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Pancreatic Transcription Factors and their Role in the Birth, Life and Survival of the Pancreatic β Cell

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Abstract

In recent years major progress has been made in understanding the role of transcription factors in the development of the endocrine pancreas in the mouse. Here we describe how a number of these transcription factors play a role in maintaining the differentiated phenotype of the β cell, and in the mechanisms that allow the β cell to adapt to changing metabolic demands that occur throughout life. Amongst these factors, Pdx1 plays a critical role in defining the region of the primitive gut that will form the pancreas, Ngn3 expression drives cells towards an endocrine lineage, and a number of additional proteins including Pdx1, in a second wave of expression, Pax4, NeuroD1/β2, and MafA act as β cell differentiation factors. In the mature β cell Pdx1, MafA, β2, and Nkx2.2 play important roles in regulating expression of insulin and to some extent other genes responsible for maintaining β cell function. We emphasise here that data from gene expression studies in rodents seldom map on to the known structure of the corresponding human promoters. In the adult the β cell is particularly susceptible to autoimmune mediated attack and to the toxic metabolic milieu associated with over-eating, and utilises a number of these transcription factors in its defence. Pdx1 has anti-apoptotic and proliferative activities that help facilitate the maintenance of β cell mass, while Ngn3 may be involved in the recruitment of progenitor cells, and Pax4 (and possibly HNF1α and Hnf4α) in the proliferation of β cells in the adult pancreas. Other transcription factors with a more widespread pattern of expression that play a role in β survival or proliferation include Foxo1, CREB family members, NFAT, FoxM1, Snail and Asc-2.

Introduction

It is universally acknowledged that an aggressor bent on destruction will always find a vulnerable individual. The pancreatic β cell is such an individual. It is a highly specialised cell that plays a pivotal role in regulating fuel metabolism and in conjunction with the glucagon secreting α cell and other counter regulatory hormones is involved primarily in the maintenance of glucose homeostasis. The β cell is unique in its ability to express, process and secrete significant amounts of insulin in a strictly regulated pulsatile manner in response to continuously varying concentrations of circulating nutrients. Glucose sensitive insulin secretion, involving the shuttling of reducing equivalents between mitochondria and the cytoplasm, is orchestrated towards providing a signal that reflects the energy status within the cell, i.e. the ratio of ATP to ADP, which in turn accurately mirrors the levels of circulating nutrients (Joseph *et al.* 2006). This, along with signals from the sympathetic nervous system and incretins secreted from the gut, affects the electrical properties of the plasma membrane leading to changes in the cytoplasmic Ca^{2+} concentration that trigger exocytosis (Wiederkehr & Wollheim 2006). It is this requirement for a high rate of mitochondrial oxidation and the energy expenditure involved in the production, storage and secretion of insulin at the expense of protective genes such as glutathione peroxidase and catalase (Tiedge *et al.* 1997)that leaves the β cell vulnerable to attack, particularly from cytokines and free radicals.

This chink in its defences can have devastating effects on the β cell leading to the spectrum of metabolic disorders known as diabetes mellitus. In type 1 diabetes, activation of the immune system leads to lymphocytic infiltration of the islets of Langerhans and selective destruction of β cells (Donath *et al.* 2003). Locally produced inflammatory cytokines such as interleukin 1β and interferon γ stimulate

nitric oxide production in the β cell leading to endoplasmic reticulum stress induced cell death (Cnop *et al.* 2005). These cytokines also up-regulate the protein FAS on the β cell surface, resulting in an increase in apoptosis. The β cell is also sensitive to high circulating lipid levels as seen in overweight or obese individuals (Unger & Zhou 2001). This causes a vicious cycle whereby impaired insulin secretion combined with insulin resistance leads to further increases in the circulating levels of glucose and lipids that accelerate the decline in β cell function and an increase in apoptosis (Gesta *et al.* 2006). Over time the β cell succumbs, leading to the metabolic derangements that are characteristic of type 2 diabetes mellitus.

The specialised features of the β cell are determined by the expression of a subset of genes controlled by a number of transcription factors, some of which are β cell specific and others that are ubiquitous. Here we will describe the role of transcription factors in the life cycle of the β cell and the way in which they contribute to the survival of the β cell within a hostile environment, where it is vulnerable to attack from the immune system and the toxic effects of nutrient excess. To understand these mechanisms it is important to begin at the birth of the β cell.

Pancreatic Development

In recent years there have been major advances in our understanding of how the pancreas develops, particularly in the mouse. The assumption (based to some extent on rare inherited mutations) is that the key factors and events will also hold true for humans, although there may well be marked differences. The reader is directed to some excellent reviews (Wilson *et al.* 2003; Servitja & Ferrer 2004; Habener *et al.* 2005; Collombat *et al.* 2006). Here we will provide an overview of the

key steps and the transcription factors involved with emphasis on those that are currently known to play a role in the differentiated β cell.

The pancreas begins life as dorsal and ventral evaginations of the foregut endoderm on embryonic day 9.5 (E9.5) in the mouse (Fig. 1). The prevailing models favour the existence of a common pool of undifferentiated, or multipotent, progenitor cells (MPCs) in the early pancreatic buds (Gu *et al.* 2003). These multipotent cells go through a period of rapid division, under the influence of FGF10 secreted by the surrounding mesenchyme (Bhushan *et al.* 2001; Hart *et al.* 2003), forming the branching epithelial network of the pancreas. At around E13 a major change occurs, the secondary transition (Pictet *et al.* 1972), at which point the two lobes of the pancreas fuse and individual cells differentiate into acinar and ductal tissue of the exocrine pancreas and the islets of Langerhans, comprising α (glucagon secreting), β (insulin secreting), δ (somatostatin secreting), PP (pancreatic polypeptide secreting) and ghrelin-expressing cells. The individual endocrine cells delaminate and cluster to form endocrine organs (islets) that continue to differentiate and only become fully functional shortly after birth (Fig. 1). A major theme of this review is that several of the transcription factors that control these events also play a role in the differentiated β cell and in particular in the mechanisms whereby the β cell can adapt to or compensate for the hostile environment to which it is particularly susceptible.

