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Review

Islet dynamics: A glimpse at beta cell proliferation

Pinar Yesil and Eckhard Lammert

Max Planck Institute of Molecular Cell Biology and Genetics (MPI-CBG), Dresden, Germany

Summary. Pancreatic islets consist of 60-80% beta cells, which secrete insulin, a hormone of profound importance in the regulation of carbohydrate, fat and protein metabolism. Beta cell death and/or dysfunction result in an insufficient amount of insulin that leads to high glucose levels in the blood, a metabolic disorder known as *Diabetes mellitus*. Many studies aiming to establish new therapeutic applications for this disorder are targeted at understanding and manipulating the mechanisms of beta cell proliferation and function. The present comprehensive review summarizes the advances in the field of beta cell renewal and focuses on three fundamental issues: (i) identification of the cellular origins of new beta cells in the adult, (ii) regulation of beta cell proliferation, and (iii) downstream signaling events controlling the cell cycle machinery. Although the source of new adult beta cells is still being debated, recent findings in mice show an important contribution of beta cell proliferation to adult beta cell mass. In conjunction with describing characterized beta cell mitogens and components of the beta cell cycle machinery, we discuss how manipulating the proliferative potential of beta cells could provide novel methods for expanding beta cell mass. Such an expansion could be achieved either through *in vitro* systems, where functional beta cells could be generated, propagated and further used for transplantation, or *in vivo*, through directed beta cell renewal from sources in the organism. Once established, these methods would have profound benefits for diabetic patients.

Key words: Pancreas, Islet, Beta cell, Proliferation, Diabetes

Offprint requests to: Eckhard Lammert, Max Planck Institute of Molecular Cell Biology and Genetics Pfotenhauerstr. 108, 01307 Dresden, Germany. e-mail: lammert@mpi-cbg.de

Introduction

Type I diabetes is caused by autoimmune destruction of pancreatic beta cells (Kukreja and Maclaren, 1999), the only cell type in the mammalian organism that synthesizes and secretes insulin. In contrast, type II diabetes is characterized by insulin resistance, as well as relative deficiency of insulin secretion (DeFronzo, 1997). Beta cells are a major target of therapeutic strategies to treat diabetes and are extensively studied in terms of their differentiation, function, and maintenance. In response to altered metabolic demands in an organism, beta cell mass dynamically changes. A marked increase in beta cell number occurs under conditions that include obesity (Kloppel et al., 1985; Pick et al., 1998), insulin resistance (Bruning et al., 1997), partial pancreatectomy (Bonner-Weir et al., 1983), and pregnancy (Parsons et al., 1992). Conversely, this number decreases in the postpartum period (Marynissen et al., 1983). The increase in beta cell mass can take place either through an increase in the cell number by neogenesis and proliferation (hyperplasia), or through an increase in the cell volume (hypertrophy) (Bonner-Weir, 2000). Currently, there is considerable interest in establishing methods to increase beta cell mass by upregulating beta cell proliferation. As discussed in detail below, proliferation of preexisting beta cells is now considered to be an important source of newly derived adult pancreatic beta cells in mice (Dor et al., 2004; Brennand et al., 2007; Teta et al., 2007). In this regard, it is crucial to identify factors regulating the cell cycle machinery of these preexisting proliferative beta cells and to be able to manipulate their proliferation. It is conceivable that this knowledge could lead to the development of methods to increase beta cell mass in diabetic patients, thus providing the missing machinery to respond to their metabolic demands.

Sources of new adult beta cells

During embryonic development, beta cells originate from a distinct population of neurogenin3 (Ngn3)-positive progenitor cells (Gradwohl et al., 2000; Edlund, 2002; Gu et al., 2002). Subsequently, during the late fetal gestation period, there is a massive increase in beta cell mass, possibly due to neogenesis from non-endocrine Ngn3-positive progenitor cells (McEvoy and Madson, 1980; Swenne and Eriksson, 1982). Beta cell mass continues to increase throughout the neonatal period, both by replication of differentiated beta cells and neogenesis (Bouwens et al., 1994). In the postnatal period, however, it has been demonstrated that the primary mechanism of beta cell mass expansion is replication rather than neogenesis (Dor et al., 2004; Georgia and Bhushan, 2004; Bouwens and Rooman, 2005). Several studies have shown that new beta cells continue to form during adulthood, but with a much slower expansion rate compared to the fetal and neonatal period (Finegood et al., 1995; Montanya et al., 2000). The sources of new adult beta cells, and the extent to which they contribute to beta cell mass turnover and expansion, are still being debated (Fig. 1).

