Role of cyclic AMP signaling in the production and function of the incretin hormone glucagon-like peptide-1

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Pancreatic cells express the proglucagon gene (gcg) and thereby produce the peptide hormone glucagon, which stimulates hepatic glucose production and thereby increases blood glucose levels. The same gcg gene is also expressed in the intestinal endocrine L cells and certain neural cells in the brain. In the gut, gcg expression leads to the production of glucagon-like peptide-1 (GLP-1). This incretin hormone stimulates insulin secretion when blood glucose level is high. In addition, GLP-1 stimulates pancreatic cell proliferation, inhibits cell apoptosis, and has been utilized in the trans-differentiation of insulin producing cells. Today, a long-term effective GLP-1 receptor agonist has been developed as a drug in treating diabetes and potentially other metabolic disorders. Extensive investigations have shown that the expression of gcg and the production of GLP-1 can be activated by the elevation of the second messenger cyclic AMP (cAMP). Recent studies suggest that in addition to protein kinase A (PKA), exchange protein activated by cAMP (Epac), another effector of cAMP signaling, and the crosstalk between PKA and Wnt signaling pathway, are also involved in cAMP-stimulated gcg expression and GLP-1 production. Furthermore, functions of GLP-1 in pancreatic cells are mainly mediated by cAMP-PKA, cAMP-Epac and Wnt signaling pathways as well.

Key words cAMP, GLP-1, Byetta, Epac, PKA, Diabetes

Introduction

Blood glucose levels are tightly controlled by a number of peptide and other hormones in our body in response to physiological and environmental changes. In addition to insulin, two other peptide hormones, namely glucagon and glucagon-like peptide-1 (GLP-1), are fundamentally impotent. There two hormone are encoded by a single gene namely proglucagon (gcg), produced in the pancreatic islet α-cells; and gut endocrine L cells, respectively[1,2]. These two hormones exert their physio-biological functions mainly via interacting with their receptors, which belong to the seven-transmembrane G-protein coupled receptor (GPCR) family. Extensive investigations during the past two decades have revealed that cyclic AMP (cAMP) signaling plays fundamentally important roles in the stimulation of gcg expression and the production of its encoded peptide hormones. cAMP pathways are also important in mediating the functions of gcg encoded peptide hormones.

GLP-1 is an important incretin hormone. GLP-1R agonist Exenatide (commercially known as Byetta) has been developed as a drug in the treatment of type II diabetes mellitus, and potentially, other metabolic disorders. In this review, we have summarized our current knowledge of the role of cAMP signaling in both the production and functions of GLP-1.

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I The proglucagon gene (gcg) and its encoded peptide hormones

In both humans and rodents, there is only one gcg gene, which is in contrast to two or more gcg genes identified in lower organisms, including Xenopus, rainbow trout, anglerfish and sea lamprey[3]. Expression of gcg is driven by the same gcg promoter in the pancreatic islet α-cells, the gut intestinal endocrine L cells, and the brain. These three cell types express the identical gcg mRNA, which is translated into the same pro-hormone proglucagon. However, cell type specific post-translational processes result in the production of different profiles of proglucagon derived peptides (PGDPs), due to the co-expression of cell type specific pro-hormone convertases (PC) in intestinal endocrine L cells versus pancreatic α-cells[4,5].

As illustrated in Fig. 1A, in the pancreatic α-cells, the pro-hormone is mainly processed to generate glucagon, due to the co-expression of pro-hormone convertase-2 (PC2). In the intestinal endocrine L cells, the pro-hormone is processed to generate GLP-1 and GLP-2, due to the co-expression of pro-hormone convertase-1/3 (PC1/3[6,7]. Fig. 1B summarizes the main biological functions of GLP-1 on different tissues. Recently, Wilson et al. reported that embryonic pancreatic α-cells also express PC1/3, indicating that during embryonic development, pancreatic α-cells may express certain amount of GLP-1, which could be important in early pancreatic islet development[8]. More recently, Wideman et al. have shown that adenovirus mediated expression of PC1/3 in a pancreatic α-cell line provoked this cell line to produce GLP-1 and such a GLP-1 expressing cell line was shown to reduce diabetic syndromes in a streptozotocin-induced mouse diabetic model[6,7]. The post-translational processes also generate several other PGDPs, such as oxyntomodulin, glicentin, glicentin related pancreatic polypeptide (GRPP), intervening peptidel (IP-1), intervening peptide 2 (IP-2), and major proglucagon fragment (MPGF) (Fig. 1A), with yet to be defined or further confirmed biological functions.

GLP-1 possesses the potent stimulatory effects on glucose-dependent insulin secretion, proinsulin gene transcription, islet cell cAMP formation, and pancreatic β-cell growth and survival. Based on these functions of GLP-1 in pancreatic β-cells and the beneficial effects of GLP-1 in other organs[9], great effort has been made for the development of new drugs in the treatment of diabetes mellitus and its complications. One approach is the generation of long-term active agonist of GLP-1R. One such agonist, namely exendin-4 (Exenatide), a peptide isolated from lizard venom, has been approved for its clinic usage with the brand name Byetta[10]. Another approach is the development of inhibitors of DPP-IV (also known as CD-26), an enzyme that inactivate native GLP-1[11]. Our understanding of the actions of GLP-1 has been summarized in several excellent review articles[1,2,9,12-16].

II PKA and Epac signaling pathway

cAMP and PKA

Early investigations by Earl Sutherland (Recipient of 1971 Nobel Physiology and Medicine prize) and colleagues on the function of epinephrine have led to a great discovery that cAMP has an intermediary role in many hormonal functions. This was followed by the studies of Edwin Krebs and Edmond Fisher (Recipients of 1992 Nobel Physiology and Medicine prize) that epinephrine and cAMP stimulate glycogen breakdown by activating glycogen phosphorylase via a protein kinase, namely cAMP-dependent protein kinase (PKA). The PKA holoenzyme consists of two regulatory subunits and two catalytic subunits. When the cAMP level is low, the holoenzyme remains intact and it is catalytically inactive. When the concentration of cAMP rises, either due to the activation of adenylate cyclases (AC) by the Gs subunit of a GPCR, or the inhibition of phosphodiesterases (PDE, which degrade cAMP), cAMP binds to the binding sites on the regulatory subunits, resulting in the release of the catalytic subunits. An important downstream effector of PKA is the transcription factor CREB (cAMP response element binding). Activated CREB is able to bind to a cAMP response element (CRE), via its basic leucine zipper domain (BZIP domain), within the promoter region of the CREB downstream target genes. This will lead to the recruitment of CREB binding protein (CBP/P300) and other nuclear coactivators and enhanced gene transcription[17].

