REVIEW

Mechanisms underlying proglucagon gene expression

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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, exenatide-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, Meeran *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 GCG gene, the CRE sequence is CAACGTCA (Nian *et al.* 1999).

and GLP-1 have been extensively reviewed elsewhere (Brubaker & Drucker 2004, Holz, 2004, Kieffer, 2004, Bonner-Weir & Weir 2005, Drucker 2006, Holst 2006, Doyle & Egan 2007).

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 (Karlsson *et al.* 1990, Vallejo *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 (Pfaff *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 (Chawengsaksohak *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 (Chawengsaksohak *et al.* 1997). Interestingly, pancreatic

Figure 2 Summary of our current understanding on the mechanisms underlying gcg expression. A neurotransmitter or growth factor, such as gastrin-releasing peptide (GRP; [Lu et al. 1996](#)), may exert its stimulatory effect via a G-protein-coupled receptor (GPCR). Insulin and IGF-1, on the other hand, activate a correspondent receptor tyrosine kinase (RTK). Wnt ligands usually bind to their receptors, frizzleds, and co-receptor LRP5/6. The elevation of cAMP in response to GPCR activation may up-regulate gcg transcription via 1) PKA-CREB, with the CRE and CRE-like elements as the correspondent cis-elements ([Knepel et al. 1990](#), [Jin & Drucker 1996](#), [Gevrey et al. 2004](#)), and 2) Epac-Rap-Raf-ERK signaling pathway, via unknown cis-elements, shown as dotted arrows. ERK is also involved in cAMP-stimulated expression of Cdx-2, a known transactivator of gcg, via the G1 element ([Chen et al. 2005](#)). Furthermore, ERK could be involved in β-cat phosphorylation and nuclear translocation ([Ding et al. 2005](#)). Insulin and IGF-1 are able to stimulate β-cat nuclear translocation and binding of cat/TCF-4 to the G2 element, a process involving PI3K and a yet to be determined signaling pathway ([Yi et al. 2008](#)). Finally, hormones and growth factors that use GPCR as their receptors may crosstalk with the Wnt signaling pathway via two potential mechanisms. First, a GPCR may stabilize β-cat via PI3K activation, presented recently by [Liu & Habener \(2008\)](#) in studying the function of GLP-1. Second, since frizzleds share structure homology with GPCRs, hormones and growth factors that use GPCR as their receptors may trigger the crosstalk with the Wnt signaling pathway at the receptor level ([Malbon 2004](#)).

development in the *Cdx-2*^{+/−} mice appeared normal. In the pancreatic InR 1-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 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 et al. 1999](#)).

Brn-4

Brn-4 is highly expressed in neural stem cells and regulates stem cell-specific gene expression ([Heller et al. 2004](#)). Brn-4 is also expressed in pancreatic α-cells but not β-cells, and its capability in activating gcg expression was first demonstrated by [Hussain 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 α-cell-specific transcription factor. [Wang 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), Henseleit *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

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

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

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. Furstenu *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.

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-1 α (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 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 α TC1-9 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 (Goncz *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

genesis and maturation of pancreatic islet cells and intestinal endocrine L cells would require cell-type-specific 'knock-out' approaches.

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.

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References

Abraham EJ, Leech CA, Lin JC, Zulewski H & Habener JF 2002 Insulinotropic hormone glucagon-like peptide-1 differentiation of human pancreatic islet-derived progenitor cells into insulin-producing cells. *Endocrinology* **143** 3152–3161.

Bell GI, Sanchez-Pescador R, Laybourn PJ & Najarian RC 1983a Exon duplication and divergence in the human preproglucagon gene. *Nature* **304** 368–371.

Verkoren-2ptBell GI, Santerre RF & Mullenbach GT 1983b Hamster preproglucagon contains the sequence of glucagon and two related peptides. *Nature* **302** 716–718.

Berthelsen J, Kilstrup-Nielsen C, Blasi F, Mavilio F & Zappavigna V 1999 The subcellular localization of PBX1 and EXD proteins depends on nuclear import and export signals and is modulated by association with PREP1 and HTH. *Genes and Development* **13** 946–953.

