REVIEW

Molecular regulation of pancreatic β-cell mass development, maintenance, and expansion

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Abstract

Pancreatic β-cells are responsible for producing all of the insulin required by an organism to maintain glucose homeostasis. Defects in development, maintenance, or expansion of β-cell mass can result in impaired glucose metabolism and diabetes. Thus, identifying the molecular regulators of these processes may provide new therapeutic targets for diabetes. Additionally, understanding the processes of β-cell differentiation and proliferation may allow for *in vitro* cultivation of β-cells in sufficient amounts to be transplanted into patients with diabetes. This review addresses many of the transcription factors and signaling pathways that play a role in early pancreatic development and endocrine cell (specifically β-cell) differentiation, conditions that influence β-cell mass development and molecular regulators of β-cell proliferation and apoptosis that are responsible for maintaining and expanding β-cell mass.

Journal of Molecular Endocrinology (2007) **38,** 193–206

Introduction

An organism's β-cell mass is determined by the number and the size of its pancreatic β-cells. This unique cell population is required for insulin production, which maintains glucose homeostasis. Diabetes is characterized by either an absolute (type I) or relative (type II) insufficiency of insulin production by β-cells. Existing treatments for diabetes primarily focus on replacing insulin and improving β-cell function. However, increasing a patient's β-cell mass could potentially improve or cure their condition. To this end, islet transplantation has been used to treat some patients with type I diabetes, but limited supply and low yield of islets from donor pancreata prevent widespread use of this therapy. Currently, efforts are being made to differentiate β-cells from precursor populations and to expand β-cells *in vitro* to generate an unlimited supply of β-cells for transplantation. Theoretically, the same could be done *in vivo* to expand a patient's existing or transplanted β-cell population. Thus, it is important to understand the molecular regulation of β-cell mass development, maintenance, and expansion.

Pancreas development

The pancreas originates from the foregut endoderm as ventral and dorsal buds, beginning at embryonic day (e) 9·5 in the mouse, and the two buds later fuse at approximately e12·5 (Kim & MacDonald 2002). The endodermal epithelium proliferates in response to various fibroblast growth factors (FGFs) produced by the adjacent mesenchyme (Bhushan *et al.* 2001), undergoes branching morphogenesis, and differentiates into ductal, exocrine, and endocrine cells. Evagination and development of the ventral pancreatic bud is slightly delayed compared with that of the dorsal bud, and the ventral bud gives rise to fewer endocrine cells than does the dorsal bud (Spooner *et al.* 1970). The ventral and dorsal buds also differ with regard to the signals they require for development. For example, the homeobox 9 (Hb9) transcription factor is required for the formation of the dorsal, but not ventral, bud (Harrison *et al.* 1999, Li *et al.* 1999). Additionally, the LIM homeodomain protein islet 1 (Isl1) is specifically expressed in the mesenchyme surrounding the dorsal, but not ventral, bud at e9·0, as well as later in differentiated endocrine cells throughout the pancreas. Isl1$^{-/-}$ mice reveal that Isl1 is required specifically in the dorsal mesenchyme for the formation of the dorsal pancreatic bud and is required in the endoderm for differentiation of all endocrine cells (Ahlgren *et al.* 1997).

Pancreatic progenitors within the foregut epithelium are marked by the expression of pancreatic-duodenal homeobox 1 (Pdx1), which is induced at e8·5 in the foregut endoderm and is expressed throughout the

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DOI: 10.1677/JME-06-0053
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pancreatic epithelium at e9·5 (Guz *et al.* 1995). Forkhead box A2 (FoxA2), previously called hepatic nuclear factor 3β-(Hnf3β), can activate transcription of Pdx1, although there are many other upstream regulators of Pdx1 (Melloul *et al.* 2002). Pdx1 is required for growth, rather than formation, of the pancreatic buds, as evidenced by Pdx1$^{-/-}$ mice in which both pancreatic buds initially form but then arrest at a very early stage of development, resulting in an apancreatic phenotype at birth (Offield *et al.* 1996). The same phenotype has been observed in a human infant with a homozygous inactivating point mutation of PDX1 (Stoffers *et al.* 1997*b*). Pdx1 expression is downregulated in acinar and ductal cells beginning at approximately e13·0, but is maintained in differentiated endocrine cells and upregulated specifically in β-cells (Guz *et al.* 1995). This expression pattern is maintained throughout life.

Pancreas transcription factor 1a (Ptf1a) is also expressed throughout the developing pancreas beginning at e9·5 and is required for the growth of the pancreatic buds (Krapp *et al.* 1998, Kawaguchi *et al.* 2002). Ptf1a is later downregulated in ductal and endocrine cells, but is maintained in acinar cells throughout life, where it induces expression of amylase and elastase. Ptf1a$^{-/-}$ mice form a rudimentary dorsal pancreas that fails to grow or produce differentiated acinar tissue (Krapp *et al.* 1998). Differentiated endocrine cells are present in these mice, although these cells are reduced in number and found scattered through the adjacent spleen.

β-Cell differentiation

No 'master regulator' of β-cell differentiation has been identified. Instead, the process of β-cell differentiation is a complex pathway requiring the specification of pancreas versus other endodermal organs, endocrine cells versus ductal or exocrine cells, and β-cells versus non-β-endocrine cells (Fig. 1). Proper differentiation of β-cells requires dynamic changes in transcription factor expression levels in appropriate sequences and within an appropriate timeline. Much of what is currently known regarding β-cell differentiation has been discovered by studying endocrine cell differentiation *in vivo*, with the hope that these findings will aid attempts to differentiate β-cells *in vitro*.

In vivo β-cell differentiation

The murine pancreas undergoes two waves of endocrine cell differentiation, the first of which gives rise to glucagon^{+}, insulin^{+}, and double-positive cells between e9·5 and e13·5. These cells appear to be a transient population, however, and lineage tracing studies show that they do not contribute to mature islets (Herrera 2000). The second wave of endocrine differentiation begins at approximately e13·5 and yields endocrine cells that contribute to mature islets (Prasadan *et al.* 2002). Second wave endocrine differentiation, unlike the first wave, relies on the transcription factors Pdx1 and Hnf6 (Offield *et al.* 1996, Jacquemin *et al.* 2000).