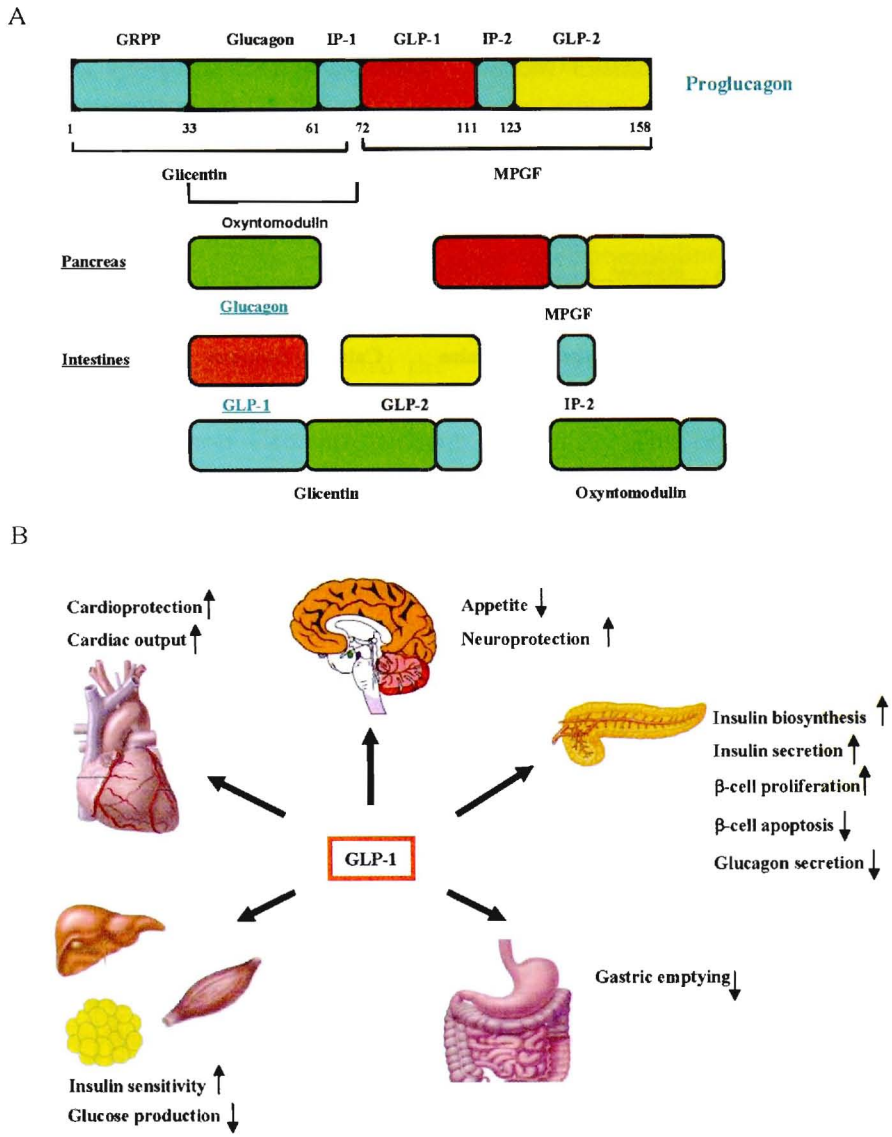

Figure 1 An illustration of gcg encoded peptide hormones in pancreas and intestines (A), and a summary of physiological functions of GLP-1 (B). A) The same pro-hormone, proglucagon is produced in pancreatic α cells and intestinal endocrine L cells. Cell type specific expression of prohormone convertases determines the generation of active hormones in each of the two cell lineages. The active hormone glucagon is generated in pancreatic islets, while GLP-1 and GLP-2 are generated in the gut. IP-1 and IP2, two intervening peptides. MPGF, major proglucagon fragment (MPGF). For more detailed description of the function of GLP-1, please see cited references[1,2,9,12–16].

cAMP and Epac signaling pathway

Extensive studies in different cell lineages suggested that PKA is not the sole mediator of the second messenger cAMP in exerting its versatile physio-biological functions[18]. In 1998, two groups have independently revealed the existence of a novel category of cAMP mediators, namely Epac-1 and Epac-2 (also known as cAMP-GEFs)[19,20]. Fig. 2A shows the overall structure of the two Epac molecules[21]. Among them, Epac-1 is highly expressed in a variety of tissues such as the heart, kidneys, ovaries, thyroid glands, and the corpus callosum of the brain, whereas Epac-2 expression is much more limited and is notably de-

tectable in the brain, pituitary, adrenal gland, and pancreas. Epac molecules are able to mediate the effect of cAMP via activating the Rap-Raf-MEK-ERK signaling pathway[18,22]. A recent study by Bos and colleagues determined the structure of Epac-2 in complex with a cAMP analogue (Sp-cAMPS) and Rap-1B by X-ray crystallography and single particle electron microscopy[23].

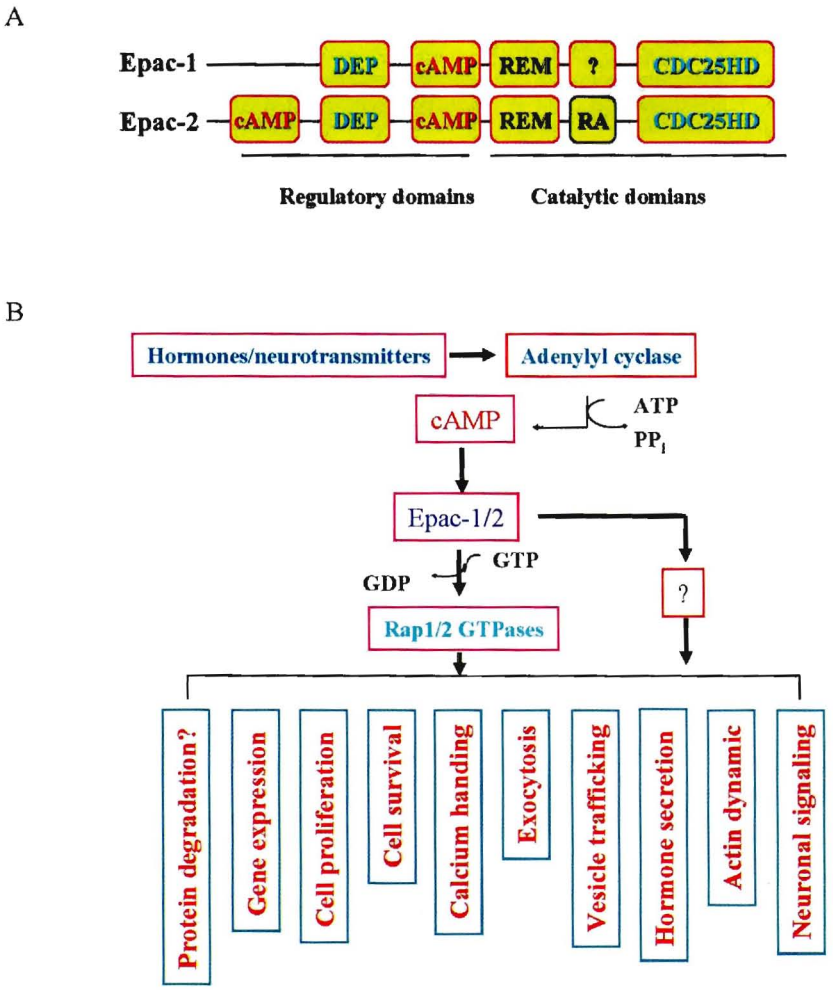

Figure 2 An illustration of the structures (A) of two Epac molecules and their versatile physiological functions (B). A) Epac-1 has one cAMP binding domain, while Epac-2 has two such binding domains. DEP, Disheveled, Egl-10-Pleckstrin; REM, Ras-exchange motif; RA, Ras-associated domain; and CDC25HD, CDX25-homology domain. ?, a domain with a yet unknown function. B) ?, whether Epac molecules exert their functions via mechanism not using Rap molecules is unknown.

As summarized in Fig. 2B, physio-biological functions of the Epac pathway have been rapidly recognized in different cell lineages in the past ten years, including calcium handling, cell proliferation, survival, differentiation and polarization, cell-cell adhesion, gene expression, hormone (including insulin) secretion, ion transport, and neuronal signaling[21,24-27]. Potential pathological effects of the Epac signaling have also been suggested in the brain and cardiovascular system and it may serve as a target of pharmacotherapy for certain diseases of the heart and brain[28,29]. Our laboratory has demonstrated the involvement of Epac signaling in mediating the stimulatory effect of

cAMP in gcg transcription and the production of its encoded hormones (see below)[30—32].

