-Myc* downregulation (Murtaugh *et al.*, 2005). In absence of Wnt/β-catenin signaling, the acinar differentiation is impaired from E16.5 (Wells *et al.*, 2007). Like in adult *Mist1* -/- mutants, the few and dysfunctional acinar cells degenerate and lead to tissue remodelling by the age of two months (Pin *et al.*, 2001, Wells *et al.*, 2007).

The stabilization of β-catenin or the inactivation of *Apc* in *Pdx1*-expressing progenitors leads to constitutively active β-catenin signaling. This results in pancreatomegaly due to specific enlargement of the postnatal exocrine pancreas (Heiser *et al.*, 2006, Strom *et al.*, 2007). The expanded acinar mass presents a *c-Myc*-dependent increased proliferation of mature acinar cells (Strom *et al.*, 2007).

Strom *et al.* have determined the existence of a temporal control of β-catenin signaling in acinar cells (Strom *et al.*, 2007).

TABLE 1

EXPRESSION PATTERN OF GENES INVOLVED IN PANCREAS DEVELOPMENT AND HOMEOSTASIS

Gene	Expressed from	in	Restricted from	to	References
Hnf6	E9-E9.5	Pdx1+ cells	E18	acinar and ductal cells	Landry et al. 1997, Rausa et al. 1997
Hlxb9	E8	two pancreatic primordia	E10.5-E12.5	β-cells	Harrison et al. 1999, Li et al. 1999
Isl1	E9-E9.5	dorsal primordium	20-25s	endocrine and mesenchymal cells	Karlsson et al. 1990
Hex	E7	ventral promordium			Bort et al. 2004, Martinez Barbera et al. 2000, Thomas et al. 1998
Pdx1	E8.5	two pancreatic primordia	E16.5	β- and some δ-cells	Jacquemin et al. 2003, Ohlsson et al. 1993, Wright et al. 1989
Ptf1a	E9.5	Pdx1+ cells	E16.5	acinar progenitors and mature cells	Krapp et al. 1996, Obata et al. 2001
Sox9	E9.5	Pdx1+ cells	E15.5	subset of Pdx1+ cells and then to centroacinar and few ductal cells in the adult	Seymour et al. 2007
Prox1	E9.5	Pdx1+ cells	E13.5	Ngn3+ cells and proliferating cells	Burke et al. 2002
Ngn3	E8.5	scattered cells	birth	hormone-negative islet cells	Gu et al. 2002
Insm1	E10.5	Ngn3+ cells	E18.5	all endocrine cells	Gierl et al. 2006, Mellitzer et al. 2006
Nkx2.2	E9.5	two pancreatic primordia	E12.5	subset of Ngn3+ cells and after birth to α-, β- and PP-cells	Schwitzgebel et al. 2000, Sussel et al. 1998
Nkx6.1	E10.5	two pancreatic primordia	E15.5	Ngn3+ and then to the β-cells	Henseleit et al. 2005, Oster et al. 1998, Sander et al. 2000
Nkx6.2	E10.5	two pancreatic primordia	E15.5	α- and acinar cells	Sander et al. 2000
Pax4	E9.5	two pancreatic primordia	E13.5	Ngn3+ and from E18.5 then to the β- and δ-cells	Smith et al. 1999, Sosa-Pineda et al. 1997, Wang et al. 2004
Arx	E9.5	two pancreatic primordia	E13.5	Ngn3+ and from E18.5 then to the α-cells	Collombat et al. 2003
Pax6	E9	scattered Pdx1+ cells	birth	all endocrine cells	St-Onge et al. 1997
MafB	E10.5 E12.5	glucagon+ cells glucagon+ and insulin+ cells	birth	α-cells	Artner et al. 2006, Nishimura et al. 2006
MafA	E10.5	insulin+ cells	-	β-cells	Kataoka et al. 2004, Nishimura et al. 2006
BETA2/ NeuroD	E9.5	scattered Pdx1+ cells	E14.5	Ngn3+ cells and then after birth to the β-cells	Itkin-Ansari et al. 2005, Naya et al. 1997
Brn4	E10.5	glucagon+ cells	E19	glucagon+ and rare PP+ cells	Heller et al. 2004
Mist1	E10.5	scattered cells of the foregut wall	E13	acinar progenitors and mature cells	Pin et al. 2001

