Transcription factors regulating β-cell function

Marlon E Cerf
Diabetes Discovery Platform, South African Medical Research Council, PO Box 19070, Tygerberg, 7505, South Africa
(Correspondence should be addressed to M E Cerf. Email: marlon.cerf@mrc.ac.za)

Abstract
Type 2 diabetes is primarily associated with insulin resistance and β-cell dysfunction. Maintenance of functional mature β-cells is imperative for ensuring glucose homeostasis. This can be achieved by optimal expression of key transcription factors that are required for normal pancreatic development and maintaining β-cell function. Defining the regulation of transcription factors as well as their regulation of important β-cell genes like insulin will provide further insight into elucidating the mechanisms leading to β-cell dysfunction.

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Introduction
In humans, mutations in specific genes lead to the various forms of maturity-onset diabetes of the young (MODY). MODY is a subtype of diabetes characterized by autosomal dominant inheritance, non-ketotic diabetes mellitus, an age of onset of <25 years and a major defect in pancreatic β-cell function (1). MODY is associated with impaired glucose-stimulated insulin secretion (GSIS), therefore it is important to further our knowledge of the transcription factors that regulate the glucose-sensing genes – the glucose transporter, GLUT-2, and the glycolytic enzyme, glucokinase (GK), as well as the principal regulator of β-cell function, insulin (2–5). The β-cell is estimated to contain up to 40 000 insulin transcripts (6–8). Several key control elements have been identified within the insulin gene, including A1, C1/RIPE3b, C2, and E1. Mutations of the GK gene lead to the manifestation of MODY2, with the remaining five types of MODY resulting from defects in genes encoding for transcription factors: Hnf-4α (MODY1), Hnf-1α (MODY3), Pdx-1 (MODY4), Hnf-1β (MODY5), and NeuroD1 (MODY6). The mutations of the GK gene in MODY2 patients have been linked to both kinetic alterations and the regulation of GK activity (9). Many novel mutations found in the MODY genes are constantly being reported in different population groups.

Most of the pancreatic transcription factors that are important for β-cell maintenance are found in other islet cell types and may also reside in pancreatic ducts. During pancreatic development, transcription factors require specific sequential regulated expression to ensure normal organogenesis. In the mature pancreas, transcription factors play a role in achieving glucose homeostasis by regulating the expression of key genes involved in maintaining the β-cell phenotype, most notably the insulin gene. Specific transcription factors are activated during early pancreatic development and β-cell differentiation, which may be switched off as the β-cell matures, providing clearance for another set of transcription factors to regulate the mature β-cell. Some transcription factors, viz., Pdx-1, Pax 4, NeuroD1, Nkx 6.1, and Nkx 2.2 are expressed in both progenitor and differentiated endocrine cells. Certain transcription factors, like Pdx-1 and Nkx 2.2, are involved in both early β-cell differentiation and mature β-cell function. This review will report briefly on transcription factors involved in the developing and differentiating endocrine pancreas, and then focus on those transcription factors crucial for maintenance of functional β-cells in the mature pancreas.

Transcription factors directing pancreatic development and β-cell differentiation
Several transcription factors are activated during early pancreatic development to ensure normal organogenesis and the subsequent differentiation of the different endocrine cell types. Pancreatic organogenesis involves a sequential cascade of inductive events in association with the activation of specific transcription factors (10). During early embryogenesis, the pancreas arises from an evagination of the foregut to form a dorsal, and then a ventral, epithelial bud (10). Subsequently, both these buds proliferate to form multiple branches and fuse together to form a functional organ (10). The organogenesis of the pancreas starts with the appearance of protodifferentiated epithelial cells, which form ductules from which both acinar and islet cells originate (11).
All islet cells are believed to originate from pluripotent progenitor cells both during development and later in life (12). Islet progenitor cells, which arise from the pancreatic ductal epithelium, undergo a series of cytodifferentiation steps that lead to the formation of mature islets (13). Islet cell differentiation depends on multiple transcription factors that display highly restricted cell-specific expression patterns (14–16). The development of the normal pancreas is a result of close interaction between mesenchymal and epithelial cells that form the initial buds (17). Signals from mesenchymal cells direct pancreatic development towards endocrine or exocrine fate (18). Mesenchymal cells of the developing pancreas express Islet-1 (19) and the neural stem cell marker, nestin (20). β-cell formation from multipotent precursors accounts for the vast majority of β-cells formed during embryogenesis (21, 22). Specific transcription factors restrict the developmental potential of the initially multipotent pancreatic progenitors and promote their differentiation into specific cell types (21).

