

Perspectives in Diabetes

Transcribing Pancreas

Helena Edlund

For ~30–35 years, our insight into some of the fundamental aspects of pancreas development has been based mainly on two independent studies performed in the 1960s by Golosow and Grobstein and Wessells and Cohen. By performing classical embryological experiments, these two reports described the morphogenesis of the pancreas and the epitheliomesenchymal interactions that are required for proper pancreas development. In the 1970s, the groups of LeDourain and associates and Rutter and associates showed, importantly, that despite their similarities with neurons, the pancreatic endocrine cells, like the exocrine and ductal cells, were of an endodermal origin. Then during the 1980s, studies pioneered by Rutter, but also performed by many other groups, were focused on the transcriptional regulation of endocrine and exocrine genes. This eventually led to the cloning of various transcription factors. By using a genetic approach to study the function of these transcription factors, new insights into pancreas development have now emerged that, on a molecular level, are beginning to explain some of the earlier observations. This review discusses our current knowledge of the mechanisms by which the various pancreatic cell types are generated. *Diabetes* 47:1817–1823, 1998

PATTERNING AND EARLY PANCREAS DEVELOPMENT

The pancreas derives from the upper, duodenal part of the foregut via a dorsal and ventral protrusion of the epithelium directly posterior to the developing stomach, and in mice, the early pancreatic buds become evident on embryonic day 9 (e9) (Fig. 1). The part of the gut from which the pancreas originates becomes committed to a pancreatic fate already at the ~10 somites stage, i.e., at e8.5 (1). At this stage, the first molecular sign that the region at this anterior-posterior level will develop into a pancreas is the restricted expression of the homeodomain protein IPF1/PDX1 (2,3) (also known as IDX-1, STF-1 [4,5]) in both the dorsal and ventral gut epithelium. A few somites later, these IPF1/PDX1-expressing regions of the duodenal epithelium will begin to evaginate, thus forming the dorsal and ventral pancreatic buds in which the majority of the cells are IPF1/PDX1⁺ (Figs. 1 and 2). Although

interactions between the gut epithelium and the surrounding mesenchyme have been demonstrated to be instrumental for proper development of the gut and its associated organs (6,7), relatively little is known about the molecular mechanisms that underlie the early regionalization of the gut tube along the anteroposterior (AP) axis. Analysis of the expression pattern of the hedgehog family of signaling molecules in the developing mouse gut tube endoderm have revealed, however, that *sonic (shh)* and *indian hedgehog (ihh)* are differentially expressed in the presumptive pancreatic epithelium, as compared with the rest of the developing gut tube endoderm (8,9). Both *shh* and *ihh* are uniformly expressed in the endoderm anterior and posterior to the pancreas. At the pancreatic AP level, hedgehog expression is, however, restricted to the lateral, intestinal part, and neither the dorsal nor the ventral pancreatic epithelium express *shh* or *ihh* (8,9). Ectopic expression of *shh* in the pancreatic epithelium under the control of the *Ipf1/Pdx1* promoter in transgenic mice clearly demonstrates that the exclusion of *shh* in the developing pancreatic anlagen (Fig. 3) is indeed a prerequisite for proper pancreas development. In *Ipf1/shh* transgenic mice, the mesenchymal component of the pancreas is completely converted into intestinal smooth muscle, and the pancreatic epithelium partly takes on an intestinal differentiation program, although still retaining the capacity to generate pancreatic cell types (9). Thus, these data provide evidence that prevention of hedgehog expression in the developing pancreatic anlagen is important for proper pancreas development. Recent work by Melton and associates (10,11) studying early pancreas development in chick has demonstrated a role for the notochord in repressing *shh* expression in committed gut epithelium, thus allowing the initiation of the pancreatic program of differentiation. In vitro cultures of early chick endoderm has also suggested that the two signaling factors activin β B and fibroblast growth factor (FGF)-2, both expressed in the notochord, may be involved in this notochord-mediated repression of *shh* expression in the pancreatic anlagen (11). Initially, however, the dorsal region of the endoderm along the entire AP axis lacks *ihh* and *shh* expression, and later, this region of the endoderm located on either side of pancreas starts to express *ihh* and *shh* (9). In contrast, the dorsal pancreatic bud never initiates the expression of *ihh* and *shh*, and thus the question seems not to be how expression of *ihh* and *shh* is repressed, but rather the how onset of *ihh* and *shh* expression in the pancreatic epithelium is selectively prevented. Because there exist no evidence of patterning of the notochord, the putative notochord-derived signals have to act on a prepatterned dorsal endoderm. The notochord is not in direct contact with the ventral gut epithelium, and it is not known whether the epithelium of the prospective ventral pancreatic bud in-

From the Department of Microbiology, University of Umea, Umea, Sweden. Address correspondence and reprint requests to Helena Edlund, Department of Microbiology, University of Umea, S-901 87 Umea, Sweden. E-mail: helena.edlund@micro.umu.se.

Received for publication 6 August 1998 and accepted 11 August 1998.

H.E. serves on an advisory board for and holds stock in Ontogeny.

AP, anteroposterior; bHLH, basic helix-loop-helix; e, embryonic day; FGF, fibroblast growth factor; HNF, hepatocyte nuclear factor; *ihh*, *indian hedgehog*; MODY, maturity-onset diabetes of the young; *shh*, *sonic hedgehog*.