Bonner-Weir S & Weir GC 2005 New sources of pancreatic beta-cells. *Nature Biotechnology* **23** 857–861.

Brubaker PL 1988 Control of glucagon-like immunoreactive peptide secretion from fetal rat intestinal cultures. *Endocrinology* **123** 220–226.

Brubaker PL & Drucker DJ 2004 Minireview: glucagon-like peptides regulate cell proliferation and apoptosis in the pancreas, gut, and central nervous system. *Endocrinology* **145** 2653–2659.

Brubaker PL, Schloos J & Drucker DJ 1998 Regulation of glucagon-like peptide-1 synthesis and secretion in the GLUTag enteroendocrine cell line. *Endocrinology* **139** 4108–4114.

Burcelin R, Katz EB & Charron MJ 1996 Molecular and cellular aspects of the glucagon receptor: role in diabetes and metabolism. *Diabetes and Metabolism* **22** 373–396.

Cai J, Qi Y, Wu R, Modderman G, Fu H, Liu R & Qiu M 2001 Mice lacking the Nkx6.2 (Gtx) homeodomain transcription factor develop and reproduce normally. *Molecular and Cellular Biology* **21** 4399–4403.

Campos RV, Lee YC & Drucker DJ 1994 Divergent tissue-specific and developmental expression of receptors for glucagon and glucagon-like peptide-1 in the mouse. *Endocrinology* **134** 2156–2164.

Chawengsaksohak K, James R, Hammond VE, Kontgen F & Beck F 1997 Homeosis and intestinal tumours in Cdx2 mutant mice. *Nature* **386** 84–87.

Chen L, Wang P, Andrade CF, Zhao IY, Dube PE, Brubaker PL, Liu M & Jin T 2005 PKA independent and cell type specific activation of the expression of caudal homeobox gene Cdx-2 by cyclic AMP. *FEBS Journal* **272** 2746–2759.

Christophe J 1996 Glucagon and its receptor in various tissues. *Annals of the New York Academy of Sciences* **805** 31–42 discussion 42–33.

Diedrich T & Knebel W 1995 Interaction of the transcription factor CREB with pancreatic islet cell-specific enhancer elements. *Biological Chemistry Hoppe-Seyler* **376** 39–44.

Ding Q, Xia W, Liu JC, Yang JY, Lee DF, Xia J, Bartholomeusz G, Li Y, Pan Y, Li Z *et al.* 2005 Erk associates with and primes GSK-3beta for its inactivation resulting in upregulation of beta-catenin. *Molecular Cell* **19** 159–170.

Doble BW & Woodgett JR 2003 GSK-3: tricks of the trade for a multi-tasking kinase. *Journal of Cell Science* **116** 1175–1186.

Dong J, Asa SL & Drucker DJ 1991 Islet cell and extrapancreatic expression of the LIM domain homeobox gene isl-1. *Molecular Endocrinology* **5** 1633–1641.

Doyle ME & Egan JM 2007 Mechanisms of action of glucagon-like peptide 1 in the pancreas. *Pharmacology and Therapeutics* **113** 546–593.

Drucker DJ 2001 Minireview: the glucagon-like peptides. *Endocrinology* **142** 521–527.

Drucker DJ 2002 Biological actions and therapeutic potential of the glucagon-like peptides. *Gastroenterology* **122** 531–544.

Drucker DJ 2006 The biology of incretin hormones. *Cell Metabolism* **3** 153–165.

Drucker DJ & Brubaker PL 1989 Proglucagon gene expression is regulated by a cyclic AMP-dependent pathway in rat intestine. *PNAS* **86** 3953–3957.

Drucker DJ, Campos R, Reynolds R, Stobie K & Brubaker PL 1991 The rat glucagon gene is regulated by a protein kinase A-dependent pathway in pancreatic islet cells. *Endocrinology* **128** 394–400.

Drucker DJ, Jin T, Asa SL, Young TA & Brubaker PL 1994 Activation of proglucagon gene transcription by protein kinase-A in a novel mouse enteroendocrine cell line. *Molecular Endocrinology* **8** 1646–1655.

Drucker DJ, Erlich P, Asa SL & Brubaker PL 1996 Induction of intestinal epithelial proliferation by glucagon-like peptide 2. *PNAS* **93** 7911–7916.