III cAMP signaling for proglucagon gene expression and the production of GLP-1

The role of the PKA and CRE element

During later 1980's and early 1990's, studies with the approaches of DNase I foot-printing, electrophoretic mobility shift assay, as well as chloramphenicol acetyltransferase (CAT) and luciferase (LUC) reporter gene analyses, have revealed the presence of pancreatic cell specific minimum promoter region and three enhancer elements within the first 300 bp 5′ flanking region of the gcg gene, namely G1, G2 and G3[33-35]. Two additional enhancer elements, namely G4 and G5, were identified in subsequent studies[36,37]. Efrat et al. found that in their transgenic mouse studies, the first 1.3 kb 5′ flanking region of the rat gcg gene can drive simian virus 40 (SV-40) large T antigen expression in pancreatic α-cells and in the brain, but not in the intestinal L cells[38]. Lee et al., however, demonstrated that when the promoter sequence was extended to 2.3 kb, it drives the expression of the same SV-40 large T antigen expression in pancreas, brain as well as intestines[39]. We have then assayed the DNA sequence between −1.3 kb to −2.3 kb, and revealed a GATA transcription factor binding site containing enhance element[40]. Within the first 300 bp 5′ flaking region of gcg, there is also a cAMP response element (CRE). Extensive examinations during the past two decades have shown that this CRE motif and enhancer elements within the gcg promoter are important in regulating gcg expression via interaction with many transcription factors, including more than a half dozens of homeodomain protein transcription factors[41].

Enormous efforts have been made for the generation of human pancreatic gcg expressing cell lines with very limited success[42]. Accordingly, studies have to be mainly conducted using cultivated cell lines derived from rodent species and in primary cultures. For studying pancreatic gcg expression, except for the use of well established αTC, HIT, and RIN cell lines, we have been using a hamster cell line namely InR1-G9[43]. Since this cell line is PKA deficient, it serves as an useful tool for studying PKA-independent stimulatory effect of cAMP in gcg transcription[31]. For studying intestinal gcg expression and GLP-1 production, examinations were mainly performed using two mouse cell lines, GLUTag and STC-1[40,44—46], as well as primary fetal rat intestinal cell (FRIC) cultures, developed by Brubaker and colleagues[47-49]. STC-1 is a mouse small intestinal endocrine cell line, derived from an intestinal endocrine tumor developed in a double transgenic mouse expressing the SV-40 large T antigen and the polyoma virus small t antigen, under the control of a rat pro-insulin gene promoter construct[50]. This cell line expresses not only the gcg mRNA, but also mRNAs that encode a few other peptide hormones[46]. STC-1, therefore, is not a typical endocrine L cell line. The GLUTag cell line was isolated from the intestinal tumor of the transgenic mice in which the expression of SV-40 large T antigen is driven by the 2.3 kb rat gcg promoter[46]. This cell line shows substantial levels of gcg mRNA expression, GLP-1 production, and its gcg mRNA expression and GLP-1 production can be activated in response to cAMP elevation. There is also a human intestinal gcg-expressing cell line, NCI-H716[51]. A recent study, however, suggested that gcg mRNA expression in NCI-H716 is aberrantly regulated[52].

The effect of cAMP in regulating gcg expression in both pancreatic α-cells and intestinal endocrine L cells has been studies intensively following the recognition of a typical CRE within the proximal gcg promoter region[45,46,53,54]. We found that in the intestinal GLUTag cells, either membrane permeable cAMP analogue or cAMP promoting agents (forskolin or cholera toxin) increased endogenous gcg mRNA expression or GLP-1 production[46]. In FRIC cultures, both cAMP elevation and PKC activation were shown to stimulate GLP-1 release[55,56]. Elevation of cAMP but not PKC activation were also shown to stimulate GLP-1 content[55,56].

The CRE motif is located at −291 bp to −298 bp of the gcg promoter. Knepel et al. found that this element mediates the stimulatory effect of cAMP on gcg expression in a pancreatic α cell line[57]. However, later studies indicated that deleting/mutating this CRE motif only moderately attenuated the stimulatory effect of forskolin/IBMX

or gastrin-releasing peptide (GRP) treatment on gcg promoter expression in the intestinal STC-1 cell line[45,53]. Fürstenau et al. have, however, identified another motif within the G2 enhancer element that mediates the stimulatory effect of both cAMP and calcium[58]. We found that within the G2 enhancer element, there is a typical binding site for members of the T cell factor family (TCF/LEF). Such a site usually mediates the binding of the bipartite transcription factor cat/TCF (formed by β-cat and a member of the TCF family), effector of the Wnt signaling pathway[47]. This notion then led to the discovery that gcg in the intestinal endocrine L cells is a downstream target of the Wnt signaling pathway[47,59—61]. β-cat can be phosphorylated by PKA at Ser675 and such a phosphorylation event will lead to β-cat nuclear translocation and increased cat/TCF mediated gene transcription[62,63]. We therefore suggest that cAMP-PKA signaling stimulates gcg transcription at least partially via utilizing cat/TCF as one of the effectors[41,47]. More recently, in studying the stimulatory effect of protein hydrolysates on gcg transcription, Gavrey et al. revealed the existence of two CRE-like elements that mediate the stimulatory effect of cAMP and amino acids[64]. Together, these observations suggest that multiple mechanisms are involved in mediating the stimulatory effect of cAMP on gcg transcription and GLP-1 production[41].

The role of Epac signaling

Studies presented by a few groups have shown that Epac signaling is evidently involved in mediating the effect of GLP-1 in stimulating insulin secretion[65—67]. Our laboratory, however, initiated the investigation of the role of Epac in GLP-1 expressing cells[30,31]. We found that cAMP stimulated the expression of Cdx-2, a transactivator of gcg, in the PKA deficient InR1-G9 cell line[31]. The activation was then demonstrated using the Epac pathway specific cAMP analogue (8-pMeOPT-2′-O-Me-cAMP, ESCA)[31]. We then reported that Epac-2 is expressed in the intestinal endocrine L cells, that the stimulatory effect of cAMP on gcg expression in the intestinal L cells could not be blocked by PKA inhibition, and that ESCA stimulated both gcg promoter and endogenous gcg mRNA expression in the L cells[30]. More recently, we have detected Epac-2 expression in two pancreatic α-cell lines and primary pancreatic islet cells. Using a dominant negative Epac-2 (Epac-2DN) expression plasmid and ESCA, we have further confirmed the role of Epac in gcg expression in both gut and pancreatic endocrine cells[32]. To investigate the downstream effector of Epac, we have shown that ERK inhibition blocked the stimulatory effect of ESCA[31,32].

