Mechanisms underlying proglucagon gene expression

Tianru Jin

1Departments of Medicine, Physiology, and Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada
2Division of Cell and Molecular Biology, Toronto General Research Institute, University Health Network, Room 10-354, 10th Floor, Toronto Medical Discovery Tower, 101 College Street Toronto, Ontario, MSG 1L7, Canada
(Correspondence should be addressed to T Jin; Email: tianru.jin@utoronto.ca)

Abstract

The proglucagon gene (gcg) encodes a number of peptide hormones that are of cell-type specifically expressed in the pancreatic islets, the distal ileum and the large intestine, as well as certain brain neuronal cells. These hormones are important in controlling blood glucose homeostasis, intestinal cell proliferation, and satiety. More importantly, the major hormone generated in the pancreas (i.e. glucagon) exerts opposite effects to the ones that are produced in the intestines (i.e. glucagon-like peptide-1 (GLP-1) and GLP-2). To understand the mechanisms underlying cell-type-specific gcg expression may lead to the identification of novel drug targets to control endogenous hormone production for therapeutic purposes. Extensive in vitro examinations have shown that more than a half dozen of homeodomain (HD) proteins are able to interact with the gcg gene promoter and activate its expression. In vivo ‘knock-out’ mouse studies, however, cannot demonstrate the role of some of them (i.e. Cdx-2, Brn-4, and Nkx6.2) in the development of pancreatic islet α-cells, suggesting that these HD proteins may exert some redundant functions in the genesis of gcg-producing cells. Investigations have also revealed that gcg expression is controlled by both protein kinase A and Epac signaling pathways in response to cAMP elevation, and cell-type specifically controlled by insulin and the effectors of the Wnt signaling pathway. This review summarizes our current understanding on the mechanisms underlying gcg transcription and presented my interpretations on how the interactions between different signaling networks regulate gcg expression. Journal of Endocrinology (2008) 198, 17–28

Introduction

The gene that encodes glucagon, namely the proglucagon gene (gcg), was isolated from rodents and humans in the early 1980s (Bell et al. 1983a, b, Lopez et al. 1983, Heinrich et al. 1984, Irwin 2001). The analysis of gcg cDNAs from these species further revealed that it encodes not only glucagon but also two glucagon-like peptide hormones, namely glucagon-like peptide-1 (GLP-1) and GLP-2 (Lund et al. 1982).

Glucagon is produced and released from the pancreatic α-cells. The major physiological function of glucagon is the stimulation of hepatic glucose release, and it has been utilized in treating patients with hypoglycemia and other disorders (Sinclair & Drucker 2005). Glucagon exerts its biological functions through binding to its receptor, which is expressed in the brain, pancreas, small and large intestines, kidney, and more importantly liver and adipose tissues (Jelinek et al. 1993, Campos et al. 1994, Burcelin et al. 1996, Christophe 1996).

GLP-1 and GLP-2 are generated and released from the enteroendocrine L cells in the distal ileum, large intestine, and certain neuronal cells of the brain (Brubaker & Drucker 2004, Kieffer 2004, Drucker 2006). GLP-1 stimulates postprandial insulin secretion and activates the transcription and biosynthesis of insulin (Brubaker & Drucker 2004, Kieffer 2004, Drucker 2006, Doyle & Egan 2007). In addition, GLP-1 may stimulate proliferation and neogenesis of pancreatic β-cells (Brubaker & Drucker 2004, List & Habener 2004, Bonner-Weir & Weir 2005, Drucker 2006, Wideman et al. 2006, 2007, Doyle & Egan 2007). The long-acting GLP-1 receptor (GLP-1R) agonist, exendin-4, has been utilized in the differentiation or trans-differentiation of insulin-producing cells from both somatic and embryonic stem cells (Abraham et al. 2002, Brubaker & Drucker 2004, Kieffer 2004, List & Habener 2004, Bonner-Weir & Weir 2005, Doyle & Egan 2007). Other physiological functions of GLP-1 include the inhibition of glucagon release and gastric emptying and the enhancement of peripheral insulin sensitivity (Drucker 2002). In addition, GLP-1 administration in the brain inhibits food intake (Turton et al. 1996, Meeren et al. 1999). Based on these findings, exenatide (commercially known as Byetta) has been developed and approved clinically for treating type II diabetes mellitus (T2D) and related
disorders (Holst 2006). GLP-2 is co-expressed with GLP-1 in the intestinal L cells and the brain. The function of GLP-2 was first identified by Drucker et al. (1996) as a growth factor of small intestines. It stimulates the proliferation of small intestinal crypt cells and inhibits cell apoptosis (Drucker 2001, Jeppesen 2003). This property makes GLP-2 a potential therapeutic agent in the treatment of intestinal injury (Jeppesen 2003). Furthermore, another intestinal product, oxyntomodulin, may prove useful as an anti-obesity drug (Murphy et al. 2006).

