0013-7227/05/$15.00/0
Printed in U.S.A.

Endocrinology 146(3):1025–1034

doi: 10.1210/en.2004-1576

Minireview: Transcriptional Regulation in Pancreatic Development

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Considerable progress has been made in the understanding of the sequential activation of signal transduction pathways and the expression of transcription factors during pancreas development. Much of this understanding has been obtained by analyses of the phenotypes of mice in which the expression of key genes has been disrupted (knockout mice). Knockout of the genes for Pdx1, Hlxb9, Isl1, or Hex results in an arrest of pancreas development at a very early stage (embryonic d 8–9). Disruption of genes encoding components of the Notch signaling pathway, *e.g.* Hes1 or neurogenin-3, abrogates development of the endocrine pancreas (islets of Langerhans). Disruption of transcription factor genes expressed more downstream in the developmental cascade (Beta2/NeuroD, Pax4, NKx2.2, and Nkx6.1) curtails the formation of insulin-producing β-cells. An understanding of the importance of transcription factor genes during pancreas development has provided insights into the pathogenesis of diabetes, in which the mass of insulin-producing β-cells is reduced. (*Endocrinology* 146: 1025–1034, 2005)

RECENT STUDIES OF the biology of pancreas development have shed new light on the cause of type 2 diabetes. The identification of transcription factors involved in regulation of the expression of key genes required in the developing and adult endocrine and exocrine pancreas has been provided by observing the phenotypic consequences of targeted disruptions of the genes (gene knockouts) encoding these factors in mice. Remarkably, almost without exception, disruption of these genes has resulted in phenotypes of impaired development of the pancreas and consequent diabetes. Furthermore, lessons learned from the gene knockouts in mice have been used to successfully identify mutations in several of the corresponding orthologous genes in individuals with familial monogenic type 2 diabetes.

In this minireview we attempt to encapsulate a rapidly growing body of knowledge that is providing insight into the genetic contributions to the development of diabetes. The thematic emphasis is on the impact that genetic polymorphisms or mutations in genes encoding transcription factors essential for pancreas development have on predisposition for diabetes. For more comprehensive, in-depth information on this topic, the reader is referred to several recent excellent reviews (1–9).

Anatomical and Morphological Development of the Pancreas

The pancreas consists of three main tissue cell types (in addition to vascular and stromal supporting tissues): the exocrine acinar tissue that produces digestive enzymes; the endocrine cells (islets of Langerhans) that produce the hormones involved in nutrient homeostasis, such as insulin and glucagon; and the elaborately branched ductal tree (10). The pancreas originates early in development [embryonic d 8.5 (e8.5) to e9.5 in the mouse] by the outcropping of two buds (ventral and dorsal) of cells from a specialized prepatterned endodermal epithelium located in the region of the foregut that is to become the duodenum (Fig. 1). By e10.5, the partially differentiated epithelium of the two buds undergoes branching morphogenesis into a ductal tree that by e12.5 results in the formation of two primordial pancreas organs consisting predominantly of an undifferentiated ductal epithelium (first developmental transition).

Between e13 and e14, the dorsal and ventral pancreata rotate and fuse into a single organ. During e14.5 and e15.5, the exocrine pancreas differentiates from the ductal epithelium; on e15.5, acini are clearly discernible from ducts. Endocrine cells are present from the very beginning of development (e9.5), but up until e14 they are arrayed as single cells within the ductal epithelium, after which they undergo extensive proliferation (second developmental transition). On e16, the endocrine cells begin to organize into islet-like clusters. The islets are not fully formed until shortly before birth on e18–e19 and undergo additional remodeling and maturation for 2–3 wk after birth (third developmental transition).

The endocrine cells of the pancreas arise from stem/progenitor cells located within the early (e9.5) gut endoderm (Fig. 1). Previous ideas that pancreatic endocrine cells are derived from the neural crest have been disproved by decisive quail-chick chimera experiments, and it is now established that they are of endodermal origin. It has been shown that isolated pancreatic endodermal-derived duct cells from embryonic rat pancreas can directly differentiate into hormone-expressing cells when cultured in the presence of

First Published Online December 16, 2004

Abbreviations: bHLH, Basic helix-loop-helix; e, embryonic day; HNF, hepatic nuclear factor; MODY, maturity-onset diabetes of the young; Ngn, neurogenin.

***Endocrinology* is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**

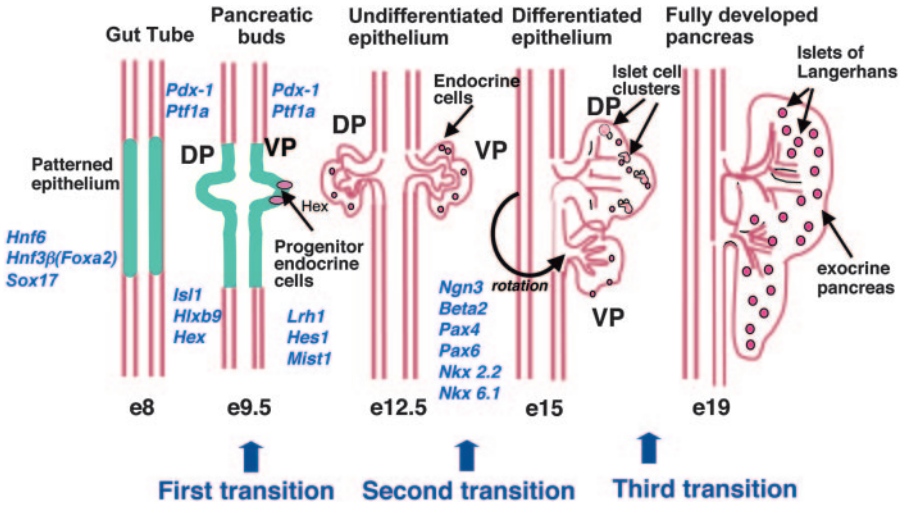

FIG. 1. Schematic diagram of pancreatic development in the mouse. On e8, prepatterned endodermal epithelium of the foregut forms dorsal (DP) and ventral (VP) buds by e9.5, which then develop into branching ducts and undifferentiated epithelium (e12.5; first development transition). Single endocrine cells are interspersed among the undifferentiated epithelium. The buds begin to differentiate into endocrine and exocrine cellular lineages by e14 and proliferate and expand extensively (second development transition). By e15, the dorsal and ventral pancreases rotate, fuse, and form a nearly fully developed pancreas by e19, containing the endocrine cells organized into isolated clusters that condense into the islets of Langerhans (third developmental transition). The third transition, consisting of maturation of endocrine cells and their acquisition of full nutrient responsiveness, continues for 2–3 wk after birth. The representative transcription factors expressed during the program of development are indicated in *blue* and are diagrammed in a simplified developmental cascade in Fig. 2. The approximate embryonic age (in days) is designated for each stage of development.

gut mesenchyme (11). The earliest endocrine cells detected in the pancreatic anlage in the foregut (e9.5) express glucagon and members of the PP-fold family of hormones, peptide YY, pancreatic polypeptide, and neuropeptide Y.

Soon thereafter (e10–e10.5), the cells coexpress glucagon and insulin and then divide into distinct lineages that express glucagon (α-cells) and insulin (β-cells). On about e14, somatostatin-expressing cells (δ-cells) arise. The precise temporal sequence of the expression of the hormones during pancreatic development remains somewhat controversial; the above description represents a consensus based on immunocytochemical examination of the mouse pancreas during embryonic development. Specific ablations of the α-, β-, and PP cell lineages have been accomplished by creating transgenic mice in which the cell-cidal diphtheria A toxin was targeted to the developing pancreas using the glucagon, insulin, and PP promoters (12). The targeted diphtheria A toxin driven by these respective promoters effectively ablated the α-, β-, and PP cell lineages, but only PP ablation alone impaired the formation of α- and β-cells, suggesting that PP-expressing cells, and not glucagon- or insulin-expressing cells, are precursors of the other endocrine cell types.

The signaling factors involved in specifying the segregation of the endocrine and exocrine compartments are of great interest. Studies of rat pancreatic rudiments grown in culture indicate that signals derived from the mesenchyme are either instructive or permissive for the determination of endocrine or exocrine cell lineages. Pancreas buds obtained from e12.5 rat embryos cultured in the presence of pancreatic mesenchyme develop into exocrine pancreatic tissue along with only a few immature insulin-producing endocrine cells. Buds cultured in the absence of pancreatic mesenchyme develop into endocrine tissue, and the development of exocrine tissue is retarded. Follistatin, present in e12.5 mesenchyme, mimics the effects of pancreatic mesenchyme to accelerate exocrine and limit endocrine differentiation (13).