Differentiation of endocrine versus exocrine cells is accomplished by lateral inhibition within the ductal epithelium, mediated by the Notch signaling pathway (Apelqvist *et al.* 1999, Jensen *et al.* 2000). The pro-endocrine basic helix–loop–helix (bHLH) transcription factor neurogenin 3 (Ngn3), a downstream target of Hnf6 (Jacquemin *et al.* 2000), induces expression of Notch ligands, which bind to Notch receptors and activate the

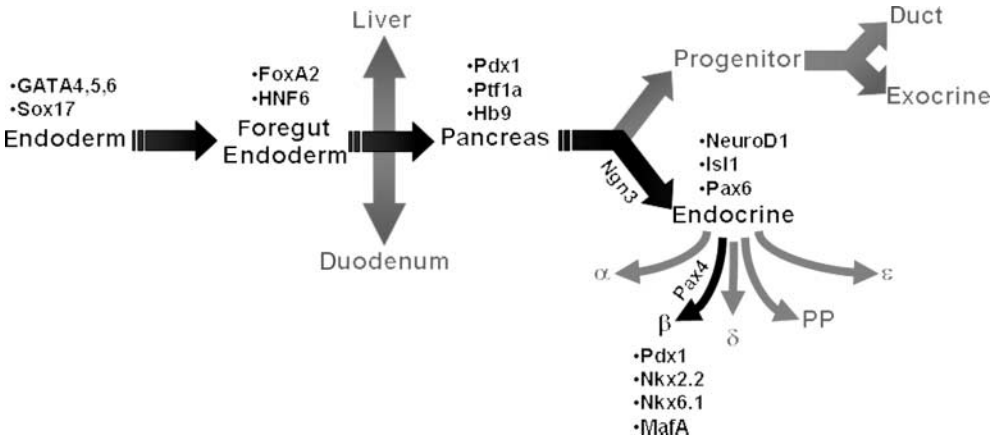

Figure 1 β-Cell differentiation from endoderm. During embryogenesis, the pancreas differentiates from foregut endoderm, which also gives rise to the liver and duodenum. Pancreatic progenitors are specified for an endocrine or non-endocrine fate, after which the non-endocrine progenitors differentiate into either duct or exocrine cells, while the endocrine progenitors differentiate into α-, β-, δ-, PP-, or ε-cells. Transcription factors expressed in each of the cell populations preceding and including β-cells are listed.

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Notch pathway in adjacent cells (Heremans *et al.* 2002). Downstream targets of the Notch pathway (e.g. Hairy/enhancer-of-split 1, Hes1) repress Ngn3 expression, which inhibits endocrine differentiation. Additionally, Hes1 represses the cell cycle inhibitor p57, which maintains a proliferative pool of pancreatic progenitor cells within the embryonic ductal epithelium (Georgia *et al.* 2006). Therefore, cells in which the Notch signaling pathway is activated, maintain their proliferative capacity, while cells in which Notch signaling is not activated, express Ngn3, exit the cell cycle, and differentiate into endocrine cells. Genetic mouse models of impaired Notch signaling (*Dll1*$^{-/-}$, *Rbp-Jκ*$^{-/-}$, *Hes1*$^{-/-}$) exhibit increased endocrine cell differentiation at the expense of the pancreatic progenitor population (Apelqvist *et al.* 1999, Jensen *et al.* 2000).

Ngn3 is required for endocrine cell differentiation, as evidenced by *Ngn3*$^{-/-}$ mice, which lack all pancreatic endocrine cell types and die postnatally due to severe diabetes (Gradwohl *et al.* 2000). Additionally, lineage tracing analysis reveals that all endocrine cells arise from Ngn3^{+} cells (Gu *et al.* 2002). Ngn3 induces expression of essential β-cell transcription factors including neurogenic differentiation 1 (Neurod1), also known as Beta2 (Huang *et al.* 2000), and paired box gene 4 (*Pax4*; Smith *et al.* 2003), but is not itself expressed in hormone-producing cells. The bHLH transcription factor NeuroD1 induces expression of several endocrine genes, including insulin, and its expression is maintained in mature endocrine cells (Naya *et al.* 1995). *NeuroD1*$^{-/-}$ mice are diabetic due to severely reduced numbers of all endocrine cell types (Naya *et al.* 1997), and humans with heterozygous mutations in NEUROD1 suffer from a type of diabetes referred to as maturity-onset diabetes of the young type 6 (MODY6; Kristinsson *et al.* 2001). In contrast, ectopic expression of Ngn3 or NeuroD1 in the pancreatic epithelium results in premature and expansive differentiation of endocrine cells, primarily glucagon-producing α-cells, at the expense of the pancreatic progenitor pool, resulting in a hypoplastic pancreas (Apelqvist *et al.* 1999, Schwitzgebel *et al.* 2000).

Endocrine cells produced by the second wave of differentiation can first be identified at e13·5, forming endocrine cords adjacent to ducts (Pictet *et al.* 1972). These cells delaminate from the ductal epithelium, differentiate, proliferate, and then cluster to form islets, which contain β-cells, α-cells, somatostatin-producing δ-cells, pancreatic polypeptide-producing PP-cells, and ghrelin-producing ε-cells. The regulation of differentiation down each of these endocrine lineages from a Ngn3^{+} cell is complex and still not completely understood. Lineage tracing analysis has revealed that the β- and α-cell lineages diverge early, while β-cells and PP-cells may differentiate from the same lineage (Herrera 2000). Additionally, β- and δ-cells share a requirement for Pax4, as exhibited by *Pax4*$^{-/-}$ mice, which have increased numbers of α-cells at the expense of β- and δ-cells (Sosa-Pineda *et al.* 1997). Pax4 is expressed in early insulin^{+}, but not glucagon^{+}, cells and is later restricted to mature β-cells, but Pax4 alone is not sufficient to drive Ngn3^{+} cells towards either a β- or δ-cell fate (Grapin–Botton *et al.* 2001). A putative antagonist of Pax4 is aristaless-related homeobox (Arx), whose expression pattern and loss-of-function phenotype directly contrast with that of Pax4 (Collombat *et al.* 2003). Arx is expressed in pancreatic progenitor cells beginning at e9·5 and is later restricted to mature α- and δ-cells. *Arx*$^{-/-}$ mice exhibit increased numbers of β- and δ-cells at the expense of α-cells. Furthermore, Arx is upregulated in *Pax4*$^{-/-}$ mice, while Pax4 is upregulated in *Arx*$^{-/-}$ mice. Thus, Pax4 and Arx function in the differential specification of endocrine cell types.

