Section 1: β-Cell Differentiation and Growth

Developmental Biology of the Pancreas

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All pancreatic cell types (endocrine, exocrine, and ductal) are derived from the same endodermal dorsal and ventral anlage, which grow together to form the definitive pancreas. Golosow and Grobstein were pioneers in the field of pancreatic developmental research, as were Wessells and Cohen, who already in the 1960s performed classic embryological experiments describing the morphogenesis of the pancreas and the epithelio-mesenchymal interactions that are instrumental for proper pancreas development. Recent findings suggest that follistatin and fibroblast growth factors represent some of these key mesenchymal factors that actively promote at least pancreatic exocrine development. The true endodermal origin of the pancreatic endocrine cells became evident by experiments performed by the groups of LeDouarin and Rutter in the 1970s. The newly acquired insights regarding the specification of pancreatic endocrine cells as controlled by the notch signaling pathway (i.e., similar to the mechanisms by which neurons are specified during neurogenesis) have provided a novel understanding of the long acknowledged similarities between neurons and the pancreatic endocrine cells. Last, the identification of a number of distinct transcription factors operating at various levels of pancreatic development and in different cell types has provided useful information both on pancreas development and on various pancreatic disorders such as diabetes. Interestingly, four of the hitherto defined five different maturity-onset diabetes of the young (MODY) genes correspond to transcription factors, and, in addition, several transcription factors have also been linked to type 2 diabetes. *Diabetes* 50 (Suppl. 1): S5–S9, 2001

The pancreas comprises endocrine, exocrine, and ductal cell types that collectively synthesize and secrete hormones and enzymes required for nutritional balance. Each of these distinct pancreatic cell types are derived from endodermal cells of the upper duodenal region of the foregut (1,2), and the development of the pancreas begins with the dorsal and ventral protrusion of a region of the primitive gut epithelium (3–5). The early steps that control the commitment of a region of localized gut epithelium to a pancreatic fate and the mechanism underlying the specification of the different pancreatic cell types are, however, not well understood. In addition, it remains unclear how the initially separate programs of dorsal and ventral pancreatic bud development are coordinated to produce a convergent developmental program.

Several homeodomain and basic helix-loop-helix (bHLH) transcription factors, notably Isl1, Nkx2.2, Pax4, Pax6, and NeuroD/β2, have been shown to exert important functions in the control of pancreatic endocrine cell differentiation (3–5). These factors are expressed at early stages of pancreas development, but in their absence, the initial steps of pancreatic development proceed normally, resulting only later in perturbation of the differentiation of pancreatic endocrine cell types (3–5). In contrast to the bHLH class, transcription factor p48, which is required for the generation of exocrine but not endocrine cells (6), and the homeodomain protein IPF1/PDX1 are required at an earlier stage in pancreas development (7–10). Mice and humans lacking *Ipf1/Pdx1* are apancreatic (7–10). Nevertheless, *Ipf1/Pdx1* appears to act at a step downstream of the initial specification of the gut endoderm to a pancreatic fate (8,9). Thus, the evagination of the epithelium and the formation of the dorsal and ventral pancreatic buds still occur in *Ipf1/Pdx1* mutant mice. Consistent with this observation, the appearance of early pancreatic markers is relatively unaffected in *Ipf1/Pdx1*–deficient mice (8,9). These findings imply the existence of additional genes involved in the regulation of earlier stages of pancreatic development.

Despite their endodermal origin (1,2) pancreatic endocrine cells share a remarkable similarity with neuronal cells with respect to expression of enzymes and hormones (11). Thus transcription factors such as Isl1, NeuroD/β2, Pax4, Pax6, and Nkx2.2, which are vital for proper pancreatic endocrine differentiation, are also expressed during, and in some cases are critical for, neuronal differentiation (12–22). In addition, during pancreatic development, the endocrine cells initially appear in a scattered manner (12,23) that resembles the process of neurogenesis (24,25). This implies that cell differentiation in the pancreas and in the nervous system may be, at least in part, under similar control. Distinct from the initiation and early stages of pancreatic development, the later stages and in particular the branching morphogenesis and exocrine differentiation critically depend on the presence of mesenchyme (12,26). Nevertheless, the identity of the mesenchymal factors acting to promote pancreatic branching morphogenesis and exocrine differentiation has remained elusive for some 30 years, and only recently have suggestions regarding the nature of some of these factors emerged. It is clear that genetic studies of transcription factors expressed in the pancreas have greatly advanced our understanding of pancreatic development. Hopefully, a similar knowledge will