New adult beta cells can form either by proliferation of preexisting beta cells or by neogenesis from stem/progenitor cells and transdifferentiated cells. Regarding neogenesis, a large body of evidence suggests that new adult beta cells are generated from pancreatic progenitor cells residing in the epithelium of the pancreatic ducts (Bouwens and Pipeleers, 1998; Bonner-Weir et al., 2000, 2004; Katdare et al., 2004). It has been demonstrated that, following partial pancreatectomy in rats, regeneration of the pancreas takes place through proliferation of preexisting beta cells (Brockenbrough et al., 1988) and the formation of new acinar and islet cells from expanded ducts (Bonner-Weir et al., 1993; Bonner-Weir, 2000). Furthermore, through adenovirus-mediated delivery of genes including NeuroD and Ngn3, the duct cells can be induced to express insulin (Noguchi et al., 2006). Considering the potential of these pancreatic duct cells to serve as progenitors for new beta cells in the

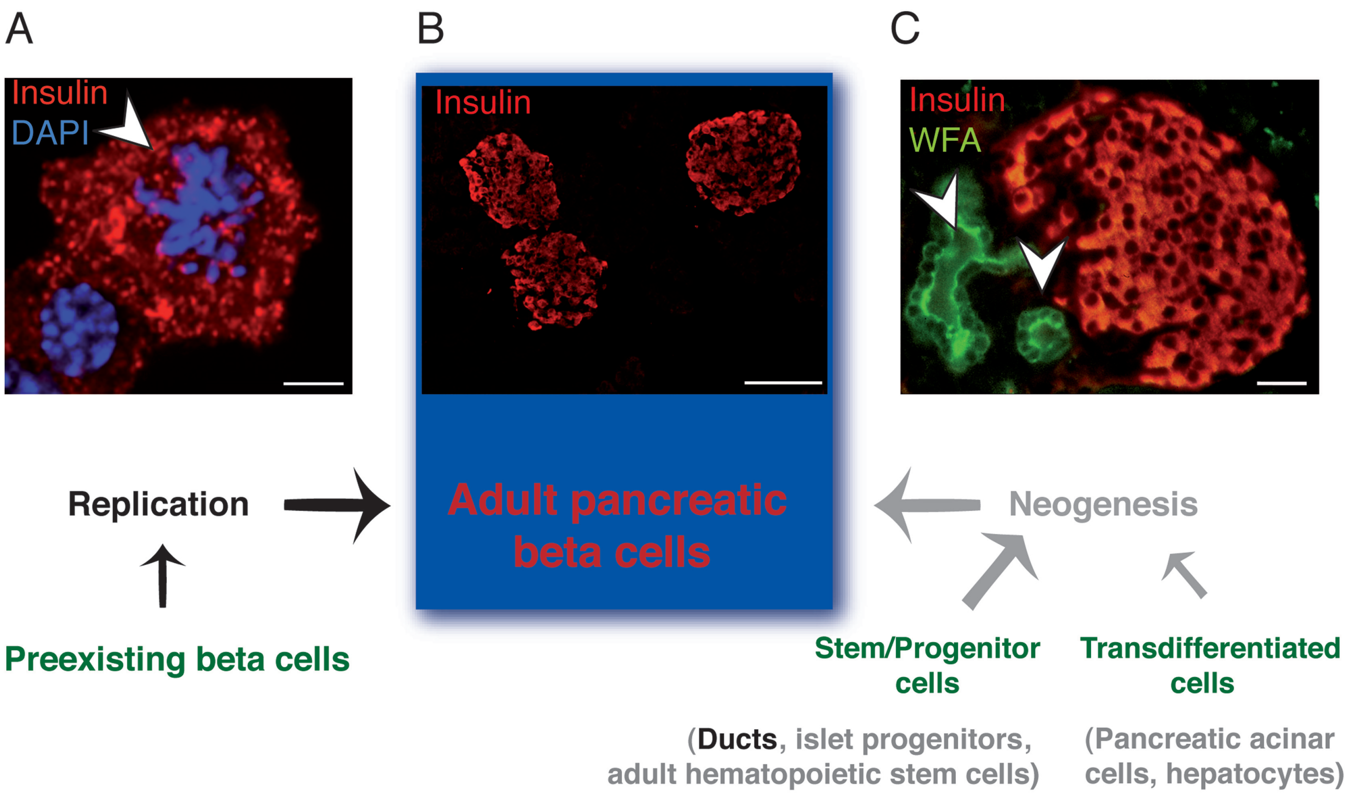

Fig. 1. Potential sources of new adult pancreatic beta cells. Pancreatic beta cell mass can expand via proliferation of already existing beta cells or neogenesis. Emerging evidence from experiments in mice suggests a crucial contribution of the preexisting beta cells to adult beta cell mass. Neogenesis is proposed to contribute through differentiation of ducts, and possibly also islet progenitor cells, adult hematopoietic stem cells, and transdifferentiation of exocrine pancreas and liver cells. (A) Confocal image of mouse insulinoma cell line MIN6 (a model cell line for beta cells) stained for cell nuclei (DAPI, blue) and insulin (red). The arrowhead points to a dividing MIN6 cell. Scale bar: 5 μm. Image by Irena Konstantinova. (B) Confocal image of pancreatic islets stained for insulin (red). Scale bar: 100 μm. Image by Pinar Yesil. (C) Confocal image of a duct (as pointed out by the arrowhead) in close proximity to an islet. Stained for *Wisteria floribunda* agglutinin (WFA) in green, and insulin in red. Scale bar: 20 μm. Image by Eckhard Lammert.

adult, their manipulation constitutes a very promising therapeutic approach for diabetes.

