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A Feat of Metabolic Proportions: Pdx1 Orchestrates Islet Development and Function in the Maintenance of Glucose Homeostasis

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Abstract

Emerging evidence over the past decade indicates a central role for transcription factors in the embryonic development of pancreatic islets and the consequent maintenance of normal glucose homeostasis. Pancreatic and duodenal homeobox 1 (Pdx1) is the best studied and perhaps most important of these factors. Whereas deletion or inactivating mutations of the *Pdx1* gene causes whole pancreas agenesis in both mice and humans, even haploinsufficiency of the gene or alterations in its expression in mature islet cells causes substantial impairments in glucose tolerance and the development of a late-onset form of diabetes known as maturity onset diabetes of the young. The study of Pdx1 has revealed crucial phenotypic interrelationships of the varied cell types within the pancreas, particularly as these impinge upon cellular differentiation in the embryo and neogenesis and regeneration in the adult. In this review, we describe the actions of Pdx1 in the developing and mature pancreas and attempt to unify these actions with its known roles in modulating transcriptional complex formation and chromatin structure at the molecular genetic level.

Keywords

Pancreas; insulin; transcription; diabetes; islet

Introduction

The β cells of the pancreatic islets of Langerhans are solely responsible for the transcription, synthesis, and release of insulin in response to elevations in extracellular glucose concentration. Although β cells comprise 70–80% of islet mass, islets themselves comprise only about 1% of total pancreatic mass [1]; this latter feature explains, in part, the relatively poor functional reserve of β cells and the consequent high prevalence of diabetes mellitus in many countries. Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and susceptibility to a host of micro- and macro-vascular complications, including cardiovascular disease, kidney disease, retinal disease, and nerve disease. The loss or impairment of β cell function appears to underlie virtually all forms of diabetes mellitus. Recently, much of the molecular and genetic

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research in the field of diabetes has focused upon ways to restore the number or function of β cells as a means of reversing the metabolic consequences of insulin deficiency. Both cell-intrinsic components (e.g. transcription factors) and cell-extrinsic components (e.g. signaling growth factors) have been implicated in the formation and maintenance of β cell mass. One cell intrinsic factor, pancreatic and duodenal homeobox 1 (Pdx1), has received attention over the years as perhaps the most crucial factor in the β cell.

Pdx1, previously referred to as STF1, IDX1, IPF1, IUF1, was identified as a β cell-specific transactivator of the insulin and somatostatin genes that appeared to be the mammalian ortholog of the endoderm-specific *Xenopus laevis* XIHbox8 homeobox protein [2–9]. As with other transcription factors, Pdx1 appears to function by binding to specific DNA sequences within the 5' enhancer regions of target genes, and subsequently recruiting complexes of proteins that ultimately regulate the rate of transcription of that gene. The gene encoding Pdx1 (*Pdx1*) is a central member of a mammalian *Parahox* gene cluster on mouse chromosome 5. This cluster is so-named because it represents a group of developmentally important genes in mammals that is found outside the classical *Hox* (homeobox) cluster of genes [10]. The *Parahox* cluster is comprised of three genes, *Gsh1*, *Pdx1*, and *Cdx2/3*, all of which are expressed in specific pancreatic cell types [11]. In this regard, the importance of Pdx1 in the pancreas is emphasized by the near-absence of pancreas formation in *Pdx1*-null mice [12–14]. This dramatic phenotype also occurs in humans with homozygous mutations of the human ortholog of the *Pdx1* gene (known as *Ipf1*) [15,16], thereby underscoring the relevance of this factor to human pancreas development. Pdx1 also appears to be crucial for the function of the mature β cell. Heterozygous missense and frameshift mutations of the *Ipf1* gene in humans (while not impairing pancreas formation) result in defective insulin secretion and the development of a form of diabetes known as maturity onset diabetes of the young 4 (MODY4) [17–21]. Similarly, studies of animal models of insulin resistance suggest that the down regulation of *pdx1* expression in the β cell may underlie the pathogenesis of β cell failure and type 2 diabetes [22–24]. Thus, Pdx1 plays both a broad role in pancreas development and a much more specific role in β cell function in the adult mammal. That Pdx1 can achieve these differential actions may arise from its ability to associate in protein complexes that have varying transcriptional outcomes. This review describes the role of Pdx1 in the differentiation of specific cell types in the developing pancreas, and how Pdx1 mechanistically achieves its transcriptional effects at target genes to maintain normal glucose homeostasis.

