INVITED REVIEW

Pancreas duodenum homeobox-1 regulates pancreas development during embryogenesis and islet cell function in adulthood

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Abstract

Pancreas duodenum homeobox-1 (PDX-1) (also known as insulin promoter factor-1, islet/duodenum homeobox-1, somatostatin transactivating factor-1, insulin upstream factor-1 and glucose-sensitive factor) is a transcription factor encoded by a Hox-like homeodomain gene. In humans and other animal species, the embryonic development of the pancreas requires PDX-1, as demonstrated by the identification of an individual with pancreatic agenesis resulting from a mutation that impaired the transcription of a functionally active PDX-1 protein. In adult subjects, PDX-1 is essential for normal pancreatic islet function as suggested by its regulatory action on the expression of a number of pancreatic genes, including insulin, somatostatin, islet amyloid polypeptide, the glucose transporter type 2 and glucokinase. Furthermore, heterozygous mutations of PDX-1 have been linked to a type of autosomal dominant form of diabetes mellitus known as maturity onset diabetes of the young type 4. The dual action of PDX-1, as a differentiation factor during embryogenesis and as a regulator of islet cell physiology in mature islet cells, underscores the unique role of PDX-1 in health and disease of the human endocrine pancreas.

Introduction

Diabetes is a metabolic disorder characterized by a hyperglycemia resulting from defects in insulin secretion and insulin action. These may coexist from the time of diagnosis or be present as isolated defects in the years preceding the clinical onset of diabetes. Over time many patients affected by diabetes present with various degrees of islet cell dysfunction and insulin resistance. The insufficient release of insulin by β-cells is caused by either an immune-mediated cell destruction (type 1 diabetes) or failure to compensate for an increasing demand for insulin (type 2 diabetes and maturity-onset diabetes of the young (MODY)). A better understanding of the molecular basis of cell dysfunction in non-immune-mediated forms of diabetes has come from genetic studies of MODY, a monogenic form of diabetes characterized by an autosomal-dominant mode of inheritance. The onset of MODY is characterized by an abnormal pattern of glucose-stimulated insulin secretion (1). Recent studies have shown a central role for various transcription factors in the etiology of this form of diabetes. These include hepatocyte nuclear factor-1 (HNF-1) α and β, HNF-3 α and β, HNF-4, as well as PDX-1 (2–6). PDX-1 is also known as insulin promoter factor-1 (IPF-1), islet/duodenum homeobox-1 (IDX-1), somatostatin transactivating factor-1, insulin upstream factor-1 and glucose-sensitive factor (6). This diverse nomenclature reflects the numerous studies on its action, and it is the result of those early reports conducted when the full sequence and the identity of the gene were not sufficiently clear. We elected to use the abbreviation PDX-1 hereafter for all reference to this gene in the various animal species in order to facilitate the reading of the manuscript.

Gene and protein structure of PDX-1

Human PDX-1 gene consists of two exons and spans a region of about 6 kb on human (7, 8) chromosome 13 (band 13q12.1). The mouse homologue, termed Ipf-1, has been mapped to the distal end of mouse (9) chromosome 5. Rat PDX-1 gene has been located on chromosome 12 (10). Human PDX-1 is a nuclear protein consisting of 282 amino acids, based on its structure features illustrated in Table 1, and it belongs to
the antp-family of homeobox proteins ipf-1/xlhbox8 superfamily (11, 12).

The transactivation domain of PDX-1 is contained within the N-terminal region (amino acids 1–79). Detailed site-directed mutagenesis of this region indicates that the gene transactivation is mediated by three highly conserved sequences, spanning amino acids 13–22 (sub-domain A), 32–38 (sub-domain B), and 60–73 (sub-domain C) (13). These sequences are required by PDX-1 to synergistically activate insulin enhancer-mediated transcription, together with other key activators, like the E2A-encoded basic helix-loop-helix (bHLH) E2-5 and E47 proteins (14, 15).

The antp-type hexapeptide present in the sequence of PDX-1 mediates its heterodimerization with the DNA-binding protein termed PBX on a regulatory element of the somatostatin promoter (16, 17). PBX is an ubiquitous homeodomain protein that by forming a complex with PDX-1 and MRG1 (another homeodomain protein) regulates the expansion of ductal, endocrine, and acinar lineages during development. While the formation of the trimeric complex that includes PDX-1, PBX and MRG1 is required for the differentiation of acinar cell lineage, this is not required for the differentiation of β-cells. Furthermore, the interaction of PDX-1 as a monomer or as part of a trimeric complex has been proposed to play a critical role in the phenotype specification of pancreatic cells (13–18).

