REVIEW

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

Amanda M Ackermann2,3 and Maureen Gannon1,2,3

Departments of Medicine, Molecular Physiology and Biophysics and Program in Developmental Biology, Vanderbilt University Medical Center, 2220 Pierce Avenue 746 PRB, Nashville, Tennessee 37232, USA

(Requests for offprints should be addressed to M Gannon; Email: maureen.gannon@vanderbilt.edu)

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.

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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 (Blushan 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
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. 1997b). 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
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-J−/−, 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 postnataally 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 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. 2005a). 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
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.
tissue utilized for islet transplantation (Heremans et al. 2002). Adenoviral infection of these cells with Ngn3 or Neurod1 results in differentiation into primarily insulin\textsuperscript{+} 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\textsuperscript{+} 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. 2005a, Zhang et al. 2006). For example, global deletion of the cell cycle inhibitor p27\textsuperscript{kip1} (p27\textsuperscript{kip1}\textsuperscript{−/−}) 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.](image-url)
### Table 1 Molecular regulators of postnatal β-cell mass in vivo

<table>
<thead>
<tr>
<th>Transcription factors</th>
<th>Mouse models</th>
<th>Effects</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pdx1</td>
<td>Pdx1−/−</td>
<td>↓ β cell mass, ↑ β cell apoptosis</td>
<td>Johnson et al. (2003)</td>
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<tr>
<td></td>
<td>Pdx1&lt;sup&gt;lox/lox&lt;/sup&gt;; Rip-Cre</td>
<td>↓ β cell mass</td>
<td>Ahlgren et al. (1998)</td>
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<td></td>
<td>Foxm1&lt;sup&gt;lox/lox&lt;/sup&gt;, Pdx1&lt;sup&gt;−/−&lt;/sup&gt;-Cre</td>
<td>↓ β cell mass, ↓ β cell proliferation, ↑ β cell size</td>
<td>Zhang et al. (2006)</td>
</tr>
<tr>
<td>Cyclic AMP response element binding protein</td>
<td>CBP&lt;sup&gt;S436A&lt;/sup&gt;; Tg (↑ CREB activity)</td>
<td>↑ β cell mass, ↑ β cell proliferation</td>
<td>Hussain et al. (2006)</td>
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<td></td>
<td>HIP-ICER-Iγ Tg (↑ CREB activity)</td>
<td>↓ β cell mass</td>
<td>Inada et al. (2004)</td>
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<tr>
<td></td>
<td>Rip-dnCREB Tg</td>
<td>↓ β cell mass, ↑ β cell proliferation</td>
<td>Jhala et al. (2003)</td>
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<tr>
<td></td>
<td>E2F1&lt;sup&gt;−/−/−&lt;/sup&gt;; E2F2&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>↓ β cell mass, ↑ β cell proliferation</td>
<td>Iglesias et al. (2004)</td>
</tr>
<tr>
<td>Nuclear factor of activated T cells, cytoplasmic, calcineurin-dependent 1</td>
<td>Rip-rTA; NFATc1&lt;sup&gt;Flox&lt;/sup&gt;</td>
<td>↑ β cell mass, ↑ β cell proliferation</td>
<td>Heit et al. (2006a)</td>
</tr>
<tr>
<td>Cyclin-dependent kinases</td>
<td>Cdk4&lt;sup&gt;R26C/R26G&lt;/sup&gt;; Tg (↑ activity)</td>
<td>↑ β cell mass, ↑ β cell size</td>
<td>Rane et al. (1999)</td>
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<tr>
<td>Cyclins</td>
<td>Rip-cyclin D1 Tg</td>
<td>↑ β cell mass, ↑ β cell proliferation, ↑ β cell apoptosis</td>
<td>Georgia &amp; Bhushan (2004)</td>
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<tr>
<td>Cyclins</td>
<td>Cyclin D2&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>↑ β cell mass, ↑ β cell proliferation, ↑ β cell apoptosis</td>
<td>and Kushner et al. (2005a)</td>
</tr>
<tr>
<td>Cyclin-dependent kinase inhibitors</td>
<td>P16&lt;sup&gt;NK4a&lt;/sup&gt;; Tg</td>
<td>↑ β cell proliferation</td>
<td>Krishnamurthy et al. (2006)</td>
