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Gastrointestinal hormones and the regulation of β-cell mass

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Type 2 diabetes occurs due to a relative deficit in β-cell mass or function. Glucagon-like peptide 1 (GLP-1), glucose-dependent insulinotropic polypeptide (GIP), cholecystokinin (CCK), and gastrin are gastrointestinal hormones that are secreted in response to nutrient intake, regulating digestion, insulin secretion, satiety, and β-cell mass. In this review, we focus upon β-cell mass regulation. β-cell mass expands through β-cell proliferation and islet neogenesis; β-cell mass is lost via apoptosis. GLP-1 and GIP are well-studied gastrointestinal hormones and influence β-cell proliferation, apoptosis, and islet neogenesis. CCK regulates β-cell apoptosis and mitogenesis, and gastrin stimulates islet neogenesis. GLP-1 and GIP bind to G protein-coupled receptors and regulate β-cell mass via multiple signaling pathways. The protein kinase A pathway is central to this process because it directly regulates proliferative and anti-apoptotic genes and transactivates several signaling cascades, including Akt and mitogen-activated protein kinases. However, the signaling pathways downstream of G protein-coupled CCK receptors that influence β-cell mass remain unidentified. Gastrointestinal hormones integrate nutrient signals from the gut to the β-cell, regulating insulin secretion and β-cell mass adaptation.

Keywords: islet; β-cell; GLP-1; GIP; CCK; gastrin; protein kinase B/Akt

Introduction

Glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are the two most well-known gut peptides that influence β-cell biology. GLP-1 is produced and secreted in response to fat and glucose from intestinal L-cells, which are located in the distal ileum and colon.[1] GIP is synthesized and secreted from intestinal K-cells, which reside in the duodenum and jejunum, also in response to fat and carbohydrate.[1] Both GLP-1 and GIP promote insulin secretion after meal ingestion by binding to their G-protein coupled receptors on the β-cell, termed *the incretin response*.[1]

Cholecystokinin (CCK) and gastrin are additional gut peptides that control β-cell biology. CCK is produced and secreted by intestinal I-cells in the duodenum in response to fat and protein.[2] CCK classically binds the CCKA receptor (CCKAR) and regulates gall bladder contraction and pancreatic exocrine secretion.[2] CCK is also a neuropeptide that binds the CCKB receptor (CCKBR) and regulates anxiety, satiety, and other behaviors.[2] Gastrin is synthesized and secreted from G cells in the gastric antrum and stimulates gastric acid secretion via the CCKBR.[2] CCK, unlike gastrin, enhances glucose-stimulated insulin secretion in mice[3] and humans[4] via the CCKAR. CCK is not considered a physiological incretin hormone because CCK receptor antagonism does not diminish meal-induced insulin secretion in humans.[5] However, CCK is a potential therapeutic for type 2 diabetes because exogenous CCK treatment enhances insulin secretion in patients with type 2 diabetes.[6]

Loss of β-cell mass plays an important role in type 2 diabetes pathogenesis. Despite β-cell mass being highly variable in both non-diabetic and diabetic human populations,[7,8] cadaveric studies demonstrate that β-cell mass declines in patients with diabetes.[9–12] In fact, a β-cell mass threshold exists, wherein reductions below this point cause hyperglycemia.[12] In non-diabetic obese humans, β-cell

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mass expands to cope with the larger demand for insulin evoked by insulin resitance.[9,10,13] Expansion of β-cell mass occurs through increased β-cell proliferation[10] and islet neogenesis.[9,10] Obese patients with diabetes have reduced β-cell mass due to increased β-cell apoptosis[9,10] and reduced β-cell proliferation.[10]

Gut peptides are excellent candidate regulators of β-cell mass expansion because they already are sensors of nutrient intake for the gastrointestinal system, the brain, and the β-cell. This review will focus upon the *in vivo* data supporting a role for gut peptides in β-cell proliferation, apoptosis, and neogenesis. We will further discuss the *in vitro* mechanisms by which gut peptides regulate β-cell mitogenesis and survival.

β-cell proliferation

GLP-1 stimulates β-cell proliferation in vivo

GLP-1 is the most studied β-cell mitogen. Exogenous GLP-1 treatment stimulates β-cell proliferation in many models, including pancreatic injury, β-cell ablation, autoimmunity, and obesity-induced diabetes. However, the role of endogenous GLP-1 in stimulating β-cell proliferation in these models is less clear.

Exogenous GLP-1 treatment enhances β-cell replication in many animal models. GLP-1 treatment of normoglycemic rats[14] and mice[15–19] stimulates β-cell proliferation. During pancreatic regeneration, GLP-1 receptor (GLP-1R) agonism increases β-cell replication after partial pancreatectomy[20,21] and after caspase 8-mediated β-cell ablation.[22] In the context of autoimmune attack, GLP-1R agonists activate β-cell mitogenesis and ameliorate diabetes in non-obese diabetic (NOD) mice[23] and BioBreeding (BB) rats.[24]

GLP-1 treatment stimulates β-cell replication in multiple models of obesity-induced diabetes. Defective leptin action, through either the Leptin^{ob} or Leptin receptor^{db} mutation, leads to severe obesity, insulin resistance, and in some mouse strains, diabetes. Leptin$^{ob/ob}$ mice treated with a GLP-1R agonist increase islet size and β-cell mass.[25] Similarly, GLP-1R agonism ameliorates diabetes and increases β-cell mitogenesis in Leptin$^{db/db}$ mice[26–29] and leptin-deficient Zucker Diabetic Fatty (ZDF) rats.[30]

GLP-1 also promotes β-cell replication in models of obesity with intact leptin signaling. The Otsuka Long Evans Tokushima Fatty (OLETF) rat is a model of obesity and diabetes, due to a null *Cckar* mutation.[31] GLP-1 treatment of the OLETF rat stimulates β-cell proliferation.[32] In a model of high-fat diet plus streptozotocin treatment to reduce β-cell mass, GLP-1R agonism increases β-cell proliferation in rats[33] and mice.[34] These data demonstrate that exogenous GLP-1 treatment promotes β-cell replication in models of β-cell regeneration and obesity-induced diabetes.

Endogenous GLP-1 may promote β-cell mitogenesis in specific models. Lean, unchallenged GLP-1 receptor (GLP-1R) knockout mice have normal β-cell mass.[35] However, after partial pancreatectomy, GLP-1R knockout mice have diminished β-cell regeneration.[36] The latter study did not differentiate between islet neogenesis and β-cell proliferation. Given that β-cell regeneration after partial pancreatectomy in mice mostly occurs through β-cell replication,[37] endogenous GLP-1 likely promotes β-cell mitogenesis after partial pancreatectomy. In the context of obesity-induced diabetes, Leptin$^{ob/ob}$ mice that are GLP-1R-deficient have equal β-cell mass as wild-type Leptin$^{ob/ob}$ controls.[38] This likely occurs because leptin-resistant and leptin-deficient animals demonstrate reduced intestinal GLP-1 secretion and plasma GLP-1 concentrations.[39] Therefore, obesity creates a relatively GLP-1-deficient state, where there is insufficient endogenous GLP-1 to expand β-cell mass.

In summary, exogenous GLP-1 treatment stimulates β-cell proliferation in multiple rodent species and models of β-cell mitogenesis. Reports of human β-cell proliferation are sparse. Does GLP-1 promote human β-cell replication? If not, why does GLP-1 not promote human β-cell proliferation? The data to support a role for endogenous GLP-1 signaling in β-cell proliferation are less clear. Future studies are necessary to clarify this issue.

Mechanisms of GLP-1-induced β-cell replication

The mechanisms of GLP-1-triggered β-cell mitogenesis have been rigorously investigated (Fig. 1). Studies have been performed *in vitro* in cell lines and islets, and sparingly *in vivo*. Due to the multiple model systems, many signaling pathways have been identified. In this review, we will present most of the pathways identified *in vitro* and describe *in vivo* corroboration of the data wherever it is available. Since

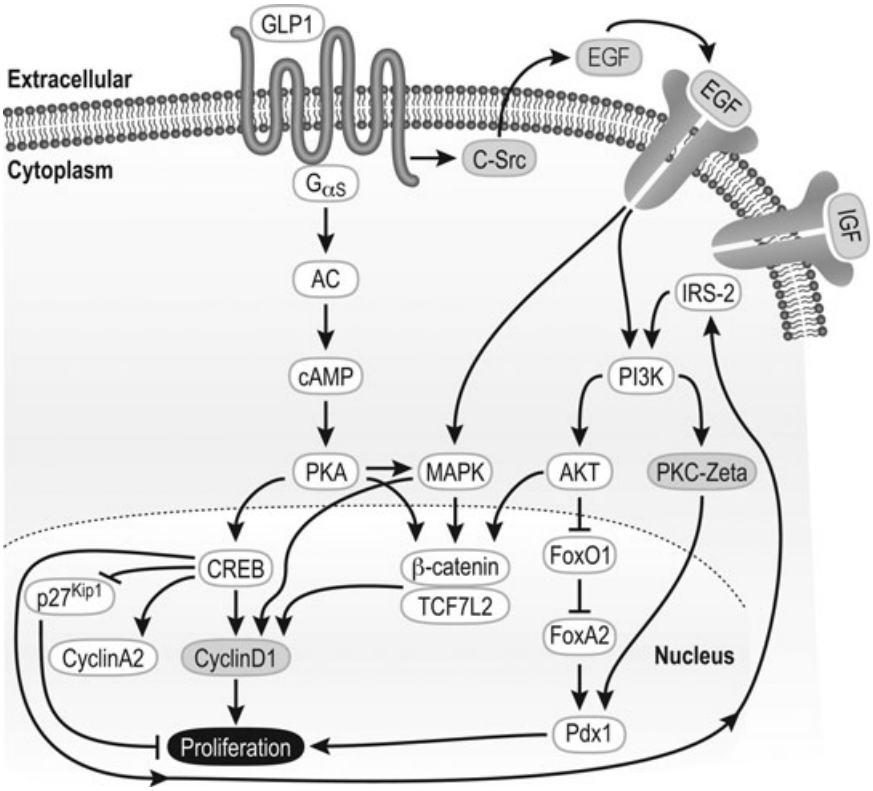

Figure 1. **Mechanisms of GLP-1-induced β-cell replication. GLP-1 stimulates PKA, Akt, MAPK, and PKC-ζ and increases β-cell proliferation. Gray bubbles indicate that *in vivo* data does not exist to support that particular pathway.**

the β-cell is poorly mitogenic, it is likely that GLP-1 uses multiple signaling mechanisms to activate cell division.

GLP-1 stimulates β-cell proliferation via cAMP-dependent signaling. The GLP-1R couples to the $G\alpha_S$ subunit, activates adenylyl cyclase (AC), which increases cAMP production, and activates protein kinase A (PKA).[1] In isolated rat islets, GLP-1 stimulates β-cell replication with efficacy equal to forskolin, suggesting the involvement of cAMP.[40] Accordingly, PKA inhibition diminishes β-cell replication.[40] GLP-1 and forskolin increase cyclin D1 expression in INS-1 cells in a manner dependent upon active PKA.[40,41] PKA-dependent phosphorylation of cAMP response element binding protein (CREB) stimulates cyclin D1 expression through a CRE site in the cyclin D1 promoter.[41] These data suggest that GLP-1R signaling in rat β-cells stimulates a cAMP→PKA→CREB→cyclin D1 cascade to activate replication. However, in mouse islets *ex vivo* and *in vivo*, GLP-1 does not increase cyclin D1 expression. Exendin-4 (a long-acting GLP-1R agonist) treatment in mice increases β-cell mass and proliferation, correlating with increased islet cyclin A2 and reduced expression of the cell cycle kinase inhibitor $p27^{kip}$.[18] In these studies, cyclin D1 and D2 levels were unchanged, in agreement with a separate report.[18,42] Enhanced nuclear CREB signaling in mouse islets phenocopies Exendin-4 treatment.[18] These data, in agreement with results from the rat islet and INS-1 studies, implicate cAMP→PKA→CREB signaling in GLP-1-triggered β-cell replication. However, mouse and rat islets apparently differ in their downstream cell cycle control of replication in response to GLP-1.

