

## Topical Review

# Plasticity of the $\beta$ cell insulin secretory competence: preparing the pancreatic $\beta$ cell for the next meal

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**It is well established that the acute rise in plasma glucose and in the incretin hormones glucose-dependent insulintropic peptide (GIP) and glucagon-like peptide-1 (7–36) amide (GLP-1), as occurs during a meal, is of pivotal importance in regulating the minute-to-minute output of insulin from pancreatic  $\beta$  cells. In addition to this well studied acute effect, both glucose and incretin hormones have been recently observed to determine the future secretory responsiveness of the cells. Such plasticity of the insulin secretory competence would imply that glucose and incretins not only act during the present meal, but also help to prepare the  $\beta$  cells to function during the subsequent meal. Evidence supporting this hypothesis is growing as a result of physiological studies of cultured  $\beta$  cells (either primary cells or  $\beta$  cell lines), as well as from an increasing number of large-scale gene expression studies, exploring transcriptional and post-transcriptional events in genes regulated by glucose and incretins. On the basis of this hypothesis, one can speculate that genetic or environmental disturbances of plasticity of the insulin secretory competence is one aspect of  $\beta$  cell dysfunction that can contribute to the aetiology of type 2 diabetes.**

(Received 19 March 2004; accepted after revision 1 June 2004; first published online 4 June 2004)

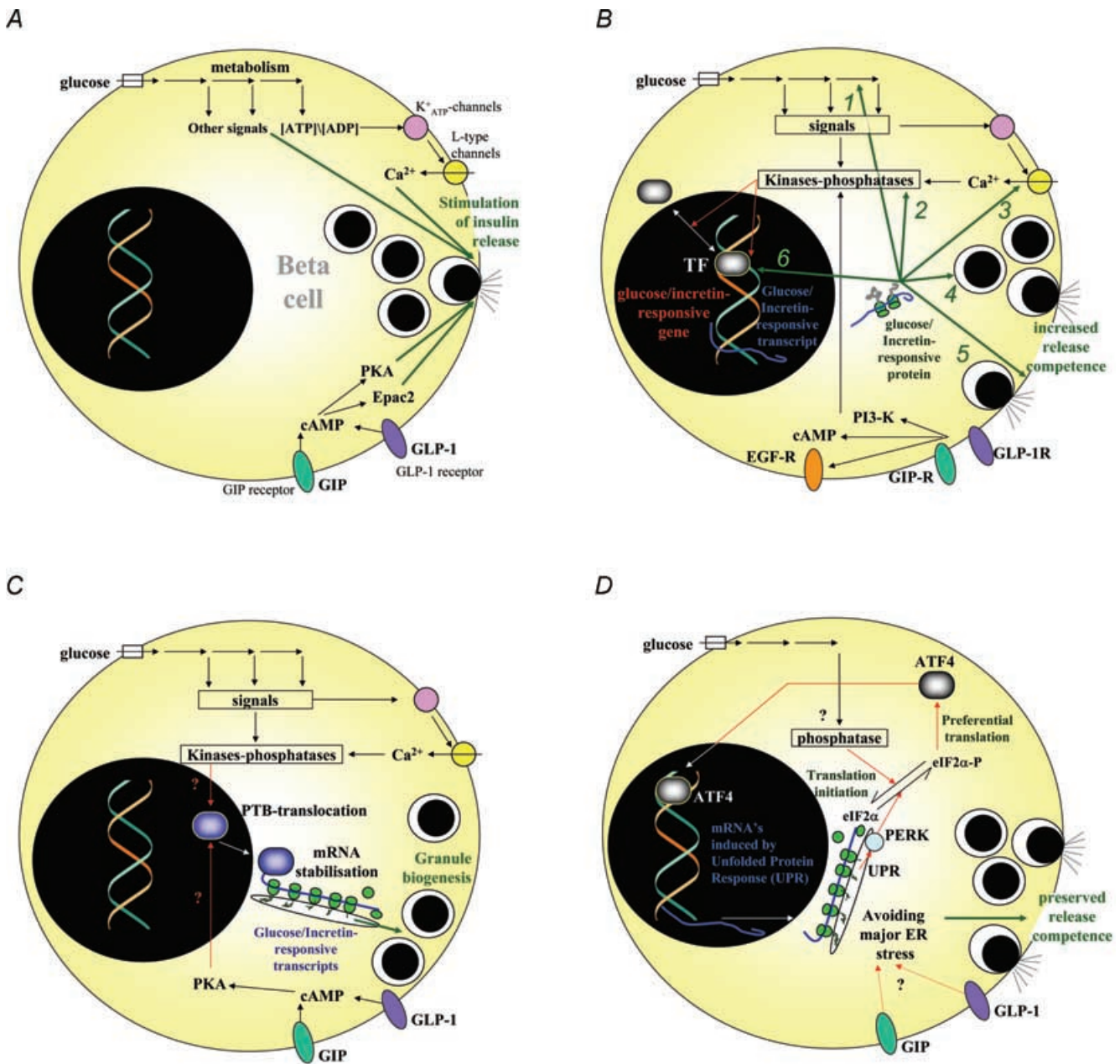
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## Different components of plasticity of the $\beta$ cell insulin secretory competence