Extensive examinations in the exploration of mechanisms underlying gcg transcription have led to the identification of a minimum promoter region (G1) and four enhancer elements (G2–G5) in the gcg gene promoter (Philippe et al. 1987a, Herzig et al. 2000; Fig. 1). In addition, gcg transcription can be regulated by cAMP, amino acids, and a number of homeodomain (HD) protein transcription factors (Knepel et al. 1990, Drucker et al. 1994, Jin & Drucker 1996, Gevrey et al. 2004). More recently, intestinal but not pancreatic gcg expression was shown to be regulated by the effectors of the Wnt signaling pathway (Ni et al. 2003, Yi et al. 2005). Furthermore, insulin at pathological concentrations was shown to stimulate intestinal gcg mRNA and GLP-1 production (Yi et al. 2008), in contrast to its known inhibitory effect on gcg expression in pancreatic α-cells (Philippe 1989, 1991). In this review, a summary of our current knowledge on the transcriptional regulation of gcg expression, focusing on the role of HD protein transcription factors, cAMP signaling, and the Wnt signaling pathway is presented. Mechanisms controlling secretion and function of glucagon

Figure 1  (A) Schematic representation of the rat gcg promoter. The diagram shows G1 and four enhancer elements (G2, G3, G4, and G5), cAMP response element (CRE), and a GATA-binding site containing gcg upstream enhancer (GUE). WGATAR, consensus GATA-binding site. W, A, or T; R, A, or G. Although this site is located within the GUE region, no evidence suggested that it functions only in the intestinal cell lines (Jin & Drucker 1995). *In pancreatic α-cells but not intestinal L cell, insulin represses gcg expression via the G3 element. (B) DNA sequence of the first 300 bp 5’ flanking region of the rat gcg gene. G1 and four enhancer elements are underlined. Trans-elements that have been shown to interact with each of the enhancer elements are indicated (Philippe et al. 1987a, Jin & Drucker 1996, Herzig et al. 2000, Ritz-Laser et al. 2005, Yi et al. 2005). TATA, TATA box. Although most of the enhancer elements are conserved between rodents and humans, in the human CGG gene, the CRE sequence is CAACGTCA (Nian et al. 1999).

**HD proteins and proglucagon gene expression**

Nearly a dozen HD proteins were shown to be expressed in pancreatic α and/or intestinal endocrine L cells, including the LIM protein Isl-1 (Dong et al. 1991, Wang & Drucker 1995), a number of paired HD proteins (Hussain & Habener 1999, Ritz-Laser et al. 1999, 2000, 2002, Zaiko et al. 2004), caudal HD protein Cdx-2 (German et al. 1992, Jin & Drucker 1996, Hussain & Habener 1999, Ritz-Laser et al. 1999), POU HD protein Brn-4 and Oct-1 (Wang et al. 2006), and members of the TALE (Herzig et al. 2000, Liu et al., 2006), and Nkx families (Sander et al. 2000, Pedersen et al. 2005, Nelson et al. 2007). Although most of them were shown to stimulate gcg promoter expression in the in vitro examinations, only a few of them were demonstrated to be essential for the development of pancreatic islets and α-cell genesis in the ‘knock-out’ mouse studies.

**Isl-1**

Isl-1 is expressed in all hormone-producing islet cells in adult mice (Dong et al. 1991, Thor et al. 1991, Ericson et al. 1992). In vitro studies have shown that Isl-1 regulates transcription of the insulin, gcg, and somatostatin genes (Carlsson et al. 1990, Vallego et al. 1992, Wang & Drucker 1995, Peng et al. 2005). Targeted disruption of Isl-1 in mice results in an early arrest of embryonic development, with the failure of the development of the dorsal pancreatic mesenchymal and the complete absence of pancreatic endocrine cells (Pfaeff et al. 1996). Wang & Drucker (1995) demonstrated that the proximal rat gcg promoter interacted with an amino-terminally-truncated Trp-E-Isl-1 fusion protein that lacked the LIM domains, or a full-length in vitro translated Isl-1 that contained intact LIM domains. Furthermore, they observed Isl-1-mediated activation of gcg promoter in the pancreatic α-cell line InR1-G9. This study not only indicates that Isl-1 is a transcriptional activator of gcg but also suggests that LIM domains are not necessarily involved in the interaction with the target promoters by the LIM HD proteins (Wang & Drucker 1995).

**Pax proteins**

Ritz-Laser et al. (2000) found that Pax-2 binds to motifs within the G3 and G1 enhancer elements of the gcg promoter and activates its transcription. Flock & Drucker (2002) found that although in cultured cell lines, Pax-2 did bind to the gcg promoter and activated its transcription, Pax-2 mRNA transcripts were not detected by RT-PCR in RNA isolated from adult rat pancreas, rat islets, embryonic d19 or adult murine pancreas, and gastrointestinal tract. Furthermore, embryonic d19 or neonatal d1 Pax-2 (1Neu) mice exhibited normal islet α-cells and intestinal endocrine L cells and no decrease in pancreatic or intestinal glucagon gene expression (Flock & Drucker 2002). In the same Pax-2 (1Neu) mutant mice, Ritz-Laser et al. found a two- to threefold increase in the average pancreas volume (Zaiko et al. 2004). Because this increase was not accompanied by significant modification in the insulin or glucagon content in the pancreas, Ritz-Laser et al. suggested that the content of these hormones per cell is decreased (Zaiko et al. 2004).