Other works that provide supporting evidence to the neogenesis theory suggest that new beta cells originate from intra-islet progenitor cells, which have a high replicative potential (Swenne, 1983; Bonner-Weir, 1992; Guz et al., 2001; Zulewski et al., 2001) or, alternatively, from hematopoietic cells (Ianus et al., 2003). There are also some reports suggesting that transdifferentiation from hepatocytes (Sapir et al., 2005) and pancreatic acinar cells (Lipsett and Finegood, 2002) represent additional pathways that may lead to adult beta cell formation.

On the other hand, lineage tracing in adult mice has shown that beta cells form by self-duplication (Dor et al., 2004). Recent studies, performed by a novel DNA-analog based lineage-tracing technique (Teta et al., 2007), as well as by label retaining and clonal analysis (Brennand et al., 2007), also support the idea that the beta cell population in adult mice forms, for the most part, by proliferation of preexisting beta cells. These results do not completely exclude neogenesis as a source of new adult beta cells, but indicate that preexisting beta cells in the mouse islet proliferate and make a major contribution to expanding or maintaining adult beta cell mass. The study by Brennand et al. (2007) also provides evidence that, at least in mice, a separate population of highly replicative beta cells that gives rise to large clones does not exist. Instead, the beta cell population is rather homogeneous regarding replicative potential and all beta cells contribute equally to beta cell mass expansion. Further and more demanding research is necessary to investigate whether these findings are relevant for adult human beta cell formation. However, the sole finding that beta cells in the pancreatic islet possess proliferative potential and are the major source of new adult beta cells encourages future research into the mechanisms regulating the rate of their proliferation.

Another viewpoint on the complex mechanism of beta cell expansion was provided by Gershengorn et al. (2004). These authors suggested that fibroblast-like cells derived from human islets have no hormone expression and divide rapidly in culture, adopting a mesenchymal state. These cells can subsequently be induced to differentiate and express insulin as well as other islet hormones, thus establishing an epithelial state. However, recent studies using mouse islets have provided evidence that the source of these fibroblast-like cells is not the beta cell population undergoing epithelial-to-mesenchymal transition (Chase et al., 2007; Morton et al., 2007).

Taken together, there has been a considerable advance in our knowledge about potential sources of new adult beta cells. However, many contradictions and debates remain. Identification of all possible sources of new pancreatic beta cells in the adult is crucial for developing regenerative therapies in diabetic patients. Beta cell mass in type I diabetes is severely destroyed, while in type II diabetes, it is diminished. Being able to renew or replenish the beta cell population in these patients might provide help for about 200 million diabetics worldwide.

Factors regulating the cell cycle machinery in beta cells

As discussed in the previous section, beta cell proliferation is a very important contributor to the dynamic nature of adult beta cell mass. Therefore, in addition to finding the sources of adult beta cells, our research should aim to explore new ways of enhancing the rate of beta cell proliferation. This is only possible by gaining a detailed knowledge at the molecular level of how the replicative machinery works. It is already well known that the downstream signaling pathways by which beta cell mitogens exert their effects are linked to cell cycle regulation. Thus, interest is rapidly being directed towards the molecular mechanisms regulating cell cycle progression in beta cells.

Members of the three fundamental classes of cell cycle related proteins, i.e. cyclins, cyclin-dependent kinases (cdk) and cyclin-dependent kinase inhibitors (CKI), are extensively studied, and their function is crucial in the cell cycle progression of various cell types. It was previously demonstrated that these molecules have similar effects in controlling the cell cycle machinery in beta cells (Cozar-Castellano et al., 2006a). Excellent reviews with detailed information for these molecules are available elsewhere (Cozar-Castellano et al., 2006a; Heit et al., 2006). In this section, we will review their function in relation to beta cell proliferation.

The G1/S cell cycle checkpoint controls the transition from the gap phase (G1) to the onset of DNA synthesis (S), and is the focus of beta cell cycle research (Pestell et al., 1999; Pagano and Jackson, 2004). This transition involves cdk4 (in complex with cyclin D) and cdk2 (in complex with cyclin E) (see Fig.2), which drive the cell cycle forward by interfering with the binding of retinoblastoma protein (Rb) to E2F (Chen et al., 1989; Chellappan et al., 1991; Hinds et al., 1992). E2F activity is regulated by Rb. Unless phosphorylated by the cdks, Rb arrests cells in the G1 phase by repressing E2F function (Weinberg, 1995; Dyson, 1998; Munger, 2003).