The pancreatic  $\beta$  cell is unique in that it is the only animal cell type that can produce, store and secrete insulin in quantities that are required for growth and survival under the most variable environmental conditions (Rorsman, 1997; Goodge & Hutton, 2000; Rutter, 2001). This physiological task is not trivial, as insulin is not only required for survival (as evidenced by ketoacidosis in untreated type 1 diabetes), but can also be lethal within minutes when circulating at inappropriately high concentrations. To match the secretion of insulin to the physiological demands, the differentiated mammalian  $\beta$  cell has evolved in such a way that it displays a sophisticated degree of plasticity that allows adaptation at various ranges of time, preventing both shortage and excesses of insulin in the circulation. On a rapid time scale, typically occurring when a meal is digested and absorbed, nutrients and the incretin hormones, glucagon-like peptide 1 (GLP-1) and glucose-dependent insulintropic peptide (GIP), synergize in the

acute stimulation of insulin secretion (Jia *et al.* 1995). This potentiation occurs via a rise in intracellular cAMP in the  $\beta$  cell (Moens *et al.* 1996) and seems to be required to obtain sufficient insulin for the proper nutrient disposal in various tissues of the organism (Holst & Orskov, 2001). A safeguard against insulin toxicity is that GIP and GLP-1 (henceforth referred to as incretins) only potentiate insulin release as long as extracellular glucose is elevated above the basal concentration (Jia *et al.* 1995). At the other extreme of the time scale, requiring days to weeks, glucose and also incretins have been proposed to determine the size of the pancreatic  $\beta$  cell pool by affecting the rates at which  $\beta$  cells are generated and destroyed, respectively, by neogenesis and apoptosis (Hoorens *et al.* 1996; Jonas *et al.* 1999; Bonner-Weir, 2000; Li *et al.* 2003; Drucker, 2003).

How the acute stimulation of exocytosis (Rorsman, 1997; Henquin, 2000; Rutter, 2001; Schuit *et al.* 2001; Newgard & McGarry, 1995), as well as the regulation of  $\beta$  cell number (Bonner-Weir, 2000; Drucker, 2003), is regulated by glucose and incretins has been reviewed elsewhere so that these aspects of plasticity are not



**Figure 1. Different components of the insulin secretory competence of pancreatic  $\beta$  cells**

A, glucose and the incretin hormones GLP-1 and GIP are known to synergize acutely at the level of exocytosis of insulin secretory granules. The exact molecular mechanisms remain to be fully elucidated. A major effect of glucose is the elevation of cytosolic calcium caused by influx via L-type calcium channels after closure of ATP-sensitive potassium channels ( $K^+$ <sub>ATP</sub> channels). The major effect of incretin receptors is elevation of cyclic AMP (cAMP). Both calcium and cAMP interact at the level of proteins that regulate or mediate exocytosis (Newgard & McGarry, 1995; Rorsman, 1997; Henquin, 2000; Rutter, 2001; Schuit *et al.* 2001). The cAMP-dependent stimulation has been shown to utilize both cAMP-dependent protein kinase (PKA) and PKA-independent signalling (Renstrom *et al.* 1997) such as via Epac2 (cAMP-GEFII; Kashima *et al.* 2000; Holz, 2004).  $K^+$ <sub>ATP</sub>-channel-independent activation of exocytosis is known to occur as well (Henquin, 2000), but is not indicated. Also not shown is the cross-talk between PKA and ion channels and the fact that calcium can activate cAMP production via adenylylase type VIII in  $\beta$  cells (Delmeire *et al.* 2003). B, at the same time that exocytosis is stimulated, glucose and incretins start to influence gene transcription via allosteric mediators or (in)activation of kinases/phosphatases that affect the activity (translocation, DNA binding, RNA-pol II activation) of transcription factors (TF). The result is altered rates of gene transcription and changes in the abundance of glucose/incretin responsive mRNAs. Some of these transcripts

discussed in detail in the current text. As for the acute stimulation, Fig. 1A gives a brief overview. In order to stimulate exocytosis, the sugar has to be taken up by the  $\beta$  cells and metabolized. Glucose metabolism in  $\beta$  cells is specialized in signal generation and contains an important mitochondrial phase (Maechler & Wollheim, 1990) with very little anaerobic glycolysis (Schuit *et al.* 1997). The mitochondrial catabolic flux causes a rise in the cellular pyridine nucleotide redox state (Malaisse *et al.* 1978), and in the ATP/ADP ratio (Detimary *et al.* 1998); in concert an anabolic flux called cataplerosis (Farfari *et al.* 2000; Flamez *et al.* 2002) is activated, resulting in the export of metabolites such as citrate and malate into the cytosol (Schuit *et al.* 1997). The further details in signalling are best understood for the rise in ATP/ADP (Fig. 1A). This change closes ATP-sensitive potassium channels ( $K^+_{ATP}$  channels; Ashcroft, 1988), which will cause depolarization of the plasma membrane and a rise in cytosolic calcium (Herchuelz *et al.* 1980). It was recently shown that opening of L-type  $Ca^{2+}$  channels calcium is responsible for about 80% of calcium-induced exocytosis (Gopel *et al.* 2004).

