REVIEW

A focus on the role of Pax4 in mature pancreatic islet β -cell expansion and survival in health and disease

Thierry Brun and Benoit R Gauthier

Department of Cell Physiology and Metabolism, University Medical Centre, 1211 Geneva 4, Switzerland

(Correspondence should be addressed to T Brun; Email: thierry.brun@medecine.unige.ch; B R Gauthier; Email: benoit.gauthier@medecine.unige.ch)

Abstract

Blood glucose homeostasis is achieved by the regulation of insulin and glucagon secretion from the pancreatic islet β - and α -cells. Diabetes mellitus, which comprises a heterogeneous group of hyperglycaemic disorders, results mainly from inadequate mass and function of islet β -cells. Autoimmune destruction of β -cells causes type 1 diabetes, while type 2 is characterized by impaired insulin secretion and is often associated with diminished insulin action on its target tissues. Interestingly, similar to type 1 diabetes, a gradual loss of β -cell mass is observed in type 2 diabetes often requiring insulin therapy. Understanding the molecular mechanism that governs β -cell mass plasticity may provide a means to develop strategies to counteract β -cell death while increasing replication. Of particular interest is the islet-specific transcription factor paired box4 (Pax4) that was previously shown to be indispensable for the establishment of the β -cell lineage during development. However, recent accumulating evidence now suggest that Pax4 is also crucial for mature β -cell expansion and survival in response to physiological cues and that mutations or polymorphisms are associated with both type 1 and type 2 diabetes. In contrast, aberrant expression of Pax4 confers protection against apoptosis to insulinomas, whereas it promotes cell growth in lymphocytes. This review summarizes promising new published results supporting the important function of Pax4 in mature islet β -cell physiology and its contribution to pathophysiology when deregulated.

Journal of Molecular Endocrinology (2008) **40**, 37–45

Introduction: diabetes, obesity and pancreatic islet plasticity

Plasma glucose levels are regulated by the action of insulin, a hormone that is produced and secreted by the pancreatic islet β -cells in response to nutrients. Diabetes mellitus, which comprises a heterogeneous group of hyperglycaemic disorders, results from inadequate mass and function of β -cells (Prentki & Nolan 2006). In 2007, over 200 million people were diagnosed with diabetes mellitus and it is now considered one of the most common non-communicable diseases in the world causing 5% of all deaths per year (www.eatlas.idf.org). Alarming, the World Health Organization predicts that this will reach epidemic proportions with at least 366 million people afflicted with the disease by 2030 (www.who.int/dietphysical-activity/publications/facts/diabetes/en). Diabetes mellitus was recently reclassified into three main entities: 1) type 1 diabetes which is linked to selective autoimmune destruction of pancreatic β -cells, 2) type 2 diabetes which is a severe disease of intermediary metabolism usually caused by both β -cell dysfunction

and resistance to the biological actions of insulin on its main target tissues (liver, muscle and adipose) and 3) gestational diabetes mellitus (GDM) which is characterized by glucose intolerance with onset or first recognition during pregnancy (Bell & Polonsky 2001, Saltiel & Kahn 2001, Expert Committee on the Diagnosis and Classification of Diabetes Mellitus 2003, Stumvoll *et al.* 2005, Shaat & Groop 2007). Long-term complications of diabetes include retinopathy and nephropathy as well as cardiovascular and cerebrovascular symptoms. Current therapy for type 2 diabetes and GDM include modification of lifestyle, such as diet and exercise, and the use of pharmacological agents that stimulate insulin secretion, decrease hepatic glucose production and increase sensitivity of target tissues to insulin. Nonetheless, often type 2 diabetic patients, like type 1 diabetics, require insulin therapy (Donath & Halban 2004).

The phenomenal rise in the incidence of diabetes is predominantly attributable to its close association with development of obesity. Indeed, ~60% of all cases of type 2 diabetes are directly linked to overweight due to shifting dietary habits and increasingly sedentary

lifestyles of humans (Yach *et al.* 2006). Studies in animal models have demonstrated that obesity-associated insulin resistance as well as increased insulin requirements during pregnancy is matched by a corresponding stimulation in insulin output through β -cell hyperplasia and hypertrophy. For instance, rat pancreatic β -cell numbers transiently increase by 50% during pregnancy before returning to normal levels *post partum* (Scaglia *et al.* 1995). This β -cell plasticity reaches remarkable levels in Zucker diabetic fatty (*fa/fa*) rats in which a fourfold increase in islet mass is observed with progression of obesity (Unger 2005). A similar increase in islet volume was shown in *ob/ob* mice when compared with control animals (Bock *et al.* 2003). The expansion of β -cell mass is also observed in human obesity as well as in pregnancy, the latter most likely mediated by increased circulating placental lactogen and prolactin (Kloppel *et al.* 1985, Brelje *et al.* 1993, Sorenson & Brelje 1997, Butler *et al.* 2003). Interestingly, up to 20% of obese individuals develop type 2 diabetes probably caused by a defective β -cell adaptation due to increased sensitivity to harmful environmental factors such as free fatty acids combined with predisposing genetic factors (Kashyap *et al.* 2003). The latter emphasizes that overall β -cell mass adaptation in response to physiological as well as to pathophysiological conditions is not only governed by the generation of new insulin-producing cells but also by susceptibility to cell death. In the extreme such as in type 1 diabetes, autoimmune-mediated cell destruction is greater than the rate of regeneration leading to hyperglycaemia and invariably requiring insulin therapy. However, in rare cases, spontaneous remission characterized by increased C-peptide secretion has been reported in type 1 diabetic patients, clearly indicating the capacity of β -cell regeneration to prevail over autoimmune annihilation (Bonfanti *et al.* 1998). Fundamental mechanisms governing β -cell replenishment remain to be identified. In this review, we discuss the potential role of the transcription factor paired box4 (Pax4) as a key player orchestrating the gene network governing β -cell mass expansion and survival under both physiological and pathophysiological conditions.