Role of Pdx1 in Pancreas Development

Pdx1 and early pancreas development

The mature pancreas is composed of endocrine (hormone-secreting), acinar (hydrolytic enzyme-secreting), and duct cells. Whereas the exocrine cells are scattered throughout the pancreas and form the bulk of pancreatic mass, the endocrine cells occur in circumscribed clusters known as islets of Langerhans. Notwithstanding the disparity in organization and function of the different cellular types, all cells within the pancreas appear to arise from *Pdx1*-expressing precursors during development [25,26]. During embryogenesis, the pancreas emanates initially as two cellular extensions of endodermal origin located dorsally and ventrally to the primitive gut tube at approximately embryonic day 9.0–9.5 (E9.0–9.5) in the mouse [1]. During this early period of pancreas development, often referred to as the “primary transition,” there is branching of the epithelial cells within the developing buds, a process that is regulated by signals from the notochord and the surrounding mesenchyme (see Fig. 1).

The notochord factors fibroblast growth factor 2 (Fgf2) and activin (transforming growth factor (TGF)- β signaling molecule) repress endodermal sonic hedgehog (Shh) protein in the pancreas-specific endoderm, thus permitting expression of pancreatic genes such as *Pdx1* [27,28]. Studies in chicken and mice have demonstrated that ectopic expression of Shh during early

development results in a loss of *Pdx1* expression (and other pancreatic β -cell markers), and in the transformation of pancreatic mesenchyme into duodenal mesenchyme [29] [30]. In addition, continued expression of hedgehog proteins (Shh and Indian hedgehog, Ihh) after initial pancreas formation (at about E12.5 in the mouse) result in reductions in pancreatic mass, including both acinar and endocrine tissues [31]. These findings point to an important role for inhibition of hedgehog signaling in early pancreas development. At least some of the phenotypes described with early ectopic hedgehog signaling can be explained by the consequent absence of *Pdx1* expression in the pancreatic endoderm. In this regard, in studies of *Pdx1*^{-/-} mice, the initial buds of the pancreas form but subsequent branching and morphogenesis of these buds is arrested [12]. By contrast, in the mature pancreas and related islet-derived cell lines, ongoing expression of hedgehog proteins appear to be important for the maintenance of both *Pdx1* and *insulin* expression [32,33]. These findings suggest a complex interplay between hedgehog proteins and *Pdx1* expression, and that regulation of *Pdx1* gene expression appears to be differentially regulated in the developing pancreas vs. the mature pancreas.

Apart from its expression throughout the early pancreatic endoderm, *Pdx1* is also expressed in regions of the developing stomach and duodenum. In addition to loss of pancreas formation, *Pdx1*^{-/-} mice also exhibit loss of Brunner's glands and deficiency of enteroendocrine differentiation in the stomach and duodenum [13,34,35]. Factors controlling the activation of the *Pdx1* gene during early embryogenesis have been identified using both mouse genetic and biochemical approaches. A phylogenetically-conserved region in mammals at ~2 kilo-basepairs (kb) upstream of the transcriptional start site of the mouse *Pdx1* gene known as Area I-II-III (or PH-1, -2, and -3 in humans) harbors binding sites for endodermal and pancreas-specific transcription factors (Foxa2, Ptf1a, HNF1 α , MafA, HNF6, Pax6, and *Pdx1* itself) [36–40]. Area III appears to control the pancreas-wide expression of *Pdx1* during early bud formation [41], whereas Areas I and II appear to control islet-specific expression in later development and adulthood [42–44]. Homozygous deletion of the entire Area I-II-III region in mice results in agenesis of the ventral pancreatic bud and hypoplasia of the dorsal bud, but without abnormalities in the stomach or duodenum [45,46]. These findings suggest that the spatial regulation of *Pdx1* gene expression in the early endoderm is regulated by discrete *cis*-elements within its promoter that are bound by *trans*-acting factors.

Beyond its importance in specifying the fate of progenitor cells within the early pancreatic buds, *Pdx1* also plays a distinct role in the proliferation of differentiating cells. This proliferation function is mediated through a discrete amino acid sequence (FPWMK) within the amino terminal domain of *Pdx1* that is believed to interact with the ubiquitously expressed TALE (three amino acid loop extension) homeodomain protein Pbx1 (pre-B cell leukemia homeobox 1) [47]. In transgenic complementation studies of *Pdx1*^{-/-} mice, mutation of the FPWMK motif results in a severely hypoplastic pancreas with presence of all cell types (endocrine, exocrine, and duct) [48]. Consistent with this finding, *Pbx1*^{-/-} mice also develop pancreatic hypoplasia, and defects in endocrine and exocrine cell formation [49]. The physical interaction between *Pdx1* and Pbx1 may also play a role in the mature pancreas, as compound heterozygotes (*Pdx1*^{+/-}; *Pbx1*^{+/-}) develop frank diabetes, suggesting a defect in islet function [49].