Pax-6 is an important factor in islet cell development (Habener et al. 2005). It is expressed early in the epithelium of both dorsal and ventral pancreatic buds of the developing pancreas. The Sey−/− mice carry a spontaneous mutation of the Pax-6 gene (Stoykova et al. 1996). These mice show abnormal organization of the islets, with decreased numbers of α, β, δ, and PP cells, associated with substantially reduced glucagon and insulin production (Sander et al. 1997). Pax-6 was shown to synergize with Cdx-2 in stimulating gcg promoter expression (Ritz-Laser et al. 1999). Pax6−/− mice die shortly after birth and fail to form pancreatic islets (St-Onge et al. 1997). Trinh et al. (2003) demonstrated that Pax-6 was able to stimulate gcg expression in intestinal endocrine L cells, following an adenovirus-mediated over-expression procedure.

**Cdx-2**

Cdx-2 is a caudal-like HD protein in mammals. Hamster Cdx-2 (=Cdx-3 or Cdx-2/3) was isolated by German et al. (1992) from the pancreatic β-cell line HIT. Cdx-2 expression was also detected in the pancreatic α-cell line InR1-G9, intestinal GLUTag and STC-1 cell lines, and in the mouse pancreas (Jin & Drucker 1996). Cdx-2 was shown to bind to two AT-rich motifs within the G1 enhancer element of the gcg promoter (Fig. 2). Cdx-2 co-transfection substantially stimulated the expression of gcg promoter and the stimulation was abolished if the Cdx-2-binding sites were mutated (Jin & Drucker 1996). Furthermore, Cdx-2 over-expression in the pancreatic InR1-G9 cell line led to an increased endogenous gcg mRNA expression (Jin et al. 1997). Laser et al. (1996) however, demonstrated that the InR1-G9 cell line expressed two alternatively spliced isoforms of Cdx-2, and the longer one functioned as the major stimulator of gcg transcription. Furthermore, Cdx-2, Pax-6, and the nuclear co-activator P300 could exert synergistically a stimulatory effect on gcg promoter expression (Hussain & Habener 1999, Ritz-Laser et al. 1999).

Cdx-2−/− mice die during 3.5–5.5 dpc, due to a fundamental role of Cdx-2 in embryo implantation (Chawengsaksophak et al. 1997). Cdx-2+−/− mice were shown to develop multiple malfunctions, including stunted growth, shortened tails, and abnormalities in their vertebral development. More importantly, 90% of the Cdx-2+−/− mice were shown to develop colorectal polyps by 3 months after birth, indicating that Cdx-2 is a potential tumor suppressor (Chawengsaksophak et al. 1997). Interestingly, pancreatic
development in the \textit{Cdx-2} \textsuperscript{+/−} mice appeared normal. In the pancreatic InR1-G9 cell line, Cdx-2 was shown to bind to two AT-rich motifs within the proximal promoter region of Cdx-2 and stimulate its own transcription (Xu \textit{et al.} 1999). Binding to a motif downstream of the transcription start site appeared to be involved in stimulating promoter expression, while binding to the TATA box appeared to attenuate the activation. We have proposed that one functional Cdx-2 allele is sufficient for maintaining its normal function in pancreatic and intestinal gcg-producing endocrine cells due to the existence of such an efficient auto-regulatory mechanism (Xu \textit{et al.} 1999).

\textbf{Brn-4}

Brn-4 is highly expressed in neural stem cells and regulates stem cell-specific gene expression (Heller \textit{et al.} 2004). Brn-4 is also expressed in pancreatic \textit{α}-cells but not \textit{β}-cells, and its capability in activating gcg expression was first demonstrated by Hussain \textit{et al.} (1997). Brn-4 expression starts at e10 in the pancreas, just before the expression of Pax-6. At time point of e19, no Brn-4 co-localization was observed with insulin or somatostatin, suggesting that Brn-4 is a pancreatic \textit{α}-cell-specific transcription factor. Wang \textit{et al.} (2001) have shown
that induced Brn-4 expression in the INS-1 β-cell line initiated detectable expression of glucagon without affecting β-cell-specific gene expression. Later, Hussain et al. (2002) reported that mis-expression of Brn-4 by the β-cell-specific Pdx-1 gene promoter resulted in ectopic expression of the gcg gene in insulin-expressing pancreatic β-cells in mice. These observations strongly suggest the role of this ‘master’ control gene in directing the development of α-cell lineage. Surprisingly, in Brn-4−/− mice, pancreatic bud formation, gcg-expressing cell numbers, and related physiological measurements all appear normal (Heller et al. 2004). Whether Brn-4 is not important for pancreatic α-cell development or the absence of abnormality in pancreatic α-cells in Brn-4−/− mice is due to the existence of redundant factors remain unresolved.

Both Cdx-2 and Brn-4 interact with motifs of the gcg promoter G1 element (Fig. 1). Their binding sites are separated by only 8 bp. Ectopic expression of either Cdx-2 or Brn-4 in the insulin-producing In111 cell line was shown to result in gcg mRNA expression (Wang et al. 2006). Brn-4 synergized with Cdx-2 in activating gcg promoter, and its activation domain was not required for this synergistic function (Wang et al. 2006). These observations further suggest that HD proteins in different families could have both synergistic and redundant effects on their target gene expression (Wang et al. 2006).