It is possible that multiple pathways for β-cell differentiation exist in the adult pancreas, perhaps differentially activated by distinct stimuli (23). Pdx-1 is a transcription factor that is expressed during early pancreatic development, during islet cell differentiation, and in the mature β-cell. Pdx-1, Pax 6, β2, and Nkx 2.2 are suggested to represent the core components of a transcription complex of an islet-enriched gene that contribute to regulating expression of genes selectively expressed in β-cells during development (24). A recent study suggested that Pax 6 functions in parallel to Pdx-1, Nkx 2.2, and Nkx 6.1 in some of the β-cells during the late stages of pancreatic development (25). MafA has recently been hypothesized to be important for the differentiation of β-cells, with Nkx 6.1 occurring upstream to MafA during pancreatic development (26).

Ngn3 is required for endocrine fate determination in the developing mouse pancreas (27). It has been suggested that a molecular pathway exists where Ngn3-positive precursor cells become committed to endocrine differentiation marked by activation of NeuroD1 and subsequently Isl-1, Pax 6, and the pancreatic hormones, followed by switching off of Ngn3 expression (28). The specification of different islet cell types and the completion of the differentiation process require the activation of transcription factors that are downstream of Ngn3 (29), which include the direct Ngn3 targets NeuroD1, Pax 4, and Nkx 2.2 (30–32). The zinc finger transcription factor IA1 (insulinoma-associated 1 or INSM1) has been recently described as a novel and direct target of Ngn3 and a positive regulator of endocrine specification (29). IA1 has an essential role for β- and α-cell differentiation in the embryonic pancreas, downstream of Ngn3, and parallel to NeuroD1 in the network of developmental transcription factors that regulate endocrine differentiation (29).

NeuroD1 is involved in the differentiation of endocrine pancreatic cells. In the mouse, NeuroD1 is first expressed in the pancreatic primordium at e9.5 and continues to be expressed in the β-cells throughout development (33) but is predominantly restricted to β-cells in mature islets. NeuroD1/β2-deficient mice develop severe diabetes and die perinatally because of a marked reduction in β-cell number and a lack of proper islet formation, demonstrating the importance of NeuroD1/β2 for the morphogenesis or differentiation of β-cells (15).

Pax 4 expression in the developing pancreas is important for ensuring normal development of the differentiating β-cell. Lineage tracing analyses, where a hormone promoter drives cre-recombinase to mark cells that activate the promoter at any point during development, indicate that α- and β-cells diverge early, before the onset of hormone expression (34), in a cell fate choice controlled by reciprocal expression of the transcription factors Arx, which is important for α-cell formation, and Pax 4, which is important for β- and δ-cell formation (35). Hnf-1α and Ngn3 cooperate in activating Pax 4 expression and β-cell differentiation in the developing pancreas, which places these factors upstream of Pax 4 in the hierarchy of factors controlling islet cell determination and differentiation (31). Pax 4 has been suggested to contribute to the maintenance of Nkx 6.1 expression in differentiating β-cells (36).

The activity of the NK-family member and homeodomain protein Nkx 2.2 is necessary for the maturation of β-cells (37), whereas its distant homologue Nkx 6.1 controls their expansion (38). The presence of incompletely differentiated islet cells led to the hypothesis that Nkx 2.2 functions in the later steps of β-cell differentiation (38). Nkx 2.2 is co-expressed with insulin (37) and Ngn3 (37, 39) during pancreatic development. Hnf-3β and Ngn3 are proposed to lie upstream from Nkx 2.2 in the hierarchy of β-cell differentiation transcription factors (32). A recent study in mice suggested that high expression levels of both Nkx 2.2 and Pax 4 are independently required to specify or maintain β-cell fate (40). The absence of either transcription factor resulted in β-cells failing to form, with the β-cells replaced by ghrelin-producing ε-cells (40). Pax 6 expression depends on Nkx 2.2 (36), thus it is probable that Nkx 2.2 acts upstream of Pax 6 in the same pathway to regulate the β- and ε-cell fate (40).