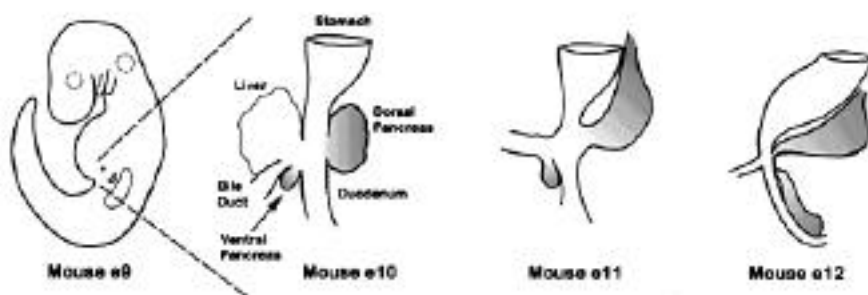


FIG. 1. Schematic drawing illustrating the morphological movements of the pancreatic anlagen during mouse development. The pancreatic buds form as a dorsal and ventral evagination of the foregut endoderm. The rotation of the stomach and duodenum positions the pancreatic buds at the same side of the duodenum, where they eventually fuse, around e13–14. In all drawings, gray fields correspond to the pancreatic primordium. (Illustration by U. Ahlgren and S. Sandström.)

tially expresses *ihh* and *shh*. Thus, the signals repressing or preventing onset of *shh* and *ihh* expression in the ventral pancreatic epithelium have to emanate from tissues other than the notochord. So far, these studies suggest that prevention of *shh* and *ihh* expression in the pancreatic endoderm is important for proper differentiation of the pancreatic mesoderm, which in turn appears to be required for correct development of the pancreatic endoderm. However, these results do not exclude the possibility that early exposure of pancreatic endoderm to SHH and/or IHH is required for the initial patterning of the pancreatic endoderm.

MORPHOGENESIS AND DIFFERENTIATION

From $-e10$ and onwards, the pancreatic epithelium subsequently proliferates and invades the surrounding mesenchyme and epitheliomesenchymal interaction(s), then stimulates proliferation, branching, and differentiation of the epithelium into endocrine and exocrine cells (Fig. 3) (1,6,12). In 1962, Golosow and Grobstein (6) had already demonstrated the importance of the mesenchyme for this process, and later Pictet and Rutter (12) also greatly contributed to the documentation of the importance of mesenchymal factors for pancreatic development. Despite extensive efforts, again mainly by Rutter and associates (13,14), to purify and identify this mesenchymal factor(s), its nature still remains largely unresolved. It is possible that a combination of mesenchymal-derived factors and/or cofactors act in concert to ensure proper development of the pancreatic epithelium, thus rendering the task of purification of the mesenchymal activity a laborious one. In vitro cultures of pancreatic rudiments derived from rat embryos have provided new insights into the role of mesenchyme with respect to pancreas development. The work carried out by Miralles et al. (15) supports the observation that mesenchyme is critically required for exocrine differentiation, but at the same time, this group provides evidence that the pancreatic mesenchyme impairs endocrine differentiation. In their in vitro system, follistatin is capable of partly mimicking the effect of mesenchyme. This suggests that blocking of activin and/or BMP7 signaling is required to obtain exocrine differentiation. This, in turn, suggests that activin and/or BMP7 acts to promote endocrine differentiation, and that differentiation of endocrine or exocrine cells occurs at the expense of the other. Interestingly, both the *BMP7* (which is expressed in the pancreatic epithelium) ($-/-$) and *Follistatin* ($-/-$) mice (16–18) seem to have a normally developed pancreas (U. Ahlgren, H. Li, E.J. Robertson, M.M. Matzuk, H.E., unpublished observations). Thus, if these factors represent important players during pancreatic development, there must exist factors that are functionally redundant.

One important aspect of morphogenesis is the branching of the pancreatic epithelium. Although the factor(s) responsible for the pancreatic epithelial growth and branching is presently unknown, there is accumulating data that FGF signaling is critically involved in controlling branching morphogenesis in both *Drosophila* and mouse. In *Drosophila*, the *Branchless* FGF induces secondary branching by activating the *Breathless* FGF receptor near the tips of growing secondary branching. In addition, *Branchless* FGF induces the newly identified FGF antagonist *sprouty*, which acts to control the degree of branching by blocking signaling to the more distant stalk cells (19). In mouse, genetic inactivation of FGFs or their receptors have, because of early embryonic lethality or

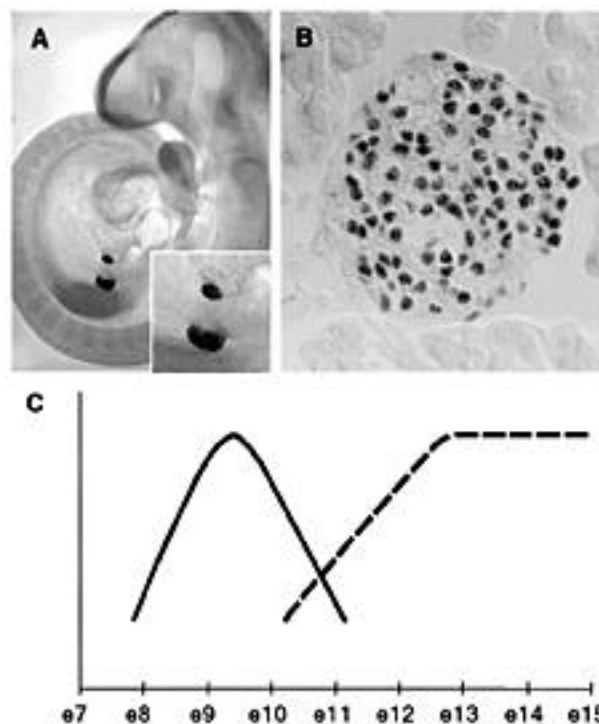


FIG. 2. A: Whole-mount immunohistochemistry of an e9.5 embryo stained with anti-IPF1 antibodies. At this stage, the majority of the epithelial cells of the buds express high levels of IPF1. B: Adult islet cell stained for IPF1 expression. C: Diagram illustrating the temporal expression of IPF1 expression. The early phase of IPF1 expression (—) is confined to the region of the gut epithelium from which the pancreatic buds are formed. Around e10.5, IPF1 expression is down-regulated in the buds but reappears again in the differentiated β -cells (- - -).

functional redundancy, failed to provide information regarding their role in vertebrate organogenesis. As an alternative approach, different transgenic mice expressing, for example, *FGF7/KGF* in the liver (20) or a dominant-negative form of the *FGF-receptor 2b* under the control of the *metallothionein* promoter (21) have been generated. In both of these experiments, the fate of the epithelium of a number of different organs, including the pancreas, where mesenchymal-epithelial interactions are known to regulate the growth and morphogenesis of the epithelium, were severely affected (20,21).