Early broad expression of Pdx1 induces expression of the NK homeodomain transcription factors Nkx2.2 and Nkx6.1, but while Nkx2.2 expression becomes restricted to α-, β-, and PP-cells (Sussel *et al.* 1998), Nkx6.1 expression is tightly restricted to β-cells (Sander *et al.* 2000). *Nkx2.2*$^{-/-}$ mice reveal that Nkx2.2 is absolutely required for β-cell differentiation and plays a lesser role in differentiation of α- and PP-cells (Sussel *et al.* 1998), while *Nkx6.1*$^{-/-}$ mice have impaired but not complete loss of β-cell differentiation (Sander *et al.* 2000). Several pieces of evidence suggest that Nkx2.2 is upstream of Nkx6.1: initiation of expression of Nkx2.2 precedes that of Nkx6.1 (e9·5 vs e10·5 respectively); all Nkx6.1^{+} cells also express Nkx2.2, while not all Nkx2.2^{+} cells express Nkx6.1; *Nkx2.2*$^{-/-}$ mice lack expression of Nkx6.1; and *Nkx2.2*$^{-/-}$; *Nkx6.1*$^{-/-}$ mice exhibit a similar phenotype to *Nkx2.2*$^{-/-}$ mice.

In addition to its early role in pancreas development, Pdx1 plays a role in the terminal differentiation of β-cells by inducing expression of insulin, glucose transporter 2 (*Glut2*), glucokinase, and islet amyloid polypeptide (*Iapp*; Edlund 2001) and is necessary for maintaining mature β-cell function. The basic-leucine zipper (bZIP) transcription factor MafA has also recently been identified as an important regulator of β-cell function and a marker of mature β-cells (Zhang *et al.* 2005*a*). *MafA*$^{-/-}$ mice undergo normal pancreatic development but develop diabetes postnatally, associated with progressively impaired insulin secretion, abnormal islet morphology, and reduced expression of insulin, Pdx1, NeuroD1, and Glut2. Additionally, insulin has been shown to be a direct transcriptional target of MafA (Kataoka *et al.* 2002, Olbrot *et al.* 2002, Matsuoka *et al.* 2004).

In vitro β-cell differentiation

As described above, β-cell differentiation *in vivo* requires that expression of specific transcription factors

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be initiated, maintained, and repressed in a precise temporal and sequential manner, which is difficult to control in an *in vitro* setting. Furthermore, simply inducing insulin expression in a progenitor population does not equate with differentiating β-cells because an important quality of mature β-cells is their ability to sense blood glucose levels and appropriately respond by synthesizing and secreting proper amounts of insulin. These functions require expression of several transporter, receptor, and exocytosis proteins. To overcome these obstacles, many different approaches are being utilized to generate β-cells, as shown in Fig. 2; proliferation of existing β-cells, differentiation of β-cells from embryonic stem (ES) cells, differentiation of β-cells from pancreatic progenitor cells (residing in pancreatic ductal epithelium), and transdifferentiation of β-cells from related cell types (e.g. pancreatic exocrine cells, hepatocytes, intestinal enteroendocrine cells; Bonner-Weir & Weir 2005). Although the prospect of deriving β-cells from ES cells is intriguing, there has been much controversy in the field, and several early studies have since become cautionary tales. For example, initial reports claiming a 10–30% efficiency rate of differentiating β-cells from ES cells failed to confirm insulin mRNA expression, which was later observed in <0·00001% of cells (Rajagopal *et al.* 2003). Improved results have been obtained by applying what has been learned about β-cell differentiation *in vivo*. For example, transfection of ES cells with *Pax4*, and to a lesser extent *Pdx1*, results in increased differentiation of insulin-producing cells and increased expression of Isl1, Ngn3, insulin, Iapp, and Glut2 (Blyszczuk *et al.* 2003). Currently, the preferred method of producing β-cells from ES cells is directed differentiation. Ku *et al.* (2004) showed that stepwise application of various growth factors known to influence β-cell development (FGF, activin, betacellulin, exendin-4, and nicotinamide) induces 2·73% of ES cells to differentiate into insulin-producing cells. More recently, D'Amour *et al.* (2006) has used a similar approach to systematically induce differentiation of endoderm, foregut endoderm, pancreatic endoderm, endocrine precursors, and finally insulin-producing cells, along with the four other endocrine cell types. This method induces 7–12% of ES cells to differentiate into insulin-producing cells, although these cells have limited glucose-induced insulin secretion. Despite these encouraging results, insulin-producing cells derived from ES cells have been shown in some cases to form teratomas after transplantation into diabetic mice (Fujikawa *et al.* 2005).

Isolation and culture of ductal epithelium from adult mouse pancreas can also yield insulin$^+$ cells, as well as islets, through islet producing stem cell (IPSC) and islet progenitor cell (IPC) intermediates (Ramiya *et al.* 2000). Increased islet yield can be obtained by culturing IPSCs with epidermal growth factor (EGF), hepatocyte growth factor (HGF), and nicotinamide. IPSCs can be maintained in culture long-term (>3 years) and can withstand freezing, and the islets derived from them can reverse streptozotocin-induced diabetes in mice. Duct cells have also been isolated from humans, from the normally discarded non-islet fraction of pancreatic

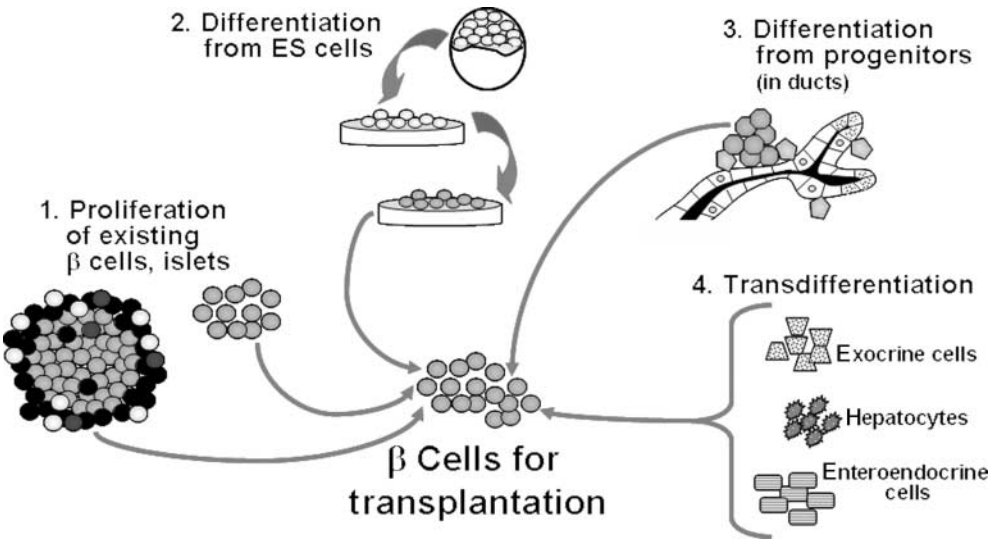

Figure 2 Sources of β-cells for transplantation. A supply of β-cells for transplantation may be derived by inducing proliferation of existing β-cells, either isolated or within islets, by inducing differentiation of ES cells into β-cells, by inducing differentiation of isolated ductal epithelium into β-cells or islets, and by inducing transdifferentiation of related cell types, such as exocrine cells, hepatocytes, or intestinal enteroendocrine cells into β-cells.