GLP-1 activates phosphoinositide 3-kinase (PI3K) and Akt signaling, and enhances β-cell replication. The stimulation of INS-1 cell proliferation by GLP-1 is abrogated by inhibtion of PI3K.[40,43] PI3K-dependent production of PI-3,4,5-triphosphate recruits phosphoinositide-dependent kinase 1 (Pdk1) and Akt to the plasma membrane, resulting in Pdk1-dependent Akt activation.[44] Inhibition of Akt also prevents GLP-1-stimulated INS-1

proliferation.[45] This pathway is supported by *in vivo* studies where Akt activation correlates with increased GLP-1-stimulated β-cell proliferation and delayed diabetes onset in Leptin$^{db/db}$ mice.[29] These data suggest that GLP-1 stimulates β-cell proliferation by a PI3K→Akt pathway.

Akt plays diverse roles in the β-cell, including phosphorylation and nuclear exclusion of FoxO1.[44] Overexpression of constitutively nuclear FoxO1, abrogates GLP-1-induced INS-1 proliferation.[15] Similarly, β-cell-specific overexpression *in vivo* of constitutively nuclear FoxO1 renders β-cells unresponsive to Exendin-4-induced replication.[15] These data suggest that FoxO1 nuclear exclusion is necessary for GLP-1-triggered β-cell mitogenesis.

FoxO1 inhibits forkhead transcription factor A2 (FoxA2) and pancreatic and duodenal homeobox 1 (Pdx-1) expression. Chromatin immunoprecipitation experiments in INS-1 cells demonstrate that GLP-1 reduces forkhead transcription factor O1 (FoXO1) occupancy on the FoxA2 gene promoter, an effect ablated by overexpression of constitutively nuclear FoxO1.[15] GLP-1 therefore enhances FoxA2 levels, which subsequently increases *Pdx-1* expression, by reducing FoxO1 transcriptional activity.[15] In agreement, GLP-1 increases *Pdx-1* expression and DNA binding ability dependent upon PI3K in INS-1 cells.[43]

A role for Pdx-1 is further supported by *in vivo* data. Exendin-4 treatment stimulates β-cell proliferation and correlates with increased *Pdx-1* expression in rats[14,46] and mice.[18] Exendin-4 treatment in β-cell-specific Pdx-1 knockout mice does not stimulate β-cell proliferation.[16] These data are consistent with a pathway whereby GLP-1 activates PI3K→Akt→FoxO1→FoxA2→Pdx-1 and stimulates β-cell proliferation.

Insulin receptor substrate 2 (IRS-2) links PKA signaling to Akt activation. As discussed above, GLP-1 activates CREB-dependent transcription via PKA. A critical target gene for CREB in the β-cell is IRS-2.[47] IRS-2 is a substrate for the insulin receptor (IR) and insulin-like growth factor 1 (IGF-1) receptor (IGF-1R) tyrosine kinases, and is necessary for islet growth. IRS-2-deficient mice develop diabetes due to reduced β-cell mass.[48] IRS-2 binds and activates signaling pathway effectors, including PI3K, via their Src homology 2 (SH2) domain. GLP-1 stimulates CREB-dependent activation of IRS-2 expression, which potentiates IGF-1 mediated activation of the PI3K→Akt pathway,[17,47] linking PKA to Akt. The importance of this pathway is demonstrated in IRS-2 deficient mice. Exendin-4 treatment does not stimulate β-cell proliferation in IRS-2 deficient mice.[17] This occurs because the absence of IRS-2 prevents GLP-1-dependent increases in *Pdx-1* expression,[17] presumably due to deficient Akt activity and FoxO1 nuclear exclusion. These data implicate IRS-2 as the link between PKA and Akt proliferative signaling evoked by GLP-1.

The epidermal growth factor receptor (EGFR) also activates PI3K signaling. In INS-1 cells and rat islets, c-Src or EGFR inhibition ablates GLP-1-induced β-cell replication.[49] It has been hypothesized that the GLP-1R activates the c-Src tyrosine kinase, which activates an endoprotease, releasing an endogenous EGF ligand, thus transactivating the EGFR.[49] This hypothesis was confirmed by addition of either a metalloproteinase inhibitor or a betacellulin (an EGF receptor ligand) neutralizing antibody. Both prevented INS-1 proliferation in the presence of GLP-1.[49] These results implicate transactivation of the EGFR in GLP-1-induced β-cell proliferation. Interestingly, c-Src or EGFR inhibition also abrogates PI3K activation.[49] These data suggest that like IRS-2, EGFR transactivation recruits and activates PI3K, likely via the SH2 domain, and stimulates β-cell replication.

Atypical protein kinase C isoform ζ (PKC-ζ) stimulates β-cell proliferation downstream of PI3K. GLP-1 treatment of INS-1 cells stimulates nuclear translocation of PKC-ζ.[50] Since PKC-ζ is a PDK1 target,[51] it is presumably activated by GLP-1 via a PI3K→PDK1→PKC-ζ pathway. Addition of a PKC-ζ pseudosubstrate or expression of dominant-negative PKC-ζ ablates GLP-1-dependent INS-1 cell proliferation.[50] These data establish that PKC-ζ is downstream of GLP-1 during the promotion of INS-1 proliferation but do not provide insight into its downstream mechanism. However, atypical PKCs have been implicated in Pdx-1 activation.[52] The data suggest a pathway where GLP-1 activates PI3K, which stimulates PKC-ζ→Pdx-1 and enhances proliferation.

Mitogen-activated protein kinase (MAPK) signaling regulates GLP-1-induced β-cell proliferation. GLP-1 stimulates MAPK activation in INS-1,[50] MIN-6,[17] rat islets,[53] and human islets.[17,54] Similarly, *in vivo* GLP-1 treatment increases β-cell proliferation and delays diabetes onset in Leptin$^{db/db}$

mice, correlating with increased MAPK activity.[29] A causal role for MAPK in INS-1 cells is supported by the observation that MAPK inhibition reduces INS-1 cell proliferation and cyclin D1 expression.[40] In addition, in rat islets, cytokines reduce β-cell proliferation by inhibiting MAPK activity.[53] GLP-1 treatment restores MAPK activity and β-cell proliferation, an effect that is sensitive to MAPK antagonism.[53] These data demonstrate that MAPK activation, potentially downstream of the EGFR[49] or PKA,[55] also regulates GLP-1-induced β-cell replication.

The Wnt signaling pathway is activated by GLP-1 and stimulates β-cell proliferation. Canonical Wnt signaling is regulated by the stability of the β-catenin transcription factor.[56] In the absence of Wnt ligands, GSK3β, other proteins, and β-catenin form a destruction complex, where GSK3β phosphorylates β-catenin, targeting it for ubiquitination and degradation.[56] In the presence of Wnt ligands, Wnt binds to the frizzled receptor, leading to activation of dishevelled and inhibition of GSK3β.[56] Inhibition of GSK3β causes accumulation and nuclear translocation of β-catenin.[56] Nuclear β-catenin then combines with other transcription factors, including TCF7L2, and activates gene transcription.[56]

GLP-1 treatment of INS-1 cells or mouse islets increases Wnt target gene expression and enhances TCF7L2 reporter gene activity.[57] This effect was ablated by GLP-1R antagonists,[57] suggesting a GLP-1R-dependent mechanism. Overexpression of dominant negative TCF7L2 (transcription factor 7-like 2) in INS-1 cells or dispersed mouse islets inhibits Exendin-4-induced β-cell proliferation.[57] Similarly, β-catenin knockdown in INS-1 cells inhibits Exendin-4-triggered replication.[57] These results demonstrate that Wnt is necessary for proliferation.

Liu and Habener[57] investigated how GLP-1R signaling leads to Wnt pathway activation in INS-1 cells. Using small molecule inhibitors, PKA, Akt, and MAPK pathways were identified as necessary for GLP-1-induced Wnt signaling activation.[57] Liu and Habener further showed that PKA phosphorylates β-catenin on Ser-675, prevents its ubiquitination and degradation.[57] GLP-1 thus activates a GLP-1R→cAMP→PKA→β-catenin pathway. Using chromatin immunoprecipitation in INS-1 cells, both β-catenin and TCF7L2 were identified on the cyclin D1 promoter after Exendin-4 treatment, coinciding with a 14-fold increase in cyclin D1 expression.[57] The importance of Wnt signaling in β-cell proliferation is supported *in vivo* by genetic studies, which both increased and reduced Wnt signaling in β-cells.[58] Increased Wnt signaling causes β-cell mass expansion through increased β-cell replication, while reduced Wnt signaling results in loss of β-cell mass.[58] In summary, GLP-1 activates Wnt signaling by stabilizing β-catenin via PKA. Increased β-catenin and TCF7L2 transcribe cyclin D1 and enhance β-cell proliferation.

In summary, the GLP-1R activates two key pathways that trigger β-cell mitogenesis (Fig. 1). The GLP-1R activates PKA signaling and transactivates the EGFR. PKA signaling directly activates MAPK signaling, Wnt signaling, and PI3K→Akt via CREB→IRS-2. The EGFR activates MAPK signaling, PI3K→Akt, and PI3K→PKC-ζ. The PKA, Akt, MAPK, and PKC-ζ pathways activate CREB, Wnt, and Pdx-1 transcription factors, stimulating β-cell replication.

The role of GIP in the stimulation of in vivo *β-cell proliferation is unclear*

Unlike GLP-1, GIP has strong effects on adipocyte biology. GIP receptor (GIPR) knockout mice fed a high-fat diet are resistant to obesity, insulin resistance, and glucose intolerance.[59] Even more strikingly, GIPR knockout mice in the Leptin$^{ob/ob}$ background resist obesity, demonstrate improved insulin sensitivity, and are more glucose tolerant.[59] This occurs because GIPR knockout mice expend more energy and use fat as their preferred energy source, resulting in reduced adiposity.[59]

The dual role of GIP in adipocytes and β-cells has complicated interpretations of GIP as a β-cell mitogen. For example, GIPR antagonist administration to Leptin$^{ob/ob}$ mice improves glucose tolerance, enhances insulin sensitivity, and reduces β-cell mass.[60] If GIP promotes β-cell proliferation during obesity, then GIP inhibition should reduce β-cell mass. However, improvements in glucose tolerance and insulin sensitivity reduce the insulin demand and need for expanded β-cell mass. Therefore, it is unclear whether the primary effect of GIP antagonism is on the adipocyte or the β-cell.