Low levels of expression of endocrine and exocrine-specific genes are detectable for 2–3 d before development of the acinar cells characteristic of the differentiated pancreas (14–16) (first developmental transition). Segregation of insulin-expressing cells from amylase-expressing cells occurs on e15 (17). Levels of mRNAs encoding endocrine hormones and exocrine proteins increase markedly from e14 to e20 (16) by several thousand-fold (18) (second developmental transition) (Fig. 1).

The observation of a coordinate regulation of several genes expressed in the exocrine pancreas led to the identification of a complex of cell-specific DNA-binding proteins (PTF1) that activate the promoters of the several enzyme genes. PTF1 is detectable in acinar cells from e15 throughout adulthood and consists of a heterooligomer comprised of three subunits (19, 20). One of the three proteins, Ptf1a/P48, is an exocrine pancreatic-specific transcription factor of the basic helix-loop-helix (bHLH) type. Ptf1a RNA is detectable in the developing pancreas on e12 and was believed to be produced exclusively in the exocrine, and not the endocrine, pancreas (21). Mice nullizygous for the Ptfla/P48 gene die within hours of birth and have no detectable exocrine pancreatic tissue. Endocrine cells develop and migrate from the intestinal mesentery to colonize the spleen (22). A subsequent study showed that Ptfla is expressed in ductal, endocrine, and exocrine progenitors (23). Inactivation of Ptf1a switches pancreatic progenitors to intestinal epithelial progenitors. Transgenic expression of Pdx1 targeted by the Ptf1a promoter in Pdx1-null mice restores the development of pancreatic tissue. The Forkhead group of proteins, hepatic nuclear factor-3β (HNF3β; Foxa2) and Hnf3γ, binds to regulatory DNA sequences in the amylase promoter and

contributes to exocrine-specific gene expression (24). Transcription of the amylase gene is stimulated by insulin, providing a possible mechanism for cross-talk between genes specific to the endocrine and exocrine compartments of the pancreas (25).

Morphogen signaling in early pancreas development

It is well recognized that the differentiation of epithelial cells during development requires their interactions with growth factors, or morphogens, produced by the mesodermal mesenchyme and by vascular endothelium in a specific temporal and spatial fashion (26). Studies of early pancreatic development in the chick embryo have provided insight into mesenchymal-derived morphogenesis in the signaling pathways involved in differentiation of the prepatterned region of the foregut endoderm that becomes the pancreas. At early stages of the developing chick pancreas, the dorsal pancreatic bud is in close proximity to the notochord (15). Removal of the notochord in early embryogenesis, before the 13-somite stage, prevents the development of adjacent endoderm otherwise destined to become the dorsal pancreas (27). Candidate mediators by which the notochord signals to the dorsal pancreatic endoderm include the growth factors activin-β and fibroblast growth factor-2 (28). When applied to chick embryo cultures of foregut, these growth factors mimic the notochord in repression of the signaling factor Sonic Hedgehog in the dorsal pancreatic endoderm and permit expression of pancreatic genes such as glucagon and insulin. Repression of Sonic Hedgehog in the pancreatic endoderm in the presence of Pdx-1 expression appears to be a prerequisite for the pancreas to develop; targeted misexpression of Sonic Hedgehog directed by the Pdx-1 promoter to the developing pancreas in mice causes the development of a disorganized mixed phenotype of intestinal and pancreatic tissues and absence of the spleen (29). Inhibition of Sonic Hedgehog signaling by the steroid alkaloid cyclopamine promotes pancreatic development in Pdx-1-expressing segments of foregut in the chick embryo (30).

Signals from developing structures other than notochord must control the development of the ventral pancreatic bud, but have yet to be completely defined. A recent study demonstrated that the homeobox protein, Hex, controls the positioning of endoderm cells beyond the cardiogenic mesoderm and thereby dictates ventral pancreatic specifications (31). The LIM (Lin11, Isl-1, and Mec-3) homeoprotein Isl1 is required for the formation of dorsal, but not ventral, pancreatic mesenchyme. Mice in which Isl1 expression is disrupted by gene knockout fail to develop a dorsal pancreas, but partially execute the differentiation of ventral exocrine pancreas (32). The dorsal pancreatic endoderm obtained from Isl1-null mice and studied *ex vivo* responds to signals transmitted from cocultured wild-type mesenchyme by differentiating into pancreatic tissue. These findings reinforce the critical requirement of mesenchymal signaling to the endoderm in allowing normal pancreatic development. The elucidation of additional signaling pathways that are critical for development of the pancreas requires additional investigation.

Transcription Factors and Pancreas Development

Several transcription factors are now recognized to be critical regulators of pancreatic development in mouse models of gene expression in which specific genes have been disrupted (gene knockouts; Table 1 and Fig. 2) (for reviews, see Refs. 1–7 and 33–38). Expression patterns of transcription factors limit the boundaries of the developing pancreas and determine the differentiation programs of individual cell lineages. Transcription factors appear to serve dual functions in determining early cellular development and later in maintaining the phenotype of terminally differentiated cells. Increasing recognition of the importance of transcription factors as a cause of human disease highlights the significance of the identification of these factors involved in the regulation of pancreatic development. The transcription factor cascade depicted in Fig. 2 is likely to be a marked oversimplification. A recent study used chromatin immunoprecipitation combined with promoter microassays to identify target genes in hepatocytes and pancreatic islets for the transcription factors Hnf6, Hnf1α, and Hnf4α (39). In islets, Hnf6, Hnf1α, and Hnf4α bound to 189, 106, and 1400 target genes, respectively. A significant number of the target genes in islets encoded transcription factors, *e.g.* eight for Hnf1α and 14 for Hnf4α.

Pdx-1

The pancreatic duodenal homeobox gene-1 (Pdx-1) is a master regulator of both pancreatic development and the differentiation of progenitor cells into the β-cell phenotype (35, 36) (Fig. 2). The transcription factor Pdx-1 was identified by several laboratories and so has multiple names, including Ipf-1 (40), Stf-1 (41), and Idx-1 (42). *Pdx-1* belongs to a ParaHox gene cluster that lies outside the major Hox cluster of homeodomain proteins (43). The ParaHox set of genes consists of Gsh, Pdx, and Cdx, expressed in anterior, middle, and posterior locations in the vertebrate axis. Because Pdx-1 is a pancreas-specific homeoprotein, it was cloned and identified as a β- and δ-cell-specific regulatory factor for transcriptional expression of insulin and somatostatin genes and has subsequently been shown to regulate the expression of other islet-specific genes, including Glut-2 (44), islet amyloid polypeptide (45), and glucokinase (46). The stable introduction and expression of Pdx-1 in a glucagon-secreting α-cell line are sufficient to induce expression of the β-cell-specific genes insulin, glucokinase, and islet amyloid polypeptide (33, 47). In the differentiated β-cell, Pdx-1 is a glucose-responsive regulator of insulin gene expression (48–51). The function of Pdx-1 in response to glucose is regulated by both its phosphorylation (50, 52) and nuclear translocation (53).