The role of D-type cyclins and cdk2/cyclin E complex

Among the D-type cyclins (D1, D2, D3), cyclin D1 and D2 are expressed in mouse islets (Kushner et al., 2005), whereas cyclin D3 expression data is contradictory (Martin et al., 2003; Cozar-Castellano et al., 2006b). Although cyclin D1-/- mice have normal islet size and number (Kushner et al., 2005), cyclin D1 overexpression in beta cells results in a higher rate of beta cell proliferation *in vivo*, consistent with a similar study performed on rat and human islets *in vitro* (Cozar-Castellano et al., 2004; Zhang et al., 2005). Cyclin D2-/- mice have reduced beta cell mass and impaired beta cell function, suggesting an important role for this molecule during postnatal beta cell expansion (Georgia and Bhushan, 2004; Kushner et al., 2005). However, the signaling pathways that lead to expression of both cyclin D1 and cyclin D2 remain to be elucidated.

It is known that cdk2 and cyclin E are expressed in mouse islets (Cozar-Castellano et al., 2006b), and both are important for progression of the beta cell cycle, since cdk2 phosphorylates and inactivates Rb. Inhibition of this complex by cdk inhibitor molecules, mentioned below, arrests cell cycle progression (Cozar-Castellano et al., 2006a).

The role of cdk4 and cdk6

Interestingly, deletion of cdk4 in mice results in a tissue-specific phenotype that severely affects beta cells, testis and ovaries (Rane et al., 1999). These mutants are characterized by pronounced beta cell hypoplasia that results in hyperglycemia and diabetes (Rane et al., 1999). Conversely, knock-in mice carrying constitutive active cdk4 have abnormalities in the same tissues, but show increased beta cell mass resulting from aberrant beta cell proliferation (Rane et al., 1999). It has also been shown that cdk4 and cyclin D1 overexpression increases Rb phosphorylation and beta cell proliferation rate (Cozar-Castellano et al., 2004). Cdk6, another cyclin dependent kinase that can form a complex with cyclin D, is not detected in mouse islets (Cozar-Castellano et al., 2006b). This absence of cdk6, which would otherwise compensate for the loss of cdk4, might explain why cdk4 deletion results in such a striking phenotype in beta cells.

The role of E2F family of transcription factors

The E2F family of transcription factors has 8 members. E2F molecules form complexes with Rb and are important for the regulation of beta cell cycle progression. Several studies have described the expression profile of E2F transcription factor family members, revealing that E2F1 and -2 (generally referred to as transcriptional activators leading to cell cycle progression), E2F4, -5, and -6 (generally referred to as transcriptional repressors suppressing cell cycle progression), and E2F7 (having no transcriptional activity) are expressed in mouse islets, whereas E2F3 expression is not detected (Cozar-Castellano et al., 2006b). Expression of E2F8 (Maiti et al., 2005) remains to be determined in the islet. The most abundantly expressed members are E2F1 and -4 (Cozar-Castellano et al., 2006b). Recent studies have shown that E2F1-/- mice have decreased beta cell mass due to suppressed proliferation, as well as insufficient insulin secretion that leads to glucose intolerance (Fajas et al., 2004). These findings demonstrate the importance of E2F1 in controlling beta cell proliferation and function. E2F1/E2F2 double knockout mice have increased DNA replication in the pancreas, in contrast to what has been observed in E2F1-/- mice (Iglesias et al., 2004). However, this effect is accompanied by increased apoptosis, which may explain the reduced beta cell mass and islet size in these mice (Iglesias et al., 2004).

The role of Rb, T-antigen and p53

Rb, an E2F partner molecule, belongs to a family of pocket proteins that also includes p107 (Ewen et al., 1991) and p130 (Hannon et al., 1993) - Rb homologues that bind different E2F family members. Apart from binding E2F transcription factors, Rb is shown to bind to a DNA virus, Simian virus 40 large T-antigen, which competes with E2F transcription factors for the binding site on Rb (Huang et al., 1991). T-antigen has been shown to be an important potentiator of cell cycle progression by relieving the inhibitory effect of Rb on E2Fs (Hanahan, 1985; Efrat et al., 1988; Kim et al., 2001). Thus, T-antigen delivery and its subsequent activation in beta cells increase the rate of beta cell proliferation (Efrat et al., 1988; Efrat, 1996).

Interestingly, the p53 tumor suppressor protein, shown to be present in mouse islets (Cozar-Castellano et al., 2006b), is demonstrated to be functionally relevant to Rb (Williams et al., 1994). p53-/- mice are viable, but rapidly develop numerous spontaneous tumors in a wide variety of organs (Donehower et al., 1992). However, no

