Human β-Cell Proliferation and Intracellular Signaling Part 2: Still Driving in the Dark Without a Road Map

Citation

Published Version
doi:10.2337/db13-1146

Permanent link
http://nrs.harvard.edu/urn-3:HUL.InstRepos:14351360

Terms of Use
This article was downloaded from Harvard University’s DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at http://nrs.harvard.edu/URN-3:HUL.InstRepos:dash.current.terms-of-use#LAA

Share Your Story
The Harvard community has made this article openly available. Please share how this access benefits you. Submit a story.

Accessibility
Enhancing β-cell proliferation is a major goal for type 1 and type 2 diabetes research. Unraveling the network of β-cell intracellular signaling pathways that promote β-cell replication can provide the tools to address this important task. In a previous Perspectives in Diabetes article, we discussed what was known regarding several important intracellular signaling pathways in rodent β-cells, including the insulin receptor substrate/phosphatidylinositol-3 kinase/Akt (IRS-PI3K-Akt) pathways, glycogen synthase kinase-3 (GSK3) and mammalian target of rapamycin (mTOR) S6 kinase pathways, protein kinase Cζ (PKCζ) pathways, and their downstream cell-cycle molecular targets, and contrasted that ample knowledge to the small amount of complementary data on human β-cell intracellular signaling pathways. In this Perspectives, we summarize additional important information on signaling pathways activated by nutrients, such as glucose; growth factors, such as epidermal growth factor, platelet-derived growth factor, and Wnt; and hormones, such as leptin, estrogen, and progesterone, that are linked to rodent and human β-cell proliferation. With these two Perspectives, we attempt to construct a brief summary of knowledge for β-cell researchers on mitogenic signaling pathways and to emphasize how little is known regarding intracellular events linked to human β-cell replication. This is a critical aspect in the long-term goal of expanding human β-cells for the prevention and/or cure of type 1 and type 2 diabetes.

Induction of proliferation in human β-cells is a major goal of current research in both types 1 and 2 diabetes. Over the last 20 years, dramatic progress has occurred in understanding transcriptional control of key genes required for mouse and human β-cell specification. More recently, advances have been made in coaxing human embryonic stem (ES) cells and induced pluripotent stem (iPS) cells to differentiate to endocrine lineage. Concurrently, major advances have been made in understanding control of cell-cycle progression in mouse and human β-cells. In contrast, one large area that remains poorly studied, particularly in human β-cells, is the network of intracellular signaling pathways that link extracellular nutrient and growth factor actions at the β-cell surface to cell-cycle machinery.

In a recent Perspectives in Diabetes article, we discussed what is known regarding several important
intracellular signaling pathways in rodent β-cells, and contrasted that ample body of data to the relative paucity of complimentary data on human β-cell intracellular signaling pathways (1). That Perspectives focused on the insulin receptor substrate/phosphatidylinositol-3 kinase/Akt (IRS-PI3K-Akt) pathway, glycogen synthase kinase-3 (GSK3) and mammalian target of rapamycin (mTOR)-S6 kinase pathways, protein kinase C (PKC) pathways, and their downstream cell-cycle molecular targets. In this article, we now turn attention to additional important signaling pathways linked to β-cell proliferation. Our goals are twofold. First, we provide a “primer” or resource for β-cell researchers on intracellular signaling pathways linked to proliferation in β-cells. Second, we emphasize how little is known regarding intracellular events in human β-cells and how important it is to understand this understudied area if we are ever going to be able to expand human β-cells ex vivo or in vitro for therapeutic exploitation.

**GLUCOSE AND METABOLIC MITOGENIC SIGNALING**

Glucose, under some physiological circumstances, is clearly a mitogenic nutrient in rodent β-cells, as glucose infusion and in vitro glucose exposure have been demonstrated repeatedly to drive replication in mouse and rat β-cells. In this model, glucose enters the β-cell via GLUT2 in rodents (or GLUT1 in humans) and is phosphorylated by glucokinase (GK). GK acts as the β-cell’s glucose sensor as a result of a K_m that lies in the center of the physiological range of blood glucose. Glucose-6-phosphate (G-6-P) generated by GK enters the glycolytic pathway to generate ATP and other metabolic signals such as pyruvate, consuming AMP, and ADP. These metabolic signals activate the three parallel downstream mitogenic pathways depicted in Fig. 1. That these downstream mitogenic effects are mediated by glucose and GK is clear, as β-cell proliferation fails to occur in hyperglycemic GK^−/− mice (2). Conversely, even hypoglycemic mice treated with pharmacologic GK activators display increases in β-cell proliferation (3).