Efrat S, Teitelman G, Anwar M, Ruggiero D & Hanahan D 1988 Glucagon gene regulatory region directs oncoprotein expression to neurons and pancreatic alpha cells. *Neuron* **1** 605–613.

Elbein SC 2007 Evaluation of polymorphisms known to contribute to risk for diabetes in African and African-American populations. *Current Opinion in Clinical Nutrition and Metabolic Care* **10** 415–419.

Ericson J, Thor S, Edlund T, Jessell TM & Yamada T 1992 Early stages of motor neuron differentiation revealed by expression of homeobox gene Islet-1. *Science* **256** 1555–1560.

Flock G & Drucker DJ 2002 Pax-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.

Florez JC 2007 The new type 2 diabetes gene TCF7L2. *Current Opinion in Clinical Nutrition and Metabolic Care* **10** 391–396.

Force T, Woulfe K, Koch WJ & Kerkela R 2007 Molecular scaffolds regulate bidirectional crosstalk between Wnt and classical seven-transmembrane-domain receptor signaling pathways. *Science's STKE* **pe41**.

Frayling TM 2007 A new era in finding type 2 diabetes genes—the unusual suspects. *Diabetic Medicine* **24** 696–701.

- Furstenau U, Schwaninger M, Blume R, Jendrusch EM & Knepel W 1999 Characterization of a novel calcium response element in the glucagon gene. *Journal of Biological Chemistry* **274** 5851–5860.
- Gajic D & Drucker DJ 1993 Multiple cis-acting domains mediate basal and adenosine 3',5'-monophosphate-dependent glucagon gene transcription in a mouse neuroendocrine cell line. *Endocrinology* **132** 1055–1062.
- German MS, Wang J, Chadwick RB & Rutter WJ 1992 Synergistic activation of the insulin gene by a LIM-homeo domain protein and a basic helix-loop-helix protein: building a functional insulin minienhancer complex. *Genes and Development* **6** 2165–2176.
- Gevery JC, Malapel M, Philippe J, Mithieux G, Chayvialle JA, Abello J & Cordier-Bussat M 2004 Protein hydrolysates stimulate proglucagon gene transcription in intestinal endocrine cells via two elements related to cyclic AMP response element. *Diabetologia* **47** 926–936.
- Goncz E, Strowski MZ, Grotzinger C, Novak KW, Kaczmarek P, Sassek M, Mergler S, El-Zayat BF, Theodoropoulou M, Stalla GK *et al.* 2008 Orexin-A inhibits glucagon secretion and gene expression through a Foxo1-dependent pathway. *Endocrinology* **149** 1618–1626.
- Grant SF, Thorleifsson G, Reynisdottir I, Benediktsson R, Manolescu A, Sainz J, Helgason A, Stefansson H, Emilsson V, Helgadóttir A *et al.* 2006 Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. *Nature Genetics* **38** 320–323.
- Grup N & Andersen G 2007 Gene-environment interactions in the pathogenesis of type 2 diabetes and metabolism. *Current Opinion in Clinical Nutrition and Metabolic Care* **10** 420–426.
- Habener JF, Kemp DM & Thomas MK 2005 Minireview: transcriptional regulation in pancreatic development. *Endocrinology* **146** 1025–1034.
- He TC, Sparks AB, Rago C, Hermeking H, Zawel L, da Costa LT, Morin PJ, Vogelstein B & Kinzler KW 1998 Identification of c-MYC as a target of the APC pathway. *Science* **281** 1509–1512.