A few studies implicate GIP in *in vivo* β-cell proliferation. Dipeptidyl Peptidase-4 (DPP-IV) degrades plasma GIP and GLP-1. DPP-IV inhibitors increase β-cell proliferation during β-cell

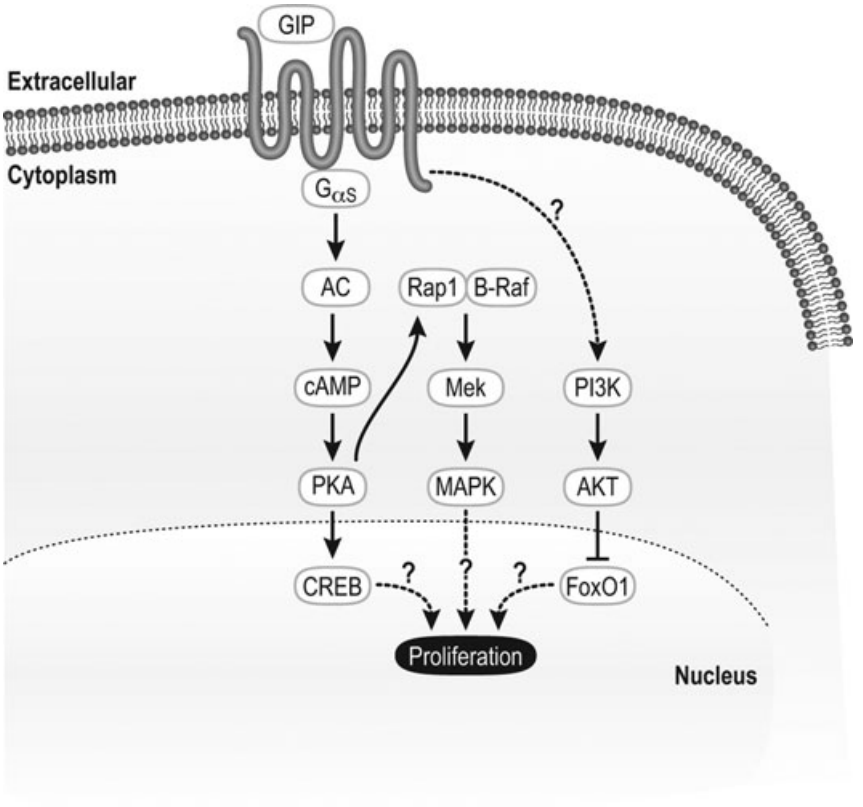

Figure 2. **Mechanisms of GIP-stimulated β-cell proliferation. GIP activates PKA, Akt, and MAPK, triggering β-cell mitogenesis. Dotted lines and question marks indicate that the mechanisms connecting the indicated arrows are unidentified.**

regeneration,[22] in the Leptin$^{db/db}$ mouse,[26] and in high-fat fed mice treated with streptozotocin.[34] These studies do not differentiate between GLP-1 and GIP. However, one report demonstrates that GIP treatment increases islet size and number in the Leptin$^{ob/ob}$ mouse.[61] The latter study does not discriminate between β-cell proliferation, apoptosis, or islet neogenesis. In a human patient, elevated fasting plasma GIP correlates with islet cell hyperplasia.[62] Despite the limited *in vivo* evidence, *in vitro* experiments demonstrate that GIP enhances β-cell mitogenesis.[40,63–65]

Mechanisms of GIP-induced β-cell replication

GIP activates the cAMP→PKA pathway in a fashion similar to GLP-1 (Fig. 2). GIP binds to its G protein-coupled receptor[1] and activates $G\alpha_S$→cAMP→CREB signaling.[1,63,65] Inhibition of PKA prevents GIP-stimulated β-cell replication in INS-1 cells[64] and rat islets.[40] These data implicate PKA signaling downstream of the GIPR.

GIP enhances MAPK signaling and stimulates β-cell proliferation. GIP treatment of INS-1 cells activates the MAPK phosphorylation cascade and MAPK-dependent transcription.[65,66] Inhibition of MAPK signaling prevents GIP from activating INS-1 cell cycle progression.[40,64] McIntosh and colleagues investigated the mechanisms of GIP-induced MAPK signaling.[66] They reported that cAMP/PKA signaling activates MAPK by influencing Rap1/B-Raf activity.[66] Increased Rap1/B-Raf triggers the MAPK cascade, activating the Mek upstream kinase, leading to Erk activation.[66] These data implicate the MAPK pathway in GIP-mediated INS-1 cellular proliferation and establish crosstalk between PKA and MAPK pathways in β-cell replication.

GIP promotes PI3K→Akt signaling and activates β-cell replication. GIP promotes activation of PI3K→Akt signaling and subsequent FoxO1 and GSK3β phosphorylation.[65] In rat islets, inhibition of PI3K prevents GIP from triggering β-cell mitogenesis.[40] However, in INS-1 cells, inhibition of PI3K

does not strongly affect GIP-mediated replication.[64] These data suggest that GIP→PI3K→Akt signaling could promote β-cell proliferation, but the data is less convincing than those implicating the PKA and MAPK pathways.

In summary, GIPR activation activates PKA, Akt, and MAPK and stimulates β-cell proliferation (Fig. 2). This pathway is quite similar to GLP-1-stimulated β-cell replication. MAPK activation occurs through PKA, but the pathway of Akt activation remains unknown. In addition, the transcription factors downstream of GIPR-dependent signaling remain unidentified. It is likely that these undetermined pathways and transcription factors are similar to those described for GLP-1.

CCK promotes rat β-cell replication

CCK stimulates rat β-cell proliferation *in vivo*. Administration of CCK-8 after streptozotocin treatment in rats increases fasting plasma insulin, reduces fasting plasma glucose, and expands β-cell mass via proliferation.[67] Similarly, in the rat partial pancreatectomy model, CCK-8 treatment increases islet cell replication during the regenerative phase.[68] The OLETF rat is a model of obesity and diabetes caused by a null *Cckar* mutation.[31] After partial pancreatectomy, the OLETF rat demonstrates reduced β-cell proliferation.[69] These data implicate CCK→CCKAR signaling *in vivo*, which promotes rat β-cell proliferation.

CCK enhances rat β-cell proliferation *ex vivo*. Neonatal rat β-cells proliferate in culture after CCK-8 treatment.[70] Similarly, overexpression of *Cck* in isolated rat islets stimulates islet cell proliferation.[71] However, overexpression of *Cck* in mouse and human islets has no effect on replication.[71] Recently, we demonstrated that CCKAR blockade, but not CCKBR antagonism, prevents CCK-8-stimulated β-cell proliferation. In addition, a specific CCKAR agonist phenocopied CCK-8-activated β-cell mitogenesis (J.A.L, unpublished data). These data demonstrate that CCK→CCKAR signaling stimulates rat β-cell proliferation.

Gastrin and β-cell proliferation

The data implicating gastrin in β-cell proliferation are sparse. Proglumide, a CCK and gastrin antagonist, diminishes β-cell regeneration after alloxan treatment in mice.[72] Given the limited involvement of CCK in mouse β-cell proliferation, this phenotype could be explained by antagonism of gastrin action. This data links gastrin to β-cell proliferation or islet neogenesis. As will be discussed later, a role for gastrin in neogenesis is more strongly supported. In humans, islet hyperplasia and β-cell proliferation have been observed in close proximity to intrapancreatic gastrinoma tumors.[73] However, despite serum gastrin levels high enough to cause gastrointestinal symptoms, β-cell proliferation was not observed >1 cm away from the gastrinoma,[73] implicating a factor other than gastrin.

Islet neogenesis

Islet neogenesis versus β-cell proliferation

The relative contributions of β-cell proliferation versus islet neogenesis to β-cell mass expansion is highly controversial. Initial studies provided histologic clues for islet neogenesis, leading to speculation about the existence of pluripotent islet stem cells. However, no unambiguous identifier for islet neogenesis exists, like β-cell BrdU incorporation in proliferation, making detection of islet neogenesis challenging.

Genetic methods to trace cell lineage have been informative, but not definitive. Irreversible β-cell labeling demonstrates that the major source of new β-cells during adult life or after partial pancreatectomy is pre-existing β-cells,[37] implying a β-cell proliferative mechanism. This result was confirmed in a β-cell regeneration model, involving diptheria toxin-induced β-cell loss.[74] These studies powerfully demonstrate the role of β-cell proliferation during β-cell mass regeneration, but they do not directly investigate the contribution of islet neogenesis to this process.

Inada and colleagues directly addressed the contribution of ductal progenitor cells to islet neogenesis during pancreatic ductal ligation.[75] They used the human carbonic anhydrase II (CAII) promoter to irreversibly label duct cells.[75] Greater than 40% of β-cell mass regeneration after pancreatic duct ligation was attributable to islet neogenesis from CAII-positive cells.[75] In a similar study, Solar and colleagues irreversibly labeled duct cells using the mouse Hnf1β promoter.[76] Fewer than 5% of β-cells were ductal in origin after pancreatic duct ligation or alloxan treatment.[76] These data suggest that CAII-positive cells and not Hnf1β-positive cells contribute to islet neogenesis. A more recent study reported α-to-β-cell transdifferentiation after β-cell ablation.[77] Taken together, these studies suggest that

significantly more plasticity exists in the endocrine and exocrine pancreas than previously expected. In addition, the progenitor cell type for islet regeneration could be model- and stimulus-specific.

GLP-1 stimulates islet neogenesis

GLP-1 promotes islet neogenesis in many model systems. In unstressed rodents, exogenous GLP-1R agonism stimulates islet neogenesis.[14,15] In models of β-cell regeneration, GLP-1R agonist administration increases markers of islet neogenesis in rats subjected to partial pancreatectomy[20,21] or streptozotocin treatment,[78] and in diabetic NOD mice.[36,79] Similar results have also been obtained in models of obesity-induced diabetes.[27,29,30] These studies provide observational and correlative data linking GLP-1 to islet neogenesis.

The data that support islet neogenesis come from histological observations. GLP-1-induced "small islets," "islet-like clusters," and/or "β-cell clusters" have been identified in many studies.[14,15,20,21,27,29,30] These very small islets have been associated with acinar[14] or duct structures.[14,20,21,30,36,78,79] The duct-associated *de novo* islets are most often associated with the smallest ducts, termed intercalated ducts. The many observations of duct-associated β-cell clusters has prompted the hypothesis that GLP-1-responsive islet progenitor cells exist within the duct cell population.

Two studies have challenged this model by transplanting duct-rich cell populations into diabetic rodents. Intravenous treatment with GLP-1 overexpressing primary duct cells of rats made diabetic with streptozotocin treatment restored glucose homeostasis via graft cell infiltration into the exocrine and endocrine pancreas.[80] Second, transplantation of low-purity human islet preparations into streptozotocin-induced diabetic NOD-Scid mice (NOD mice without a competent immune system) improved glycemia, increased graft insulin content, and increased insulin-positive graft cells during gastrin and GLP-1 combination therapy.[81] In addition, the insulin-positive graft cells were commonly cytokeratin 19 (CK19)-positive.[81] These studies suggest that GLP-1-responsive islet progenitors may exist within the duct cell population, but do not exclude extra-graft effects of GLP-1 treatment.

In vitro differentiation studies demonstrate that GLP-1 promotes β-cell differentiation. Several studies show that GLP-1 treatment induces the differentiation of duct cells into insulin-producing cells,[53,82,83] which are capable of secreting insulin in response to glucose.[82,83] In addition, embryonic stem cell treatment with GLP-1 and other factors stimulates differentiation of insulin-positive cells and increases glucose responsiveness.[84,85] Furthermore, transplantation of these cells into diabetic NOD-Scid mice improves glycemic control.[84,85]

In conclusion, substantial evidence supports GLP-1-stimulated islet neogenesis. During unstressed conditions, obesity-induced diabetes, or after β-cell ablation, GLP-1R agonism increases the number of small islets and duct-associated β-cells. In culture, GLP-1 can trigger the differentiation of duct or embryonic stem cells into insulin-producing cells. Genetic labeling studies are controversial, both supporting and denying the existence of ductal islet progenitor cells.[75,76] Future studies to identify and genetically label the islet progenitor cells before GLP-1 treatment will be necessary to conclude that GLP-1 causes islet neogenesis.