The process by which incretin hormones potentiate the nutrient-induced process described above is essentially caused by the fact that both GLP-1 receptors and GIP receptors are functionally coupled to adenylate cyclases,

raising cAMP in the  $\beta$  cell (Moens *et al.* 1996). A signalling cascade initiated by cAMP interacts with the rise in cytosolic  $Ca^{2+}$  to induce exocytosis (Ammala *et al.* 1993; Takahashi *et al.* 1999). This effect has been shown to require both cAMP-dependent protein kinase (PKA) and a PKA-independent mechanism (Renstrom *et al.* 1997). The latter has been identified as Epac2 (cAMP-GEFII), a guanine nucleotide exchange factor that interacts with Rim2 and Rab3 in islets (Kashima *et al.* 2001; Holz, 2004). Notably, generation of cyclic AMP and stimulation of downstream kinase modules by incretin receptor activation has been shown to be independent of ambient glucose concentrations (Hinke *et al.* 2000; Ehses *et al.* 2002), yet for incretin potentiation of insulin release, glucose must be elevated. The precise molecular mechanism of this phenomenon is not clearly understood, and although probably an oversimplification, it has been proposed that the glucose dependence of incretin action on exocytosis is a combined manifestation of the need for a high ATP/ADP ratio and energy dependence of granule mobilization by PKA (Gromada *et al.* 1998). Within a time frame of minutes, the acute insulin secretory competence can be intensified by repetition of the glucose stimulation, either in intact perfused pancreata (Moens *et al.* 2002) or in perfused isolated islets of Langerhans (Zawalich *et al.* 1988). As this

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are believed to encode for proteins that alter the insulin secretory competence (dark green arrows) via different mechanisms such as enhancing metabolism (1), amplifying signalling pathways (2 and 3), causing genesis of new dense core granules (4), mediating exocytosis (5), or being regulators of transcription (6), causing a second wave of transcription. One site of synergism of glucose and incretin hormones at this level is NFAT, a transcription factor that promotes insulin gene expression (Lawrence *et al.* 2001). Known cyclic AMP-mediated effects on transcription proceed via PKA and the phosphorylation of cAMP-responsive element binding protein (CREB) and cAMP response element modulator (ICER; CREM). Incretin receptors may also induce gene transcription via PI-3 kinase signalling and *trans*-activation of epidermal growth factor receptors (EGF-R; Buteau *et al.* 2003). C, insulin granule biogenesis has to occur in a balanced manner together with accelerated exocytosis in order to prevent degranulation of  $\beta$  cells, as such depletion would jeopardize chronic insulin secretory competence. One molecular mechanism that has recently been elucidated is that glucose regulates polypyrimidine track-binding protein (PTB), promoting its nucleo-cytoplasmic translocation, thus enhancing the stability of transcripts with a 3'-pyrimidine-rich motif (Knoch *et al.* 2004). The kinases/phosphatases involved in this process remain to be elucidated. As such mRNAs in the  $\beta$  cell encode insulin as well as secretory granule components (IA-2, PC1/3, PC2, chromogranin A, among others), synthesis of new granules is favoured preferentially. As cyclic AMP induces a similar effect on PTB in PC-12 cells by steps involving PKA-dependent phosphorylation (Xie *et al.* 2003), it is conceivable but not yet demonstrated that glucose and incretins synergize at the level of this molecular target in primary  $\beta$  cells. D, the biosynthetic stimulation of insulin secretory granule components, including insulin itself, greatly enhances the protein folding load of the ER compartment. In order to prevent accumulation of unfolded protein, an integrated cellular response (unfolded protein response, UPR; Kaufman, 2002) induces coordinated changes in gene expression resulting in greater protein folding capacity. In pancreatic  $\beta$  cells, this response is critically dependent (Delepine *et al.* 2000; Harding *et al.* 2001; Scheuner *et al.* 2001) on the pancreatic ER kinase (PERK) which phosphorylates the initiation factor eIF-2 $\alpha$ . The result is dual: (i) acute arrest of translational initiation; and (ii) preferential translation of ATF4, a transcription factor that induces genes that enhance the protein-folding capacity of the  $\beta$  cell (Ron, 2002). This response will prevent major ER stress in the  $\beta$  cell under conditions of increased physiological biosynthetic demand. Control of translational initiation may be further assisted by balance between PERK-inactivation (phosphorylation) and glucose activation (dephosphorylation) of eIF2 $\alpha$ .

short-term glucose memory occurs so rapidly, it is likely that this adaptation occurs either via post-translational modifications of already existing protein(s) involved in exocytosis or by the cellular accumulation of signals such as calcium or lipid mediators (Zawalich *et al.* 1988) that are responsible for triggering the release process.

This Topical Review focuses on an intermediate level of plasticity, taking place within minutes following the onset of stimulation and extending up to many days. At this level, gene expression in existing differentiated  $\beta$  cells is modified, changing the functional behaviour of the cells. During a particular meal, when physiological stimuli acutely trigger exocytosis during the whole period of digestion and absorption (60–120 min), glucose and incretins simultaneously affect multiple other steps in  $\beta$  cell function. The outcome of this effect is that release of insulin granules is balanced (Schuit *et al.* 1991) by a process of granule formation; the responsible mechanisms comprise transcription and translation of the insulin gene(s), accelerated insulin folding and formation of other insulin secretory granule components (Goode & Hutton, 2000; Ohneda *et al.* 2000). It is expected that such adaptation involves enhanced expression of a large number of other genes in addition to the insulin gene(s).