The homeodomain of PDX-1, which contains the nuclear localization signal (NLS), not only mediates DNA binding, but also acts as a protein–protein interaction domain for other transcription factors like tcf3 (e47), neurod1 and hmg-i(y) (18). The coordinated activity of those proteins is required for the full differentiation of precursor endocrine cells into hormone-secreting cells (18).

The NLS of PDX-1 is necessary for its transport into the nucleus. Point mutations of basic amino acid residues within helix 3 have led to identification of an NLS domain, consisting of six amino acids (RRMKWKK) (Table 1) that are necessary for the nuclear translocation of PDX-1 (19, 20). Because this NLS does not match known examples of NLSs, the PDX-1 NLS may represent a novel class of NLS (19).

As shown in Table 2, the PDX-1 sequences of different species show a very high degree of homology throughout evolution. Indeed, comparing the amino acid sequence of human PDX-1 with the homologous sequence from hamster, rat, mouse and frog, it could be appreciated that respectively a 90% (1-283aa:1-283aa), 88% (1-283aa:1-283aa), 87% (1-283aa:1-284aa) and 68% (1-239aa:1-232aa) of amino acid identity is present for core regulatory regions (Table 2). Interestingly, even in a species distant in evolution from humans, like the zebrafish, the PDX-1 sequence shares a 95% of homology with the mammalian PDX-1 in the homeodomain region (21).

### Cell distribution of PDX-1

While during organogenesis PDX-1 is widely expressed in all cells differentiating towards the exocrine and endocrine components of the pancreas, in the adult...
Table 2 Alignment of PDX-1 sequence from various species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>V.N.P.O.A.T.</td>
</tr>
<tr>
<td>Mouse</td>
<td>V.N.W.L.P.O.A.T.</td>
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<tr>
<td>Rat</td>
<td>G.V.A.I.R.L.</td>
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<td>Xenopus</td>
<td>S.G.V.A.I.R.L.</td>
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</table>

human pancreas its expression is restricted primarily to β- and δ-cells, where it regulates the expression of cell-specific genes (22, 23).

While some homeobox transcription factors are important in defining gradients of cellular differentiation and are expressed throughout the gastrointestinal apparatus, others show a more specialized distribution in specific regions of the intestine (duodenum, jejunum and ileum) (24). PDX-1 appears to meet the criteria of the first group during development and to acquire in adult subjects a more cell-specific expression, which is characteristic of the second group of homeobox/transcription proteins.

The 5′-untranslated sequence extending from 6.5 kb to 4.5 kb of the rat and the mouse PDX-1 gene is known to contain the information necessary to target PDX-1 expression to islet cells (25–27). Stoffers et al. (28) used transgenic mice expressing the Escherichia coli lacZ gene under control of the 5′-proximal 4.6 kb of the PDX-1 promoter to track the developmental expression of PDX-1. During development the expression of lacZ was detected in the exocrine and endocrine pancreas, in pancreatic ducts, common bile and cystic ducts, pyloric glands of the distal segment of the stomach, Brunner’s glands, the intestinal epithelium of the duodenum, and the spleen. This observed spatial pattern of lacZ expression under the control of PDX-1 promoter supports the important role of this gene in specifying the development of several endodermal structures located within the mid-segment of the body.

In addition to the gastrointestinal tract, PDX-1 is expressed in the embryonic brain at a time of active neurogenesis (29). Immunohistochemical staining of rat embryos showed PDX-1-positive cells located near the ventricular surface in germinative areas of the developing central nervous system. Cellular co-localization of PDX-1 and somatostatin has been demonstrated in several areas of the developing brain, including the cortex, the ganglionic eminence, the hypothalamus, and the inferior colliculus.

**Regulation of PDX-1 gene expression**

The expression of the rat PDX-1 gene is regulated by a proximal E box and a distal enhancer element that is activated by the cooperative action of the endodermal transcription factors HNF-3 and BETA2 (15, 29). HNF-3, a member of the forkhead and winged-helix family of transcription factors, is essential for the differentiation of cells of the endodermal lineage. It is structurally related to histone H5, which can alter the nucleosomal structure and thus is capable of priming target genes for their expression by opening the chromatin structure to provide promoter access to other transcription factors. A study of the mouse PDX-1 promoter revealed that this HNF-3-like region is involved
in regulating cell-specific transcription and directing appropriate developmental processes, and cell-specific gene expression in adult subjects. Thus HNF-3 appears to be necessary for the transcriptional regulation of PDX-1 (30).