The patterns of expression of Pdx-1 in the developing pancreas are maintained throughout development and provide both spatial and temporal contributions to the commitment of endoderm to a pancreatic phenotype (54). Pdx-1 expression is first detected in murine cells on e8.5 in a narrow band of foregut endoderm. On e9.5, Pdx-1 expression is seen in both ventral and dorsal pancreatic buds (54, 55). From e11.5 to e13.5, Pdx-1 is expressed throughout the developing ductal tree. As the exocrine pancreas appears and the islets begin to form into the hormone-producing cells (e14–e15),

TABLE 1. Endocrine pancreatic phenotypes of transcription factor knockouts

Gene knockout	Onset of pancreatic expression	Alterations in phenotype	Ref. no.
Sox17	5.5–6.5	Expressed throughout endoderm after gastrulation; required for pancreas specification	110
HNF3β	5.5–6.5	Embryonic lethal (e11); lack of foregut formation	59, 111
HNF3β (cre lox)		Hyperinsulinemic hypoglycemia	60
Hlxb9	8	Failure of dorsal bud to develop; relative reduction in β-cell number in remnant pancreas	56, 60
Hex	8.5	Failure in ventral pancreatic specification	31
PDX-1	8.5	Pancreatic agenesis; initial dorsal bud formation	57, 112, 113
PDX-1 (cre lox)		Diabetes	112
Isl-1	9	Embryonic lethal (e9.5); lack of dorsal mesenchyme formation and exocrine differentiation, complete lack of differentiated islet cells	114
Hes-1	9.5	Pancreatic hypoplasia; depletion of pancreatic precursors; precocious, premature endocrine differentiation (α-cell)	115
HNF6	9.5	Impaired endocrine cell differentiation; severely reduced *ngn3* expression	116
Ngn3	9–9.5	Complete absence of endocrine cells and endocrine precursors	71
Beta2/NeuroD	9.5	Reduction in endocrine cell number (especially β-cell); arrested islet morphology; increased apoptosis of endocrine cells	72
Pax6	9–9.5	Absence of α-cells	69, 70
Pax4	9.5	Absence of β- and δ-cells; increase in α-cells	73
Nkx2.2	9.5	Lack of insulin-producing β-cells; increased number of incompletely differentiated β-cells; reduction of α- and PP cells	75
Nkx6.1	9–9.5	Profound inhibition of β-cell formation	77
HNF3α	7.5	Reduction of proglucagon gene expression (70%)	78
Ptf1a(P48)	10	Complete absence of exocrine pancreatic tissue; endocrine cells found in spleen	59, 60
Mist1	10.5	Extensive disorganization of exocrine tissue	93
MafA	14	?	91, 92
MafB	15	?	92

Pdx-1 expression shifts to the endocrine compartment. Co-localization of Pdx-1 and the exocrine cell-specific enzyme amylase is observed on e13.5, diminishes markedly by e16.5, and is nearly undetectable in the exocrine pancreas of adult mice. During the later stages of islet development, by e18.5, the expression of Pdx-1 becomes mostly restricted to the mature β-cells of the endocrine pancreas (54, 55). In the adult pancreas, subpopulations of somatostatin-producing and pancreatic polypeptide-producing cells also express Pdx-1. Only a few glucagon-producing cells express Pdx-1 (40–42, 54).

Targeted disruption of the *pdx-1* gene in mice results in agenesis of the pancreas (55, 56). Nullizygous *pdx-1* mice are viable, but die within days after birth (56). In these animals rudimentary pancreatic buds form, but then regress. Defects in development of the rostral duodenal epithelium and enteroendocrine cells also occur (55). The pancreatic mesenchyme is visibly normal in *pdx-1*-null mice, but the pancreatic

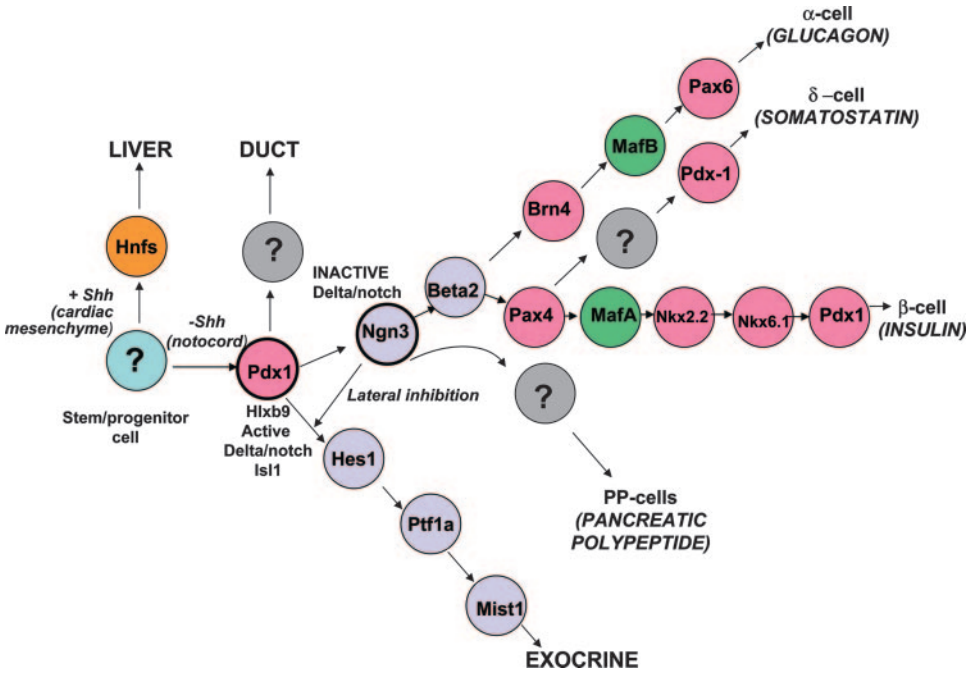

FIG. 2. A simplified hierarchy of transcription factor expression in the developing pancreas based on the temporal expression and phenotypic results of gene-specific knockouts of the factors in mice. The color scheme designates the type (class) of transcription factors involved in the proposed hierarchal cascade: *pink*, homeodomain proteins; *purple*, bHLH; *green*, Maf proteins; *orange*, Hnfs; and *gray*, unidentified factors. The model does not strictly represent direct signaling pathways, but, rather, a consensus of gene expression requirements that permits continued progression toward the terminal differentiated state.

epithelium is defective in its ability to respond to mesenchymal signals required to complete the differentiation program (57). A few insulin-expressing cells are detected in Pdx-1-null mice early in pancreas development, suggesting that a population of insulin-positive, Pdx-1-negative cells may occur distinct from the mature Pdx-1-expressing β-cells of the developed pancreas (57). Notably, a child born without a pancreas (pancreatic agenesis) is homozygous for an inactivating mutation in *pdx-1* (ipf-1) (58), underscoring the importance of the Pdx-1 transcription factor in the development of the human, as well as the mouse, pancreas.

Hlxb9

Hlxb9 is a bHLH transcriptional activator protein expressed early in pancreas development on e8. In mice homozygous for a null mutation of Hlxb9, the dorsal lobe of the pancreas fails to develop (59, 60). The remnant Hlxb9-null pancreas has small islets with reduced numbers of insulin-producing β-cells.

Isl1

The LIM homeodomain protein Isl1 is expressed in the developing pancreas and the central nervous system (61, 62). In adult animals, Isl1 is expressed in all of the hormone-producing cells of pancreatic islet cells, but its low level of expression in β-cells argues against it having an important function to regulate insulin gene transcription. Targeted disruption of the *isl-1* gene results in an early arrest of embryonic development on approximately e9.5 (63). Analysis of the pancreatic anlage reveals failure of development of the dorsal pancreatic mesenchyme and the complete absence of endocrine cells. Notably, development of the ventral pancreatic epithelium and associated mesenchyme in Isl-1-null mice is unaffected (32). The addition of mesenchyme derived from pancreas or lung to explants of dorsal pancreatic epithelium of *isl-1*-null animals results in the development of exocrine tissue, but no endocrine cells are generated. These findings suggest that Isl1 is required for development of the mesenchyme of the dorsal pancreatic bud and also for differentiation of the dorsal pancreatic epithelium to endocrine cells.

Hes1, Neurogenin-3 (Ngn3), and Notch Signaling

Notch signaling is an important factor in the periodicity of somatogenesis in the vertebrate embryo (64). Although its role as a key participant in the biological clock of embryonic development is slowly emerging, evidence of notch signaling in the developing pancreas suggests a primary role in governing cell fate. Signals exchanged between neighboring cells through the Notch receptor can amplify and consolidate differences in cellular phenotypes; thus, Notch signaling provides a developmental tool to influence morphogenesis and organ formation. Several recent studies suggest the involvement of Notch in the regulation of pancreatic exocrine and endocrine cell fate, reflecting the well established regulatory functions of Notch in neural development by a mechanism known as lateral inhibition (65). In the neural crest, expression of the bHLH transcription factor Ngn leads to the expression of extracellular ligands such as δ (dl), Serrate (Ser), and Jagged (Jag) in neural precursor cells that activate Notch receptors on adjacent cells. Intracellular recombination signal sequence binding protein mediates Notch receptor-dependent expression of Hes genes in the adjacent cell. Hes genes encode bHLH factors that function to repress the expression of Ngn and other target genes, thereby preventing neuronal differentiation in cells adjacent to developing neuroblasts. In essence, the lateral inhibition model provides a mechanism by which the cell that will differentiate into the endocrine lineage (*ngn3* positive) inhibits its neighboring cells, forcing them to adopt a nonendocrine fate. In the generation of the pancreas, Ngn3 is required for the development of all endocrine cell lineages of the pancreas (9, 66) and has been designated as a marker of islet precursor cells (see below). Overexpression of Ngn3 in the developing pancreas results in accelerated differentiation of endocrine progenitor cells. Hes-1, although expressed in the pancreas, is absent from endocrine cells, in agreement with this model. Hes-1 knockout mice exhibit a rapid depletion of precursor cells and a resultant precocious development of endocrine cells (67). Furthermore, mice deficient for δ-like ligand-1 (dll1) display accelerated differentiation of pancreatic epithelial cells expressing Ngn3 (68).