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bHLH, basic helix-loop-helix; FGF, fibroblast growth factor; HNF, hepatocyte nuclear factor; Ihh, Indian hedgehog; IPF1, insulin promoter factor-1; MODY, maturity-onset diabetes of the young; ngn, neurogenin; PDX1, pancreatic duodenal homeobox factor-1; Shh, Sonic hedgehog.

be achieved with respect to key extrinsic molecules in the years to follow.

INITIATION OF THE PANCREATIC PROGRAM

Little is known about the signals that control the early regionalization of the gut tube along its anteroposterior and dorsal-ventral axes. Two members of the hedgehog family of signaling molecules, Sonic hedgehog (Shh) and Indian hedgehog (Ihh), are together expressed uniformly, albeit in a partially overlapping manner, in regions of the gut endoderm anterior and posterior to the position at which the pancreas develops (27,28). At the level of the pancreatic anlage, the expression of both *Shh* and *Ihh* is restricted to the lateral prospective intestinal part of the epithelium and is excluded from both the dorsal and ventral pancreatic epithelium (27,28). Moreover, in transgenic mice, which ectopically express *Shh* in the prospective pancreatic epithelium, there is in an impairment of pancreatic development and a compensatory enhancement of intestinal development (28).

The region of the gut epithelium fated to form the dorsal pancreatic bud is initially in direct contact with the notochord (29,30), and notochord-derived factors, such as activin-β and fibroblast growth factor (FGF)-2, have been implicated in the initial repression of *Shh* and *Ihh* expression in the presumptive dorsal pancreatic endoderm (31–33). In contrast, the ventral gut epithelium destined to form the ventral pancreatic bud is never contacted by the notochord, implying that the exclusion of *Shh* and *Ihh* gene expression from the ventral pancreatic epithelium is achieved by a distinct notochord-independent mechanism. Additional differences have been described between the development of the dorsal and ventral pancreatic buds. The mesenchymal tissue that surrounds the dorsal pancreatic bud can be distinguished from more ventrally located mesenchyme by expression of the LIM homeodomain protein Isl1 (12). There is also a temporal delay in the appearance of endocrine cells in the ventral bud compared with the dorsal bud. In the mouse, the first endocrine cells appear in the dorsal bud at the 15-somite stage on embryonic day 9 at least 36 h before the overt differentiation of endocrine cells in the ventral bud (23,34). Regions of the adult pancreas that are derived from the ventral bud also contain fewer endocrine cells—notably glucagon cells (34).

Thus, despite increasing information on the identity and function of factors involved in pancreatic cell differentiation, the molecular steps that occur before the onset of *Ipf1/Pdx1* function to specify early stages in the program of differentiation of the dorsal and ventral pancreatic buds remain unclear. Recent findings on the role of the homeobox gene *Hlxb9* (encoding Hb9 [35]) during pancreatic development add interesting information both to the early events of pancreatic specification and the dorsal-ventral difference (36,37). *Hlxb9* is transiently expressed in regions of endoderm that give rise to the respiratory and digestive tubes as well as to the dorsal and ventral pancreatic anlage (36,37). Later, Hb9 expression in the pancreas becomes restricted to the insulin-producing β-cells (36,37). Dorsal pancreatic development is blocked in mice lacking *Hlxb9* function. In contrast, the ventral pancreas develops and contains both endocrine and exocrine cells (36,37). However, the relative proportions and spatial organization of the various endocrine cells in the ventral pancreas are perturbed. Thus, the requirement for *Hlxb9* in pancreatic development reveals a molecular distinction in the dorsal and ventral differentiation programs and sequential *Hlxb9* functions at both early and late stages of pancreatic differentiation.