Nkx 6.1 is expressed in three cell populations during pancreatic development, first broadly in the undifferentiated epithelial cells of the early pancreatic bud, then in a subset of proliferating islet cell progenitors, and finally in differentiated β-cells (38). Nkx 6.1 and Pdx-1 gene expression occur in parallel in the developing rat pancreas (41). Conclusive genetic evidence showed that Nkx 6.1 lies downstream to Nkx 2.2 in the pathway for β-cell formation as Nkx 2.2/Nkx 6.1 double mutants displayed a similar phenotype to Nkx 2.2 single mutants (38). A recent study in Nkx 6.1/Nkx 6.2 double nullizygous mice showed that proper β- and α-cell
development requires the combined activities of Nkx 6.1 and its paralog, Nkx 6.2 (42).

After pancreatic organogenesis and the regulation of endocrine cell differentiation by transcription factors, the process of pancreatic development is complete. New β-cells can be formed by either mitotic division of a pre-existing differentiated β-cell (replication), or by differentiation from an undifferentiated precursor or stem cells (neogenesis) (43). The mature pancreas is composed of the endocrine hormone-producing islets, the exocrine acini that produce various digestive enzymes (such as amylases, proteases, and nucleases), and the ductal tree comprising the centroacinar cells, ductules, and ducts that transport these enzymes into the intestine. The normal human adult pancreas is highly vascularized, richly innervated, and contains about one million islets, which constitute 2% of the total pancreas weight. The main cell types are the β-, α-, δ-, PP−, and newly discovered ε-cells that produce the hormones insulin, glucagon, somatostatin, pancreatic polypeptide, and ghrelin respectively, with the core of the islet containing the β-cells and the remaining cell types dispersed in the periphery of the islets. Regulation of the key β-cell genes in the mature pancreas, by transcription factors, is important for maintaining the β-cell phenotype.

Transcription factors regulating gene expression in the mature β-cell

**Pdx-1**

Pdx-1 (also known as lfP-1, Idx-1, Iuf-1, and Stf-1) is considered to be the key transcription factor involved in early pancreatic development, β-cell differentiation, and maintenance of the mature β-cell (44). In adult β-cells, Pdx-1 regulates the transcription of the insulin (45), GLUT-2 (46), GK (47), and Nkx 6.1 (48, 49) genes (Table 1). However, Pdx-1 regulation of GK remains controversial as heterozygous Pdx-1 mutant mice showed no change in GK expression in the islets isolated from these mice (50), suggesting that Pdx-1 does not regulate the GK gene as previously reported (51). Unaltered GK levels were also demonstrated in the islets of mice with β-cell specific deletion of Pdx-1 (52, 53). Furthermore, Pdx-1 was reported not to bind to the β-cell GK promoter in vivo (53). Further studies are required to comprehensively demonstrate that Pdx-1 does in fact regulate GK expression. The possibility exists that if Pdx-1 does not directly regulate GK gene expression, it may have an indirect effect by interacting with other genes that modulate expression of the GK gene. The transcription factor NeuroD1 has been reported to regulate GK (54). A recent study highlighted the role of the novel transcription factor, MafA, as a key regulator of GSIS in vivo. MafA-deficient mice displayed reduced insulin 1, insulin 2, Pdx-1, NeuroD1, and GLUT-2 transcripts (55). Perhaps, MafA may regulate GK indirectly via its modulation of NeuroD1. This remains to be proven as GK mRNA levels were not altered in MafA-deficient mice (55).