The majority of information presented above is derived from rodent models (Fig. 1A), but it is important to emphasize two points relating to the human β-cell (Fig. 1B). First, in rodents, GLUT2 is the principal β-cell glucose transporter. In contrast, in humans, GLUT1 serves as the major glucose transporter (4,5). Second, additional support for the importance of GK in human β-cell proliferation comes from human neonates with activating GK mutations who demonstrate increases in β-cell proliferation (1). The effect of LKB1 on cell growth and proliferation are mediated, in part, by AMPK modulation of mTOR signaling and by PTEN regulation of PI3K/Akt (17–19). In addition, it is possible that the interaction of LKB1 with GSK3 and PKCζ (1) may also play a role, although it remains unclear if this occurs in β-cells.

Insights into the functions of this kinase in β-cells emerge from conditional as well as constitutive
Figure 1—Glucose signaling pathways to β-cell proliferation via mTOR, via ChREBP/cMyc, and via NFATs. A: Signaling mechanisms in rodent β-cells. B: Signaling molecules confirmed in human β-cells. Molecules and arrows in gray denote pathways that are known to exist in rodents, but are unstudied in human β-cells. Briefly, glucose enters the β-cell via GLUT2 (in rodents) or GLUT1 (in humans) whose kinetics ensure that phosphorylation and subsequent catabolism is proportional to blood glucose levels. Glucose is phosphorylated by GK to G-6-P and enters glycolysis. In the right side of the figure, this generates ATP, depleting ADP and AMP, which permits suppression of AMP-kinase, with resultant activation of mTOR signaling to proliferation. In parallel, in the middle of the figure, metabolism of glucose activates ChREBP, which leads to activation of cMyc and then cyclins.
inactivation of LKB1. In both models, deletion of LKB1 in adult or developing β-cells resulted in enhanced insulin secretion and β-cell mass (20–22). The changes in β-cell mass resulted from both increased proliferation and cell size and were associated with both reduced AMPK activity as well as enhanced mTOR C1 signaling. These studies make it clear that LKB1 acts on AMPK/mTOR C1 signaling to modulate β-cell proliferation, size, and mass.

In human β-cells, LKB1 expression has been demonstrated in islets at the mRNA level, but little is known about the role of this kinase in regulation of human β-cell proliferation. Mutations of LKB1 in patients with Peutz-Jeghers syndrome are associated with an increased susceptibility to endocrine tumors, but insulinoma has not been described in these patients.

**AMPK**

AMPK is an energy-sensing heterotrimeric serine/threonine kinase that mediates the adaptation to decreased nutrients (low ATP/ADP + AMP ratio) by promoting energy production and limiting energy utilization. AMPK contains a catalytic α-subunit (AMPKα-1 or -2), a scaffold β-subunit (β-1 or -2) and a regulatory γ-subunit (γ-1, -2, or -3). Activation of AMPK inhibits mTOR C1 activity by acting on its upstream negative regulators, tuberous sclerosis complex proteins 1 and 2 (TSC1/2), and thereby inhibits β-cell proliferation and hypertrophy.

The importance of AMPK in regulation of mature β-cell function has recently been demonstrated in conditional mice in which AMPKα-1 and -2 were disrupted in β-cells (23). These mice displayed impaired glucose tolerance, decreased insulin secretion, and enhanced insulin sensitivity. These mice also showed normal β-cell mass, decreased β-cell size, and increased β-cell proliferation. The mechanisms responsible for this surprising proliferative response were not explored. Experiments in transgenic mice overexpressing either constitutively active or dominant negative AMPKα in β-cells failed to show major defects in mass but underscored the role of AMPK on insulin secretion (22). The phenotypes of models with loss of AMPK function are in marked contrast with the observations in LKB1-deficient mice described above. Thus, it is possible that AMPK-independent effects downstream of LKB1 regulate β-cell proliferation, but further studies using inducible mice and β-cell–specific Cre lines could determine the role of AMPK on β-cell proliferation.