The events that precede emergence of the pancreatic bud include the anterior-posterior patterning of the endoderm, which occurs from as early as E7.5 (Moore-Scott *et al.* 2007), and the subsequent specification that takes place during the transformation of the endoderm sheet into a gut tube. On the dorsal side of the gut, this specification involves contact of the endoderm cells with the notochord, somatic mesoderm and aorta, while on the ventral side, it requires signals from the splancnic

mesoderm, visceral endoderm and the septum transversum (Spagnoli 2007) (Fig. 1). At around E8.5, the pancreatic endoderm domain is marked by the homeodomain transcription factor Pdx1 (McKinnon & Docherty 2001) in a sub-region of the fore-midgut where Hedgehog signalling is repressed (Apelqvist *et al.* 1997). In Pdx1 null mice the pancreatic buds form, but further development of the pancreas fails at about E10.5 (Jonsson *et al.* 1994; Offield *et al.* 1996), while in humans with homozygous mutations in Pdx1 (the human gene is called *Ipf1*) the pancreas also fails to form (Stoffers *et al.* 1997) . Members of the hepatocyte nuclear factor family (Hnfβ, Hnf1β, and Hnf6) have also been shown to delineate regions of the foregut endoderm from which the pancreas forms, and inactivation of any of these genes leads to abnormalities in the endoderm and pancreas (Maestro *et al.* 2007). Hnf1β (Tcf2) has been specifically implicated in bud formation as shown by the early rescued null mutants for this gene (null mutants die before gastrulation), which only form a dorsal bud rudiment that transiently expresses the transcription factors Pdx1 and the homeodomain protein Hlxb9 (Haumaitre *et al.* 2005). Hlxb9 is of particular importance for the dorsal bud formation. In mice homologous for a null mutation of this gene, the dorsal lobe of the pancreas fails to develop (Harrison *et al.* 1999). Loss of the LIM-domain protein Isl1 also leads to impaired formation of the dorsal bud (Ahlgren *et al.* 1997). On the ventral side, *Hex*, another homeobox gene, has been shown to position endoderm cells beyond the cardiogenic mesoderm and, therefore, enable ventral pancreatic specification (Bort *et al.* 2004). The basic helix loop helix factor (bHLH) Ptf1a also plays an important role in bud formation. In the Ptf1a knockout mouse, the Pdx1 positive domain of the foregut adopts a duodenal fate, suggesting that Ptf1a may act along with Pdx1 to drive endoderm towards a pancreatic fate (Kawaguchi *et al.* 2002; Afelik *et al.* 2006). Under some conditions

Pdx1 can interact physically with the TALE homeodomain protein Pbx1(Peers *et al.* 1995) and Pdx1:Pbx1 complexes may be important for normal proliferation of cells during pancreatic development (Dutta *et al.* 2001). Pbx1 null mice display pancreatic hypoplasia and marked defects in exocrine and endocrine cell differentiation prior to death at E15 or E16 (Kim *et al.* 2002).

Within the emerging pancreatic bud the putative multipotent progenitor cell (MPC) is marked by co-expression of Pdx1 as well as Hnf1β, Hnf3β and HNF6 and a number of transcription factors that are subsequently restricted to the endocrine pancreas (Lynn *et al.* 2007b). Ptf1a, which is later restricted to exocrine cells is also expressed in these MPCs (Chiang & Melton 2003; Burlison *et al.* 2008). As the epithelial network expands the MPCs become localised at the tip of the tree with the differentiated progeny forming the trunk of the branches (Zhou *et al.* 2007). At around E14.5 the commitment to expand further or differentiate is governed by Delta-Notch signaling on adjacent cells. Notch-mediated signalling ensures maintenance of the progenitor cell population through activation of the bHLH factor Hes1 (Jensen *et al.* 2000). In a population of $Pdx1^{+}$ cells that evades the Notch ligand, repression of Hes1 leads to activation of the bHLH factor neurogenin 3 (Ngn3) that specifies the endocrine lineage (Gradwohl *et al.* 2000; Schwitzgebel *et al.* 2000). Ptf1a expression on the other hand becomes restricted to cells that will form the exocrine pancreas (Krapp *et al.* 1998). The HMG box transcription factor Sox9 is also expressed in the developing pancreas during the expansion period (Piper *et al.* 2002). Its expression is restricted to a mitotically active population of $Pdx1^{+}$ cells and inactivation of Sox9 results in depletion of the progenitor pool similar to that seen in the Hes-1 null embryos (Seymour *et al.* 2007). Sox9 may regulate the progenitor pool by governing its release into a programme of differentiation (Lynn *et al.* 2007b).

The expression of Ngn3, insulinoma 1 (Insm1, which is also known as IA1) (Lan *et al.* 1994; Mellitzer *et al.* 2006) and NeuroD1/β2 (a bHLH protein which forms a heterodimeric complex with bHLH partners such as E47) (Lee *et al.* 1995; Naya *et al.* 1995) at around E14 specifies the endocrine lineage. Within this lineage NKx2.2, Nkx6.1, Pax4, Isl1, MafA and Pdx1 (in a second wave of expression) drive formation of β cells, while Pax6, Arx and Brn4 (Collombat *et al.* 2005) drive formation of α cells (Servitja & Ferrer 2004). Very little is known about the transcription factors that drive formation of δ , PP and ghrelin cells, although it is likely that the exact timing of expression of key transcription factors such as Ngn3 will be important in determining which type of endocrine cell is produced (Johansson *et al.* 2007). In the adult mouse expression of Pdx1 is restricted to β cells, some (~20%) δ cells and rare endocrine cells in the gut, while Nkx6.1 is restricted to β cells and some neurones. The leucine zipper protein MafA is also enriched in β cells (Zhao *et al.* 2005). Interestingly, MafB is expressed early in pancreatogenesis where it regulates the expression of key factors that are required for the production of mature α and β cells (Artner *et al.* 2007). There is a switch to MafA at a late stage of differentiation concomitant with the acquisition of glucose sensing and other characteristics of the mature β cell (Nishimura *et al.* 2006).