Pdx1 and mid to late pancreas development

After formation of the initial dorsal and ventral pancreatic buds at E8.5 in mice, these structures undergo branching morphogenesis and proliferation as they evaginate into the surrounding mesenchyme. Between E13.5 and E15.5, endocrine and exocrine gene products dramatically magnify their levels of expression, coincident with the exponential increase in cellular mass; this period of time is referred to as the “secondary transition.” By E15.5, gut rotation causes

the ventral and dorsal buds to fuse, forming a single pancreatic organ as seen in adults [50] (see Fig. 1). The role of *Pdx1* in the development of the embryonic pancreas beyond the elaboration of the initial buds has been addressed in elegant studies of R. MacDonald and colleagues. Using a mouse model in which the expression of *Pdx1* can be selectively repressed by administration of doxycycline, they studied the effect of *Pdx1* deletion at various time points after the formation of the initial pancreatic buds [51]. Deletion of *Pdx1* at E12.5 led to the formation of a pancreas nearly devoid of acinar cells and islets; the precursor pancreatic epithelium was observed to grow and branch out, producing a truncated ductal tree with immature duct-like cells. The lack of acinar cell formation coincided with the loss of Ptf1a/p48, a transcription factor necessary for acinar cell development [52]. Deletion of *Pdx1* at the start of the secondary transition (E13.5) results in the formation of incompletely differentiated acinar cells [52]. Thus, although expression of *Pdx1* is necessary for the initial differentiation and growth of precursors within the developing pancreatic buds at E8.5–9.5, ongoing expression of *Pdx1* during the secondary transition (after E12.5) appears to be necessary for the subsequent differentiation and expansion of acinar tissue and islets, whereas ducts appear to be only partially dependent upon *Pdx1* after this stage. These findings are also consistent with lineage tracing studies, which demonstrate *Pdx1* expression in duct precursors until about E12.5, but not thereafter [26]. However, it should be noted that these lineage tracing approaches relied upon transgenic expression of a reporter protein, a technique that may not fully and accurately recapitulate the expression pattern of the endogenous gene.

After the secondary transition, *Pdx1* protein is downregulated in acinar cells [53], suggesting that the maintenance of acinar cells in their differentiated state is not dependent upon *Pdx1*. In fact, continued expression of *Pdx1* in acinar cells appears detrimental; studies of Heller, et al. demonstrated that ongoing expression of *Pdx1* in acinar cells after the secondary transition (using an *elastase-1* promoter-driven *Pdx1* transgene in mice) results in dysmorphogenesis of the acinar compartment with a high rate of cellular turnover (both proliferation and apoptosis) and eventual fatty infiltration [54]. The importance of selective expression of *Pdx1* during pancreas development is dramatically emphasized in studies by Miyastuka, et al., which showed that forced expression of *Pdx1* throughout all cells of the developing pancreas from ~E10 onward results in pancreatic hypoplasia and an acinar compartment that has been replaced by duct-like structures; this phenotype appears to be a consequence of the ectopic activation by *Pdx1* of the signal transducer and activator of transcription 3 (STAT3) [55]. On the one hand, these findings appear contradictory to the studies described above showing that *Pdx1* is necessary for acinar, but not ductal, differentiation and growth. On the other hand, these findings emphasize that *Pdx1* action is heavily nuanced by the cellular context; thus, in early pancreatic precursors, *Pdx1* is necessary to drive proliferation and pancreatic outgrowth, and in subsequent time points, it is necessary for lineage determination, proliferation, and maintenance of selected cell types. The molecular mechanisms underlying this selectivity may be influenced by both the physical accessibility (“chromatin structure”) of potential downstream target genes and the nature of the transcriptional protein complexes formed by *Pdx1*. These mechanisms will be addressed later in this review.