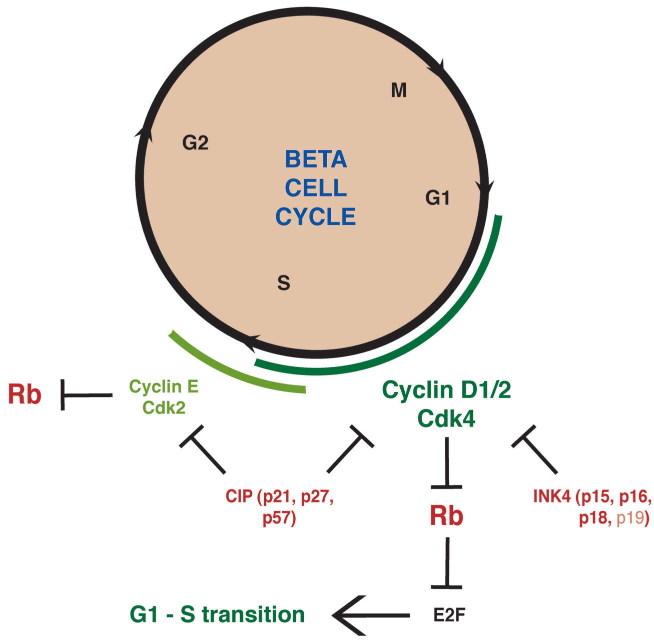

Fig. 2. Regulation of cell cycle machinery in beta cells. Experiments regarding the cell cycle machinery of beta cells focus on molecules regulating the G1/S phase transition, since this period is absolutely crucial for commitment to progression of the cell cycle. The figure shows key molecules for this regulation process. The periods during which different cyclin/cdks are expressed are shown with light and dark green arcs. The two cyclin/cdk complexes, in the transition from G1 to S are shown in green as their activity leads to cell cycle progression. The inhibitory molecules and their corresponding targets are depicted in red.

insulinomas were detected in these mice (Donehower et al., 1992). Previously, insulinoma development was shown to result from the combined loss of Rb and p53 (Williams et al., 1994; Harvey et al., 1995). However, more recently, beta-cell-specific knockout of Rb (full Rb knockouts are embryonic lethal (Wu et al., 2003)) showed that these mice have little or no alterations in the beta cell proliferation rate and mass (Vasavada et al., 2007). This result was quite unexpected since, for a long time, Rb has been believed to be a major repressor of beta cell cycle progression. This outcome may indicate either the presence of an as yet unidentified protein that compensates for the loss of Rb in beta cells, or a role for Rb in controlling beta cell cycle that differs from the original proposal. Therefore, further exploration of the role of Rb in regulating beta cell mass dynamics is required.

The role of cdk inhibitors

Other participants in the complex system regulating the cell cycle are cdk inhibitors, which include inhibitory kinases (INKs) and cyclin inhibitory proteins (CIPs; also referred to as kinase inhibitory proteins (KIP)). These molecules negatively regulate cell cycle progression, although in some systems, such as mouse fibroblasts, two members of the CIP family have been shown to accelerate the cell cycle (Cheng et al., 1999; Cozar-Castellano et al., 2006a). All four members of the INK family (p15ink4b, p16ink4a, p18ink4c, p19ink4d) (Cozar-Castellano et al., 2006b) and the three CIP family members (p21cip1, p27kip1, p57kip2) are expressed in mouse islets (Kassem et al., 2001; Uchida et al., 2005; Cozar-Castellano et al., 2006b). Several studies in mice, using various genetic approaches, have demonstrated a crucial role for some of the INK family members (p15ink4b and p18ink4c) in inhibiting beta cell cycle progression (Franklin et al., 2000; Latres et al., 2000; Pei et al., 2004). Previously, p16ink4a and p19ink4d have not been found to affect beta cell proliferation (Kamijo et al., 1997). However, a study by Krishnamurthy et al. (2006) demonstrated that p16ink4a plays an important role in inhibiting islet cell proliferation in an age-dependent way. The expression of this molecule was found to increase in the islets with age (Krishnamurthy et al., 2004) and p16ink4a-deficient old mice are shown to have enhanced islet proliferation (Krishnamurthy et al., 2006). Further research is needed to determine the mechanism of this effect.

Regarding CIP family members, p21cip1-/- mouse islets treated with beta cell mitogens *in vitro* show higher DNA synthesis compared to p21-expressing islets that were treated with the same mitogens (Cozar-Castellano et al., 2006b). However, *in vivo* studies conducted by the same group in p21cip1-/- mice demonstrated that beta cell function and proliferation rate are not altered in these mice (Cozar-Castellano et al., 2006c). This result suggests that either p21cip1 does not act alone to arrest the beta cell cycle, or that it is not crucial for the regulation of beta cell cycle progression. In addition, a number of gain-of-function and loss-of-function studies have shown that the two other members of the CIP family, p27kip1 and p57kip2, regulate beta cell proliferation by arresting the cell cycle in the adult or during embryonic development, respectively (Kassem et al., 2001; Karnik et al., 2005; Uchida et al., 2005). A recent study has shown that p27kip1 is also important for the regulation of beta cell cycle during development (Rachdi et al., 2006), suggesting a critical role for this molecule in determining whether a beta cell remains quiescent or will divide. A scheme illustrating the regulators of the beta cell cycle described here is shown in Fig. 2.