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tissue utilized for islet transplantation (Heremans *et al.* 2002). Adenoviral infection of these cells with *Ngn3* or *Neurod1* results in differentiation into primarily insulin$^+$ cells (tenfold over that observed in control cells), although intracellular insulin content is relatively low. Similar results are observed when immortalized ductal cell lines from mice or humans are transfected with *Ngn3* or *Neurod1* (Gasa *et al.* 2004). Whether or not the insulin$^+$ cells and islets that are derived from these various sources are fully differentiated and fully functioning is unknown.

Establishing an organism's β-cell mass

β-Cell mass is increased by β-cell neogenesis (differentiation from precursor cells), β-cell proliferation, and β-cell hypertrophy (increased cell size), and is decreased by β-cell death, primarily through apoptosis, and β-cell atrophy (decreased cell size; Fig. 3). From embryogenesis to adulthood, there is a net increase in β-cell mass as the organism's size increases. β-Cell differentiation, as described above, gives rise to the initial β-cells of an organism during embryogenesis, but there is much debate regarding whether and to what extent β-cell neogenesis occurs in the postnatal and adult stages under normal circumstances. However, β-cell neogenesis has been reported in models of pancreatic injury.

β-Cell proliferation proceeds at a high rate (approximately 10% per day in mice) during late embryogenesis (Bernard-Kargar & Ktorza 2001) but begins to decline postnatally (Scaglia *et al.* 1997). During adulthood, β-cells proliferate at a low rate that may gradually decline with age. Approximately, 1–4% of β-cells replicate per day in rats between 30 and 100 days old (Finegood *et al.* 1995), while <1% of β-cells replicate per day in mice at 1 year of age (Teta *et al.* 2005). The differences observed in the 'rates' of β-cell proliferation at these timepoints is thought to be due to differences in the percentage of β-cells that are able to be recruited to enter the cell cycle, rather than differences in cell cycle lengths. The mechanism by which more β-cells are recruited to enter the cell cycle during embryonic versus postnatal and adult stages is currently unknown. However, evidence from several genetic mouse models indicates that the factors that regulate β-cell proliferation during embryogenesis may differ from those that regulate β-cell proliferation postnatally (Rane *et al.* 1999, Georgia & Bhushan 2004, 2006, Kushner *et al.* 2005*a*, Zhang *et al.* 2006). For example, global deletion of the cell cycle inhibitor $p27^{Kip1}$ ($p27^{Kip1-/-}$) increases β-cell proliferation during embryogenesis and adulthood, but not during the early postnatal period, resulting in increased β-cell mass at birth and throughout life (Georgia & Bhushan 2006).

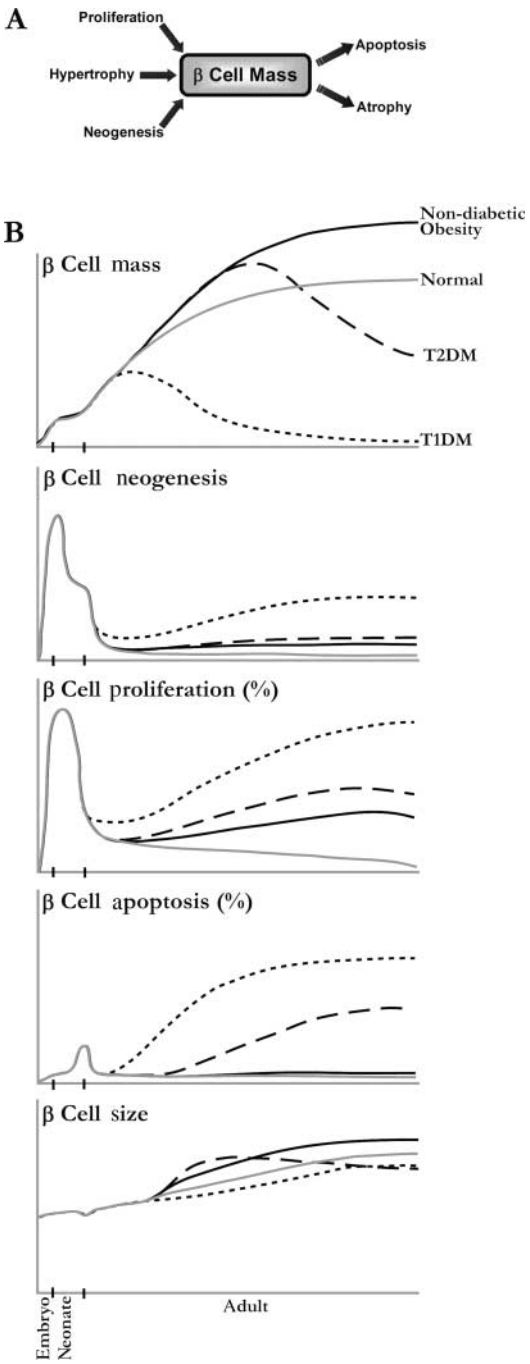

Figure 3 β-Cell mass dynamics. (A) β-Cell proliferation, neogenesis, and hypertrophy (enlarged cell size) increase β-cell mass, while β-cell apoptosis and atrophy (reduced cell size) decrease β-cell mass. (B) Graphs represent approximate changes in these processes over the course of a lifetime in normal individuals (gray solid line), in non-diabetic obesity (black solid line), in type II diabetes mellitus (T2DM; black dashed line), and in type I diabetes mellitus (T1DM; black dotted line), based on rodent and human studies. Embryo denotes the period of time prior to birth. Neonate denotes the period of time between birth and weaning (approximately 3 weeks in the rodent). Figure adapted with permission from Rhodes (2005).