Analysis of the DNA sequence located upstream of the human and mouse PDX-1 promoter reveals that there are three highly conserved domains. These are termed PH1 (PDX-1 homology regions 1), PH2 and PH3 (31). They span DNA fragments of the PDX-1 gene extending from kb 2.8809 to 2.655 (PH1), from 2.233 to 2.097 (PH2), and 1.952 to 1.668 (PH3). These key regulatory domains exhibit a very high degree of similarity between human and mouse, with a nucleotide homology of 94, 81 and 73% for each of the three regions respectively. The PH1 enhancer element has been shown to be capable of binding both HNF-3 and PDX-1. Indeed, the PH1 DNA domain has been proposed to participate in a feedback mechanism that controls the expression of PDX-1 at different stages of development (31), while HNF-3 may mediate the transcriptional activation of the PH2 domain.

The sequences associated with the, so-called, hypersensitive site 1 of PDX-1 represent the principal areas of homology between different species. This is particularly evident in three sub-domains termed area I (from 2694 to 2561 bp), area II (2139 to 1958) and area III (1879 to 1799). In those domains the nucleotide identity between mouse, chicken and human ranges from 78 to 89%, although only areas I and III are present in the chicken PDX-1 gene. Pancreatic cell-specific expression of PDX-1 has been shown to be controlled in mouse and human by area I and area II, but not area III, and they represent the binding sites for transcription factors such as HNF-3 (26).

In addition to the cis-regulation of PDX-1 transcription, Stoffers et al. (27) have proposed the existence of a post-transcriptional regulation of PDX-1. Insulin and glucose, for example, have been shown to regulate the PDX-1 level, as well as its nuclear translocation via the phosphatidylinositol 3-kinase (PI3K)-dependent pathway (Fig. 1). Exposure of islet β-cells to elevated glucose concentrations (30 mmol/l vs 3 mmol/l) has been shown to enhance preproinsulin (PPI) gene transcription and the translocation to the nucleoplasm of PDX-1 (Fig. 2). Rafiq et al. (32) have shown that in β-cell lines, the overexpression of p110 CAAX (a constitutively active form of PI3K), mimics the stimulatory effect of glucose on PPI promoter activity. On the other hand, δ85, a dominant negative form of the p85 sub-unit lacking the p110-binding domain, as well as the PI3K inhibitor LY 294002, has been shown to block these effects (32). Similarly, glucose-stimulated nuclear translocation of endogenous PDX-1 is blocked by δ85, and PI3K inhibitors (like wortmannin or LY 294002) block the translocation from the nuclear membrane to the nucleoplasm of epitope-tagged PDX-1-c-myc (32).

The insulinotropic hormone glucagon-like peptide-1 (GLP-1) when administered to diabetic or not diabetic subjects stimulates insulin secretion and effectively lowers blood glucose levels (Fig. 1). GLP-1 also enhances β-cell mass in animal models of diabetes. These effects have been shown to be mediated by the transcription of PDX-1, and by its nuclear translocation (33).

Elevated fatty acid levels have been shown to down regulate the expression of PDX-1. Gremlich et al. (34) demonstrated that the exposure of isolated rat islets to palmitic acid induces a 70% decrease in PDX-1 mRNA and protein levels, and it is responsible for a 40–65% decrease in the binding activity of PDX-1 to the promoter regions of glucose transporter type 2 (GLUT-2) and insulin (34).

**Regulation of β-cell-specific gene expression by PDX-1**

The endocrine pancreas consists primarily of three cell types, which are distinguished by their selective expression of insulin, glucagon or somatostatin. Approximately 90% of the islet β-cell and 15% of δ-cells express PDX-1 (35). In the islets of Langerhans, PDX-1 regulates the expression of insulin, somatostatin, islet amyloid polypeptide (IAPP), glucokinase (GK) and GLUT-2.

**Insulin**

Insulin gene expression is controlled by its promoter activity via binding of transcription factors to the enhancer region, located between nucleotides −340 and −91 (Fig. 3) (35). The characterization of the gene-enhancing region indicates that its β-cell-specific expression is mediated predominantly by A3-, C1–A2- and E-elements whose core binding motifs are
present within the transcription units of all characterized insulin genes of various animal species. Binding of PDX-1 and HNF-1 to A-elements (A1 – A3) is a critical regulator of insulin gene expression, as well as of pancreatic development. This transactivation of the insulin gene is mediated by the N-terminal sequences of PDX-1, especially by the three highly conserved sequences spanning amino acids 13–22, 32–38 and 60–73 (36).