IV cAMP signaling in mediating the function of GLP-1

The incretin effect of GLP-1: insulin secretion

Endocrinologists have learned for many years that glucose administration via the gastrointestinal tract induces a much greater stimulatory effect on insulin secretion than a comparable glucose challenge intravenously[68]. Two gastrointestinal hormones, GLP-1 and glucose-dependent insulinotropic polypeptide (originally called gastric inhibitory polypeptide, GIP) mediate such an incretin effect[14,69—71]. GLP-1 exerts its physiological function via binding to its receptor GLP-1R, a GPCR, and thereby increasing cAMP production through the activation of AC[72]. It was assumed that the potentiating effects of incretins on insulin secretion and other functions are mediated by the cAMP-PKA signaling pathway in the pancreatic β-cells[73]. However, how PKA activation leads to increased insulin secretion is not clear, although PKA was shown to phosphorylate a few proteins that are expressed in pancreatic β-cells, including the glucose transport GLUT2; Kir6.2 and SUR1, two subunits of the β-cell ATP-sensitive potassium channel (K_{ATP} channel); and α-SNAP, a vesicle-associated protein. The roles of these phosphorylation events in insulin secretion are unknown[74—77]. Other investigations suggested that incretins may also exert their effects in β-cells in cAMP-independent manners[78—80]. For example, Wheeler et al. demonstrated that a single GLP-I receptor species is able to mediate the effects of GLP-I-(7—37) through multiple G-protein-coupled signaling pathways, including the AC system and phospholipase-C[78]. Bode et al. reported that GLP-1-induced cytosolic free Ca^{2+} elevation was mediated independently of PKA[79]. Furthermore, Skoglund et al. observed that GLP-1 stimulated rat pro-insulin I

gene transcription was also on a PKA-independent manner[80].

Prior to the discovery of the Epac signaling[19,20], utilizing electrophysiological measurements, Renstrom et al. reported that cAMP can promote exocytosis in pancreatic β-cells by both a PKA-dependent as well as a PKA-independent mechanism[81]. Shortly after the discovery of Epac, Ozaki et al. reported that Epac-2 is a direct target of cAMP in regulating β-cell exocytosis[82]. They demonstrated that Epac-2 interacts with Rim2, a target of the small G-protein Rab3, and mediates cAMP-dependent and PKA-independent exocytosis in an in vitro reconstituted system[82]. Kashima et al. obtained similar results in native pancreatic β-cells. They found that either Epac-2 knockdown or PKA inhibition attenuated approximately 50% of incretin-potentiated insulin secretion, while a combination of Epac-2 knockdown and PKA inhibition resulted in ~80%—90% repression[83]. Taken the advantage of using an ESCA, Kang et al. observed that Epac is involved in mediating the effect of cAMP in calcium induced calcium release (CICR) and exocytosis in pancreatic β-cells[84]. Epac-2 is able to interact with isolated nucleotide-binding fold-1 (NBF-1) of the β-cell sulphonyl urea receptor-1 (SUR1)[82]. Holz and colleagues, therefore, proposed that cAMP might act via Epac to inhibit β-cell K_{ATP} channel[66,85]. They found that indeed, an ESCA but not a cGMP analogue, inhibited the function of K_{ATP} channels in human β-cells and in the rat INS-1 cell line[85]. Munc 13 family constitutes of three highly homologous members (Munc 13-1, Munc 13-2 and Munc 13-3). These proteins may exert a central priming function in synaptic vesicle exocytosis[86]. Munc 13-1 was shown to interact with both Epac-2 and Rim-2, while β-cells from Munc 13-1 haplodeficient mice (*Munc 13-1*$^{+/-}$) exhibit reduced insulin secretion and the mice show glucose intolerance[87]. To investigate which exocytotic steps caused by Munc 13-1 deficiency are rescued by cAMP-Epac or cAMP-PKA signaling activation, Kwan et al. conducted their examinations with patch-clamp capacitance measurements. They found that the addition of cAMP restored the reduced readily releasable pool (RRP) and partially restored refilling of a releasable pool of vesicles in the β-cells isolated from *Munc 13-1*$^{+/-}$ mice. Epac activation showed partial restoration, while PKA blockade showed impaired restoration by cAMP. Conversely, a PKA-selective agonist was able to completely restored RRP and partially restored refilling of a releasable pool of vesicles. These observations suggest that cAMP rescues of priming defects caused by Munc 13-1 deficiency via both Epac and PKA signaling pathways[87]. This group has also demonstrated that the rescue requires downstream Munc 13-1-Rim2 interaction[87].

Proliferative effect of GLP-1, crosstalk with the Wnt signaling pathway

Acute or chronic administration of GLP-1 or exendin-4 was shown to increase β-cell mass in both normal mice and diabetic mouse models[13,88,89]. Exendin-4 administration in the neonatal period of rats following the induction of experimental intrauterine growth retardation is associated with an increased β-cell proliferation and expansion of β-cell mass in adult animals, along with reduced incidence of diabetes[90]. Mechanisms by which GLP-1 modulates β-cell mass has been intensively investigated, focusing on three potential means: 1) enhancement of cell proliferation, 2) inhibition of apoptosis, and 3) differentiation of stem cells via islet neogenesis[13]. Potential signaling pathways that are involved in mediating the effect of GLP-1 include PKA, phosphatidylinositol-3 kinase (PI3K), Akt, MAPK and protein kinase Cζ[13].

More recently, the role of Wnt signaling pathway in pancreatic islets has drawn our attention[61]. This pathway was initially characterized through colon cancer research and studies of embryonic development in *Drosophila*, *Xenopus* and other organisms[91,92]. Wnt signaling exerts many important physiological and patho-physiological functions in different cell lineages and organs. The key effector of the canonical Wnt signaling pathway (defined as the Wnt pathway hereafter) is the bipartite transcription factor cat/TCF, formed by β-cat and a member of the TCF family [TCF-1/TCF7, LEF-1, TCF-3/TCF7L1 and TCF-4/TCF7L2]. The concentration of β-cat in cytosol in a resting cell is tightly controlled by the proteasome-mediated degradation process with the participation of APC, Axin/conductin, the serine/threonine kinases glycogen synthase kinase-3 (GSK-3),

and casein kinase-1α (CK-1α). Wnt glycoproteins, as the ligands, exert their effect via the seven transmembrane domain frizzled receptors and the LRP5/6 co-receptors. Following receptor binding, Wnt signals are transmitted by an association between the Wnt receptors and Dishevelled (Dvl), an event that triggers the disruption of the complex that contains APC, Axin, GSK-3, CK-1α, and β-cat, thus preventing the phosphorylation-dependent degradation of β-cat. β-cat then enters the nucleus to form the cat/TCF complex and the activation of cat/TCF (or Wnt) downstream target genes[61].

Liu and Habener demonstrated recently that in both isolated islets and the pancreatic islet Ins-1 cell line, the expression of cyclin D1 and c-Myc, both are known downstream targets of the Wnt signaling pathway and the determinants of cell proliferation, can be activated by GLP-1 or exendin-4[93]. Utilizing a cat/TCF responsive LUC reporter gene system (Topflash) as the read out, they demonstrated the stimulatory effect of exendin-4 on Wnt mediated transcriptional activity[93]. The authors suggest that basal endogenous Wnt signaling activity depends on Wnt frizzled receptors, as well as the Akt-GSK-3β signaling cascade, but not PKA. In contrast, GLP-1 agonists enhance Wnt signaling via GLP-1 receptor-mediated activation is independent of GSK-3β[93]. This investigation suggests that the incretin hormone GLP-1 is able to utilize the effector of Wnt signaling pathway to exert its stimulatory effect on β-cell proliferation. Whether cAMP-PKA and/or cAMP-Epac signaling is involved in mediating this important biological function of GLP-1 requires further investigations.