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Table 1 Molecular regulators of postnatal β-cell mass *in vivo*

	Mouse models	Effects	References
Transcription factors			
Pdx1	$Pdx1^{+/-}$	↓β cell mass, ↑β cell apoptosis	Johnson *et al.* (2003)
	$Pdx1^{flox/flox}$; Rip-Cre	↓β cell mass	Ahlgren *et al.* (1998)
Foxm1	$Foxm1^{flox/flox}$; $Pdx1^{4.3}$-Cre	↓β cell mass, ↓β cell proliferation, ↑−↓β cell size, ↔β cell apoptosis	Zhang *et al.* (2006)
Cyclic AMP response element binding protein	$CBP^{S436A/+}$ Tg (↑CREB activity)	↑β cell mass, ↑β cell proliferation, ↔β cell size, ↔β cell apoptosis	Hussain *et al.* (2006)
	HIP-ICER-Iγ Tg (↓CREB activity)	↓β cell mass, ↓β cell proliferation, ↔β cell apoptosis	Inada *et al.* (2004)
	Rip-dnCREB Tg	↓β cell mass, ↑β cell apoptosis	Jhala *et al.* (2003)
E2Fs	$E2F1^{-/-}$	↓β cell mass, ↓β cell proliferation, ↔β cell apoptosis	Fajas *et al.* (2004)
	$E2F1^{-/-}$;$E2F2^{-/-}$	↓β cell mass, ↑β cell proliferation	Iglesias *et al.* (2004)
Nuclear factor of activated T cells, cytoplasmic, calcineurin-dependent 1	Rip-rtTA; $NFATc1^{nuc}$	↑β cell mass, ↑β cell proliferation	Heit *et al.* (2006*a*)
Cell cycle proteins			
Cyclin-dependent kinases	$Cdk4^{R26C/R26C}$ Tg (↑activity)	↑β cell mass	Rane *et al.* (1999)
	$Cdk4^{-/-}$	↓β cell mass	Rane *et al.* (1999)
Cyclins	Rip-cyclin D1 Tg	↑β cell mass, ↑β cell proliferation, ↔β cell apoptosis	Zhang *et al.* (2005*a*,*b*)
	Cyclin $D2^{-/-}$	↓β cell mass, ↓β cell proliferation, ↔β cell apoptosis	Georgia & Bhushan (2004) and Kushner *et al.* (2005*a*)
	Cyclin $D1^{+/-}$; cyclin $D2^{-/-}$	↓β cell mass, ↓β cell proliferation, ↔β cell apoptosis	Kushner *et al.* (2005*a*)
Cyclin-dependent kinase inhibitors	$P16^{INK4a}$ Tg	↓β cell proliferation	Krishnamurthy *et al.* (2006)
	$P16^{INK4a-/-}$	↑β cell proliferation	Krishnamurthy *et al.* (2006)
	$P18^{INK4c-/-}$	↑β cell mass	Pei *et al.* (2004)
	Rip-$p27^{Kip1}$ Tg	↓β cell mass, ↓β cell proliferation, ↔β cell size	Uchida *et al.* (2005)
	$P27^{Kip1-/-}$	↑β cell mass, ↑β cell proliferation, ↔β cell size	Georgia & Bhushan (2006)
Tumor suppressors	$pRb^{flox/flox}$;Rip-Cre	↔β cell mass, ↔β cell proliferation	Vasavada *et al.* (2006)
	$pRb^{+/-}$; $p53^{+/-}$	↑β cell mass	Williams *et al.* (1994)
	$pRb^{+/-}$; $p53^{-/-}$	↑β cell mass	Williams *et al.* (1994)
Growth factors			
Lactogens	Rip-PL1 Tg	↑β cell mass, ↑β cell proliferation, ↑β cell size	Vasavada *et al.* (2000) and Cozar-Castellano *et al.* (2006*a*)
	$PrlR^{-/-}$	↓β cell mass	Freemark *et al.* (2002)
Parathyroid hormone-related protein	Rip-PTHrP Tg	↑β cell mass, ↔β cell proliferation, ↔β cell apoptosis, ↔β cell size	Porter *et al.* (1998)
Growth hormone	$GHR^{-/-}$	↓β cell mass, ↓β cell proliferation, ↓β cell size	Liu *et al.* (2004)
Hepatocyte growth factor	Rip-HGF Tg	↑β cell mass, ↑β cell proliferation	Garcia-Ocaña *et al.* (2000, 2001) and Cozar-Castellano *et al.* (2006*a*)
	c-$Met^{flox/flox}$; Rip-Cre	↔β cell mass, ↔β cell proliferation	Roccisana *et al.* (2005)
Epidermal growth factor	HIP-EGF Tg	↑β cell mass, ↑β cell proliferation	Krakowski *et al.* (1999)
Keratinocyte growth factor	HIP-KGF Tg	↑β cell mass, ↑β cell proliferation	Krakowski *et al.* (1999)
Insulin, insulin-like growth factors	$IR^{flox/flox}$; Rip-Cre	↓β cell mass	Otani *et al.* (2004)
	Rip-IGF-I Tg	↔β cell mass, ↑β cell proliferation, ↔β cell apoptosis, ↔β cell neogenesis	George *et al.* (2002)
	IGF-$I^{flox/flox}$; $Pdx1^{4.3}$-Cre	↑β cell mass, ↑β cell size	Lu *et al.* (2004)
	IGF-$IR^{flox/flox}$; Rip-Cre	↔β cell mass	Kulkarni *et al.* (2002)
	IGF-II Tg	↑β cell mass, ↑β cell proliferation, ↓β cell apoptosis, ↔β cell size	Petrik *et al.* (1999)
	Rip-IGF-II Tg	↑β cell mass	Devedjian *et al.* (2000)
Fibroblast growth factors	Pdx1-dnFGFR1c Tg	↓β cell mass, ↔β cell apoptosis	Hart *et al.* (2000)
	Pdx1-dnFGFR2b Tg	↔β cell mass	Hart *et al.* (2000)