- Heinrich G, Gros P, Lund PK, Bentley RC & Habener JF 1984 Pre-proglucagon messenger ribonucleic acid: nucleotide and encoded amino acid sequences of the rat pancreatic complementary deoxyribonucleic acid. *Endocrinology* **115** 2176–2181.
- Heller RS, Klein T, Ling Z, Heimberg H, Katoh M, Madsen OD & Serup P 2003 Expression of Wnt, Frizzled, sFRP, and DKK genes in adult human pancreas. *Gene Expression* **11** 141–147.
- Heller RS, Stoffers DA, Liu A, Schedl A, Crenshaw EB, III, Madsen OD & Serup P 2004 The role of Brn4/Pou3f4 and Pax6 in forming the pancreatic glucagon cell identity. *Developmental Biology* **268** 123–134.
- Henseleit KD, Nelson SB, Kuhlbrodt K, Hennings JC, Ericson J & Sander M 2005 NKX6 transcription factor activity is required for α - and β -cell development in the pancreas. *Development* **132** 3139–3149.
- Herzig S, Fuzesi L & Knepel W 2000 Heterodimeric Pbx1 homeodomain protein binding to the glucagon gene restricting transcription in a cell type-dependent manner. *Journal of Biological Chemistry* **275** 27989–27999.
- Holst JJ 2006 Glucagon-like peptide-1: from extract to agent. The Claude Bernard Lecture, 2005. *Diabetologia* **49** 253–260.
- Holz GG 2004 New insights concerning the glucose-dependent insulin secretagogue action of glucagon-like peptide-1 in pancreatic β -cells. *Hormone and Metabolic Research* **36** 787–794.
- Hussain MA & Habener JF 1999 Glucagon gene transcription activation mediated by synergistic interactions of pax-6 and cdx-2 with the p300 co-activator. *Journal of Biological Chemistry* **274** 28950–28957.
- Hussain MA, Lee J, Miller CP & Habener JF 1997 POU domain transcription factor brain 4 confers pancreatic alpha-cell-specific expression of the proglucagon gene through interaction with a novel proximal promoter G1 element. *Molecular and Cellular Biology* **17** 7186–7194.
- Hussain MA, Miller CP & Habener JF 2002 Brn-4 transcription factor expression targeted to the early developing mouse pancreas induces ectopic glucagon gene expression in insulin-producing beta cells. *Journal of Biological Chemistry* **277** 16028–16032.
- Irwin DM 2001 Molecular evolution of proglucagon. *Regulatory Peptides* **98** 1–12.
- Jelinek LJ, Lok S, Rosenberger GB, Smith RA, Grant FJ, Biggs S, Bensch PA, Kuijper JL, Sheppard PO, Sprecher CA *et al.* 1993 Expression cloning and signaling properties of the rat glucagon receptor. *Science* **259** 1614–1616.
- Jeppesen PB 2003 Clinical significance of GLP-2 in short-bowel syndrome. *Journal of Nutrition* **133** 3721–3724.
- 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.
- Jin T & Drucker DJ 1996 Activation of proglucagon gene transcription through a novel promoter element by the caudal-related homeodomain protein cdx-2/3. *Molecular and Cellular Biology* **16** 19–28.
- Jin T, Trinh DK, Wang F & Drucker DJ 1997 The caudal homeobox protein cdx-2/3 activates endogenous proglucagon gene expression in InR1-G9 islet cells. *Molecular Endocrinology* **11** 203–209.