GIP and islet neogenesis

Three reports suggest that endogenous and exogenous GIP stimulate islet neogenesis. Overexpression of a dominant-negative GIPR in mouse β-cells causes diabetes due to severely reduced β-cell mass.[86] Even as early as 10 days of age, the pancreas of GIPR dominant-negative mice demonstrates fewer islets of normal size and fewer β-cell clusters,[86] suggesting impaired endogenous islet neogenesis. Similarly, after streptozotocin treatment, GIPR antagonism worsens diabetes and severely reduces β-cell mass.[87] Exogenous GIPR agonists increase islet number and islet size in Leptin$^{ob/ob}$ mice.[61] Greater islet number might be an indication of neogenesis, but association of new islets with ducts has not been reported in these studies. GIP addition to differentiation cocktails for embryonic stem cells enhances differentiation, insulin production, and glucose responsiveness.[88] These studies implicate a potential for GIP in the regulation of islet neogenesis and warrant further investigation.

Gastrin promotes islet neogenesis

Gastrin signaling stimulates islet neogenesis in unstressed mice. Pancreatic overexpression of transforming growth factor α (TGFα) in mice causes metaplastic-ductule formation, including some insulin-positive cells.[89] These insulin-positive

cells are not sufficient to increase β-cell mass.[89] β-cell-specific overexpression of gastrin alone leads to no demonstrable phenotype.[89] However, combined overexpression of gastrin and TGFα increases islet mass and reduces duct mass when compared to transgenic mice overexpressing TGFα only.[89] These findings suggest that gastrin promotes the differentiation of ductule cells into islets. Similarly, pancreatic overexpression of the CCKBR via the *Elastase* promoter increases insulin-positive single cells and cell clusters both adjacent to ducts and interspersed within the acinar tissue.[90] These changes increase whole pancreas insulin content and improve glucose tolerance,[90] suggesting increased functional β-cell mass.

Gastrin promotes islet neogenesis during islet regeneration. After pancreatic duct ligation in rats, gastrin treatment doubles β-cell mass by increasing duct-associated single β-cells and β-cell clusters without affecting β-cell proliferation or apoptosis.[91] Similarly, gastrin and EGF treatment restores normoglycemia by increasing islet number and islet mass in alloxan- and streptozotocin-treated mice.[92,93] Histologically, more insulin-positive duct cells[93] and cytokeratin-positive β-cells were observed in ducts, small β-cell clusters, and islets.[92] Insulin and cytokeratin co-staining suggest duct-to-islet transdifferentiation. In support of this inference, culture of isolated human islets with gastrin and EGF increases the number of insulin-, glucagon-, and CK19-positive cells.[94] Transplantation of these cultured human islets into diabetic NOD-Scid mice improves glucose tolerance and increases glucagon-, insulin-, and CK19-positive graft cells.[94] These data suggest, in agreement with those conducted in alloxan-treated mice, that gastrin and EGF treatment can stimulate islet neogenesis from cytokeratin-positive cells.

Gastrin therapy, in combination with an EGF ligand, stimulates islet neogenesis. The data support a model wherein the EGF ligand increases ductule mass through acinar transdifferentation or ductule proliferation. Gastrin therapy, via CCKBR signaling, promotes the differentiation of new ductule structures into single β-cells and eventually, duct-associated islets.

CCK and islet neogenesis

A small number of reports suggest a role for CCK in islet neogenesis. One report suggests that both CCK and GLP-1 enhance glucose-stimulated insulin secretion and the number of β-cells in fetal pig islet-cell like clusters.[95] These data suggest that CCK, like GLP-1, stimulates functional maturation and differentiation of fetal β-cells. As discussed previously, in one study, simultaneous CCKAR and CCKBR antagonism worsens diabetes in alloxan-treated mice.[72] This study does not differentiate between CCK and gastrin nor their role in islet neogenesis versus β-cell proliferation. Since CCK is as good an agonist for the CCKBR as gastrin, it is likely that combination treatment of CCK with an EGF-like ligand could promote islet neogenesis.

β-cell apoptosis

GLP-1 promotes β-cell survival in vivo

GLP-1 prevents β-cell death in multiple models of β-cell loss. In unstressed mice, GLP-1 reduces β-cell apoptosis.[16] In streptozotocin-induced β-cell toxicity, GLP-1 receptor agonism reduces β-cell apoptosis and improves glycemic control,[96,97] while GLP-1R knockout mice demonstrate increased β-cell apoptosis and worsened diabetes.[96] In models of autoimmunity, GLP-1 treatment prevents β-cell apoptosis in the NOD mouse[23,79] and the BB rat.[24] In models of obesity-induced diabetes, GLP-1 receptor agonism reduces β-cell apoptosis and ameliorates diabetes in the Leptin$^{db/db}$ mouse,[29,98] the ZDF rat,[30] the OLETF rat,[32] and *Psammomys obesus*.[99] These studies provide strong evidence for GLP-1 receptor signaling as an anti-apoptotic therapeutic for the β-cell, and suggest a possible application in islet transplantation therapy. In streptozotocin-induced diabetic mice receiving islet transplants, GLP-1R agonists reduce graft β-cell apoptosis.[100–102] The strength of these studies has prompted the use of GLP-1R agonists in human patients with type 1 diabetes receiving islet transplantations. GLP-1R agonism after islet transplantation reduces both the number of islets required for and extends the duration of insulin-independence.[103–105]

Anti-apoptotic mechanisms of GLP-1

GLP-1 can promote β-cell survival in many models of β-cell apoptosis. GLP-1 prevents the cytotoxic effects of cytokines, ER stress, glucolipotoxicity, staurosporine, hydrogen peroxide, and streptozotocin. In this section, we will focus upon the signaling pathways downstream of the GLP-1 receptor (Fig. 3).

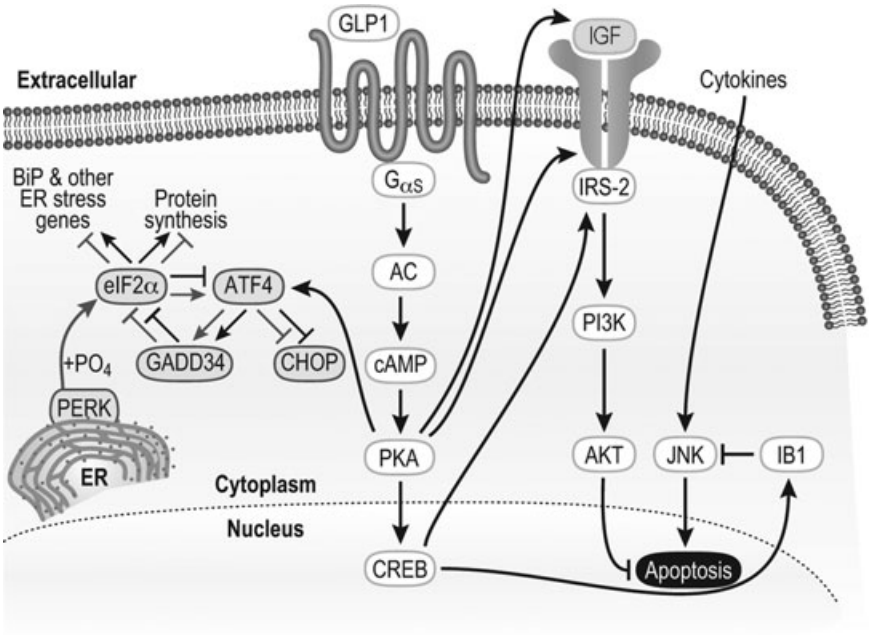

Figure 3. Mechanisms whereby GLP-1 protects β-cells from apoptosis. GLP-1 increases PKA signaling, which activates Akt and prevents β-cell apoptosis. Gray arrows indicate normal unfolded protein response pathways. Black arrows indicate how GLP-1 influences ER stress.

GLP-1 protects β-cells from cytokine-mediated cell death. In mouse,[106,107] rat,[96,108] and human[109] islet cultures, GLP-1R agonists prevent cytokine-mediated β-cell apoptosis. Several reports detail the involvement of both the PKA and the Akt pathways in this process.

PKA signaling is necessary and sufficient for GLP-1 to protect β-cells from cytokines. Direct activation of PKA by forskolin in rat islets mimics the cytoprotective effects of GLP-1.[108] Accordingly, dominant-negative CREB overexpression in human islets prevents GLP-1 from protecting β-cells from cytokine-induced apoptosis.[109] Abderrahmani and colleagues further elucidated the mechanisms downstream of CREB.[110] Islet-brain 1 (IB1) is a c-Jun N-terminal kinase (JNK) pathway scaffold protein, where IB1 expression negatively regulates JNK activation.[111] IB1 has been identified as a type 2 diabetes candidate gene[112] and its expression is critical for JNK activity and apoptosis in the β-cell.[113] Ferdaoussi and colleagues showed that GLP-1-stimulated PKA activity causes CREB-dependent IB1 expression via a *cis*-acting CRE site in the IB1 promoter.[110] Increased IB-1 expression prevents cytokine-mediated JNK activation and apoptosis.[110] These data suggest a pathway wherein GLP-1 activates CREB-dependent IB1 expression and prevents JNK activation. A role for PKA signaling *in vivo* is supported by the $G\alpha_S$ β-cell-specific knockout mouse[114] and the β-cell-specific transgenic overexpressing dominant-negative CREB.[47] Both of these mice are deficient in CREB activity and are diabetic due to reduced β-cell mass and increased β-cell apoptosis.[47,114]

PKA activates the Akt pathway and prevents cytokine-mediated β-cell apoptosis. In human islets, GLP-1-induced protection from cytokine-mediated apoptosis correlates with increased phosphorylated Akt.[109] In rat islets and INS-1 cells, inhibition of Akt signaling by antagonist treatment[108] or dominant-negative Akt constructs[115] abolishes GLP-1-dependent protection from cytokines. Cornu and colleagues further investigated the mechanisms by which Akt activation prevents cytokine-mediated β-cell apoptosis.[106] GLP-1 receptor signaling induces the expression of the IGF-1R and the expression and secretion of IGF-2.[106] A reduction in IGF-1R signaling through ablation of IGF-2 or IGF-1R expression prevents GLP-1-dependent cytoprotection.[106] Recently, Thorens and colleages

further demonstrated that IGF-1R expression is PKA-dependent.[116] These studies highlight a pathway whereby GLP-1 activates PKA, which induces the expression of IGF-2 and the IGF-1R and increases Akt-dependent signaling and prevent apoptosis. Similarly, β-cell-specific overexpression of a dominant-negative CREB causes diabetes due to reduced β-cell mass, correlating with reduced IRS-2 expression.[47] Therefore, PKA-dependent signaling increases Akt activation and promotes β-cell survival by increasing IGF-2 expression and secretion, IGF-1R expression, and IRS-2 expression.

GLP-1 protects β-cells from ER stress-induced death by directly modulating the ER stress pathway via PKA. In the Leptin$^{db/db}$ mouse, Exendin-4 treatment reduces endoplasmic reticulum (ER) stress and pro-apoptotic CCAAT/-enhancing-binding protein homologous protein (CHOP) expression, increases β-cell mass and ameliorates diabetes.[98] In isolated rat islets, Exendin-4 protects β-cells from ER stress-induced cell death via PKA activity.[98] Drucker and colleagues further determined the mechanism whereby GLP-1 signaling mediates ER stress and the unfolded protein response.[98] GLP-1R signaling regulates the protein kinase-like endoplasmic reticulum kinase (PERK) arm of the unfolded protein response, restoring translation, and promoting synthesis of proteins involved in the ER stress gene expression program, which maintain ER homeostasis.[98] PKA enhances activating transcription factor 4 (ATF-4) translation early during ER stress.[98] Early upregulation of ATF-4, promotes growth arrest and DNA-damage-inducible 34 (GADD34) expression, which promotes dephosphorylation of eIF2α, thus increasing translation of ER stress response genes and decreasing expression of pro-apoptotic CHOP.[98] A role for GLP-1R signaling in modulation of ER stress and promotion of β-cell survival has been confirmed in other animal models. Exendin-4 treatment reduces ER stress, decreases β-cell apoptosis, and increases β-cell mass in both β-cell-specific calmodulin overexpression[117] and after partial pancreatectomy[20] in mice.