In human β-cells, AMPKα-1 and -2 are present and AMPK activity is negatively regulated by glucose treatment (24). While activation of AMPK inhibits insulin secretion, its role in β-cell proliferation is unclear and warrants research given the extensive therapeutic use of metformin.

**Calcium, Nuclear Factor of Activated T Cells, Calcineurin, and Proliferation**

In addition to the LKB1 and ChREBP glucose-driven mitogenic pathways, a third calcium-mediated signaling pathway related to β-cell proliferation exists (Fig. 1). In this pathway, the same canonical signals that link glucose entry into cells, activating calcium-mediated insulin secretion, also can activate β-cell proliferation. In the β-cell mitogenic pathway, this increment in intracellular calcium binds to and activates calmodulin, which then phosphorylates and activates the catalytic (CnA) and regulatory (CnB) subunits of the phosphatase, calcineurin (25–31). Calcineurin has a number of substrates. One of the substrates is CREB-regulated transcription coactivator-2 (CRTC2) (also called Transducer of Regulated CREB activity-2 [TORC2]) (32), which is bound to and retained in the cytoplasm by the scaffolding protein 14-3-3. Dephosphorylation of CRTC2 by calcineurin releases TORC2 from 14-3-3 and permits nuclear translation and association of CRTC2 with other transcription factors and coactivators such as CREB, CAMP-response element modulator (CREM), and ATF1 on the promoters of cell-cycle–activating genes, such as the cyclin A promoter (32,33).

A similar scenario applies to a second calcineurin substrate, the nuclear factor of activated T cells (NFAT) family of transcription factors (25,26,28–30,34). NFATs are phosphorylated and thereby constrained to the cytoplasm in quiescent cells. With a rise in intracellular calcium, calmodulin phosphorylates and activates calcineurin, which dephosphorylates NFATs, allowing them to translocate to the nucleus. There, they bind to the promoter of a number of cell-cycle activators (e.g., D and A cyclins, cyclin-dependent kinase 2 and 4, cMyc, and FoxM1 among others) (26,30), thereby activating proliferation.

This calcium-mitogenic paradigm may employ multiple additional variations. For example, NFAT action is often enhanced by association with transcription factors such as CREM, CREB, and AP1, Jun, or Fos (34). NFATs

---

with β-cell proliferation. In the right side of the figure, in parallel with the other pathways, glucose metabolism to generate ATP blocks potassium entry via the potassium inward rectifier/sulfonylurea receptor complex, which leads to depolarization of voltage-dependent calcium channels, and resultant calcium entry. This leads to activation of calmodulin, and thus the phosphatase, calcineurin, with resultant dephosphorylation of proliferative molecules such as the NFAT and CRTC2 families. Upon growth factor and insulin stimulation, Akt and ERK phosphorylates and inactivates TSC2, releasing the inhibition of Rheb and activation of mTOR complex 1 (mTORC1). In contrast, phosphorylation and activation of TSC2 by AMPK and GSK3β inhibits mTOR signaling. mTORC1 controls growth (cell size) and proliferation (cell number) by modulating mRNA translation through phosphorylation of 4E-BP 1, 2, and 3 and the ribosomal protein S6 kinases (S6K1 and 2). Phosphorylation of the 4E-BPs triggers their release from eIF4E and initiates cap-dependent translation. See the text for more detail.
may also transcriptionally repress the promoters of certain cell-cycle inhibitors, with p21 and p27 being examples. Also, they may bind to the promoter of IRS-2, which can then recruit PI3K, Akt/ PKB, and Ras/Raf/MAP kinase pathways to activate proliferation (25). In another variation, ChREBP is retained in the cytoplasm in an inactive state, bound to the binding protein, sorcin, under conditions of low glucose and low calcium, thereby preventing its nuclear localization. Intracellular calcium increments release ChREBP from sorcin, and allow it to enter the nucleus and drive proliferation. In a third variant of calcium-mediated β-cell mitogenesis, activation of GABA receptors, which activate extracellular calcium entry, can also serve to activate these pathways (35).