Pbx proteins

Pbx proteins are able to associate with most members of the Hox HD protein family and certain Hox–related HD proteins, including Cdx-2 (Liu et al. 2006). This association may enhance the binding selectivity and affinity of Hox and Hox-related proteins to their target promoters. It has been suggested that a penta-peptide motif, localized in many Hox and Hox-related HD proteins, is involved in the interaction with Pbx proteins (Berthelsen et al. 1999).

The expression of Pbx1 in the embryonic pancreas starts at e10-5. After that Pbx-1 is expressed in a gradient manner with a high level of expression in pancreatic mesenchyme and vascular endothelium, a modest level in central ductal epithelia, and low expression level in more peripheral exocrine pancreatic epithelium cells. In adult mice, Pbx-1 is detectable in all four islet hormone-producing cells, acinar cells, and at the highest level in the ductal cells. The expression pattern of Pbx-1 suggested that it may play a role in pancreatic development and in maintaining normal pancreatic functions (Kim et al. 2002). Pbx-1−/− mice die at e15–e16 with hypoplasia or aplasia of many organs, including the pancreas where marked defects of exocrine and endocrine cells were observed (Kim et al. 2002). At e10-5, Pbx-1−/− mice showed less compact and disordered dorsal pancreatic mesenchyme. At e13-5–e14, dorsal and ventral pancreatic hypoplasia were found. Further abnormalities in both dorsal and ventral pancreas were observed at e15. Detailed analysis of these embryos revealed cell morphological defects and suggested that Pbx-1 was required for embryonic pancreas cell growth and survival. At e14-5, ~35–40% reduction in pancreatic cell proliferation in the Pbx-1−/− embryo was recorded. Isl-1 and another transcription factor, Atoh5, which are important for pancreatic cell differentiation, were found to be repressed in the Pbx-1−/− mice, followed by impaired pancreatic cell differentiation. Interestingly, Pbx-1+/− mice also showed islet malformation and hyperinsulinemia (Kim et al. 2002). These observations indicate an essential role for Pbx-1 in maintaining normal pancreatic development and physiology.

Since the penta-peptide motif involved in the interaction with Pbx proteins is also present in Cdx-2 and other caudal HD proteins, Liu et al. (2006) have detected the interaction between Cdx-2 and Pbx-1 and examined whether Pbx-1 functions as a co-factor of Cdx-2 in regulating gcg expression. Pbx-1 co-transfection was shown to enhance the activation of gcg promoter by Cdx-2 and mutating the penta-peptide motif attenuated the stimulatory effect of Cdx-2 (Liu et al. 2006).

Nkx family

Nkx2.2 is expressed in α, β and PP cells, but not in the δ cells. Sussel et al. (1998) demonstrated that Nkx2.2−/− mice develop severe hyperglycemia and die shortly after birth. The mutant embryos lack insulin-producing cell and have fewer glucagon- or PP-producing cells. Sander et al. (2000) found that disruption of Nkx6.1 leads to the loss of β-cell precursor and the lack of β-cell neogenesis during the secondary transition period (e13 and after). Because Nkx6.1/Nkx2.2 double mutant mice showed identical phenotypes with that of the Nkx2.2−/− mice, Sander et al. (2000) suggested that Nkx6.1 is downstream of Nkx2.2. More recently, Schisler et al. (2005) reported that Nkx6.1 binds to the gcg promoter and represses endogenous gcg mRNA expression. The Nkx6.1 paralog, Nkx6.2, is also expressed in pancreatic islet cells. Although Nkx6.2−/− mice were shown to be fertile and lack a detectable defect (Cai et al. 2001), Henesleit et al. (2005) demonstrated that Nkx6.2 could exert redundant functions to Nkx6.1 in the development of both pancreatic α- and β-cells. Nelson et al. (2007) assessed the capability for rescuing insulin-producing β-cells in the Nkx6.1−/− mice with different Nkx6-expressing transgenes. The formation and maturation of β-cells was restored when Nkx6.1 expression was driven by the Pdx-1 gene promoter, but not by the Neurog3 (Ngn3) promoter. More interestingly, Pdx-1-driven Nkx6.2 also rescued insulin-producing β-cells in the Nkx6.1−/− mice (Nelson et al. 2007).

In conclusion, despite great efforts by multiple laboratories, the spatiotemporal aspects of pancreatic and intestinal endocrine subtype specification have remained largely elusive. Although many HD proteins were shown to stimulate gcg promoter or endogenous gcg mRNA expression in cultured cell lines, ‘knock-out’ studies can only directly verify the involvement of Pax-6 and Pbx-1 in pancreatic α-cell development (Table 1). Since Cdx-2−/− mice are embryonic
lethal, the involvement of Cdx-2 in the genesis and functional maintenance of pancreatic α-cells can be assessed only if α-cell-specific ‘knock-out’ mice are generated. Biochemical studies have also revealed physical interactions between different HD proteins and between HD proteins and other transcription factors (which are not covered in this review). This, along with the observations that the same cis-element can be bound by different HD proteins, suggests that HD proteins exert synergistic and redundant effects on the genesis of pancreatic α-cells and the expression of the gcg gene.