The transcription of insulin depends on multiple nuclear proteins that interact with each other, as well as with nucleotide sequences on the insulin promoter so as to build a transcriptional activation complex (37). Harrington & Sharma (38) demonstrated that the β-cell-specific transcription regulator RIPE3b1 and A2.2 recognize overlapping DNA-binding sites located within the insulin enhancer region. Both C1- and A2-elements together constitute the binding site for RIPE3b1. In addition to C1–A2 binding complexes, three binding complexes that specifically recognize A2-elements have been identified in nuclear extracts from insulinoma cell lines (38). Transient transfection of those domains indicates that both C1–A2- and A2-specific binding activators are necessary to cooperatively activate the expression of the insulin gene. Interestingly, C1–A2- and A2-specific activators have been shown to respond differently to glucose, suggesting that their overlapping binding specificity and functional cooperation may play an important role in the physiological regulation of insulin gene expression.

PDX-1 binds to the A3/4 region of the rat insulin I promoter and activates insulin gene transcription by cooperating with the bHLH protein E47/Pan1; this in turn binds to the adjacent E2 site. Ohneda et al. (39) have demonstrated that the homeodomain region of the PDX-1 gene acts as a protein–protein interaction domain to recruit multiple proteins, including E47/Pan1, BETA2/NeuroD1, and high-mobility group protein I, to an activation complex on the E2A3/4 mini-enhancer (15). These studies indicate that the transcriptional activity of this complex results from the clustering of multiple activation domains capable of interacting with co-activators and with the basal transcriptional machinery.

To examine the underlying mechanisms by which the insulin gene is efficiently expressed only in pancreatic β-cells, Glick et al. (15) expressed β-cell transcription factors in non-β-cells. Expression of BETA2, E2A, or PDX-1 alone led to modest (<10-fold) activation of the insulin promoter, whereas co-expression of the three proteins produced synergistic, high level activation (160-fold). Of the three factors studied, BETA2 appeared to play a dominant role and it appeared to cooperate primarily with PDX-1.

The decline of insulin expression observed in isolated β-cells cultured in high glucose has been shown to be associated with a decrease in binding activity of two essential β-cell transcription factors, PDX-1 and RIPE-3b1. Harmon et al. (40) observed that the loss of RIPE-3b1 occurs much earlier (79% decrease by passage 81) than the loss of PDX-1 (65% by passage 104), with disappearance of both factors by later culture passages (passage 122). Transfection of those
cells with either PDX-1 alone, or co-transfection with E2-5, an E-box factor known to be synergistically associated with PDX-1, was capable of restoring some of the characteristics of differentiated β-cells. An increase in insulin promoter activity was observed in intermediate passages, after transfection with PDX-1 (1.43-fold), and this become more significant when E2-5 was co-transfected (1.78-fold). Co-transfection of PDX-1 and E2-5 in late-passage cells induced a 2.8-fold increase in insulin promoter activity.

Although the evidence here described support the notion that PDX-1 univocally promotes insulin gene expression, it has been shown that high levels of ectopic PDX-1 have an inhibitory effect on the expression of the human insulin gene. This repression might occur by a protein–protein interaction. Indeed, it has been proposed that PDX-1 may compete for a co-activator present only in limited amounts in normal adult islets (41, 42). Kajimoto et al. (43) culturing a mouse insulinoma cell line with an antisense PDX-1 oligonucleotide, complementary to a sequence matching the translation initiation codon of PDX-1, described a potent and dose-dependent reduction in PDX-1 expression. Surprisingly, this study did not reveal a decrease in the mRNA levels of insulin, GK and IAPP.

**IAPP**

IAPP, also known as amylin, is a 37-amino acid peptide and is a member of the calcitonin gene family. IAPP was originally isolated from the amyloid deposits present in the pancreas of subjects with type 2 diabetes, as well as from the extracellular component of human insulinomas (44). In pancreatic β-cells, IAPP is co-stored with insulin in the secretory granules and co-released with insulin in response to a variety of secretagogues. The amylin gene, which is also expressed in islet δ-cells, contains AT-rich sequences in its regulatory region similarly to those present in the insulin promoter. The expression of amylin is principally regulated by promoter proximal sequences, mapped between nucleotides −222 and +450. Indeed, linker-scanning mutations of the 5’-promoter proximal region have defined several key distinct domains that control the transcription of IAPP (Fig. 4). Those include a negative-acting element at −111/−102 bp, as well as positive-acting elements like the bHLH-like binding site at −138/−131 bp, and three A/T-rich, homeobox-like sites at −172/−163, −154/−142 and −91/−84 bp. Site-directed mutagenesis within any one of these elements eliminates the transcription of IAPP.

PDX-1 interacts specifically with the −154/−142 bp and −91/−84 bp 5’-untranslated regions of the IAPP gene (45). In the study by Watada et al. (46), all three characteristic A-element-like sequences of the IAPP gene, that matched the CT-box (consensus AT-1, −207/−202; AT-2, −154/−142; and AT-3, −88/−83), were shown to be capable of binding PDX-1. On the other hand, when the promoter activity was examined in the mouse insulinoma cell line MIN-6 cells, the disruption of either AT-1 or AT-3, but not of AT-2, caused a marked reduction in the IAPP gene promoter activity (Fig. 4). Thus, despite the observation that all the three A-elements could bind to PDX-1, the AT-2 site may not be involved in mediating the PDX-1-dependent expression of IAPP (46).