Defining the regenerative unit of the pancreatic islet: the β -cell at centre stage

The search for the regenerative β -cell or precursor pool that responds to metabolic demands has proved more challenging and controversial than anticipated (Bock 2004, Bonner-Weir *et al.* 2004, Bonner-Weir & Sharma 2006). Indeed, depending on the experimental model and degree of injury inflicted to the pancreas, new β -cells were shown to be generated by neogenesis of ductal epithelium cells (Peshavaria *et al.* 2006), by

transdifferentiation of acinar cells (Lipsett & Finegood 2002, Minami *et al.* 2005) and from intra-islet nestin-positive precursor cells (Zulewski *et al.* 2001). Bone marrow stem cells also appear to indirectly contribute to islet regeneration by promoting proliferation of resident islet cells (Ianus *et al.* 2003, Hasegawa *et al.* 2007). Alternatively, lineage-tracing studies elegantly demonstrated that pre-existing mouse adult pancreatic β -cells, rather than specialized progenitors, were the major source of new insulin-producing cells during adult life and after pancreatectomy (Dor *et al.* 2004, Nir *et al.* 2007, Teta *et al.* 2007). Furthermore, it was recently reported that all β -cells contributed equally to islet growth and maintenance (Brennand *et al.* 2007). Thus, similar to other organs such as the liver, all of the aforementioned sources most likely contribute to regeneration according to the severity of the injury (Corcelle *et al.* 2006). However, new evidence showing sustained β -cell apoptosis in human patients with long-standing type 1 diabetes along with the demonstration of β -cell mitogenesis in a diagnosed type 1 diabetic patient clearly argues in favour of β -cell replication as the predominant mechanism of *in vivo* islet regeneration in humans (Meier *et al.* 2005, 2006b). Consistent with this premise, β -cell proliferation was observed in the vicinity of intrapancreatic gastrinomas in humans (Meier *et al.* 2006a). Therefore, harnessing key regulatory factors responsible for mature β -cell replication would certainly facilitate development of a regenerative therapy for the treatment of diabetes.

Pax4 as a master regulator of islet development

Pax genes encode a family of transcription factors that are key regulators of tissue development and cellular differentiation in embryos acting to promote cell proliferation, migration and survival. Pax proteins comprise nine members divided into four groups based on the specific assembly of three structural domains: the paired domain, the homeodomain and the octapeptide (Lang *et al.* 2007). DNA binding activity is conferred by either the paired or homeodomain. Pax6 and Pax4, which form subgroup IV characterized by the absence of the octapeptide, are predominantly expressed in the pancreatic islet as well as in the central nervous system for Pax6. Pax4 was shown to be essential for the generation of islet cell progenitors and subsequent β - and somatostatin-producing δ -cell maturation, while Pax6 was found to be crucial for α -cell fate lineage during embryogenesis (Sosa-Pineda *et al.* 1997, St-Onge *et al.* 1997, Greenwood *et al.* 2006). Interestingly, Pax4 expression is predominantly restricted to β - and δ -cells, whereas Pax6 is ubiquitously expressed in all islet cell types (Sosa-Pineda *et al.* 1997, St-Onge *et al.* 1997). During development, the

Pax4 transcript is initially detected in the pancreatic bud at embryonic day (E) 9.5, becoming maximal at E13.5–15.5 and thereafter declining to low expression levels. Lineage-tracing studies performed on transgenic mice bearing a *pax4* promoter/cre recombinase gene DNA cassette confirmed confinement of the Pax4 expression domain exclusively to endocrine cells of the islets (Greenwood *et al.* 2006). Consistent with its tissue and cell-specific expression pattern, targeted disruption of the *pax4* gene in mice results in the absence of mature β - and δ -cells with a commensurate increase in the α -cells (Sosa-Pineda *et al.* 1997, Wang *et al.* 2004). This increase was attributed to the α -cell-specific transcription factor Arx that is repressed by Pax4 during development (Collombat *et al.* 2003, 2005). Substantiating the latter finding, conditional expression of Arx in either embryonic or adult β -cells was recently found to convert β -cells into α - and pancreatic polypeptide (PP)-producing cells (Collombat *et al.* 2007). In mouse mutant Pax4 embryos, scattered insulin-staining cells are apparent at early stages of development (E8.5–9), indicating that Pax4 expression is most likely not mandatory for the generation of β -cell precursors. However, the strong induction of *pax4* gene expression between E13.5 and E15.5 indicates that Pax4 is critical for sustaining the phenotype as well as the proliferation and/or survival of these early committed insulin-producing cells (Sosa-Pineda *et al.* 1997). Consistent with this premise, the time interval between E13.5 and E15.5 corresponds to a period of massive proliferation and differentiation of β -cells, the so-called secondary transition phase (Pictet & Rutter 1972).

Pax4 as a key player coordinating mature islet β -cell plasticity

Expression of Pax4 in mature islets has been a matter of debate nourished by the inability to detect low endogenous transcript levels as well as the lack of specific antibodies (Sosa-Pineda *et al.* 1997, Smith *et al.* 1999, 2000). The failure to generate Pax4 antibodies most likely stems from its high homology to other Pax members, rendering it difficult to delineate specific