IPF1 AND PANCREAS DEVELOPMENT

During embryogenesis, IPF1/PDX1 expression is initiated at the 10–12 somite stage, i.e., before hormone gene expression, and is restricted to the dorsal and ventral walls of the primitive foregut at the positions where pancreas will later form. The pattern of IPF1/PDX1 expression (Figs. 2 and 3) and its ability to stimulate insulin gene transcription suggested that IPF1/PDX1 functions both in the regionalization of the primitive gut endoderm and in the maturation of the pancreatic β -cell (2,3). Homozygosity for mutations in the *Ipf1/Pdx1* gene in mice and humans results in a complete pancreas agenesis (22–24). IPF1/PDX1 does not seem to be required for the evagination and initial bud formation, but rather it appears to specify the early pancreatic epithelium, permitting its proliferation, branching, and subsequent differentiation (2,23). Thus, the initial stages of pancreas ontogeny involving the early inductive events leading to the formation of the pancreatic buds and the appearance of a few early insulin, and in particular glucagon cells, still occurs in the *Ipf1/Pdx1*-deficient embryos (Fig. 3). These results suggest that additional transcriptional factors exist that act upstream of IPF1/PDX-1 in the initial specification of the pancreatic endoderm (Fig. 4). However, the subsequent morphogenesis of the pancreatic epithelium and the progression of differentiation of the endocrine cells are arrested in the *Ipf1/Pdx1* ($-/-$) embryos. In contrast, the pancreatic mesenchyme grows and develops both morphologically and functionally independent of the epithelium (2). The pancreatic epithelium in the *Ipf1/Pdx1* mutants is, however, unable to respond to the mesenchymal-derived signal(s) that normally promotes pancreatic morphogenesis. Together, these data provide evidence that IPF1/PDX-1 acts cell-autonomously and that the lack of a pancreas in the *Ipf1/Pdx1* ($-/-$) mice is due to a defect in the pancreatic epithelium.

IPF1 FUNCTION IN DIFFERENTIATED β -CELLS

Mice in which the *Ipf1/Pdx1* gene has been disrupted specifically in β -cells initially appear healthy but, on aging, develop diabetes (25). Analysis of these mice revealed that IPF1/PDX1 is required for maintaining the hormone-producing phenotype of the β -cell by positively regulating insulin and islet amyloid polypeptide expression and by repressing glucagon expression. IPF1/PDX1 is also required for the expression of glucose transporter type 2 (GLUT2) in β -cells. IPF1/PDX1 seems to regulate the expression of GLUT2 in a dosage-dependent manner, suggesting that lowered IPF1/PDX1 activity may contribute to the development of type 2 diabetes by causing impaired expression of both GLUT2 and insulin (25). Interestingly, heterozygosity for mutations in the human *Ipf1* gene causes maturity-onset diabetes of the young (MODY) 4 (26), a form of diabetes that results from defects in insulin

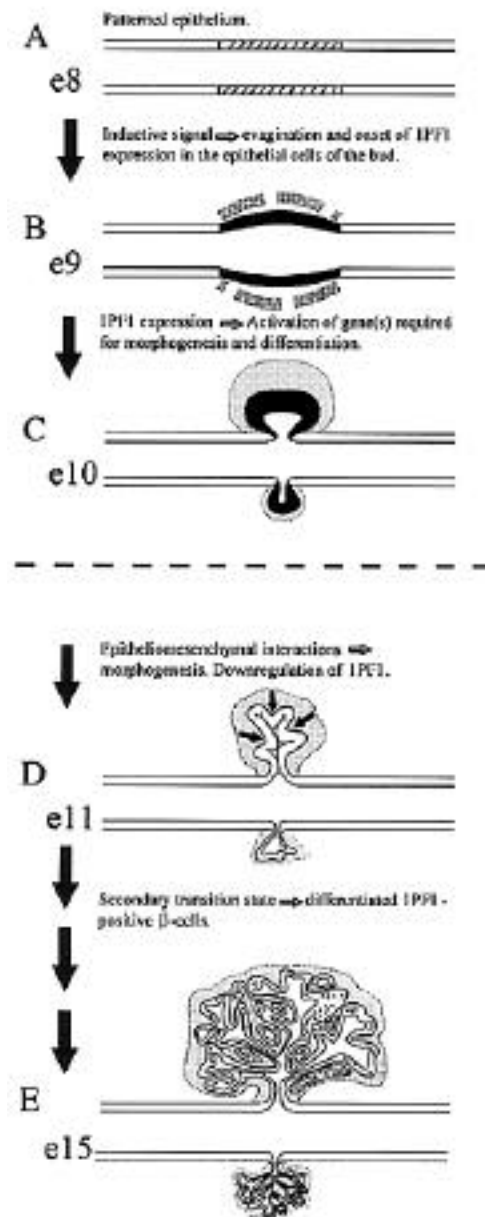


FIG. 3. Summary of the expression profile of IPF1 and different inductive events involved in the development of the pancreas from e8 to e15. This model proposes a dual role for the homeobox gene *Ipf1* during pancreas development. **A:** e8: Patterned embryonic gut epithelium (striped) receives an inductive signal before 10 somites. This leads to the onset of IPF1 in the dorsal and ventral *shh*-negative part of the gut wall and the initiation of evagination. At this stage, no pancreatic mesenchyme is associated with the dorsal gut tissue, which instead is in direct contact with the notochord. **B:** e9: High levels of IPF1 expression (black) in the protruding dorsal and ventral pancreatic diverticulum (see also Fig. 2). Mesenchymal cells (gray) gradually accumulate adjacent to the dorsal gut epithelium. Glucagon-expressing cells appear. **C:** e10: IPF1 has activated gene(s) that makes the pancreatic epithelium able to grow, branch, and differentiate. Early insulin cells appear. In the *Ipf1* ($-/-$) mice, pancreas development is arrested at this stage. **D:** e11: Epitheliomesenchymal interactions lead to morphogenesis and differentiation of the pancreas. IPF1 expression is downregulated. During the following days, the secondary transition state occurs. This leads to a marked increase in the relative numbers of insulin-positive cells that are now IPF1 positive. **E:** e15: The pancreatic buds are heavily lobulated, and differentiated exocrine cells have appeared. The majority of the insulin-expressing cells (dark gray dots) are now IPF1 positive and produce high amounts of the hormone.