cAMP and proglucagon expressions

CRE and other cAMP response elements on the proglucagon promoter

The effect of cAMP signaling in stimulating gcg expression in both pancreatic islet α-cells and intestinal endocrine L cells has been studied extensively following the recognition of a typical cAMP response element (CRE) within the proximal gcg promoter (Drucker & Brubaker 1989, Gajic & Drucker 1993, Drucker et al. 1994, Lu et al. 1996) (Fig. 1). Many studies have been conducted using cultured pancreatic α-cell lines or primary pancreatic islet cell cultures of the rodent species (Philippe et al. 1987b, Drucker & Brubaker 1989, Knepel et al. 1990, Drucker et al. 1991, Schwaninger et al. 1993, Diedrich & Knepel 1995, Wang et al. 2003). It has been found that in the intestinal GLUTag cell line, either membrane-permeable cAMP analog or cAMP-promoting agents (forskolin or cholera toxin) increased endogenous gcg mRNA expression or GLP-1 production (Drucker et al. 1994). In the fetal rat intestine cell (FRIC) cultures, Brubaker (1988) and Brubaker et al. (1998) demonstrated that both cAMP elevation and PKC activation stimulated glucagon-like immunoreactive peptide (GLI) release. Elevation of cAMP but not PKC activation also stimulated GLI or GLP-1 content (Brubaker 1988, Brubaker et al. 1998).

As shown in Fig. 1, a typical CRE is located between −291 and −298 bp of the rat gcg promoter. Knepel et al. (1990) found that this element mediated the stimulatory effect of cAMP elevation on gcg expression in the pancreatic α-cells. However, a later study by Gajic & Drucker (1993) indicated that the deletion of this element only moderately attenuated the stimulatory effect of cAMP on gcg promoter expression when the mouse intestinal STC-1 cell line was examined. Similarly, Lu et al. (1996) found that either deleting or mutating this CRE motif generated only a partial repression on the stimulatory effect of gastrin-releasing peptide on gcg promoter expression in the STC-1 cell line. Furstenau et al. (1999) however, have identified another motif within the G2 enhancer element that could mediate the stimulatory effect of both cAMP and calcium. In addition, Wang et al. 2003 found that activated transcription factor 3 also exerted its stimulatory effect on gcg transcription via interacting with this CRE motif. In studying the stimulatory effect of protein hydrolysates on gcg transcription, Gevrey et al. (2004) revealed the existence of two CRE-like elements that could mediate the stimulatory effect of cAMP and amino acids.

It should be pointed out that although the CRE motif is 100% conserved among rodent species, in the human gcg promoter this site is CAACGTCA instead of TGACGTCA (Nian et al. 1999). Whether this motif in human gcg gene promoter is capable of interacting with CREB is unknown. Researchers have generated transgenic mice in which the expression of the growth hormone reporter was driven by the 1-6 kb human gcg promoter (Nian et al. 1999, 2002, Irwin 2001). They demonstrated the evidence of divergence in the mechanisms utilized for tissue-specific regulation of the human and rodent gcg genes (Nian et al. 1999, 2002). Nevertheless, when the human gcg promoter–LUC reporter was transfected into the mouse gcg-expressing cell lines, cAMP elevation significantly stimulated LUC reporter expression (personal communication). This, along with the observations that cAMP elevation may utilize other cis-elements to activate gcg promoter transcription, indicates that the typical CRE motif in gcg promoter of the rodent species may not be physiologically important.

Table 1 Phenotypes of knock-out mice in which a candidate pancreatic α-cell homeodomain protein transcription factor is genetically deleted

<table>
<thead>
<tr>
<th>Gene knock-out</th>
<th>Alterations in phenotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cdx-2−/−</td>
<td>Embryonic lethal (e3.5–e5.5)</td>
<td>Chawengsaksophak et al. (1997)</td>
</tr>
<tr>
<td>Cdx-2+/−</td>
<td>Multiple abnormalities in most of the mice</td>
<td>Chawengsaksophak et al. (1997)</td>
</tr>
<tr>
<td>Isl-1</td>
<td>Complete absence of pancreatic endocrine cells</td>
<td>Pfaff (1996)</td>
</tr>
<tr>
<td>Pax-6</td>
<td>Die shortly after the birth, fail to form pancreatic islet</td>
<td>St-Onge et al. (1997)</td>
</tr>
<tr>
<td>Brn-4−/−</td>
<td>No detectable defect in pancreatic islet cells</td>
<td>Heller et al. (2004)</td>
</tr>
<tr>
<td>Pbx-1+/−</td>
<td>Die at e15–e16, hypoplasia and aplasia for many organs, including pancreas</td>
<td>Kim et al. (2002)</td>
</tr>
<tr>
<td>Nkx2.2−/−</td>
<td>Pancreatic islet malformation and hyperinsulinemia</td>
<td>Kim et al. (2002)</td>
</tr>
<tr>
<td>Nkx6.2−/−</td>
<td>Arrested differentiation of β cells, fewer glucagon-producing cells</td>
<td>Sussel et al. (1998)</td>
</tr>
<tr>
<td>Pax-2 (1 Neu mutant)</td>
<td>Increased pancreatic islet volume</td>
<td>Cai et al. (2001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Zaike et al. (2004)</td>
</tr>
</tbody>
</table>
Epac signaling and the gcg gene expression

It has been noticed that the stimulatory effect of cAMP elevation on both gcg promoter and endogenous gcg mRNA expression in the pancreatic α-cell line InR1-G9 was much lower compared with that in the GLUTag and STC-1 cell lines (Drucker et al. 1991, 1994). Later, Chen et al. (2005) reported that InR1-G9 cell line was deficient in protein kinase A (PKA) activity.