Finally, glucose has been shown to require PDX-1 and calcium influx to induce IAPP promoter activity, as demonstrated by overexpression of the two K(ATP) channel sub-units SUR1 and Kir6.2 in a human β-cell line (NES2Y), which lacks PDX-1 and operational K(ATP) channels (47).

**GLUT-2**

Islet-derived insulin-secreting cells have been shown to lose glucose responsiveness as a result of a decreased PDX-1 expression, which in turn would down regulate the intracellular level of GLUT-2 (48). Waerber et al. (49) have proposed that the action of PDX-1 on the GLUT-2 promoter is mediated via interaction with a TAAT motif (5’-TAATA-ATAACA-3’), which is a very conserved nucleotide sequence among various species.

GLUT-2 expression has been shown to be reduced in the β-cells of several animal models of diabetes. The transcriptional control of GLUT-2 is regulated by at least two islet-specific DNA-binding proteins, GTIIa and PDX-1. Bonny et al. (48) assessed the DNA-binding activities of GTIIa and PDX-1 to their respective cis-elements of the GLUT-2 promoter by using nuclear extracts prepared from pancreatic islets of diabetic db/db mice. They demonstrated that the decreased GLUT-2 mRNA expression correlates with a decrease of the GTIIa DNA-binding activity, whereas PDX-1-1-binding activity is increased. They proposed that the decreased activity of GTIIa might represent a major initial step in the development of the β-cell dysfunction, which may be followed by a down regulation of PDX-1 leading to a greater degree of β-cell dysfunction.

A PPRE (peroxisomal proliferator response element) has been identified in the +68/+89 region of the rat GLUT-2 gene. Kim et al. (50) demonstrated that the GLUT-2-PPRE domain is functionally responsive to ligand binding and plays a significant role in the gene expression of GLUT-2.
expression of GLUT-2. The relationship between PDX-1 and PPRE in the control of GLUT-2 transcription has not yet been elucidated.

**GK**

The glycolytic enzyme GK plays a primary role in the glucose-sensitive secretion of insulin, and defects of this enzyme have been shown to be responsible for a MODY-2 (51). Watada et al. (52) demonstrated that the promoter region of the human GK gene comprises multiple cis-acting elements, including two very important cis-motifs: the palindrome structure h6Pal-1, and the insulin gene cis-motif-A-element-like hUPE3. PDX-1 binds the hUPE3 motif to activate the transcription of GK. The impaired ability of mutant PDX-1 alleles to activate the transcription of GK has been associated with the insulin secretory defects in subjects affected by MODY-4, as hereafter discussed.

**Somatostatin**

In adult subjects, in addition to the large majority of β-cells, PDX-1 is detected in 15% of the δ-cells, where it transactivates the somatostatin gene (53). The promoter region of somatostatin contains four homeodomain-binding sites with a common TAAT core motif; they are termed SMS-UE-B, SMS-TAAT1, SMS-TAAT2 and SMS-TAAT3. PDX-1 regulates the expression of somatostatin by binding to SMS-UE-B (TSE-I) and SMS-TAAT1 (TSE-II) (Fig. 5) (54).

The specific role of the different PDX-1 domains involved in the transactivation of the somatostatin gene has not been fully elucidated. Lu et al. (55) have demonstrated that the deletion of the amino-proximal sequence of the homeodomain of PDX-1 enhances DNA-binding to the TAAT-1 transcriptional control element of the somatostatin promoter. This results in a substantial decrease in the transactivation of a transcriptional reporter containing the TAAT-1 element (55).

The distal A-element of the somatostatin gene, upstream to the enhancer element (SMS-UE), contains overlapping binding sites for PDX-1 and PBX (56). A point mutation in the A-element that abolishes both PDX-1 and PBX binding has been shown to be unable to reduce the promoter activity (57). In contrast, a point mutation that selectively eliminates PDX-1 binding to a proximal B-element reduces the promoter activity. The B-element completely overlaps with a Pax6-binding site, termed the C-element. Pax6 binding is essential for the promoter activity and a stop codon mutation in the A-element has been shown to reduce both Pax6 binding and somatostatin gene transcription. Andersen et al. (57) proposed that the β/δ-cell-specific activity of the SMS-UE is achieved through simultaneous binding of PDX-1 and Pax6 to the B- and C-elements respectively. It has been demonstrated that PDX-1 forms a heterodimeric complex with PBX, the mammalian homologue of the *Drosophila* extradenticle. This heterodimer has been shown to bind the TAAT sequence of the somatostatin promoter, although not to the same sequence present in the insulin promoter, suggesting that this preference may represent the basis for target site selection in developing islet cells and that the transcriptional function of the gene may thus be context-dependent (58).