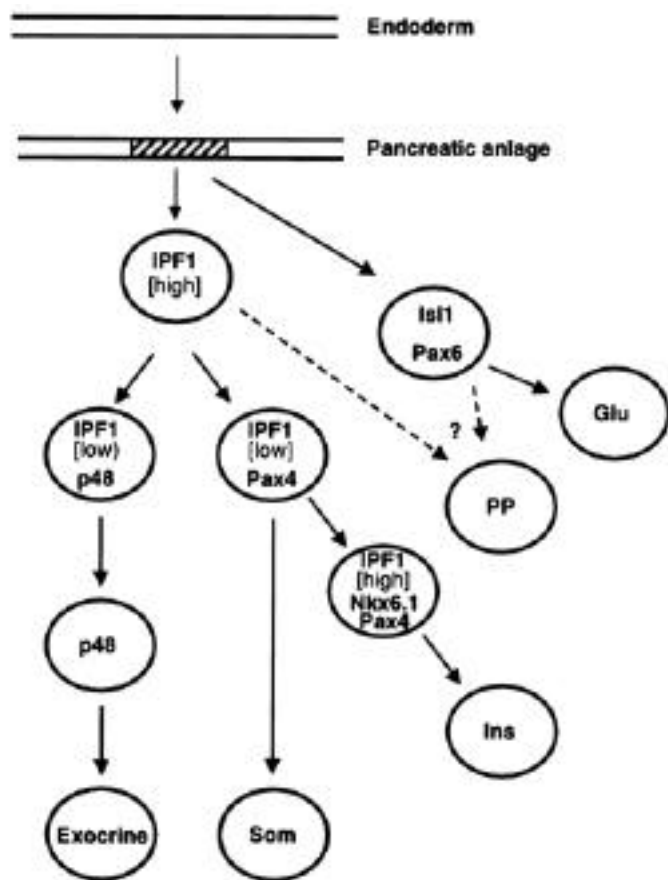


FIG. 4. Model for pancreatic cell differentiation based on genetic analysis of transcription factors.

secretion, suggesting that the late function of IPF1/PDX1 is also conserved from mice to humans. Together, these results point toward a role for IPF1 in ensuring normal β -cell homeostasis, and suggest that decreased IPF1/PDX1 expression and/or function may contribute in a more general way to type 2 diabetes. Enticingly, four of five genes presently linked to MODY represent transcription factors: MODY4, as already mentioned, is linked to mutations in the *Ipf1* gene; MODY1 and -3 to mutations in hepatocyte nuclear factor (HNF)-4 α and -1 α , respectively; MODY to mutations in HNF-1 β ; and MODY2 to glucokinase (26–30). It is thus plausible to assume that mutations in other transcription factors (see below) regulating the expression of pancreatic β -cell genes will turn out to be linked to other forms of MODY.

OTHER TRANSCRIPTION FACTORS LINKED TO PANCREAS DEVELOPMENT

ISL1. During pancreas ontogeny, the expression of the LIM homeodomain protein ISL1 is initiated soon after the islet cells have left the cell cycle, and in the adult pancreas, ISL1 is expressed in all classes of islet cells (31,32). ISL1 is also expressed in mesenchymal cells that surround the dorsal, but not ventral, evagination of the gut endoderm and that together comprise the pancreatic anlagen. In mice lacking ISL1 function, the dorsal pancreatic mesenchyme does not form, and there is an associated failure of exocrine cell differentiation in the dorsal but, not the ventral, pancreas (32).

In addition, there is a complete loss of differentiated islet cells. Exocrine, but not endocrine, cell differentiation in the dorsal pancreas can be rescued *in vitro* by the provision of mesenchyme derived from wild-type embryos. These results indicate that ISL1, by virtue of its requirement for the formation of dorsal mesenchyme, is necessary for the development of the dorsal exocrine pancreas and that ISL1 function in pancreatic endodermal cells is required for the generation of all endocrine islet cells (32).

PAX6. *Pax6* belongs to the *Pax* gene family and, along with ISL1, is expressed in all endocrine cells both during development and in the adult pancreas, but unlike ISL1, PAX6 is not expressed in the surrounding mesenchyme (33,34). In mice homozygous for deletions of the *Pax6* gene, the numbers of all differentiated endocrine cell types are markedly reduced, and in addition, there is a significant decrease in hormone production (33,34). The latter is in agreement with the observation that PAX6 binds to a conserved element common to the *glucagon*, *insulin*, and *somatostatin* promoters (33). The endocrine phenotype partly mimics that of *Isl1* mutants, although the *Pax6* phenotype seems less severe because a small number of cells expressing hormones still occur in the *Pax6* mutants. Interestingly, *Pax6* expression cannot be detected in e9.5 *Isl1* ($-/-$) embryos, although the expression of other endocrine-specific genes like *Nkx2.2* and *NeuroD* appears normal (U. Ahlgren, H.E., unpublished observations). This suggests that ISL1 may act upstream of PAX6 during pancreatic endocrine differentiation.

PAX4. *Pax4* expression is evident as early as e9.5 in both pancreatic buds, but later in the development, *Pax4* expression becomes restricted to the developing β -cells (35). Mice lacking a functional *Pax4* gene lack differentiated insulin and somatostatin cell types (35). In addition, there is an associated increase in the number of glucagon-producing cells. This could possibly suggest that *Pax4* is responsible for an *Ins*⁺ cell fate choice and that in absence of *Pax4*, the default fate would be to become a *Glu*⁺ cell. The increase in *Glu*⁺ cells is, however, reminiscent of the islet phenotype of the β -cell-specific *Ipf1* mutants, and it is thus possible that *Pax4* is directly or indirectly involved in repressing glucagon expression. It would be interesting to analyze the expression of other types of β -cell-associated genes like *Glut2*, *glucokinase*, *IAPP*, *prohormone convertase 1/3* (*PC1/3*), and *Nkx6.1* in the *Pax4*-deficient mice to more fully elucidate the β -cell phenotype of the *Pax4* ($-/-$) mice (see below).