While the majority of information described above is derived from rodent β-cells or cell lines, it is likely that many of the phenomena also apply to human β-cells (Fig. 1B). For example, it is known from human GK mutations and experiments using calcium-channel blockers, calcium-channel inhibitors, and calcineurin inhibitors that glucose activates intracellular calcium in human β-cells, that human β-cells contain calcineurins and NFATs, and that calcium entry is associated with, and to some extent required for, human β-cell proliferation (6,31,34). Indeed, calcineurin inhibitors such as cyclosporin and tacrolimus, used as immunosuppressive agents in human organ transplantation, lead to diabetes, in part as a result of loss of proliferation, failure of insulin secretion, and de-differentiation in rodent as well as human β-cells (34,36,37).

**EPIDERMAL GROWTH FACTOR AND PLATELET-DERIVED GROWTH FACTOR AND PROLIFERATION**

The epidermal growth factor (EGF) family of proteins has attracted much attention in the β-cell replication field since 1993, when betacellulin (BTC) was identified in mouse insulinoma cell lines (38), suggesting that BTC could be a contributor to the development of the insulinoma phenotype. Indeed, BTC is a potent mitogen in vitro for INS-1 insulinoma cells (39) and rodent pancreatic β-cells in vivo (40,41). BTC-mediated mitogenic effects in β-cells require activation of both EGF/ErbB1 and ErbB2 receptors and also upregulation of IRS2 (42) (Fig. 2A).

EGF receptor (EGFR) deficiency in mouse models causes markedly reduced β-cell proliferation and diabetes, associated with reductions in extracellular signal–related kinase (ERK) and Akt activity (43–45). Further, EGFR-deficient mice fail to expand β-cell mass following high-fat feeding or during pregnancy, in which β-cell replication is the primary mechanism for compensatory β-cell growth (43). In addition, the mitogenic effects of glucagon-like peptide 1 (GLP-1) in INS-1 cells require EGFR activation (46). Therefore, an intact EGFR pathway is required for β-cell proliferation postnatally, when induced by GLP-1 and in insulin-resistant states. However, and in contrast to BTC, two EGFR ligands, EGF or transforming growth factor (TGF)-α only modestly increase rodent β-cell proliferation (39,46). In addition, transgenic overexpression of heparin-binding (HB)-EGF did not lead to increased β-cell proliferation (47). Taken together, these results suggest that although EGFR activation is required for normal β-cell proliferation and expansion, EGF, HB-EGF, or TGFα may not be the natural ligands that activate this receptor. Recently, another EGFR ligand, epieregulin, has been shown to increase β-cell proliferation in vitro (48). These studies highlight the complicated nature of β-cell mitogenic effects involving this large family of ligands and receptors and point out the need to analyze the role of ErbB receptors and ligands in β-cell proliferation and expansion.

In vitro experiments in the 1990s demonstrated that cotransfection of platelet-derived growth factor (PDGF) with PDGF receptor (PDGFR) increases islet cell proliferation (49,50). More recently, an elegant study by Chen et al. (51) has shown that PDGFR expression decreases in an age-dependent manner, reducing the proliferative potential of this growth factor in adult β-cells. Exogenous addition of PDGF-AA to juvenile mouse islets leads to increased expression of the histone methyltransferase, Ezh2 (a repressor of p16INK4a), and increased β-cell proliferation (51). However, these effects were not observed in adult mouse islets. Conditional inactivation of PDGFR-α in β-cells of young mice leads to a remarkable decrease in β-cell proliferation and mass and the development of hyperglycemia and glucose intolerance (51). Importantly, PDGFR inactivation impairs β-cell regeneration and restoration of β-cell mass after streptozotocin treatment in adult mice. On the other hand, overexpression of an active form of the PDGFR-α in the β-cell of transgenic mice leads to remarkable β-cell proliferation in old mice. Collectively, these studies demonstrate that PDGFR signaling is required for physiologic β-cell proliferation and expansion, and its activation is sufficient to sustain adult β-cell expansion in vivo in mice. Activation of Erk1/2, but not PI3K or phospholipase C-γ, is responsible for PDGFR signaling–mediated β-cell proliferation (Fig. 2A).