Extensive examinations in many different cell lineages have shown that PKA is not the sole mediator of the second messenger cAMP in exerting its versatile biological functions (Richards 2001). In 1998, two groups independently revealed the existence of novel cAMP mediators, namely Epac-1 and Epac-2 (also known as cAMP-GEFs; Kawasaki et al. 1998, de Rooij et al. 1998). Epac molecules are able to mediate the effect of cAMP via activating the Rap-1–Raf–MEK–ERK signaling pathway (Richards 2001, Holz 2004).

The identification of additional cis-elements in gcg promoter that mediate the stimulatory effect of cAMP partially explained why deleting or mutating the CRE motif in the gcg promoter only partially attenuated the effect of cAMP elevation (Furstenau et al. 1999, Gevrey et al. 2004). However, it cannot explain moderate but significant activation of gcg promoter and endogenous gcg mRNA expression by cAMP elevation in the PKA-deficient InR1-G9 cell line. Lotfi et al. (2006) have recently reported that Epac-2 was also expressed in the intestinal endocrine L cells. They found that the stimulatory effect of cAMP in the intestinal GLUTag and STC-1 cell lines cannot be blocked by PKA inhibition, and Epac pathway-specific cAMP analog stimulated both gcg promoter and endogenous gcg mRNA expressions in these two cell lines. More recently, Islam & Jin have also detected Epac-2 expression in pancreatic α-cell lines and primary pancreatic islet cells. Using a dominant negative Epac-2 expression plasmid and the Epac pathway-specific cAMP analog, Islam & Jin (personal communication) found that Epac signaling was also involved in glucagon production. Ma et al. (2005) have reported that the Epac signaling pathway is involved in glucagon secretion.

It should be pointed out that Epac signaling could be involved in the regulation of the expression and function of transcriptional regulators of the gcg gene, such as the HD protein Cdx-2, whose activation appeared to be dependent on the MEK–ERK signaling cascade (Chen et al. 2005).

Wnt signaling pathway and intestinal gcg gene expression

Wnt signaling pathway

The Wnt signaling pathway was initially identified in colon cancer research and embryological studies in Drosophila, Xenopus, and other species (Moon et al. 1997, He et al. 1998, Peifer & Polakis 2000). Wnt ligands exert their regulatory effect mainly via binding to their receptor Frizzleds and the co-receptor LRP5/6. Many Wnt genes and their receptors are expressed in the pancreatic islets (Heller et al. 2003). The key effector of the canonical Wnt signaling pathway (defined as Wnt pathway hereafter) is the bipartite transcription factor cat/TCF, formed by β-catenin (β-cat) and one of the four TCF factors (TCF-1, LEF-1, TCF-3, and TCF7L2, previous known as TCF-4). Among them, TCF7L2 is the major partner of β-cat in the intestinal epithelia (Morin et al. 1997, Yi et al. 2005). Under non-stimulating conditions, the concentration of free β-cat is tightly controlled by the proteasome-mediated degradation process, with the participation of the tumor suppressor adenomatous polyposis coli, axin, glycogen synthase kinase-3 (GSK-3), and casein kinase-1α (Doble & Woodgett 2003, Zeng et al. 2005). In response to Wnt ligand stimulation, free β-cat accumulates, leading to the formation of the cat/TCF complex and the activation of cat/TCF downstream target genes. In addition, lithium and other inhibitors of GSK-3 may also stimulate the expression of downstream target genes of the Wnt signaling pathway, mainly through free β-cat accumulation (Stambolic et al. 1996).

Wnt signaling pathway and intestinal gcg gene expression

The expression of gcg mRNA in the intestinal STC-1 and GLUTag cell lines and in the primary FRIC cultures could be activated by lithium (Ni et al. 2003). The activation evidently involves the effector of the Wnt signaling pathway because a constitutively active β-cat molecule (S33Y mutant) stimulated gcg promoter expression, and dominant negative TCF7L2 blocked the stimulatory effect of lithium on gcg promoter and gcg mRNA expression and GLP-1 production in the GLUTag cell line (Yi et al. 2005). In addition, the expression of dominant negative TCF-4 also substantially inhibited basal gcg mRNA expression in the GLUTag cell line (Yi et al. 2005). They then determined the role of a TCF factor-binding motif within the G2 enhancer element of the gcg promoter. Because this region was previously demonstrated by Furstenau et al. as a cis-element that mediates the effect of cAMP and calcium on gcg promoter expression this raised the interesting question as to whether Wnt signaling pathway crosstalks with the cAMP-PKA signaling in the intestinal endocrine L cells (Yi et al. 2005).