Potential novel function of GLP-1 in protecting β-cells from oxidative stress

Pancreatic β-cells are one of the most fragile cell types, sensitive to various stresses. Both oxidative stress and ER stress have been shown to cause β-cell damage, especially in diabetic conditions. GLP-1, in addition to attenuating ER stress, also plays an important role in protecting β-cells from oxidative stress[94]. A recent study suggested that this could be achieved through a novel mechanism. Txnip, also known as vitamin D3 up-regulated protein-1 (VDUP-1), was initially isolated from a hemotopoietic cell line[95]. The role of Txnip in reduction/oxidation (redox) regulation was recognized later and further studied after the demonstration of the interaction between Txnip and Thioredoxin (TRX, a major components of the thiol reducing system) by a yeast-2 hybridication system[96]. Txnip binds to reduced TRX but not to oxidized TRX nor to mutant TRX, in which two redox active cystine residues are substituted by serine[96]. Schulze et al. reported that hyperglycemia promotes oxidative stress through inhibition of thioredoxin function via Txnip[97]. In the pancreatic β-cells, Txnip functions as an excellent sensor for blood glucose levels, and its expression can be elevated by various stresses[98-100]. Minn et al. found that Txnip expression is stimulated by glucose through a carbohydrate response element and it induces β-cell apoptosis[99]. A very recent study by Chen et al. shows that in the pancreatic β-cell line Ins-1, Exendin-4 down-regulated Txnip protein levels[101]. Although mechanism/s underlying this down-regulation has yet to be investigated, this observation indicates that GLP-1 may protect β-cells from oxidative stress and apoptosis by reducing Txnip expression levels. Again, whether cAMP signaling is involved in this degradation process deserves further investigations.

Summary and perspective

Following the recognition of the fundamental incretin effect of GLP-1 in maintaining blood glucose homeostasis, extensive investigations have shown that both the production of GLP-1 and its stimulatory effect on insulin secretion could be activated by the second messenger cAMP. Both cAMP-PKA and cAMP-Epac signaling pathways are involved in stimulating gcg expression and thereby GLP-1 production, as well as the incretin effect of GLP-1. As shown in Fig. 3A, in the gut endocrine L cells, cAMP elevation may occur in response to the stimulation of a hormone, such as GRP. We suggest that cAMP-Epac activation leads to stimulated expression of transcriptional activators of gcg, including Cdx-2[31]. cAMP-PKA-CREB activation, however, will stimulate gcg transcription via the CRE motif, CRE-like motifs, and yet to be identified additional cis-elements within the gcg promoter. In addition, cAMP-PKA may utilize cat/TCF, an essential mediator of the

Wnt signaling, as the effector in stimulating gcg transcription and thereby GLP-1 production. As shown in Fig. 3B, in pancreatic β-cells, elevated cAMP production in response to the native hormone GLP-1 or the GLP-1R agonist Extendin-4/Byetta will also stimulate both PKA and Epac signaling pathways, and both of them are participated in stimulating insulin secretion. These two pathways may also be involved in stimulating β-cell proliferation via enhancing cat/TCF mediated expression of cyclin D1 and c-Myc, and possibly other cell proliferation determinants. Finally, GLP-1 may protect β-cells from oxidative stress via reducing Txnip expression via a yet to be determined mechanism/signaling pathway.

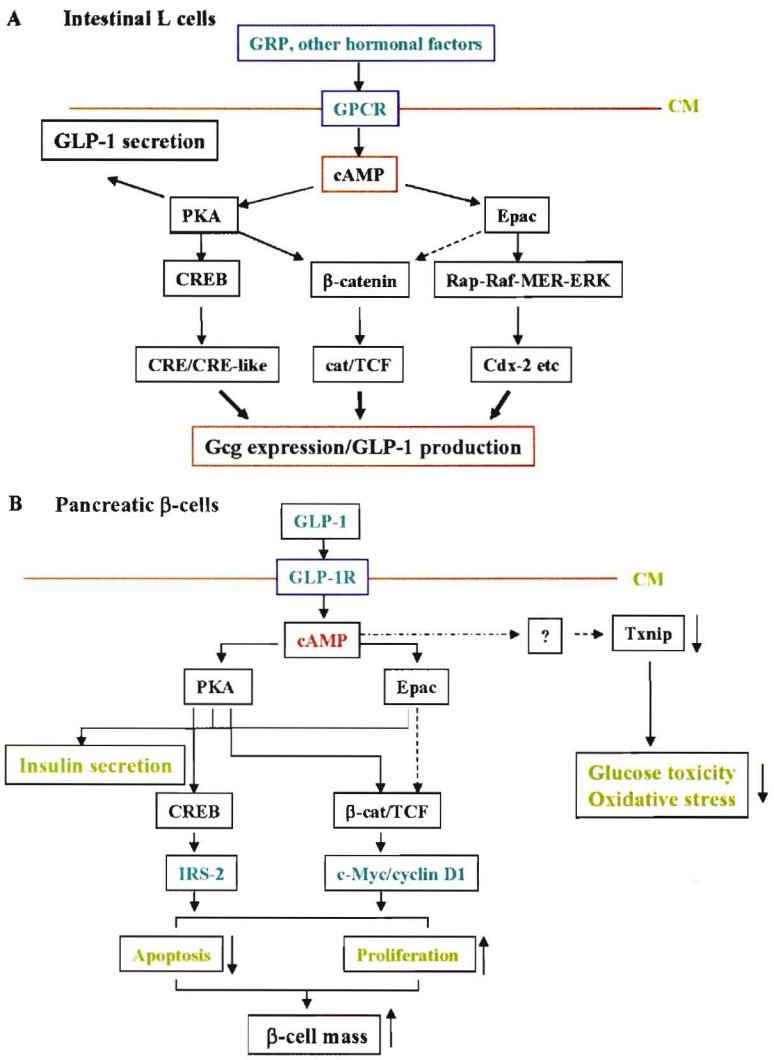

Figure 3 A summarization of our current understanding of the role of cAMP signaling in the production (A) and functions (B) of GLP-1. A) In the intestinal L cells, PKA activates gcg transcription (and thereby GLP-1 production) via phosphorylating CREB, which will stimulate gcg transcription via binding to CRE or CRE-like elements. Epac, however, may stimulate the expression of transcriptional activators of the gcg gene. In addition, cAMP may utilize PKA and Epac (unknown, indicated by dotted line) to affect phosphorylation status and nuclear translocation of β-cat, the effect of the Wnt signaling pathway. B) In the pancreatic α-cells, both PKA and Epac are implicated in GLP-1 stimulated insulin secretion. PKA is able to stimulate IRS-2 via CREB, which will lead to increased cell proliferation and reduced cell apoptosis. PKA may also cross-talk with the effector of Wnt signaling (cat/TCF) and stimulate cell proliferation. Whether Epac is able to exert such a crosstalk is unknown (indicated by dotted line). In addition, Exendin-4 is able to reduce Txnip expression level and thereby protect b-cells from oxidative stress. Whether cAMP-Epac and/or cAMP-PKA is involved in this novel function is currently unknown.

A peptide hormone, like insulin, may function as both a metabolic regulator and a proliferative stimulator of their target cells or tissues. Obviously the incretin hormone GLP-1 possesses the features as a metabolic regulator as well as a proliferative stimulator in the pancreatic β-cells. The investigation of the contributions of cAMP-PKA, cAMP-Epac, as well as the crosstalk between GLP-1 pathway and the effector of the Wnt signaling pathway in these two features deserves our close attention in the near future.

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References

[1] Kieffer TJ, Habener JF. The glucagon-like peptides. Endocr Rev 1999;20:876—913.

[2] Drucker DJ. Minireview: the glucagon-like peptides. Endocrinology 2001;142:521—7.