More detailed studies of the beta cell cycle will provide valuable information about the molecules responsible for regulating the cell cycle machinery. This will hopefully address why beta cells divide at such a low rate. Moreover, it is particularly important to identify signaling pathways that lead to the activation or inhibition of cell cycle regulatory molecules in beta cells. More knowledge and an understanding of the mechanisms of beta cell cycle progression will facilitate an appropriate manipulation of beta cells and will help to design novel diabetes therapies.

Other factors involved in beta cell proliferation

Factors responsible for regulating beta cell proliferation and their downstream signaling are linked to the cell cycle machinery and have long been a focus of intense investigation. Increasing knowledge in this field is particularly beneficial, since the factors themselves and their receptors may be easily targeted by drug therapy. In this section, we will discuss major factors implicated in regulating beta cell proliferation and the mechanisms by which they exert their effects.

In beta cells, cell proliferation relies on several ways of transducing signals to the nucleus upon the binding of a ligand to its corresponding receptor. These include the signaling pathways through phosphoinositide-3 kinase (PI3K)-protein kinase B (PKB)/acute transforming retrovirus thymoma (Akt); janus kinase (JAK)/ signal transducer and activator of transcription (STAT); mitogen-activated protein kinase (MAPK); insulin receptor substrate-1, -2 (IRS1 and IRS2); adenylate cyclase/protein kinase A (PKA); and calcineurin/nuclear factor of activated T-cells (NFAT) (Cozar-Castellano et al., 2006a; Heit et al., 2006). Some key molecules involved are FoxO1, Menin and NFAT, which are described in detail in the review by Heit et al. (2006). The different factors implicated in upregulating beta cell proliferation are classified according to the type of their receptors in Table 1.

Factors acting through receptor tyrosine kinases (RTKs)

Factors acting through RTKs include insulin, IGF-1, -2, EGF, HGF and PDGF. Insulin, itself, is a crucial growth factor that upregulates beta cell proliferation (Kulkarni, 2005). Upon binding to the insulin receptor (IR), insulin promotes activation of the PI3K and extracellular signal-regulated kinase (Erk1/2) signaling pathways, which includes the activation of p70 ribosomal S6 kinase (Kulkarni, 2005; Morioka and Kulkarni, 2006). These signaling molecules, in turn, activate the serine/threonine protein kinase Akt (Pende et al., 2000; Tuttle et al., 2001). Activated Akt, through repressing the function of its targets such as FoxO1 and p27kip1, can upregulate beta cell proliferation (Nakae et al., 2002; Czech, 2003; Matsumoto and Accili, 2005; Uchida et al., 2005). Consistent with the role of insulin as an enhancer of beta cell growth, beta-cell-specific IR knockout mice have an age-dependent reduction in islet size and impaired glucose tolerance (Kulkarni et al., 1999). Similarly, studies using insulin receptor substrate (IRS)-1, -2 single knockout mice, as well as IRS1 and IR double heterozygous mice, support a role for insulin in upregulating beta cell proliferation (Bruning et al., 1997; Withers et al., 1998; Kushner et al., 2002). Similar to insulin, IGF-1, acting through the PI3K-PKB/Akt pathway, represses FoxO1 transcriptional activity and is suggested to enhance beta cell proliferation (Kitamura et al., 2002; Holz and Chepurny, 2005; Kulkarni, 2005). IGF-2, however, has mostly been referred to as a beta cell survival factor, suppressing beta cell apoptosis in the early postnatal period (Petrik et al., 1998; Hill et al., 2000).

A number of studies have shown that another growth factor, epidermal growth factor (EGF), is important for beta cell mass expansion. A recent study reports that signaling through the EGF-receptor (EGFR) is crucial for postnatal beta cell growth (Miettinen et al., 2006). One member of the EGF family, betacellulin, has been reported to act as a beta cell mitogen, since it enhances DNA synthesis in INS-1 cells, a rat beta cell line (Huotari et al., 1998). A study by Yamamoto et al. (2000) using glucose-intolerant mice (induced by alloxan) treated with recombinant human betacellulin shows that betacellulin induces neogenesis of beta cells *in vivo*. Transforming growth factor alpha (TGF-alpha) also acts through EGFR and it is known to have a combined action with gastrin (Wang et al., 1993) as stated below.

Similarly, hepatocyte growth factor (HGF) has been shown to activate MAPK and PI3K-PKB/Akt signaling pathways and upregulate beta cell proliferation (Garcia-Ocana et al., 2001; Cozar-Castellano et al., 2006a). HGF overexpression in mouse beta cells increases beta cell proliferation. However, when the c-met receptor for HGF was deleted in mouse islets, signaling through this receptor was proven to be essential for insulin secretion, but not for beta cell growth (Roccisana et al., 2005). Another potential beta cell mitogen, platelet-derived growth factor (PDGF), induces fetal rat islet cell proliferation (Swenne et al., 1988). Additionally, transfection of the PDGF beta-receptor gene into islet cell suspensions rich in beta cells increases DNA synthesis in these cells (Welsh et al., 1990). However, both of these studies were conducted *in vitro*, and further research is necessary to show the effect of PDGF on regulation of beta cell proliferation *in vivo*.

