

Review

New insights into the role of cAMP in the production and function of the incretin hormone glucagon-like peptide-1 (GLP-1)

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ARTICLE INFO

Article history:

Received 27 April 2009

Received in revised form 10 September 2009

Accepted 13 September 2009

Available online 19 September 2009

Keywords:

cAMP

cat/TCF

Diabetes

Epac

GLP-1

PKA

Wnt

ABSTRACT

The proglucagon gene (gcg) encodes both glucagon and glucagon-like peptide-1 (GLP-1), produced in pancreatic α cells and intestinal endocrine L cells, respectively. The incretin hormone GLP-1 stimulates insulin secretion and pro-insulin gene transcription. GLP-1 also enhances pancreatic β -cell proliferation, inhibits cell apoptosis, and has been utilized in the trans-differentiation of insulin producing cells. A long-term effective GLP-1 receptor agonist, Byetta, has now been developed as the drug in treating type II diabetes and potentially other metabolic disorders. 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, and the crosstalk between PKA and the Wnt signaling pathway, are involved in cAMP-stimulated gcg transcription and GLP-1 production as well. Finally, functions of GLP-1 in pancreatic β cells are also mediated by PKA, Epac, as well as the effector of the Wnt signaling pathway. Together, these novel findings bring us a new insight into the role of cAMP in the production and function of the incretin hormone GLP-1.

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1. Introduction

Many peptide and other hormones participated in maintaining blood glucose homeostasis in response to physiological, pathological, and environmental changes. Among them, insulin and two other peptide hormones, glucagon and glucagon-like peptide-1 (GLP-1), are

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fundamentally impotent. Both glucagon and GLP-1 are encoded by the proglucagon gene (*gcg*), produced in pancreatic islet α cells and intestinal endocrine L cells, respectively [1,2]. Glucagon and GLP-1 exert their functions mainly via interacting with their receptors, which belong to the glucagon/VIP seven-transmembrane G-protein coupled receptor (GPCR) super-family. Therefore the second messenger cyclic AMP (cAMP) is among the effectors of *gcg* encoded peptide hormones. The elevation of cAMP was also shown to play important roles in the activation of *gcg* transcription and the production of its encoded peptide hormones. The effectors of cAMP signaling, including PKA and Epac, are important in regulating the secretion of GLP-1 as well.

GLP-1 is an important incretin hormone. It stimulates insulin secretion from pancreatic β cells after eating, even before blood glucose levels become elevated. GLP-1R agonist Exenatide (Exendin-4, commercially known as Byetta) has been developed as a drug for treating type II diabetes mellitus (T2D), and potentially, other metabolic disorders. To further explore mechanisms underlying the production and function of GLP-1 may lead to the recognition of novel therapeutic targets for the treatment and the prevention of T2D and its related complications. In this review, we have summarized the current knowledge of the role of cAMP signaling in both the production of GLP-1 in gut endocrine L cells and the function of GLP-1 in pancreatic β cells, focusing on the involvement of Epac and the crosstalk between Wnt and cAMP signaling pathways.

2. *Gcg* encodes the incretin hormone GLP-1

In mammals, the expression of *gcg* is driven by the same *gcg* promoter in pancreatic islet α cells, gut intestinal endocrine L cells, and certain neuronal cells in the brain [3–5]. These three types of cells express the identical *gcg* mRNA, which is translated into the same pro-hormone, pre-proglucagon. Several studies have identified a number of cis-elements within the first 300 bp rat *gcg* promoter region, including a typical cAMP response element (CRE) and two CRE-like elements [3]. This typical CRE motif, however, is not 100% conserved in humans [6,7].

Although the same pro-hormone, pre-proglucagon, is produced in the pancreas, the gut, and the brain, cell type specific post-translational processes, with the participation of pro-hormone convertases (PC), result in the production of different profiles of proglucagon derived peptides (PGDPs) in these tissues [8,9]. As illustrated in Fig. 1, in pancreatic α cells, the pro-hormone is mainly processed to generate glucagon, due to the co-expression of 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 PC1/3 [10,11]. The half-life of GLP-1 is very short, due to ubiquitous expression of DPP-IV (also known as CD-26), an enzyme that inactivates GLP-1 (Fig. 1). Fig. 2 summarizes the main biological functions of GLP-1 on different tissues. Embryonic pancreatic α cell was also shown to express PC1/3, indicating that during embryonic development, pancreatic islet α cells may express certain amount of GLP-1, which might be important in early pancreatic islet development [12]. Adenovirus-delivered expression of PC1/3 in a hamster pancreatic α cell line was shown to provoke this cell line to produce GLP-1 and such a GLP-1 expressing cell line was able to reduce diabetic syndromes in a streptozotocin (STZ) induced mouse diabetic model [10,11]. The post-translational processes also generate several other PGDPs, including oxyntomodulin, glicentin, glicentin related pancreatic polypeptide (GRPP), intervening peptide1 (IP-1), intervening peptide 2 (IP-2), and major proglucagon fragment (MPGF) (Fig. 1). The functions of these PGDPs are yet to be defined or further confirmed.