Hnf6 (Landry *et al.*, 1997, Rausa *et al.*, 1997); *Hlxb9* (Harrison *et al.*, 1999, Li *et al.*, 1999); *Isl1* (Karlsson *et al.*, 1990); *Hex* (Bort *et al.*, 2004, Martinez Barbera *et al.*, 2000, Thomas *et al.*, 1998); *Pdx1* (Jacquemin *et al.*, 2003, Ohlsson *et al.*, 1993, Wright *et al.*, 1989); *Ptf1a* (Krapp *et al.*, 1996, Obata *et al.*, 2001); *Sox9* (Seymour *et al.*, 2007); *Prox1* (Burke and Oliver, 2002); *Ngn3* (Gu *et al.*, 2002); *Insm1* (Gierl *et al.*, 2006, Mellitzer, 2006 #66); *Nkx2.2* (Schwitzgebel *et al.*, 2000, Sussel *et al.*, 1998); *Nkx6.1* (Henseleit *et al.*, 2005, Oster *et al.*, 1998, Sander *et al.*, 2000); *Nkx6.2* (Sander *et al.*, 2000); *Pax4* (Smith *et al.*, 1999, Sosa-Pineda *et al.*, 1997, Wang *et al.*, 2004); *Arx* (Collombat *et al.*, 2003); *Pax6* (St-Onge *et al.*, 1997); *MafB* (Artner *et al.*, 2006, Nishimura *et al.*, 2006); *MafA* (Kataoka *et al.*, 2004, Nishimura *et al.*, 2006); *BETA2/NeuroD* (Itkin-Ansari *et al.*, 2005, Naya *et al.*, 1997); *Brn4* (Heller *et al.*, 2004); *Mist1* (Pin *et al.*, 2001).

In the absence of *Apc* from E10.5, there is an upregulation of *c-Myc* associated with acinar cell hyperproliferation from birth to six months of age. This suggests that the sensitivity to β-catenin is restricted to mature acinar cells during a window of competence, after which *c-Myc* is downregulated. This explains the lack of tumorigenesis in the pancreas after the loss of *Apc* (Strom *et al.*, 2007), contrary to what happens in other organs with the very same *Apc* mutation.

Notch signaling

Adequate Notch signaling is absolutely required for exocrine pancreas commitment and differentiation.

Alteration of Notch signaling in Pdx1$^+$ progenitors by overexpressing the intracellular domain of *Notch 1 receptor* ($N1^{ICD}$) or *Notch3 repressor* ($N3^{ICD}$), hampers the formation of the pancreatic buds, such that only poorly ramified evaginations form. In these mutants, no *Mist1*-expressing acinar progenitors or amylase-positive cells appear.

TGFβ signaling

Appropriate TGFβ signaling is required for the renewal of acinar cells. The ubiquitous expression of *Activin βE subunit* results in pancreas hypoplasia from two weeks of age (Hashimoto *et al.*, 2006). In these mice, mature acinar cells progressively disappear due to insufficient proliferation, being replaced by adipose tissue. Intriguingly, the reverse experiment, i.e. the disruption of TGFβ signaling by expressing a dominant-negative mutant *type II TGFβ receptor* in acinar cells, also leads to postnatal pancreas hypoplasia: from 5 months of age, acinar cells have increased apoptosis and decreased proliferation. Like in chronic pancreatitis, there is ductal metaplasia and fibrosis, and acini are progressively replaced by adipose tissue (Bottinger *et al.*, 1997).

Srf

Srf is also required for the postnatal maintenance of acinar cells. The MCM1-agamous-deficient serum response factor, Srf, is a transcription factor ubiquitously. Srf is involved in many cellular mechanisms, such as cell growth, differentiation and prevention of apoptosis. In the pancreas, broad *Srf* expression starts from E11 in the pancreatic epithelium, and persists during development and in the adult.

The conditional inactivation of *Srf* in Pdx1$^+$ cells alters pancreas development. After birth, *Srf*-/- mice have severely reduced acinar cell proliferation rates. From 6 weeks of age, mutant pancreata further undergo acinar cell deficit and chronic pancreatitis (Miralles *et al.*, 2006). At 4 months of age, almost all acinar cells are replaced by adipose tissue, which contains ductal and endocrine cells.