</tr>
<tr>
<td>Tumor suppressors</td>
<td>pRb&lt;sup&gt;Dox/fox&lt;/sup&gt;; Rip-Cre</td>
<td>↑ β cell mass, ↑ β cell proliferation, ↑ β cell apoptosis, ↑ β cell size</td>
<td>Pei et al. (2004)</td>
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<tr>
<td>Growth factors</td>
<td>Rip-PL1 Tg</td>
<td>↑ β cell mass, ↑ β cell proliferation, ↑ β cell apoptosis</td>
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<td>Growth factors</td>
<td>Rip-PL1 Tg</td>
<td>↑ β cell mass, ↑ β cell proliferation, ↑ β cell apoptosis, ↑ β cell size</td>
<td>Vasavada et al. (2000) and Cozar-Castellano et al. (2006a)</td>
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<td>Growth factors</td>
<td>Rip-ALK Tg</td>
<td>↑ β cell mass, ↑ β cell proliferation, ↑ β cell apoptosis, ↑ β cell size</td>
<td>Vasavada et al. (2004)</td>
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<td>Growth factors</td>
<td>GHR&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>↑ β cell mass, ↑ β cell proliferation, ↑ β cell apoptosis, ↑ β cell size</td>
<td>Liu et al. (2004)</td>
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<td>Growth factors</td>
<td>Rip-HGF Tg</td>
<td>↑ β cell mass, ↑ β cell proliferation, ↑ β cell apoptosis, ↑ β cell size</td>
<td>Garcia-Ocaña et al. (2000), 2001 and Cozar-Castellano et al. (2006a)</td>
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<td>Growth factors</td>
<td>c-Met&lt;sup&gt;fox/fox&lt;/sup&gt;; Rip-Cre</td>
<td>↑ β cell mass, ↑ β cell proliferation, ↑ β cell apoptosis, ↑ β cell size</td>
<td>Roccisana et al. (2005)</td>
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<td>Growth factors</td>
<td>HIP-EGF Tg</td>
<td>↑ β cell mass, ↑ β cell proliferation, ↑ β cell apoptosis, ↑ β cell size</td>
<td>Krakowski et al. (1999)</td>
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<td>Growth factors</td>
<td>HIP-KGF Tg</td>
<td>↑ β cell mass, ↑ β cell proliferation, ↑ β cell apoptosis, ↑ β cell size</td>
<td>Krakowski et al. (1999)</td>
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<tr>
<td>Insulin, insulin-like growth factors</td>
<td>IR&lt;sup&gt;fox/fox&lt;/sup&gt;; Rip-Cre</td>
<td>↑ β cell mass, ↑ β cell proliferation, ↑ β cell apoptosis, ↑ β cell size</td>
<td>Otani et al. (2004)</td>
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<td>Insulin, insulin-like growth factors</td>
<td>Rip-IGF-I Tg</td>
<td>↑ β cell mass, ↑ β cell proliferation, ↑ β cell apoptosis, ↑ β cell size</td>
<td>George et al. (2002)</td>
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<td>Insulin, insulin-like growth factors</td>
<td>Pdx1&lt;sup&gt;14.3/CRE&lt;/sup&gt;, Pdx1&lt;sup&gt;−/−&lt;/sup&gt;-Cre</td>
<td>↑ β cell mass, ↑ β cell proliferation, ↑ β cell apoptosis, ↑ β cell size</td>
<td>Lu et al. (2004)</td>
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<tr>
<td>Insulin, insulin-like growth factors</td>
<td>Pdx1&lt;sup&gt;−/−/−&lt;/sup&gt;-Cre</td>
<td>↑ β cell mass, ↑ β cell proliferation, ↑ β cell apoptosis, ↑ β cell size</td>
<td>Kulkarni et al. (2002)</td>
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<tr>
<td>Insulin, insulin-like growth factors</td>
<td>RIP-IR&lt;sup&gt;3/fox&lt;/sup&gt;; Rip-Cre</td>
<td>↑ β cell mass, ↑ β cell proliferation, ↑ β cell apoptosis, ↑ β cell size</td>
<td>Petrik et al. (1999)</td>
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<tr>
<td>Fibroblast growth factors</td>
<td>Rip-IGF-II Tg</td>
<td>↑ β cell mass, ↑ β cell proliferation, ↑ β cell apoptosis, ↑ β cell size</td>
<td>Devedjian et al. (2000)</td>
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<tr>
<td>Fibroblast growth factors</td>
<td>Pdx1-dnFGFR1c Tg</td>
<td>↑ β cell mass, ↑ β cell proliferation, ↑ β cell apoptosis, ↑ β cell size</td>
<td>Hart et al. (2000)</td>
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<td>Fibroblast growth factors</td>
<td>Pdx1-dnFGFR2b Tg</td>
<td>↑ β cell mass, ↑ β cell proliferation, ↑ β cell apoptosis, ↑ β cell size</td>
<td>Hart et al. (2000)</td>
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(continued)
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.