- Karlsson O, Thor S, Norberg T, Ohlsson H & Edlund T 1990 Insulin gene enhancer binding protein Isl-1 is a member of a novel class of proteins containing both a homeo- and a Cys-His domain. *Nature* **344** 879–882.
- Kawasaki H, Springett GM, Mochizuki N, Toki S, Nakaya M, Matsuda M, Housman DE & Graybiel AM 1998 A family of cAMP-binding proteins that directly activate Rap1. *Science* **282** 2275–2279.
- Kieffer TJ 2004 Gastro-intestinal hormones GIP and GLP-1. *Annales d'Endocrinologie* **65** 13–21.
- Kim SK, Selleri L, Lee JS, Zhang AY, Gu X, Jacobs Y & Cleary ML 2002 Pbx1 inactivation disrupts pancreas development and in *Ipfl*-deficient mice promotes diabetes mellitus. *Nature Genetics* **30** 430–435.
- Knepel W, Chafitz J & Habener JF 1990 Transcriptional activation of the rat glucagon gene by the cyclic AMP-responsive element in pancreatic islet cells. *Molecular and Cellular Biology* **10** 6799–6804.
- Korinek V, Barker N, Moerer P, van Donselaar E, Huls G, Peters PJ & Clevers H 1998 Depletion of epithelial stem-cell compartments in the small intestine of mice lacking Tcf-4. *Nature Genetics* **19** 379–383.
- Laser B, Meda P, Constant I & Philippe J 1996 The caudal-related homeodomain protein Cdx-2/3 regulates glucagon gene expression in islet cells. *Journal of Biological Chemistry* **271** 28984–28994.
- Lee YC, Asa SL & Drucker DJ 1992 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. *Journal of Biological Chemistry* **267** 10705–10708.
- List JF & Habener JF 2004 Glucagon-like peptide 1 agonists and the development and growth of pancreatic beta-cells. *American Journal of Physiology. Endocrinology and Metabolism* **286** E875–E881.
- Liu Z & Habener JF 2008. Glucagon-like peptide-1 activation of TCF7L2-dependent Wnt signaling enhances pancreatic β -cell proliferation. *Journal of Biological Chemistry* **283** 8723–8735.
- 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.
- Loos RJ, Franks PW, Francis RW, Barroso I, Gribble FM, Savage DB, Ong KK, O'Rahilly S & Wareham NJ 2007 TCF7L2 polymorphisms modulate proinsulin levels and beta-cell function in a British European population. *Diabetes* **56** 1943–1947.
- Lopez LC, Frazier ML, Su CJ, Kumar A & Saunders GF 1983 Mammalian pancreatic preproglucagon contains three glucagon-related peptides. *PNAS* **80** 5485–5489.
- Lotfi S, Li Z, Sun J, Zuo Y, Lam PP, Kang Y, Rahimi M, Islam D, Wang P, Gaisano HY & Jin T 2006 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* **147** 3727–3736.
- Lu F, Jin T & Drucker DJ 1996 Proglucagon gene expression is induced by gastrin-releasing peptide in a mouse enteroendocrine cell line. *Endocrinology* **137** 3710–3716.
- Lund PK, Goodman RH, Dee PC & Habener JF 1982 Pancreatic preproglucagon cDNA contains two glucagon-related coding sequences arranged in tandem. *PNAS* **79** 345–349.
- Lysenko V, Lupi R, Marchetti P, Del Guerra S, Orho-Melandier M, Almgren P, Sjogren M, Ling C, Eriksson KF, Lethagen AL *et al.* 2007 Mechanisms by which common variants in the TCF7L2 gene increase risk of type 2 diabetes. *Journal of Clinical Investigation* **117** 2155–2163.