Hnf6

Hnf6 promotes the formation of Pdx1$^+$ and Ngn3$^+$ progenitors and plays a determinant role in the differentiation of ductal cells. *Hnf6* is expressed from E13.5 in the ductal lineage and persists in mature cells (Pierreux *et al.*, 2006). The inactivation of *Hnf6* disrupts ductal morphogenesis and leads to disorganization of the ductal network, with the formation of cysts (Pierreux *et al.*, 2006). This ductal epithelium has dysmorphic traits: primary cilia are absent, *Mucin* and *Hnf1β* are not homogeneously induced, and there is persistence of *Glut2* and *Pdx1* expression (Jacquemin *et al.*, 2000, Pierreux *et al.*, 2006).

Conclusion and perspectives

The identification of key regulators of pancreatic development has progressed very rapidly in recent years. Understanding their role in commitment, progenitor cell expansion and differentiation represents an important challenge that should help devising new treatments for diabetes, pancreatitis or pancreatic cancer. For instance, such knowledge could be used to promote tissular maintenance or regeneration by blocking inappropriate cell death or survival, or by stimulating differentiation of newly formed cells or expansion of newly differentiated cells.

References

AFELIK, S., CHEN, Y. and PIELER, T. (2006). Combined ectopic expression of Pdx1 and Ptf1a/p48 results in the stable conversion of posterior endoderm into endocrine and exocrine pancreatic tissue. *Genes Dev* 20: 1441-6.

AHLGREN, U., PFAFF, S.L., JESSELL, T.M., EDLUND, T. and EDLUND, H. (1997). Independent requirement for ISL1 in formation of pancreatic mesenchyme and islet cells. *Nature* 385: 257-60.

APELQVIST, A., LI, H., SOMMER, L., BEATUS, P., ANDERSON, D.J., HONJO, T., HRABE DE ANGELIS, M., LENDAHL, U. and EDLUND, H. (1999). Notch signalling controls pancreatic cell differentiation. *Nature* 400: 877-81.

ARTNER, I., LE LAY, J., HANG, Y., ELGHAZI, L., SCHISLER, J.C., HENDERSON, E., SOSA-PINEDA, B. and STEIN, R. (2006). MafB: an activator of the glucagon gene expressed in developing islet alpha- and beta-cells. *Diabetes* 55: 297-304.

ASHERY-PADAN, R., ZHOU, X., MARQUARDT, T., HERRERA, P., TOUBE, L., BERRY, A. and GRUSS, P. (2004). Conditional inactivation of Pax6 in the pancreas causes early onset of diabetes. *Dev Biol* 269: 479-88.

BORT, R., MARTINEZ-BARBERA, J.P., BEDDINGTON, R.S. and ZARET, K.S. (2004). Hex homeobox gene-dependent tissue positioning is required for organogenesis of the ventral pancreas. *Development* 131: 797-806.

BOTTINGER, E.P., JAKUBCZAK, J.L., ROBERTS, I.S., MUMY, M., HEMMATI, P., BAGNALL, K., MERLINO, G. and WAKEFIELD, L.M. (1997). Expression of a dominant-negative mutant TGF-beta type II receptor in transgenic mice reveals essential roles for TGF-beta in regulation of growth and differentiation in the exocrine pancreas. *EMBO J* 16: 2621-33.

BURKE, Z. and OLIVER, G. (2002). Prox1 is an early specific marker for the developing liver and pancreas in the mammalian foregut endoderm. *Mech Dev* 118: 147-55.

COCKELL, M., STEVENSON, B.J., STRUBIN, M., HAGENBUCHLE, O. and WELLAUER, P.K. (1989). Identification of a cell-specific DNA-binding activity that interacts with a transcriptional activator of genes expressed in the acinar pancreas. *Mol Cell Biol* 9: 2464-76.

COCKELL, M., STOLARCZYK, D., FRUTIGER, S., HUGHES, G.J., HAGENBUCHLE, O. and WELLAUER, P.K. (1995). Binding sites for hepatocyte nuclear factor 3 beta or 3 gamma and pancreas transcription factor 1 are required for efficient expression of the gene encoding pancreatic alpha-amylase. *Mol Cell Biol* 15: 1933-41.

COLLOMBAT, P., HECKSHER-SORENSEN, J., BROCCOLI, V., KRULL, J., PONTE, I., MUNDIGER, T., SMITH, J., GRUSS, P., SERUP, P. and MANSOURI, A. (2005). The simultaneous loss of Arx and Pax4 genes promotes a somatostatin-producing cell fate specification at the expense of the alpha- and beta-cell lineages in the mouse endocrine pancreas. *Development* 132: 2969-80.