Pdx-1 has been reported to function in concert with other transcription factors in regulating the expression of the insulin gene and several other islet-specific genes (4, 15, 56, 57). VP16, a fusion protein of Pdx-1, enhances Pdx-1 function as a transdifferentiation factor from the liver to the pancreas (58, 59). Studies have shown that a modified form of XIHbox8, the Xenopus homolog of Pdx-1, carrying the VP16 trans scripting activation domain from the herpes simplex virus, efficiently induces insulin gene expression in the liver of the tadpole (58, 59). Pdx-1/VP16 expression, together with NeuroD1 or Ngn3 markedly induced insulin gene transcription, ameliorated glucose tolerance, and substantially induced insulin biosynthesis and various β-cell factors necessary for β-cell function, viz., GK, Sur1, and Kir6.2 in the liver (60). Bridge-1, a PDZ co-activator for E2A isolated from pancreatic insulinoma cells, was suggested to participate in the regulation of insulin gene transcription in β-cells (61). Bridge-1 was shown to be a protein interaction partner to modify transcriptional action functions of Pdx-1 (62). The zinc finger transcription factor, Egr-1 (also known as zif268, NGFI-A, Mrox24, and TIS8) does not directly interact with sequences within the insulin promoter. However, Egr-1 regulates insulin gene expression by regulating Pdx-1 expression levels in response to changes in glucose concentrations in order for the β-cells to meet metabolic demands for insulin production (63). Egr-1 may also be an important regulator of the glucagon promoter in α-cells (64).

The mechanism of insulin gene regulation by Pdx-1 was recently studied (8). Pdx-1 directly regulates insulin transcription (i.e., the insulin gene is a direct downstream target of Pdx-1) through formation of a complex with transcriptional co-activators on the proximal insulin promoter (8). The complex leads to enhancement of elongation by basal transcriptional machinery (8). The co-activator, p300, interacts with Pdx-1 and is believed to enhance insulin transcriptional activity through multiple mechanisms, including the recruitment and activation of components of the basal transcriptional machinery and histone/protein acetylation (65–69). Previous studies using the rat insulin 1 promoter constructs showed that Pdx-1, E47 (a ubiquitous bHLH protein, which forms a heterodimer complex with NeuroD1 (70)) and NeuroD1 could interact synergistically to stimulate promoter activity (71). Pdx-1, MafA, and NeuroD1/E47 acting through the promoter proximal A1, C1, and E1 sites respectively, play an important role in maintaining basal promoter activity of the human insulin gene, with evidence of the transcription factors having a synergistic effect on the human promoter (72). Pdx-1 had the strongest stimulatory effect on the insulin promoter, particularly, in the human insulin promoter, relative to the weaker effects of
MafA, NeuroD1, and E47, while mutating the human insulin promoter to resemble the rat insulin 1 promoter generated more synergy (72). The study emphasized the differences in regulation of the human promoter and the well-characterized rodent insulin promoter (72). Pdx-1/VP16 was shown to activate both the mouse insulin 1 and insulin 2 genes, whereas Pdx-1 alone was only able to activate the mouse insulin 1 gene (72). Two highly conserved sequences in the 5′-flanking region of the Pdx-1 gene (PH1/area1 and PH2/area2) confer β-cell-specific transcriptional activity on a heterologous promoter (73, 74). Pdx-1 itself binds to the PH1/area1 element and co-operates with Hnf-3β to activate transcription (74). Another study reported that Pdx-1, Pax 6, and Nkx 2.2 regulate the insulin gene by binding to intact β-cells in the regulatory control regions of other genes selectively transcribed in islet cells (24).

Pdx-1 may be directly activated by the transcription factors NeuroD1 (75), Hnf-1α and Hnf-3β (76). In type 2 diabetic islets, Pdx-1 mRNA expression was increased, despite augmented expression of Forkhead box O1 (Foxo-1) (77), which is considered an inhibitor of Pdx-1 gene expression (78, 79). Pdx-1 autoregulates its transcription (80) and was shown to bind to its own promoter region using ChIP assay (24).