GLP-1 possesses the potent stimulatory effects on the secretion of insulin, transcription of the pro-insulin gene, islet cell cAMP formation, and pancreatic β -cell growth and survival. Based on these features of GLP-1 in pancreatic β cells and the beneficial effects of GLP-1 on certain

other organs [13], great effort has been made for the development of new drugs in treating T2D and its complications. One approach is the generation of long-term active agonist of GLP-1R. An agonist, namely Exendin-4 (exenatide), a peptide isolated from lizard venom, has been approved for its clinical usage with the brand name Byetta [14]. Another approach is the development of DPP-IV inhibitors [15]. Our current understanding on the functions of GLP-1 has been summarized in many excellent review articles [1,2,13,16–20].

3. Epac as another major effector of cAMP

Studies by Earl Sutherland and colleagues in 1960s on the function of the epinephrine have led to a great discovery that cAMP exerts an intermediary role in many hormonal functions. This was followed by the studies of Edwin Krebs and Edmond Fisher 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 cAMP level is low, the holoenzyme remains intact and catalytically inactive. When the concentration of cAMP rises, either due to the activation of adenylate cyclases (AC), or the inhibition of phosphodiesterases (PDE), 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 (bZIP) domain, within the promoter region of the cAMP/CREB downstream target genes. This will lead to the recruitment of CREB binding protein (CBP/p300) and possibly other nuclear co-activators, enhancing gene transcription [21].

Accumulated evidence in different research disciplines suggested that PKA is not the sole mediator of cAMP [22]. In 1998, two research groups have independently revealed the existence of a novel category of cAMP mediators, namely Epac (also known as cAMP-GEFs) [23,24]. Fig. 3A shows the overall structure of the two Epac proteins [25]. Epac-1 possesses one cAMP binding motif, while two such motifs are located in Epac-2.

Epac-1 is highly expressed in tissues including the heart, kidneys, ovaries, thyroid glands, and the corpus callosum of the brain, whereas Epac-2 expression is much more limited and is notably detectable in the brain, pituitary, adrenal gland, and pancreatic islets. Epac proteins are able to mediate the effect of cAMP via activating the Ras-related small GTPases Rap1 and Rap2 [25]. Several early studies have suggested that this activation would lead to the stimulation of the MEK-ERK signaling pathway [22,26]. However, Enserink et al. reported that the Epac pathway specific cAMP analogue 8CPT-2Me-cAMP does not activate ERK, nor does it inhibit growth-factor-induced ERK activation. They suggest that Rap1 is not involved in regulating ERK activity, while PKA and Ras mediate cAMP-induced ERK activation [27]. Nevertheless, in a *gcg*-expressing endocrine cell line, 8CPT-2Me-cAMP was shown to induce ERK phosphorylation [28], suggesting that ERK activation by cAMP is very complicated and the involvement of Epac might be cell type specific. More recently, Bos and colleagues has determined the structure of Epac-2 in complex with a cAMP analogue (Sp-cAMPS) and Rap1B by X-ray crystallography and single particle electron microscopy [29].

As summarized in Fig. 3B, physio-biological functions of the Epac signaling 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, hormone (including insulin) secretion, ion transport, and neuronal signaling [25,30–33]. Epac activation may also lead to increased gene transcription. cAMP is known to inhibit interleukin-6 responses in the immune system, due to the induction of suppressor of cytokine signaling-3 (SOCS-3). Yarwood et al. found that this induction is mediated by Epac, via increasing C/EBP DNA binding activity and