The various cell lineages within the developing pancreas are determined by feed-forward and feed-back as well as autocrine control of transcription factor gene expression. Thus, the Ngn3 promoter contains binding sites for and is activated by the upstream factors Hnf3β, Hnf1β and Hnf6 and inhibited by Hes-1 and Ngn3 itself (Lee *et al.* 2001). Pax4 in turn is regulated by Hnf1β and Ngn3 (Smith *et al.* 2000), while NKx2.2 is regulated by Hnf3β, Ngn3 and Pax4 (Wilson *et al.* 2003). Nkx6.1 is an exception in that, although the mRNA is broadly expressed in the pancreatic

epithelium amongst various lineages starting at early stages of development (E10.5), the protein is found only in β cells at very late stages of differentiation. This suggests that post-translational control involving *cis*-acting elements in the mRNA (and perhaps microRNAs) may also play a role in β cell differentiation (Sander *et al.* 2000; Lynn *et al.* 2007a).

Maintenance of β cell function

While many of the transcription factors described above, e.g. Ngn3 and Hnf1β, only appear transiently during development of the pancreas, several of the others involved in the birth of the β cell are present in mature β cells, where they are assumed to play a role in the maintenance of the β cell phenotype. Others can be induced under special circumstances, allowing the β cell to respond to stressful conditions (see below). The term β cell phenotype applies to the expression of genes in the mature β cell that enable its specialised function, i.e. the expression, processing, storage and regulated secretion of insulin. There are a huge number of genes involved in these processes, and it is likely that defects in many of these genes will contribute to the development of type 2 diabetes. We know very little about how most of these genes are regulated. Almost all the published studies have focused on genes that have a specific function in β cells or related cells within the neuroendocrine system. These include studies on the genes encoding insulin, glucokinase (a key regulator of glycolytic flux in the β cell), Kir 6.1 (an ATP-dependent K^+ channel involved in insulin secretion), GLUT2 (a high Km glucose transporter that is expressed in rodent β cells) and islet amyloid polypeptide (IAPP or amylin). They contain relatively short promoters that generally harbour binding sites for Pdx1, β2, MafA and other transcription factors that appear in the foetal pancreas (Fig. 2). For example, within the insulin promoter there exists a

highly conserved arrangement of *cis*-acting elements (see box in Fig. 2) comprising the C1, E1, and A1 boxes that bind MafA, E47/β2 and Pdx1 respectively (Docherty *et al.* 2005).

While the regulatory elements in the insulin promoter and the transcription factors that bind them have been studied in great detail, our understanding of the promoters of the other genes involved in the maintenance of β cell phenotype lags far behind. This is compounded by the fact that most work has been carried out on rodent genes, and it is emerging that the significant differences which exist between rodent and human insulin gene promoters (Hay & Docherty 2006) are also reflected in the other β cell specific genes. Given the limited number of comprehensive studies on the human promoters, we have focused on the insulin, IAPP and glucokinase genes (Fig. 2).

The insulin promoter of humans and other primates contains a GG1 element adjacent to the highly conserved C1, E1, and A1 boxes that emphasises the importance of Pdx1 in controlling insulin gene expression in these species. In lower mammals Pdx1 may have a less important role since a 50% reduction in Pdx1 in mice is still compatible with insulin gene expression (Johnson *et al.* 2003), and Pdx1 expression appears to peter out with age (by age 8 weeks) in mice with no effect on the levels of insulin mRNA (Kitamura *et al.* 2002). Members of the CREB/ATF (bZIP) family also play an important role in regulating the insulin gene. In fact, the cAMP responsive element (CRE) is one of the most highly conserved sequences in the insulin promoter. CREB/ATF proteins can exist as multiple isoforms that can be activated by cAMP and diacylglycerol signalling pathways to form complexes with other transcription factors to create activators, non-activators or repressors. It is likely that the β cell has adopted these signalling pathways to adjust the level of insulin gene

expression to various extracellular signals over relatively short periods. The fact that the number of CRE binding sites has increased from one in rodents and lower mammals through two in lower primates and to four in higher primates and humans (see Fig. 2) emphasises the importance of these regulatory mechanisms and could reflect the increased fruit (i.e. sugar) consumption in the primate diet (Hay & Docherty 2006). The human CRE2 sequence for example can bind several members of the family but has preference for Atf-2 (Hay *et al.* 2007). It will be of interest to see whether, amongst the bZIP family members, Atf-2 also plays a pre-eminent role in the developing pancreas. Other CRE-binding factors may play important roles in β cell physiology. Goto-Kakizaki rats (a model for type 2 diabetes) express increased levels of repressor isoforms of CREM compared to non-diabetic rats with a concomitant reduction in expression of insulin (Inada *et al.* 1998). Similarly, overexpression of CREM repressor in transgenic mice impairs β cell proliferation leading to early diabetes (Inada *et al.* 2004).

The islet amyloid polypeptide (IAPP) promoter shares roughly similar juxtapositions of E and A boxes with the insulin promoter (Bretherton-Watt *et al.* 1996; Carty *et al.* 1997). It has 3 A boxes, all of which can bind Pdx1. The possible role of the homeodomain transcription factor Isl1 at the important A2 box, which contains 3 TAAT motifs, remains unclear. Isl1 has been shown to bind to the human IAPP A3 regulatory element and to stimulate the equivalent sequence in the rat IAPP promoter in reporter assays (Wang & Drucker 1996). Other studies, however, have concluded that Isl1 does not bind to the site (Carty *et al.* 1997). Like the insulin gene, the IAPP gene can be upregulated by cAMP through a CRE site, although this signaling pathway does not seem to be involved in glucose up regulation of IAPP expression (Ding *et al.* 2001). A major species difference is exemplified by the human

promoter lacking the important CCAAT box which is present in both mouse and rat promoters (-120 to -115) (Ekawa *et al.* 1997).