antigenic peptide to Pax4. However, novel approaches in antibody production may provide a means to circumvent this caveat in the close future. Nonetheless, recent studies, including ours, have provided evidence for the expression of the Pax4 transcript in adult human, rat and mouse pancreatic islets (Zhang *et al.* 2001, Heremans *et al.* 2002, Kojima *et al.* 2003, Zalzman *et al.* 2003, Brun *et al.* 2004, Theis *et al.* 2004). Furthermore, several human genetic studies have highlighted the importance of Pax4 on islet physiology (Table 1). Indeed, mutations in the *pax4* gene were shown to be associated with type 2 diabetes in the Japanese population as well as in Afro-Americans (Shimajiri *et al.* 2001, 2003, Kanatsuka *et al.* 2002, Mauvais-Jarvis *et al.* 2004, Tokuyama *et al.* 2006). More recently, two mutations in Pax4 were also linked to a subform of type 2 diabetes, maturity onset diabetes of the young (MODY) in the Thai population (Plengvidhya *et al.* 2007). In addition, two haplotypes of Pax4 have been correlated with type 1 diabetes in Scandinavian families (Holm *et al.* 2004). Consistent with the latter, an independent study identified two variants of the *pax4* gene that were differentially distributed among normal individuals and type 1 diabetic children in the Swiss and German populations. The Pax4C variant was frequent in type 1 diabetic children (73%) and rare in the control population (32%). In contrast, the heterozygote Pax4A/C combination was prevalent in controls (62%) and in islet antibody-positive subjects (73.6%) that did not develop diabetes but rare in patients that became diabetic (17.5%; Biason-Lauber *et al.* 2005). However, no correlation between this polymorphism and type 1 diabetes could be established in Finnish, Hungarian and UK populations (Hermann *et al.* 2005, Martin *et al.* 2006). Discrepancies in these studies most likely arise from genetic heterogeneity or population differences in gene–gene as well as gene–environment interactions. As the common denominator between type 1 and type 2 diabetes is a gradual destruction of β -cells, we have recently proposed the working hypothesis that Pax4 is likely critical for the expansion as well as the survival of β -cell mass. Dysfunction in the *pax4* gene during

Table 1 Mutations and polymorphisms in the paired box4 (*pax4*) genes that are associated with diabetes in specific populations

	Diabetic phenotype	Population	Reference
Nucleotide change^a			
C109T	Ketosis-prone diabetes	Afro-American	Mauvais-Jarvis <i>et al.</i> (2004)
C363T	Type 2	Japanese	Shimajiri <i>et al.</i> (2001, 2003)
C397T	Ketosis-prone diabetes	Afro-American	Mauvais-Jarvis <i>et al.</i> (2004)
C492T	Type 2, MODY	Thai	Plengvidhya <i>et al.</i> (2007)
A1168C	Type 1	Swiss/German	Biason-Lauber <i>et al.</i> (2005)
Nucleotide 1 (intron 7) G/A	Type 2, MODY	Thai	Plengvidhya <i>et al.</i> (2007)

^aThe nucleotide position is relative to the transcriptional initiation site defined as +1.

development and/or in mature islets would contribute to increased susceptibility to apoptosis coincident with decreased cell proliferation leading to a gradual loss of β -cells and ultimately to diabetes in adulthood.

To understand the mechanisms that control *pax4* gene expression and thereby impacting its function in mature β -cells, several studies have mapped and characterized the regulatory regions of both the human and mouse *pax4* genes (Smith *et al.* 2000, Xu & Murphy 2000, Brink *et al.* 2001). Sequence deletion analysis revealed that the optimal mouse *pax4* gene promoter region required for pancreatic islet expression was found within the first 400 bp upstream of the transcription initiation site. This region shares 88% similarity with the human sequence (Brink *et al.* 2001, Brink & Gruss 2003). Pax4 expression was shown to be dependent on the concerted action of the key pancreatic transcription factors Pan1, Beta2/NeuroD, hepatic nuclear factor (HNF)-1 α , HNF-4 α , pancreatic duodenal homeobox (Pdx)1 and E47/E12 interacting with the proximal promoter (Smith *et al.* 2000, 2004, Kanno *et al.* 2006). A further increase in transcription was observed when Beta2/NeuroD was substituted by the early pancreatic committing transcription factor Ngn3 (Smith *et al.* 2000, 2004). The action of Beta2/NeuroD, HNF-1 α , HNF-4 α and Pdx1 on Pax4 gene expression is interesting, as mutations in these transcription factors have been associated with MODY (Hani *et al.* 1999, Fajans *et al.* 2001, Love-Gregory *et al.* 2004). Thus, Pax4 may participate as a downstream target of these -diabetes-linked transcription factors and most likely aggravates the MODY phenotype by its inability to promote cell replication and to protect against apoptosis.

Evidence for the implication of Pax4 in β -cell adaptation in response to physiological cues emerges from studies performed with the proinflammatory cytokine interleukin (IL)-1 β . Indeed, low concentration of IL-1 β stimulates *pax4* gene expression, correlating with increased human β -cell proliferation induced by the cytokine. In contrast, higher concentrations inhibited Pax4 mRNA levels with a concomitant increase in apoptosis (Maedler *et al.* 2006). This bimodal effect was shown to involve the Fas signalling pathway, which can switch from a mitogenic signal to induction of apoptosis when the FLICE-inhibitory protein (FLIP) is inactive (Maedler *et al.* 2006). This pathway was also found to be important in sustaining the secretory function of β -cells, as Fas-deficient mice exhibited impaired glucose-induced insulin secretion (Schumann *et al.* 2007). It would therefore appear that Pax4 is an integrated factor of the Fas/FLIP signal transduction pathway, which relays either the positive or negative effects of IL-1 β pending levels of the cytokine. The latter most likely mimics the *in vivo* conditions observed in both type 1 and type 2 diabetic patients (Donath & Halban 2004). More recently, the cytokine ciliary neurotrophic factor was also found to

significantly increase Pax4 mRNA levels and to promote the survival of neonatal rat islets (Rezende *et al.* 2007), substantiating the notion that Pax4 is an important mediator of cytokine signalling pathways and a regulator of β -cell plasticity in mature islets.