GLP-1 protects β-cells from many toxic stresses via PKA and Akt signaling pathways. In human[118] and rat islets,[108] GLP-1 protects β-cells from high glucose and palmitate-triggered apoptosis. Activation of PKA by forskolin mimics GLP-1-induced cytoprotection from glucolipotoxicity.[108] Inhibition of Akt by antagonist treatment[108] or dominant-negative Akt expression[118] ablates GLP-1-mediated protection from high glucose and palmitate. Similarly, PKA and Akt signaling protect β-cells from staurosporine[45,119] and hydrogen peroxide[15,120] induced β-cell apoptosis.

In summary, GLP-1-dependent prevention of β-cell apoptosis is directly controlled by PKA signaling (Fig. 3). The PKA pathway directly inhibits the pro-apoptotic JNK pathway by increasing IB-1 expression. In addition, PKA signaling increases Akt activation by stimulating IGF-2, IGF-1R, and IRS-2 expression and activation. Finally, PKA enhances ATF-4 translation and ameliorates ER stress during the unfolded protein response. By regulating these pathways, GLP-1 protects β-cells from cytokines, glucolipotoxicity, ER stress, staurosporine, and hydrogen peroxide.

GIP prevents β-cell apoptosis in vivo

The role of GIP in reduction of β-cell apoptosis *in vivo* is more clearly defined than its role in β-cell proliferation. In models of obesity-induced diabetes, GIPR agonism reduces β-cell apoptosis and increase β-cell mass, ameliorating diabetes in the Leptin$^{ob/ob}$ mouse,[61] the ZDF rat,[121] and the Vancouver ZDF rat.[121,122] In streptozotocin-treated mice, GIPR agonist treatment reduces β-cell apoptosis,[97,121] while GIPR antagonist treatment worsens diabetes.[87] These studies demonstrate that exogenous GIP treatment prevents β-cell apoptosis during severe obesity and streptozotocin-induced diabetes.

Anti-apoptotic mechanisms of GIP

GIP prevents β-cell death through multiple signaling pathways (Fig. 4). PKA, Akt, p38 MAPK, and JNK are all influenced by GIPR-dependent signaling. Crosstalk between all of these pathways likely mediates the effects of GIP in preventing apoptosis in severe obesity and streptozotocin toxicity.

GIP protects β-cells from ER stress-induced apoptosis via cAMP-dependent pathways. McIntosh and colleagues showed that CREB activates *Bcl-2* expression via a *cis*-acting CRE site in the *Bcl-2* promoter.[123] Inhibition of CREB or *Bcl-2* expression prevented GIP-mediated cytoprotection from ER stress.[123] In addition, both GIP and forskolin phenocopied the effect of GLP-1 in modulating the unfolded protein response via the PERK pathway,

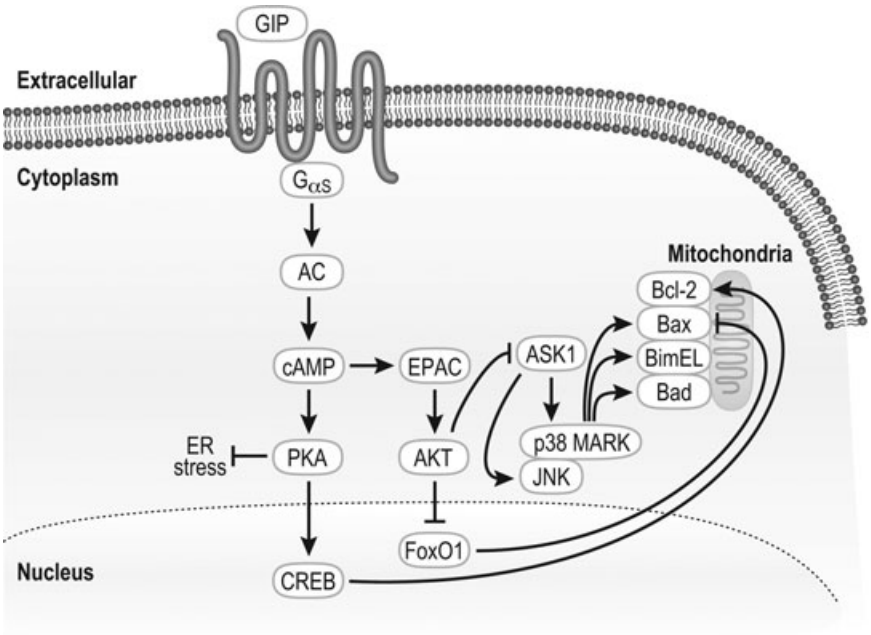

Figure 4. **Mechanisms whereby GIP prevents β-cell apoptosis. GIP stimulates PKA signaling, which activates Akt and inhibits p38 MAPK and JNK, thereby reducing β-cell apoptosis.**

ameliorating ER stress, and promoting β-cell survival via PKA signaling.[98] These studies suggest a direct role for both PKA and CREB, downstream of the GIPR, in reducing ER stress-induced β-cell apoptosis.

GIP activates the Akt pathway, reducing β-cell apoptosis. Combined high glucose/free fatty acid treatment of INS-1 cells and mouse islets stimulates β-cell apoptosis by reducing Akt signaling, increasing nuclear FoxO1, and increasing FoxO1-dependent pro-apoptotic *Bax* expression.[122] GIP treatment increases Akt signaling, preventing nuclear FoxO1 accumulation and reducing *Bax* expression.[122] McIntosh and colleagues further elucidated the mechanism of Akt activation by GIP.[119] Akt inhibition, but not PI3K inhibition, ablates GIP-mediated protection from staurosporine-induced INS-1 cell death.[119] Furthermore, direct exchange protein activated by cAMP (EPAC) activation phenocopies GIP, preventing staurosporine-mediated INS-1 cell death.[119] Unlike GLP-1R signaling, pathways by which PKA signaling increases IGF signaling (via the IGF-1R, IGF-2, and/or IRS-2) are unlikely to mediate Akt activation downstream of the GIPR because PI3K is dispensable. These studies suggest that cAMP activates Akt signaling through EPAC, preventing β-cell death likely via reducing *Bax* expression.

The p38 MAPK and JNK pathways are inhibited by GIP, preventing β-cell apoptosis. GIP-mediated protection from staurosporine-induced INS-1 cell death is suppressed by AC, Akt, p38 MAPK, and JNK inhibition.[124] Inhibition of Akt signaling prevents GIP-mediated reduction in active p38 MAPK and JNK signaling,[124] suggesting that GIP activates Akt and supresses p38 MAPK and JNK activation. This occurs through Akt-dependent phosphorylation and inhibition of apoptosis signal-regulating kinase 1 (ASK1).[124] Finally, activation of p38 MAPK and JNK trigger apoptosis by increasing mitochondrial BimEL, Bad, and Bax phosphorylation, which trigger cytochrome C release and apoptosis.[124] These data implicate a pathway whereby GIP activates Akt, which inactivates ASK1, reducing p38 MAPK and JNK activity. Reduced p38 MAPK and JNK activity prevent mitochondrial accumulation of pro-apoptotic Bcl-2 family member proteins. Since AC is also necessary for this pathway, it is likely that EPAC regulates Akt activation, as discussed previously.

In summary, GIP prevents β-cell apoptosis by increasing cAMP (Fig. 4). GIPR agonism increases

AC activity and cAMP production. Cyclic AMP activates PKA and EPAC. PKA prevents apoptosis directly by regulating the PERK arm of the unfolded protein response. PKA also increases CREB-dependent transcription, increasing *Bcl-2* expression. EPAC activates Akt signaling. Akt prevents apoptosis by phosphorylating FoxO1, preventing FoxO1 nuclear accumulation, and reducing FoxO1-dependent *Bax* expression. Akt also phosphorylates ASK1, which reduces p38 MAPK and JNK activity, preventing mitochondrial pro-apoptotic signaling.

CCK reduces β-cell apoptosis

A few reports exist demonstrating that CCK reduces β-cell apoptosis. The OLETF rat becomes diabetic due to reduced β-cell mass, resulting from increased β-cell apoptosis.[125,126] These studies suggest that signaling through the CCKAR prevents β-cell apoptosis during obesity. Recently, we reported that whole-body CCK-deficiency in the Leptin$^{ob/ob}$ background increases hyperglycemia due to relative hypoinsulinemia.[127] CCK-deficient obese mice display reduced β-cell mass due to increased β-cell death.[127] Furthermore, exogenous CCK treatment *ex vivo* or *in vitro* rescues β-cells from thapsigargin- and cytokine-induced β-cell death.[127] Taken together, these data suggest that CCK binds the CCKAR and reduces β-cell apoptosis during obesity.

Gastrin and β-cell apoptosis

We are aware of only one report linking gastrin to the regulation of β-cell apoptosis. Combination treatment of gastrin and GLP-1 reduced β-cell apoptosis and restored euglycemia in the NOD mouse.[79] Given the sparse data supporting a role for gastrin in β-cell apoptosis and the virtual absence of the CCKBR on the β-cell, it is most likely that GLP-1 was responsible for the prevention of β-cell apoptosis in this study. The combination treatment of GLP-1 with gastrin likely increased neogenesis and restored euglycemia in conjunction with GLP-1, preventing β-cell apoptosis. The role for gastrin in β-cell mass regulation is likely only through islet neogenesis.

Conclusions and future directions

GLP-1 promotes β-cell proliferation, islet neogenesis, and increases β-cell survival. The data demonstrating a role for GLP-1 in islet neogenesis are incomplete because no reliable identifier of islet neogenesis exists. A key future direction will be to determine the islet progenitor cell and use genetic lineage tracing techniques to more strongly demonstrate a role for GLP-1 in islet neogenesis. The signaling pathways underlying GLP-1-induced islet neogenesis have not been fully elucidated. However, the cAMP→PKA→CREB pathway is central to β-cell proliferation and survival, due to its direct effects and its ability to influence Akt, Wnt, PKC-ζ, p38 MAPK, JNK, and MAPK pathways. Most of these signaling networks have been elucidated *in vitro* using cell lines. A significant hurdle will be to determine what are the relative contributions of each pathway to *in vivo* β-cell proliferation.

GIP promotes β-cell survival *in vivo* but only promotes β-cell proliferation *in vitro*. The effects of GIP on adipocyte biology make conclusions about GIP biology in the islet challenging. An important future direction will be the development of adipocyte- and β-cell-specific GIPR knockout mice to elucidate the role of GIP in β-cell proliferation and islet neogenesis *in vivo*. Next, determination of the contributions of each signaling pathway to *in vivo* β-cell proliferation, neogenesis, and survival will be important. Still to be answered is, what are the overlaps, synergies, and differences between GLP-1- and GIP-mediated signaling in the promotion of adaptive β-cell mass expansion?

Gastrin promotes islet neogenesis via the CCKBR. Like GLP-1, definitive cell lineage tracing experiments to conclusively demonstrate neogenesis will be an important advance. In addition, the signaling pathways are not yet fully elucidated and will help determine the extent of overlap between gastrin and GLP-1 in the context of islet neogenesis.