COLLOMBAT, P., MANSOURI, A., HECKSHER-SORENSEN, J., SERUP, P., KRULL, J., GRADWOHL, G. and GRUSS, P. (2003). Opposing actions of Arx and Pax4 in endocrine pancreas development. *Genes Dev* 17: 2591-603.

DEUTSCH, G., JUNG, J., ZHENG, M., LORA, J. and ZARET, K.S. (2001). A bipotential precursor population for pancreas and liver within the embryonic endoderm. *Development* 128: 871-81.

FUJITANI, Y., FUJITANI, S., BOYER, D.F., GANNON, M., KAWAGUCHI, Y., RAY,

M., SHIOTA, M., STEIN, R.W., MAGNUSON, M.A. and WRIGHT, C.V. (2006). Targeted deletion of a cis-regulatory region reveals differential gene dosage requirements for Pdx1 in foregut organ differentiation and pancreas formation. *Genes Dev* 20: 253-66.

GIERL, M.S., KAROULIAS, N., WENDE, H., STREHLE, M. and BIRCHMEIER, C. (2006). The zinc-finger factor Insm1 (IA-1) is essential for the development of pancreatic beta cells and intestinal endocrine cells. *Genes Dev* 20: 2465-78.

GRADWOHL, G., DIERICH, A., LEMEUR, M. and GUILLEMOT, F. (2000). neurogenin3 is required for the development of the four endocrine cell lineages of the pancreas. *Proc Natl Acad Sci USA* 97: 1607-11.

GRAPIN-BOTTON, A., MAJITHIA, A.R. and MELTON, D.A. (2001). Key events of pancreas formation are triggered in gut endoderm by ectopic expression of pancreatic regulatory genes. *Genes Dev* 15: 444-54.

GU, G., DUBAUSKAITE, J. and MELTON, D.A. (2002). Direct evidence for the pancreatic lineage: NGN3+ cells are islet progenitors and are distinct from duct progenitors. *Development* 129: 2447-57.

HALE, M.A., KAGAMI, H., SHI, L., HOLLAND, A.M., ELSASSER, H.P., HAMMER, R.E. and MACDONALD, R.J. (2005). The homeodomain protein PDX1 is required at mid-pancreatic development for the formation of the exocrine pancreas. *Dev Biol* 286: 225-37.

HARMON, E.B., APELQVIST, A.A., SMART, N.G., GU, X., OSBORNE, D.H. and KIM, S.K. (2004). GDF11 modulates NGN3+ islet progenitor cell number and promotes beta-cell differentiation in pancreas development. *Development* 131: 6163-74.

HARRISON, K.A., THALER, J., PFAFF, S.L., GU, H. and KEHRL, J.H. (1999). Pancreas dorsal lobe agenesis and abnormal islets of Langerhans in Hlxb9-deficient mice. *Nat Genet* 23: 71-5.

HASHIMOTO, O., USHIRO, Y., SEKIYAMA, K., YAMAGUCHI, O., YOSHIOKA, K., MUTOH, K. and HASEGAWA, Y. (2006). Impaired growth of pancreatic exocrine cells in transgenic mice expressing human activin betaE subunit. *Biochem Biophys Res Commun* 341: 416-24.

HEBROK, M., KIM, S.K. and MELTON, D.A. (1998). Notochord repression of endodermal Sonic hedgehog permits pancreas development. *Genes Dev* 12: 1705-13.

HEISER, P.W., LAU, J., TAKETO, M.M., HERRERA, P.L. and HEBROK, M. (2006). Stabilization of beta-catenin impacts pancreas growth. *Development* 133: 2023-32.

HELLER, R.S., STOFFERS, D.A., LIU, A., SCHEDL, A., CRENSHAW, E.B., 3RD, MADSEN, O.D. and SERUP, P. (2004). The role of Brn4/Pou3f4 and Pax6 in forming the pancreatic glucagon cell identity. *Dev Biol* 268: 123-34.

HENSELEIT, K.D., NELSON, S.B., KUHLBRODT, K., HENNINGS, J.C., ERICSON, J. and SANDER, M. (2005). NKX6 transcription factor activity is required for alpha- and beta-cell development in the pancreas. *Development* 132: 3139-49.