Consistent with this premise, we previously demonstrated that Pax4 is indeed a key regulator of β -cell mass (Brun *et al.* 2004). We found that activin A and betacellulin stimulated *pax4* gene expression with a concomitant increase in rat islet β -cell replication. Wortmannin suppressed betacellulin-induced Pax4 expression, implicating the PI3 kinase signalling pathway. Our results corroborated previous studies demonstrating that activin A could induce *pax4* gene expression in pancreatic cell lines (Ueda 2000) and stimulate growth and differentiation of human foetal pancreatic cells in combination with betacellulin (Demeterco *et al.* 2000). Furthermore, endogenous Pax4 mRNA levels were also induced in human islets by glucose, activin A and betacellulin. In addition, the incretin glucagon-like peptide (GLP)-1, a new therapeutic agent for the treatment of diabetes, which has been shown to increase β -cell mass in mouse and rat pancreas (Xu *et al.* 1999, Stoffers *et al.* 2000), also induced Pax4 expression in human islets in the presence of glucose (Brun *et al.* In press). Overexpression of mouse Pax4 (mPax4) in rat islets resulted in the induction of the c-myc/Id2 proliferation pathway and of the anti apoptotic gene *bcl-xl*. Two independent studies have demonstrated that forced expression of c-myc requires concomitant induction of Bcl-xL to promote β -cell proliferation rather than apoptosis (Pelengaris *et al.* 2002, Cheung *et al.* 2004). Taken together, these studies suggest that Pax4 coordinates the activation of both genes in order to promote cell survival and mitogenesis (Fig. 1). Overexpression of mPax4 in human islets also induced proliferation and conferred protection against cytokine-mediated apoptosis, whereas the diabetes-linked mutant identified in the Japanese population (R121W; Shimajiri *et al.* 2001) was less efficient (Brun *et al.* 2004). An elegant study recently demonstrated that the mouse Pax4 protein could permeate islet cells as well as Min6 cells through a novel protein transduction domain located in the paired domain and confer protection against tumour necrosis factor α -induced apoptosis. Furthermore, both c-myc and Bcl-xL expression were increased in transduced cells, indicating that similar proliferative and survival pathways are implicated in both rodent and human islets (Lu *et al.* 2007). These studies pave the way to exciting new therapeutic strategies using Pax4 transduction to improve islet cell survival in culture prior to transplantation in type 1 diabetic patients (Shapiro *et al.* 2006). However, an immediate challenge is to identify additional downstream Pax4 target genes that may also prove useful for therapeutics.

Figure 1 Pax4 controls the expression of the c-myc/Id2 proliferation pathway as well as the expression of the anti-apoptotic gene *bcl-xl*. Physiological cues activate Pax4, which will then increase c-myc and Bcl-xL gene transcription. c-myc will promote *Id2* gene expression and activate the cell-cycle replication programme. Bcl-xL-increased expression will promote survival by preventing mitochondria from initiating the apoptotic programme.

Pax4 as an oncogene

Pax gene family members have also been associated with cancer development (Robson *et al.* 2006). Consistent with the latter, elevated expression levels of Pax4 are found in both human and rat insulinomas when compared with mature β -cells (Miyamoto *et al.* 2001, Brun *et al.* 2007). Furthermore, a novel Pax4 spliced variant, lacking the carboxy-terminal (C-terminal) end of the protein involved in mediating repression of gene transcription, was also identified in human insulinomas (Miyamoto *et al.* 2001). Ablation of this C-terminal end may therefore confer tumorigenicity. In contrast, its presence may result in the interaction with cofactors and/or post-translational modifications of Pax4 that limit cell proliferation. Precedents for this premise were demonstrated for other Pax proteins such as Pax3, 5 and 8 that interact with the tumour suppressor pRb and regulate cell cycle (Buckingham & Relaix 2007). To address the potential oncogenic function of Pax4, the latter was suppressed by RNA interference in the insulinoma INS-1E cell line. Inhibition of Pax4 provoked spontaneous apoptosis and further sensitized cells to cytokine-mediated cell death without altering cell proliferation, suggesting that the transcription factor acts as a survival gene rather than promoting replication in this cell line (Brun *et al.* 2007). The latter study highlights potential pitfalls of using transformed cell lines to delineate the

functional role of factors implicated in the regulation of cell replication and apoptosis. Further evidence for the potential mitogenic function of Pax4 in β -cells was provided by the recent discovery that primary lymphoma and haematologic malignancies expressed high levels of Pax4 that correlated with tumorigenesis. Aberrant expression of the transcription factor in these cells which normally do not express Pax4 was caused by the demethylation of CpG islands with subsequent promoter activation (Li *et al.* 2006). Accordingly, we have recently demonstrated that similar epigenetic modifications are imposed on the *pax4* gene in mature pancreatic islets, thus refraining high expression levels when compared with INS-1E cells (Brun *et al.* In press). Perturbation in the expression of other closely related members of the Pax family can also lead to cancer (Robson *et al.* 2006). Increased levels of Pax3 were observed in human tumours of neural crest origin, while Pax2 expression was shown to be indispensable for survival of ovarian and bladder cancer cell lines (Muratovska *et al.* 2003, Parker *et al.* 2004). Pax5 was also identified as a key factor for the maintenance of the tumorigenic phenotype of neuroblastoma (Baumann Kubetzko *et al.* 2004). Similar to Pax4, suppression of Pax2 or Pax7 in tumour cell lines resulted in programmed cell death (Margue *et al.* 2000, Muratovska *et al.* 2003). Pax4 may thus qualify as a potential oncogene joining other Pax family members as an important transcriptional regulator involved in development and cancer (Robson *et al.* 2006).

Conclusion: Pax4 a gene of all trades in islet plasticity

The alarming spread of obesity worldwide has entailed a dramatic increase in type 2 diabetes. The mechanism is considered to involve inadequate insulin secretion to meet increasing insulin requirements due to obesity-related resistance to the hormone in the subgroup of individual with inherited susceptibility for type 2 diabetes. The genetic defects are thought to include inappropriate β -cell proliferative capacity and increased susceptibility to apoptosis. Insufficient regeneration of destroyed β -cells in the autoimmune process of type 1 diabetes may also be determined by genetic predisposition. The transcription factor Pax4 could be a common denominator of β -cell growth and survival in the two diabetic conditions. Accordingly, we would like to propose the working model outlined in Fig. 2 to integrate the various functions that Pax4 may have on β -cell physiology and pathophysiology (Fig. 2). Under physiological conditions in which β -cell mass is called upon to compensate for increasing insulin demands such as in pregnancy or obesity, Pax4 acts as an *adaptive gene* that permits β -cell replication. In contrast,