### MafA

MafA is one of the more recently discovered transcription factors and has been demonstrated to play an important role in maintenance of β-cell function. This basic leucine zipper transcription factor is reportedly found only in the β-cells of the pancreas (81, 82). MafA expression in the liver, together with Pdx-1 and NeuroD1, markedly induces insulin gene transcription and dramatically ameliorates glucose tolerance in animal models of diabetes (83). MafA overexpression, together with Pdx-1 and NeuroD1, drastically induced insulin production in the liver (83). The triple infection with adenovirus for MafA, Pdx-1, and NeuroD1 was more effective than single or soluble infection postulated to be due to either marked induction of the insulin mRNA expression to increase insulin promoter activity, since insulin promoter activity per se was most significantly increased by triple infection (83). Another possibility is that the transcription factors are recruited to the insulin promoter region by MafA, and together with Pdx-1 and NeuroD1, this enables these transcription factors to exert strong synergistic effects and to markedly induce insulin gene expression (83). MafA, Pdx-1, and NeuroD1 also control glucose-regulated transcription of the insulin gene (2–5) (Table 1). MafA was found to interact functionally with Pdx-1 and NeuroD1 to promote synergistic activation of the insulin enhancer-driven reporter cell in non-β-cells (84) and shown to play a direct and principal role in insulin gene activation in β-cell lines (84). MafA, NeuroD1/E47, and Pdx-1 were recently reported to bind to the mouse insulin 2 promoter in a cyclic manner for about 10–15 min using MIN6 β-cells and ChIP assays (85). MafA appears to act downstream to Nkx 6.1 and is only found in terminally differentiated β-cells (82). This novel β-cell transcription factor may provide valuable information in the regulation of key β-cell genes and in the hierarchy of transcription factors in the mature β-cell. It also appears to have the potential for revealing important information that will aid our understanding of type 2 diabetes.

### NeuroD1

NeuroD1 (or β2) is a key transcription factor required for pancreatic development and endocrine cell differentiation. The human NeuroD1 gene (86) is identical to

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Table 1 Transcription factors regulating β-cell function.
the hamster β2 gene (87) that was cloned as a regulator of insulin gene expression. The ubiquitous E2A family of proteins, including E12 and E47, function either as homo- or heterodimers with tissue-specific class B bHLH proteins to bind and transactivate promoters via conserved sequence elements known as E-boxes (61). bHLH proteins are transcription factors involved in the determination of cell type and differentiation during development (88–92). NeuroD1 is a class B bHLH factor, expressed in pancreatic endocrine cells, the intestine, and brain (87, 88, 93). Mutations in NeuroD1 are linked to MODY6 in humans (94). One mutation is an Arg 111 missense mutation within the DNA-binding domain that diminishes the ability of NeuroD1 to bind to DNA and is associated with type 2 diabetes in the heterozygous state (94). Another mutation causes C-terminal truncation of NeuroD1 and leads to a more severe clinical phenotype of type 2 diabetes (94).

The heterodimer of NeuroD1 and ubiquitous bHLH protein, E47, binds insulin E-box complex with a high affinity and also activates the transcription of the insulin gene in β-cells (87). The heterodimer β2/E47 represents an islet-specific transcription factor that controls both insulin (65, 70, 87, 95, 96) and glucagon gene transcriptions (70) (Table 1). E47 overexpression inhibits glucagon gene expression, but activates insulin gene transcription suggesting that the β2/E47 ratio may be critical for the regulation of both insulin and glucagon gene expression (70). A mutation in p300, a co-activator of NeuroD1, abolished binding to NeuroD1 and destroyed the ability of p300 to activate insulin E-box-directed transcription in β-cells, showing the importance of p300 interactions with E-box activators in insulin gene transcription (96). Strong promoter activity of a 2 kb fragment containing 5’ upstream region of mouse NeuroD1 gene was found in the β-cell lines, β-HC3, β-HC9, and NIT-1 (97). Small heterodimer partner (SHP) is one of the unique members of the orphan nuclear receptor superfamily that lacks a conventional DNA-binding domain (98). SHP directly regulates the transcriptional activity of Hnf-4α (99–101) and acts as a co-activator of β2 via physical interaction (102). SHP represses β2 by competing with p300 for binding to β2 (102). β2/E47 specifically has been reported to bind and activate the upstream GK promoter in the islet (54). NeuroD1 binds and activates the promoter of Sur1 (103), which plays a major role in insulin activation upon glucose stimulation (104), and may be controlled at the transcriptional and post-transcriptional level by an increase in intracellular Ca2+ concentration (103). The principal role of NeuroD1 may be in the maintenance of insulin expression in mature β-cells by active repression of the somatostatin promoter (23).