HERRERA, P.L. (2000). Adult insulin- and glucagon-producing cells differentiate from two independent cell lineages. *Development* 127: 2317-22.

HOLLAND, A.M., GONEZ, L.J., NASELLI, G., MACDONALD, R.J. and HARRISON, L.C. (2005). Conditional expression demonstrates the role of the homeodomain transcription factor Pdx1 in maintenance and regeneration of beta-cells in the adult pancreas. *Diabetes* 54: 2586-95.

HOLLAND, A.M., HALE, M.A., KAGAMI, H., HAMMER, R.E. and MACDONALD, R.J. (2002). Experimental control of pancreatic development and maintenance. *Proc Natl Acad Sci USA* 99: 12236-41.

ITKIN-ANSARI, P., MARCORA, E., GERON, I., TYRBERG, B., DEMETERCO, C., HAO, E., PADILLA, C., RATINEAU, C., LEITER, A., LEE, J.E. *et al.* (2005). NeuroD1 in the endocrine pancreas: localization and dual function as an activator and repressor. *Dev Dyn* 233: 946-53.

JACQUEMIN, P., DURVIAUX, S.M., JENSEN, J., GODFRAIND, C., GRADWOHL, G., GUILLEMOT, F., MADSEN, O.D., CARMELIET, P., DEWERCHIN, M., COLLEN, D. *et al.* (2000). Transcription factor hepatocyte nuclear factor 6 regulates pancreatic endocrine cell differentiation and controls expression of the proendocrine gene Ngn3. *Mol Cell Biol* 20: 4445-54.

JACQUEMIN, P., LEMAIGRE, F.P. and ROUSSEAU, G.G. (2003). The Onecut transcription factor HNF-6 (OC-1) is required for timely specification of the pancreas and acts upstream of Pdx-1 in the specification cascade. *Dev Biol* 258: 105-16.

JACQUEMIN, P., YOSHITOMI, H., KASHIMA, Y., ROUSSEAU, G.G., LEMAIGRE, F.P. and ZARET, K.S. (2006). An endothelial-mesenchymal relay pathway regulates early phases of pancreas development. *Dev Biol* 290: 189-99.

JENSEN, J., PEDERSEN, E.E., GALANTE, P., HALD, J., HELLER, R.S., ISHIBASHI, M., KAGEYAMA, R., GUILLEMOT, F., SERUP, P. and MADSEN, O.D. (2000). Control of endodermal endocrine development by Hes-1. *Nat Genet* 24: 36-44.

JOHANSSON, K.A., DURSUN, U., JORDAN, N., GU, G., BEERMANN, F., GRADWOHL, G. and GRAPIN-BOTTON, A. (2007). Temporal control of neurogenin3 activity in pancreas progenitors reveals competence windows for the generation of different endocrine cell types. *Dev Cell* 12: 457-65.

JONSSON, J., CARLSSON, L., EDLUND, T. and EDLUND, H. (1994). Insulin-promoter-factor 1 is required for pancreas development in mice. *Nature* 371: 606-9.

KARLSSON, O., THOR, S., NORBERG, T., OHLSSON, H. and EDLUND, T. (1990). Insulin gene enhancer binding protein Isl-1 is a member of a novel class of proteins containing both a homeo- and a Cys-His domain. *Nature* 344: 879-82.

KATAOKA, K., SHIODA, S., ANDO, K., SAKAGAMI, K., HANDA, H. and YASUDA, K. (2004). Differentially expressed Maf family transcription factors, c-Maf and MafA, activate glucagon and insulin gene expression in pancreatic islet alpha- and beta-cells. *J Mol Endocrinol* 32: 9-20.

KAWAGUCHI, Y., COOPER, B., GANNON, M., RAY, M., MACDONALD, R.J. and WRIGHT, C.V. (2002). The role of the transcriptional regulator Ptf1a in converting intestinal to pancreatic progenitors. *Nat Genet* 32: 128-34.

KIM, S.K., HEBROK, M. and MELTON, D.A. (1997). Notochord to endoderm signaling is required for pancreas development. *Development* 124: 4243-52.

KIM, S.K. and MELTON, D.A. (1998). Pancreas development is promoted by cyclopamine, a hedgehog signaling inhibitor. *Proc Natl Acad Sci USA* 95: 13036-41.

