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
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 Pdx1 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. Hebrot and others determined that TGFβ ligand (activin B) 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 B. A similar effect was observed when FGF2 concentrations was low. On the contrary, disruption of activin B-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 repression 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 Flik-/− em-
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**Hepatocyte nuclear factor 6 (Hnf6)**

The One-cut homeodomain transcription factor *Hepatocyte nuclear factor 6* (Hnf6) is expressed from 8s, shortly prior to *Pdx1* induction, in the foregut/midgut region of the endoderm, which gives rise to the pancreatic diverticula. 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 factor 1 (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 (Aeflik 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 endoderm, the ventral pancreatic primordium, which only expresses *Pdx1*, is specified by unknown mechanisms.
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.

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-
transient. This was shown by ectopically expressing deficient proliferation of endodermal cells (Bort foregut, which gives rise to the ventral pancreas and liver. Fushi Tarazu class, is expressed from E7.0 in the ventral-lateral is necessary for endocrine pancreas differentiation. This suggests a non-cell autonomous effect for Hoxb9 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 mediated 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 pancreatic progenitors 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).

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,
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 PAX2/NeuroD1 prior to hormone production. Cell tracing studies demonstrated that while all pancreatic endocrine cell derived 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, Schweigert et al., 2000). On the other 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 PAX2/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 time-points, 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.
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).

MafBs 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**

Differently differentiated endocrine cells maintain their characteristics thanks to the permanent expression of maturation genes. The transcription factor Brn4 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 BET2/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 Bmn4 is determinant for glucagon expression.

**Paired homebox 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, p27kip1, 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 BET2/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, BET2/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 bHLH 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 Sfr. More specifically, Hnf6 is required for the ducal 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 bHLH 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 MistMB 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 FrzB 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 Apcin 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).
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 (N1ICD) or Notch3 repressor (N3ICD), 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., 2006).

**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 ducal network, with the formation of cysts (Pierreux et al., 2006). This ducal 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.

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