Figure 2 Proposed model of Pax4 action in mature β -cell health and disease. Under physiological conditions, transient stimulation of Pax4 by mitogens such as activin A and betacellulin or by low concentrations of cytokines evokes a burst in β -cell proliferation (depicted as black circles) as well as increasing survival. The net result is the increased insulin output to compensate for demands (upper panel). However, chronic exposure to cytokines or potentially to hyperglycaemia or hyperlipidaemia results in the inhibition of *pax4* gene expression with a concomitant decrease in cell replication and increase susceptibility to apoptosis (lower left panel). Dysfunction in Pax4 due to mutations or polymorphisms in the gene would have similar consequences. The gradual decline in β -cell mass (represented by open circles) over time would ultimately lead to either type 1 or type 2 diabetes. In contrast, alterations in Pax4 that increase activity confer resistance to apoptosis in insulinomas or promote mitogenesis in lymphomas, resulting in overall augmentation in cell mass (lower right panel; black circles, new cells).

mutations and polymorphisms that weaken Pax4 transcriptional activity would initially contribute to reduction in β -cell survival and/or proliferation during pancreatic development. Subsequently, the inability of β -cells to be replenished in mature islets coincident with unabated apoptosis would result in the gradual loss of insulin-producing cells. This will cause relative insulin deficiency, hyperglycaemia and ultimately diabetes. Therefore, Pax4 would qualify

as a *survival gene*. In contrast, gain-of-function mutations conferring resistance against apoptosis would provide a selective advantage for tumour development. In these circumstances, Pax4 would be considered an *oncogene*. Validation of this working model will ultimately require the generation of transgenic mice that can specifically and conditionally express either Pax4 wild-type or diabetes-associated variants in islet β -cells.

Acknowledgements

We would like to acknowledge past and present members of the laboratory as well as the numerous collaborators who have contributed to the achievement of these studies. Special thanks to Drs K Maedler, M Donath, B Thorens, P Halban and A Lauber-Biason for their fruitful discussion. We are indebted to Professor Claes B Wollheim (C B W) for his guidance and support in this project. This work was funded by the Swiss National Science Foundation (#3100A0-107682/1 to B R G). The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

References

- Baumann Kubetzko FB, Di Paolo C, Maag C, Meier R, Schafer BW, Betts DR, Stahl RA & Himmelman A 2004 The PAX5 oncogene is expressed in N-type neuroblastoma cells and increases tumorigenicity of a S-type cell line. *Carcinogenesis* **25** 1839–1846.
- Bell GI & Polonsky KS 2001 Diabetes mellitus and genetically programmed defects in β -cell function. *Nature* **414** 788–791.
- Biason-Lauber A, Boehm B, Lang-Muritano M, Gauthier BR, Brun T, Wollheim CB & Schoenle EJ 2005 Association of childhood diabetes mellitus with a genomic variant of Pax4: possible link to β cell regenerative capacity. *Diabetologia* **48** 900–905.
- Bock T 2004 The source(s) for new pancreatic β cells in adult life. *BioEssays* **26** 1156–1159.
- Bock T, Pakkenberg B & Buschard K 2003 Increased islet volume but unchanged islet number in ob/ob mice. *Diabetes* **52** 1716–1722.
- Bonfanti R, Boggetti E, Meschi F, Brunelli A, Riva MC, Pastore MR, Calori G & Chiumello G 1998 Residual β -cell function and spontaneous clinical remission in type 1 diabetes mellitus: the role of puberty. *Acta Diabetologica* **35** 91–95.
- Bonner-Weir S & Sharma A 2006 Are there pancreatic progenitor cells from which new islets form after birth? *Nature Clinical Practice. Endocrinology and Metabolism* **2** 240–241.
- Bonner-Weir S, Toschi E, Inada A, Reitz P, Fonseca SY, Aye T & Sharma A 2004 The pancreatic ductal epithelium serves as a potential pool of progenitor cells. *Pediatric Diabetes* **5** 16–22.
- Brelje TC, Scharp DW, Lacy PE, Ogren L, Talamantes F, Robertson M, Friesen HG & Sorenson RL 1993 Effect of homologous placental lactogens, prolactins, and growth hormones on islet B-cell division and insulin secretion in rat, mouse, and human islets: implication for placental lactogen regulation of islet function during pregnancy. *Endocrinology* **132** 879–887.
- Brennan K, Huangfu D & Melton D 2007 All β cells contribute equally to islet growth and maintenance. *PLoS Biology* **5** 1520–1529.
- Brink C & Gruss P 2003 DNA sequence motifs conserved in endocrine promoters are essential for Pax4 expression. *Developmental Dynamics* **228** 617–622.