KRAPP, A., KNOFLER, M., FRUTIGER, S., HUGHES, G.J., HAGENBUCHLE, O. and WELLAUER, P.K. (1996). The p48 DNA-binding subunit of transcription factor PTF1 is a new exocrine pancreas-specific basic helix-loop-helix protein. *EMBO J* 15: 4317-29.

LAMMERT, E., CLEAVER, O. and MELTON, D. (2001). Induction of pancreatic differentiation by signals from blood vessels. *Science* 294: 564-7.

LANDRY, C., CLOTMAN, F., HIOKI, T., ODA, H., PICARD, J.J., LEMAIGRE, F.P. and ROUSSEAU, G.G. (1997). HNF-6 is expressed in endoderm derivatives and nervous system of the mouse embryo and participates to the cross-regulatory network of liver-enriched transcription factors. *Dev Biol* 192: 247-57.

LI, H., ARBER, S., JESSELL, T.M. and EDLUND, H. (1999). Selective agenesis of the dorsal pancreas in mice lacking homeobox gene Hlxb9. *Nat Genet* 23: 67-70.

LI, H. and EDLUND, H. (2001). Persistent expression of Hlxb9 in the pancreatic epithelium impairs pancreatic development. *Dev Biol* 240: 247-53.

MARTINEZ BARBERA, J.P., CLEMENTS, M., THOMAS, P., RODRIGUEZ, T., MELOY, D., KIOUSSIS, D. and BEDDINGTON, R.S. (2000). The homeobox gene Hex is required in definitive endodermal tissues for normal forebrain, liver and thyroid formation. *Development* 127: 2433-45.

MELLITZER, G., BONNE, S., LUCO, R.F., VAN DE CASTEELE, M., LENNE-SAMUEL, N., COLLOMBAT, P., MANSOURI, A., LEE, J., LAN, M., PIPELEERS, D. *et al.* (2006). IA1 is NGN3-dependent and essential for differentiation of the endocrine pancreas. *EMBO J* 25: 1344-52.

MIRALLES, F., LAMOTTE, L., COUTON, D. and JOSHI, R.L. (2006). Interplay between FGF10 and Notch signalling is required for the self-renewal of pancreatic progenitors. *Int J Dev Biol* 50: 17-26.

MURTAUGH, L.C., LAW, A.C., DOR, Y. and MELTON, D.A. (2005). Beta-catenin is essential for pancreatic acinar but not islet development. *Development* 132: 4663-74.

NAYA, F.J., HUANG, H.P., QIU, Y., MUTOH, H., DEMAYO, F.J., LEITER, A.B. and TSAI, M.J. (1997). Diabetes, defective pancreatic morphogenesis, and abnormal enteroendocrine differentiation in BETA2/NeuroD-deficient mice. *Genes Dev* 11: 2323-34.

NEVE, B., FERNANDEZ-ZAPICO, M.E., ASHKENAZI-KATALAN, V., DINA, C., HAMID, Y.H., JOLY, E., VAILLANT, E., BENMEZROUA, Y., DURAND, E., BAKAHER, N. *et al.* (2005). Role of transcription factor KLF11 and its diabetes-associated gene variants in pancreatic beta cell function. *Proc Natl Acad Sci USA* 102: 4807-12.

NISHIMURA, W., KONDO, T., SALAMEH, T., EL KHATTABI, I., DODGE, R.,

BONNER-WEIR, S. and SHARMA, A. (2006). A switch from MafB to MafA expression accompanies differentiation to pancreatic beta-cells. *Dev Biol* 293: 526-39.

OBATA, J., YANO, M., MIMURA, H., GOTO, T., NAKAYAMA, R., MIBU, Y., OKA, C. and KAWAICHI, M. (2001). p48 subunit of mouse PTF1 binds to RBP-Jkappa/CBF-1, the intracellular mediator of Notch signalling, and is expressed in the neural tube of early stage embryos. *Genes Cells* 6: 345-60.

OHLSSON, H., KARLSSON, K. and EDLUND, T. (1993). IPF1, a homeodomain-containing transactivator of the insulin gene. *EMBO J* 12: 4251-9.

OSTER, A., JENSEN, J., EDLUND, H. and LARSSON, L.I. (1998). Homeobox gene product Nkx 6.1 immunoreactivity in nuclei of endocrine cells of rat and mouse stomach. *J Histochem Cytochem* 46: 717-21.