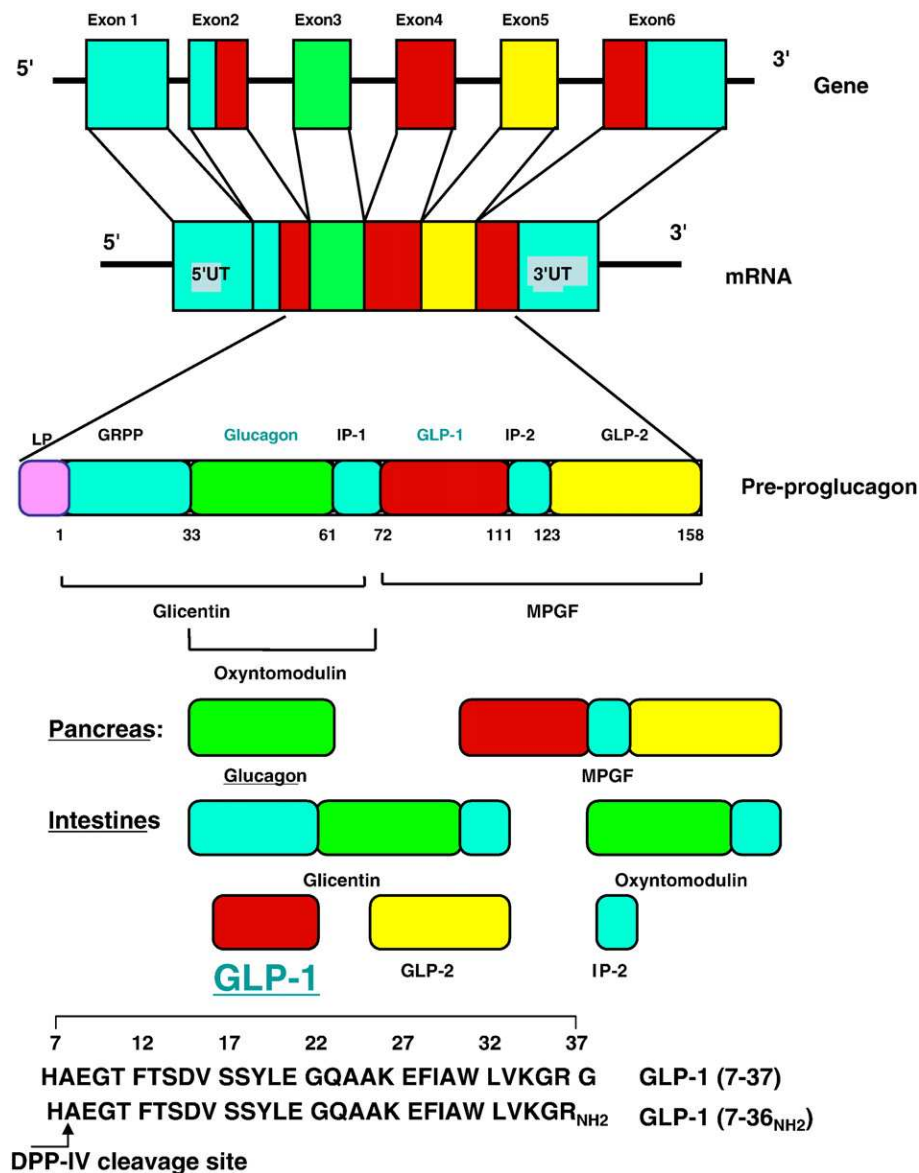


Fig. 1. The proglucagon gene encodes the incretin hormone GLP-1. The same pro-hormone, pre-proglucagon is produced in pancreatic α cells and intestinal endocrine L cells. Cell type specific expression of prohormone convertases (PC) determines the generation of active hormones in each of the two cell lineages. The active hormone glucagon is generated in pancreatic islets, while GLP-1 and GLP-2 are generated in the gut. LP, leader peptide; IP-1 and IP2, two intervening peptides. MPGF, major proglucagon fragment (MPGF). The DPP-IV cleavage site on GLP-1 is shown by an arrow.

recruitment of C/EBP β to the SOC3 gene promoter [34]. Notably, potential pathological effects of Epac signaling activation have also been recently suggested in the brain and cardiovascular system, suggesting that Epac may serve as a target of pharmacotherapy for certain diseases of the heart and brain [35,36].

4. The involvement of cAMP in stimulating gcg expression and GLP-1 production in intestinal endocrine L cells

4.1. The role of PKA

Shortly after the isolation of gcg cDNAs from rodents and humans in 1980s, studies with the methods 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 rat gcg gene promoter, namely G1, G2 and G3 [37–39]. Two additional enhancer elements, namely G4 and G5, were identified in subsequent studies [40,41].

Within the first 300 bp 5' flanking region of gcg, there is also a cAMP response element (CRE). Examinations have shown that this CRE motif and enhancer elements within the gcg promoter play their roles in regulating gcg transcription via interacting with many transcription factors, including a number of homeodomain transcription factors [3].

For studying pancreatic gcg expression, except for the use of well established α TC, HIT, and RIN cell lines, a hamster cell line namely InR1-G9 was also utilized in a number of studies [42]. Since InR1-G9 is PKA deficient, it serves as a useful tool for studying PKA-independent stimulation of cAMP on gcg transcription [28]. For studying intestinal gcg expression and GLP-1 production, examinations have been performed using two mouse cell lines, GLUTag and STC-1 [43–46], as well as primary fetal rat intestinal cell (FRIC) cultures [47–49]. The GLUTag cell line was isolated from the intestinal tumor of the transgenic mice in which the expression of SV-40 large T is driven by the 2.3 kb rat gcg promoter [46,50]. 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 [46].