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Table 1 *Continued*

	Mouse models	Effects	References
Vascular endothelial growth factors	Rip-VEGF-A Tg	↔β cell mass	Gannon *et al.* (2002)
	VEGF-A$^{flox/flox}$; Rip-Cre	↔β cell mass, ↑β cell size	Inoue *et al.* (2002)
Incretins	GLP-1R$^{-/-}$	↔β cell mass	Ling *et al.* (2001)
	GIPR$^{-/-}$	↑β cell mass	Pamir *et al.* (2003)
Gastrin	Rip-Gastrin Tg	↔β cell mass	Wang *et al.* (1993)
	Gastrin$^{-/-}$	↔β cell mass, ↔β cell proliferation	Boushey *et al.* (2003)
Cell signaling proteins			
Insulin response substrate	Rip-Irs2 Tg	↑β cell mass, ↔β cell proliferation, ↔β cell size	Hennige *et al.* (2003)
	Irs2$^{-/-}$	↓β cell mass, ↑β cell apoptosis	Withers *et al.* (1998, 1999) and Kubota *et al.* (2000)
	Irs2$^{flox/flox}$; Rip-Cre	↑β cell mass, ↑β cell proliferation, ↔β cell apoptosis	Kubota *et al.* (2004)
Protein kinase B/Akt	caAkt Tg	↓β cell mass, ↓β cell proliferation, ↑β cell size	Fatrai *et al.* (2006)
	Rip-caAkt Tg	↑β cell mass, ↑β cell proliferation, ↑β cell size, ↑β cell neogenesis	Bernal-Mizrachi *et al.* (2001)
	Rip-caAkt Tg	↑β cell mass, ↑β cell size, ↑β cell apoptosis, ↔β cell proliferation	Tuttle *et al.* (2001)
	Rip-kdAkt Tg	↔β cell mass, ↔β cell apoptosis, ↔β cell size	Bernal-Mizrachi *et al.* (2004)
p70S6K	P70$^{S6K1-/-}$	↓β cell mass, ↓β cell size	Pende *et al.* (2000)
Phosphoinositide-dependent kinase	PDK1$^{flox/flox}$; Rip-Cre	↓β cell mass, ↓β cell proliferation, ↓β cell size, ↑β cell apoptosis	Hashimoto *et al.* (2006)
Others			
Menin	Men1$^{+/-}$	↑β cell mass, ↑β cell proliferation	Karnik *et al.* (2005)
	Men1$^{flox/flox}$; Rip-Cre	↑β cell mass, ↑β cell proliferation, ↔β cell apoptosis	Crabtree *et al.* (2003)
Pkr-like ER kinase	PERK$^{-/-}$	↓β cell mass, ↑β cell apoptosis	Harding *et al.* (2001) and Zhang *et al.* (2002)
Phosphatase and tensin homologue	PTEN$^{+/-}$	↓β cell mass, ↓β cell proliferation	Kushner *et al.* (2005*b*)
	PTEN$^{flox/flox}$; Rip-Cre	↑β cell mass, ↑β cell proliferation, ↓β cell apoptosis, ↔β cell size	Stiles *et al.* (2006)
Calcineurin b1	Cnb1$^{flox/flox}$; Rip-Cre	↓β cell mass, ↓β cell proliferation, ↔β cell apoptosis	Heit *et al.* (2006*a*)

Cre, Cre recombinase; Rip, rat insulin promoter; HIP, human insulin promoter; rtTA, reverse tetracycline transactivator; Tg, transgenic; dn, dominant negative; ca, constitutively active; kd, kinase dead; ↔, no change.

β-Cell apoptosis occurs at very low rates during embryogenesis, but there is evidence that a transient burst of β-cell apoptosis occurs at weaning, which may be associated with islet remodeling and/or changes in β-cell maturation (Scaglia *et al.* 1997). During adulthood, β-cell apoptosis also normally occurs at very low rates. Average β-cell size is fairly stable during the postnatal period (Scaglia *et al.* 1997), but it increases with age during later adulthood (Montanya *et al.* 2000).

Proper development of an organism's β-cell mass requires appropriate nutrition during embryogenesis. Poor maternal nutrition results in intrauterine growth retardation (IUGR), low birth weight, and underdeveloped β-cell mass in newborn rats, which predisposes them to glucose intolerance and diabetes later in life (Breant *et al.* 2006). When IUGR is caused by total caloric restriction, the reduction in β-cell mass is due to reduced β-cell differentiation, with decreased expression of Pdx1, Pax6, and Nkx6.1, rather than due to reduced β-cell proliferation. These changes are associated with increased levels of glucocorticoids, which can independently reduce fetal β-cell mass when exogenously administered to the mother. IUGR caused by protein restriction also results in underdeveloped β-cell mass in newborn rats, but this is due to reduced β-cell proliferation and increased β-cell apoptosis, associated with reduced levels of insulin-like growth factors (IGFs; Reusens & Remacle 2006). In contrast, maternal obesity and/or diabetes results in newborn macrosomia (large birth weight) and increased β-cell mass associated with increased β-cell proliferation, likely in response to maternal hyperglycemia. These offspring are predisposed to obesity, insulin resistance, and diabetes, associated with early β-cell exhaustion.

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Maintaining an organism's β-cell mass

Although it was once thought that an organism was born with all of the β-cells it would ever have, prevailing evidence now shows that new β-cells can form throughout life. Several studies have revealed that the primary mechanism by which new β-cells form during adulthood is through proliferation rather than neogenesis (Dor *et al.* 2004, Georgia & Bhushan 2004). Thus, organisms born with reduced β-cell mass, as discussed above, have fewer β-cells available to enter the cell cycle later in life. Under normal circumstances during adulthood, β-cells are a slowly renewing population, with steady low levels of proliferation and apoptosis. However, β-cell mass continuously expands over the lifespan of an organism (Montanya *et al.* 2000), likely due to age-related increases in body weight and insulin resistance. In rats, β-cells achieve this progressive increase in β-cell mass by increasing their cell size, rather than increasing proliferation (Montanya *et al.* 2000).

The ability of an organism to maintain its β-cell mass during adulthood is paramount to maintaining glucose homeostasis and preventing diabetes. Table 1 summarizes the mouse models in which molecular regulators of postnatal β-cell mass are perturbed. Several genetic mouse models of cell cycle dysregulation impair postnatal β-cell proliferation, and cause a progressive decline in β-cell mass, associated with a progressive glucose intolerant and diabetic phenotype. For example, global inactivation of cyclin-dependent kinase 4 in mice ($Cdk4^{-/-}$) specifically affects endocrine cells within the pancreas, causing diabetes by 2 months of age, and within the pituitary, causing reduced body size and infertility (Rane *et al.* 1999). The diabetes observed in these mice is associated with severely reduced β-cell mass, although β-cell mass is comparable with that of wild-type mice at postnatal days (P) 1 and 2. Progression from G_1 to S phase in the cell cycle requires phosphorylation of the retinoblastoma protein (Rb) by Cdk4/6 complexed with a D cyclin, which releases E2F allowing it to activate transcription of necessary cell cycle target genes. Thus, Cdk6 function is redundant with Cdk4; however, β-cells do not express detectable levels of Cdk6 (Martin *et al.* 2003), making them uniquely susceptible to cell cycle perturbations caused by loss of Cdk4. Similarly, global deletion of cyclin D2 ($CyclinD2^{-/-}$), the predominant D cyclin expressed in β-cells, fails to stimulate adequate compensatory up-regulation of cyclin D1 or D3 within islets and drastically impairs postnatal β-cell proliferation (Georgia & Bhushan 2004, Kushner *et al.* 2005*a*). $CyclinD2^{-/-}$ mice exhibit normal β-cell mass at e17·5 but significantly reduced β-cell mass postnatally, which causes progressive glucose intolerance and diabetes.