For human β-cells (Fig. 2B), information on EGF/EGFR family members relating to proliferation is almost nonexistent. It is known that BTC and EGFR are expressed specifically in human α-cells, β-cells, and ductal cells (52). In addition, it has been shown that human insulinomas also express BTC, suggesting that BTC might be important for human islet growth (52). Interestingly, the PDGFR-α is present in juvenile human islets, but not in adult human islets, suggesting that PDGF signaling attenuation is a preserved feature of aging human β-cells (51). PDGF induces a remarkable increase in the proliferation of juvenile human β-cells, an effect potentially mediated by Erk1/2 activation, but was ineffective in adult human β-cells (51). Unfortunately, it is unknown whether the transfer of PDGFR to adult human...
Figure 2—Signaling by EGF and PDGF in the regulation of β-cell proliferation. Schematic representation of the signaling pathways activated by EGF family of proteins and PDGF in rodent (A) and human (B) β-cells. Multiple members of the EGF family of proteins, including BTC, EGF, HB-EGF, TGF-α, and epiregulin, have been shown to act in β-cells. ErbB family of receptors is expressed in β-cells and BTC binding to ErbB1, also called EGFR, and ErbB2 has been reported to activate the IRS2/PI3K pathway, which in turn signals via PDK-1 to
islets could lead to enhanced β-cell proliferation in the presence of PDGF.

Wnt, β-CATENIN, AND PROLIFERATION

Genome-wide association studies have implicated a role for the Wnt signaling pathway in the pathogenesis of type 2 diabetes and variants of the transcription factor TCF7L2 convey the strongest genetic risk factor for type 2 diabetes. The canonical Wnt pathway is activated by binding of Wnt ligands to the FzrRed receptor, inducing a cascade of events that result in nuclear localization of β-catenin and transcriptional activation by interacting with T-cell–specific factor/lymphoid enhancer–binding factor (TCF/LEF) transcription factors (53,54). Absence of Wnt signaling permits β-catenin proteosomal degradation by activation of a complex of proteins that include axins, GSK3β, and APC (55). In addition to responses to Wnt ligands, this pathway is induced by activation of the GLP-1 receptor in β-cells (56). This signaling pathway plays a major role in pancreas development and β-cell proliferation (57–62). Wnt3a induces expression of cyclin D2, D1, and cdk4 and leads to increased β-cell proliferation in vitro (63). Increased proliferation by Wnt appeared to be mediated by Pitx2-induced cyclin D2 transcription. In vivo studies demonstrated that overexpression of an active mutant of β-catenin increases β-cell proliferation (63). In contrast, inhibition of Wnt signaling by overexpression of axin prevented β-cell expansion and deletion of β-catenin in β-cells using Rip-Cre or Pdx1-Cre mice also had a detrimental impact on islet mass and proliferation (57,63,64). Interestingly, conditional deletion of the Wnt coreceptor TCF7L2 in the pancreas using Pdx1-Cre or Rip2-CreERT2 failed to show a clear defect in β-cell mass in normal conditions (65,66). However, it is possible that TCF7 could play a role in β-cell proliferation and regeneration during diabetogenic conditions but the mechanisms and cell-cycle components are largely unexplored (65,67,68).

In humans, most of the studies implicating a role for Wnt signaling in β-cells have focused on TCF7L2 (Fig. 3B). Depletion of TCF7L2 in human islets was associated with reduced β-cell proliferation under basal conditions (69). In contrast, TCF7L2 overexpression reversed the decrease in β-cell proliferation induced by chronic high glucose (69). Exposure of human islets to Wnt3a stimulated expression of Pitx2 and cyclin D2, although β-cell expansion has not been demonstrated (63).

LEPTIN AND PROLIFERATION

Leptin receptors have been reported to be expressed in primary murine β-cells and in insulinoma cell lines (70–72). The major isoform, ObRb, is considered to mediate the actions of leptin in β-cells. Leptin has been shown to inhibit insulin gene transcription by directly or indirectly (via Janus kinase/signal transducers and activators of transcription [JAK-STAT]) activating SOCS3, which, in turn, inhibits insulin promoter activity. The effects of leptin on β-cell proliferation occur by inhibition of PTEN that activates PI3K/Akt. Alternatively, leptin activation of JAK-STAT has also been reported to directly modulate β-cell proliferation (Fig. 3A).