Table 1 Continued

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<thead>
<tr>
<th>Mouse models</th>
<th>Effects</th>
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<tr>
<td>Vascular endothelial growth factors</td>
<td>Rip-VEGF-A Tg</td>
<td>++β cell mass</td>
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<td></td>
<td>VEGF-A/−/−, Rip-Cre</td>
<td>++β cell mass, ↑β cell size</td>
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<td>GLP-1R+/−</td>
<td>++β cell mass</td>
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<td>GIPR+/−</td>
<td>↑β cell mass</td>
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<td>Rip-Gastrin Tg</td>
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<td></td>
<td>Gastrin−/−</td>
<td>++β cell mass, ↔β cell proliferation</td>
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<td>Incretins</td>
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<td>Cell signaling proteins</td>
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<td>Insulin response substrate</td>
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<td>Rip-Irs2 Tg</td>
<td>↑β cell mass, ↔β cell proliferation, ↑β cell size</td>
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<td></td>
<td>Irs2−/−</td>
<td>↓β cell mass, ↑β cell apoptosis</td>
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<td></td>
<td>Irs2+/−, Rip-Cre</td>
<td>↑β cell mass, ↑β cell proliferation, ↔β cell apoptosis</td>
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<td></td>
<td>caAkt Tg</td>
<td>↑β cell mass, ↑β cell proliferation, ↑β cell size</td>
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<td></td>
<td>Rip-caAkt Tg</td>
<td>↑β cell mass, ↑β cell proliferation, ↑β cell size, ↑β cell neogenesis</td>
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<td>Rip-kdAkt Tg</td>
<td>↑β cell mass, ↑β cell size, ↑β cell apoptosis, ↔β cell proliferation</td>
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<td>P70S6K</td>
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<td></td>
<td>P70S6Kt+/−</td>
<td>↑β cell mass, ↑β cell size</td>
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<tr>
<td>Phosphoinositide-dependent kinase</td>
<td>PDK1+/−, Rip-Cre</td>
<td>↑β cell mass, ↑β cell proliferation, ↑β cell size, ↑β cell apoptosis</td>
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<tr>
<td>Others</td>
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<tr>
<td>Menin</td>
<td>Men1+/−</td>
<td>↑β cell mass, ↑β cell proliferation</td>
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<tr>
<td></td>
<td>Men1+/−, Rip-Cre</td>
<td>↑β cell mass, ↑β cell proliferation</td>
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<tr>
<td>Pkr-like ER kinase</td>
<td>PERK+/−</td>
<td>↑β cell mass, ↑β cell apoptosis</td>
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<tr>
<td>Phosphatase and tensin homologue</td>
<td>PTEN+/−</td>
<td>↑β cell mass, ↑β cell proliferation</td>
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<td>PTEN+/−, Rip-Cre</td>
<td>↑β cell mass, ↑β cell proliferation, ↑β cell size</td>
</tr>
<tr>
<td>Calcineurin b1</td>
<td>Cnb1+/−, Rip-Cre</td>
<td>↑β cell mass, ↑β cell proliferation, ↔β cell apoptosis</td>
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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.
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 G1 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. 2005a). 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 p27Kip1 within β-cells using the rat insulin promoter (Rip-p27Kip1) 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 p27Kip1 (p27Kip1−/−) 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 p57Kip2, 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 p16INK4a (Cdk4R24C/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 G2 to M phase progression; Cdc25A and B phosphatases, which dephosphorylate and activate Cdk5; 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 p27Kip1 and p21Cip1 for proteasomal degradation. Pancreas-specific deletion of Foxm1 using a Cre-lox strategy (Foxm1flox/flox;Pdx1Cre;CckCre) 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 p27Kip1, 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 (Men1flox/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 p18INK4c and p27Kip1, both of which are direct targets of menin-mediated histone methylation (Karnik et al. 2005). Furthermore, a MEN1-like phenotype is observed in p18INK4c−/−;
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. (2006b) and Heit et al. (2006b).

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\(_{xx}\) 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\(^{lox/lox}\); 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. 1997a), 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. 2006a). 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-Ocana et al. 2000, 2001, Cozar-Castellano et al. 2006a). 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).
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 (Irs2flox/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.

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