The selective ablation of the dorsal pancreas is striking in view of the early widespread pattern of *Hlxb9* expression throughout the primitive dorsal endoderm (36). Both Hb9 and IPF1/PDX1 are expressed transiently by the early-forming pancreatic buds, but in the dorsal pancreatic anlage, the initiation of Hb9 expression appears to precede that of IPF1/PDX1 expression, whereas in the ventral anlage, Hb9 and IPF1/PDX1 appear to be expressed concurrently (36). The expression of both proteins reappears later in differentiated β-cells. Nevertheless, comparison of the phenotype of *Hlxb9*- and *Ipf1/Pdx1*–deficient mice reveals important differences in the function of these two genes. Most significantly, *Hlxb9* appears to control an earlier step in the specification of the dorsal pancreatic program, whereas *Ipf1/Pdx1* acts at a subsequent step in pancreatic development in both dorsal and ventral regions of the pancreas.

It still remains an open question whether the dependence of dorsal pancreatic differentiation on *Hlxb9* reflects a function intrinsic to the gut epithelium or whether it is a consequence of *Hlxb9* also being transiently expressed by notochord cells from approximately embryonic day 8 to embryonic day 10. As already mentioned above, the notochord has been suggested to be instrumental for the initiation of pancreatic development (31,32), and it is possible that *Hlxb9* acts to control dorsal pancreatic specification by regulating the expression of inductive or repressive factors secreted from the notochord. The expression of both *Shh* and *Ihh* is, however, restricted normally at the pancreatic level in *Hlxb9* mutant embryos (36). These results suggests that *Hlxb9* is not involved in establishing the suggested notochord-mediated zone of exclusion of *Shh* or *Ihh* expression in the presumptive dorsal pancreatic region. Nevertheless, the activation or repression of other genes required for pancreatic development could normally be controlled by signals from the notochord that are missing in *Hlxb9*-deficient mice. Thus, dorsal pancreatic development seems to be dependent both on the expression of *Hlxb9* and on the exclusion of hedgehog gene expression.

The specification of the ventral pancreatic differentiation program is independent of *Hlxb9* function, and hence a ventral pancreas develops with both exocrine and endocrine cell types. Nevertheless, there is a decreased number of insulin-positive cells, which is paralleled by an increase in the number of somatostatin-positive cells, together with lack of GLUT2 expression in insulin-positive cells present in the ventral pancreas of *Hlxb9* mutant mice. This suggests that *Hlxb9* is required for terminal differentiation and/or maturation of β-cells.

SPECIFICATION OF PANCREATIC CELL FATE

As already mentioned above, both *Isl1* and *NeuroD*/β2 are expressed in neurons and in all differentiated pancreatic endocrine cells as they appear. Nevertheless, neither of these two genes seems to function as a bona fide proendocrine gene, because endocrine progenitor cells seem to appear in the *Isl1* mutant embryos, and differentiated endocrine cells still form in *NeuroD*/β2 mutant mice (4). The mammalian *neurogenin* (*ngn*)*-1* and *-2* genes both function as determination genes during mammalian neurogenesis (38,39) analogous to

the proneural genes in *Drosophila* (24,25). Interestingly, a third member of the *ngn* gene family, *ngn3*, is expressed in the developing pancreas (40).

Generation of scattered differentiated cells from an initially homogenous field of cells, as in neuronal differentiation, is often executed by a process called lateral specification mediated by the notch signaling pathway (24,25). Differentiating cells express high levels of the ligands (delta or serrate) and signal to activate notch receptors on the adjacent cells to suppress the same fate in these cells. The activated intracellular domain of notch receptors interacts with the DNA-binding protein RBP-Jκ to activate expression of the bHLH *Hes* genes, which in turn repress expression of downstream target genes (24,25), including the *ngn* genes (38,39).

We have recently shown that similar to the generation of neurons during neurogenesis, the endocrine cells of the pancreas are specified by lateral specification via notch signaling (41). This study involved the analyses of pancreas development in mice genetically altered at several steps in the notch signaling pathway. Mice deficient for *delta-like gene 1* (*Dll1*) or the intracellular mediator *RBP-Jκ* showed accelerated differentiation of pancreatic endocrine cells paralleled by a depletion of the pool of pancreatic precursor cells (41). A similar phenotype was observed in mice overexpressing *ngn3* or the intracellular form of *Notch3*, which act as repressors of notch signaling (41,42). Collectively, these data show that by altering the notch signaling pathway at the ligand, receptor, and intracellular mediator level, the pancreatic cell differentiation fate is changed, providing evidence that notch signaling is critical for the decision between the endocrine and progenitor/exocrine fates in the developing pancreas.