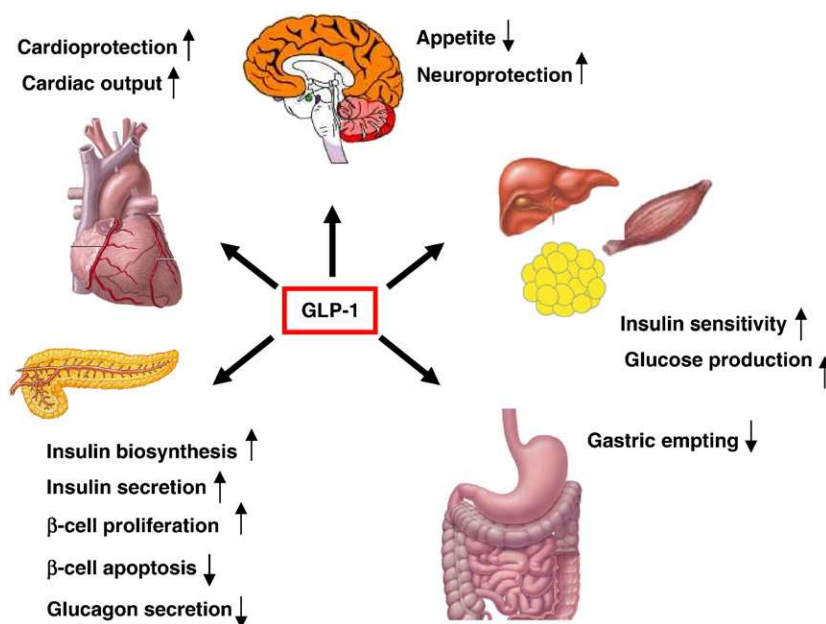


Fig. 2. A summary of physiological functions of GLP-1.

Following the recognition of the existence of the typical CRE motif within the rat *gcg* promoter, several studies have shown that in both pancreatic α and intestinal endocrine L cells, cAMP elevation led to increased *gcg* mRNA expression and promoter transcription [4,44,46,51]. In the intestinal GLUTag cell line, either membrane permeable cAMP analogue or cAMP promoting agents (forskolin or cholera toxin) increased endogenous *gcg* mRNA expression and the production of GLP-1 [46]. In FRIC cultures, elevations of cAMP but not PKC activation were also shown to stimulate GLP-1 content [52,53].

The CRE motif is located within 291 bp to 298 bp of the rat *gcg* promoter. Knepel et al. found that this CRE motif mediates the stimulation of cAMP on *gcg* expression in the pancreatic α cells [54]. However, a few following studies showed that deleting or mutating this CRE motif only moderately attenuated the stimulation of forskolin/IBMX or gastrin-releasing peptide (GRP) on the expression of *gcg* promoter [44,51]. A motif within the G2 enhancer element was later shown to mediate the effect of cAMP and calcium on *gcg* promoter activity [55]. Within the G2 enhancer element, there is a typical binding site for members of the T cell factor family (TCF/LEF). Such a site may mediate the stimulatory effect of Wnt signaling, via interacting with the bipartite transcription factor cat/TCF, formed by free β -cat and a member of the TCF family [47]. This notion led to the discovery that *gcg* in the intestinal endocrine L cells is a downstream target of the Wnt signaling pathway [47,56–58]. β -cat can be phosphorylated at Ser675 in response to PKA activation. Such phosphorylation event may lead to the stimulation of cat/TCF mediated gene transcription [59,60], suggesting that cAMP-PKA signaling could stimulate *gcg* transcription via utilizing cat/TCF as an effector [3,47]. 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 [61]. Together, these observations, along with the fact that the typical CRE motif is not conserved in the human *gcg* gene promoter [6], indicate that multiple mechanisms are involved in mediating the stimulatory effect of cAMP on *gcg* transcription and GLP-1 production [3].

4.2. The role of Epac signaling

Considering that Epac is evidently involved in mediating the effect of GLP-1 in stimulating insulin secretion from pancreatic β cells [62–64], and the presence of PKA-independent activity of cAMP on *gcg* transcription [44,51], Lotfi et al. [65] and Islam et al. [66] have investigated the role of Epac in stimulating *gcg* transcription, glucagon and GLP-1 production. The expression of Epac-2 in *gcg*-expressing pancreatic α and intestinal L cells has been detected by both RT-PCR and immunostaining [65,66]. The stimulation of Epac on the *gcg* promoter and the expression of endogenous *gcg* mRNA was also demonstrated, utilizing the Epac pathway specific cAMP analogue [66]. It should be pointed out that Epac activation also leads to increased