[3] Irwin DM, Sivarajah P. Proglucagon cDNAs from the leopard frog, Rana pipiens, encode two GLP-1-like peptides. Mol Cell Endocrinol 2000;162:17—24.

[4] Rouille Y, Martin S, Steiner DF. Differential processing of proglucagon by the subtilisin-like prohormone convertases PC2 and PC3 to generate either glucagon or glucagon-like peptide. J Biol Chem 1995;270:26488—96.

[5] Dhanvantari S, Seidah NG, Brubaker PL. Role of prohormone convertases in the tissue-specific processing of proglucagon. Mol Endocrinol 1996;10:342—55.

[6] Wideman RD, Yu IL, Webber TD, Verchere CB, Johnson JD, Cheung AT, Kieffer TJ. Improving function and survival of pancreatic islets by endogenous production of glucagon-like peptide 1 (GLP-1). Proc Natl Acad Sci U S A 2006;103:13468—73.

[7] Wideman RD, Covey SD, Webb GC, Drucker DJ, Kieffer TJ. A Switch from PC2 to PC1/3 Expression in Transplanted (alpha)-cells is Accompanied by Differential Processing of Proglucagon and Improved Glucose Homeostasis in Mice. Diabetes 2007.

[8] Wilson ME, Kalamaras JA, German MS. Expression pattern of IAPP and prohormone convertase 1/3 reveals a distinctive set of endocrine cells in the embryonic pancreas. Mech Dev 2002;115:171—6.

[9] Drucker DJ. Biologic actions and therapeutic potential of the proglucagon-derived peptides. Nat Clin Pract Endocrinol Metab 2005;1:22—31.

[10] Holst JJ 2006 Glucagon-like peptide-1: from extract to agent. The Claude Bernard Lecture. Diabetologia 2005;49:253—60.

[11] McIntosh CH, Demuth HU, Kim SJ, Pospisilik JA, Pederson RA. Applications of dipeptidyl peptidase IV inhibitors in diabetes mellitus. Int J Biochem Cell Biol 2006;38:860—72.

[12] Kieffer TJ. Gastro-intestinal hormones GIP and GLP-1. Ann Endocrinol (Paris) 2004;65:13—21.

[13] Brubaker PL, Drucker DJ. Minireview: Glucagon-like peptides regulate cell proliferation and apoptosis in the pancreas, gut, and central nervous system. Endocrinology 2004;145:2653—9.

[14] Fehmann HC, Goke R, Goke B. Cell and molecular biology of the incretin hormones glucagon-like peptide-I and glucose-dependent insulin releasing polypeptide. Endocr Rev 1995;16:390—410.

[15] Baggio LL, Drucker DJ. Biology of incretins: GLP-1 and GIP. Gastroenterology 2007;132:2131—57.

[16] Drucker DJ. The biology of incretin hormones. Cell Metab 2006;3:153—65.

[17] Mayr B, Montminy M. Transcriptional regulation by the phosphorylation-dependent factor CREB. Nat Rev Mol Cell Biol 2001;2:599—609.

[18] Richards JS. New signaling pathways for hormones and cyclic adenosine 3′,5′-monophosphate action in endocrine cells. Mol Endocrinol 2001;15:209—18.

[19] Kawasaki H, Springett GM, Mochizuki N, Toki S, Nakaya M, Matsuda M, Housman DE, Graybiel AM. A family of cAMP-binding proteins that directly activate Rap1. Science 1998;282:2275—9.

[20] de Rooij J, Zwartkruis FJ, Verheijen MH, Cool RH, Nijman SM, Wittinghofer A, Bos JL. Epac is a Rap1 guanine-nucleotide-exchange factor directly activated by cyclic AMP. Nature 1998;396:474—7.

[21] Bos JL. Epac proteins: multi-purpose cAMP targets. Trends Biochem Sci 2006;31:680—6.

[22] Holz GG. New insights concerning the glucose-dependent insulin secretagogue action of glucagon-like peptide-1 in pancreatic beta-cells. Horm Metab Res 2004;36:787—94.

[23] Rehmann H, Arias-Palomo E, Hadders MA, Schwede F, Llorca O, Bos JL. Structure of Epac2 in complex with a cyclic AMP analogue and RAP1B. Nature 2008;455:124—7.

[24] Roscioni SS, Elzinga CR, Schmidt M. Epac: effectors and biological functions. Naunyn Schmiedebergs Arch Pharmacol 2008;377:345—57.

[25] Borisov AB, Raeker MO, Kontrogianni-Konstantopoulos A, Yang K, Kurnit DM, Bloch RJ, Russell MW. Rapid response of cardiac obscurin gene cluster to aortic stenosis: differential activation of Rho-GEF and MLCK and involvement in hypertrophic growth. Biochem Biophys Res Commun 2003;310:910—8.

[26] Bos JL. Epac: a new cAMP target and new avenues in cAMP research. Nat Rev Mol Cell Biol 2003;4:733—8.

[27] Bos JL, de Bruyn K, Enserink J, Kuiperij B, Rangarajan S, Rehmann H, Riedl J, de Rooij J, van Mansfeld F, Zwartkruis F. The role of Rap1 in integrin-mediated cell adhesion. Biochem Soc Trans 2003;31:83—6.

[28] Ster J, de Bock F, Bertaso F, Abitbol K, Daniel H, Bockaert J, Fagni L. Epac mediates PACAP-dependent long-term depression in the hippocampus. J Physiol 2009;587:101—13

[29] Metrich M, Lucas A, Gastineau M, Samuel JL, Heymes C, Morel E, Lezoualc'h F. Epac mediates beta-adrenergic receptor-induced cardiomyocyte hypertrophy. Circ Res 2008;102:959—65.

[30] Lotfi S, Li Z, Sun J, Zuo Y, Lam PP, Kang Y, Rahimi M, Islam D, Wang P, Gaisano HY, Jin T. Role of the exchange protein directly activated by cyclic adenosine 5′-monophosphate (Epac) pathway in regulating proglucagon gene expression in intestinal endocrine L cells. Endocrinology 2006;147:3727—36.

[31] Chen L, Wang P, Andrade CF, Zhao IY, Dube PE, Brubaker PL, Liu M, Jin T. PKA independent and cell type specific activation of the expression of caudal homeobox gene Cdx-2 by cyclic AMP. Febs J 2005;272:2746—59.

[32] Islam D, Zhang N, Wang P, Li H, Brubaker PL, Gaisano HY, Wang Q, Jin T. Epac is involved in cAMP-stimulated proglucagon expression and hormone production but not hormone secretion in pancreatic (alpha)-and intestinal L-cell lines. Am J Physiol Endocrinol Metab 2009;296:E174—81.

[33] Drucker DJ, Philippe J, Jepeal L, Habener JF. Glucagon gene 5′-flanking sequences promote islet cell-specific gene transcription. J Biol Chem 1987;262:15659—65.

[34] Philippe J, Drucker DJ, Chick WL, Habener JF. Transcriptional regulation of genes encoding insulin, glucagon, and angiotensinogen by sodium butyrate in a rat islet cell line. Mol Cell Biol 1987;7:560—3.

[35] Morel C, Cordier-Bussat M, Philippe J. The upstream promoter element of the glucagon gene, G1, confers pancreatic alpha cell-specific expression. J Biol Chem 1995; 270: 3046—55.

[36] Herzig S, Fuzesi L, Knepel W. Heterodimeric Pbx-Prep1 homeodomain protein binding to the glucagon gene restricting transcription in a cell type-dependent manner. J Biol Chem 2000;275:27989—99.