In vivo models also provide evidence for a direct effect of leptin on islet biology without impacting hypothalamic function. Mice with islet-specific deletion of the leptin receptor (73,74) do not show changes in body weight or food intake, but exhibit improved glucose tolerance indicating the removal of a tonic inhibitory effect of leptin on insulin release. These studies indicate that absence of leptin action promotes β-cell growth by affecting phosphorylation of p70S6k and Akt. These data are consistent with an increase in β-cell mass in Zucker fatty rats, which also lack leptin action (75).

Human β-cells are also known to express ObRb receptors and exogenous leptin is known to suppress insulin secretion in vitro in human islets (71,76). However, leptin effects on β-cell mass remain unexplored (Fig. 3B). The effects of leptin in vivo in humans with type 2 diabetes and obesity is not explicit given the effects of hyperinsulinemia that can in turn lead to leptin release from adipocytes and promote leptin resistance (77).

ESTROGEN, PROGESTERONE, AND PROLIFERATION

Estrogen

In rodent models, the main female estrogen, 17β-estradiol (E2), protects functional β-cell mass against injury associated with both type 1 and type 2 diabetes, including oxidative stress, amyloid polypeptide toxicity, glucolipotoxicity, and apoptosis (78). The role of E2 in β-cell proliferation is less clear, although E2 may also promote β-cell proliferation under specific physiological and experimental conditions (Fig. 4). Historically, an effect of estrogen on islet regeneration was suggested 60 years ago by Houssay et al. (79), who observed that subtotal pancreatectomy followed by implantation of an...
Figure 3—Signaling by leptin, Wnt, and β-catenin in the regulation of β-cell proliferation. A: In murine models, leptin acts via the JAK-STAT pathway to inhibit PTEN and also modulates Akt/PKB and p70S6K. Akt/PKB, which is also activated by growth factor (insulin/IGF-1) signaling, modulates GSK3β. The Wnt/frizzled pathway also regulates GSK3β, which blocks phosphorylation of β-catenin to control the expression of Lef/Tcf7L2 and cyclin D2 and potentially cyclin D1 and cMyc to activate the cell cycle and regulate proliferation. GLP-1 receptor signaling activated by GLP-1 or exendin-4 leads to elevation of cAMP and activation of protein kinase A, which can directly or indirectly via the MEK/ERK1/2 pathway phosphorylate β-catenin. Activation of the insulin or IGF-1 receptors leads to phosphorylation of serine/threonine residues in IRS2, activation of PI3K and Akt/PKB, which can, in turn, phosphorylate and inactivate GSK3β. B: In human β-cells, leptin receptors, GLP-1 receptors, and elements of the Wnt signaling pathway have been reported. However, the downstream proteins (marked in gray) that link to the proliferation response are not fully understood.
Figure 4—Estrogen/progesterone signaling pathways involved in proliferation. A: In rodent β-cells, GPER has been implicated in β-cell proliferation during pregnancy. In rodents, GPER expression is upregulated during pregnancy, which leads to a decrease in the expression of the islet microRNA, miR-338-3p, leading to increased mRNA expression of IRS-2, Pdx1, FOXM1, and cyclin D2 and stimulation of β-cell proliferation. These effects of E2/GPER are cAMP- and PKA-dependent. E2 has also been reported to increase β-cell proliferation in ovariectomized rodents with subtotal pancreatectomy. This effect was associated with an increase in the expression of IRS-2 and PDX1.
estrogen pellet in the remaining pancreas induced regeneration of surrounding islets. Further, a stimulatory effect of estrogen on islet and β-cell regeneration was also observed in the alloxan and streptozotocin diabetic rat models (80). E2 also increases cultured rat islet cell proliferation (81). In these studies, however, estrogen was used at pharmacological concentrations, so it is unclear if these observations are relevant to physiology. Nevertheless, in one study, physiological doses of estrogen have been reported to increase β-cell proliferation and restore the decrease in β-cell mass observed in ovariectomized rodents with subtotal pancreatectomy (82). This effect was associated with an increase in the expression of IRS-2 and Pdx1 proteins via the activation of CREB (Fig. 4A) (82). Thus, in classical models of β-cell regeneration, or at high doses, E2 can induce β-cell proliferation. In contrast, in most studies, E2 used at doses leading to physiological serum concentrations in either male or female rodents, β-cell proliferation was not significantly induced (83–85).