Additional studies of the EGF, HGF and PDGF signaling pathways with respect to beta cell proliferation may provide new strategies to enhance beta cell proliferation.

Factors acting through JAK binding receptors:

Growth hormone (GH), prolactin (PRL) and placental lactogen (PL) are three essential beta cell mitogens that have been extensively studied. All three are suggested to act through JAK/STAT pathways, in particular JAK2/STAT5, inducing upregulation of cyclin D2 expression (Nielsen et al., 2001; Friedrichsen et al.,

Table 1. Factors upregulating beta cell proliferation.

Through receptor tyrosine kinases (RTKs)	Through G-protein coupled receptors (GPCRs)
• Insulin • Insulin-like growth factor (IGF-1, -2) • Epidermal growth factor (EGF) • Hepatocyte growth factor (HGF) • Platelet-derived growth factor (PDGF) • Transforming growth factor alpha (TGF-alpha)	• Parathyroid hormone-related protein (PTHrP) • Gastrin Incretins: • Glucagon-like peptide 1 (GLP-1) • Exendin-4 (GLP-1 agonist) • Glucose-dependent insulinotropic polypeptide (GIP)
Through JAK binding receptors	**Through other signaling pathways**
• Growth hormone (GH) • Prolactin (PRL) • Placental lactogen (PL)	• Extracellular matrix proteins (ECM proteins) • Amino acids • Glucose • ICA512 • Wnt3a

Factors important for beta cell proliferation are classified according to the type of their receptors.

2003). During pregnancy, PRL and PL upregulate beta cell proliferation due to the increased metabolic demand in the organism. All three hormones lead to increased DNA synthesis in pancreatic islets (Nielsen, 1982). Additionally, GH enhances mitosis in cultured beta cells (Rabinovitch et al., 1983). With respect to its effect on pancreas, PRL receptor-/- mice have reduced beta cell mass and islet number (Freemark et al., 2002). PL overexpression in beta cells increases beta cell mass (Fleenor et al., 2000). Detailed reviews of these three growth factors are available elsewhere (Sorenson and Brelje, 1997; Nielsen et al., 1999; Nielsen et al., 2001).

Factors acting through G-protein coupled receptors (GPCRs)

The most extensively studied factors increasing beta cell proliferation involve molecules acting through GPCRs, such as parathyroid hormone-related protein (PTHrP), gastrin and the incretin hormones.

Upon binding to its receptor (PTH1R), PTHrP upregulates beta cell proliferation through adenylate cyclase/PKA and MAPK pathways (Gaich et al., 1993; Zhang et al., 2003; Cozar-Castellano et al., 2006a). PTHrP stimulates islet growth in mice by enhancing DNA synthesis, and its overexpression in beta cells leads to islet hyperplasia (Villanueva-Penacarrillo et al., 1999; Fujinaka et al., 2004).

Gastrin is a hormone secreted from the G cells of the stomach, and increased plasma levels can be detected upon food uptake. Upon binding to its cholecystokinin-B (CCK-B) receptor, gastrin has been suggested to take part in islet growth and beta cell neogenesis in combination with TGF-alpha (Wang et al., 1993). Studies have shown that combined manipulation of EGF and gastrin leads to islet regeneration, increased beta cell mass and neogenesis (Rooman and Bouwens, 2004; Suarez-Pinzon et al., 2005). There is growing interest about the mode of action of gastrin because it markedly expands beta cell mass when it is combined with other factors that upregulate beta cell proliferation.

Incretins are gastrointestinal hormones that cause enhanced insulin secretion in response to food uptake. Undoubtedly, the incretin hormone glucagon-like peptide-1 (GLP-1) is a major beta cell mitogen. GLP-1 not only induces beta cell proliferation in the rodent pancreas (Perfetti et al., 2000), but it also plays a role in the differentiation of pancreatic ductal cells into beta-cell-like phenotype (Hui et al., 2001; Bulotta et al., 2002). Treatment with human GLP-1 of cultured pancreatic ductal cells induces expression of GLUT-2, insulin and glucokinase in these cells (Bulotta et al., 2002). Furthermore, signaling through GLP-1 receptor decreases beta cell apoptosis (Farilla et al., 2003; Li et al., 2003). The receptor for glucose-dependent insulinotropic polypeptide (GIP), another intestinal incretin hormone, is expressed at high levels in beta cells (Maletti et al., 1984; Amiranoff et al., 1986; Usdin et al., 1993) and is crucial for beta cell function and proliferation. Synergistically, in concert with glucose, GIP is shown to increase INS-1 cell proliferation (Trumper et al., 2001). Another factor acting through GPCRs is exendin-4, a long-lasting GLP-1 agonist, which has been reported to increase beta cell mass by inducing beta cell replication and neogenesis upon binding to GLP-1 receptor (Xu et al., 1999; Tourrel et al., 2001).