- Brink C, Chowdhury K & Gruss P 2001 Pax4 regulatory elements mediate beta cell specific expression in the pancreas. *Mechanisms of Development* **100** 37–43.
- Brun T, Franklin I, St-Onge L, Biason-Lauber A, Schoenle E, Wollheim CB & Gauthier BR 2004 The diabetes-linked transcription factor Pax4 promotes β -cell proliferation and survival in rat and human islets. *Journal of Cell Biology* **167** 1123–1135.
- Brun T, Duhamel DL, Hu He KH, Wollheim CB & Gauthier BR 2007 The transcription factor Pax4 acts as a survival gene in the insulinoma INS1E cells. *Oncogene* **26** 4261–4271.
- Brun T, Hu He KH, Lupi R, Boehm B, Wojtuszczyk A, Sauter N, Donath M, Marchetti P, Maedler K & Gauthier BR In press.
- The diabetes-linked transcription factor Pax4 is expressed in human pancreatic islets and is activated by mitogens and GLP-1 Human Molecular Genetics In press.
- Buckingham M & Relaix F 2007 The role of Pax genes in the development of tissues and organs: Pax3 and Pax7 regulate muscle progenitor cell functions. *Annual Review of Cell and Developmental Biology* **23** 645–673.
- Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA & Butler PC 2003 β -cell deficit and increased β -cell apoptosis in humans with type 2 diabetes. *Diabetes* **52** 102–110.
- Cheung WC, Kim JS, Linden M, Peng L, Van Ness B, Polakiewicz RD & Janz S 2004 Novel targeted deregulation of c-Myc cooperates with Bcl-X(L) to cause plasma cell neoplasms in mice. *Journal of Clinical Investigation* **113** 1763–1773.
- Collombat P, Mansouri A, Hecksher-Sorensen J, Serup P, Krull J, Gradwohl G & Gruss P 2003 Opposing actions of Arx and Pax4 in endocrine pancreas development. *Genes and Development* **17** 2591–2603.
- Collombat P, Hecksher-Sorensen J, Broccoli V, Krull J, Ponte I, Mundiger T, Smith J, Gruss P, Serup P & Mansouri A 2005 The simultaneous loss of Arx and Pax4 genes promotes a somatostatin-producing cell fate specification at the expense of the α - and β -cell lineages in the mouse endocrine pancreas. *Development* **132** 2969–2980.
- Collombat P, Hecksher-Sorensen J, Krull J, Berger J, Riedel D, Herrera PL, Serup P & Mansouri A 2007 Embryonic endocrine pancreas and - mature β cells acquire α and PP cell phenotypes upon Arx misexpression. *Journal of Clinical Investigation* **117** 961–970.
- Corcelle V, Stieger B, Gjinovci A, Wollheim CB & Gauthier BR 2006 Characterization of two distinct liver progenitor cell subpopulations of haematopoietic and hepatic origins. *Experimental Cell Research* **312** 2826–2836.
- Demeterco C, Beattie GM, Dib SA, Lopez AD & Hayek A 2000 A role for activin A and betacellulin in human fetal pancreatic cell differentiation and growth. *Journal of Clinical Endocrinology and Metabolism* **85** 3892–3896.
- Donath MY & Halban PA 2004 Decreased β -cell mass in diabetes: significance, mechanisms and therapeutic implications. *Diabetologia* **47** 581–589.
- Dor Y, Brown J, Martinez OI & Melton DA 2004 Adult pancreatic β -cells are formed by self-duplication rather than stem-cell differentiation. *Nature* **429** 41–46.
- Expert Committee on the Diagnosis and Classification of Diabetes Mellitus 2003 Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care* **26** S5–S20.
- Fajans SS, Bell GI & Polonsky KS 2001 Molecular mechanisms and clinical pathophysiology of maturity-onset diabetes of the young. *New England Journal of Medicine* **345** 971–980.
- Greenwood AL, Li S, Jones K & Melton DA 2006 Notch signaling reveals developmental plasticity of Pax4(+) pancreatic endocrine progenitors and shunts them to a duct fate. *Mechanisms of Development* **124** 97–107.
- Hani EH, Stoffers DA, Chevre JC, Durand E, Stanojevic V, Dina C, Habener JF & Froguel P 1999 Defective mutations in the insulin promoter factor-1 (IPF-1) gene in late-onset type 2 diabetes mellitus. *Journal of Clinical Investigation* **104** R41–R48.
- Hasegawa Y, Ogihara T, Yamada T, Ishigaki Y, Imai J, Uno K, Gao J, Kaneko K, Ishihara H, Sasano H *et al.* 2007 Bone marrow (BM) transplantation promotes β -cell regeneration after acute injury through BM cell mobilization. *Endocrinology* **148** 2006–2015.
- Heremans Y, Van De Castele M, in't Veld P, Gradwohl G, Serup P, Madsen O, Pipeleers D & Heimberg H 2002 Recapitulation of embryonic neuroendocrine differentiation in adult human pancreatic duct cells expressing neurogenin 3. *Journal of Cell Biology* **159** 303–312.
- Hermann R, Mantere J, Lipponen K, Veijola R, Soltesz G, Otonkoski T, Simell O, Knip M & Ilonen J 2005 Lack of association of PAX4 gene with type 1 diabetes in the Finnish and Hungarian populations. *Diabetes* **54** 2816–2819.