As discussed above, the initiating step toward endocrine cell fate appears to depend on regulation of the bHLH transcription factor Ngn3. The role of Ngn3 as an early marker of cells differentiating toward a primary endocrine fate was clearly demonstrated by its ectopic expression under the regulatory control of the *pdx-1* promoter in transgenic mice (68). Small and poorly branched pancreatic buds were evident on e12, followed by premature and precocious differentiation of pancreatic progenitor cells into endocrine cells, and the resultant depletion in precursor cells with the capacity to proliferate, branch, and differentiate into exocrine cells. Expression of Ngn3 starts on e9–e9.5, peaks on e15.5 during the major wave of endocrine cell genesis, and is greatly diminished at birth, with little or no detection of Ngn3 in the adult pancreas. Significantly, mice homozygous for an *ngn3*-null mutation failed to generate any endocrine cells or putative endocrine precursors during development (66). These observations led to the assumption that *ngn3* expression is a functional marker of an islet cell precursor population in the developing pancreas.

Because Ngn3 is both necessary and sufficient to drive the differentiation program of islet cells during pancreatic development, it may be inferred that the activities of specific transcription factors that regulate *cis*-acting elements within the promoter region of the *ngn3* gene, collectively dictate the endocrine fate of the cell. In the distal region of the *ngn3* promoter, a cluster of binding sites for HNF1α, HNF3β, and HNF6 was recently identified (69). Concurrent with the roles of these transcription factors in regulating *ngn3* gene expression and the subsequent initiation of the endocrine program, disruption of their respective genes in the developing embryo results in corroborative phenotypes, affecting the progression of early pancreatic development and endocrine specification. Mice homozygous for a disrupted *hnf1α* gene have smaller islets and reduced insulin secretion (70). HNF3β-null mice show a lack of foregut formation (71, 72), and in mouse embryos lacking *hnf6* expression, a marked

reduction in endocrine cell differentiation is observed along with severely reduced levels of *ngn3* gene expression (73). However, the independent expression of HNF factors appears not to be sufficient for *ngn3* expression, but, rather, a cooperative mechanism exists between these accessory proteins in the cell type-restricted activation of the *ngn3* gene promoter (69). Hes-1 represses the expression of the *ngn3* gene promoter by binding to several silencer sites located close to the transcription initiation site and thus suppresses endocrine precursor patterning through the Notch signaling pathway.

Beta2/NeuroD

The bHLH transcription factor Beta2/NeuroD is a key regulator of both insulin gene transcription in pancreatic β-cells and the terminal differentiation of neurons (74, 75). Beta2/NeuroD expression occurs in a subset of pancreatic epithelial cells as early as e9.5, colocalizing with glucagon expression (76). On e14.5, expression appears within and in proximity to ductal epithelium, and by e17.5, expression is restricted to islets (77). Beta2/NeuroD heterodimerizes with ubiquitous bHLH proteins of the E2A family to regulate transcription of the insulin gene and other β-cell-specific genes. Expression of the Beta2/NeuroD gene is activated by Ngn3 (78). Mice homozygous for a targeted disruption of the *β2/neuroD* gene survive to birth, but die within 3–5 d postpartum of severe hyperglycemia. The islets of these mice are dysmorphic and have markedly diminished numbers of endocrine cells arranged in streaks and irregular aggregates and reduced numbers of β-cells (77).

Pax4

Pax4 is a paired-box homeoprotein whose expression is restricted to the central nervous system and the developing pancreas. By e9.5, Pax4 is expressed in both ventral and dorsal buds of the developing pancreas, and by the time of birth, expression is restricted to β-cells. Targeted disruption of Pax4 in mice results in a striking pancreatic phenotype (79). The *pax4*-null mice survive birth, but die within 3 d of hyperglycemia and dehydration. There is a virtual absence of β- and δ-cells, but α-cells are increased. PDX-1 expression in *pax4*-null pancreas is absent, a finding consistent with the absence of differentiated β-cells. Thus, Pax4 functions early in the development of islet cells to promote the differentiation of β- and δ-cells.

Pax6

Another paired-box homeoprotein, Pax6, is important for islet cell development. The expression of Pax6 is restricted to the eye, the central nervous system, the nose, and the endocrine pancreas (80, 81). Pax6 is expressed early in the epithelium of the developing pancreas on e9.0 in both dorsal and ventral pancreatic buds and is expressed in differentiated α-, β-, δ-, and PP cells. A spontaneous mutation of the *pax6* gene in mice, known as *Small eye* ($Sey^{-/-}$), has defined the importance of Pax6 in the development of the lens placode of the eye and portions of the forebrain (82). $Small\ eye^{Neu}$ mice have abnormal organization of islets, with decreased α-, β-, δ-, and PP cells (83). The production of glucagon and insulin is substantially reduced in these animals. Pax6 regulates the promoters of the glucagon, insulin, and somatostatin genes, suggesting a mechanism to explain the decreased hormone production observed in *Small eye* mice (83). Knockout *pax6* nullizygous mice die within minutes of birth. These animals fail to form islets (84). The disorganization of islets is proposed to relate to a possible function of Pax6 in the regulation of cell adhesion molecules (84, 85). These mouse models of gene knockouts implicate both Pax4 and Pax6 as key regulators of the terminal steps in cellular differentiation of the endocrine pancreas.

Nkx2.2 and Nkx6.1

Members of the NK2 family of homeoprotein transcription factors, Nkx2.2 and Nkx6.1, are regulators of the differentiation of pancreatic endocrine cells (86). Nkx2.2 is expressed early in developing pancreatic buds and is later restricted to α-, β-, and PP cells of islets. Nkx6.1 is expressed primarily in β-cells of adult islets (87, 88). Disruption of either the Nkx2.2 or Nkx6.1 gene in mice results in death soon after birth due to severe diabetes (86). The pancreata of Nkx2.2 and Nkx6.1 knockout mice have no insulin-producing cells, and glucagon-producing cells are diminished, although the exocrine pancreas is histologically normal. Nkx2.2-null mice have a large number of hormone-negative, arrested precursor cells that express endocrine pancreas-specific proteins, such as synaptophysin, neuroendocrine cell adhesion molecule, amyloid polypeptide, and prohormone convertase 1/3, but no hormones. Nkx6.1-null mice have a selective reduction of β-cells with a normal complement of other endocrine cell types (89). Notably, it appears that in both Nkx2.2- and Pax4-null mice that fail to develop insulin-producing cells, the arrested progenitor β-cell expresses the hormone ghrelin (90).

MafA and MafB

MafA and MafB are transcription factors previously recognized to be important in development of the lens of the eye. During pancreatic development, MafA is expressed in early β-cells during the second developmental transition on e14 (91, 92). MafB appears to be expressed in glucagon-producing α-cells on e15. The phenotypic consequences of MafA and MafB gene knockouts in mice have not yet been reported.

Mist1

Mist1 is a bHLH transcription factor expressed in pancreatic acinar cells (93). Mist1-null mice exhibit extensive disorganization of exocrine tissue and intracellular enzyme activation, and acinar tissue acquires a ductal phenotype. It has been proposed that Mist1-null mice represent a genetic model for chronic pancreatic injury and that Mist1 serves as a key regulator of acinar cell function and stability.