Opposite effect of insulin on gcg expression in pancreatic α-cells versus intestinal L cells

Insulin has been shown to repress pancreatic gcg mRNA expression by Philippe (1989, 1991). This repression is physiologically important because gcg expression in pancreatic islets leads to the production of glucagon, the major counter-regulatory hormone of insulin. Philippe (1991) showed that the repression is mediated by a cis-element within the G3 element of the rat gcg promoter. Schinner et al. (2005) found that PKB was sufficient to mimic the inhibitory effect.
of insulin in the pancreatic α-cells on gcg expression. More recently, McKinnon et al. (2006) found that insulin treatment in the αTC1-9 cell line caused endogenous FOXO1 to translocate from the nucleus to the cytoplasm. Furthermore, in the absence of insulin, FOXO1 silencing by RNA interference reduced gcg mRNA expression by >40% (McKinnon et al. 2006). The neuropeptide hormone orexin A, however, was shown to inhibit gcg expression in the InR1-G9 cells, possibly through 1) decreasing cAMP and calcium levels and 2) increasing PKB, PDK-1, and FOXO1 phosphorylations (Gonzé et al. 2007).

More recently, Yi et al. (2008) reported that in the intestinal endocrine L cells, insulin at pathological dosages stimulated gcg mRNA expression and GLP-1 production. Furthermore, in the hyperglycemic and insulin resistance MKR mice, gcg mRNA expression and GLP-1 content in the distal ileum were shown to be significantly higher than that of sex- and age-matched controls (Yi et al. 2008). More interestingly, insulin was demonstrated to utilize the same cis- and trans-elements in stimulating intestinal gcg expression, supporting the existence of crosstalk between insulin and Wnt signaling pathways (Yi et al. 2008).

Exactly how insulin exerts opposite effects on the expression of the same gcg gene in the intestinal endocrine L cells versus pancreatic α-cells is not clear. However, it appears that different signaling cascades are involved in these two cell lineages. As demonstrated by Schinner et al. (2005) in the pancreatic α-cells, the repressive effect of insulin on gcg expression relies on PKB activity. In the intestinal L cells, it is unlikely that PKB activity is involved in insulin-stimulated gcg promoter transcription (Yi et al. 2008).

TCF7L2 as a potential T2D susceptible gene

A study by Grant et al. (2006) indicated that in three different ethnic groups, individuals carrying certain single nucleotide polymorphisms (SNP) in the TCF7L2 gene are more susceptible to the development of T2D. This discovery has drawn great attention globally. Detailed information on this line of research has been summarized in a few review articles in 2007 (Elbein 2007, Florez 2007, Frayling 2007, Grarup & Andersen 2007, Owen & McCarthy 2007). To date, studies have indicated that T2D susceptible TCF7L2 SNP carriers show reduced postprandial insulin secretion (Schafer et al. 2007). In addition, detection of TCF7L2 expression in human pancreatic β-cells has been reported recently and this transcription factor plays a role in insulin production and secretion, as well as pancreatic β-cell growth (Loos et al. 2007, Lyssenko et al. 2007, Shu et al. 2007, Liu & Habener 2008). Interestingly, to date, all of the identified T2D susceptible TCF7L2 SNPs are located within the intron regions of the TCF7L2 gene; therefore, it is likely that the polymorphisms affect TCF7L2 gene expression, but not its function. Since TCF7L2”−/−” mice die shortly after their birth, due to extensive defects of intestinal stem cell development (Korinek et al. 1998); to further investigate the role of TCF7L2 in the

Summary and perspective

We have learned that nearly a dozen homeobox genes are potentially involved in the expression of the gcg gene, although ‘knock-out’ approaches have only directly confirmed the roles for Pax-6 and Pbx-1. It is likely that this is due to the existence of redundant mechanisms for compensation. To fully understand the spatiotemporal aspects of pancreatic α-cell specification, more transgenic mouse work using the approaches including ‘conditional knock-out’ (Cdx-2, for example) and ‘double knock-out’ (Cdx-2 and Brn-4, for example) needs to be conducted.

We have learned that multiple cis-elements are implicated in mediating the stimulatory effect of cAMP elevation on gcg expression in pancreatic and intestinal gcg-producing cells, possibly involving both the PKA and Epac signaling pathways. It should be pointed out that crosstalk between Wnt signaling and signaling pathways that are activated by G-protein-coupled receptors have drawn our attention recently (Force et al. 2007, Liu & Habener 2008). Whether certain hormones and neurotransmitters exert their effects on gcg expression via crosstalk with the Wnt signaling pathway remains to be investigated. Figure 2 summarizes the interpretations of how the signaling molecules and HD proteins are involved in regulating gcg transcription, including the newly recognized Wnt signaling.

GLP-1 is a powerful therapeutic agent for T2D and potentially for several other disorders. In T2D patients, GLP-1 secretion could be reduced (Vilsboll et al. 2001). Expanding our understanding on the mechanisms underlying endogenous gcg expression, therefore, will be helpful for the development of novel approaches in treating T2D and other disorders. These studies should also lead to the discovery of additional diagnostic and prognostic factors. It also should be further emphasized that during embryonic stages, pancreatic gcg expression may lead to the production of GLP-1, which could play a role in pancreatic β-cell genesis and maturation (Wilson et al. 2002). Indeed, Thyssen et al. (2006) have shown that in streptozotocin (STZ)-treated rats, β-cell regeneration is associated with increased pancreatic levels of GLP-1. Therefore, the exploration of spatiotemporal aspects of pancreatic α-cell generation and maturation should not be left behind too much. Interestingly, both GLP-1R−/− and PC1/3−/− mice develop pancreatic β-cells (Scrocchi et al. 1996, Zhu et al. 2002), suggesting that although pancreatic GLP-1 may be important, GLP-1 signaling is not absolutely required for pancreatic islet development.