Hnf-1α

Hnf-1α (or Tcf-1) has been characterized as the gene responsible for MODY3 (105), the most common form of MODY. The Hnf-1α and Hnf-1β homeodomain-containing transcription factors share > 90% sequence homology in their DNA-binding sites and can function as homo- or heterodimers (106). Hnf-1α has been proposed to regulate the genetic expression of insulin and GLUT-2 (107, 108) (Table 1). It has been reported that Hnf-1α recruits p300 to regulate expression of the human GLUT-2 gene (109). In the murine pancreas, Hnf-4α mRNA was found to be transcribed from a distant upstream promoter that is directly controlled by Hnf-1α (110). This was confirmed in the human pancreas where genetic evidence showed that Hnf-1α is a major regulator of Hnf-4α expression, acting directly through a distinct essential cis element in the Hnf-4α P2 promoter (111).

Hnf-3β

Hnf-3β (also known as also known as Foxa2, Lhx1, and Lim1) is believed to regulate transcription of the Nkx 6.1 gene (48) (Table 1). Hnf-6 transactivates Hnf-3β gene expression (112). In mice with pancreatic β-cell-specific deletion of Foxa2, the mRNA levels of GLUT-2, GK, and glutamate dehydrogenase did not significantly change (113). However, steady-state mRNA levels of the ATP-sensitive potassium channel, Sur1, and the inward rectifier potassium channel member, Kir6.2, were reduced by approximately 75% (113).

Pax 4

Pax 4 is one of the key transcription factors involved in the formation of β-cells during pancreatic development and islet cell differentiation. Mutations in the Pax 4 gene are associated with type 2 diabetes (114, 115), with heterozygous mutations in the Pax 4 gene linked to glucose intolerance in human carriers of these mutations (116). Four of the MODY transcription factors, Hnf-4α (MODY1), Hnf-1α (MODY3), Pdx-1 (MODY4), and NeuroD1 (MODY6) interact with the Pax 4 regulatory region, thus the simultaneous expression of these transcription factors are required for efficient Pax 4 transcription and may play a role in regulating tissue-specific regulation of Pax 4 (117). In a study using a rat glucagon-producing cell line, Pax 4 was shown to act as a repressor of glucagon gene expression (118). Furthermore, Pax 4 can inhibit the insulin promoter in the absence of Pax 6, suggesting an active repression mechanism of Pax 4 (118) (Table 1). Another study showed that Pax 4 binds with high affinity to Pax 6 target sites of the glucagon gene promoter suggesting a competition mechanism of transcriptional inhibition (119). The Pax 4 gene promoter contains several binding sites for Pax 4 itself, suggesting that Pax 4 inhibits its own expression i.e. a strong negative autoregulatory effect (120).
Pax 6

Pax 6 appears to be necessary for the correct execution of β-cell differentiation (36) and is essential for the normal expression of final differentiation markers such as insulin and GLUT-2 (25). Pax 6 regulates the C2 element of the insulin gene (121) (Table 1). GLUT-2 protein was not detected in the Pax 6-deficient pancreas suggesting that Pax 6 plays a role in GLUT-2 regulation (25). Pax 6 has been proposed to regulate Pdx-1 expression as Pax 6-binding sites have been detected in the Pdx-1 promoter (122). Pax 6-deficient islets maintained expression of PC1/3, a proinsulin processing enzyme, suggesting that the reduction in insulin in Pax 6-deficient mice may be due to direct changes in insulin expression and/or the inability of these cells to respond to elevated glucose levels because of reduced GLUT-2 expression (25).

Conclusions

There is increasing evidence that transcription factors act synergistically to achieve normal pancreatic development and function. Rapid progress has been made elucidating regulatory mechanisms of key β-cell genes by transcription factors. Improving our knowledge of these important transcription factors, establishing their hierarchy, finding novel transcription factors, and ultimately regulating their expression to ensure glucose homeostasis may be the key to prevent or correct β-cell dysfunction. Novel cofactors (p300 and Bridge-1) and fusion proteins (VP16) have already been identified and demonstrated to enhance expression of Pdx-1. Therapeutic agents like the incretin, glucagon-like peptide-1 (GLP-1), and the more potent GLP-1 receptor agonist, exenatide (or exendin-4), are promising as they improve Pdx-1 expression (72, 123, 124). Novel drugs targeted against key transcription factors could restore β-cell function in diabetes patients.

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