Relevance of pancreatic development to pathogenesis of diabetes mellitus

Diabetes mellitus results from a combination of insulin resistance and deficient or dysregulated insulin secretion

TABLE 2. Transcription factors involved in pancreas development associated with diabetes

Gene/protein	Phenotype
Monogenic diabetes	
HNF4α	MODY1
HNF1α	MODY3
IPF1	MODY4
HNF1β	MODY5
Beta2	MODY6
Polygenic contribution to diabetes	
Pax4	Late-onset diabetes
Isl1	Late-onset diabetes
Ngn3	Late-onset diabetes

by pancreatic β-cells. Human genetic studies indicate that dysfunction of β-cell transcriptional regulators causes some forms of type 2 diabetes. Maturity-onset diabetes of the young (MODY) is a type of autosomal dominant monogenic diabetes with early onset (age of 30 yr or less). The prevalence of MODY-type diabetes is higher than initially proposed (94), now accounting for 5–10% of type 2 diabetes. The autosomal dominant inheritance pattern of this type of diabetes has facilitated genetic analysis of familial type 2 diabetes. The first genetic mutations linked with MODY were in the glucokinase gene (MODY2), an enzyme essential for glucose-sensing in β-cells (95, 96). The five additional types of MODY identified to date are linked to mutations in transcription factors relevant to pancreatic β-cell function (Table 2), although additional MODY genes remain to be defined (97). These transcription factors function in both pancreatic development and the differentiated β-cell. MODY3 results from mutations in the Hnf1α gene. Hnf1α is a homeodomain and POU-like DNA-binding protein that transactivates insulin and IGF-I gene transcription (98, 99). Disruption of the Hnf1α gene in mice results in Laron dwarfism, decreased insulin gene expression, and diabetes. Humans with Hnf1α (MODY3) mutations have defective glucose utilization, insulin secretion, and glucose disposal (100). MODY1 is a result of mutations in the transcription factor Hnf4α. Hnf4α activates the HNF1α promoter (101) (Fig. 3), which is relevant in light of the linkage of Hnf1α mutations to MODY3 (102). Both Hnf4α and Hnf1α are essential for normal pancreatic β-cell function, and human subjects with mutations in either gene develop impaired glucose tolerance and/or diabetes (103, 104). MODY5 results from mutations in Hnf1β, a POU (Pit-1, Oct-1, and Unc-86)-type homeoprotein, structurally related to Hnf1α. Selective deletion of the Hnf1β gene in mice manifests in impaired glucose tolerance and impaired glucose-dependent insulin release (105). In addition, the pancreata of Hnf1β mice have increased Pdx1 and Hnf1α mRNA levels and decreased levels of Hnf4α mRNA.

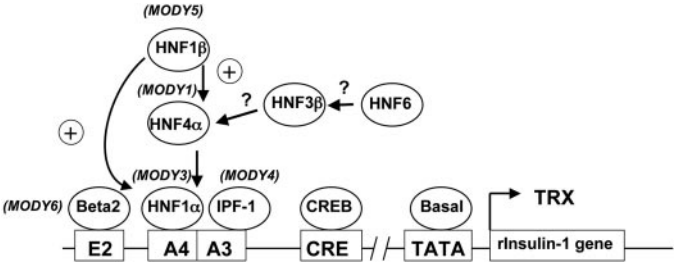

FIG. 3. Transcription factor cascade involved in the regulation of the rat insulin-1 gene promoter. The enhancers E2-A4/A3 (and E1/A1, not shown) bind and are activated by bHLH proteins and the homeodomain protein Ipf-1 (Pdx-1). HNF1α also binds to the E2-A4/A3 complex. Hnf4α, in turn, is regulated by Hnf1β and possibly Hnf3β (activated by Hnf6). Mutations in Hnf4α, Hnf1α, Ipf-1, Hnf1β, and Beta2 are responsible for MODY1, -3, -4, -5, and -6, respectively. CREB, cAMP response element-binding protein; Basal, basal transcription factors; Trx, transcriptional activation of rat insulin-1 gene promoter (rInsulin-1 gene).

Mutations in the Beta2/NeuroD gene appear to have defined a MODY6. At least two distinct mutations in the Beta2/NeuroD gene appear to be responsible for the onset of type 2 diabetes (MODY6) (106). The linkage of MODY with three members of the Hnf regulatory cascade of transcription factors suggests that additional genes in the Hnf signaling cascade may be involved in causing diabetes. For example, Hnf6 is expressed in the developing exocrine pancreatic cells and in islets and may be important in stimulating the transcriptional expression of Hnf4α and Hnf3β. Hnf3β is a transcription factor expressed in embryonic pancreatic buds and islets and functions as an activator of the expression of the Hnf4α, Hnf1α, and Pdx-1 genes.

An inactivating mutation in transcription factor Pdx-1 (Ipf1) causes MODY4. Pdx-1 is required for pancreatic development. Individuals heterozygous for the inactivating mutation in the Pdx-1 gene develop diabetes. Evaluation of this inactivating Pdx-1 mutation *in vitro* suggests that diabetes results from both diminished gene dosage (haploinsufficiency) and dominant negative inhibition of transcriptional activation of the intact PDX-1 allele. In the mouse model of Pdx-1 inactivation, Pdx-1 hemizygous animals are glucose intolerant, and conditional inactivation of Pdx-1 after the pancreas has developed results in diabetes. Several missense and insertional mutations in the Pdx-1 gene are now shown to be associated with the development of late-onset type 2 diabetes in the French and United Kingdom populations (107, 108). β-Cell dysfunction due to altered cellular levels of Pdx-1 may occur in the absence of mutations. Suppression of Pdx-1 expression in transgenic mice expressing a conditional Pdx-1 antisense ribozyme targeted by the rat insulin promoter develop age-dependent diabetes (109). In β-cell models of glucotoxicity, sustained exposure to hyperglycemia downregulates Pdx-1 expression concordant with decreased insulin gene transcription.

Several additional transcription factors essential for pancreatic development or for differentiated β-cell function, including the regulation of insulin gene transcription, are candidate diabetes genes. These transcription factors include Isl-1, Pax4, Nkx2.2, Nkx6.1, and Ngn3, based on the findings that targeted disruptions of the genes encoding these factors in mice result in impaired development of the pancreas and/or pancreatic islets (β-cells). A subset of these transcription factors, such as Pdx-1, may be critical in mediating both β-cell development and differentiated β-cell function. Investigations of pancreatic development, islet gene regulation, and the genetics of diabetes mellitus are rapidly converging,

with the potential to generate novel and potent therapies for pancreatic disease.

Acknowledgments

We thank the members of the Laboratory of Molecular Endocrinology for their helpful suggestions, and Melissa Fannon and Kimberly MacDonald for help with the preparation of the manuscript.

Received December 6, 2004. Accepted December 8, 2004.

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This work was supported in part by United States Public Health Service Grants DK-55365 (to J.F.H.) and DK-02476 (to M.K.T.). J.F.H. is an investigator with the Howard Hughes Medical Institute.