Three estrogen receptors (ERs), ERα, ERβ, and the G-protein–coupled ER (GPER) (also called GPR30), have been identified in rodent β-cells. Unlike the classical nuclear ERs that function as ligand-activated transcription factors in the nucleus, β-cell ERs reside mainly in extranuclear locations. They exert their effect via cytosolic interactions with kinases such as Src, ERK, and AMPK or with transcription factors such as STAT3 (78,86,87). Interestingly, GPER has recently been implicated in β-cell proliferation (88). Pregnancy is associated with an expansion of the functional β-cell mass to adapt to the increased metabolic demand. In rodents, GPER expression is strongly upregulated during pregnancy. In addition, β-cell mass expansion during pregnancy is associated with a decrease in the expression of the islet microRNA, miR-338–3p. In isolated rat islets, exposure to E2 or the GPER agonist, G1, decreased miR-338–3p to levels observed in gestation, which was associated with increased β-cell proliferation. These effects of E2 are cAMP-dependent and are blocked by cAMP-dependent protein kinase (PKA) inhibitors.

In human β-cells, the three ERs identified in rodents are also present (78,84,85,89,90) (Fig. 4B). Beneficial effects of E2 and ER ligands on β-cell survival, function, and nutrient homeostasis are all observed in cultured human islets (84,85,89,90). Further, the antiapoptotic action of physiological doses of E2 and ER agonists is maintained in human islets transplanted into mice in an in vivo hyperglycemic environment (84). However, in these conditions, there is no proliferation of human β-cells (84). Exposure to E2 reduces the level of miR-338–3p in human islet cells (88). However, neither E2 nor silencing of miR-338–3p elicited replication of human β-cells in culture. Thus, as in the case of other molecules described in this review, the impressive effect of E2, GPER, and miR-338–3p observed in rodent β-cell proliferation is not observed in human β-cells. Finally, E2 has been reported to promote the proliferation and inhibit the differentiation of adult human islet-derived precursor cells via ERα (91).

**Progesterone**

Progesterone treatment in vivo stimulates α- and β-cell proliferation in male and female mice. This effect is not observed in gonadectomized mice, suggesting that progesterone-induced islet cell proliferation requires intact gonadal function (92). Indeed, this effect is not observed in cultured rat islet cells (81). In contrast, Picard et al. (93) have shown that female progesterone receptor-deficient mice have lower fasting blood glucose and higher fasting insulin associated with greater glucose clearance. The enhanced pancreatic function in these mice is attributed to higher islet mass with enhanced β-cell proliferation. This is not associated with differences in the islet expression level of the cell-cycle regulators, p21, p27, cyclin D1, cyclin B1, and cyclin E (Fig. 4A). In contrast, the protein levels of the tumor-suppressor p53 were markedly decreased in progesterone receptor-deficient islets. Progesterone did not affect miR-338–3p levels in INS-1 cells (88).

Although the presence of progesterone receptors in the endocrine pancreas in humans suggests a direct role of progesterone on pancreatic islet function (94), no effect of progesterone or its receptor in human β-cell proliferation has been described (Fig. 4B).

**CONCLUSIONS**

As is clear from the preceding sections, intracellular signaling pathways connecting cell surface receptors and channels to proliferative machinery in the β-cell are complex. Interestingly, as emphasized previously (1), signaling pathways can access cell-cycle machinery in multiple, as well as distinct ways, with some pathways activating cdk5, others activating early or late cyclins, and others principally repressing cell-cycle inhibitors, and still others acting on several of these targets. An aspect of signaling that has not been emphasized in these two Perspectives, but is very important, is the complex cross talk between and among signaling pathways. For example, GSK3β is a constitutively active kinase that is involved in both PI3K/Akt signaling as well as Wnt–β-catenin signaling.