The regulation of somatostatin expression by PDX-1 in organs other than the islet of Langerhans, like brain and gastrointestinal tract, is not discussed in the present review (59, 60).

PDX-1 also regulates the transcription of the somatostatin receptors (SSTRs). The expression pattern of the five known SSTR genes has been shown to be substantially different among the various segments of the gastrointestinal tract. While SSTR1 mRNA is relatively abundant throughout the entire digestive apparatus, the levels of SSTR2, 3 and 4 mRNAs vary in different regions within the gastrointestinal tract. On the other hand, SSTR5 mRNA has not been detected in the gastrointestinal tract (61). Sequence analyses of the SSTR1 gene promoter, the best characterized of the SSTR genes, revealed the absence of the characteristic TATA and CAAT motifs and the presence of a variety of potential binding sites for various transcriptional regulators. Among these are binding sites for GCF, AP-2, AP-4, response elements for somatostatin, epidermal growth factor and the cytokines GAS and NFIL, as well as for tissue-specific transcription factors such as Pit-1 (pituitary) and PDX-1 (pancreatic cells). Mobility shift assays have confirmed that nuclear proteins obtained from the pancreatic β-cells line RIN1046-38, and from pituitary GH3 tumor cells bind to oligonucleotides containing the overlapping Pit-1- and PDX-1-binding sites. Thus, the Pit-1/ PDX-1 sites may be critical for the activation of the SSTR1 gene in both cell types (60).

**Role of PDX-1 in the developing pancreas**

The human pancreas containing primarily two distinct populations of cells, the exocrine cells that secrete
enzymes into the digestive tract, and the endocrine cells that secrete hormones into the bloodstream. It arises from the endoderm as a dorsal and a ventral bud, which eventually fuse together to form the adult pancreas. Mammals, birds, reptiles and amphibians have a pancreas with a very similar histology and mode of development. On the other hand in some fish the islet cells are segregated as Brockmann bodies, and they are in contact with exocrine cells (62). Invertebrates do not have a pancreas; however, functionally comparable endocrine cells may be found in the gut and in the brain (63).

Recently, considerable progress in understanding of the embryogenesis of the pancreas has been made with the identification of genes that control the embryonic development of the islet. Most of them are transcription factors such as isl-1, PDX-1, BETA2 (also called NeuroD), Nkx2.2, Pax4 and Pax6 (64–66). Among those, PDX-1 appears to serve as a master switch for the expression of genes that lead to the development of both the exocrine and endocrine population of cells.

First detected in dorsal endoderm cells at embryonic day 8.5 of the mouse, PDX-1 is initially expressed by exocrine and endocrine cells. As the pancreatic morphogenesis proceeds, PDX-1 expression is eventually restricted to β- and δ-cells of the islets, where it primarily regulates the expression of insulin and somatostatin (67). Jonsson et al. (68) reported that targeted disruption of PDX-1 in mice results in a failure of the entire pancreas (endocrine and exocrine component) to develop. Stoffers et al. (69) identified a single homozygous nucleotide deletion within codon 63 of the human PDX-1 gene in a patient with pancreatic agenesis. This single nucleotide deletion caused a frameshift at the C-terminal border of the transactivation domain of PDX-1, resulting in the synthesis of a functionally inactive protein.

Insights into the role of PDX-1 in islet cell differentiation have also been obtained using animal models of diabetes. After 90% pancreatectomy (Px), the adult pancreas regenerates in a process recapitulating embryonic development, starting with a burst of proliferation in the epithelium of the common pancreatic duct. Using this animal model, it was demonstrated that PDX-1 protein was only faintly detected in ducts at day 1 after Px, but was easily detected at day 2 and 3 after Px. The increase of PDX-1 expression followed that of bromodeoxyuridine (BrdU) incorporation into the cell nuclei (a marker of cell proliferation). Finally, islets isolated 3–7 days after Px showed higher PDX-1 protein expression than control islets, indicating that not only during embryogenesis but also during pancreatic regeneration there is an up regulation of PDX-1 in actively divided ductal cells (70).