References

1. **Servitja JM, Ferrer J** 2004 Transcriptional networks controlling pancreatic development and β cell function. Diabetologia 47:597–613
2. **Jensen J** 2004 Gene regulatory factors in pancreatic development. Dev Dyn 229:176–200
3. **Kemp DM, Thomas MK, Habener JF** 2003 Developmental aspects of the endocrine pancreas. Rev Endocr Metab Disord 4:5–17
4. **Wilson ME, Scheel D, German MS** 2003 Gene expression cascades in pancreatic development. Mech Dev 120:65–80
5. **Zhang YQ, Sarvetnick N** 2003 Development of cell markers for the identification and expansion of islet progenitor cells. Diabetes Metab Res Rev 19:363–374
6. **Kim SK, MacDonald RJ** 2002 Signaling and transcriptional control of pancreatic organogenesis. Curr Opin Genet Dev 12:540–547
7. **Thomas MK, Habener JF** 2002 Pancreas Development. In: Gill RG, ed. Immunologically mediated endocrine diseases. Philadelphia: Lippincott Williams & Wilkins; 141–166
8. **Murtaugh LC, Melton DA** 2003 Genes, signals, and lineages in pancreas development. Annu Rev Cell Dev Biol 19:71–89
9. **Schwitzgebel VM** 2001 Programming of the pancreas. Mol Cell Endocrinol 185:99–108
10. **Slack JMW** 1995 Developmental biology of the pancreas. Development 121: 1569–1580
11. **Dudek RW, Lawrence IE, Hill RS, Johnson RC** 1991 Induction of islet cytodifferentiation by fetal mesenchyme in adult pancreatic ductal epithelium. Diabetes 40:1014–1048
12. **Herrera PL, Huarte J, Zufferey R, Nichols A, Mermillod B, Philippe J, Muniesa P, Sanvito F, Orci L, Vassalli JD** 1994 Ablation of islet endocrine cells by targeted expression of hormone promoter-driven toxigenes. Proc Natl Acad Sci USA 91:12999–13003
13. **Miralles F, Czernichow P, Scharfmann R** 1998 Follistatin regulates the relative proportions of endocrine versus exocrine tissue during pancreatic development. Development 125:1017–1024
14. **Rutter WJ, Kemp JD, Bradshaw WS, Clark WR, Ronzio RA, Sanders TG** 1968 Regulation of specific protein synthesis in cytodifferentiation. J Cell Physiol 72:1–18
15. **Pictet R, Rutter WJ** 1972 Development of the embryonic endocrine pancreas. Washington DC: American Physiological Society
16. **Han JH, Rall L, Rutter WJ** 1986 Selective expression of rat pancreatic genes during embryonic development. Proc Natl Acad Sci USA 83:110–114
17. **Alpert S, Hanahan D, Teitelman G** 1988 Hybrid insulin genes reveal a developmental lineage for pancreatic endocrine cells and imply a relationship with neurons. Cell 53:295–308
18. **Sanders TG, Rutter WJ** 1974 The developmental regulation of amylolytic and proteolytic enzymes in the embryonic rat pancreas. J Biol Chem 249:3500–3509
19. **Cockell M, Stevenson BJ, Strubin M, Hagenbüchle O, Wellauer PK** 1989 Identification of a cell-specific DNA-binding activity that interacts with a transcriptional activator of genes expressed in the acinar pancreas. Mol Cell Biol 9:2464–2476
20. **Roux E, Strubin M, Hagenbuchle O, Wellauer PK** 1989 The cell-specific transcription factor PTF1 contains two different subunits that interact with the DNA. Genes Dev 3:1613–1624
21. **Krapp A, Knöfler M, Frutiger S, Hughes GJ, Hagenbüchle O, Wellauer PK** 1996 The p48 DNA-binding subunit of transcription factor PTF1 is a new exocrine pancreas-specific basic helix-loop-helix protein. EMBO J 15:4317–4329
22. **Krapp A, Knöfler M, Ledermann B, Burki K, Berney C, Zoerkler N, Hagenbuchle O, Wellauer PK** 1998 The bHLH protein PTF1–p48 is essential for the formation of the exocrine and the correct spatial organization of the endocrine pancreas. Genes Dev 12:3752–3763
23. **Kawaguchi Y, Cooper B, Gannon M, Ray M, MacDonald RJ, Wright CV** 2002 The role of the transcriptional regulator Ptf1a in converting intestinal to pancreatic progenitors. Nat Genet 32:128–134
24. **Cockell M, Stolarczyk D, Frutiger S, Hughes GJ, Hagenbuchle O, Wellauer PK** 1995 Binding sites for hepatocyte nuclear factor 3β or 3γ and pancreas transcription factor 1 are required for efficient expression of the gene encoding pancreatic α-amylase. Mol Cell Biol 15:1933–1941
25. **Johnson TM, Rosenberg MP, Meisler MH** 1993 An insulin-responsive element in the pancreatic enhancer of the amylase gene. J Biol Chem 268:464–468
26. **Lammert E, Cleaver O, Melton D** 2003 Role of endothelial cells in early pancreas and liver development. Mech Dev 120:59–64
27. **Kim SK, Hebrok M, Melton DA** 1997 Notochord to endoderm signaling is required for pancreas development. Development 124:4243–4252
28. **Hebrok M, Kim SK, Melton DA** 1998 Notochord repression of endodermal sonic hedgehog permits pancreas development. Genes Dev 12:1705–1713
29. **Apelqvist A, Ahlgren U, Edlund H** 1997 Sonic hedgehog directs specialised mesoderm differentiation in the intestine and pancreas. Curr Biol 7:801–804
30. **Kim SK, Melton DA** 1998 Pancreas development is promoted by cyclopamine, a Hedgehog signaling inhibitor. Proc Natl Acad Sci USA 95:13036–13041
31. **Bort R, Martinez-Barbera JP, Beddington RS, Zaret KS** 2004 Hex homeobox gene-dependent tissue positioning is required for organogenesis of the ventral pancreas. Development 131:797–806
32. **Ahlgren U, Pfaff SL, Jessell TM, Edlund T, Edlund H** 1997 Independent requirement for ISL1 in formation of pancreatic mesenchyme and islet cells. Nature 385:257–260
33. **Madsen OD, Jensen J, Petersen HV, Pedersen EE, Oster A, Andersen FG, Jorgensen MC, Jensen PB, Larsson LI, Serup P** 1997 Transcription factors contributing to the pancreatic β-cell phenotype. Horm Metab Res 29:265–270
34. **Sander M, German MS** 1997 The β cell transcription factors and development of the pancreas. J Mol Med 75:327–340
35. **Stoffers DA, Thomas MK, Habener JF** 1997 Homeodomain protein IDX-1: a master regulator of pancreas development and insulin gene expression. Trends Endocrinol Metab 8:145–151
36. **Habener JF, Stoffers DA** 1998 A newly discovered role of transcription factors involved in pancreas development and the pathogenesis of diabetes mellitus. Proc Assoc Am Physicians 110:12–21
37. **Edlund H** 1998 Transcribing pancreas. Diabetes 47:1817–1823
38. **St-Onge L, Wehr R, Gruss P** 1999 Pancreas development and diabetes. Curr Opin Genet Dev 9:295–300
39. **Odom DT, Zizlsperger N, Gordon DB, Bell GW, Rinaldi NJ, Murray HL, Volkert TL, Schreiber J, Rolfe PA, Gifford DK, Fraenkel E, Bell GI, Young RA** 2004 Control of pancreas and liver gene expression by HNF transcription factors. Science 303:1378–1381
40. **Ohlsson H, Karlsson K, Edlund T** 1993 IPF1, a homeodomain-containing transactivator of the insulin gene. EMBO J 12:4251–4259
41. **Leonard J, Peers B, Johnson T, Ferreri K, Lee S, Montminy MR** 1993 Characterization of somatostatin transactivating factor-1, a novel homeobox factor that stimulates somatostatin expression in pancreatic islet cells. Mol Endocrinol 7:1275–1283
42. **Miller CP, McGehee RE, Habener JF** 1994 IDX-1: a new homeodomain transcription factor expressed in rat pancreatic islets and duodenum that transactivates the somatostatin gene. EMBO J 13:1145–1156
43. **Brooke NM, Carcia-Fernandez J, Holland PWH** 1998 The parahox gene cluster is an evolutionary sister of the Hox gene cluster. Nature 392:920–922
44. **Waeber G, Thompson N, Nicod P, Bonny C** 1996 Transcriptional activation of the GLUT2 gene by the IPF-1/STF-1/IDX-1 homeobox factor. Mol Endocrinol 10:1327–1334
45. **Watada H, Kajimoto Y, Kaneto H, Matsuoka T, Fujitani Y, Miyazaki J, Yamasaki Y** 1996 Involvement of the homeodomain-containing transcription factor PDX-1 in islet amyloid polypeptide gene transcription. Biochem Biophys Res Commun 229:746–751
46. **Watada H, Kajimoto Y, Miyagawa J, Hanafusa T, Hamaguchi K, Matsuoka T, Yamamoto K, Matsuzawa Y, Kawamori R, Yamasaki Y** 1996 PDX-1 induces insulin and glucokinase gene expressions in αTC1 clone 6 cells in the presence of betacellulin. Diabetes 45:1826–1831
47. **Serup P, Jensen J, Andersen FG, Jorgensen MC, Blume N, Holst JJ, Madsen OD** 1996 Induction of insulin and islet amyloid polypeptide production in pancreatic islet glucagonoma cells by insulin promoter factor 1. Proc Natl Acad Sci USA 93:9015–9020
48. **Melloul D, Ben-Neriah Y, Cerasi E** 1993 Glucose modulates the binding of an islet-specific factor to a conserved sequence within the rat I and the human insulin promoters. Proc Natl Acad Sci USA 90:3865–3869
49. **Petersen HV, Serup P, Leonard J, Michelsen BK, Madsen OD** 1994 Transcriptional regulation of the human insulin gene is dependent on the homeodomain protein STF1/IPF1 acting through CT boxes. Proc Natl Acad Sci USA 91:10465–10469
50. **MacFarlane W, Read ML, Gilligan M, Bujalska I, Docherty K** 1994 Glucose modulates the binding activity of the β cell transcription factor IUF-1 in a phosphorylation-dependent manner. Biochem J 303:625–631
51. **Petersen HV, Peshavaria M, Pedersen AA, Philippe J, Stein R, Madsen OD,**