The glucokinase gene is unusual in having two promoters (Magnuson & Shelton 1989) that are separated by 35 Kbp. The upstream glucokinase promoter region, which lacks a TATA box, is active in both pancreatic cells and the anterior pituitary, while the downstream promoter is active only in hepatocytes, thus permitting the glucokinase gene to be regulated by insulin in the liver and by glucose in the β cell. There are marked differences between the rat and human glucokinase β cell promoters. The 3 A boxes characteristic of the insulin and IAPP promoters are present in the mouse glucokinase β cell promoter (Postic *et al.* 1995) and are conserved in the rat, however the human promoter has a single A box (Fig. 2). The equivalent sequences to A1 and A3 contain changes resulting in loss of the homeodomain binding sequence and deletion of these sites has no effect on transcription (Watada *et al.* 1996). An even more dramatic divergence of the human glucokinase β cell promoter from both the rodent forms and other β cell specific genes is the absence of E boxes. The rat glucokinase promoter binds β2/E47 at an E box (Moates *et al.* 2003) but the equivalent human sites have lost the consensus sequences and their deletion has no significant effect on expression (Watada *et al.* 1996). The rat glucokinase promoter contains a Foxo1 response element (FRS) at position -550 which binds the forkhead transcription factor Foxo1 (Yoshida *et al.* 2007)and represents the final step in the signalling pathway of Insulin-like growth factor-1 (IGF1) stimulation of glucokinase expression. In contrast to the rat promoter, the human glucokinase promoter lacks this regulatory element and computational analysis of 1 Kbp of promoter sequence failed to detect an alternative site or other Foxo consensus sequence. Unlike the insulin or IAPP promoters, the glucokinase β cell

promoter contains 2 identical palindromic regulatory elements termed Pal1 and Pal2. These sites are conserved in rodents and human and contain 4 bp inverted repeats which up regulate glucokinase expression through unidentified transcription factors (Moates *et al.* 1996).

Nkx2.2 may also be important in the maintenance of mature β cell function (Doyle & Sussel 2007). Thus transgenic mice, in which a repressor form of Nkx2.2 was expressed in β cells, exhibited reduced β cell levels of MafA, GLUT2 and insulin, and the islets displayed a disrupted architecture. The observation that the repressor form of Nkx2.2 could substitute for Nkx2.2 in the developing pancreas (Doyle *et al.* 2007), suggests that Nkx2.2 functions as a repressor of gene expression in the developing pancreas but as an activator of gene transcription in mature β cells. Its effect on the insulin gene may be through binding sites in the promoter (Cissell *et al.* 2003), or indirectly through its ability to activate expression of MafA (Raum *et al.* 2006).

Of particular importance to this discussion is the role of HNF1α and HNF4 α in the adult β cell. Mutations in these genes result in MODY (maturity-onset diabetes of the young) a form of diabetes that develops in young people and is inherited in an autosomal dominant manner (Frayling *et al.* 2001). The clinical phenotype in MODY3 (Hnf1 α) is very similar to that of MODY1 (Hnf4 α), i.e. a progressive form of diabetes with a blunted insulin response to glucose. There is no convincing evidence that either HNF1α or Hnf4α have an effect on embryonic growth of the β cell. It is more likely that these genes play a role in regulating key genes in the adult β, and although chromatin immunoprecipitaion studies have shown that HNF1 α can bind more than 10,000 promoters (Odom *et al.* 2004), we still know very little about their targets in the human β cell (Maestro *et al.* 2007). It is also probable that these

genes contribute, along with others described below, to the proliferative response of adult β cells that forms part of a compensatory or survival strategy.

Activating signal cointegrator-2 (Asc2), a transcriptional coactivator of nuclear receptors has also been shown to be involved in glucose sensitive insulin secretion and the maintenance of β cell mass (Yeom *et al.* 2006). The mechanisms are unclear, although there is evidence that the liver X receptor (LXR), which is also involved in glucose sensing in β cells (Efanov *et al.* 2004), and the MODY1 gene Hnf4α might be involved.

Survival and regeneration of β cells

Shortly after birth the endocrine pancreas undergoes remodelling through a process that involves substantial apoptosis and β cell replication (Bonner-Weir 2001). In the adult, under normal conditions, the β cell has a slow turnover rate (Finegood *et al.* 1995) whereby (at least in rodents) β cell death (by apoptosis) is balanced by the replication of existing β cells (Fig. 3) as demonstrated by in vivo lineage tracing using an insulin promoter driven Cre recombinase (Dor *et al.* 2004), and subsequently confirmed using a DNA-analogue-based lineage tracing technique (Teta *et al.* 2007).