CCK, via the CCKAR, promotes β-cell survival in rodents and β-cell proliferation in rats only. Although CCK does not stimulate human β-cell proliferation, does CCK promote human β-cell survival? Since CCK can bind the CCKBR with similar affinity as gastrin, does CCK promote islet neogenesis? In addition, the signaling pathways that CCK stimulates in the β-cell resulting in proliferation (in rat islets) and survival (in mice) remain undetermined. It is likely that CCK signals similarly to GLP-1 and GIP because the CCK receptors are G protein-coupled and increase cAMP levels.[2] Elucidation of these mechanisms could answer why the

effects of CCK on β-cells are species-specific and could help to design new therapeutics.

Gut peptides regulate β-cell mass by affecting islet neogenesis, β-cell proliferation, and apoptosis by centrally regulating the cAMP→PKA→CREB pathway. In addition, gut peptides stimulate insulin secretion[1,3,4,6] and slow gastric emptying.[1] Reduced gastric motility prolongs nutrient absorption and reduces appetite, stimulating weight loss. These effects have prompted the use of Byetta (Exenatide) and Januvia (Sitagliptin, a DPP-IV inhibitor) as diabetes therapeutics. Their combined effects upon insulin secretion, gastric motility, and β-cell mass make these drugs powerful anti-diabetogenic agents. Improved understanding of the pathways downstream of gut peptide receptors will allow for design of new therapeutics. Potential new therapeutics include (1) GIP receptor ligands with diminished adipogenic and improved insulinotropic effects, (2) CCK and gastrin receptor ligands, and (3) β-cell-specific cAMP→PKA→CREB pathway activators.

Conflicts of interest

The authors declare no conflicts of interest.

References

1. Baggio, L.L. & D.J. Drucker. 2007. Biology of incretins: GLP-1 and GIP. *Gastroenterology*. **132:** 2131–2157.
2. Dufresne, M., C. Seva & D. Fourmy. 2006. Cholecystokinin and gastrin receptors. *Physiol. Rev*. **86:** 805–847.
3. Karlsson, S. & B. Ahren. 1992. CCK-8-stimulated insulin secretion in vivo is mediated by CCKA receptors. *Eur. J. Pharmacol*. **213:** 145–146.
4. Ahren, B., M. Pettersson, K. Uvnas-Moberg, *et al*. 1991. Effects of cholecystokinin (CCK)-8, CCK-33, and gastric inhibitory polypeptide (GIP) on basal and meal-stimulated pancreatic hormone secretion in man. *Diabetes Res. Clin. Pract*. **13:** 153–161.
5. Hildebrand, P., J.W. Ensinck, S. Ketterer, *et al*. 1991. Effect of a cholecystokinin antagonist on meal-stimulated insulin and pancreatic polypeptide release in humans. *J. Clin. Endocrinol. Metab*. **72:** 1123–1129.
6. Ahren, B., J.J. Holst & S. Efendic. 2000. Antidiabetogenic action of cholecystokinin-8 in type 2 diabetes. *J. Clin. Endocrinol. Metab*. **85:** 1043–1048.
7. Matveyenko, A.V. & P.C. Butler. 2008. Relationship between beta-cell mass and diabetes onset. *Diabetes Obes. Metab*. **10**(Suppl 4): 23–31.
8. Henquin, J.C., E. Cerasi, S. Efendic, *et al*. 2008. Pancreatic beta-cell mass or beta-cell function? That is the question!. *Diabetes Obes. Metab*. **10**(Suppl 4): 1–4.
9. Butler, A.E., J. Janson, S. Bonner-Weir, *et al*. 2003. Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. *Diabetes*. **52:** 102–110.
10. Hanley, S.C., E. Austin, B. Assouline-Thomas, *et al*. 2010. {beta}-Cell Mass Dynamics and Islet Cell Plasticity in Human Type 2 Diabetes. *Endocrinology*. **151:** 1462–1472.
11. Rahier, J., Y. Guiot, R.M. Goebbels, *et al*. 2008. Pancreatic beta-cell mass in European subjects with type 2 diabetes. *Diabetes Obes. Metab*. **10**(Suppl 4): 32–42.
12. Ritzel, R.A., A.E. Butler, R.A. Rizza, *et al*. 2006. Relationship between beta-cell mass and fasting blood glucose concentration in humans. *Diabetes Care*. **29:** 717–718.
13. Kloppel, G., M. Lohr, K. Habich, *et al*. 1985. Islet pathology and the pathogenesis of type 1 and type 2 diabetes mellitus revisited. *Surv. Synth. Pathol. Res*. **4:** 110–125.
14. Perfetti, R., J. Zhou, M.E. Doyle & J.M. Egan. 2000. Glucagon-like peptide-1 induces cell proliferation and pancreatic-duodenum homeobox-1 expression and increases endocrine cell mass in the pancreas of old, glucose-intolerant rats. *Endocrinology*. **141:** 4600–4605.
15. Buteau, J., M.L. Spatz & D. Accili. 2006. Transcription factor FoxO1 mediates glucagon-like peptide-1 effects on pancreatic beta-cell mass. *Diabetes*. **55:** 1190–1196.
16. Li, Y., X. Cao, L.X. Li, *et al*. 2005. beta-Cell Pdx1 expression is essential for the glucoregulatory, proliferative, and cytoprotective actions of glucagon-like peptide-1. *Diabetes* **54:** 482–491.
17. Park, S., X. Dong, T.L. Fisher, *et al*. 2006. Exendin-4 uses Irs2 signaling to mediate pancreatic beta cell growth and function. *J. Biol. Chem*. **281:** 1159–1168.
18. Song, W.J., W.E. Schreiber, E. Zhong, *et al*. 2008. Exendin-4 stimulation of cyclin A2 in beta-cell proliferation. *Diabetes* **57:** 2371–2381.
19. Tschen, S.I., S. Dhawan, T. Gurlo & A. Bhushan. 2009. Age-dependent decline in beta-cell proliferation restricts the capacity of beta-cell regeneration in mice. *Diabetes* **58:** 1312–1320.
20. Kwon, D.Y., Y.S. Kim, I.S. Ahn, *et al*. 2009. Exendin-4 potentiates insulinotropic action partly via increasing beta-cell proliferation and neogenesis and decreasing apoptosis in association with the attenuation of endoplasmic reticulum stress in islets of diabetic rats. *J. Pharmacol. Sci*. **111:** 361–371.
21. Xu, G., D.A. Stoffers, J.F. Habener & S. Bonner-Weir. 1999. Exendin-4 stimulates both beta-cell replication and neogenesis, resulting in increased beta-cell mass and improved glucose tolerance in diabetic rats. *Diabetes* **48:** 2270–2276.
22. Wang, Z.V., J. Mu, T.D. Schraw, *et al*. 2008. PANIC-ATTAC: a mouse model for inducible and reversible beta-cell ablation. *Diabetes* **57:** 2137–2148.
23. Zhang, J., Y. Tokui, K. Yamagata, *et al*. 2007. Continuous stimulation of human glucagon-like peptide-1 (7–36) amide in a mouse model (NOD) delays onset of autoimmune type 1 diabetes. *Diabetologia* **50:** 1900–1909.
24. Perez-Arana, G., Blandino-Rosano, M., Prada-Oliveira, A., *et al*. 2010. Decrease in {beta}-cell proliferation precedes apoptosis during diabetes development in biobreeding/worcester rat: beneficial role of Exendin-4. *Endocrinology* **151:** 2538–2546.
25. Green, B.D., K.S. Lavery, N. Irwin, *et al*. 2006. Novel glucagon-like peptide-1 (GLP-1) analog (Val8)GLP-1

results in significant improvements of glucose tolerance and pancreatic beta-cell function after 3-week daily administration in obese diabetic (ob/ob) mice. *J. Pharmacol. Exp. Ther*. **318:** 914–921.
26. Cheng, Q., P.K. Law, M. de Gasparo & P.S. Leung. 2008. Combination of the dipeptidyl peptidase IV inhibitor LAF237 [(S)-1-[(3-hydroxy-1-adamantyl)ammo]acetyl-2-cyanopyrrolidine] with the angiotensin II type 1 receptor antagonist valsartan [N-(1-oxopentyl)-N-[[2′-(1H-tetrazol-5-yl)-[1,1′-biphenyl]-4-yl]methyl]-L- valine] enhances pancreatic islet morphology and function in a mouse model of type 2 diabetes. *J. Pharmacol. Exp. Ther*. **327:** 683–691.
27. Kim, J.G., L.L. Baggio, D.P. Bridon, *et al*. 2003. Development and characterization of a glucagon-like peptide 1-albumin conjugate: the ability to activate the glucagon-like peptide 1 receptor in vivo. *Diabetes* **52:** 751–759.
28. Ma, X., H. Hui, Z. Liu, *et al*. 2009. Poly-GLP-1, a novel long-lasting glucagon-like peptide-1 polymer, ameliorates hyperglycaemia by improving insulin sensitivity and increasing pancreatic beta-cell proliferation. *Diabetes Obes. Metab*. **11:** 953–965.
29. Wang, Q. & P.L. Brubaker. 2002. Glucagon-like peptide-1 treatment delays the onset of diabetes in 8 week-old db/db mice. *Diabetologia* **45:** 1263–1273.
30. Farilla, L., H. Hui, C. Bertolotto, *et al*. 2002. Glucagon-like peptide-1 promotes islet cell growth and inhibits apoptosis in Zucker diabetic rats. *Endocrinology* **143:** 4397–4408.
31. Takiguchi, S., Y. Takata, A. Funakoshi, *et al*. 1997. Disrupted cholecystokinin type-A receptor (CCKAR) gene in OLETF rats. *Gene* **197:** 169–175.
32. Wang, Y. & X.H. Guo. 2006. [Effects of GLP-1 treatment on protection of B cells in Otsuka Long-Evans Tokushima fatty rats]. *Beijing Da Xue Xue Bao*. **38:** 375–380.
33. Li, Y., C. Shi, Q. Lv, *et al*. 2008. GLP-1 C-terminal structures affect its blood glucose lowering-function. *J. Pept. Sci*. **14:** 777–785.
34. Mu, J., A. Petrov, G.J. Eiermann, *et al*. 2009. Inhibition of DPP-4 with sitagliptin improves glycemic control and restores islet cell mass and function in a rodent model of type 2 diabetes. *Eur. J. Pharmacol*. **623:** 148–154.
35. Ling, Z., D. Wu, Y. Zambre, *et al*. 2001. Glucagon-like peptide 1 receptor signaling influences topography of islet cells in mice. *Virchows Arch*. **438:** 382–387.
36. De Leon, D.D., S. Deng, R. Madani, *et al*. 2003. Role of endogenous glucagon-like peptide-1 in islet regeneration after partial pancreatectomy. *Diabetes* **52:** 365–371.
37. Dor, Y., J. Brown, O.I. Martinez & D.A. Melton. 2004. Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation. *Nature* **429:** 41–46.
38. Scrocchi, L.A., M.E. Hill, J. Saleh, *et al*. 2000. Elimination of glucagon-like peptide 1R signaling does not modify weight gain and islet adaptation in mice with combined disruption of leptin and GLP-1 action. *Diabetes* **49:** 1552–1560.
39. Anini, Y. & P.L. Brubaker. 2003. Role of leptin in the regulation of glucagon-like peptide-1 secretion. *Diabetes* **52:** 252–259.
40. Friedrichsen, B.N., N. Neubauer, Y.C. Lee, *et al*. 2006. Stimulation of pancreatic beta-cell replication by incretins involves transcriptional induction of cyclin D1 via multiple signalling pathways. *J. Endocrinol*. **188:** 481–492.
41. Kim, M.J., J.H. Kang, Y.G. Park, *et al*. 2006. Exendin-4 induction of cyclin D1 expression in INS-1 beta-cells: involvement of cAMP-responsive element. *J. Endocrinol*. **188:** 623–633.
42. He, L.M., D.J. Sartori, M. Teta, *et al*. 2009. Cyclin D2 protein stability is regulated in pancreatic beta-cells. *Mol. Endocrinol*. **23:** 1865–1875.
43. Buteau, J., R. Roduit, S. Susini & M. Prentki. 1999. Glucagon-like peptide-1 promotes DNA synthesis, activates phosphatidylinositol 3-kinase and increases transcription factor pancreatic and duodenal homeobox gene 1 (PDX-1) DNA binding activity in beta (INS-1)-cells. *Diabetologia* **42:** 856–864.
44. Lawlor, M.A. & D.R. Alessi. 2001. PKB/Akt: a key mediator of cell proliferation, survival and insulin responses. *J. Cell Sci*. **114:** 2903–2910.
45. Wang, Q., L. Li, E. Xu, *et al*. 2004. Glucagon-like peptide-1 regulates proliferation and apoptosis via activation of protein kinase B in pancreatic INS-1 beta cells. *Diabetologia* **47:** 478–487.
46. Stoffers, D.A., B.M. Desai, D.D. DeLeon & R.A. Simmons. 2003. Neonatal exendin-4 prevents the development of diabetes in the intrauterine growth retarded rat. *Diabetes* **52:** 734–740.
47. Jhala, U.S., G. Canettieri, R.A. Screaton, *et al*. 2003. cAMP promotes pancreatic beta-cell survival via CREB-mediated induction of IRS2. *Genes Dev*. **17:** 1575–1580.
48. Withers, D.J., J.S. Gutierrez, H. Towery, *et al*. 1998. Disruption of IRS-2 causes type 2 diabetes in mice. *Nature* **391:** 900–904.
49. Buteau, J., S. Foisy, E. Joly & M. Prentki. 2003. Glucagon-like peptide 1 induces pancreatic beta-cell proliferation via transactivation of the epidermal growth factor receptor. *Diabetes* **52:** 124–132.
50. Buteau, J., S. Foisy, C.J. Rhodes, *et al*. 2001. Protein kinase Czeta activation mediates glucagon-like peptide-1-induced pancreatic beta-cell proliferation. *Diabetes* **50:** 2237–2243.
51. Le Good, J.A., W.H. Ziegler, D.B. Parekh, *et al*. 1998. Protein kinase C isotypes controlled by phosphoinositide 3-kinase through the protein kinase PDK1. *Science* **281:** 2042–2045.
52. Furukawa, N., T. Shirotani, E. Araki, *et al*. 1999. Possible involvement of atypical protein kinase C (PKC) in glucose-sensitive expression of the human insulin gene: DNA-binding activity and transcriptional activity of pancreatic and duodenal homeobox gene-1 (PDX-1) are enhanced via calphostin C-sensitive but phorbol 12-myristate 13-acetate (PMA) and Go 6976-insensitive pathway. *Endocr. J*. **46:** 43–58.
53. Blandino-Rosano, M., G. Perez-Arana, J.M. Mellado-Gil, *et al*. 2008. Anti-proliferative effect of pro-inflammatory cytokines in cultured beta cells is associated with extracellular signal-regulated kinase 1/2 pathway inhibition: protective role of glucagon-like peptide -1. *J. Mol. Endocrinol*. **41:** 35–44.
54. Trumper, J., D. Ross, H. Jahr, *et al*. 2005. The Rap-B-Raf signalling pathway is activated by glucose and glucagon-like peptide-1 in human islet cells. *Diabetologia* **48:** 1534–1540.