Serup P 1998 Glucose stimulates the activation domain potential of the PDX-1 homeodomain transcription factor. FEBS Lett 431:362–366
52. **MacFarlane WM, Smith SB, James RF, Clifton AD, Doza YN, Cohen P, Docherty K** 1997 The p38/reactivating kinase mitogen-activated protein kinase cascade mediates the activation of the transcription factor insulin upstream factor 1 and insulin gene transcription by high glucose in pancreatic β-cells. J Biol Chem 272:20936–20944
53. **Rafiq I, Kennedy HJ, Rutter GA** 1998 Glucose-dependent translocation of insulin promoter factor-1 (IPF-1) between the nuclear periphery and the nucleoplasm of single MIN6 β-cells. J Biol Chem 273:23241–23247
54. **Guz Y, Montminy MR, Stein R, Leonard J, Gamer LW, Wright CV, Teitelman G** 1995 Expression of murine STF-1, a putative insulin gene transcription factor, in β cells of pancreas, duodenal epithelium and pancreatic exocrine and endocrine progenitors during ontogeny. Development 121:11–18
55. **Offield MF, Jetton TL, Labosky PA, Ray M, Stein RW, Magnuson MA, Hogan BL, Wright CV** 1996 PDX-1 is required for pancreatic outgrowth and differentiation of the rostral duodenum. Development 122:983–995
56. **Jonsson J, Carlsson L, Edlund T, Edlund H** 1994 Insulin-promoter-factor 1 is required for pancreas development in mice. Nature 371:606–609
57. **Ahlgren U, Jonsson J, Edlund H** 1996 The morphogenesis of the pancreatic mesenchyme is uncoupled from that of the pancreatic epithelium in IPF1/PDX1-deficient mice. Development 122:1409–1416
58. **Stoffers DA, Zinkin NT, Stanojevic V, Clarke WL, Habener JF** 1997 Pancreatic agenesis attributable to a single nucleotide deletion in the human *IPF1* coding region. Nat Genet 15:106–110
59. **Li H, Arber S, Jessell TM, Edlund H** 1999 Selective agenesis of the dorsal pancreas in mice lacking homeobox gene Hlxb9. Nat Genet 23:67–70
60. **Harrison KA, Thaler J, Pfaff SL, Gu H, Kehrl JH** 1999 Pancreas dorsal lobe agenesis and abnormal islets of Langerhans in Hlxb9-deficient mice. Nat Genet 23:71–75
61. **Karlsson O, Thor OS, Nor berg T, Ohlsson H, Edlund T** 1990 Insulin gene enhancer binding protein Isl-1 is a member of a novel class of proteins containing a homeo- and a Cys-His domain. Nature 344:879–882
62. **Thor S, Ericson J, Brannstrom T, Edlund T** 1991 The homeodomain LIM protein isl-1 is expressed in subsets of neurons and endocrine cells in the adult rat. Neuron 7:881–889
63. **Pfaff SL, Mendelsohn M, Stewart CL, Edlund T, Jessell TM** 1996 Requirement for LIM homeobox gene Isl1 in motor neuron generation reveals a motor neuron-dependent step in interneuron differentiation. Cell 84:309–320
64. **Dale KJ, Pourquie O** 2000 A clock-work somite. Bioessays 22:72–83
65. **Artavanis-Tsakonas S, Rand MD, Lake RJ** 1999 Notch signaling: cell fate control and signal integration in development. Science 284:770–776
66. **Gradwohl G, Dierich A, LeMeur M, Guillemot F** 2000 Neurogenin3 is required for the development of the four endocrine cell lineages of the pancreas. Proc Natl Acad Sci USA 97:1607–1611
67. **Jensen J, Pedersen EE, Galante P, Hald J, Heller RS, Ishibashi M, Kageyama R, Guillemot F, Serup P, Madsen OD** 2000 Control of endodermal endocrine development by Hes-1. Nat Genet 24:36–44
68. **Apelqvist A, Li H, Sommer L, Beatus P, Anderson DJ, Honjo T, Hrabe de Angelis M, Lendahl U, Edlund H** 1999 Notch signalling controls pancreatic cell differentiation. Nature 400:877–881
69. **Lee JC, Smith SB, Watada H, Lin J, Scheel D, Wang J, Mirmira RG, German MS** 2001 Regulation of the pancreatic pro-endocrine gene neurogenin3. Diabetes 50:928–936
70. **Pontoglio M, Sreenan S, Roe M, Pugh W, Ostrega D, Doyen A, Pick AJ, Baldwin A, Velho G, Froguel P, Levisetti M, Bonner-Weir S, Bell GI, Yaniv M, Polonsky KS** 1998 Defective insulin secretion in hepatocyte nuclear factor 1α-deficient mice. J Clin Invest 101:2215–2222
71. **Ang SL, Rossant J** 1994 HNF-3β is essential for node and notochord formation in mouse development. Cell 78:561–574
72. **Weinstein DC, Ruiz i Altaba A, Chen WS, Hoodless P, Prezioso VR, Jessell TM, Darnell Jr JE** 1994 The winged-helix transcription factor HNF-3β is required for notochord development in the mouse embryo. Cell 78:575–588
73. **Jacquemin P, Durviaux SM, Jensen J, Godfraind C, Gradwohl G, Guillemot F, Madsen OD, Carmeliet P, Dewerchin M, Collen D, Rousseau GG, Lemaigre FP** 2000 Transcription factor hepatocyte nuclear factor 6 regulates pancreatic endocrine cell differentiation and controls expression of the proendocrine gene ngn3. Mol Cell Biol 20:4445–4454
74. **Naya FJ, Stellrecht CMM, Tsai M-J** 1995 Tissue-specific regulation of the insulin gene by a novel basic helix-loop-helix transcription factor. Genes Dev 9:1009–1019
75. **Lee JE, Hollenberg SM, Snider L, Turner DL, Lipnick N, Weintraub H** 1995 Conversion of *Xenopus* ectoderm into neurons by NeuroD, a basic helix-loop-helix protein. Science 268:836–844
76. **Mutoh H, Fung BP, Naya FJ, Tsai M-J, Nishitani J, Leiter AB** 1997 The basic helix-loop-helix transcription factor BETA2/NeuroD is expressed in mammalian enteroendocrine cells and activates secretin gene expression. Proc Natl Acad Sci USA 94:3560–3564
77. **Naya FJ, Huang H-P, Qiu Y, Mutoh H, DeMayo FJ, Leiter AB, Tsai MJ** 1997 Diabetes, defective pancreatic morphogenesis, and abnormal enteroendocrine differentiation in BETA2/NeuroD-deficient mice. Genes Dev 11:2323–2334
78. **Huang HP, Liu M, El-Hodiri HM, Chu K, Jamrich M, Tsai MJ** 2000 Regulation of the pancreatic islet-specific gene BETA2 (neuroD) by neurogenin 3. Mol Cell Biol 20:3292–3307
79. **Sosa-Pineda B, Chowdhury K, Torres M, Oliver G, Gruss P** 1997 The *Pax4* gene is essential for differentiation of insulin-producing β-cells in the mammalian pancreas. Nature 386:399–402
80. **Walther C, Gruss P** 1991 Pax-6, a murine paired box gene, is expressed in the developing CNS. Development 113:1435–1449
81. **Turque N, Plaza S, Radvanyl F, Carriere C, Saule S** 1994 Pax-QNR/Pax-6, a paired box- and homeobox-containing gene expressed in neurons, is also expressed in pancreatic endocrine cells. Mol Endocrinol 8:929–938
82. **Stoykova A, Fritsch R, Walther C, Gruss P** 1996 Forebrain patterning defects in Small eye mutant mice. Development 122:3453–3465
83. **Sander M, Neubüser A, Kalamaras J, Ee HC, Martin GR, German MS** 1997 Genetic analysis reveals that PAX6 is required for normal transcription of pancreatic hormone genes and islet development. Genes Dev 11:1662–1673
84. **St.-Onge L, Sosa-Pineda B, Chowdhury K, Mansouri A, Gruss P** 1997 Pax6 is required for differentiation of glucagon-producing α-cells in mouse pancreas. Nature 387:406–409
85. **Chalepakis G, Wijnholds J, Giese P, Schachner M, Gruss P** 1994 Characterization of Pax-6 and Hoxa-1 binding to the promoter region of the neural cell adhesion molecule L1. DNA Cell Biol 13:891–900
86. **Sussel L, Kalamaras J, Hartigan-O'Connor DJ, Meneses JJ, Pedersen RA, Rubenstein JL, German MS** 1998 Mice lacking the homeodomain transcription factor Nkx2.2 have diabetes due to arrested differentiation of pancreatic β cells. Development 125:2213–2221
87. **Rudnick A, Ling TY, Odagiri H, Rutter W, German MS** 1994 Pancreatic β cells express a diverse set of homeobox genes. Proc Natl Acad Sci USA 91:12203–12207
88. **Jensen J, Serup P, Karlsen C, Nielsen TF, Madsen OD** 1996 mRNA profiling of rat islet tumors reveals nkx 6.1 as a β-cell-specific homeodomain transcription factor. J Biol Chem 271:18749–18758
89. **Sander M, Sussel L, Conners J, Scheel D, Kalamaras J, Dela Cruz F, Schwitzgebel V, Hayes-Jordan A, German M** 2000 Homeobox gene Nkxb.1 lies downstream of Nkx2.2 in the major pathway of β-cell formation in the pancreas. Development 127:5533–5540
90. **Prado CL, Pugh-Bernard AE, Elghazi L, Sosa-Pineda B, Sussel L** 2004 Ghrelin cells replace insulin-producing β cells in two mouse models of pancreas development. Proc Natl Acad Sci USA 101:2924–2929
91. **Olbrot M, Rud J, Moss LG, Sharma A** 2002 Identification of β-cell-specific insulin gene transcription factor RIPE3b1 as mammalian MafA. Proc Natl Acad Sci USA 99:6737–6742
92. **Matsuoka TA, Artner I, Henderson E, Means A, Sander M, Stein R** 2004 The MafA transcription factor appears to be responsible for tissue-specific expression of insulin. Proc Natl Acad Sci USA 101:2930–2933
93. **Pin CL, Rukstalis JM, Johnson C, Konieczny SF** 2001 The bHLH transcription factor Mist1 is required to maintain exocrine pancreas cell organization and acinar cell identity. J Cell Biol 155:519–530
94. **Ledermann HM** 1995 Is maturity onset diabetes at young age (MODY) more common in Europe than previously assumed? Lancet 345:648
95. **Froguel P, Vaxillaire M, Sun F, Velho G, Zouali H, Butel MO, Lesage S, Vionnet N, Clément K, Fougerousse F, Tanizawa Y, Weissenbach J, Beckmann JS, Lathrop GM, Passa P, Permutt MA, Cohen D** 1992 Close linkage of glucokinase locus on chromosome 7p to early-onset non-insulin-dependent diabetes mellitus. Nature 356:162–164
96. **Froguel P, Zouali H, Vionnet N, Velho G, Vaxillaire M, Sun F, Lesage S, Stoffel M, Takeda J, Passa P, Permutt MA, Beckmann JS, Bell GI, Cohen D** 1993 Familial hyperglycemia due to mutations in glucokinase. N Engl J Med 328:697–702
97. **Chèvre J-C, Hani EH, Boutin P, Vaxillaire M, Blanche H, Vionnet N, Pardini VC, Timsit J, Larger E, Charpentier G, Beckers D, Maes M, Bellanne-Chantelot C, Velho G, Froguel P** 1998 Mutation screening in 18 Caucasian families suggest the existence of other MODY genes. Diabetologia 41:1017–1023
98. **Emens L, Landers DW, Moss LG** 1992 Hepatocyte nuclear factor 1α is expressed in a hamster insulinoma line and transactivates the rat insulin I gene. Proc Natl Acad Sci USA 89:7300–7304
99. **Nolten LA, Steenbergy PH, Sussenbach JS** 1995 Hepatocyte nuclear factor 1α activates promoter 1 of the human insulin-like growth factor I gene via two distinct binding sites. Mol Endocrinol 9:1488–1499
100. **Surmely J-F, Guenat E, Philippe J, Dussoix P, Schneiter P, Temler E, Vaxillaire M, Froguel P, Jequier E, Tappy L** 1998 Glucose utilization and production in patients with maturity-onset diabetes of the young caused by a mutation of the hepatocyte nuclear factor-1α gene. Diabetes 47:1459–1463
101. **Kuo CJ, Conley PB, Chen L, Sladek FM, Darnell Jr JE, Crabtree GR** 1992 A transcriptional hierarchy involved in mammalian cell-type specification. Nature 355:457–461
102. **Yamagata K, Oda N, Kalsaki 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, Bell GI, et al.** 1996 Mu-