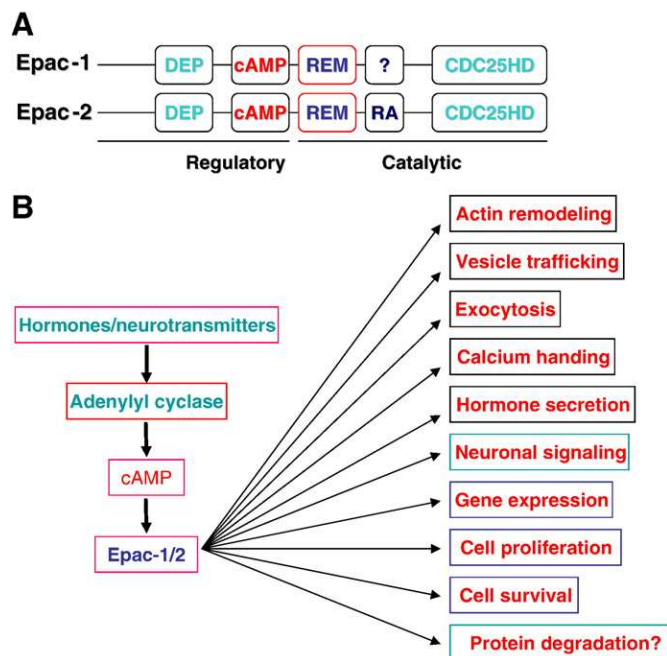


Fig. 3. An illustration of the structure (A) of two Epac proteins and their versatile physiological functions (B). A) Epac-1 has one cAMP binding domain, while Epac-2 has two such binding domains. DEP, Dishevelled, Egl-10-Pleckstrin; REM, Ras-exchange motif; RA, Ras-associated domain; and CDC25HD, CDX25-homology domain. ?, a domain with a yet unknown function. B) ?, whether Epac molecules exert their functions via mechanism not using Rap molecules is unknown.

expression of the homeobox gene *Cdx-2*, a known transactivator of *gcg* [28,65]. This activation can be effectively attenuated by MEK inhibition [28]. Finally, Islam et al. have recently verified the role of Epac in *gcg* expression, utilizing a dominant negative Epac-2 [66].

5. Both cAMP-PKA and cAMP-Epac are effectors of GLP-1 in stimulating insulin secretion

Endocrinologists have learned for many years that glucose administration via the gastrointestinal tract provokes a much greater stimulation on insulin secretion than a comparable intravenously glucose administration [67]. This incretin effect is due to the presence of two gastrointestinal hormones, GLP-1 and glucose-dependent insulinotropic polypeptide (GIP) [18,68–70]. GLP-1 exerts its function via binding to its receptor GLP-1R, a GPCR, and thereby increasing cAMP production via AC activation [71]. It was assumed that the potentiating effects of incretins on insulin secretion are mediated by PKA in pancreatic β cells [72]. However, how PKA activation leads to increased insulin secretion is not clear, although PKA activation may lead to the phosphorylation of a few proteins that are expressed in pancreatic β cells. These proteins are: glucose transport-2 (GLUT2); two subunits of the β cell ATP-sensitive potassium channel (K_{ATP} channel), Kir6.2 and SUR1; and α -SNAP, a vesicle-associated protein. How these phosphorylation events affect insulin secretion, nevertheless, is not clear [73–76]. A few observations suggested that incretins may also exert their effects in PKA-independent manners [77–79]. For example, GLP-1-induced cytosolic free Ca^{2+} elevation was shown to be mediated independently of PKA [78]. A single GLP-1 receptor species was found to mediate the effects of GLP-1 through multiple G-protein-coupled signaling pathways, including the AC system and phospholipase-C [77]. Furthermore, GLP-1 stimulated pro-insulin I gene transcription was also shown to be mediated via a PKA-independent manner [79].

Prior to the discovery of Epac [23,24], utilizing electrophysiological measurements, Renstrom et al. reported that cAMP can promote exocytosis in β cells by both a PKA-dependent and a PKA-independent mechanism [80]. Shortly after the discovery of Epac, Ozaki et al. reported that Epac-2 is a direct target of cAMP in regulating β -cell exocytosis [81]. 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 [81]. 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 [82]. Taking the advantage of using 8CPT-2Me-cAMP, Kang et al. observed that Epac is involved in mediating the effect of cAMP in calcium induced calcium release (CICR) and exocytosis in β cells [83]. Epac-2 is able to interact with isolated nucleotide-binding fold-1 (NBF-1) of the β -cell sulphonyl urea receptor-1 (SUR1) [81]. Holz et al., therefore, proposed that cAMP may act via Epac to inhibit β cell K_{ATP} channel [63,84]. They found that indeed, 8CPT-2Me-cAMP but not a cGMP analogue, inhibited the function of K_{ATP} channels in human β cells and the rat INS-1 cell line [84].