The overall mass can, however, be modulated to compensate for the increased metabolic demands that occur with age and during pregnancy, and in response to the insulin resistance associated with obesity (Unger 1997). The regulation of β cell growth and proliferation occurs mainly through insulin/IGF signalling acting through IRS-2. Thus mice in which the *IRS-2* gene has been inactivated in β cells develop β cell failure due to decreased proliferation and an increased rate of apoptosis (Withers *et al.* 1998; Kubota *et al.* 2000). Not surprisingly, given its importance in the development of the pancreas, the differentiation of β cells, and in gene expression in

the mature β cell, Pdx1 turns out also to be a major player in the maintenance of an adequate pool of healthy β cells in adults (Johnson *et al.* 2003). In mice with a 50% reduction in Pdx1, isolated islets are significantly more susceptible to apoptosis at basal glucose concentrations and there is an impaired ability to maintain β cell mass with age. The survival functions of Pdx1 may be mediated by insulin/IGF signalling acting through the forkhead transcription factor Foxo1. When *IRS2-/-* mice were crossed with *Foxo1+/-* mice, the reduced levels of Foxo1 were able to partially rescue the phenotype with a concomitant increase in Pdx1 levels, suggesting that insulin or IGF1 regulate β cell mass by relieving Foxo1 inhibition of Pdx1 expression (Kitamura *et al.* 2002). It turns out that insulin (or IGF1) inhibits the actions of Foxo1 through Akt- mediated phosphorylation of Foxo1, which induces movement of the transcription factor from the nucleus to the cytoplasm.

Pdx1 also acts as a critical regulator of β cell replication that occurs during the compensatory response to insulin resistance (Kulkarni *et al.* 2004). This was demonstrated by crossing insulin receptor deficient (*IR-/-)* or IRS-1 deficient (*IRS-1 -/-)* mice with *PDX1+/-* mice. Evidence that Foxo1 was also involved in this process was provided by using mice that harboured a mutant Foxo1 transgene that is retained in the nucleus. Thus in two models of insulin resistance, i.e. *IR-/-* mice, and mice in which the elastase promoter is used to achieve local expression of IGF2, the presence of the mutant Foxo1 transgene that is retained in the nucleus (and thus inhibits expression of Pdx-1) blocked IGF-induced β cell proliferation (Okamoto *et al.* 2006).

Other transcription factors that have been implicated in the survival and maintenance of β cells include CREB, NFAT, FoxM1, Pax4 and PPARα. Mice in which CREB has been inactivated specifically in the β cell develop diabetes in adulthood due to increased apoptosis and reduced β cell proliferation (Jhala *et al.*

2003). The mechanisms are unclear, although it is possible that it may involve the CREB co-activator TORC2/CRTC2, which is normally retained in the cytoplasm but upon signal mediated dephosphorylation translocates to the nucleus where it potentiates gene expression through a direct interaction with CREB (Bittinger *et al.* 2004). Mice with a β cell specific deletion of the calcium-dependent phosphatase calcineurin, which regulates the intracellular localisation of NFAT, develop age-dependent diabetes characterised by decreased β cell proliferation and mass (Heit *et al.* 2006). This effect on β cell proliferation in the adult could be rescued by overexpression of an active form of NFAT. The forkhead transcription factor FoxM1 acts as a cell cycle regulator in proliferating cells. FoxM1 appears to affect β cell proliferation in adult mice but has no effect on embryonic β cell proliferation. Thus specific inactivation of FoxM1 in pancreatic cells had no effect on the developing pancreas, i.e. mice were born with normal pancreatic and β cell mass, however the resultant adults displayed a gradual decline in β cell mass with age (Zhang *et al.* 2006). PAX4 is expressed at very low levels in adult β cells. It has been shown that treatment of adult isolated rat or human islets of Langerhans with the mitogens activin A or betacellulin substantially increased the levels of Pax4 (Brun *et al.* 2004). Furthermore, adenoviral-mediated overexpression of Pax4 in β cells increased the rate of proliferation. This effect may be mediated by the growth-promoting transcription factor c-myc. Bcl-xl, which can act by suppressing apoptosis, may also play a role in the protective effects of PAX4 on the. Transcription factors also play a role in the adaptive response of β cells to fasting. During fasting the levels of PPARα (proliferator-activated receptor α) in β cells increases (Gremlich *et al.* 2005). This leads to PPARα- mediated transcriptional activation of fatty acid oxidation, resulting in reduced insulin secretion and the avoidance of hypoglycaemia.

Neogenesis, i.e. the generation of β cells from undifferentiated or partially differentiated precursors, may also be involved in the maintenance of β cell mass (Xu *et al.* 2006). Very little is known about these putative stem cells, which may reside within the islet itself (Zulewski *et al.* 2001; Petropavlovskaia & Rosenberg 2002)or in the pancreatic ducts (Bonner-Weir *et al.* 2000; Zhao *et al.* 2007). The convincing data demonstrating that β cells maintain their mass through the replication of pre-existing β cells (Dor *et al.* 2004; Teta *et al.* 2007) has questioned the need for islet progenitor cells under normal physiological conditions (Lee *et al.* 2006). Recent studies, however, have demonstrated that in adult mice new β cells can be formed from non β cells located in the lining of the duct during regeneration of the pancreas in response to duct ligation. Shortly after duct ligation there was an increased number of cells expressing Ngn3, which is not normally expressed in the adult pancreas (Xu *et al.* 2008). These Ngn3-positive cells were sorted by flow cytometry and implanted into pancreatic buds from Ngn3 -/- mice. Under these conditions the Ngn3 positive cells from the regenerating adult pancreas differentiated into β and other endocrine cell types. This important study will certainly stimulate further efforts to identify the source, frequency and nature of the cells that give arise to the Ngn3 positive islet precursors and the extent to which these cells exist in the human pancreas.

It has also been suggested that under certain circumstances β cells can arise through transdifferentiation of acinar cells. Thus treatment of an acinar enriched cell population in vitro with epidermal growth factor (EGF) or leukaemia inhibitory factor (LIF) promoted the formation of new β cells (Baeyens *et al.* 2005). Similarly, adult acinar cells genetically labelled with an amylase promoter-driven Cre recombinase gave rise to new insulin-secreting cells following treatment in vitro with EGF and nicotinamide (Minami *et al.* 2005). The mechanisms are not well understood but may

involve disruption and remodelling of cadherin-mediated intercellular contacts by phosphatidyl inositol 3-kinase mediated pathways (Minami *et al.* 2008). The physiological relevance of these in vitro studies is unclear since in vivo support has been indirect (Lardon *et al.* 2004), and recent in vivo lineage tracing studies provide strong evidence that pre-existing acinar cells can give rise to acinar cells but not β cells using several models of pancreatic injury in mice (Desai *et al.* 2007). The conflicting outcome of the in vitro and in vivo studies emphasises the effect of the in vitro environment on cellular plasticity and differentiation potential (Ferrer *et al.* 2007).