### Glucose and incretins regulate plasticity of $\beta$ cell insulin secretory competence *in vitro*

In serum-free conditions and using primary rat  $\beta$  cells, Ling *et al.* (1996) showed that the functional status of  $\beta$  cells was much better preserved in culture medium at 10 mM glucose than in 5 mM glucose, although cellular viability and the size of the insulin storage pool were constant. Translational activity was also significantly higher in the cells cultured at 10 mM glucose, indicating that overall translational rates are correlated to glucose competence. This idea was further supported by two studies using the non-specific protein translation blocker cycloheximide (Garcia-Barrado *et al.* 2001; Schuit *et al.* 2002), as such treatment leads to rapid loss of insulin secretory competence. In mouse islets, within 2 h of incubation with cycloheximide, the secretory response to glucose was diminished (Garcia-Barrado *et al.* 2001). In fluorescence-activated cell sorting (FACS)-purified  $\beta$  cells, 6 h of cycloheximide treatment significantly suppressed insulin secretory competence, without measurable effects upon cellular viability or insulin content (Schuit *et al.* 2002). This indicates that protein(s) with rapid turnover are implicated in regulating glucose competence of the  $\beta$  cells in a manner that is independent of the amount of stored insulin granules per cell. As glucose itself is a

major stimulus for translational activity in  $\beta$  cells (Schuit *et al.* 1988) and glucose can affect gene transcription as well as mRNA stability in  $\beta$  cells (see next section), it can be proposed that a history of glucose-induced gene expression (transcriptional or post-transcriptional) is necessary for the glucose competence of the cells. Similar evidence is accumulating for the incretin hormones. GLP-1 has been reported to induce several genes in  $\beta$  cells lines, promoting the growth of the cells and inhibiting apoptosis (Li *et al.* 2003; Drucker, 2003). In a recent study (Delmeire *et al.* 2004), it was shown that the secretory competence of cultured primary rat  $\beta$  cells was enhanced by intermittent addition of 0.5 nM of GLP-1 and GIP together to the tissue culture medium. As longer incubation with incretins resulted in receptor desensitization, the stimulation was applied as three 1-h periods together with 10 mM glucose, attempting to mimic the periodicity of nutrients and hormones observed *in vivo*. The enhanced secretory capacity of these terminally differentiated cells could not be explained by altered viability of the cells or a change in the size of the insulin granule storage pool. One can hypothesize therefore that the incretin-induced changes in secretory competence are caused by changes in gene expression in the  $\beta$  cell.

### Molecular mechanisms involved in plasticity of $\beta$ cell secretory competence

Over the time frame of minutes to several hours, insulin secretory competence can be altered by changes at the level of signalling or altered gene transcription and protein synthesis. Some pathways by which this can be achieved are shown in Fig. 1B–D. It can be assumed that changes in this plasticity are mediated by critical protein regulator(s) that are up-regulated (stimulators) or suppressed (inhibitors) following glucose and incretin stimulation. A number of microarray experiments have suggested (Webb *et al.* 2000; Shalev *et al.* 2002; Flamez *et al.* 2002; Schuit *et al.* 2002) that glucose alters the abundance of a large number of transcripts (depending on the chosen conditions perhaps as many as a few hundred) that are involved in synthesis of new insulin granules, metabolic signalling, second messenger production, signal amplification, exocytosis as well as (post)transcriptional regulation. It is becoming increasingly clear that the specialized phenotype of insulin-secreting  $\beta$  cells is the work of number of  $\beta$  cell-specific proteins from which expression is regulated by a  $\beta$  cell-specific transcription factor network (Fig. 1B). Microarrays searching for gene promoters binding to particular transcription factors

present in the  $\beta$  cell, such as HNF1 $\alpha$ , HNF4 $\alpha$  and HNF6, suggest that a large part of the set of transcripts present in  $\beta$  cells are expressed in this way (Odom *et al.* 2004). The tight link between maturity onset diabetes of the young (MODY; a monogenic form of diabetes) and a series of transcription factors expressed in the  $\beta$  cell (Fajans *et al.* 2001) is a medical consequence of this phenomenon. While transcriptional regulation seems extremely important, the  $\beta$  cell secretory competence seems also to be regulated at the post-transcriptional level. This section will discuss some of these mechanisms.

### Transcriptional modulation in $\beta$ cells by hormones and nutrients.

Glucose and incretins can alter mRNA abundance of critical transcripts by changing rates of transcription (Fig. 1B), either by altering the activity of regulators of transcription that are already present in the cell or by net synthesis of novel transcription factors. Via the latter mechanism, up-regulation of mRNA encoding the sterol element binding protein 1c (SREBP1c) and down-regulation of peroxisome proliferation-activated receptor  $\alpha$  (PPAR $\alpha$ ) in glucose-stimulated  $\beta$  cells might explain suppression of transcripts encoding enzymes of fatty acid oxidation and induction of mRNA encoding lipogenic enzymes (Flamez *et al.* 2002). It is not clear whether such a coordinated response enhances metabolic signalling in the  $\beta$  cell or whether it serves a greater need for secretory granule membrane synthesis. Probably the most often encountered transcriptional regulation occurs via changing the functional status of existing transcription factors that are already present or have to be translocated into initiation complexes of transcription. This change can be caused either by second messengers that act allosterically upon transcription factors or by kinases/phosphatases that cause changes in the phosphorylation status of transcription factors (Fig. 1B). Allosteric interactions and (de)phosphorylation can modify the subcellular localization, e.g. translocating the transcription factor from cytosol to the nucleus, or (in)activate transcription factors that are already residing in the nucleus, waiting for a signal to respond. Glucose and incretins synergize at the level of a rise in intracellular calcium ( $[Ca^{2+}]_i$ ) via effects on ATP-sensitive potassium channels ( $K^+$ <sub>ATP</sub> channels) and L-type voltage-dependent calcium channels (VDCCs) (Suga *et al.* 1997a,b), and potentially also via the process of calcium-induced calcium release (Holz *et al.* 1999). Furthermore, glucose and GLP-1 synergize at the level of intracellular cAMP production, as  $\beta$  cells express adenylate cyclase type VIII (Delmeire *et al.* 2003). So it seems conceivable, and even

likely, that many of the effects of glucose/incretins on the activity of transcription factors may be the result of changes in  $[Ca^{2+}]_i$  and in intracellular production of cAMP.