- Ma X, Zhang Y, Gromada J, Sewing S, Berggren PO, Buschard K, Salehi A, Vikman J, Rorsman P & Eliasson L 2005 Glucagon stimulates exocytosis in mouse and rat pancreatic α -cells by binding to glucagon receptors. *Molecular Endocrinology* **19** 198–212.
- Malbon CC 2004 Frizzleds: new members of the superfamily of G-protein-coupled receptors. *Frontiers in Bioscience* **9** 1048–1058.
- McKinnon CM, Ravier MA & Rutter GA 2006 FoxO1 is required for the regulation of preproglucagon gene expression by insulin in pancreatic α TC1-9 cells. *Journal of Biological Chemistry* **281** 39358–39369.
- Meeran K, O'Shea D, Edwards CM, Turton MD, Heath MM, Gunn I, Abusnana S, Rossi M, Small CJ, Goldstone AP *et al.* 1999 Repeated intracerebroventricular administration of glucagon-like peptide-1-(7–36) amide or exendin-(9–39) alters body weight in the rat. *Endocrinology* **140** 244–250.
- Moon RT, Brown JD & Torres M 1997 WNTs modulate cell fate and behavior during vertebrate development. *Trends in Genetics* **13** 157–162.
- Morin PJ, Sparks AB, Korinek V, Barker N, Clevers H, Vogelstein B & Kinzler KW 1997 Activation of β -catenin-Tcf signaling in colon cancer by mutations in β -catenin or APC. *Science* **275** 1787–1790.
- Murphy KG, Dhillon WS & Bloom SR 2006 Gut peptides in the regulation of food intake and energy homeostasis. *Endocrine Reviews* **27** 719–727.
- Nelson SB, Schaffer AE & Sander M 2007 The transcription factors Nkx6.1 and Nkx6.2 possess equivalent activities in promoting beta-cell fate specification in Pdx1+ pancreatic progenitor cells. *Development* **134** 2491–2500.
- Ni Z, Anini Y, Fang X, Mills G, Brubaker PL & Jin T 2003 Transcriptional activation of the proglucagon gene by lithium and beta-catenin in intestinal endocrine L cells. *Journal of Biological Chemistry* **278** 1380–1387.
- Nian M, Drucker DJ & Irwin D 1999 Divergent regulation of human and rat proglucagon gene promoters *in vivo*. *American Journal of Physiology* **277** G829–G837.
- Nian M, Gu J, Irwin DM & Drucker DJ 2002 Human glucagon gene promoter sequences regulating tissue-specific versus nutrient-regulated gene expression. *American Journal of Physiology Regulatory, Integrative and Comparative Physiology* **282** R173–R183.
- Owen KR & McCarthy MI 2007 Genetics of type 2 diabetes. *Current Opinion in Genetics and Development* **17** 239–244.
- Pedersen JK, Nelson SB, Jorgensen MC, Henseleit KD, Fujitani Y, Wright CV, Sander M & Serup P 2005 Endodermal expression of Nkx6 genes depends differentially on Pdx1. *Developmental Biology* **288** 487–501.
- Peifer M & Polakis P 2000 Wnt signaling in oncogenesis and embryogenesis – a look outside the nucleus. *Science* **287** 1606–1609.
- Peng SY, Wang WP, Meng J, Li T, Zhang H, Li YM, Chen P, Ma KT & Zhou CY 2005 ISL1 physically interacts with BETA2 to promote insulin gene transcriptional synergy in non-beta cells. *Biochimica et Biophysica Acta* **1731** 154–159.
- Pfaff SL, Mendelsohn M, Stewart CL, Edlund T & Jessell TM 1996 Requirement for LIM homeobox gene Isl1 in motor neuron generation reveals a motor neuron-dependent step in interneuron differentiation. *Cell* **84** 309–320.
- Philippe J 1989 Glucagon gene transcription is negatively regulated by insulin in a hamster islet cell line. *Journal of Clinical Investigation* **84** 672–677.
- Philippe J 1991 Insulin regulation of the glucagon gene is mediated by an insulin-responsive DNA element. *PNAS* **88** 7224–7227.
- Philippe J, Drucker DJ, Chick WL & Habener JF 1987a Transcriptional regulation of genes encoding insulin, glucagon, and angiotensinogen by sodium butyrate in a rat islet cell line. *Molecular and Cellular Biology* **7** 560–563.
- Philippe J, Drucker DJ & Habener JF 1987b Glucagon gene transcription in an islet cell line is regulated via a protein kinase C-activated pathway. *Journal of Biological Chemistry* **262** 1823–1828.
- Richards JS 2001 New signaling pathways for hormones and cyclic adenosine 3',5'-monophosphate action in endocrine cells. *Molecular Endocrinology* **15** 209–218.
- Ritz-Laser B, Estreicher A, Klages N, Saule S & Philippe J 1999 Pax-6 and Cdx-2/3 interact to activate glucagon gene expression on the G1 control element. *Journal of Biological Chemistry* **274** 4124–4132.
- Ritz-Laser B, Estreicher A, Gauthier B & Philippe J 2000 The paired homeodomain transcription factor Pax-2 is expressed in the endocrine pancreas and transactivates the glucagon gene promoter. *Journal of Biological Chemistry* **275** 32708–32715.