Role of *Pdx1* in the Adult Pancreas

Pdx1 in the maintenance of functional β cell mass

At the end of gestation (about e18.5 in the mouse), there is formation of distinct circumscribed structures of hormone-producing cells known as islets of Langerhans. In the late gestational and mature pancreas, *Pdx1* expression is restricted primarily to insulin-producing β cells of the islet, with expression in some somatostatin-producing δ cells, and low-level expression in subpopulations of acinar and duct cells [53] (see Fig. 2). *Pdx1* haploinsufficiency does not appear to physically or functionally impinge upon pancreas development in *Pdx1*^{+/-} mice [12,13], however, such mice exhibit strikingly impaired glucose tolerance and glucose-

stimulated insulin secretion with age [56,57]. Similarly impaired β cell function in the setting of Pdx1 deficiency has been observed in several other animal models (rat, fish, and sand rat [58–62]), as well as in humans with single allelic mutations in *Ipfl* (where the development of impaired glucose responsiveness and frank diabetes occurs) [17–21]. The sand rat (*Psammomys obesus*) is a particularly an interesting case, as Pdx1 is not normally found in islets from these animals; however, gene transfer experiments show that repletion of Pdx1 in these islets greatly enhances insulin content and glucose-stimulated insulin transcription [62]. Complete or near-complete deletion of Pdx1 in late embryonic [63] or adult mice [64] also result in a later diabetic phenotype. Although subtle developmental defects cannot be ruled out, these observations suggest a dramatically different requirement for Pdx1 gene dosage in the developing pancreas vs. the mature β cell, the latter of which may require more tightly regulated transcription and/or translation. In this regard, in some mouse models (and quite possibly in obese humans) the progressive loss of Pdx1 protein levels with age might account for the increasing incidence of β cell dysfunction and impaired glucose tolerance [22,57,65, 66].

The cause of β cell functional impairment in Pdx1 deficiency has been studied in multiple systems, including both cell line and mouse models. Based upon these studies, several mechanisms have been postulated: (a) increased β cell apoptosis via and Bcl-2), with resulting loss of downregulation of anti-apoptotic genes (Bcl_{XL} functional cell mass [56,67], (b) loss of activity of key *Pdx1* target genes whose products are involved in glucose-stimulated insulin transcription and secretion (including *Glut2*, *glucokinase*, *MafA*, *Nkx6.1*, *insulin*) [57,63,68–76], and (d) loss of new β cell formation/regeneration [64,77]; in this regard, studies suggest that the action of glucagon-like peptide 1 (GLP1) in enhancing β cell growth and formation in the adult may rely upon activation of *Pdx1* in β cells and potential precursor cell types, such as those residing within ducts [74,78–82].

Role of Pdx1 in adaptive β cell hyperplasia and β cell regeneration

The effects of Pdx1 on β cell growth and survival noted above raise the tantalizing possibility that it may play a central role in the β cell hyperplasia and hyperinsulinemia observed in states of insulin resistance, including obesity and severe stress [22,66]. Amongst the most well-studied pathways that regulate β cell mass include the insulin receptor substrate 1 and 2 (IRS1 and IRS2) branch of insulin receptor (IR)/insulin-like growth factor 1 receptor (IGF1R) signaling [83,83,84]. Several studies suggest that Pdx1 may act downstream of the IR/IGF1R signaling to promote β cell proliferation [83,85–89]. Studies of Kulkarni, et al. reveal that haploinsufficiency of *Pdx1* in two mouse models of insulin resistance—insulin receptor/IRS1 double heterozygous mice and liver insulin receptor knockout mice—virtually completely abrogated the adaptive islet hyperplastic response, resulting instead in β cell apoptosis and premature death of the animals [88]. Importantly, there is also loss of β cell proliferation in these models, suggesting that an important component of islet hyperplasia in insulin resistant states may be an enhanced replication rate of pre-existing β cells (rather than β cell neogenesis). β catenin signaling, a pathway mediating pancreas growth, may in part underlie proliferation of mature β cells. In this regard, it appears that a full complement of Pdx1 is necessary to promote the dissociation of β catenin from its membranous E cadherin anchor, thereby allowing the cytoplasmic and nuclear translocation of β catenin [88]. Precisely how Pdx1 action impinges upon β catenin release is unclear; nonetheless, this pathway may not represent an exclusive mechanism of Pdx1-mediated proliferation, as overexpression of active β catenin alone in late pancreas development appears to have minimal, if any, effects on β cell proliferation ([90]). These latter data suggest that the confluence of pathways (IR/IRS and β catenin signaling) in the setting of a normal Pdx1 complement is necessary for adaptive β cell proliferation.