Munc 13 proteins exert a central priming function in synaptic vesicle exocytosis [85]. Munc 13-1 is able to interact with both Epac-2 and Rim-2, while β cells from *Munc 13-1^{+/-}* mice exhibit reduced insulin secretion and the mice show glucose intolerance [86]. To investigate which exocytotic steps caused by Munc 13-1 deficiency can be rescued by Epac or PKA activation, Kwan et al. conducted the study using 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 *Munc 13-1^{+/-}* β cells. Epac activation showed partial restoration, while PKA blockade showed impaired restoration by cAMP. Conversely,

a PKA-selective agonist was able to completely restore RRP and partially restored refilling of a releasable pool of vesicles. Together, these observations suggest that cAMP rescues the defects caused by Munc 13-1 deficiency via both Epac and PKA [86].

6. Function of GLP-1 in maintaining β cell mass, the involvement of the cAMP signaling

GLP-1 is able to enhance β cell proliferation and hence β cell mass. This has been attributed, at least partially, to the activation of epidermal growth factor (EGF) signaling [87]. Pancreatic β cells are very sensitive to both ER stress and oxidative stress, while GLP-1 exerts a protective role against these stresses [88]. Recent studies have revealed two novel mechanisms underlying this protection, both of them involving the cAMP signaling.

6.1. The reduction of the expression level of thioredoxin interacting protein (TxNIP)

TxNIP was initially identified as a vitamin D-3 stimulated protein (VDUP-1) [89]. The role of TxNIP in reduction/oxidation (redox) regulation was recognized later and was further studied after the demonstration of the physical interaction between TxNIP and thioredoxin (TRX, a major components of the thiol reducing system) by a yeast-2 hybrid system [90]. Hyperglycemia was shown to promote oxidative stress through the inhibition of the function of TRX via increasing TxNIP expression [91]. In pancreatic β cells, TxNIP is an excellent sensor for blood glucose levels, and it induces β cell apoptosis [92–94]. TxNIP transcription was shown to be stimulated by glucose through a carbohydrate response element (ChoRE) [93]. A very recent study reveals that the transcription factor ChREBP binds the ChoRE within the TxNIP gene promoter and activates its transcription, involving the recruitment of the nuclear co-activator p300 [95]. The expression level of TxNIP in pancreatic β cells, however, can be significantly attenuated by Exendin-4 treatment [96]. Because the function of ChREBP can be inhibited by cAMP via promoting its nuclear export [97], it is quite possible that GLP-1 utilizes cAMP as the effector in reducing TxNIP production. Whether this involves PKA and Epac has yet to be investigated.

6.2. The effector of the Wnt signaling pathway for the function of GLP-1

Both acute and chronic administration of GLP-1 or Exendin-4 increased β -cell mass in normal and diabetic mouse models [17,98,99]. Furthermore, 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 the expansion of β -cell mass in adult mice, along with reduced incidence of the development of diabetes [100]. Mechanisms by which GLP-1 increases β cell mass have been intensively investigated in the past, focusing on three potential means: 1) enhancement of cell proliferation, 2) inhibition of cell apoptosis, and 3) differentiation of stem cells via islet neogenesis [17]. Signaling pathways that are potentially involved in mediating the effect of GLP-1 include EGFR, PKA, PI3K, PKB, MAPK and PKC ζ [17].

More recently, the role of the Wnt signaling pathway in the development and function of pancreatic islets has drawn the attention globally, partly due to the fact that genome wide association studies have revealed the strong association between certain single nucleotide polymorphisms (SNPs) of TCF7L2 (=TCF-4) and the risk of T2D [58]. The Wnt signaling pathway was initially characterized through colon cancer research and studies of embryonic development in *Drosophila*, *Xenopus* and other organisms [101,102]. Wnt signaling exerts many important physiological and patho-physiological functions in different cell lineages and organs, and aberrant activation of Wnt signaling may lead to the development of colon cancer and other

types of tumors. The key effector of the Wnt signaling pathway is the bipartite transcription factor cat/TCF, formed by free β -cat and a member of the TCF family [TCF-1/TCF7, LEF-1, TCF-3/TCF7L1 and TCF7L2]. The concentration of free 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-1 α (CK-1 α). Wnt ligands exert their effect via the seven transmembrane 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-1 α , and β -cat, thus preventing the phosphorylation-dependent degradation of β -cat. Free β -cat then enters the nucleus to form the cat/TCF complex and the activation of cat/TCF (or Wnt) downstream target genes [58].

It has been demonstrated recently by Liu and Habener that in pancreatic β cells, the expression of cyclin D1 and c-Myc, both are known downstream targets of the Wnt signaling and the determinants of cell proliferation, can be activated by Exendin-4 [103]. Utilizing the cat/TCF responsive LUC reporter gene system as the read out, Liu and Habener demonstrated the stimulatory effect of Exendin-4 on Wnt mediated transcriptional activity [103]. The authors suggested that basal endogenous Wnt signaling activity depends on Wnt frizzled receptors, as well as the Akt-GSK-3 β signaling cascade, but not PKA. In contrast, GLP-1 agonists enhance Wnt signaling via GLP-1 receptor-mediated activation is independent of GSK-3 β [103]. GLP-1 also stimulated β -cat phosphorylation at Ser675, and this is likely mediated by PKA activation [103]. This investigation suggests that GLP-1 utilizes the effector of the Wnt signaling pathway to exert its stimulatory effect on β cell proliferation. Whether Epac is involved in mediating this important biological function of GLP-1 requires further investigations.