Instead of a comprehensive review of all such known regulations of this type in the  $\beta$  cell, we chose to discuss one example of the sophisticated interplay between glucose and incretins in the stimulation of insulin gene transcription. nuclear factor of activated T cells (NFAT) is a well characterized calcium-dependent transcription factor in lymphocytes (Rao *et al.* 1997; Okamura *et al.* 2000) that was proposed to mediate a synergistic interplay between glucose and incretin-induced insulin gene transcription (Lawrence *et al.* 2001, 2002). In resting T cells, NFAT resides in the cytoplasm and is fully phosphorylated; following stimulation, it rapidly becomes dephosphorylated by calcineurin (calcium/calmodulin-dependent protein phosphatase 2B) activated by an increase in cytosolic calcium concentration. Dephosphorylation results in the exposure of a nuclear localization sequence promoting the rapid translocation of NFAT to the nucleus (Luo *et al.* 1996). In  $\beta$  cells, NFAT has been shown to bind in a calcium/calcineurin-dependent manner to three distinct NFAT-response elements within the insulin promoter and to activate insulin gene transcription (Lawrence *et al.* 2001; Lawrence *et al.* 2002). As the major NFAT-binding site in the insulin promoter overlaps with an inverted responsive element for C/EBP $\beta$ , a repressor of insulin gene transcription during prolonged exposure of  $\beta$  cells to high glucose (Seufert *et al.* 1998), competition between different transcription factors for binding to common responsive elements activated by different second messengers further illustrates the plasticity of the secretory response of  $\beta$  cells. Inhibition of calcineurin during post-transplantation therapy by the immunosuppressant calcineurin inhibitors FK506 and cyclosporin A has been shown to decrease pre-proinsulin mRNA, depleting the cells of insulin and increasing the risk for post-transplant diabetes mellitus (Drachenberg *et al.* 1999), perhaps illustrating the clinical relevance of NFAT-regulated insulin gene expression.

### Post-transcriptional control of mRNA abundance and $\beta$ cell plasticity.

In addition to transcriptional regulation, changes in mRNA abundance can be regulated post-transcriptionally, for example, differential effects upon mRNA splicing, nuclear export or stability. Following glucose stimulation, exocytotic events are responsible for the loss of granules from the storage pool. To

replenish this pool and keep a balance between release and storage (Schuit *et al.* 1991), the  $\beta$  cell should rapidly enhance the synthesis of new granule proteins and produce new membrane at the level of the endoplasmic reticulum (ER). Protein and mRNA analysis suggest that the abundance of a large number of secretory granule-related genes is up-regulated by glucose in a coordinated manner (Guest *et al.* 1989; Knoch *et al.* 2004). As for accelerated protein synthesis in primary  $\beta$  cells, this effect is achieved primarily by a powerful acceleration of general translation (Schuit *et al.* 1988; Schuit *et al.* 1991). In addition, in primary  $\beta$  cells, insulin mRNA is translated preferentially as compared to non-insulin encoding mRNAs in these cells (Schuit *et al.* 1988). Increased translation of proinsulin mRNA results from the stimulation of translation initiation and elongation (Permutt, 1974), as well as from a reduced degradation of insulin mRNA (Welsh *et al.* 1985) (Fig. 1C). This latter mechanism has recently been explored and involves the rapid nucleo-cytoplasmic translocation of polypyrimidine tract-binding protein (PTB) upon  $\beta$  cell stimulation with glucose (Knoch *et al.* 2004). Activated PTB has been shown to increase the stability of insulin mRNA by binding to its 3'-untranslated region, which contains a pyrimidine-rich consensus sequence (Tillmar *et al.* 2002). The coordinated glucose-stimulated synthesis of insulin and other components of dense-core secretory granules (Guest *et al.* 1989) can be explained (at least in part) by the fact that PTB also binds and stabilizes mRNAs encoding different transcripts related to granule biogenesis, in particular prohormone convertases 1/3 or 2 (PC1/3, PC2), pre-chromogranin A, secretogranin II, synaptobrevin 2, synaptophysin and the tyrosine phosphatase like molecule I-A2 (Knoch *et al.* 2004). The importance of PTB stabilization for the regulation of dense core biogenesis was further supported by silencing of the endogenous PTB mRNA expression in the  $\beta$  cell line INS-1 (Knoch *et al.* 2004). Four days after addition of siRNA, transfected cells had virtually no insulin granules left, and the expression of a number of granule-proteins was strongly reduced. It is assumed that an unidentified mechanism, perhaps involving protein kinases and proteases, mediates the effect of glucose/metabolites upon PTB, converting part of the 59 K full length protein into a 27 kDa protein fragment, but the exact molecular events promoting the nucleo-cytoplasmic translocation remain to be elucidated. Interestingly, in neuroendocrine PC-12 cells, cAMP-dependent phosphorylation of PTB has been reported, resulting in its nucleo-cytoplasmic transport (Xie *et al.* 2003); if this process also occurs in primary  $\beta$  cells, PTB would represent another regulator of gene expression by

which glucose and incretins exert their control of  $\beta$  cell plasticity.