Cdk inhibitor proteins (Cips, Kips, INKs) also play important roles in β-cell proliferation. Transgenic overexpression of $p27^{Kip1}$ within β-cells using the rat insulin promoter ($Rip\text{-}p27^{Kip1}$) impairs β-cell proliferation, resulting in decreased β-cell mass and diabetes in mice (Uchida *et al.* 2005). In contrast, as mentioned before, global deletion of $p27^{Kip1}$ ($p27^{Kip1-/-}$) increases β-cell proliferation under normal circumstances (Georgia & Bhushan 2006), as well as in genetic models of insulin resistance and diabetes ($Irs2^{-/-}$ or *db/db*), the latter of which restores glucose homeostasis (Uchida *et al.* 2005). β-Cell hyperplasia is also observed in humans with focal loss of heterozygosity of $p57^{Kip2}$, and these subjects suffer from hyperinsulinism of infancy (Kassem *et al.* 2001). Additionally, mice that express a mutant Cdk4, which cannot be bound and inhibited by $p16^{INK4a}$ ($Cdk4^{R24C/R24C}$), exhibit postnatal increases in β-cell proliferation and β-cell mass that improve glucose regulation (Rane *et al.* 1999).

Our laboratory has recently found that FoxM1 also plays an important role in β-cell proliferation (Zhang *et al.* 2006). FoxM1 is known to regulate expression levels of several cell cycle proteins: cyclin B, which complexes with Cdk1 to promote G_2 to M phase progression; Cdc25A and B phosphatases, which dephosphorylate and activate Cdks; and S-phase kinase-associated protein 2 (Skp2) and Cdk subunit 1 (Cks1), which together form the Skp1-Cullin1-F-box protein (SCF) ubiquitin ligase complex that targets $p27^{Kip1}$ and $p21^{Cip1}$ for proteasomal degradation. Pancreas-specific deletion of *Foxm1* using a Cre-lox strategy ($Foxm1^{flox/flox}$; $Pdx1^{4.3}$-*Cre*) in mice results in postnatal defects in β-cell proliferation, which contribute to a postnatal deficiency in β-cell mass and a progressive glucose intolerant and diabetic phenotype. However, β-cell mass and islet morphology are normal at P1, despite inactivation of *Foxm1* early in embryogenesis. Increased nuclear $p27^{Kip1}$, an indirect target of FoxM1, is associated with impaired β-cell proliferation in these mice. Thus, FoxM1 is critical for maintaining β-cell mass during adulthood by properly coordinating cell cycle progression.

Another regulator of cell cycle genes is the transcriptional co-activator menin, encoded by *Men1*, mutation of which results in multiple endocrine neoplasia type 1 (MEN1) in humans (Crabtree *et al.* 2003). This syndrome is characterized by hyperplasia of endocrine cell types primarily within the parathyroids, anterior pituitary, and pancreas, resulting in tumor formation, most commonly insulinomas composed of β-cells. $Men1^{+/-}$ mice display a similar phenotype. β-Cell-specific deletion of *Men1* ($Men1^{flox/flox}$; *Rip-Cre*) also results in β-cell hyperplasia, insulinomas, hyperinsulinemia, and hypoglycemia. Significantly increased rates of β-cell proliferation are observed, and this is associated with reduced levels of $p18^{INK4c}$ and $p27^{Kip1}$, both of which are direct targets of menin-mediated histone methylation (Karnik *et al.* 2005). Furthermore, a MEN1-like phenotype is observed in $p18^{INK4c-/-}$;

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$p27^{Kip1-/-}$ mice (Franklin *et al.* 2000). Other mouse models of impaired and enhanced β-cell proliferation have been reviewed by Cozar-Castellano *et al.* (2006*b*) and Heit *et al.* (2006*b*).

Just as impaired β-cell proliferation causes a net loss of β-cells, increased β-cell death can have the same effect. Inherent defects that make β-cells more susceptible to apoptosis, for example, result in a negative balance of β-cell turnover, as observed in $Pdx1^{+/-}$ mice, which exhibit normal β-cell mass at 3 months of age, but are unable to appropriately increase their β-cell mass as they age (Johnson *et al.* 2003). Haploinsufficiency of *Pdx1* makes β-cells more susceptible to undergoing apoptosis, associated with reduced expression levels of the antiapoptotic genes Bcl_{XL} and *Bcl-2*. Increased β-cell apoptosis results in insufficient β-cell mass and progressive glucose intolerance. A more dramatic phenotype is observed in mice with β-cell-specific deletion of *Pdx1* ($Pdx1^{flox/flox}$; *Rip-Cre*; Ahlgren *et al.* 1998). These mice suffer from worsening glucose intolerance due to both progressive loss of β-cell mass and impaired β-cell function. Mutations of *PDX1* in humans result in similar phenotypes; a dominant-negative mutation of *PDX1* causes MODY4 (Stoffers *et al.* 1997*a*), while a heterozygous inactivating mutation of *PDX1* predisposes to late-onset type II diabetes (Macfarlane *et al.* 1999). Thus, Pdx1 is a critical regulator of β-cell survival and maintenance of β-cell mass and function during adulthood.

Dynamic changes in an organism's β-cell mass

In addition to maintaining β-cell mass under normal circumstances, as just discussed, an organism must also be able to alter its β-cell mass in accordance with its requirements for insulin. In states of insulin resistance, such as pregnancy and obesity, β-cell mass is known to increase (Rhodes 2005). Such β-cell mass expansion is accomplished primarily by increasing β-cell proliferation, although neogenesis may also contribute. However, when compensatory β-cell mass expansion is inadequate, diabetes ensues – gestational diabetes in the case of pregnancy, and type II diabetes in the case of obesity. Although the majority of humans do not become diabetic in these circumstances, a significant portion of the population is predisposed to β-cell failure, for currently unknown reasons. It is likely that factors that regulate β-cell proliferation may play a role, although whether the factors that regulate β-cell mass expansion are the same as those that regulate β-cell mass maintenance is unclear.