There is some evidence that certain non- β cells, such as duct cells of the pancreas, may also contribute to adaptive β cell hyperplasia in a Pdx1-dependent fashion. For example, in *Irs2*^{-/-} mice there is diminished β cell mass with impaired glucose tolerance [89,91]; in this model, superposition of haploinsufficiency of the Forkhead transcription factor Foxo1 causes activation of *Pdx1* expression in duct cells, and the subsequent recovery of β cell mass [92]. In support of this observation, overexpression of *Pdx1* alone in *Irs2*^{-/-} animals promotes recovery of β cell mass and correction of glucose tolerance throughout life [93]. Whether duct cells *per se* give rise to β cells in this model, however, was not directly demonstrated in these studies and must await more rigorous lineage tracing studies. Activation of *Pdx1* in duct cells of the pancreas has also been observed following partial pancreatectomy in mice [85] and in insulin resistant Zucker fatty (ZF) rats [83]. Importantly in this regard, Pdx1 expression is often observed in proliferating duct cells [77,94,95], and such cells have been suggested to serve as substrate for β cell neogenesis, particularly in cases of adaptive hyperplasia or pancreas regeneration [92,96–99]. A recent study suggests that Pdx1, in complex with TALE transcription factors, negatively regulates the duct-specific keratin 19 gene in mature duct cells [100]. This finding raises the possibility that enhanced levels of Pdx1 protein in duct cells may promote β cell transdifferentiation by direct repression of the duct cell phenotype (through suppression of keratin 19 and related genes) and simultaneous activation of β cell-specific genes [101–103]. Currently, there is little evidence that any other cell type contributes to β cell neogenesis. Although some studies had suggested the potential role of Pdx1 in promoting β cell transdifferentiation from acinar tissue [83,104–106], a recent elegant lineage tracing analysis suggests that such cells do not contribute to new β cells in models of regeneration *in vivo* [107].

Mechanism of Pdx1 Action

Posttranslational modifications of Pdx1

As a member of the homeodomain class of proteins, Pdx1 is believed to exert its actions almost exclusively within the nucleus as a result of regulating gene transcription. Although some reports in the literature have described physiologic circumstances where the distribution of Pdx1 might be cytoplasmic, it is believed that cytoplasmic sequestration may represent more a mechanism to attenuate the nuclear action of Pdx1 under physiologic or pathologic conditions, rather than to promote a specific cytoplasmic function. For example, the negative effect of fatty acids on β cell function may be related to their eventual sequestration of Pdx1 in the cytoplasm [76], whereas the positive effect of glucose on insulin transcription may be a result of its enhancement of Pdx1 nuclear translocation [108]. A basic amino acid sequence with the homeodomain of Pdx1, RRMKWKK, is believed to function as the nuclear targeting sequence [109]. Teleologically, regulation at the level of Pdx1 protein compartmentation (e.g. nuclear-cytoplasmic shuttling) or function would allow for acute alterations to target gene transcription, where control on the order of seconds to minutes might be crucial. At least 3 different reversible post-translational modifications, including phosphorylation [86,108,110–114], sumoylation [115], and glycosylation [116], have been proposed to explain nuclear-cytoplasmic shuttling and/or other functions of Pdx1. Consistent with these modifications, different molecular weight species of Pdx1 have been described in immunoblots [115]. Of the three modifications, phosphorylation is perhaps the best-studied. Elevated glucose levels have been suggested to promote Pdx1 phosphorylation through one or a combination of several pathways, including the stress-activated protein kinase (SAPK) and extracellular signal-regulated kinase (ERK) 1/2 pathways [86,108,112], phosphatidylinositol 3-kinase pathway [117,118], and/or the Per-Arnt-Sim (PAS) kinase [110]. Although the role of phosphorylation of specific residues within Pdx1 has not been rigorously analyzed, phosphorylation of Ser61 and Ser66 of Pdx1 via glycogen synthase kinase 3 appears to target Pdx1 for proteosomal degradation, thereby decreasing its half-life. This modification could partially explain

diminished β cell function during endoplasmic reticulum stress and oxidative stress (both of which occur in the diabetic state), where GSK3 activity is enhanced [111,119]. Similarly, diminished β cell function following DNA damage might be explained by phosphorylation of Thr11 of Pdx1 by DNA-dependent kinases; this modification has also been proposed to lead to its more rapid intracellular degradation [114].