7. Summary and perspective

The fundamental incretin effect of GLP-1 was recognized more than 20 years ago, and this discovery has led to the development of a novel therapeutic drug for T2D treatment. During the past two

decades, extensive investigations have shown that both the production of GLP-1 and its stimulation on insulin secretion could be activated by cAMP, involving both PKA and Epac. As summarized in Fig. 4, in the gut endocrine L cells, cAMP elevation may occur in response to the stimulation of a hormone or neurotransmitter, such as GRP. We suggest that Epac activation leads to stimulated expression of transcriptional activators of gcg, such as Cdx-2 [28]. cAMP-PKA-CREB activation, however, stimulates gcg transcription via the CRE motif or the CRE-like motifs [3]. In addition, PKA is also able to utilize cat/TCF, an essential effector of the Wnt signaling pathway, in stimulating gcg transcription and thereby GLP-1 production. As summarized in Fig. 5, in the pancreatic β cells, elevated cAMP production in response to the native hormone GLP-1 or the GLP-1R agonist Exendin-4/Byetta will also stimulate both PKA and Epac, and both of them participated in stimulating insulin secretion. More importantly, cAMP and Epac are also involved in stimulating β cell proliferation via enhancing cat/TCF mediated Wnt target gene expression. Finally, GLP-1 is able to protect β cells from oxidative stress or glucose toxicity through reducing TxNIP levels, possibly involving PKA and/or Epac.

A significant advancement in this research area is the recognition of the importance of the Wnt signaling and the crosstalk between Wnt and cAMP signaling, in the production and function of GLP-1. Certain peptide hormones, such as insulin, are known to function as both a metabolic regulator and a proliferative stimulator. Obviously the incretin hormone GLP-1 possesses the features as a metabolic regulator and a proliferative stimulator of β cells. The contributions of PKA, Epac, as well as the crosstalk between GLP-1 and the effector of the Wnt signaling in these two features deserves our close attention in the near future.

An important feature of the function of Epac is that it is frequently interconnected with the cAMP signaling through PKA [25]. Epac and PKA mediate many sub-cellular processes in very complicated manners. They could exert additive or sometime completely opposite effects on a given process, and this complexity is mainly due to the fact that cAMP signaling is compartmentalized in cells [104]. The cAMP gradients are interpreted through the selective activation of sub-populations of effector molecules, including not only PKA and Epac, but also PDEs [104]. To explore in detail how PKA and Epac mediates

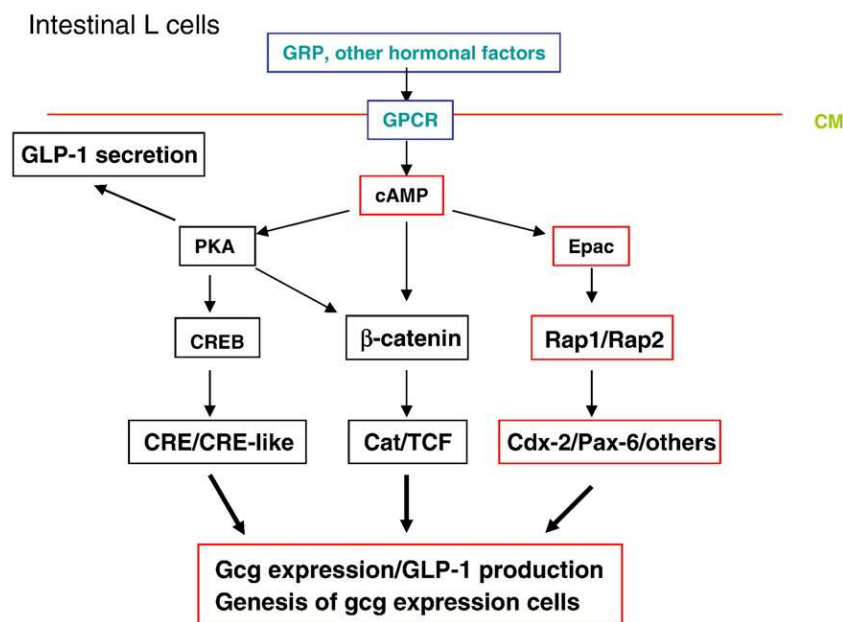


Fig. 4. A summary of current understanding of the role of cAMP signaling in GLP-1 production. In the intestinal L cells, PKA activates gcg transcription and thereby GLP-1 production via phosphorylating CREB, which stimulates gcg transcription via binding to CRE or CRE-like elements. Epac stimulates the expression of transcriptional activators of gcg, such as Cdx-2. In addition, cAMP may utilize PKA and Epac (unknown, indicated by dotted line) to affect phosphorylation status and nuclear translocation of β -cat, the effector of Wnt signaling.