proteins. β: Human β-cells. The gray lines are molecules and pathways that are known to exist in rodents but are unknown in human β-cells. The human β-cell signaling road map is underdeveloped. Although FOXM1, IRS-2, cyclin D2, PDX-1 are known to be present in human β-cell, their involvement in estrogen signaling has not been studied. Exposure to E2 reduces the level of miR-338–3p in human islet cells. However, neither E2 nor silencing of miR-338–3p elicited replication of cultured human β-cells. In addition, progesterone signaling has not been studied in human β-cells.
And IRS2 may activate downstream signals in both the PI3K and RAS-MAPK pathways. Prolactin receptor–signaling may activate not only its canonical downstream JAK2-STAT5 pathway, but also PI3K and MAPK signaling. Moreover, these pathways, which we have depicted as being linear, top-down pathways, in fact are replete with autoinhibitory as well as amplifying limbs. Thus, insulin and IGF2 signaling via IRS2 activates PI3K/mTOR pathways that feed back to inhibit IRS2, to attenuate IRS2 signaling. This complexity is challenging, but also provides multiple and rich targets for small molecules that can activate β-cell proliferation.

It is also important to emphasize that additional important intracellular signaling pathways have not been covered in these two Perspectives because of space limitations. Examples include GH, Epo, ICA-512, JAK2, STAT5, SOCS-CISH signaling; stem cell factor, c-kit signaling; GPCR, cAMP, PKA, CREB, CREM signaling; TGF-β, BMP, activin, inhibin, myostatin, SMAD signaling; details of RAS-MAPK signaling; muncaricin, adrenergic, and cannabinoid signaling; cadherins, integrins, and focal adhesion kinases; and signaling via purinergic receptor and adenosine kinase pathways that have recently become relevant as a result of high-throughput small molecule β-cell screens. These and others merit attention in future reviews.

Finally, it is important to underscore how little research has been done on these pathways in the adult human β-cell. While broadly similar in juvenile rodent and adult human β-cells, these pathways differ in important details. Elucidating these differences may provide clues explaining why adult human β-cells are recalcitrant to induction of proliferation, as well as therapeutic opportunities for inducing human β-cell proliferation. With the advent of techniques to purify human β-cells, there is great current opportunity to explore and define the unique detailed “anatomy” of human β-cell proliferation. Thus, in human β-cells, we are still driving in the dark without headlights. It is time to turn on the floodlights.

Acknowledgments. The authors apologize to the many authors whose important publications were not cited because of lack of space. The authors would like to thank Corentin Cras-Meneur for assistance in preparing the figures.

Funding. The authors wish to thank the funding agencies for their essential contribution to this work, which was supported by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) grants DK084236 and DK073716 and the Juvenile Diabetes Research Foundation (JDRF) International grant 46-2010-758 to E.B.-M.; NIDDK grants DK067536 and DK055523, the Harvard Stem Cell Institute, the JDRF/Sanofi Strategic Alliance, and AstraZeneca to R.N.K.; NIDDK grant DK065149, American Diabetes Association grant 7-11-BS-128, and JDRF grant 17-2011-598 to D.K.S.; NIDDK grants DK093626, DK074970, and HD044405, JDRF grant 1-2006-837, and the March of Dimes grant 6-FY07-678 to F.M.-J.; the NIDDK Beta Cell Biology Consortium grants U01 DK089538 and DK55023, JDRF grants 1-2008-39, 17-2011-598, and 34-2008-630 to A.F.S.; and NIDDK grants DK77096 and DK67351 and JDRF grant 47-2012-750 to A.G.-O.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. E.B.-M., R.N.K., D.K.S., F.M.-J., A.F.S., and A.G.-O. wrote the manuscript, reviewed and edited the manuscript, and contributed to the discussion. E.B.-M., R.N.K., D.K.S., F.M.-J., A.F.S., and A.G.-O. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

References
17. Corradetti MN, Inoki K, Bardeesy N, DePinho RA, Guan KL. Regulation of the TSC pathway by LKB1: evidence of a molecular link between tuberous


76. Houssay BA, Foglia VG, Rodriguez RR. Production or prevention of some types of experimental diabetes by oestrogens or corticosteroids. Acta Endocrinol (Copenh) 1954;17:146–164
78. Sorensen RL, Breilje TC, Roth C. Effects of steroid and lactogenic hormones on islets of Langerhans: a new hypothesis for the role of pregnancy steroids in the adaptation of islets to pregnancy. Endocrinology 1993;133:2227–2234