The interaction among different homeodomain proteins, expressed in a spatially and temporally restricted manner during development, determines the pattern of organogenesis. Heller et al. (71) generated transgenic mice in which the expression of PDX-1 was misdirected by a promoter of the mesoderm-specific homeodomain protein (Hoxa-4) known to be expressed in the stomach and hindgut during development. This resulted in an altered midgut–hindgut union and agenesis of the cecum. A possible mechanism for the dysmorphogenesis of the proximal colon has been proposed to be due to an inhibition of Cdx-2 actions mediated by PDX-1 transactivation (72).

The development of the pancreas appears to depend on various growth factors that are believed to be, at least in part, controlled by PDX-1. Kaneto et al. (73) demonstrated that most of the cluster-forming cells and the primitive ducts or ductal-like cells express heparin-binding epidermal growth factor-like growth factor (HB-EGF) and that PDX-1 is a specific and dominant binding factor for an A-element-like sequence in the HB-EGF gene. Hart et al. (74) studying the role of fibroblast growth factor (FGF) in the function of insulin-secreting cells, demonstrated that PDX-1 acts upstream of FGF-dependent signaling in β-cells to maintain proper glucose sensing, insulin processing and glucose homeostasis.

Itkin-Ansari et al. (75) demonstrated that PDX-1 and cell–cell interaction act in synergy to promote the development of β-cells from endocrine precursor cells. Cell lines obtained from fetal and adult pancreas, developed by retroviral transfer of the SV40 T and ras (val12) oncogenes, have been shown to lose insulin expression but retain extremely low levels of somatostatin and glucagon mRNA. These cell lines share a common biological feature, the loss of PDX-1 expression. When PDX-1 was transfected into those cells there was a 10- to 100-fold increase in somatostatin gene

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**Table 3** Identified mutations of PDX-1 in humans.

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<th>Gene Bank No.</th>
<th>Position</th>
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<td>Type 2 diabetes</td>
<td>Hani et al. (95)</td>
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<td>3. CI993094</td>
<td>243</td>
<td>3bp ins (CCG)</td>
<td>PRO243</td>
<td>Type 2 diabetes</td>
<td>Hani et al. (95)</td>
</tr>
<tr>
<td>4. CM992899</td>
<td>18</td>
<td>aTGC-CGC</td>
<td>CYS18ARG</td>
<td>Type 2 diabetes</td>
<td>Macfarlane et al. (94)</td>
</tr>
<tr>
<td>5. CM992902</td>
<td>197</td>
<td>CGC-CAC</td>
<td>ARG197HIS</td>
<td>Type 2 diabetes</td>
<td>Macfarlane et al. (94)</td>
</tr>
<tr>
<td>6. CM992901</td>
<td>76</td>
<td>GAC-AAC</td>
<td>ASP76ASN</td>
<td>Type 2 diabetes</td>
<td>Macfarlane et al. (94), Hani et al. (95)</td>
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expression. Perhaps even more interesting is the observation that promotion of cell–cell contact, by means of aggregation of TRM-6/PDX-1 into islet-like clusters, produced a further 10- to 100-fold increase in somatostatin mRNA, a level similar to that observed in freshly isolated islets.

In addition to the pancreatic tissue, PDX-1 is expressed in discrete cells of the rat central nervous system during embryonic development (76, 77). The studies describing those findings are not discussed in the present review.

**Role of PDX-1 in the proliferation and differentiation of β-cells**

Mature rat β-cells have a lifespan of a few weeks and are replaced by the replication of pre-existing β-cells or by the differentiation and proliferation of precursor cells present in the pancreatic ducts (78–80). *In vitro*, β-cell have been produced from the differentiation of ductal (81, 82) and exocrine cells (83). The pancreatic exocrine cell line termed AR42J has been reported to differentiate towards an endocrine phenotype when stimulated with various growth factors, such as activin A, hepatocyte growth factor, β-cellsulin or GLP-1 (84–86). The acquisition of a β-cell-like phenotype has been shown to be associated with the expression of PDX-1 (87, 88) and with a down regulation of the leucine zipper transcription factor CCAAT/enhancer-binding protein beta (C/EBPβ), which is an inhibitor of insulin gene transcription (89).

Using the sub-pancreatectomized rat model, Sharma et al. (70) found that PDX-1 was expressed in the pancreatic ducts of sham or unoperated rats but was strongly induced in the ducts of sub-pancreatectomized rats, at day 2–3 after surgery. The increase of PDX-1 protein levels followed that of BrdU, suggesting that PDX-1 may be more directly involved in the differentiation process, rather that with the early events characterized by cell proliferation.