Nkx2.2. *Nkx2.2* belongs to the mammalian *NK2* homeobox family of transcription factors (36) and, as revealed by *in situ* hybridization, is expressed from at least the ~14 somite stage (e8.75–9.0) in both the dorsal and ventral pancreatic epithelium (H.E., unpublished observations). Later in development, *Nkx2.2* is expressed in all islet cells except the somatostatin-producing δ -cells, and *Nkx2.2* ($-/-$) mice lack *Ins*⁺ cells and have reduced numbers of *Glu*⁺ and *PP*⁺ cells (36). Interestingly, presumptive β -cells seem to form in these mice, and although failing to express insulin, these cells still express, for example, *IAPP* and *PC1/3*. This suggests that *Nkx2.2* is required for the terminal differentiation of β -cells or, alternatively, that *Nkx2.2* is critically required for the expression of the insulin gene but not for the expression of other β -cell-specific genes (36).

Nkx6.1. Another *NK2* family member, *Nkx6.1* (37,38) is initially (~e9.0–9.5) expressed predominantly in the dorsal pan-

creatic epithelium in a subset of the IPF1/PDX1⁺ cells (H.E., unpublished observations). Later in development, Nkx6.1, which is analogous to IPF1/PDX1, becomes restricted to the differentiated β -cells (37,38), and preliminary analysis of Nkx6.1 (-/-) mice show that they have drastically reduced numbers of Ins⁺ cells but normal numbers of the other islet cells (38).

NeuroD/Beta2. NeuroD/Beta2 is a cell-type restricted basic helix-loop-helix (bHLH) transcription factor that is expressed in all pancreatic endocrine cells (39,40); together with its ubiquitous counterpart, the *E2A* gene product E12, NeuroD/Beta2 binds to the E-boxes of the insulin regulatory region. In *NeuroD/Beta2*-deficient mice, endocrine cells still appear, but at markedly reduced numbers: β -cells are reduced by ~75%, α -cells by 40%, and δ -cells by 20%, and the remaining endocrine cells fail to form mature islets (41).

p48. All of the above described transcription factors, many of them first identified as endocrine gene transcription regulators, are or become endocrine specific, and mutations in either of these genes affect various aspects of pancreatic endocrine differentiation. In contrast, relatively little is known about transcription factors controlling pancreatic exocrine gene expression and/or exocrine cell differentiation. To fully understand pancreas development, it is crucial to identify genes involved in the specification and/or differentiation of exocrine cells. p48 is a bHLH transcription factor that, together with E12 and HEB/REB/Alf1, constitutes the exocrine transcription complex PTF1, which binds to a conserved element of the regulatory region of exocrine genes (42–44 and O. Hagenbüchle, P. Wellauer, personal communication). p48 mRNA expression is restricted to the exocrine part of the pancreas. However, during development, p48 mRNA expression can be detected as early as e10 in both pancreatic buds (U. Ahlgren, H.E., unpublished observations), and thus p48 mRNA expression precedes exocrine enzyme expression, which first occurs at e12–14. It is noteworthy, though, that PTF1 DNA-binding activity is first detectable around e15 (42), i.e., the time at which a high level of exocrine gene expression becomes conceived. *p48* (-/-) mice generated by O. Hagenbüchle, P. Wellauer, and coworkers point toward a crucial role for p48 in exocrine cell differentiation, since it is completely impaired in these mice, whereas endocrine cell differentiation is not (43 and O. Hagenbüchle, P. Wellauer, personal communication). p48 is thus the first exocrine-specific transcription factor that has been isolated and whose function has been genetically tested. Further analysis of p48 function will be of vital importance for our understanding of pancreatic cell differentiation. Two other exocrine transcription factors, although not yet genetically analyzed, will probably add to our understanding of pancreas development and are thus worth mentioning.

Mist1. Mist1 also belongs to the bHLH family of transcription factors and can, at least in vitro, bind to E-box elements either as a homo- or a heterodimer together with E12 (45). In the pancreas, *Mist1* is expressed in the exocrine cells from e14.5 and onward, suggesting that Mist1 is acting late in exocrine differentiation.

HNF-6. HNF-6 is a member of the *winged helix/fork head* family of transcription factors. HNF-6 expression can be detected as early as e10.5 in both pancreatic buds, but later, the expression becomes restricted to the exocrine pancreas (46,47). HNF-6 expression also remains in the pancreatic

epithelium, but the endocrine cells are HNF-6-negative throughout development.

AN EPIGENETIC CODE FOR THE PANCREAS?

The pancreas has rapidly become one of the most studied organs with respect to function of transcription factors, and many pancreatic phenotypes have been described. However, the present genetic data only allow some more general comments to be made about the epigenetic control of pancreas development. One major reason for this is that most genetic studies have so far failed to address whether a given transcription factor exerts its function in pancreatic progenitor and/or in postmitotic cells.

Isl1 and *Pax6* show a very similar pattern of expression and appear to be selectively expressed in postmitotic endocrine cells. No terminally differentiated endocrine cells are found in *Isl1* (-/-) mice (32), and in *Pax6* (-/-) mutants, the number of hormone-producing cells are severely reduced. *Pax6* is not expressed in *Isl1* (-/-) mice, suggesting that it acts downstream of *Isl1* in postmitotic endocrine cells. *Ipf1/Pdx1*, *Pax4*, *NeuroD*, *Nkx2.2*, and *Nkx6.1* are expressed both in progenitor cells and in differentiated endocrine cells. *Nkx2.2* and *NeuroD* are expressed in most endocrine cells. Nevertheless, in mice deficient in either of these genes, endocrine progenitor cells and terminally differentiated islet cells still appear. This provides evidence that neither of these transcription factors is involved in the generation of a multipotent progenitor cell for endocrine cells. Both *Pax4* and *Nkx6.1* are expressed in a manner strikingly similar to that of *IPF1/PDX1*. The β -cell specific loss of IPF1/PDX1 in adult mice (25), together with preliminary analysis of e10.5 *Ipf1/Pdx1* null mutants performed in our laboratory, indicates that at least *Nkx6.1*, but also possibly *Pax4*, is acting downstream of *IPF1/PDX1*. At present, it is not possible to distinguish whether these genes exert their functions in progenitor cells and/or in postmitotic endocrine cells.