- Ritz-Laser B, Estreicher A, Gauthier BR, Mamin A, Edlund H & Philippe J 2002 The pancreatic β -cell-specific transcription factor Pax-4 inhibits glucagon gene expression through Pax-6. *Diabetologia* **45** 97–107.
- Ritz-Laser B, Mamin A, Brun T, Avril I, Schwitzgebel VM & Philippe J 2005 The zinc finger-containing transcription factor Gata-4 is expressed in the developing endocrine pancreas and activates glucagon gene expression. *Molecular Endocrinology* **19** 759–770.
- de Rooij J, Zwartkruis FJ, Verheijen MH, Cool RH, Nijman SM, Wittinghofer A & Bos JL 1998 Epac is a Rap1 guanine-nucleotide-exchange factor directly activated by cyclic AMP. *Nature* **396** 474–477.
- Sander M, Neubuser A, Kalamaras J, Ee HC, Martin GR & German MS 1997 Genetic analysis reveals that PAX6 is required for normal transcription of pancreatic hormone genes and islet development. *Genes and Development* **11** 1662–1673.
- Sander M, Sussel L, Connors J, Scheel D, Kalamaras J, Dela Cruz F, Schwitzgebel V, Hayes-Jordan A & German M 2000 Homeobox gene Nkx6.1 lies downstream of Nkx2.2 in the major pathway of beta-cell formation in the pancreas. *Development* **127** 5533–5540.
- Schafer SA, Tschrirer O, Machicao F, Thamer C, Stefan N, Gallwitz B, Holst JJ, Dekker JM, T'Hart LM, Nijpels G *et al.* 2007 Impaired glucagon-like peptide-1-induced insulin secretion in carriers of transcription factor 7-like 2 (TCF7L2) gene polymorphisms. *Diabetologia* **50** 2443–2450.
- Schinner S, Barthel A, Dellas C, Grzeskowiak R, Sharma SK, Oetjen E, Blume R & Knebel W 2005 Protein kinase B activity is sufficient to mimic the effect of insulin on glucagon gene transcription. *Journal of Biological Chemistry* **280** 7369–7376.
- Schisler JC, Jensen PB, Taylor DG, Becker TC, Knop FK, Takekawa S, German M, Weir GC, Lu D, Mirmira RG & Newgard CB 2005 The Nkx6.1 homeodomain transcription factor suppresses glucagon expression and regulates glucose-stimulated insulin secretion in islet beta cells. *PNAS* **102** 7297–7302.
- Schwaninger M, Lux G, Blume R, Oetjen E, Hidaka H & Knebel W 1993 Membrane depolarization and calcium influx induce glucagon gene transcription in pancreatic islet cells through the cyclic AMP-responsive element. *Journal of Biological Chemistry* **268** 5168–5177.
- Scrocchi LA, Brown TJ, MacClusky N, Brubaker PL, Auerbach AB, Joyner AL & Drucker DJ 1996 Glucose intolerance but normal satiety in mice with a null mutation in the glucagon-like peptide 1 receptor gene. *Nature Medicine* **2** 1254–1258.
- Shu L, Sauter NS, Schulthess FT, Matveyenko AV, Oberholzer J & Maedler K 2008 TCF7L2 regulates β -cell survival and function in human pancreatic islets. *Diabetes* **57** 645–653.
- Sinclair EM & Drucker DJ 2005 Proglucagon-derived peptides: mechanisms of action and therapeutic potential. *Physiology* **20** 357–365.
- Stambolic V, Ruel L & Woodgett JR 1996 Lithium inhibits glycogen synthase kinase-3 activity and mimics wingless signalling in intact cells. *Current Biology* **6** 1664–1668.
- St-Onge L, Sosa-Pineda B, Chowdhury K, Mansouri A & Gruss P 1997 Pax6 is required for differentiation of glucagon-producing α -cells in mouse pancreas. *Nature* **387** 406–409.
- Stoykova A, Fritsch R, Walther C & Gruss P 1996 Forebrain patterning defects in small eye mutant mice. *Development* **122** 3453–3465.
- Sussel L, Kalamaras J, Hartigan-O'Connor DJ, Meneses JJ, Pedersen RA, Rubenstein JL & German MS 1998 Mice lacking the homeodomain transcription factor Nkx2.2 have diabetes due to arrested differentiation of pancreatic β -cells. *Development* **125** 2213–2221.
- Thor S, Ericson J, Brannstrom T & Edlund T 1991 The homeodomain LIM protein Isl-1 is expressed in subsets of neurons and endocrine cells in the adult rat. *Neuron* **7** 881–889.
- Thyssen S, Arany E & Hill DJ 2006 Ontogeny of regeneration of beta-cells in the neonatal rat after treatment with streptozotocin. *Endocrinology* **147** 2346–2356.
- Trinh DK, Zhang K, Hossain M, Brubaker PL & Drucker DJ 2003 Pax-6 activates endogenous proglucagon gene expression in the rodent gastrointestinal epithelium. *Diabetes* **52** 425–433.
- Turton MD, O'Shea D, Gunn I, Beak SA, Edwards CM, Meeran K, Choi SJ, Taylor GM, Heath MM, Lambert PD *et al.* 1996 A role for glucagon-like peptide-1 in the central regulation of feeding. *Nature* **379** 69–72.