During pregnancy, rats exhibit a greater than 50% increase in β-cell mass, which is accomplished primarily through an approximate threefold increase in β-cell proliferation (Scaglia *et al.* 1995). The chief stimuli of β-cell proliferation during pregnancy are placental lactogens (PLs), although prolactin (Prl) and growth hormone (GH) also have similar effects on β-cells and are also elevated during pregnancy. After delivery, β-cell mass returns to normal levels within 10 days through increased β-cell apoptosis, decreased β-cell proliferation, and β-cell atrophy.

To determine the direct role of PLs on β-cell mass in non-pregnant animals, transgenic mice expressing PL1 within their β-cells (*Rip-Pl1*) were developed (Vasavada *et al.* 2000, Cozar-Castellano *et al.* 2006*a*). These mice exhibit hypoglycemia and improved glucose clearance due to hyperinsulinemia, which is associated with a doubling of β-cell mass. This expansion of β-cell mass is attributed to a twofold increase in β-cell proliferation and a 20% increase in β-cell size. Similar results are observed in transgenic mice expressing HGF within their β-cells (*Rip-Hgf*; Garcia-Ocaña *et al.* 2000, 2001, Cozar-Castellano *et al.* 2006*a*). These mice also exhibit a doubling of β-cell mass, but the increase in β-cell proliferation is not as significant as that in *Rip-Pl1* mice. Interestingly, islet number is significantly increased in *Rip-Hgf* mice but not in *Rip-Pl1* mice versus wild-type mice, suggesting that HGF may stimulate neogenesis.

Diet-induced obesity results in insulin resistance and β-cell mass expansion in humans and mice. The C57Bl/6 mouse strain is notoriously susceptible to these effects, exhibiting a 2·2-fold increase in β-cell mass and proliferation after 4 months on a high-fat diet versus a control diet (Sone & Kagawa 2005). However, these mice eventually become diabetic and lose their β-cell mass due to increased β-cell apoptosis and reduced β-cell proliferation.

In genetic models of obesity and insulin resistance, there is also a compensatory expansion of β-cell mass. For example, *db/db* mice, which lack a functional leptin receptor, exhibit a twofold increase in β-cell mass by 8 weeks of age (Wang & Brubaker 2002). This timepoint correlates with the onset of diabetes, which progresses from glucose intolerance that is first observed between 4 and 6 weeks of age. A similar rat model, the Zucker diabetic fatty (ZDF) rat (*fa/fa*), also has a homozygous mutation in the gene encoding the leptin receptor. ZDF rats exhibit increased β-cell mass and increased β-cell proliferation prior to the onset of diabetes, but increased β-cell apoptosis prevents them from adequately expanding their β-cell mass after the onset of diabetes, despite continued high rates of β-cell proliferation (Pick *et al.* 1998). This phenotype contrasts with what is observed in non-diabetic Zucker fatty (ZF) rats, which possess the same mutation as ZDF rats and also become obese and insulin resistant but do not develop diabetes due to sufficient β-cell mass expansion through increased β-cell proliferation, neogenesis, and hypertrophy (Pick *et al.* 1998).

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Another model of insufficient β-cell mass expansion is the insulin receptor substrate two null mouse ($Irs2^{-/-}$; Kubota *et al.* 2004). Global inactivation of Irs2 results in severe insulin resistance, both centrally in the brain causing obesity, and peripherally, for which β-cell mass expansion should be able to compensate. However, because β-cells require Irs2 for proper proliferation and function, $Irs2^{-/-}$ mice are unable to expand their β-cell mass, and they develop diabetes by 10 weeks of age. This phenotype is not observed in $Irs1^{-/-}$ mice, despite the fact that these mice exhibit similar insulin resistance, because Irs1 is not required for β-cell mass expansion. Deletion of *Irs2* specifically within β-cells and the hypothalamus ($Irs2^{flox/flox}$; *Rip-Cre*) causes central insulin resistance and obesity, with glucose intolerance developing at 8 weeks of age but without progression to diabetes, likely due to the lack of peripheral insulin resistance (Kubota *et al.* 2004). These mice also exhibit reduced β-cell proliferation and β-cell mass at 8 weeks of age, although these impairments are not observed before the onset of insulin resistance (between 4 and 8 weeks). These experiments provide additional evidence that Irs2 is required for β-cell mass expansion in response to insulin resistance. Furthermore, overexpression of Irs2 in β-cells (*Rip-Irs2*) is sufficient to prevent β-cell failure in diet-induced obesity and streptozotocin-induced diabetic models (Hennige *et al.* 2003).

Several downstream effectors of the insulin signaling pathway have been shown to play a role in β-cell mass expansion in models of insulin resistance. For example, haploinsufficiency of FoxO1 ($Foxo1^{+/-}$) restores β-cell mass and proliferation to nearly normal levels in $Irs2^{-/-}$ mice, possibly due to reduced FoxO1-mediated repression of *Pdx1* (Kitamura *et al.* 2002). In contrast, expression of constitutively nuclear FoxO1 prevents β-cell mass expansion in two other models of insulin resistance (Okamoto *et al.* 2006).

Therapeutic implications

In understanding how β-cell mass is developed, maintained, and manipulated, we seek to better understand diabetes etiology, identify new and optimal therapeutic targets, and develop new therapeutic techniques. Out of necessity, much of the work in this field has been and is being performed in lower mammals, and thus much of it must still be confirmed in humans. Furthermore, outside fields, such as gene therapy and immunology, must make substantial progress before some clinical interventions can be feasible. However, there remains great optimism regarding the future ability to manipulate β-cell differentiation and proliferation *in vitro* to provide an unlimited supply of β-cells for transplantation into patients with diabetes. Although studies in this area have revealed that it is difficult to achieve full differentiation and maturation of β-cells, which is essential for clinical application, techniques are continuously being improved. Tumor formation, either from ES cell-derived cells or from induction of β-cell proliferation, which may induce transient de-differentiation, is another concern that must be considered. However, ultimately, the processes of β-cell differentiation and proliferation will one day likely be controlled *in vivo* as a means to treat or prevent diabetes.

Acknowledgements

We would like to thank Elizabeth Tweedie for critical reading of the manuscript and helpful suggestions. Due to space restrictions, we were unable to include all studies relevant to β cell mass regulation. There are many laboratories that are contributing greatly to this field of research, and we regret that we could not refer to all of them. M G is supported by a grant from the NIH/NIDDK (R01 DK071052-01) The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

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Received in final form 10 November 2006
Accepted 4 December 2006
Made available online as an Accepted Preprint 12 December 2006

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