These and other studies have not yet provided sufficient information to establish the lineage relationship between the different endocrine cells, the existence of lineage-restricted endocrine progenitor cell populations or identified transcription factors that selectively control the generation of progenitor populations and individual islet cell types. However, based on the recent genetic data, a working model for the role of transcription factors in pancreatic cell differentiation can be proposed (Fig. 4). On e8.0, patterning of the embryonic endoderm by a yet unknown transcription factor, gene X, commits a region of the endoderm to a pancreatic fate (Fig. 4), partly by preventing the onset of hedgehog expression. By e8.5 IPF1/PDX1 expression has been induced, and shortly thereafter, the pancreatic epithelium evaginates to form the pancreatic buds (Figs. 3 and 4). The first endocrine cells to appear are the glucagon cells that appear around e9.5. These cells require *Isl1* and *Pax6*, but are independent of *Ipf1/Pdx1*, since although there is already an arrest in the pancreatic development around e9.5–10 in the *Ipf1/Pdx1* null mutants, postmitotic *Isl1*⁺ glucagon cells still appear. This is consistent with the observation that IPF1/PDX1 is not normally expressed in glucagon cells (2), and suggests that glucagon cells originate from a separate pool of early IPF1/PDX1-negative progenitor cells. In the absence of *Isl1* expression, the loss of IPF1/PDX1 in progenitor cells is not sufficient to generate ectopic glucagon

cells (2). In contrast, loss of IPF1/PDX1 in differentiated β -cells seems to allow glucagon expression in β -cells (25), which concomitantly lose insulin expression. It is possible that this switch in hormone production is enabled by the expression of *Isl1* in these postmitotic endocrine cells.

High levels of IPF1/PDX1 expression persist until \sim e10.5, and this initial phase of IPF1/PDX1 expression is required for pancreas development to proceed beyond this stage (Figs. 3 and 4). The high-level IPF1/PDX1 expression is then down-regulated, and in a majority of pancreatic epithelial cells, *p48* mRNA expression is now initiated. These *p48*⁺ cells are the progenitors of the exocrine lineage. The phenotype of the *p48*^{-/-} mice partly mimics that of the *Ipf1/Pdx1* mice, and *p48* seems to be downstream of IPF1/PDX1 (U. Ahlgren, H.E., unpublished observations). Hence, the full analysis of the *p48*^{-/-} mice will be critical for our understanding of when and how exocrine precursor cells are generated. The insulin and somatostatin cells seem to segregate relatively late in development, and the differentiation of both cell types requires *Pax4*. The insulin cells appear around e14.5 and are dependent on high-level expression of *Ipf1/Pdx1* and *Nkx6.1*, whereas the somatostatin-cells appear a few days later from the remaining *Pax4*⁺ cells (Fig. 4). All endocrine cells depend on *Isl1* for their terminal differentiation.

CONCLUSION

The work done by Pictet et al. (48) and by Fontaine and Le Douarin (49) unequivocally showed that the different pancreatic endocrine cells, like the exocrine and ductal cells, are of true endodermal origin. However, how these various cell types are generated from a pool of endodermal cells fated to become the pancreas still remains poorly understood. The identification and loss of function analysis of transcription factors expressed in the pancreas have provided some insight into this process. However, to fully understand the principle of pancreas development, we need to identify transcription factors that initially specify gut endoderm for a pancreatic fate, to find out when exocrine, ductal, and endocrine lineages segregate and to identify the transcription factors controlling these events. We also need to isolate transcription factors selectively expressed in ductal cells and to identify the mechanism underlying the generation of the different endocrine cell types. The fact that a variety of additional transcription factors already have been associated with the pancreas (50), but have not yet been genetically analyzed, makes such a goal a prosperous one. Whether the key event in the generation of the various islet cells is the early induction of different populations of lineage-restricted progenitor cells or whether the different endocrine cells are generated from multipotent precursor cells by later-acting instructive signals remains to be established. Also, although a requirement has been established for many transcription factors by loss of function analysis, sufficiency has rarely been tested by gain of function experiments. It seems evident that to understand the principles behind these processes, more attention has to be given to the latter approach.

A major reason for our limited understanding of pancreas development is the relative lack of information on proliferative and inductive signals operating in the pancreas. In contrast to many other organs or tissues, little or almost no genetic evidence exists for the involvement of different classes of proliferative or patterning signals in the pancreas.

Whether this reflects that unique signals or common, but redundant, signals are used in the pancreas remains to be established. Still, as exemplified in other systems, the identification of signal molecules and transcription factors expressed in the pancreas and the elucidation of their functions should be a powerful way to rapidly unravel the principles of pancreas development. Finally, the prospect of establishing a system where β -cells might be selectively induced and amplified, *in vivo* or *in vitro*, and subsequently used as cell replacement therapy in both type 1 and type 2 diabetes, will be critically dependent on the identification of factors controlling the generation of precursor cells and their differentiation into β -cells.

ACKNOWLEDGMENTS

Work in my laboratory is supported by Swedish Medical Research Council Grant 13XB-10367 and by Juvenile Diabetes Foundation Grant 197015. H.E. is a scientist of the Swedish Medical Research Council (13P-12462).

I wish to thank Dr. Thomas Edlund for helpful discussions and valuable criticism of this article, Sara Sandström for artwork, Mark Dopson for editing my English, and Dr. Ulf Ahlgren for help with the figures.