It should be noted that expression of PDX-1 is not *per se* sufficient for the expression of insulin by β-cells. *Ex vivo* expansion of human β-cells is an important step toward the development of cell-based insulin delivery systems in type 1 diabetes. Beattie *et al.* (90) reported that human pancreatic endocrine cells could be expanded through 15 cell doublings *in vitro* for an estimated total 30,000-fold increase in cell number. Although the expanded β-cells continued to express PDX-1, insulin expression was lost over time. A similar observation has been reported by Palgi *et al.* (87) using AR42J cells, which while expressing both PDX-1 and Nkx2.2 had very low levels of insulin mRNA. Whether expression of islet-specific genes, other than PDX-1, is essential for successful end-stage cell differentiation of β-cells, although intuitively important, remains to be characterized.

**Mutation and MODY-4**

Five different proteins whose genetic absence or functional defects cause that form of diabetes termed MODY have been identified. Those include the enzyme GK (GK/MODY-2) and four transcription factors: HNF-4/MODY-1, HNF-1α/MODY-3, PDX-1 (MODY-4) and HNF-1β/MODY-5 (91).

Mutations of PDX-1 have been shown to have a wide range of effects on the development of the pancreas and on the function of insulin-secreting cells (Table 3). Stoffers *et al.* (69) identified a frameshift of the PDX-1 gene resulting from a bp deletion in a Caucasian female infant in whom the diagnosis of pancreatic agenesis was made by autopsy, after a survival of 18 days. The infant presented with neonatal diabetes mellitus at birth and with pancreatic exocrine insufficiency (92). There was a strong family history for type 2 diabetes. While in the proband, the homozygosity for the FS123TER mutation of PDX-1 impaired the development of the pancreas, in other family members the heterozygosity for the same mutation was associated with a MODY-like form of diabetes. The family pedigree showed that the presence of diabetes could be traced back for six generations. The average age at onset was 35 years (with a range from 17 to 67 years of age). Six of eight affected heterozygous relatives were treated with diet or oral hypoglycemic agents. None showed ketosis or other indications of severe insulin deficiency. Gene expression of the mutant PDX-1 in eukaryotic cells allowed for the detection of a second PDX-1 isoform (93). The reading frame of this isoform crossed over to the wild-type allele, at the site of the point deletion, in close proximity to the transactivation domain. Thus, the single mutated allele resulted in the translation of two PDX-1 proteins. The first consisted of the N-terminal transactivation domain and was sequestered in the cytoplasm, the second PDX-1 isoform contained the C-terminal DNA-binding domain but lacked the transactivation domain. The C-terminal domain of the second PDX-1 isoform did not activate insulin gene transcription, and inhibited the transactivation functions of the wild-type PDX-1.

Three novel PDX-1 missense mutations (C18R, D76N and R197H) have been recently identified by Macfarlane *et al.* (94) in patients with type 2 diabetes (Table 3). Functional analyses of these mutations demonstrated a decreased binding activity of PDX-1 to the insulin promoter and a reduced transcription of insulin in response to hyperglycemia in the human β-cell line Nes2y. These mutations are believed to be present in 1% of the population and may predispose individuals to overt diabetes (102). The relative risk of developing diabetes in the subjects carrying any mutation of the PDX-1 gene is 3.0. Those appear not to be highly penetrant MODY mutations, as several non-diabetic carriers of 25–53 years of age have been identified.
Hani et al. (95) investigating 192 French, non-MODY type 2 diabetic families, have identified three additional PDX-1 mutations, including two substitutions (Q59L and D76N) and an in-frame proline insertion (insCCG243) (Table 3). Functional transactivation assays of these PDX-1 mutants demonstrated a significant inhibition of basal insulin promoter activity. The strongest inhibition was observed in cells transfected with the insCCG243 mutant. It appeared that the insCCG243 mutation was linked, in two families, to an autosomal dominant form of type 2 diabetes, in which insulin secretion became progressively impaired, as individuals aged. The low-penetrant mutants termed D76N and Q59L were more prevalent in the population studied by Hani et al. (95) and were associated with a relative risk to develop diabetes 12.6-fold higher than controls. The authors demonstrated that even in the non-diabetic population those mutations were associated with a decreased glucose-stimulated insulin secretion.

Conclusion

The study of PDX-1 represents an infrequent and fortunate example in which molecular investigation and clinical observation led investigators to identify a novel protein, characterize its mechanism of action, and demonstrate its relevance in human diseases.

In addition to the lesson regarding the methodology of conducting biomedical research, PDX-1 may represent one of the best examples of key regulatory genes that acquire novel functional properties depending on the age of the individual. Indeed, the action of PDX-1 evolves during the lifespan from one of a growth/differentiation factor during embryogenesis, to the regulation of gene transcription of proteins regulating glucose-responsiveness of the islets of Langerhans in adult subjects; and it retains the possibility of gaining back its pro-differentiative properties at a time in which an expansion of islet-cell mass may be required to preserve euglycemia during adulthood.

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