In summary, the intestinal incretins and the GLP-1 agonist exendin-4 are of fundamental importance for beta cell proliferation and function. Synthetic exendin-4, exenatide, is approved as a diabetes treatment and has been available for clinical use since 2005.

Factors acting through other signaling pathways

Our lab has previously demonstrated that beta cells secrete vascular endothelial growth factor A (VEGF-A) to attract VEGFR-2 expressing endothelial cells, which provide beta cells with a vascular basement membrane (Nikolova et al., 2006). The components of the vascular basement membrane, in particular laminin-411, interact with alpha-6 beta-1 integrin on the surface of beta cells to support their proliferation and insulin gene expression (Nikolova et al., 2006). In addition, islets or beta cells exposed to extracellular matrix proteins show upregulated beta cell proliferation and function *in vitro*, as well as an increased survival (Bosco et al., 2000).

Many studies have reported the importance of glucose in promoting beta cell expansion and survival (Chick, 1973; Swenne, 1982; Bonner-Weir et al., 1989; Bernard et al., 1998, 1999; Paris et al., 2003; Topp et al., 2004). In rat, glucose infusion leads to an increase in beta cell number. This increase is mainly through neogenesis from the stem cells residing in the pancreatic ducts (Bernard et al., 1999). Glucose exerts its effect through various direct and indirect mechanisms, including activation of NFAT signaling or through increased secretion of insulin, which then signals in an autocrine manner to enhance proliferation (Frodin et al., 1995; Heit et al., 2006). Amino acids are also known to indirectly affect beta cell proliferation through stimulating insulin secretion and subsequent insulin autocrine signaling (Floyd et al., 1966; Blachier et al., 1989; Sener et al., 1990).

Apart from these three factors, a well-studied signaling pathway that is crucial for embryonic development, the Wnt pathway, has recently been reported to be involved in regulating beta cell proliferation. Upon treatment with Wnt3a, cultured mouse islets have increased Ki67 labeling and cyclin D2 levels (Rulifson et al., 2007), demonstrating a role of this molecule in stimulating beta cell proliferation.

In addition to these factors known to induce beta cell proliferation, ICA512, an inactive receptor tyrosine phosphatase on beta cells, is a promising candidate. ICA512 is cleaved by Calpain-1 upon glucose-induced exocytosis of insulin granules (Ort et al., 2001; Trajkovski et al., 2004). The cytoplasmic tail of this molecule translocates to the nucleus, where it enhances STAT5 levels and insulin gene transcription (Mziaut et al., 2006). Since STAT5 is an important signaling component in beta cell proliferation, this molecule might be involved in upregulation of beta cell proliferation.

In conclusion, the identification of factors that are involved in regulating beta cell proliferation and the pathways through which they are linked to cell cycle machinery are crucial for designing novel therapeutic approaches to increase beta cell mass in diabetic patients. It is important to point out that some of these factors may have profound effects only when applied in combination with others. These molecules and their receptors are promising potential drug targets for the treatment of diabetes.

Outlook and future perspectives

Pancreatic beta cells, the only source of insulin in the mammalian organism, are either lost or become dysfunctional during the progression of the metabolic disorder diabetes. Beta cells have long been known to have a very low mitogenic index. However, beta cell mass, as shown by a large body of evidence, is dynamic and maintained through a delicate balance of regeneration and apoptosis. Although it is known that the massive increase in beta cell mass during the fetal and neonatal period stems both from neogenesis and self-renewal, the source of new beta cells in the adult is hotly debated. A number of recent studies suggest that the proliferation of preexisting beta cells makes a very important contribution to adult beta cell mass. However, this does not exclude possible neogenesis through stem/progenitor cells or transdifferentiation from other cell types. Identifying the sources of new beta cells in the adult is extremely important for regenerating beta cells in the diabetic patient. The increasing number of diabetic patients worldwide adds urgency to this problem.

Another approach for increasing beta cell mass is through manipulating the cell cycle machinery of beta cells. This is only possible through a detailed understanding of the molecular mechanisms involved, in order to provide targets that can be manipulated in patients to drive the cell cycle forward.

To achieve this aim, cell cycle manipulation experiments must be designed and analyzed with great consideration before any further applications. The few molecules currently known to regulate the cell cycle are not beta cell specific, and stimulation or inhibition of these targets is thus prone to yield unexpected outcomes. Despite this, the factors implicated in the regulation of beta cell proliferation, though most of them are not beta cell specific either, are currently proving to be valuable in the clinic.

Studies on the source of new beta cells in the adult, on the beta cell cycle, and on factors upregulating beta cell proliferation are clearly fundamental to deciphering the mechanisms that increase adult beta cell mass. Importantly, novel strategies to treat patients, or to generate and expand beta cells *in vitro* for transplantation, will only become a reality once we know how to specifically control beta cell dynamics.

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