tations in the hepatocyte nuclear factor-1α gene in maturity-onset diabetes of the young (MODY3). Nature 384:455–460
103. **Byrne MM, Sturis J, Fajans SS, Ortiz FJ, Stoltz A, Stoffel M, Smith MJ, Bell GI, Halter JB, Polonsky KS** 1995 Altered insulin secretory responses to glucose in subjects with a mutation in the *MODY1* gene on chromosome 20. Diabetes 44:699–704
104. **Byrne MM, Sturis J, Menzel S, Yamagata K, Fajans SS, Dronsfield MJ, Bain SC, Hattersley AT, Velho G, Froguel P, Bell GI, Polonsky KS** 1996 Altered insulin secretory responses to glucose in diabetic and nondiabetic subjects with mutations in the diabetes susceptibility gene MODY3 on chromosome 12. Diabetes 45:1503–1510
105. **Wang L, Coffinier C, Thomas MK, Gresh L, Eddu G, Manor T, Levitsky LL, Yaniv M, Rhoads DB** 2004 Selective deletion of the Hnf1β (MODY5) gene in β-cells leads to altered gene expression and defective insulin release. Endocrinology 145:3941–3949
106. **Malecki MT, Jhala US, Antonellis A, Fields L, Doria A, Orban T, Saad M, Warram JH, Montminy M, Krolewski AS** 1999 Mutations in NEUROD1 are associated with the development of type 2 diabetes mellitus. Nat Genet 23:323–328
107. **Macfarlane WM, Frayling TM, Ellard S, Evans JC, Allen LI, Bulman MP, Ayres S, Shepherd M, Clark P, Millward A, Demaine A, Wilkin T, Docherty K, Hattersley AT** 1999 Missense mutations in the insulin promoter factor-1 gene predispose to type 2 diabetes. J Clin Invest 104:R33–R39
108. **Hani EH, Stoffers DA, Chevre JC, Durand E, Stanojevic V, Dina C, Habener JF, Froguel P** 1999 Defective mutations in the insulin promoter factor-1 (IPF-1) gene in late-onset type 2 diabetes mellitus. J Clin Invest 104:R41–R48
109. **Thomas MK, Devon ON, Lee JH, Peter A, Schlosser DA, Tenser MS, Habener JF** 2001 Development of diabetes mellitus in aging transgenic mice following suppression of pancreatic homeoprotein IDX-1. J Clin Invest 108: 319–329
110. **Kanai-Azuma M, Kanai Y, Gad JM, Tajima Y, Taya C, Kurohmaru M, Sanai Y, Yonekawa H, Yazaki K, Tam PP, Hayashi Y** 2002 Depletion of definitive gut endoderm in Sox17-null mutant mice. Development 129:2367–23679
111. **Rauskolb C, Irvine KD** 1999 Notch-mediated segmentation and growth control of the *Drosophila* leg. Dev Biol 210:339–350
112. **Gerrish K, Gannon M, Shih D, Henderson E, Stoffel M, Wright CV, Stein R** 2000 Pancreatic β cell-specific transcription of the pdx-1 gene. The role of conserved upstream control regions and their hepatic nuclear factor 3β sites. J Biol Chem 275:3485–3492
113. **Ahlgren U, Jonsson J, Jonsson L, Simu K, Edlund H** 1998 β-Cell-specific inactivation of the mouse *Ipf1/Pdx1* gene results in loss of the β-cell phenotype and maturity onset diabetes. Genes Dev 12:1763–1768
114. **Gittes GK, Galante PE, Hanahan D, Rutter WJ, Debas HT** 1996 Lineage-specific morphogenesis in the developing pancreas: role of mesenchymal factors. Development 122:439–447
115. **Hebrok M, Kim SK, St Jacques B, McMahon AP, Melton DA** 2000 Regulation of pancreas development by hedgehog signaling. Development 127: 4905–4913
116. **Ben-Shushan E, Marshak S, Shoshkes M, Cerasi E, Melloul D** 2001 A pancreatic β-cell-specific enhancer in the human PDX-1 gene is regulated by hepatocyte nuclear factor 3β (HNF-3β), HNF-1α, and SPs transcription factors. J Biol Chem 276:17533–17540

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