REFERENCES

- Wessels NK, Cohen JH: Early pancreas organogenesis: morphogenesis, tissue interactions and mass effects. *Dev Biol* 15:237–270, 1967
- Ahlgren U, Jonsson J, Edlund H: Arrested development of the pancreas in IPF1/PDX1 deficient mice reveals that the pancreatic mesenchyme develops independently of the pancreatic epithelium. *Development* 122:1409–1416, 1996
- Ohlsson H, Karlsson K, Edlund T: IPF1, a homeodomain-containing transactivator of the insulin gene. *EMBO J* 12:4251–4259, 1993
- Leonard J, Peers B, Johnson T, Ferreri K, Lee S, Montminy MR: Characterization of somatostatin transactivating factor-1, a novel homeobox factor that stimulates somatostatin expression in pancreatic islet cells. *Mol Endocrinol* 7:1275–1283, 1993
- Miller CP, McGehee RE Jr, Habener JF: IDX-1: a new homeodomain transcription factor expressed in rat pancreatic islets and duodenum that transactivates the somatostatin gene. *EMBO J* 13:1145–1158, 1994
- Golosow N, Grobstein C: Epitheliomesenchymal interaction in pancreatic morphogenesis. *Dev Biol* 4:242–255, 1962
- Haffen K, Keding K, Simon-Assman P: Mesenchyme-dependent differentiation of epithelial cells in the gut. *J Pediatr Gastroenterol Nutr* 6:14–23, 1987
- Bitgood MJ, McMahon AP: Hedgehog and Bmp genes are coexpressed at many diverse sites of cell-cell interaction in the mouse embryo. *Dev Biol* 172:126–138, 1995
- Apelqvist Å, Ahlgren U, Edlund H: Sonic hedgehog directs specialised mesoderm differentiation in the intestine and pancreas. *Curr Biol* 7:801–804, 1997
- Kim SK, Hebrok M, Melton DA: Notochord to endoderm signaling is required for pancreas development. *Development* 124:4243–4252, 1997
- Hebrok M, Kim SK, Melton DA: Notochord repression of endodermal sonic hedgehog permits pancreas development. *Genes Dev* 12:1705–1713, 1998
- Pictet R, Rutter WJ: Development of the embryonic endocrine pancreas. In *Handbook of Physiology*. Steiner DF, Frenkel N, Eds. Washington, DC, Williams and Wilkins, 1972, p. 25–66
- Ronzio RA, Rutter WJ: Effects of a partially purified factor from chick embryos on macromolecular synthesis of embryonic pancreatic epithelia. *Dev Biol* 30:307–320, 1973
- Levine S, Pictet R, Rutter WJ: Control of cell proliferation and cytodifferentiation by a factor reacting with the cell surface. *Nature New Biol* 246:49–52, 1973
- Miralles F, Czernichow P, Scharfmann R: Follistatin regulates the relative proportions of endocrine versus exocrine tissue during pancreas development. *Development* 125:1017–1024, 1998
- Dudley AT, Lyons KM, Robertson EJ: A requirement for bone morphogenetic protein-7 during development of the mammalian kidney and eye. *Genes Dev* 9:2795–2807, 1995
- Luo G, Hofmann C, Bronckers ALJ, Sohocki M, Bradley A: BMP7 is an inducer of nephrogenesis and is also required for eye development and skeletal patterning. *Genes Dev* 9:2808–2820, 1995