- Vallejo M, Penchuk L & Habener JF 1992 Somatostatin gene upstream enhancer element activated by a protein complex consisting of CREB, Isl-1-like, and alpha-CBF-like transcription factors. *Journal of Biological Chemistry* **267** 12876–12884.
- Vilsboll T, Krarup T, Deacon CF, Madsbad S & Holst JJ 2001 Reduced postprandial concentrations of intact biologically active glucagon-like peptide 1 in type 2 diabetic patients. *Diabetes* **50** 609–613.
- Wang M & Drucker DJ 1995 The LIM domain homeobox gene *isl-1* is a positive regulator of islet cell-specific proglucagon gene transcription. *Journal of Biological Chemistry* **270** 12646–12652.
- Wang H, Maechler P, Ritz-Laser B, Hagenfeldt KA, Ishihara H, Philippe J & Wollheim CB 2001 Pdx1 level defines pancreatic gene expression pattern and cell lineage differentiation. *Journal of Biological Chemistry* **276** 25279–25286.
- Wang J, Cao Y & Steiner DF 2003 Regulation of proglucagon transcription by activated transcription factor (ATF) 3 and a novel isoform, ATF3b, through the cAMP-response element/ATF site of the proglucagon gene promoter. *Journal of Biological Chemistry* **278** 32899–32904.
- Wang P, Liu T, Li Z, Ma X & Jin T 2006 Redundant and synergistic effect of Cdx-2 and Brn-4 on regulating proglucagon gene expression. *Endocrinology* **147** 1950–1958.
- Wideman RD, Yu IL, Webber TD, Verchere CB, Johnson JD, Cheung AT & Kieffer TJ 2006 Improving function and survival of pancreatic islets by endogenous production of glucagon-like peptide 1 (GLP-1). *PNAS* **103** 13468–13473.
- 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.
- Wilson ME, Kalamaras JA & German MS 2002 Expression pattern of IAPP and prohormone convertase 1/3 reveals a distinctive set of endocrine cells in the embryonic pancreas. *Mechanism of Development* **115** 171–176.
- Xu F, Li H & Jin T 1999 Cell type-specific autoregulation of the Caudal-related homeobox gene *Cdx-2/3*. *Journal of Biological Chemistry* **274** 34310–34316.
- Yi F, Brubaker PL & Jin T 2005 TCF-4 mediates cell type-specific regulation of proglucagon gene expression by β -catenin and glycogen synthase kinase-3beta. *Journal of Biological Chemistry* **280** 1457–1464.
- Yi F, Sun J, Lim GE, Fantus IG, Brubaker PL & Jin T 2008 Crosstalk between the insulin and Wnt signaling pathways: evidence from intestinal endocrine L cells. *Endocrinology* (In Press).
- Zaiko M, Estreicher A, Ritz-Laser B, Herrera P, Favor J, Meda P & Philippe J 2004 Pax2 mutant mice display increased number and size of islets of Langerhans but no change in insulin and glucagon content. *European Journal of Endocrinology* **150** 389–395.
- Zeng X, Tamai K, Doble B, Li S, Huang H, Habas R, Okamura H, Woodgett J & He X 2005 A dual-kinase mechanism for Wnt co-receptor phosphorylation and activation. *Nature* **438** 873–877.
- Zhu X, Zhou A, Dey A, Norrbom C, Carroll R, Zhang C, Laurent V, Lindberg I, Ugleholdt R, Holst JJ & Steiner DF 2002 Disruption of PC1/3 expression in mice causes dwarfism and multiple neuroendocrine peptide processing defects. *PNAS* **99** 10293–10298.

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