**The role of regulating protein translation in  $\beta$  cell function.** A final important step in the regulation of gene expression is mediated by the fact that glucose has a profound effect on overall efficiency of translation in the  $\beta$  cell (Schuit *et al.* 1988), implying that the mRNAs that are present at a given time point are more rapidly translated as extracellular glucose rises. The exact molecular mechanisms are still partially unknown. Early studies on insulin biosynthesis pointed to accelerated translational initiation (Permutt, 1974) and elongation at the level of signal peptide-mediated arrest (Welsh *et al.* 1986). Glucose control of initiation may involve phosphorylation of IF4E binding protein (Xu *et al.* 1998) and eIF2 $\alpha$ , the latter being a sensor of the balance between the total protein folding load and folding capacity in the ER compartment (Kaufman, 2002; Ron, 2002). Accelerated synthesis of insulin, other secretory proteins and components of secretory granules (see previous paragraph) impose a burden at the level of protein folding in the ER lumen. Physiologically, such a condition activates the so-called unfolded protein response (Kaufman, 2002). When the biosynthetic load exceeds folding capacity, the ER chaperone BiP is reallocated from the eIF2 $\alpha$ -kinase PERK to the ER lumen, complexing the unfolded protein. By dissociation of BiP from the luminal domain of PERK, this kinase is activated (Ron, 2002). PERK activation then rapidly attenuates overall translation by phosphorylating the initiation factor eIF2 $\alpha$ . Phosphorylated eIF2 $\alpha$ , however, specifically favours translation of mRNA encoding the transcription factor ATF4, thereby increasing ATF4 protein in the cell (Fig. 1D). As ATF4 is involved in transcription of a number of genes that are relevant for protein folding in the ER compartment, the cell can adapt with time to a higher biosynthetic load by increasing the ER folding capacity (Ron, 2002). Recent data have indicated that without such compensatory translational and transcriptional response, the increased load on the biosynthetic apparatus in the  $\beta$  cell would result in severe ER stress and loss of insulin secretory competence (Harding *et al.* 2001; Scheuner *et al.* 2001). The pivotal roles of PERK and eIF2 $\alpha$ -phosphorylation have been underlined in PERK-deficient mice (Harding *et al.* 2001) and mice in which the serine-51 phosphorylation site was mutated into an alanine (Scheuner *et al.* 2001). A direct clinical correlation was provided by the observation that a mutation of the gene encoding PERK in man causes diabetes (Delepine

*et al.* 2000). Thus, translational control of the  $\beta$  cell and its connection to the molecular pathology of diabetes is an interesting emerging field of research. It seems relevant to investigate whether incretin hormones can interact with this pathway and whether nutritional variables can modulate the integrated response to ER stress in the  $\beta$  cell.

### **Do glucose and incretins regulate plasticity of $\beta$ cell insulin secretory competence *in vivo*?**

A major question which has not been effectively addressed is whether the *in vitro* observations of glucose and incretin priming effects occur *in vivo*. The primary difficulty would be to experimentally differentiate these effects from the well documented effects of nutrients and hormones on  $\beta$  cell neogenesis and proliferation (Drucker, 2003). Furthermore, in intact animals, it may be hard to separate direct from indirect effects, also considering that paracrine effects of other islet hormones may alter  $\beta$  cell glucose competence (Huypens *et al.* 2000), and that innervation of the endocrine pancreas also plays a large part in controlling the secretory response (Gilon & Henquin, 2001). Two approaches have been made to examine how the nutritional state of an animal alters its future secretory responsiveness: glycaemic clamp studies (*in vivo* analysis) and perfusion of freshly isolated islets (*ex vivo* study), in both cases comparing the fed and fasted state. Virtually all reports find greater responsiveness of pancreatic  $\beta$  cells which are exposed to periodic elevations of nutrients and incretin hormones (i.e. *ad libitum* food access) compared to the fasted state. The molecular mechanism underlying this phenomenon has been a matter of debate for several decades (Zawalich & Zawalich, 2000). It can generally be stated that it does not correlate well to changes in insulin content. The molecular underpinnings of this observation probably occur as the result of a number of alterations in the  $\beta$  cell caused by periodic elevations in nutrients and incretin hormones which induce intermediate and long-term alterations in the secretory phenotype that are based upon altered gene and protein expression in the  $\beta$  cell.

In healthy individuals, glucose is strictly maintained between narrow limits as a result of insulin action during and following a meal, and counter-regulatory mechanisms during fasting. Similarly, bioactive incretin hormone concentrations are also tightly controlled, by the rapid inactivation by dipeptidyl peptidase IV and renal elimination of peptide metabolites over a longer time course (Kieffer *et al.* 1995; Meier *et al.* 2004). The truncated metabolites of GIP and GLP-1 appear to have no effect on

glucose homeostasis (Hinke *et al.* 2002; Vahl *et al.* 2003); however, it cannot be discounted that these metabolites have physiological effects, but the appropriate parameter and experimental model have not yet been examined. It is perhaps an important observation that despite their rapid degradation, measurable intact bioactive incretins can be detected even following prolonged fasting (Meier *et al.* 2004), indicating that in addition to acting during the postprandial phase, they also may be exerting tonic effects on the  $\beta$  cell. Support for this notion is given by the biological effects described for so-called 'sustained release' formulations of GLP-1 or metabolically stable GLP-1 analogues, which have been shown to exert long-term beneficial effects on glucose homeostasis after single injection (Chang *et al.* 2003; Nielsen *et al.* 2004). Implicit to this hypothesis is that although the acute actions of incretins are dependent upon elevated glucose concentrations, many studies have shown that the receptor activations of signal transduction cascades do not require glucose.