[37] Cordier-Bussat M, Morel C, Philippe J. Homologous DNA sequences and cellular factors are implicated in the control of glucagon and insulin gene expression. Mol Cell Biol 1995;15: 3904—16.

[38] Efrat S, Teitelman G, Anwar M, Ruggiero D, Hanahan D. Glucagon gene regulatory region directs oncoprotein expression to neurons and pancreatic alpha cells. Neuron 1988;1: 605—13.

[39] Lee YC, Asa SL, Drucker DJ. Glucagon gene 5′-flanking sequences direct expression of simian virus 40 large T antigen to the intestine, producing carcinoma of the large bowel in transgenic mice. J Biol Chem 1992;267:10705—8.

[40] Jin T, Drucker DJ. The proglucagon gene upstream enhancer contains positive and negative domains important for tissue-specific proglucagon gene transcription. Mol Endocrinol 1995;9:1306—20.

[41] Jin T. Mechanisms underlying proglucagon gene expression. J Endocrinol 2008;198:17—28.

[42] de la Tour D, Halvorsen T, Demeterco C, Tyrberg B, Itkin-Ansari P, Loy M, Yoo SJ, Hao E, Bossie S, Levine F. Beta-cell differentiation from a human pancreatic cell line in vitro and in vivo. Mol Endocrinol 2001;15:476—83.

[43] Takaki R, Ono J, Nakamura M, Yokogawa Y, Kumae S, Hiraoka T, Yamaguchi K, Hamaguchi K, Uchida S. Isolation of glucagon-secreting cell lines by cloning insulinoma cells. In Vitro Cell Dev Biol 1986;22:120—6.

[44] Jin T, Drucker DJ. Activation of proglucagon gene transcription through a novel promoter element by the caudal-related homeodomain protein cdx-2/3. Mol Cell Biol 1996; 16: 19—28.

[45] Lu F, Jin T, Drucker DJ. Proglucagon gene expression is induced by gastrin-releasing peptide in a mouse enteroendocrine cell line. Endocrinology 1996;137:3710—6.

[46] Drucker DJ, Jin T, Asa SL, Young TA, Brubaker PL. Activation of proglucagon gene transcription by protein kinase-A in a novel mouse enteroendocrine cell line. Mol Endocrinol 1994;8:1646—55.

[47] Ni Z, Anini Y, Fang X, Mills G, Brubaker PL, Jin T. Transcriptional activation of the proglucagon gene by lithium and beta-catenin in intestinal endocrine L cells. J Biol Chem 2003; 278:1380—7.

[48] Brubaker PL, Drucker DJ, Greenberg GR. Synthesis and secretion of somatostatin-28 and-14 by fetal rat intestinal cells in culture. Am J Physiol 1990;258:G974—81.

[49] Anini Y, Hansotia T, Brubaker PL. Muscarinic receptors control postprandial release of glucagon-like peptide-1: in vivo and in vitro studies in rats. Endocrinology 2002; 143: 2420—6.

[50] Rindi G, Grant SG, Yiangou Y, Ghatei MA, Bloom SR, Bautch VL, Solcia E, Polak JM. Development of neuroendocrine tumors in the gastrointestinal tract of transgenic mice. Heterogeneity of hormone expression. Am J Pathol 1990; 136:1349—63.

[51] Reimer RA, Darimont C, Gremlich S, Nicolas-Metral V, Ruegg UT, Mace K. A human cellular model for studying the regulation of glucagon-like peptide-1 secretion. Endocrinology 2001;142:4522—8.

[52] Cao X, Flock G, Choi C, Irwin DM, Drucker DJ. Aberrant regulation of human intestinal proglucagon gene expression in the NCI-H716 cell line. Endocrinology 2003;144:2025—33.

[53] Gajic D, Drucker DJ. Multiple cis-acting domains mediate basal and adenosine 3′,5′-monophosphate-dependent glucagon gene transcription in a mouse neuroendocrine cell line. Endocrinology 1993;132:1055—62.

[54] Drucker DJ, Brubaker PL. Proglucagon gene expression is regulated by a cyclic AMP-dependent pathway in rat intestine. Proc Natl Acad Sci U S A 1989;86:3953—7.

[55] Brubaker PL. Control of glucagon-like immunoreactive peptide secretion from fetal rat intestinal cultures. Endocrinology 1988;123:220—6.

[56] Brubaker PL, Schloos J, Drucker DJ. Regulation of glucagon-like peptide-1 synthesis and secretion in the GLUTag enteroendocrine cell line. Endocrinology 1998;139:4108—14.

[57] Knepel W, Chafitz J, Habener JF. Transcriptional activation of the rat glucagon gene by the cyclic AMP-responsive element in pancreatic islet cells. Mol Cell Biol 1990; 10: 6799—804.

[58] Furstenau U, Schwaninger M, Blume R, Jendrusch EM, Knepel W. Characterization of a novel calcium response element in the glucagon gene. J Biol Chem 1999;274:5851—60.

[59] Yi F, Brubaker PL, Jin T. TCF-4 mediates cell type-specific regulation of proglucagon gene expression by beta-catenin and glycogen synthase kinase-3beta. J Biol Chem 2005; 280: 1457—64.

[60] Yi F, Sun J, Lim GE, Fantus IG, Brubaker PL, Jin T. Cross talk between the insulin and Wnt signaling pathways: evidence from intestinal endocrine L cells. Endocrinology 2008;149:2341—51.

[61] Jin T, Liu L. Minireview: the Wnt signaling pathway effector TCF7L2 and type 2 diabetes mellitus. Mol Endocrinol 2008;22:2383—92.

[62] Taurin S, Sandbo N, Yau DM, Sethakorn N, Dulin NO. Phosphorylation of beta-catenin by PKA promotes ATP-induced proliferation of vascular smooth muscle cells. Am J Physiol Cell Physiol 2008;294:C1169—74.

[63] Taurin S, Sandbo N, Qin Y, Browning D, Dulin NO. Phosphorylation of beta-catenin by cyclic AMP-dependent protein kinase. J Biol Chem 2006;281:9971—6.

[64] Gevrey JC, Malapel M, Philippe J, Mithieux G, Chayvialle JA, Abello J, Cordier-Bussat M. Protein hydrolysates stimulate proglucagon gene transcription in intestinal endocrine cells via two elements related to cyclic AMP response element. Diabetologia 2004;47:926—36.

[65] Holz GG, Chepurny OG, Schwede F. Epac-selective cAMP analogs: new tools with which to evaluate the signal transduction properties of cAMP-regulated guanine nucleotide exchange factors. Cell Signal 2008;20:10—20.

[66] Holz GG, Kang G, Harbeck M, Roe MW, Chepurny OG. Cell physiology of cAMP sensor Epac. J Physiol 2006;577: 5—15.

[67] Seino S, Shibasaki T. PKA-dependent and PKA-independent pathways for cAMP-regulated exocytosis. Physiol Rev 2005; 85:1303—42.

[68] Perley MJ, Kipnis DM. Plasma insulin responses to oral and intravenous glucose: studies in normal and diabetic sujbjects. J Clin Invest 1967;46:1954—62.

[69] Brown JC, Dryburgh JR, Ross SA, Dupre J. Identification and actions of gastric inhibitory polypeptide. Recent Prog Horm Res 1975;31:487—532.

[70] Drucker DJ. Glucagon-like peptides. Diabetes 1998;47:159—69.

[71] Seufert J, Kieffer TJ, Habener JF. Leptin inhibits insulin gene transcription and reverses hyperinsulinemia in leptin-deficient ob/ob mice. Proc Natl Acad Sci U S A 1999;96:674—9.