55. Briaud, I., M.K. Lingohr, L.M. Dickson, *et al*. 2003. Differential activation mechanisms of Erk-1/2 and p70(S6K) by glucose in pancreatic beta-cells. *Diabetes* **52:** 974–983.
56. Welters, H.J. & R.N. Kulkarni. 2008. Wnt signaling: relevance to beta-cell biology and diabetes. *Trends Endocrinol. Metab.* **19:** 349–355.
57. Liu, Z. & J.F. Habener. 2008. Glucagon-like peptide-1 activation of TCF7L2-dependent Wnt signaling enhances pancreatic beta cell proliferation. *J. Biol. Chem.* **283:** 8723–8735.
58. Rulifson, I.C., S.K. Karnik, P.W. Heiser, *et al*. 2007. Wnt signaling regulates pancreatic beta cell proliferation. *Proc. Natl Acad. Sci. USA* **104:** 6247–6252.
59. Miyawaki, K., Y. Yamada, N. Ban, *et al*. 2002. Inhibition of gastric inhibitory polypeptide signaling prevents obesity. *Nat. Med*. **8:** 738–742.
60. Gault, V.A., N. Irwin, B.D. Green, *et al*. 2005. Chemical ablation of gastric inhibitory polypeptide receptor action by daily (Pro3)GIP administration improves glucose tolerance and ameliorates insulin resistance and abnormalities of islet structure in obesity-related diabetes. *Diabetes* **54:** 2436–2446.
61. Irwin, N., G.C. Clarke, B.D. Green, *et al*. 2006. Evaluation of the antidiabetic activity of DPP IV resistant N-terminally modified versus mid-chain acylated analogues of glucose-dependent insulinotropic polypeptide. *Biochem. Pharmacol*. **72:** 719–728.
62. Kidd, G.S., Donowitz, M., O'Dorisio, T., *et al*. 1979. Mild chronic watery diarrhea-hypokalemia syndrome associated with pancreatic islet cell hyperplasia. Elevated plasma and tissue levels of gastric inhibitory polypeptide and successful management with nicotinic acid. *Am. J. Med.* **66:** 883–888.
63. Ehses, J.A., V.R. Casilla, T. Doty, *et al*. 2003. Glucose-dependent insulinotropic polypeptide promotes beta-(INS-1) cell survival via cyclic adenosine monophosphate-mediated caspase-3 inhibition and regulation of p38 mitogen-activated protein kinase. *Endocrinology* **144:** 4433–4445.
64. Trumper, A., K. Trumper & D. Horsch. 2002. Mechanisms of mitogenic and anti-apoptotic signaling by glucose-dependent insulinotropic polypeptide in beta(INS-1)-cells. *J. Endocrinol*. **174:** 233–246.
65. Trumper, A., K. Trumper, H. Trusheim, *et al*. 2001. Glucose-dependent insulinotropic polypeptide is a growth factor for beta (INS-1) cells by pleiotropic signaling. *Mol. Endocrinol*. **15:** 1559–1570.
66. Ehses, J.A., S.L. Pelech, R.A. Pederson & C.H. McIntosh. 2002. Glucose-dependent insulinotropic polypeptide activates the Raf-Mek1/2-ERK1/2 module via a cyclic AMP/cAMP-dependent protein kinase/Rap1-mediated pathway. *J. Biol. Chem.* **277:** 37088–37097.
67. Kuntz, E., M. Pinget & P. Damge. 2004. Cholecystokinin octapeptide: a potential growth factor for pancreatic beta cells in diabetic rats. *JOP* **5:** 464–475.
68. Chen, S., S. Turner, E. Tsang, *et al*. 2007. Measurement of pancreatic islet cell proliferation by heavy water labeling. *Am. J. Physiol. Endocrinol. Metab.* **293:** E1459–E1464.
69. Shima, K., M. Zhu & A. Mizuno. 1999. Pathoetiology and prevention of NIDDM lessons from the OLETF rat. *J. Med. Invest*. **46:** 121–129.
70. Brelje, T.C. & R.L. Sorenson. 1991. Role of prolactin versus growth hormone on islet B-cell proliferation in vitro: implications for pregnancy. *Endocrinology* **128:** 45–57.
71. Lavine, J.A., P.W. Raess, D.B. Davis, *et al*. 2010. Contamination with E1A-positive wild-type adenovirus accounts for species-specific stimulation of islet cell proliferation by CCK: a cautionary note. *Mol. Endocrinol*. **24:** 464–467.
72. Parmar, N.S., M. Tariq & A.M. Ageel. 1987. Proglumide, a cholecystokinin receptor antagonist, exacerbates alloxan-induced diabetes mellitus in Swiss mice. *J. Pharm. Pharmacol*. **39:** 1028–1030.
73. Meier, J.J., A.E. Butler, R. Galasso, *et al*. 2006. Increased islet beta cell replication adjacent to intrapancreatic gastrinomas in humans. *Diabetologia*. **49:** 2689–2696.
74. Nir, T., D.A. Melton & Y. Dor. 2007. Recovery from diabetes in mice by beta cell regeneration. *J. Clin. Invest*. **117:** 2553–2561.
75. Inada, A., C. Nienaber, H. Katsuta, *et al*. 2008. Carbonic anhydrase II-positive pancreatic cells are progenitors for both endocrine and exocrine pancreas after birth. *Proc. Natl. Acad. Sci. USA* **105:** 19915–19919.
76. Solar, M., C. Cardalda, I. Houbracken, *et al*. 2009. Pancreatic exocrine duct cells give rise to insulin-producing beta cells during embryogenesis but not after birth. *Dev. Cell*. **17:** 849–860.
77. Thorel, F., V. Nepote, I. Avril, *et al*. 2010. Conversion of adult pancreatic alpha-cells to beta-cells after extreme beta-cell loss. *Nature* **464:** 1149–1154.
78. Tourrel, C., D. Bailbe, M.J. Meile, *et al*. 2001. Glucagon-like peptide-1 and exendin-4 stimulate beta-cell neogenesis in streptozotocin-treated newborn rats resulting in persistently improved glucose homeostasis at adult age. *Diabetes* **50:** 1562–1570.
79. Suarez-Pinzon, W.L., R.F. Power, Y. Yan, *et al*. 2008. Combination therapy with glucagon-like peptide-1 and gastrin restores normoglycemia in diabetic NOD mice. *Diabetes* **57:** 3281–3288.
80. Lee, J., J. Wen, J.Y. Park, *et al*. 2009. Reversal of diabetes in rats using GLP-1-expressing adult pancreatic duct-like precursor cells transformed from acinar to ductal cells. *Stem Cells Dev*. **18:** 991–1002.
81. Suarez-Pinzon, W.L., J.R. Lakey & A. Rabinovitch. 2008. Combination therapy with glucagon-like peptide-1 and gastrin induces beta-cell neogenesis from pancreatic duct cells in human islets transplanted in immunodeficient diabetic mice. *Cell Transplant*. **17:** 631–640.
82. Bulotta, A., H. Hui, E. Anastasi, *et al*. 2002. Cultured pancreatic ductal cells undergo cell cycle re-distribution and beta-cell-like differentiation in response to glucagon-like peptide-1. *J. Mol. Endocrinol*. **29:** 347–360.
83. Hui, H., C. Wright & R. Perfetti. 2001. Glucagon-like peptide 1 induces differentiation of islet duodenal homeobox-1-positive pancreatic ductal cells into insulin-secreting cells. *Diabetes* **50:** 785–796.
84. Mao, G.H., G.A. Chen, H.Y. Bai, *et al*. 2009. The reversal of hyperglycaemia in diabetic mice using PLGA scaffolds