Target gene recognition by Pdx1

In the nucleus, the action of Pdx1 relies upon its binding to specific genes and subsequently regulating their rates of transcription. The homeodomain of Pdx1 recognizes A/T-rich DNA sequences (characteristically 5'-TAAT-3') with affinity in the nanomolar range [120,121]. Interestingly, the range of DNA sequences bound by Pdx1 within the nucleus cannot be entirely predicted by its affinity for DNA *in vitro*; thus, some sequences that show preferential binding *in vitro* by electrophoretic mobility shift assays demonstrate little or no association within the nucleus by chromatin immunoprecipitation, and vice-versa [70,71]. At least two factors could account for this discrepancy. First, amino acid sequences outside of the homeodomain of Pdx1 itself may contribute to DNA binding either directly or through interactions with other proteins [120,121]. For example, the interaction of Pdx1 with the bHLH factor BETA2/NeuroD1 would allow for selective targeting to genes whose regulatory regions contain appropriately-spaced binding sites for both factors [122,123]. Second, the chromatin structure or conformation of DNA at specific genes within the nucleus could alter DNA site accessibility or binding affinity, respectively. In this regard, Pdx1 displays little or no ability to dynamically alter chromatin structure, and therefore appears to be highly susceptible to the nature chromatin accessibility [124]. Studies in our laboratory demonstrate that chromatin accessibility at the insulin gene in two different cultured pancreatic cell types (β cells vs. duct cells) is dramatically different, permitting the occupancy of Pdx1 in only one cell type (β cells) but not the other (duct cells) [124]. The limited accessibility in duct cells in that study could be accounted for by the presence of a nucleosome (DNA complexed with histone proteins) at a key Pdx1 binding site in the insulin promoter, and binding in the presence of this nucleosome can only be overcome by high concentrations of Pdx1 protein [124]. These data raise the attractive hypothesis that the higher levels of Pdx1 associated with transdifferentiating duct cells is a necessary trigger to initially overcome limited chromatin accessibility at β cell gene targets.

Even in the presence of accessible or “open” chromatin, the local conformation (or “bending”) of DNA may subtly influence binding affinity and subsequent transcriptional rates. A recent crystallographic structure of the Pdx1 homeodomain by Longo, et al. demonstrates two distinctly different structures of Pdx1 when bound to DNA, each showing distinct side chain contacts depending upon the conformation of the DNA [125]. This “induced fit” model suggests that transcription factors bound at more distant sites (and possibly not even interacting with Pdx1) could alter the nature of the DNA conformation at the Pdx1 binding region. Thus, the nuclear composition of proteins (hence cellular context), may affect occupancy and consequent activity of Pdx1 at any given gene.

Mechanisms of transcriptional regulation by Pdx1

Based on studies using transient reporter gene assays in cell lines and electrophoretic mobility shift assays, a host of genes in the β cell initially emerged as potential candidates for direct regulation by Pdx1. These included the genes encoding insulin [68,70,71,126], Glut2 [127, 128], glucokinase [129,130], islet amyloid polypeptide (IAPP) [63,75,131,132], Pax4 [133], Nkx6.1 [69,134], MafA [72], liver receptor homolog (Lrh) 1 [135], and Pdx1 itself [37]. In recent years, the direct regulation of many of these genes by Pdx1 has been confirmed using loss-of-function studies in islets and intact animals, and occupancy by Pdx1 of the endogenous genes in islets and β cell lines has been confirmed using the chromatin immunoprecipitation (ChIP) assay [70]. The insulin gene represents the best and most definitively characterized

gene with respect to Pdx1 action. As such, much of our knowledge of the mechanism by which Pdx1 regulates target genes has emerged from study of the *insulin* enhancer. The general consensus from these studies has been that Pdx1 is an activator of gene transcription, with the activation function residing with the amino terminal domain of the protein, between amino acids 13–73 [136–138]. However, despite years of research, remarkably little is still known about the mechanisms by which Pdx1 directly influences gene transcription. For example, how does Pdx1 communicate across the gene with other transcription factors to achieve transcriptional synergy? How do proteins necessary for mRNA initiation and elongation interrelate with these transcription factors? How are all of these events achieved in the context of potentially repressive chromatin structure? Studies in our laboratory and others have elucidated that a more “dynamic” and intricate series of events ultimately contributes to transcriptional activation by Pdx1. These include (a) the formation of an activation complex involving other transcription factors and transcriptional coactivators, (b) the modulation of local chromatin structure to ensure a more “accessible” state of the gene, and (c) communication with components of the basal transcriptional machinery to alter final transcriptional responsiveness. Each of these events are briefly discussed below.

Formation of protein complexes—The participation of Pdx1 in a transcriptional complex is suggested by functional studies in cell lines, whereby addition of Pdx1 in conjunction with other β cell-specific transcription factors results in the synergistic activation of *insulin* gene-based reporter plasmids [139]. These interacting factors include bHLH proteins that bind to E-boxes (BETA2/NeuroD1 and E2A proteins) [122,123,140,141], the basic leucine zipper factor MafA that binds to the C-box [73,142], the homeodomain proteins Pbx1 and MEIS2 [47,48,143,144], and high mobility group (HMG) proteins that bind to the DNA backbone [123]. The free energy gained from these interactions may increase the likelihood that Pdx1 targets genes with binding sites for these factors [123,144]. Alternatively, these interactions may cause conformational changes in Pdx1 and/or DNA that enhance DNA binding affinity [125].