- Holm P, Rydlander B, Luthman H & Kockum I 2004 Interaction and association analysis of a type 1 diabetes susceptibility locus on chromosome 5q11-q13 and the 7q32 chromosomal region in Scandinavian families. *Diabetes* **53** 1584–1591.
- Ianus A, Holz G, Theise N & Hussain M 2003 *In vivo* derivation of glucose-competent pancreatic endocrine cells from bone marrow without evidence of cell fusion. *Journal of Clinical Investigation* **111** 843–850.
- Kanatsuka A, Tokuyama Y, Nozaki O, Matsui K & Egashira T 2002 β -cell dysfunction in late-onset diabetic subjects carrying homozygous mutation in transcription factors NeuroD1 and Pax4. *Metabolism* **51** 1161–1165.
- Kanno R, Ogihara T, Igarashi Y, Tanaka Y, Smith SB, Kojima I, German MS, Kawamori R & Watada H 2006 Activin A-induced expression of PAX4 in AR42J-B13 cells involves the increase in transactivation of E47/E12. *Biochimica et Biophysica Acta* **1759** 44–50.
- Kashyap S, Belfort R, Gastaldelli A, Pratipanawatr T, Berria R, Pratipanawatr W, Bajaj M, Mandarino L, DeFronzo R & Cusi K 2003 A sustained increase in plasma free fatty acids impairs insulin secretion in nondiabetic subjects genetically predisposed to develop type 2 diabetes. *Diabetes* **52** 2461–2474.
- Kloppel G, Lohr M, Habich K, Oberholzer M & Heitz PU 1985 Islet pathology and the pathogenesis of type 1 and type 2 diabetes mellitus revisited. *Survey and Synthesis of Pathology Research* **4** 110–125.
- Kojima H, Fujimiya M, Matsumura K, Younan P, Imaeda H, Maeda M & Chan L 2003 NeuroD-betacellulin gene therapy induces islet neogenesis in the liver and reverses diabetes in mice. *Nature Medicine* **9** 596–603.
- Lang D, Powell SK, Plummer RS, Young KP & Ruggeri BA 2007 PAX genes: roles in development, pathophysiology, and cancer. *Biochemical Pharmacology* **73** 1–14.
- Li Y, Nagai H, Ohno T, Ohashi H, Murohara T, Saito H & Kinoshita T 2006 Aberrant DNA demethylation in promoter region and aberrant expression of mRNA of PAX4 gene in hematologic malignancies. *Leukemia Research* **30** 1547–1553.
- Lipsett M & Finegood DT 2002 β -cell neogenesis during prolonged hyperglycemia in rats. *Diabetes* **51** 1834–1841.
- Love-Gregory LD, Wasson J, Ma J, Jin CH, Glaser B, Suarez BK & Permutt MA 2004 A common polymorphism in the upstream promoter region of the hepatocyte nuclear factor-4 α gene on chromosome 20q is associated with type 2 diabetes and appears to contribute to the evidence for linkage in an Ashkenazi Jewish population. *Diabetes* **53** 1134–1140.
- Lu J, Li G, Lan MS, Zhang S, Fan W, Wang H & Lu D 2007 Pax4 paired domain mediates direct protein transduction into mammalian cells. *Endocrinology* **148** 5558–5565.
- Maedler K, Schumann DM, Sauter N, Ellingsgaard H, Bosco D, Baertschiger R, Iwakura Y, Oberholzer J, Wollheim CB, Gauthier BR *et al.* 2006 Low concentration of interleukin-1 β induces FLICE-inhibitory protein-mediated β -cell proliferation in human pancreatic islets. *Diabetes* **55** 2713–2722.
- Margue CM, Bernasconi M, Barr FG & Schafer BW 2000 Transcriptional modulation of the anti-apoptotic protein BCL-XL by the paired box transcription factors PAX3 and PAX3/FKHR. *Oncogene* **19** 2921–2929.
- Martin RJ, Savage DA, Carson DJ, Maxwell AP & Patterson CC 2006 The PAX4 gene variant A1168C is not associated with early onset type 1 diabetes in a UK population. *Diabetic Medicine* **23** 927–928.
- Mauvais-Jarvis F, Smith SB, Le May C, Leal SM, Gautier JF, Molokhia M, Riveline JP, Rajan AS, Kevorkian JP, Zhang S *et al.* 2004 PAX4 gene variations predispose to ketosis-prone diabetes. *Human Molecular Genetics* **13** 3151–3159.
- Meier JJ, Bhushan A, Butler AE, Rizza RA & Butler PC 2005 Sustained β cell apoptosis in patients with long-standing type 1 diabetes: indirect evidence for islet regeneration? *Diabetologia* **48** 2221–2228.
- Meier JJ, Butler AE, Galasso R, Rizza RA & Butler PC 2006a Increased islet β cell replication adjacent to intrapancreatic gastrinomas in humans. *Diabetologia* **49** 2689–2696.
- Meier JJ, Lin JC, Butler AE, Galasso R, Martinez DS & Butler PC 2006b Direct evidence of attempted β cell regeneration in an 89-year-old patient with recent-onset type 1 diabetes. *Diabetologia* **49** 1838–1844.
- Minami K, Okuno M, Miyawaki K, Okumachi A, Ishizaki K, Oyama K, Kawaguchi M, Ishizuka N, Iwanaga T & Seino S 2005 Lineage tracing and characterization of insulin-secreting cells generated from adult pancreatic acinar cells. *PNAS* **102** 15116–15121.
- Miyamoto T, Kakizawa T, Ichikawa K, Nishio S, Kajikawa S & Hashizume K 2001 Expression of dominant negative form of PAX4 in human insulinoma. *Biochemical and Biophysical Research Communications* **282** 34–40.
- Muratovska A, Zhou C, He S, Goodyer P & Eccles MR 2003 Paired-Box genes are frequently expressed in cancer and often required for cancer cell survival. *Oncogene* **22** 7989–7997.
- Nir T, Melton DA & Dor Y 2007 Recovery from diabetes in mice by β cell regeneration. *Journal of Clinical Investigation* **117** 2553–2561.
- Parker CJ, Shawcross SG, Li H, Wang QY, Herrington CS, Kumar S, MacKie RM, Prime W, Rennie IG, Sisley K *et al.* 2004 Expression of PAX 3 alternatively spliced transcripts and identification of two new isoforms in human tumors of neural crest origin. *International Journal of Cancer* **108** 314–320.
- Pelengaris S, Khan M & Evan G 2002 Suppression of Myc-induced apoptosis in β cells exposes multiple oncogenic properties of Myc and triggers carcinogenic progression. *Cell* **109** 321–334.
- Peshavaria M, Larmie BL, Lausier J, Satish B, Habibovic A, Roskens V, Larock K, Everill B, Leahy JL & Jetton TL 2006 Regulation of pancreatic β -cell regeneration in the normoglycemic 60% partial-pancreatectomy mouse. *Diabetes* **55** 3289–3298.
- Pictet R & Rutter WJ 1972 In *Development of the Embryonic Endocrine Pancreas*, Eds SE Samaras, MA Cissell, K Gerrish, CVE Wright, M Gannon & R Stein. Washington, DC: American Physiological Society.
- Plengvidhya N, Kooptiwut S, Songtawe N, Doi A, Furuta H, Nishi M, Nanjo K, Tantibhedhyangkul W, Boonyasrisawat W, Yenchitsomanus PT *et al.* 2007 PAX4 mutations in Thais with maturity onset diabetes of the young. *Journal of Clinical Endocrinology and Metabolism* **92** 2821–2826.
- Prentki M & Nolan CJ 2006 Islet β cell failure in type 2 diabetes. *Journal of Clinical Investigation* **116** 1802–1812.
- Rezende L, Stoppiglia L, Souza KL, Negro A, Langone F & Boschero A 2007 Ciliary neurotrophic factor promotes survival of neonatal rat islets via the BCL-2 anti-apoptotic pathway. *Journal of Endocrinology* **195** 157–165.
- Robson EJ, He SJ & Eccles MR 2006 A PANorama of PAX genes in cancer and development. *Nature Reviews. Cancer* **6** 52–62.
- Saltiel AR & Kahn CR 2001 Insulin signalling and the regulation of glucose and lipid metabolism. *Nature* **414** 799–806.
- Scaglia L, Smith FE & Bonner-Weir S 1995 Apoptosis contributes to the involution of β cell mass in the *post partum* rat pancreas. *Endocrinology* **136** 5461–5468.
- Schumann DM, Maedler K, Franklin I, Konrad D, Stirling J, Boni-Schnetzler M, Gjinovci A, Kurrer MO, Gauthier BR, Bosco D *et al.* 2007 The Fas pathway is involved in pancreatic β cell secretory function. *PNAS* **104** 2861–2866.
- Shaat N & Groop L 2007 Genetics of gestational diabetes mellitus. *Current Medicinal Chemistry* **14** 569–583.
- Shapiro AM, Ricordi C, Hering BJ, Auchincloss H, Lindblad R, Robertson RP, Secchi A, Brendel MD, Berney T, Brennan DC *et al.* 2006 International trial of the Edmonton protocol for islet transplantation. *New England Journal of Medicine* **355** 1318–1330.
- Shimajiri Y, Sanke T, Furuta H, Hanabusa T, Nakagawa T, Fujitani Y, Kajimoto Y, Takasu N & Nanjo K 2001 A missense mutation of Pax4 gene (R121W) is associated with type 2 diabetes in Japanese. *Diabetes* **50** 2864–2869.
- Shimajiri Y, Shimabukuro M, Tomoyose T, Yogi H, Komiya I & Takasu N 2003 PAX4 mutation (R121W) as a prodiabetic variant in Okinawans. *Biochemical and Biophysical Research Communications* **302** 342–344.