It seems likely that prolonged stimulation *in vivo* with bioaccumulating incretin agonists would also cause down-regulation of the receptors on islets (although this has not been shown experimentally); however, most of the data regarding desensitization is based upon *in vitro* observations. The first studies examining desensitization of the GIP and GLP-1 receptors were reported in 1991, examining HIT-T15 cells by perfusion; results demonstrated both homologous and heterologous desensitization for the incretin receptors (Fehmann & Habener, 1991). Subsequent studies have used both insulinoma cell models and over-expression systems to examine the molecular nature of incretin receptor desensitization. The desensitization of the GLP-1R during prolonged stimulation was found to be PKA independent (Gromada *et al.* 1996), and homologous and heterologous desensitization (and secondary endocytosis) were shown to occur following phosphorylation of serine doublets found in the intracellular C-terminal tail of the receptor, probably mediated by PKC (Widmann *et al.* 1996, 1997). Homologous desensitization of the GIP receptor was similarly shown to be PKA independent, and although PKC was implicated in modulating GIP receptor responsiveness from kinase inhibitor studies, its involvement in GIPR desensitization was not supported by the experimental evidence (Hinke *et al.* 2000). Analysis of the carboxyl tail of the GIPR by site directed mutagenesis was examined in two independent studies, and identified several potential regulatory target residues for receptor internalization (over the course of minutes to hours of stimulation) or chronic down-regulation

(24 h of continuous stimulation) (Tseng & Zhang, 1998; Wheeler *et al.* 1999). The acute control of GIP receptor responsiveness is thought to be primarily controlled by phosphorylation mediated by G-protein receptor kinase 2 (GRK-2) (Tseng & Zhang, 2000); however, similar studies have not been published for the GLP-1 receptor. Recent studies have shown that desensitization of both incretin receptors also occurs in primary purified rat  $\beta$  cells (Delmeire *et al.* 2004). In addition, as receptor desensitization is unlikely ever to be 100%, a chronic low stimulation of the  $\beta$  cell, such as with incretin analogues with improved biological and circulating half-lives, may foster appropriate handling of glycaemic excursions via priming, recruitment and sensitization of the  $\beta$  cell, which may be mediated by direct post-translational modification (phosphorylation/dephosphorylation) or positive long-term changes in gene expression. Alternatively, the process of receptor desensitization itself may improve the capacity of the endocrine pancreas to respond to glucose, as receptor internalization has been demonstrated to be one of the mechanisms used by G-protein-coupled receptors to transactivate the epidermal growth factor receptor (Pierce *et al.* 2000; Buteau *et al.* 2003), and thus may promote  $\beta$  cell function and/or expansion via activation of genes controlling  $\beta$  cell differentiation and proliferation or activation of kinases/phosphatases which mediate the intermediate phase of islet competence.

In coverage of this topic, it must be noted that some studies have been unable to reproduce the priming effects of incretins on subsequent insulin release in human *in vivo* experiments (Rachman *et al.* 1997; Brandt *et al.* 2001; Meier *et al.* 2003). It is unclear whether the discrepancy between these clinical studies and results from rodents (*in vitro* and *in vivo*) are due to experimental design, alteration in neural tone, the result of receptor desensitization (or failure to re-sensitize), or due to differences in the proliferative or regenerative capacity of human endocrine tissue relative to rodents. One possibility which warrants further examination is reproduction of periodicity of stimulation, as this is likely to be a critical element in this process. Thus, in studies using sustained release incretin analogues (Chang *et al.* 2003; Nielsen *et al.* 2004), it is likely that the tonic stimulation of  $\beta$  cells was greater than usually present during the inter-digestive phase, but periodicity of endogenously secreted incretins was normal, and thus mediated future positive effects on  $\beta$  cell responsiveness. Further examination of the secretory competence of the  $\beta$  cell, especially comparing *ex vivo* data on gene expression and *in vivo* function must resolve these questions.