One of the major scientific breakthroughs of recent years has been the discovery that adult differentiated cells can be reprogrammed into pluripotent cells (IPCs), that exhibit many of the properties of embryonic stem cells (Takahashi & Yamanaka 2006; Okita *et al.* 2007; Aoi *et al.* 2008). The concept of cellular plasticity and epigenetic programming is now an area of intensive research activity. The recent finding that a single transcription factor Arx can cause adult β cells to transdifferentiate into α and PP cells (Collombat *et al.* 2007) has emphasised the potential for transdifferentiation within the endocrine pancreas. The fact that human IPCs are not so very different from rodent IPCs (Yu *et al.* 2007; Nakagawa *et al.* 2008) suggests that the recent exciting data on the transdifferentiation of islet cells may translate well towards novel therapies for the treatment of the two major forms of diabetes mellitus. The zinc finger transcription factor Snail has also been shown to affect the differentiated state of islet cell lines in culture (Rukstalis *et al.* 2006). During passage and growth Snail is expressed in a sub-set of cells along with decreased levels of hormone expression. As the cells begin to cluster and assume a differentiated appearance the levels of Snail decreases as hormone expression returns.

Snail may act to regulate the balance between differentiated and dedifferentiated cells and as such may also have a therapeutic role in diabetes.

Conclusion

This review has emphasised the key role played by pancreatic transcription factors in the life and struggle to survive of the β cell. The major conclusion is that factors such as Pdx1, β2, MafA, and Nkx2.2 that play important roles in the early stages of pancreatic development and the later stages of β cell differentiation are also important in maintaining the function of the β cell in adulthood. Pdx1 appears to have adopted a particularly important role in the survival of the β cell. This is possibly illustrated best by the sand rat *Psammomys obesus,* which has undetectable levels of Pdx1 in adult islets and is particularly susceptible to nutrient stress induced β cell failure (Leibowitz *et al.* 2001). Other transcription factors that may play an important role in maintaining β cell mass through their effects on β cell replication and neogenesis include Pax4, Ngn3 (and possibly HNF1α and HNF4α), and the more ubiquitously expressed NFAT, FoxM1, Foxo1, and the CREB family of proteins. PPARα, on the other hand, seems to enable the β cell to adapt to the fasting state. Recent exciting data have shown that Ngn3 may play an important role in the recruitment of islet progenitor cells in response to injury to the pancreas, while the ability to reprogramme islet cells using a single transcription factor, Arx (and possibly Snail), has further emphasised the reversible nature of the fully differentiated state. The overriding theme, however, is that transcription factors that are present during the formation of islet cells are either retained or induced in adult islets to allow the islet to withstand the hostile attack to which it is so particularly vulnerable.

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Figure Legends

Figure 1: Development of the pancreas in mouse

Schematic showing the various stages in the development of the pancreas in the mouse. Further details are provided in the text.

Figure 2: The human insulin, IAPP and glucokinase gene promoters.

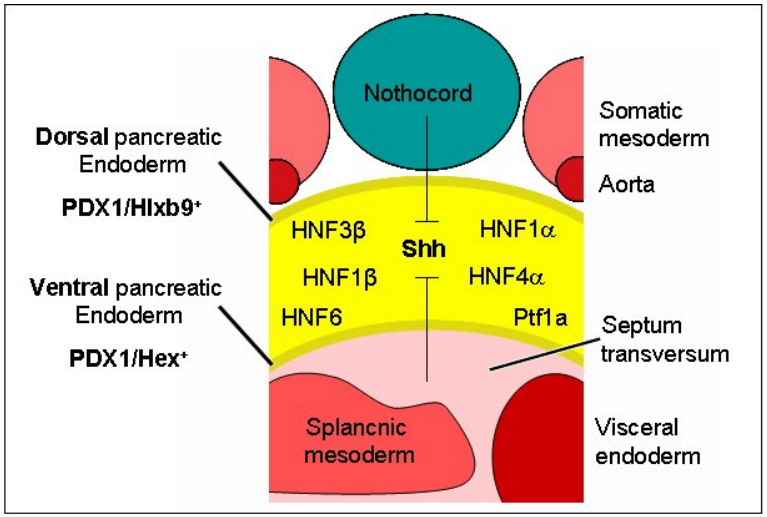
Schematic representation of the human insulin, IAPP and glucokinase promoters. The transcription start site is shown by the arrow and base pairs upstream and downstream of this point are shown on the scale above. The round symbols represent transcription factor binding sites with the name of the element. The boxed area highlights the C1-E1-A1 region which is described in the text. The principle binding sites for PDX-1, MafA, and β2/E47 are also shown.

Figure 3: Schematic depicting the mechanisms whereby β cell mass is maintained in the adult.

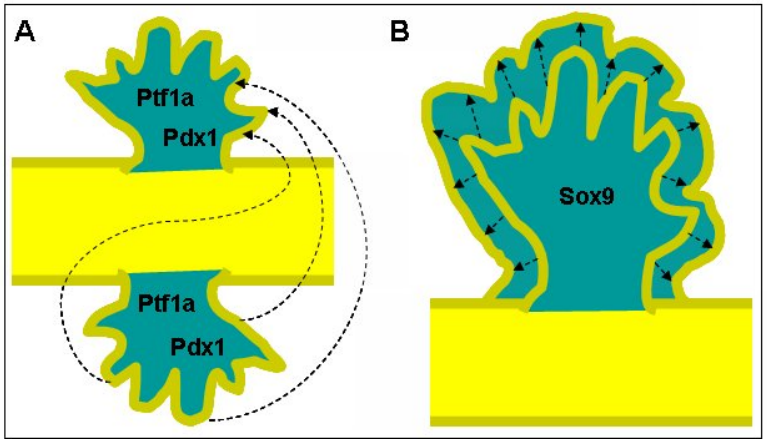
Under normal conditions β cell turnover is balanced by β cell replication. Under conditions of stress during which the β cell is destroyed by the immune system new β cells can arise from progenitor cells located in the gut through a process of neogenesis. The transcription factors involved in these various processes are described in the text.