- Smith S, Ee H, Connors J & German M 1999 Paired-homeodomain transcription factor PAX4 acts as a transcriptional repressor in early pancreatic development. *Molecular and Cellular Biology* **19** 8272–8280.
- Smith SB, Watada H, Scheel DW, Mrejen C & German MS 2000 Autoregulation and maturity onset diabetes of the young transcription factors control the human PAX4 promoter. *Journal of Biological Chemistry* **275** 36910–36919.
- Smith SB, Watada H & German MS 2004 Neurogenin3 activates the islet differentiation program while repressing its own expression. *Molecular Endocrinology* **18** 142–149.
- Sorenson RL & Brelje TC 1997 Adaptation of islets of Langerhans to pregnancy: β -cell growth, enhanced insulin secretion and the role of lactogenic hormones. *Hormone and Metabolic Research* **29** 301–307.
- Sosa-Pineda B, Chowdhury K, Torres M, Oliver G & Gruss P 1997 The Pax4 gene is essential for differentiation of insulin-producing β cells in the mammalian pancreas. *Nature* **386** 399–402.
- Stoffers DA, Kieffer TJ, Hussain MA, Drucker DJ, Bonner-Weir S, Habener JF & Egan JM 2000 Insulinotropic glucagon-like peptide 1 agonists stimulate expression of homeodomain protein IDX-1 and increase islet size in mouse pancreas. *Diabetes* **49** 741–748.
- St-Onge L, Sosa-Pineda B, Chowdhury K, Mansouri A & Gruss P 1997 Pax6 is required for differentiation of glucagon-producing α -cells in mouse pancreas. *Nature* **387** 406–409.
- Stumvoll M, Goldstein BJ & van Haeften TW 2005 Type 2 diabetes: principles of pathogenesis and therapy. *Lancet* **365** 1333–1346.
- Teta M, Rankin MM, Long SY, Stein GM & Kushner JA 2007 Growth and regeneration of adult β cells does not involve specialized progenitors. *Developmental Cell* **12** 817–826.
- Theis M, Mas C, Doring B, Degen J, Brink C, Caille D, Charollais A, Kruger O, Plum A, Nepote V *et al.* 2004 Replacement by a lacZ reporter gene assigns mouse connexin 36, 45 and 43 to distinct cell types in pancreatic islets. *Experimental Cell Research* **294** 18–29.
- Tokuyama Y, Matsui K, Ishizuka T, Egashira T & Kanatsuka A 2006 The Arg121Trp variant in PAX4 gene is associated with β -cell dysfunction in Japanese subjects with type 2 diabetes mellitus. *Metabolism* **55** 213–216.
- Ueda Y 2000 Activin A increases Pax4 gene expression in pancreatic β cell lines. *FEBS Letters* **480** 101–105.
- Unger RH 2005 Longevity, lipotoxicity and leptin: the adipocyte defense against feasting and famine. *Biochimie* **87** 57–64.
- Wang J, Elghazi L, Parker SE, Kizilocak H, Asano M, Sussel L & Sosa-Pineda B 2004 The concerted activities of Pax4 and Nkx2.2 are essential to initiate pancreatic β -cell differentiation. *Developmental Biology* **266** 178–189.
- Xu W & Murphy L 2000 Cloning of the mouse Pax4 gene promoter and identification of a pancreatic β cell specific enhancer. *Molecular and Cellular Endocrinology* **170** 79–89.
- Xu G, Stoffers DA, Habener JF & Bonner-Weir S 1999 Exendin-4 stimulates both β -cell replication and neogenesis, resulting in increased β -cell mass and improved glucose tolerance in diabetic rats. *Diabetes* **48** 2270–2276.
- Yach D, Stuckler D & Brownell KD 2006 Epidemiologic and economic consequences of the global epidemics of obesity and diabetes. *Nature Medicine* **12** 62–66.
- Zalzman M, Gupta S, Giri RK, Berkovich I, Sappal BS, Karnieli O, Zern MA, Fleischer N & Efrat S 2003 Reversal of hyperglycemia in mice by using human expandable insulin-producing cells differentiated from fetal liver progenitor cells. *PNAS* **100** 2426–2431.
- Zhang YQ, Mashima H & Kojima I 2001 Changes in the expression of transcription factors in pancreatic AR42J cells during differentiation into insulin-producing cells. *Diabetes* **50** S10–S14.
- Zulewski H, Abraham E, Gerlach M, Daniel P, Moritz W, Muller B, Vallejo M, Thomas M & Habener J 2001 Multipotential nestin-positive stem cells isolated from adult pancreatic islets differentiate *ex vivo* into pancreatic endocrine, exocrine, and hepatic phenotypes. *Diabetes* **50** 521–533.

Received in final form 8 November 2007

Accepted 23 November 2007

Made available online as an Accepted Preprint
23 November 2007