[72] Thorens B. Expression cloning of the pancreatic beta cell receptor for the gluco-incretin hormone glucagon-like peptide 1. Proc Natl Acad Sci U S A 1992;89:8641—5.

[73] Jones PM, Persaud SJ. Protein kinases, protein phosphorylation, and the regulation of insulin secretion from pancreatic beta-cells. Endocr Rev 1998;19:429—61.

[74] Oho C, Seino S, Takahashi M. Expression and complex formation of soluble N-ethyl-maleimide-sensitive factor attachment protein (SNAP) receptors in clonal rat endocrine cells. Neurosci Lett 1995;186:208—10.

[75] Thorens B, Deriaz N, Bosco D, DeVos A, Pipeleers D, Schuit F, Meda P, Porret A. Protein kinase A-dependent phosphorylation of GLUT2 in pancreatic beta cells. J Biol Chem 1996;271:8075—81.

[76] Hirling H, Scheller RH. Phosphorylation of synaptic vesicle proteins: modulation of the alpha SNAP interaction with the core complex. Proc Natl Acad Sci U S A 1996;93:11945—9.

[77] Beguin P, Nagashima K, Nishimura M, Gonoi T, Seino S. PKA-mediated phosphorylation of the human K(ATP) channel: separate roles of Kir6.2 and SUR1 subunit phosphorylation. Embo J 1999;18:4722—32.

[78] Wheeler MB, Lu M, Dillon JS, Leng XH, Chen C, Boyd AE, 3rd. Functional expression of the rat glucagon-like peptide-I receptor, evidence for coupling to both adenylyl cyclase and phospholipase-C. Endocrinology 1993;133:57—62.

[79] Bode HP, Moormann B, Dabew R, Goke B. Glucagon-like peptide 1 elevates cytosolic calcium in pancreatic beta-cells independently of protein kinase A. Endocrinology 1999; 140: 3919—27.

[80] Skoglund G, Hussain MA, Holz GG. Glucagon-like peptide 1 stimulates insulin gene promoter activity by protein kinase A-independent activation of the rat insulin I gene cAMP response element. Diabetes 2000;49:1156—64.

[81] Renstrom E, Eliasson L, Rorsman P. Protein kinase A-dependent and -independent stimulation of exocytosis by cAMP in mouse pancreatic B-cells. J Physiol 1997;502 (Pt 1):105—18.

[82] Ozaki N, Shibasaki T, Kashima Y, Miki T, Takahashi K, Ueno H, Sunaga Y, Yano H, Matsuura Y, Iwanaga T, Takai Y, Seino S. cAMP-GEFII is a direct target of cAMP in regulated exocytosis. Nat Cell Biol 2000;2:805—11.

[83] Kashima Y, Miki T, Shibasaki T, Ozaki N, Miyazaki M, Yano H, Seino S. Critical role of cAMP-GEFII-Rim2 complex in incretin-potentiated insulin secretion. J Biol Chem 2001;276:46046—53.

[84] Kang G, Joseph JW, Chepurny OG, Monaco M, Wheeler MB, Bos JL, Schwede F, Genieser HG, Holz GG. Epac-selective cAMP analog 8-pCPT-2′-O-Me-cAMP as a stimulus for Ca^{2+}-induced Ca^{2+} release and exocytosis in pancreatic beta-cells. J Biol Chem 2003;278:8279—85.

[85] Kang G, Chepurny OG, Malester B, Rindler MJ, Rehmann H, Bos JL, Schwede F, Coetzee WA, Holz GG. cAMP sensor Epac as a determinant of ATP-sensitive potassium channel activity in human pancreatic beta cells and rat INS-1 cells. J Physiol 2006;573:595—609.

[86] Koch H, Hofmann K, Brose N. Definition of Munc13-homology-domains and characterization of a novel ubiquitously expressed Munc13 isoform. Biochem J 2000;349:247—53.

[87] Kwan EP, Xie L, Sheu L, Ohtsuka T, Gaisano HY. Interaction between Munc13-1 and RIM is critical for glucagon-like peptide-1 mediated rescue of exocytotic defects in Munc13-1 deficient pancreatic beta-cells. Diabetes 2007;56:2579—88.

[88] Stoffers DA, Kieffer TJ, Hussain MA, Drucker DJ, Bonner-Weir S, Habener JF, Egan JM. Insulinotropic glucagon-like peptide 1 agonists stimulate expression of homeodomain protein IDX-1 and increase islet size in mouse pancreas. Diabetes 2000;49:741—8.

[89] Drucker DJ. Glucagon-like peptides: regulators of cell proliferation, differentiation, and apoptosis. Mol Endocrinol 2003; 17:161—71.

[90] Stoffers DA, Desai BM, DeLeon DD, Simmons RA. Neonatal exendin-4 prevents the development of diabetes in the intrauterine growth retarded rat. Diabetes 2003;52:734—40.

[91] Peifer M, Polakis P. Wnt signaling in oncogenesis and embryogenesis—a look outside the nucleus. Science 2000;287: 1606—9.

[92] Moon RT, Brown JD, Torres M. WNTs modulate cell fate and behavior during vertebrate development. Trends Genet 1997;13:157—62.

[93] Liu Z, Habener JF. Glucagon-like peptide-1 activation of TCF7L2-dependent Wnt signaling enhances pancreatic beta cell proliferation. J Biol Chem 2008;283:8723—35.

[94] Yusta B, Baggio LL, Estall JL, Koehler JA, Holland DP, Li H, Pipeleers D, Ling Z, Drucker DJ. GLP-1 receptor activation improves beta cell function and survival following induction of endoplasmic reticulum stress. Cell Metab 2006; 4: 391—406.

[95] Chen KS, DeLuca HF. Isolation and characterization of a novel cDNA from HL-60 cells treated with 1,25-dihydroxyvitamin D-3. Biochim Biophys Acta 1994;1219:26—32.

[96] Nishiyama A, Matsui M, Iwata S, Hirota K, Masutani H, Nakamura H, Takagi Y, Sono H, Gon Y, Yodoi J. Identification of thioredoxin-binding protein-2/vitamin D(3) up-regulated protein 1 as a negative regulator of thioredoxin function and expression. J Biol Chem 1999;274:21645—50.

[97] Schulze PC, Yoshioka J, Takahashi T, He Z, King GL, Lee RT. Hyperglycemia promotes oxidative stress through inhibition of thioredoxin function by thioredoxin-interacting protein. J Biol Chem 2004;279:30369—74.

[98] Shalev A, Pise-Masison CA, Radonovich M, Hoffmann SC, Hirshberg B, Brady JN, Harlan DM. Oligonucleotide microarray analysis of intact human pancreatic islets: identification of glucose-responsive genes and a highly regulated TGFbeta signaling pathway. Endocrinology 2002; 143: 3695—8.

[99] Minn AH, Hafele C, Shalev A. Thioredoxin-interacting protein is stimulated by glucose through a carbohydrate response element and induces beta-cell apoptosis. Endocrinology 2005; 146:2397—405.

[100] Cheng DW, Jiang Y, Shalev A, Kowluru R, Crook ED, Singh LP. An analysis of high glucose and glucosamine-induced gene expression and oxidative stress in renal mesangial cells. Arch Physiol Biochem 2006;112:189—218.

[101] Chen J, Couto FM, Minn AH, Shalev A. Exenatide inhibits beta-cell apoptosis by decreasing thioredoxin-interacting protein. Biochem Biophys Res Commun 2006;346:1067—74.

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