Since glucagon produced in pancreatic α-cells and GLP-1 produced in intestinal L cells exert opposite effects on blood glucose homeostasis, one may expect the existence of cell-type-specific mechanisms underlying gcg transcription. Comparing reporter gene expression of the transgenic mice generated by
Efrat et al. (1988) with those generated by Lee et al. (1992) led to the suggestion that the DNA sequence from \(-1.3\) to \(-2.3\) kb of rat gcg promoter is required for its expression in intestinal L cells (Jin & Drucker 1995). In vitro transfection studies, however, could not locate a cis-element within this region that mediates gcg promote expression via an intestinal L-cell-specific trans-element (Jin & Drucker 1995). Nevertheless, recent studies have revealed cell-type-specific activation of gcg expression by the effectors of the Wnt signaling pathway and the crosstalk between Wnt and insulin signaling pathways (Yi et al. 2005). Whether Wnt signaling and crosstalk between Wnt and other signaling pathways are involved in the genesis of intestinal L cells, and the process involves the DNA sequence between \(-1.3\) and \(-2.3\) kb remain to be investigated.

**Declaration of Interest**

The author declares that there is no conflict of interest that would prejudice the impartiality of this scientific work.

**Funding**

The Canadian Institutes of Health Research (CIHR, 68991 and 62746), Canadian Diabetes Association (CDA), and Bantering and Best Diabetes Centre (BBDC) provided operating funding support, as well as fellowship and studentship support for the author’s researcher trainees.

**Acknowledgements**

The author would like to thank Dr David Irwin for his valuable suggestions.

**References**


Flock G & Drucker DJ 2002 Paa-2 activates the proglucagon gene promoter but is not essential for proglucagon gene expression or development of proglucagon-producing cell lineages in the murine pancreas or intestine. *Molecular Endocrinology* **16** 2349–2359.


Gajic D & Drucker DJ 1993 Multiple cis-acting domains mediate basal and
Furstenau U, Schwaninger M, Blume R, Jendrusch EM & Knepel W 1999
German MS, Wang J, Chadwick RB & Rutter WJ 1992 Synergistic activation
Grarup N & Andersen G 2007 Gene-environment interactions in the
Goncz E, Strowski MZ, Grotzinger C, Nowak KW, Kaczmarek P, Sassek M,
Grant SF, Thorleifsson G, Reynisdottir I, Benediktsson R, Manolescu A,
Habener JF, Kemp DM & Thomas MK 2005 Minireview: transcriptional
Heinrich G, Gros P, Lund PK, Bentley RC & Habener JF 1984 Pre-
Holz GG 2004 New insights concerning the glucose-dependent insulin
Henseleit KD, Nelson SB, Kuhlbrodt K, Hennings JC, Ericson J & Sander M
Jelinek LJ, Lok S, Rosenberg GB, Smith RA, Grant FJ, Biggs S, Bensch PA,
Irwin DM 2001 Molecular evolution of proglucagon. Regulatory Peptides 98
Jelinski Lj, Lok S, Rosenberg GB, Smith RA, Grant FJ, Biggs S, Bensch PA,
Journal of Biological Chemistry 274 5851–5860.
Genes and Development 6 2165–2176.
proinsulin levels and β-cell function in a British Europid population. Journal of Biological Chemistry 280 25989–25999.
serum glucagon levels and expression targeted to the early developing mouse pancreas induces ectopic glucagon cell identity.
homeodomain protein binding to the glucagon gene resulting in repression of a cell-type-dependent manner. Journal of Biological Chemistry 275 27589–27599.
expression, which common variants in the TCF7L2 gene increase risk of type 2 diabetes. Nature Genetics 38 320–323.
Heinrich G, Gros P, Lund PK, Bentley RC & Habener JF 1984 Pre-
Hosoda T, Branch DR & Jin T 2006 Pbx1 is a co-factor for Cdx-2 in regulating proglucagon gene expression in pancreatic A cells. Molecular and Cellular Endocrinology 249 140–149.
Jin T & Drucker DJ 1995 The proglucagon gene upstream enhancer contains positive and negative domains important for tissue-specific proglucagon gene transcription. Molecular Endocrinology 9 1306–1320.
Liu T, Branch DR & Jin T 2006 Pbx1 is a co-factor for Cdx-2 in regulating proglucagon gene expression in pancreatic A cells. Molecular and Cellular Endocrinology 249 140–149.


Wideman RD, Covey SD, Webb GC, Drucker DJ & Kieffer TJ 2007 A switch from prohormone convertase (PC)-2 to PC1/3 expression in transplanted alpha-cells is accompanied by differential processing of proglucagon and improved glucose homeostasis in mice. Diabetes 56 2744–2752.


Received in final form 20 March 2008
Accepted 3 April 2008
Made available online as an Accepted Preprint 3 April 2008