PAPADOPOULOU, S. and EDLUND, H. (2005). Attenuated Wnt signaling perturbs pancreatic growth but not pancreatic function. *Diabetes* 54: 2844-51.

PIERREUX, C.E., POLL, A.V., KEMP, C.R., CLOTMAN, F., MAESTRO, M.A., CORDI, S., FERRER, J., LEYNS, L., ROUSSEAU, G.G. and LEMAIGRE, F.P. (2006). The transcription factor hepatocyte nuclear factor-6 controls the development of pancreatic ducts in the mouse. *Gastroenterology* 130: 532-41.

PIN, C.L., RUKSTALIS, J.M., JOHNSON, C. and KONIECZNY, S.F. (2001). The bHLH transcription factor Mist1 is required to maintain exocrine pancreas cell organization and acinar cell identity. *J Cell Biol* 155: 519-30.

RAUSA, F., SAMADANI, U., YE, H., LIM, L., FLETCHER, C.F., JENKINS, N.A., COPELAND, N.G. and COSTA, R.H. (1997). The cut-homeodomain transcriptional activator HNF-6 is coexpressed with its target gene HNF-3 beta in the developing murine liver and pancreas. *Dev Biol* 192: 228-46.

RUKSTALIS, J.M., KOWALIK, A., ZHU, L., LIDINGTON, D., PIN, C.L. and KONIECZNY, S.F. (2003). Exocrine specific expression of Connexin32 is dependent on the basic helix-loop-helix transcription factor Mist1. *J Cell Sci* 116: 3315-25.

SANDER, M., SUSSEL, L., CONNERS, J., SCHEEL, D., KALAMARAS, J., DELA CRUZ, F., SCHWITZGEBEL, V., HAYES-JORDAN, A. and GERMAN, M. (2000). Homeobox gene Nkx6.1 lies downstream of Nkx2.2 in the major pathway of beta-cell formation in the pancreas. *Development* 127: 5533-40.

SANVITO, F., HERRERA, P.L., HUARTE, J., NICHOLS, A., MONTESANO, R., ORCI, L. and VASSALLI, J.D. (1994). TGF-beta 1 influences the relative development of the exocrine and endocrine pancreas in vitro. *Development* 120: 3451-62.

SCHWITZGEBEL, V.M., SCHEEL, D.W., CONNERS, J.R., KALAMARAS, J., LEE, J.E., ANDERSON, D.J., SUSSEL, L., JOHNSON, J.D. and GERMAN, M.S. (2000). Expression of neurogenin3 reveals an islet cell precursor population in the pancreas. *Development* 127: 3533-42.

SERUP, P., PETERSEN, H.V., PEDERSEN, E.E., EDLUND, H., LEONARD, J., PETERSEN, J.S., LARSSON, L.I. and MADSEN, O.D. (1995). The homeodomain protein IPF-1/STF-1 is expressed in a subset of islet cells and promotes rat insulin 1 gene expression dependent on an intact E1 helix-loop-helix factor binding site. *Biochem J* 310 (Pt 3): 997-1003.

SEYMOUR, P.A., FREUDE, K.K., TRAN, M.N., MAYES, E.E., JENSEN, J., KIST, R., SCHERER, G. and SANDER, M. (2007). SOX9 is required for maintenance of the pancreatic progenitor cell pool. *Proc Natl Acad Sci USA* 104: 1865-70.

SHALABY, F., ROSSANT, J., YAMAGUCHI, T.P., GERTSENSTEIN, M., WU, X.F., BREITMAN, M.L. and SCHUH, A.C. (1995). Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice. *Nature* 376: 62-6.

SMART, N.G., APELQVIST, A.A., GU, X., HARMON, E.B., TOPPER, J.N., MACDONALD, R.J. and KIM, S.K. (2006). Conditional expression of Smad7 in pancreatic beta cells disrupts TGF-beta signaling and induces reversible diabetes mellitus. *PLoS Biol* 4: e39.

SMITH, S.B., EE, H.C., CONNERS, J.R. and GERMAN, M.S. (1999). Paired-homeodomain transcription factor PAX4 acts as a transcriptional repressor in early pancreatic development. *Mol Cell Biol* 19: 8272-80.