seeded with islet-like cells derived from human embryonic stem cells. *Biomaterials* **30:** 1706–1714.
85. Yue, F., L. Cui, K. Johkura, *et al*. 2006. Glucagon-like peptide-1 differentiation of primate embryonic stem cells into insulin-producing cells. *Tissue Eng*. **12:** 2105–2116.
86. Herbach, N., B. Goeke, M. Schneider, *et al*. 2005. Overexpression of a dominant negative GIP receptor in transgenic mice results in disturbed postnatal pancreatic islet and beta-cell development. *Regul. Pept*. **125:** 103–117.
87. McClean, P.L., V.A. Gault, N. Irwin, *et al*. 2008. Daily administration of the GIP-R antagonist (Pro3)GIP in streptozotocin-induced diabetes suggests that insulin-dependent mechanisms are critical to anti-obesity-diabetes actions of (Pro3)GIP. *Diabetes Obes. Metab*. **10:** 336–342.
88. Marenah, L., J.T. McCluskey, Y.H. Abdel-Wahab, *et al*. 2006. A stable analogue of glucose-dependent insulinotropic polypeptide, GIP(LysPAL16), enhances functional differentiation of mouse embryonic stem cells into cells expressing islet-specific genes and hormones. *Biol. Chem.* **387:** 941–947.
89. Wang, T.C., S. Bonner-Weir, P.S. Oates, *et al*. 1993. Pancreatic gastrin stimulates islet differentiation of transforming growth factor alpha-induced ductular precursor cells. *J. Clin. Invest*. **92:** 1349–1356.
90. Gigoux, V., P. Clerc, D. Sanchez, *et al*. 2008. Reg genes are CCK2 receptor targets in ElasCCK2 mice pancreas. *Regul. Pept*. **146:** 88–98.
91. Rooman, I., J. Lardon & L. Bouwens. 2002. Gastrin stimulates beta-cell neogenesis and increases islet mass from transdifferentiated but not from normal exocrine pancreas tissue. *Diabetes* **51:** 686–690.
92. Rooman, I. & L. Bouwens. 2004. Combined gastrin and epidermal growth factor treatment induces islet regeneration and restores normoglycaemia in C57Bl6/J mice treated with alloxan. *Diabetologia* **47:** 259–265.
93. Brand, S.J., S. Tagerud, P. Lambert, *et al*. 2002. Pharmacological treatment of chronic diabetes by stimulating pancreatic beta-cell regeneration with systemic co-administration of EGF and gastrin. *Pharmacol. Toxicol*. **91:** 414–420.
94. Suarez-Pinzon, W.L., J.R. Lakey, S.J. Brand & A. Rabinovitch. 2005. Combination therapy with epidermal growth factor and gastrin induces neogenesis of human islet {beta}-cells from pancreatic duct cells and an increase in functional {beta}-cell mass. *J. Clin. Endocrinol. Metab.* **90:** 3401–3409.
95. Hardikar, A.A., X.Y. Wang, L.J. Williams, *et al*. 2002. Functional maturation of fetal porcine beta-cells by glucagon-like peptide 1 and cholecystokinin. *Endocrinology* **143:** 3505–3514.
96. Li, Y., T. Hansotia, B. Yusta, *et al*. 2003. Glucagon-like peptide-1 receptor signaling modulates beta cell apoptosis. *J. Biol. Chem.* **278:** 471–478.
97. Maida, A., T. Hansotia, C. Longuet, *et al*. 2009. Differential importance of glucose-dependent insulinotropic polypeptide vs glucagon-like peptide 1 receptor signaling for beta cell survival in mice. *Gastroenterology* **137:** 2146–2157.
98. Yusta, B., L.L. Baggio, J.L. Estall, *et al*. 2006. GLP-1 receptor activation improves beta cell function and survival following induction of endoplasmic reticulum stress. *Cell Metab.* **4:** 391–406.
99. Uckaya, G., P. Delagrange, A. Chavanieu, *et al*. 2005. Improvement of metabolic state in an animal model of nutrition-dependent type 2 diabetes following treatment with S 23521, a new glucagon-like peptide 1 (GLP-1) analogue. *J. Endocrinol.* **184:** 505–513.
100. Lin, C.C. & K.S. Anseth. 2009. Glucagon-like peptide-1 functionalized PEG hydrogels promote survival and function of encapsulated pancreatic beta-cells. *Biomacromolecules* **10:** 2460–2467.
101. Merani, S., W. Truong, J.A. Emamaullee, *et al*. 2008. Liraglutide, a long-acting human glucagon-like peptide 1 analog, improves glucose homeostasis in marginal mass islet transplantation in mice. *Endocrinology* **149:** 4322–4328.
102. Toyoda, K., T. Okitsu, S. Yamane, *et al*. 2008. GLP-1 receptor signaling protects pancreatic beta cells in intraportal islet transplant by inhibiting apoptosis. *Biochem. Biophys. Res. Commun.* **367:** 793–798.
103. Faradji, R.N., T. Tharavanij, S. Messinger, *et al*. 2008. Long-term insulin independence and improvement in insulin secretion after supplemental islet infusion under exenatide and etanercept. *Transplantation* **86:** 1658–1665.
104. Froud, T., R.N. Faradji, A. Pileggi, *et al*. 2008. The use of exenatide in islet transplant recipients with chronic allograft dysfunction: safety, efficacy, and metabolic effects. *Transplantation* **86:** 36–45.
105. Gangemi, A., P. Salehi, B. Hatipoglu, *et al*. 2008. Islet transplantation for brittle type 1 diabetes: the UIC protocol. *Am. J. Transplant*. **8:** 1250–1261.
106. Cornu, M., J.Y. Yang, E. Jaccard, *et al*. 2009. Glucagon-like peptide-1 protects beta-cells against apoptosis by increasing the activity of an IGF-2/IGF-1 receptor autocrine loop. *Diabetes* **58:** 1816–1825.
107. Wideman, R.D., I.L. Yu, T.D. Webber, *et al*. 2006. Improving function and survival of pancreatic islets by endogenous production of glucagon-like peptide 1 (GLP-1). *Proc. Natl Acad. Sci. USA* **103:** 13468–13473.
108. Bregenholt, S., A. Moldrup, N. Blume, *et al*. 2005. The long-acting glucagon-like peptide-1 analogue, liraglutide, inhibits beta-cell apoptosis in vitro. *Biochem. Biophys. Res. Commun.* **330:** 577–584.
109. Sarkar, S.A., J. Gunter, R. Bouchard, *et al*. 2007. Dominant negative mutant forms of the cAMP response element binding protein induce apoptosis and decrease the anti-apoptotic action of growth factors in human islets. *Diabetologia* **50:** 1649–1659.
110. Ferdaoussi, M., S. Abdelli, J.Y. Yang, *et al*. 2008. Exendin-4 protects beta-cells from interleukin-1 beta-induced apoptosis by interfering with the c-Jun NH2-terminal kinase pathway. *Diabetes* **57:** 1205–1215.
111. Bonny, C., A. Oberson, M. Steinmann, *et al*. 2000. IB1 reduces cytokine-induced apoptosis of insulin-secreting cells. *J. Biol. Chem.* **275:** 16466–16472.
112. Waeber, G., J. Delplanque, C. Bonny, *et al*. 2000. The gene MAPK8IP1, encoding islet-brain-1, is a candidate for type 2 diabetes. *Nat. Genet*. **24:** 291–295.

113. Haefliger, J.A., T. Tawadros, L. Meylan, *et al*. 2003. The scaffold protein IB1/JIP-1 is a critical mediator of cytokine-induced apoptosis in pancreatic beta cells. *J. Cell Sci.* **116:** 1463–1469.
114. Xie, T., M. Chen, Q.H. Zhang, *et al*. 2007. Beta cell-specific deficiency of the stimulatory G protein alpha-subunit Gsalpha leads to reduced beta cell mass and insulin-deficient diabetes. *Proc. Natl Acad. Sci. USA* **104:** 19601–19606.
115. Li, L., W. El-Kholy, C.J. Rhodes & P.L. Brubaker. 2005. Glucagon-like peptide-1 protects beta cells from cytokine-induced apoptosis and necrosis: role of protein kinase B. *Diabetologia* **48:** 1339–1349.
116. Cornu, M., H. Modi, D. Kawamori, *et al*. 2010. Glucagon-like peptide-1 increases beta-cell glucose competence and proliferation by translational induction of insulin-like growth factor-1 receptor expression. *J. Biol. Chem.* **285:** 10538–10545.
117. Tsunekawa, S., N. Yamamoto, K. Tsukamoto, *et al*. 2007. Protection of pancreatic beta-cells by exendin-4 may involve the reduction of endoplasmic reticulum stress; in vivo and in vitro studies. *J. Endocrinol*. **193:** 65–74.
118. Buteau, J., W. El-Assaad, C.J. Rhodes, *et al*. 2004. Glucagon-like peptide-1 prevents beta cell glucolipotoxicity. *Diabetologia* **47:** 806–815.
119. Widenmaier, S.B., A.V. Sampaio, T.M. Underhill & C.H. McIntosh. 2009. Noncanonical activation of Akt/protein kinase B in {beta}-cells by the incretin hormone glucose-dependent insulinotropic polypeptide. *J. Biol. Chem.* **284:** 10764–10773.
120. Hui, H., A. Nourparvar, X. Zhao & R. Perfetti. 2003. Glucagon-like peptide-1 inhibits apoptosis of insulin-secreting cells via a cyclic 5′-adenosine monophosphate-dependent protein kinase A- and a phosphatidylinositol 3-kinase-dependent pathway. *Endocrinology* **144:** 1444–1455.
121. Widenmaier, S.B., Kim, S.J., Yang, G.K., *et al*. 2010. A GIP receptor agonist exhibits beta-cell anti-apoptotic actions in rat models of diabetes resulting in improved beta-cell function and glycemic control. *PLoS One* **5:** e9590.
122. Kim, S.J., K. Winter, C. Nian, *et al*. 2005. Glucose-dependent insulinotropic polypeptide (GIP) stimulation of pancreatic beta-cell survival is dependent upon phosphatidylinositol 3-kinase (PI3K)/protein kinase B (PKB) signaling, inactivation of the forkhead transcription factor Foxo1, and down-regulation of bax expression. *J. Biol. Chem.* **280:** 22297–22307.
123. Kim, S.J., C. Nian, S. Widenmaier & C.H. McIntosh. 2008. Glucose-dependent insulinotropic polypeptide-mediated up-regulation of beta-cell antiapoptotic Bcl-2 gene expression is coordinated by cyclic AMP (cAMP) response element binding protein (CREB) and cAMP-responsive CREB coactivator 2. *Mol. Cell Biol*. **28:** 1644–1656.
124. Widenmaier, S.B., Z. Ao, S.J. Kim, *et al*. 2009. Suppression of p38 MAPK and JNK via Akt-mediated inhibition of apoptosis signal-regulating kinase 1 constitutes a core component of the beta-cell pro-survival effects of glucose-dependent insulinotropic polypeptide. *J. Biol. Chem.* **284:** 30372–30382.
125. Huang, Q., S. Bu, Y. Yu, *et al*. 2007. Diazoxide prevents diabetes through inhibiting pancreatic beta-cells from apoptosis via Bcl-2/Bax rate and p38-beta mitogen-activated protein kinase. *Endocrinology* **148:** 81–91.
126. Zhao, J., N. Zhang, M. He, *et al*. 2008. Increased beta-cell apoptosis and impaired insulin signaling pathway contributes to the onset of diabetes in OLETF rats. *Cell Physiol. Biochem.* **21:** 445–454.
127. Lavine, J.A., P.W. Raess, D.S. Stapleton, *et al*. 2010. Cholecystokinin is up-regulated in obese mouse islets and expands beta-cell mass by increasing beta-cell survival. *Endocrinology* **151:** 3577–3588.