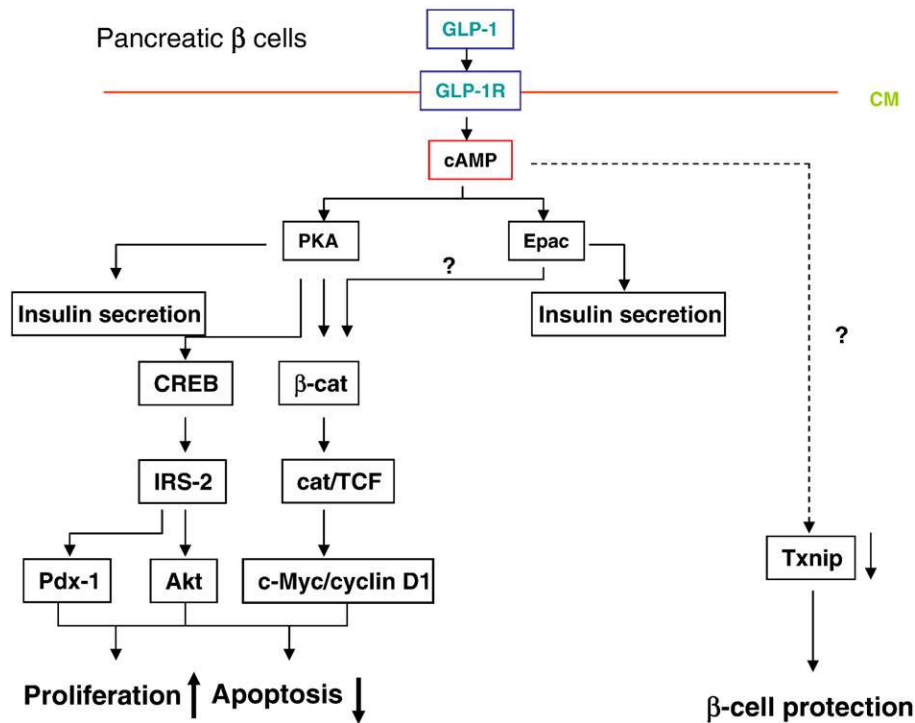


Fig. 5. A summary of current understanding of the role of cAMP in mediating the function of GLP-1. In pancreatic β cells, both PKA and Epac are implicated in GLP-1 stimulated insulin secretion. PKA is also able to stimulate IRS-2 via CREB, which will lead to increased cell proliferation and reduced apoptosis. PKA may 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, GLP-1/Exendin-4 may reduce TxNIP expression level and thereby protect β cells from oxidative stress. Whether Epac and/or PKA are involved in this novel function is currently unclear.

the metabolic and proliferative role of GLP-1 in β cells, it is necessary to study the sub-cellular compartmentalization of PKA, Epac and PDE in response to cAMP elevation in time dependent manners.

It should be pointed out that recent studies in other cell lineages have demonstrated the crosstalk between cAMP and PKB signaling pathways [104–108]. For example, Brennesvik et al. found that adrenaline potentiates insulin-stimulated PKB activation via cAMP and Epac [108]. Houston et al. found that cAMP regulates the nuclear/cytoplasmic trafficking of DNA-dependent protein kinase (DNA-PK), an important kinase that repairs double-stranded breaks of damaged DNA and phosphorylates PKB. It has been shown cAMP employs Epac to transduce a positive effect on the activation of DNA-PK, whereas PKA provides the negative input by antagonizing these actions [104]. In adipocytes, the increase of intracellular cAMP leads to increased activity of AMP activated protein kinase (AMPK). Omar et al. found that the stimulatory effect of adrenergic agonist isoproterenol on AMPK phosphorylation (T172) can be diminished by insulin co-incubation. This diminishing effect of insulin can be reversed by PDE3B inhibition, but not by PDE4 inhibition. Furthermore, 8-pCPT-2'-O-Me-cAMP treatment increased AMPK phosphorylation [106]. These observations collectively suggest that PKA and Epac may selectively regulate the function of PKB in cell type specific manners, possibly involving selected cAMP degradation by PDEs. Obviously, to explore the potential crosstalk between cAMP and PKB in pancreatic β cells will further deepen our understanding of the physiological function of the incretin hormone GLP-1.

Acknowledgements

Tianru Jin would like to thank the Canadian Institutes of Health Research (CIHR, MOP-89987) and Canadian Diabetes Association (CDA, 2341) for providing funding support in conducting his current research on this topic. We regret that due to space limitation, many excellent studies cannot be cited and discussed.

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