SORIANO, P. (1999). Generalized lacZ expression with the ROSA26 Cre reporter strain. *Nat Genet* 21: 70-1.

SOSA-PINEDA, B. (2004). The gene Pax4 is an essential regulator of pancreatic beta-cell development. *Mol Cells* 18: 289-94.

SOSA-PINEDA, B., CHOWDHURY, K., TORRES, M., OLIVER, G. and GRUSS, P. (1997). The Pax4 gene is essential for differentiation of insulin-producing beta cells in the mammalian pancreas. *Nature* 386: 399-402.

ST-ONGE, L., SOSA-PINEDA, B., CHOWDHURY, K., MANSOURI, A. and GRUSS, P. (1997). Pax6 is required for differentiation of glucagon-producing alpha-cells in mouse pancreas. *Nature* 387: 406-9.

STANGER, B.Z., TANAKA, A.J. and MELTON, D.A. (2007). Organ size is limited by the number of embryonic progenitor cells in the pancreas but not the liver. *Nature* 445: 886-91.

STOFFERS, D.A., ZINKIN, N.T., STANOJEVIC, V., CLARKE, W.L. and HABENER, J.F. (1997). Pancreatic agenesis attributable to a single nucleotide deletion in the human IPF1 gene coding sequence. *Nat Genet* 15: 106-10.

STROM, A., BONAL, C., ASHERY-PADAN, R., HASHIMOTO, N., CAMPOS, M.L., TRUMPP, A., NODA, T., KIDO, Y., REAL, F.X., THOREL, F. *et al.* (2007). Unique mechanisms of growth regulation and tumor suppression upon Apc inactivation in the pancreas. *Development* 134: 2719-25.

SUSSEL, L., KALAMARAS, J., HARTIGAN-O'CONNOR, D.J., MENESES, J.J., PEDERSEN, R.A., RUBENSTEIN, J.L. and GERMAN, M.S. (1998). Mice lacking the homeodomain transcription factor Nkx2.2 have diabetes due to arrested differentiation of pancreatic beta cells. *Development* 125: 2213-21.

THOMAS, M.K., LEE, J.H., RASTALSKY, N. and HABENER, J.F. (2001). Hedgehog signaling regulation of homeodomain protein islet duodenum homeobox-1 expression in pancreatic beta-cells. *Endocrinology* 142: 1033-40.

THOMAS, M.K., RASTALSKY, N., LEE, J.H. and HABENER, J.F. (2000). Hedgehog signaling regulation of insulin production by pancreatic beta-cells. *Diabetes* 49: 2039-47.

THOMAS, P.Q., BROWN, A. and BEDDINGTON, R.S. (1998). Hex: a homeobox gene revealing peri-implantation asymmetry in the mouse embryo and an early transient marker of endothelial cell precursors. *Development* 125: 85-94.

WANG, J., ELGHAZI, L., PARKER, S.E., KIZILOCAK, H., ASANO, M., SUSSEL, L. and SOSA-PINEDA, B. (2004). The concerted activities of Pax4 and Nkx2.2 are essential to initiate pancreatic beta-cell differentiation. *Dev Biol* 266: 178-89.

WANG, J., KILIC, G., AYDIN, M., BURKE, Z., OLIVER, G. and SOSA-PINEDA, B. (2005). Prox1 activity controls pancreas morphogenesis and participates in the production of «secondary transition» pancreatic endocrine cells. *Dev Biol* 286: 182-94.

WELLS, J.M., ESNI, F., BOIVIN, G.P., ARONOW, B.J., STUART, W., COMBS, C., SKLENKA, A., LEACH, S.D. and LOWY, A.M. (2007). Wnt/beta-catenin signaling is required for development of the exocrine pancreas. *BMC Dev Biol* 7: 4.

WRIGHT, C.V., SCHNEGELSBERG, P. and DE ROBERTIS, E.M. (1989). XlHbox 8: a novel Xenopus homeo protein restricted to a narrow band of endoderm. *Development* 105: 787-94.

ZHANG, C., MORIGUCHI, T., KAJIHARA, M., ESAKI, R., HARADA, A., SHIMOHATA, H., OISHI, H., HAMADA, M., MORITO, N., HASEGAWA, K. *et al.* (2005). MafA is a key regulator of glucose-stimulated insulin secretion. *Mol Cell Biol* 25: 4969-76.