Apart from interaction with transcription factors, Pdx1 also appears to recruit transcriptional co-activators (proteins that bridge Pdx1 to the basal transcriptional machinery), including CBP/p300 [68,113,141,145,146], Set7/9 [147], Bridge-1 [148], PCIF1 [149], and histone deacetylases (HDACs) [150]. Once recruited, cofactors are believed to serve as a physical or functional “bridge” that allows Pdx1 to communicate with components of the basal transcriptional machinery. Although the majority of these cofactor interactions with Pdx1 are believed to enhance the activation function of Pdx1, there is the distinct possibility that certain cofactor interactions may allow Pdx1 to aid in the repression of some genes. In this regard, the well-defined repression of genes such as glucagon [63,64,70,134] and K19 [100] by Pdx1 may reflect a repression complex formed in association with corepressor molecules.

Modulation of chromatin structure—Although packaging of DNA in the context of chromatin is responsible for the efficient storage of genetic material within the nucleus, it also has the capacity to impede the accessibility of DNA to transcription factors and the transcriptional machinery [151]. In this regard, basic unit of chromatin is formed by the complex between DNA and histone proteins; the covalent modifications of the amino terminal tails of histone proteins (including acetylation and methylation of Lys residues) have been demonstrated to alter chromatin packaging, and hence directly affect rates of gene transcription [152]. Interestingly, although Pdx1 itself does not appear to have intrinsic ability to either modify histones or directly alter chromatin structure, its depletion in β cell lines results in the loss of histone H3-Lys4 dimethylation (a mark of active, open chromatin) at the *insulin* gene. This result may be secondary to the recruitment of the histone methyltransferase Set7/9 by Pdx1 [147]. Similarly, the glucose-stimulated increase in histone H4 acetylation (another marker of active, open chromatin) at the *insulin* gene in both cell lines and islets by Pdx1 is consistent with its recruitment of the histone acetyltransferase p300 [145,153,154]. These

results suggest that an important component of gene activation by the Pdx1 protein complex may be its direct alteration of chromatin to a more open state, thereby permitting enhanced accessibility to other transcription factors and the basal transcriptional machinery.

Communication with basal transcriptional machinery—The term “basal transcriptional machinery” is applied to a group of proteins responsible for the elongation of mRNAs and their subsequent processing. In eukaryotes, these include several general transcription factors (TFIIA, TFIIB, TFIID, TFIIE, TFIIF, TFIIH) and the RNA polymerase II holoenzyme [155]. Few studies in β cells have attempted to address a link between Pdx1 and the basal transcriptional machinery. Our laboratory has demonstrated that in the absence of Pdx1, the RNA polymerase II complex continues to be recruited to the *insulin* promoter, but transcriptional elongation by the complex is impaired [68]. Consistent with this observation, it appears that Pdx1 is necessary to ensure that the Ser5-phosphorylated isoform (the “initiation isoform”) is appropriately converted to the Ser2-phosphorylated isoform (the “elongation isoform”) [147]. These findings raise the possibility that the linkage between the Pdx1 transcriptional complex and the basal transcriptional machinery may involve an as yet unidentified kinase. Importantly, in yeast systems, these phosphorylation events (thought to be mediated by cyclin-dependent kinases) are inextricably linked to the presence of histone H3-Lys4 methylation [156]. Thus, the link between Pdx1 and the putative kinase may be indirect, and possibly secondary to the maintenance of the appropriate chromatin state by Pdx1.