Primary Transition

Stage 1: Emergence of bud

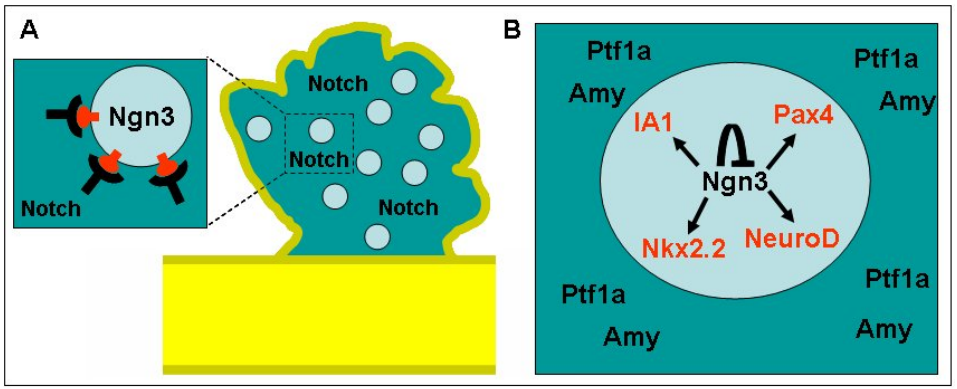


Stage 2: Fusion and expansion



Secondary Transition

Stage 1: Specification of endocrine and exocrine lineages