18. Matzuk MM, Lu N, Vogel H, Sellheyer K, Roop DR, Bradley A: Multiple defects and perinatal death in mice deficient in follistatin. *Nature* 374:360–363, 1995
19. Hacohen N, Kramer S, Sutherland D, Hiromi Y, Krasnow MA: Sprouty encodes a novel antagonist of FGF signaling that patterns apical branching of the *Drosophila* airways. *Cell* 92:253–263, 1998
20. Nguyen HQ, Danilenko DM, Bucay N, DeRose ML, Van GY, Thomason A, Simonet WS: Expression of keratinocyte growth factor in embryonic liver of transgenic mice causes changes in epithelial growth and differentiation resulting in polycystic kidneys and other organ malformations. *Oncogene* 12:2109–2119, 1996
21. Celli G, LaRochelle WJ, Mackem S, Sharp R, Merlino G: Soluble dominant-negative receptor uncovers essential roles for fibroblast growth factors in multi-organ induction and patterning. *EMBO J* 17:1642–1655, 1998
22. Jonsson J, Carlsson L, Edlund T, Edlund H: Insulin-promoter-factor 1 is required for pancreas development in mice. *Nature* 371:606–609, 1994
23. Offield MF, Jetton TL, Labosky PA, Ray M, Stein R, Magnuson MA, Hogan BL, Wright CVE: PDX-1 is required for pancreatic outgrowth and differentiation of the rostral duodenum. *Development* 122:983–995, 1996
24. Stoffers DA, Zinkin NT, Stanojevic V, Clarke WL, Habener JF: Pancreatic agenesis attributable to a single nucleotide deletion in the human IPF1 gene coding sequence. *Nat Genet* 15:106–110, 1997
25. Ahlgren U, Jonsson J, Jonsson L, Simu K, Edlund H: β -cell specific inactivation of the mouse *Ipf1/Pdx1* gene results in impaired glucose transporter expression and late onset diabetes. *Genes Dev* 12:1763–1768, 1998
26. Stoffers DA, Ferrer J, Clarke WL, Habener JF: Early-onset type-II diabetes mellitus (MODY4) linked to IPF1. *Nat Genet* 17:138–139, 1997
27. Yamagata K, Furuta H, Oda N, Kaisaki PJ, Menzel S, Cox NJ, Fajans SS, Signorini S, Stoffel M, Bell GI: Mutations in the hepatocyte nuclear factor-4 α gene in maturity-onset diabetes of the young. *Nature* 384:458–460, 1996
28. Yamagata K, Oda N, Kaisaki PJ, Menzel S, Furuta H, Vaxillaire M, Southam L, Cox RD, Lathrop GM, Boriraj VV, Chen X, Cox NJ, Oda Y, Yano H, Le Beau MM, Yamada S, Nishigori H, Takeda J, Fajans SS, Hattersley AT, Iwasaki N, Hansen T, Pedersen O, Polonsky KS, Turner RC, Verlho G, Chevre J-C, Froguel P, Bell GI: Mutations in the hepatocyte nuclear factor-1 α gene in maturity-onset diabetes of the young. *Nature* 384:455–458, 1996
29. Horikawa Y, Iwasaki N, Hara M, Furuta H, Hinokio Y, Cockburn BN, Lindner T, Yamagata K, Ogata M, Tomonaga O, Kuroki H, Kasahara T, Iwamoto Y, Bell GI: Mutation in hepatocyte nuclear factor-1 beta gene (TCF2) associated with MODY. *Nat Genet* 17:384–385, 1997
30. Froguel P, Vaxillaire M, Sun F, Velho G, Zouali H, Butel MO, Lesage S, Vionnet N, Clement K, Fougerousse F, Tanizawa Y, Weissenbach J, Beckmann JS, Lathrop GM, Passa P, Permutt MA, Cohen D: Close linkage of glucokinase locus on chromosome 7p to early-onset non-insulin-dependent diabetes mellitus. *Nature* 356:162–164, 1992
31. Thor S, Ericson J, Brannstrom T, Edlund T: The homeodomain LIM protein *Isl-1* is expressed in subsets of neurons and endocrine cells in the adult rat. *Neuron* 7:881–889, 1991
32. Ahlgren U, Pfaff S, Jessel TM, Edlund T, Edlund H: Independent requirement for *ISL1* in the formation of the pancreatic mesenchyme and islet cells. *Nature* 385:257–260, 1997
33. Sander M, Neubuser A, Kalamaras J, Ee HC, Martin GR, German MS: Genetic analysis reveals that *PAX6* is required for normal transcription of pancreatic hormone genes and islet development. *Genes Dev* 11:1662–1673, 1997
34. St-Onge L, Sosa-Pineda B, Chowdhury K, Mansouri A, Gruss P: *Pax6* is required for differentiation of glucagon-producing α -cells in mouse pancreas. *Nature* 387:406–409, 1997
35. Sosa-Pineda B, Chowdhury K, Torres M, Oliver G, Gruss P: The *Pax4* gene is essential for differentiation of insulin-producing β cells in the mammalian pancreas. *Nature* 386:399–402, 1997
36. Sussel L, Kalamaras J, Hartigan-O'Connor DJ, Meneses JJ, Pedersen RA, Rubenstein JL, German MS: Mice lacking the homeodomain transcription factor *Nkx2.2* have diabetes due to arrested differentiation of pancreatic β cells. *Development* May 125:2213–2221, 1998
37. Jensen J, Serup P, Karlsen C, Nielsen TF, Madsen OD: mRNA profiling of rat islet tumors reveals *Nkx 6.1* as a β -cell-specific homeodomain transcription factor. *J Biol Chem* 271:18749–18758, 1996
38. Sander M, Kalamaras J, German MS: The homeobox gene *Nkx6.1* is essential for differentiation of insulin-producing β -cells in the mouse pancreas. Abstract from The Keystone Symposium on Vertebrate Development, Steamboat Springs, Colorado, April 3–8, 1998
39. Lee JE, Hollenberg SM, Snider L, Turner DL, Lipnick N, Weintraub H: Conversion of *Xenopus* into neurons by *NeuroD*, a basic helix-loop-helix protein. *Science* 268:836–844, 1995
40. Naya FJ, Stellrecht CM, Tsai MJ: Tissue-specific regulation of the insulin gene by a novel basic helix-loop-helix transcription factor. *Gene Dev* 9:1009–1019, 1995
41. Naya FJ, Huang HP, Qiu Y, Mutoh H, DeMayo FJ, Leiter AB, Tsai MJ: Diabetes, defective pancreatic morphogenesis, and abnormal enteroendocrine differentiation in *BETA2/neuroD*-deficient mice. *Gene Dev* 11:2323–2334, 1997
42. Petrucco S, Wellauer PK, Hagenbuchle O: The DNA-binding activity of transcription factor *PTF1* parallels the synthesis of pancreas-specific mRNAs during mouse development. *Mol Cell Biol* 10:254–264, 1990
43. Krapp A, Knofler M, Frutiger S, Hughes GJ, Hagenbuchle O, Wellauer PK: The p48 DNA-binding subunit of transcription factor *PTF1* is a new exocrine pancreas-specific basic helix-loop-helix protein. *EMBO J* 15:4317–4329, 1996
44. Hagenbuchle O, Krapp A, Knofler M, Wellauer PK: The bHLH p48, a DNA-binding subunit of *PTF1*, acts as a morphogen during pancreas development. *Endocrinol Diabetes* 105:A1–A51, 1997
45. Lemerrier C, To RQ, Swanson BJ, Lyons GE, Konieczny SF: *Mist1*: a novel basic helix-loop-helix transcription factor exhibits a developmentally regulated expression pattern. *Dev Biol* 182:101–113, 1997
46. Rausa F, Samadani U, Ye H, Lim L, Fletcher CF, Jenkins NA, Copeland NG, Costa RH: The cut-homeodomain transcriptional activator *HNF-6* is coexpressed with its target gene *HNF-3 beta* in the developing murine liver and pancreas. *Development* 192:228–246, 1997
47. Landry C, Clotman F, Hioki T, Oda H, Picard JJ, Lemaigre FP, Rousseau GG: *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* 192:247–257, 1997
48. Pictet RL, Rall LB, Phelps P, Rutter WJ: The neural crest and the origin of the insulin-producing and other gastrointestinal hormone producing cells. *Science* 191:191–192, 1976
49. Fontaine J, Le Douarin NM: Analysis of endoderm formation in the avian blastoderm by the use of quail-chick chimaeras: the problem of the neuroectodermal origin of the cells of the APUD series. *J Embryol Exp Morphol* 41:209–222, 1977
50. Sander M, German MS: The β cell transcription factors and the development of the pancreas. *J Mol Med* 75:327–340

Author Queries (please see Q in margin and underlined text)

Q1: Please make sure that use of italics for gene names throughout text is correct and consistent. Please italicize all gene names.

Q2: OK to add “and associates” twice in phrase beginning “the groups of...”? If not, please reword for clarity.

Q4: Should the “s” in “*shh*” be lowercase here, as elsewhere?

Q5: Fig. 2 C: Please indicate what each line type (whole or dashed) refers to.

Q5a: Figure 3: Do you mean “black” here?

Q5b: Figure 3A, the “stripes” have been retouched because they were not showing in the original. OK as retouched?

Q7: Since you are talking about Ref. 12 here, “Pictet and Rutter” OK for “Rutter and co-workers”?

Q8: ADA lists the first author of a reference for in-text citation. “Miralles et al.” OK for “Scharfmann and co-workers” here?>

Q9: Please spell out LIM.

Q 10: “H.E.” OK for “our own”? If not, please provide names of all researchers.

Q11: Please include a citation for Ref. 39 before this one for Ref. 40.

Q12: To avoid repetition of information, changes to first two sentences of section headed “NeuroD/Beta2” OK? If not, please reword.

Q13: Correct that this is a list of genes (and should therefore be italicized)?

Q14: Do you mean “dependent on” or “independent of” here?