Our findings were later confirmed by two different studies involving analyses of *Hes1*- and *ngn3*-deficient mice (43 and 44, respectively). In the *Hes1* mutant mice, there is accelerated differentiation of pancreatic endocrine cells resulting in severe pancreatic hypoplasia due to a depletion of the pool of pancreatic progenitor cells (43). The *ngn3* mutant mice develop a grossly normal pancreas, but histological analysis revealed a complete lack of pancreatic endocrine cells (44). Thus, these studies collectively demonstrate that in the developing pancreas, notch signaling controls the choice between differentiated endocrine and progenitor cell fates so that the lack of notch pathway signaling, resulting in high *ngn3* levels, promotes the endocrine fate. In contrast, cells with active notch signaling, resulting in an upregulation of *Hes1* expression, remain as undifferentiated progenitor cells, which would allow the subsequent proliferation, morphogenesis, and differentiation of the pancreatic epithelial precursor cells analogous to the function of notch signaling during early mammalian neurogenesis (24,25).

As differentiated endocrine cells appear, they delineate from the epithelium and migrate into the adjacent mesenchyme where they cluster. Thus, this migration is likely to result in a decrease in notch signaling (i.e., lateral inhibition) among progenitor cells and in addition allows the formation of islet structures. This would allow a continued appearance of cells with a primary (endocrine) fate that can respond to later-appearing inductive signals and thus generate distinct endocrine cells throughout the development of the pancreas depending on the inductive milieu. Progenitor cells that are not singled out to become endocrine cells will subsequently differentiate into exocrine cells or alternatively turn into ductal cells. The mesenchyme appears to be critically involved in promoting the differentiation of the exocrine pancreas, while at the same time negatively influencing endocrine differentiation. Scharfmann and colleagues (45–47), by performing a series of in vitro experiments involving culturing of pancreatic rudiments, provided evidence that follistatin and FGFs are capable of partly mimicking the effect of mesenchyme with respect to exocrine differentiation.

GENERATION OF FUNCTIONAL β-CELLS

To investigate the role of *Ipf1/Pdx1* in adult β-cells, we generated mice in which the *Ipf1/Pdx1* gene was disrupted specifically in β-cells by means of the Cre-lox approach. Mice in which *Ipf1/Pdx1* was inactivated in β-cells initially appear healthy but, upon aging, develop diabetes (48). 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 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 (48). Interestingly, heterozygosity for a nonsense mutation that results in a dominant-negative frameshift in the human *Ipf1* gene causes maturity-onset diabetes of the young (MODY)-4 (49), a form of diabetes that results from defects in insulin secretion. These findings provide evidence that also the late function of IPF1/PDX1 is conserved from mice to humans and point toward a role for IPF1 in ensuring normal β-cell homeostasis. Moreover, these data collectively suggest that decreased IPF1/PDX1 expression and/or function may contribute in a more general way to type 2 diabetes. Recent findings describing missense mutations in the human *Ipf1* gene support this because such mutations appear to represent a predisposing factor to type 2 diabetes development (50,51). It is intriguing that 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 MODY3 to mutations in hepatocyte nuclear factor (HNF)-4α and -1α, respectively, and MODY to mutations in HNF-1β, whereas MODY2 is linked to glucokinase (49,52–55). Additional transcription factors linked to diabetes are NeuroD/β2 and Islet-brain-1 (IB1) (56,57). It is therefore possible to assume that mutations in other transcription factors regulating the expression of β-cell genes will turn out to be linked to other forms of MODY and/or type 2 diabetes.