Stage 2: Differentiation of β cells

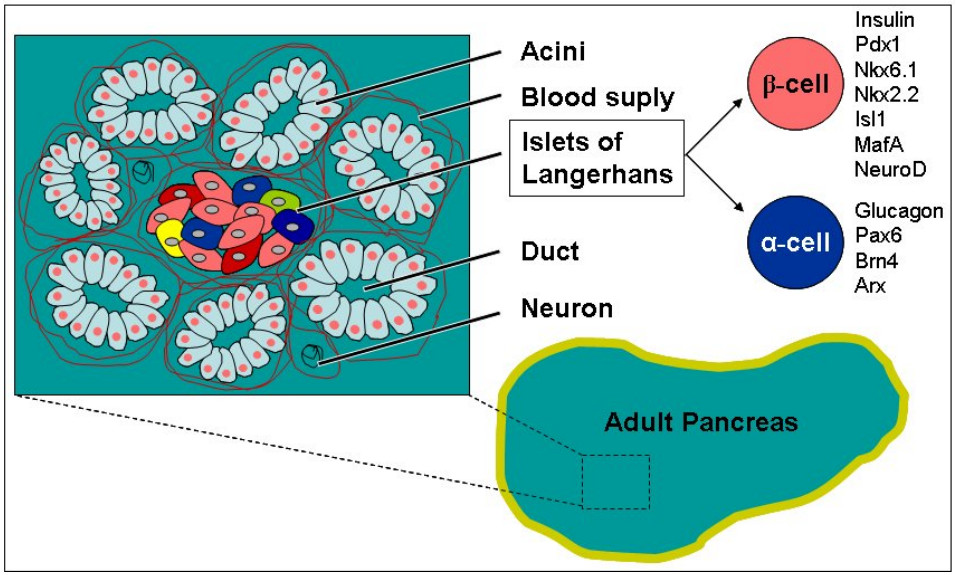


Figure 1

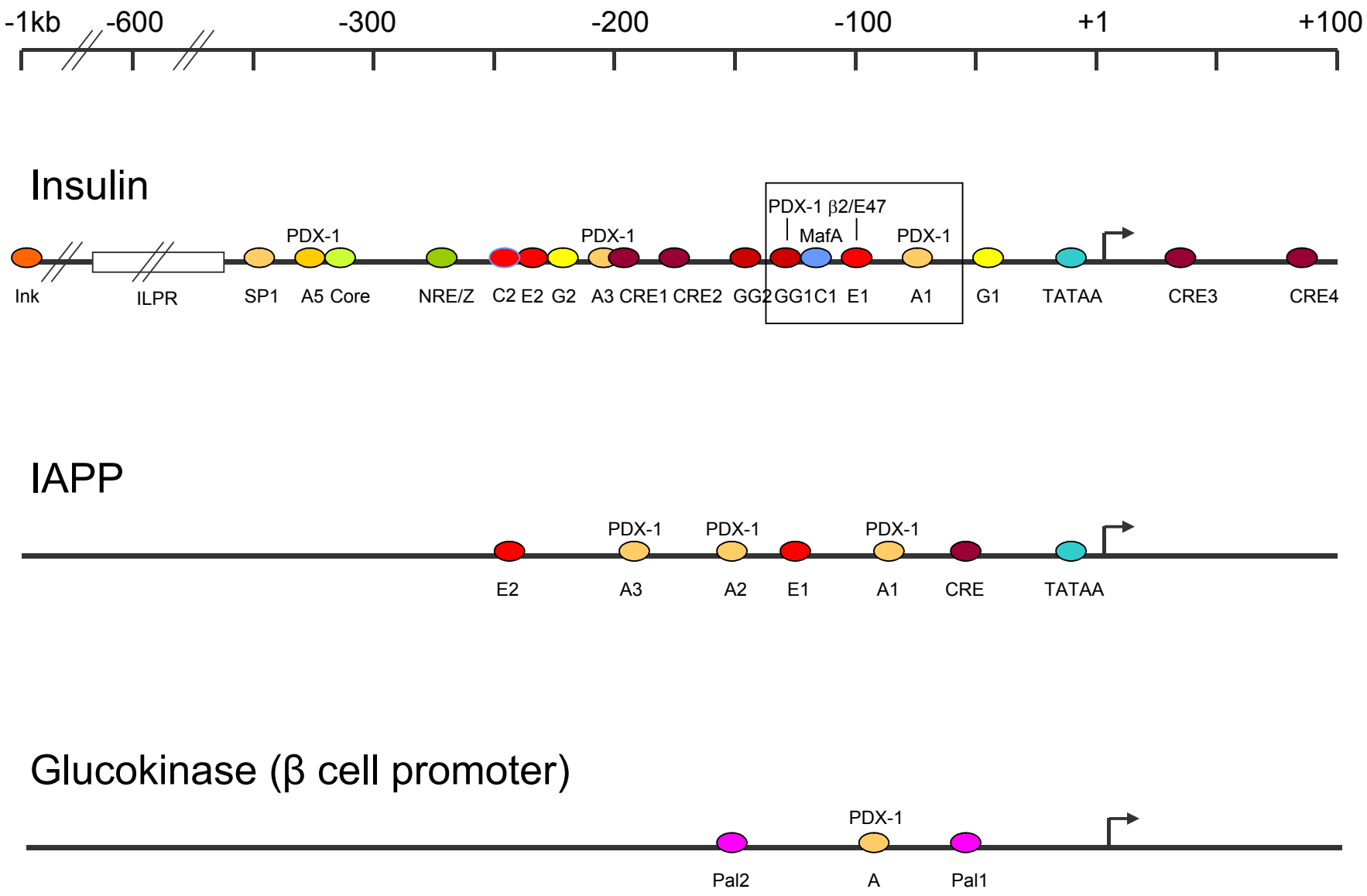


Figure 2

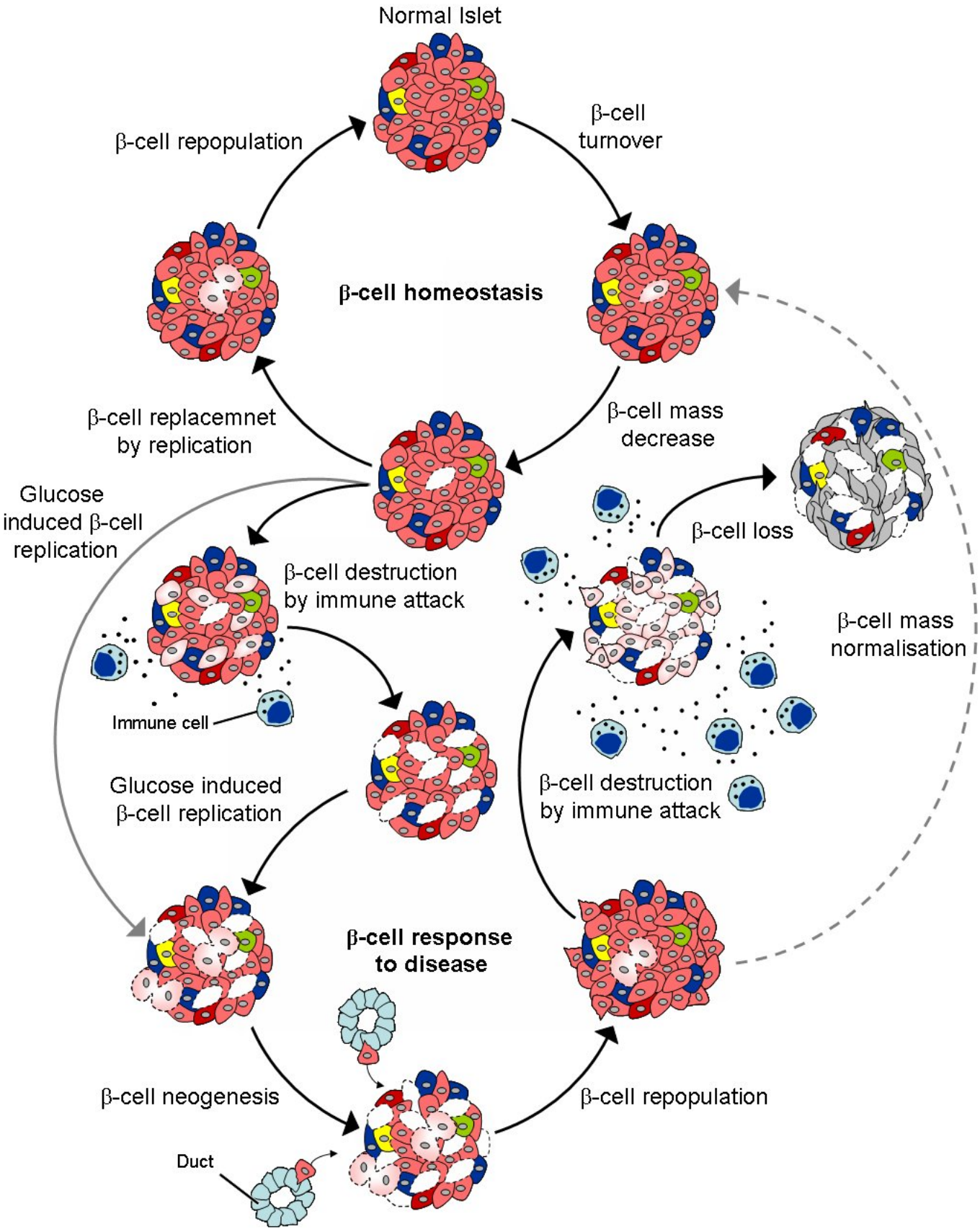


Figure 3