Conclusions

The prevalence of diabetes mellitus is increasing to alarming proportions. Current estimates from the Centers for Disease Control predict that 1 in 3 Americans born in the year 2000 will develop diabetes, and similar trends are predicted around the world [157]. Underlying all forms of diabetes is dysfunction at the level of the β cell. Given the broad role of Pdx1 in the differentiation of cell types with the pancreas and in the maintenance of β cell function and mass (see summary in Table I), preclinical studies have recently focused on the therapeutic potential for Pdx1 and related factors to reverse Type 1 diabetes. For example, viral-mediated expression of *Pdx1* in liver cells appears to stimulate a host of pancreas-related genes (including *insulin*), leading to reversal of chemically-induced diabetes in mice [158–161]. From a delivery perspective, transduction of Pdx1 using viruses may not be requisite. Pdx1 also has been shown to harbor a protein transduction domain, thereby enabling delivery of the protein directly into potential precursor cells to induce the pancreatic gene program [162,163]. Thus, leveraging of the protein transduction domain may open therapeutic avenues via delivery of Pdx1 protein *in vivo* to stimulate potential β cell precursors. The results of these therapeutic intervention studies emphasize the central role of Pdx1 in the pancreatic transcriptional hierarchy (see Fig. 3) and suggest a relatively simplistic approach to cellular “reprogramming” for the generation of new β cells. Just as importantly, however, the disparate effects of Pdx1 in the context of different cell types emphasize our limited understanding of the mechanisms and pathways encompassing Pdx1 action. For example, what upstream signaling pathways are necessary for initiation and maintenance of *Pdx1* gene expression? What factors distinguish why one cell type responds to Pdx1 by activating acinar genes, another responds by activating β cell genes, and still others activate neither acinar nor β cell genes? How does the level of Pdx1 protein in a given cell type nuance the activity of a given gene or, ultimately, the global gene expression pattern in that cell? The answers to these and related mechanistic questions may ultimately hold the clues to the identification and/or engineering of a renewable supply of β cells for individuals with diabetes.

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ABBREVIATIONS

bHLH	basic helix-loop-helix
Pbx1	pre-B cell leukemia homeobox 1
Pdx1	pancreatic and duodenal homeobox 1
Shh	sonic hedgehog
TALE	three amino acid loop extension

Figure 1. Pdx1 expression and gross pancreatic morphology during mouse embryonic development

Shown are schematic representations of the developing mouse pancreas at different embryonic ages. At approximately embryonic day 8.5 (E8.5) signals from the notochord, including fibroblast growth factor 2 (FGF2) and activin, repress expression of hedgehog proteins (HH) in the region of the gut endoderm that is destined to develop into pancreatic buds (dark blue). Initial pancreatic bud outgrowth occurs between E9–9.5 (primary transition), and subsequent branching morphogenesis is dependent upon the expression of Pdx1 (see text). During the secondary transition (between E11–15), there is differentiation of islet cell types (e.g. α and β cells) and acinar cells that is dependent upon the action of Pdx1. After E15, the two buds

fuse to form the single organ seen in adult mammals. In adults, Pdx1 expression persists at highest levels in islets (particularly β cells) and at low levels in duct cells and acinar cells.

Figure 2. Pdx1 protein expression in the adult pancreas

Pancreas from a 10 week-old mouse was fixed in paraformaldehyde and embedded in paraffin. Sections were then hematoxylin stained and subject to immunohistochemistry or immunofluorescence. *A*, horseradish peroxidase-based immunostaining for Pdx1. Pdx1 (dark brown color) demonstrates an islet-specific expression pattern with nuclear localization. Occasional duct cells are also observed to express Pdx1 (*yellow arrowheads*). *B*, dual immunofluorescence of an islet using antibodies against Pdx1 (*green*) and insulin (*red*). Pdx1 is seen to localize within the nuclei of insulin-producing β cells. *C*, dual immunofluorescence of an islet using antibodies against Pdx1 (*green*) and glucagon (*red*). Pdx1 is not expressed in glucagon-positive α cells. In *panels B and C* nuclei are counterstained with hoechst H33342 dye (*blue*).

Figure 3. Upstream regulators and direct downstream targets of Pdx1

The figure emphasizes the central role of Pdx within the pancreas. Cell-extrinsic factors emanating from the notochord (FGF2 and activin) as well as cell-intrinsic factors (transcription factors Foxa2 and HNF6) collaborate to activate Pdx1 expression in the primitive gut endoderm. During pancreas development, Pdx1 is believed to directly activate or repress a subset of genes that lead to differentiation of islet and acinar cells. In the mature pancreas, Pdx1 directly activates or represses genes that confer the β cell phenotype. The “+” sign indicates that the gene is activated by Pdx1, and the “-” sign indicates that the gene is repressed by Pdx1.

TABLE I
Expression Pattern and Function of Pdx1 in the Developing and Mature Mouse Pancreas

Developmental Period	Pdx1 Expression Pattern
Early Development (E8.5 – E12)	All cells of the early pancreatic endoderm, and portions of the stomach and duodenum
Mid to Late Pancreas Development (E12.5 – E18)	Endocrine and acinar cells
Adult Pancreas	Primarily β (insulin) and δ (somatostatin) endocrine cells of the islets of Langerhans; occasional duct cells and acinar cells.