CONCLUDING REMARKS

Figure 1 summarizes the general pathways involved in islet cell ontogeny. Genetic studies in mice have shown that several transcription factors exhibit important roles in pancreatic development and/or glucose homeostasis (3–5). These studies have provided critical information regarding the development of the pancreas in both humans and mice, and many of these genes have been linked to human diabetic or pancreatic syndromes, thus demonstrating conservation in the function of genes that control pancreatic development (3–5). Our knowledge regarding proliferative and inductive signals operating during the different stages of pancreas development remain limited, however, and to fully unravel the principles

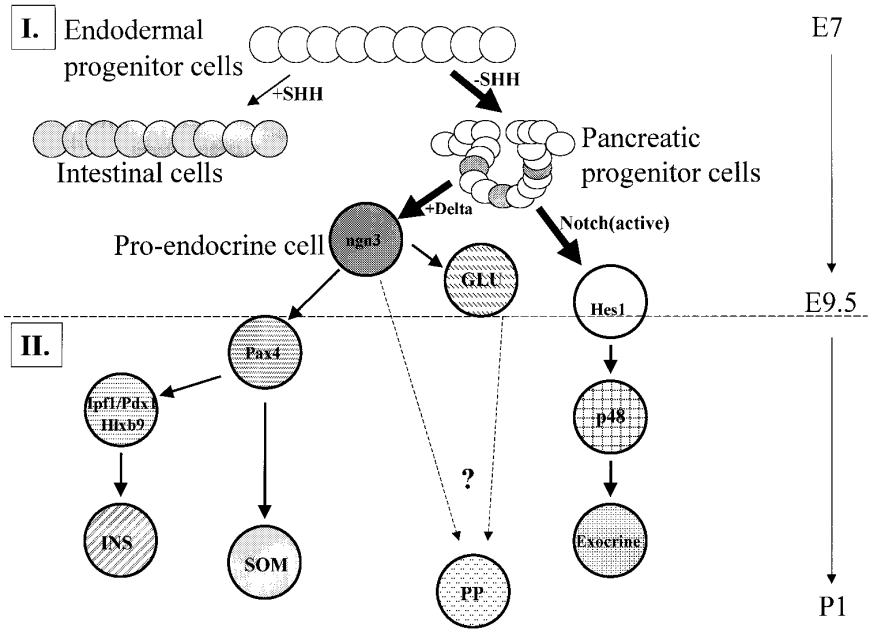

FIG. 1. Model for pancreatic cell differentiation. In contrast to the prospective intestinal cells, early endodermal cells destined to become pancreatic progenitor cells do not express the hedgehog signaling molecules that have been shown to negatively influence pancreatic development while promoting intestinal differentiation. The initiation of the dorsal, but not ventral, pancreatic program is also dependent on *Hlxb9* function. The initial phase (I) of pancreatic development, i.e., evagination and bud formation, appears to be independent of both *Ipf1/Pdx1* function and presence of mesenchyme. The further growth (i.e., from embryonic day 9.5 [E9.5] to embryonic day 10 [II]), morphogenesis, and differentiation of the pancreatic anlage critically require, however, *Ipf1/Pdx1* function as well as signals from the mesenchyme. During pancreas development, notch signaling appears to control the choice between endocrine and exocrine fates so that the lack of notch signaling pathway, resulting in high *ngn3* levels, promotes an endocrine fate. The endocrine cells appear in a temporal order in which the glucagon cells are the first to appear. Results obtained from the analysis of *Pax4*- and *Hlxb9*-deficient mice suggest that the establishment of insulin and somatostatin cell types may be coupled. Thus, it is possible that β- and δ-cells originate from a common *Pax4*-dependent progenitor cell and that *Hlxb9* might function at a later stage to promote β-cell fate and characteristics. Exocrine cells seem to be derived from cells with active notch signaling that consequently express high levels of *Hes1*, and *p48*. *p48* has been shown to be critically required for the generation of differentiated exocrine cells. GLU, glucagon; INS, insulin; P1, postnatal day 1; PP, pancreatic polypeptide; SOM, somatostatin.

of pancreas development, it is pivotal that these factors are also identified and characterized. In parallel, efforts focusing on defining and isolating pancreatic stem and/or progenitor cells need to be intensified to materialize the vision in which β-cells will be selectively induced and amplified, thus providing us with a simple manageable approach toward generating sufficient amounts of functional β-cells for cell replacement therapy in both type 1 and type 2 diabetes.

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