## Perspective

Five out of six known forms of MODY are caused by mutations in transcription factors expressed in the  $\beta$  cell (Fajans *et al.* 2001). Because of this clinical information as well as mechanistic studies in  $\beta$  cell lines (see for example Wobser *et al.* 2002) the multiple links between diabetes,  $\beta$  cell dysfunction and abnormalities in the operation of transcription factor networks are starting to become understood (Ferrer, 2002). It will be important to search in a systematic manner, e.g. by genome-wide mRNA or proteome analysis, for transcription factors from which the abundance is regulated by glucose, as such factors may be high in the hierarchy of glucose control of gene expression, regulating in turn the expression of numerous glucose-dependent genes. The recently published microarray study on chromatin immunoprecipitated from human islets (Odom *et al.* 2004) is an important example of this type of research. Mechanistic studies on HNF1 $\alpha$  (Wobser *et al.* 2002; Ferrer, 2002), as well as SREBP1c (Flamez *et al.* 2002; Wang *et al.* 2003) and PPAR $\alpha$  (Roduit *et al.* 2000; Flamez *et al.* 2002) should shed more light on the function of the encoded proteins. A difficult task will be to identify the true master switch genes of glucose control in a complicated interacting network. Perhaps the concept of a 'master switch' will prove to be incorrect, while the multiple and complex functionality states of the network itself predominate. As compared to glucose control of gene transcription, relatively few studies have been carried out on post-transcriptional regulation of  $\beta$  cell genes, as well as the influence of incretin hormones on gene expression in the  $\beta$  cell. Consequently, it can be anticipated that these topics may become fascinating areas of future  $\beta$  cell research. Analogous to the MODY paradigm, molecular defects in post-transcriptional regulation in  $\beta$  cells may turn out to be predisposing factors for human diabetes as has been already illustrated by the Wolcott-Rallison syndrome (Delepine *et al.* 2000). Finally, new pharmacological tools could be designed on the basis of the physiological emerging principle that a synergism between glucose and incretin hormones is required to direct  $\beta$  cell gene expression in preparation for the next meal.

## References

- Ammala C, Ashcroft FM & Rorsman P (1993). Calcium-independent potentiation of insulin release by cyclic AMP in single beta-cells. *Nature* **363**, 356–358.
- Ashcroft FM (1988). Adenosine 5'-triphosphate-sensitive potassium channels. *Annu Rev Neurosci* **11**, 97–118.

- Bonner-Weir S (2000). Perspective: Postnatal pancreatic beta cell growth. *Endocrinology* **141**, 1926–1929.
- Brandt A, Katschinski M, Arnold R, Polonsky KS, Goke B & Byrne MM (2001). GLP-1-induced alterations in the glucose-stimulated insulin secretory dose–response curve. *Am J Physiol Endocrinol Metab* **281**, E242–E247.
- Buteau J, Foisy S, Joly E & Prentki M (2003). Glucagon-like peptide 1 induces pancreatic beta-cell proliferation via transactivation of the epidermal growth factor receptor. *Diabetes* **52**, 124–132.
- Chang AM, Jakobsen G, Sturis J, Smith MJ, Bloem CJ, An B, Galecki A & Halter JB (2003). The GLP-1 derivative NN2211 restores beta-cell sensitivity to glucose in type 2 diabetic patients after a single dose. *Diabetes* **52**, 1786–1791.
- Delepine M, Nicolino M, Barrett T, Golamaully M, Lathrop GM & Julier C (2000). EIF2AK3, encoding translation initiation factor 2-alpha kinase 3, is mutated in patients with Wolcott–Rallison syndrome. *Nat Genet* **25**, 406–409.
- Delmeire D, Flamez D, Hinke SA, Cali JJ, Pipeleers D & Schuit F (2003). Type VIII adenylyl cyclase in rat beta cells: coincidence signal detector/generator for glucose and GLP-1. *Diabetologia* **46**, 1383–1393.
- Delmeire D, Flamez D, Moens K, Hinke SA, Van Schravendijk CFH, Pipeleers D & Schuit F (2004). Prior *in vitro* exposure to GLP-1 with or without GIP can influence the subsequent beta cell responsiveness. *Biochem Pharmacol* (in press).
- Detimary P, Dejonghe S, Ling Z, Pipeleers D, Schuit F & Henquin JC (1998). The changes in adenine nucleotides measured in glucose-stimulated rodent islets occur in beta cells but not in alpha cells and are also observed in human islets. *J Biol Chem* **273**, 33905–33908.
- Drachenberg CB, Klassen DK, Weir MR, Wiland A, Fink JC, Bartlett ST, Cangro CB, Blahut S & Papadimitriou JC (1999). Islet cell damage associated with tacrolimus and cyclosporine: morphological features in pancreas allograft biopsies and clinical correlation. *Transplantation* **68**, 396–402.
- Drucker DJ (2003). Glucagon-like peptides: regulators of cell proliferation, differentiation, and apoptosis. *Mol Endocrinol* **17**, 161–171.
- Ehse JA, Pelech SL, Pederson RA & McIntosh CH (2002). Glucose-dependent insulintropic polypeptide activates the Raf-Mek1/2-ERK1/2 module via a cyclic AMP/cAMP-dependent protein kinase/Rap1-mediated pathway. *J Biol Chem* **277**, 37088–37097.
- Fajans SS, Bell GI & Polonsky KS (2001). Molecular mechanisms and clinical pathophysiology of maturity-onset diabetes of the young. *New Engl J Med* **345**, 971–980.
- Farfari S, Schulz V, Corkey B & Prentki M (2000). Glucose-regulated anaplerosis and cataplerosis in pancreatic beta-cells: possible implication of a pyruvate/citrate shuttle in insulin secretion. *Diabetes* **49**, 718–726.
- Fehmann HC & Habener JF (1991). Homologous desensitization of the insulintropic glucagon-like peptide-I (7–37) receptor on insulinoma (HIT-T15) cells. *Endocrinology* **128**, 2880–2888.
- Ferrer J (2002). A genetic switch in pancreatic beta-cells: implications for differentiation and haploinsufficiency. *Diabetes* **51**, 2355–2362.
- Flamez D, Berger V, Kruhoffer M, Orntoft T, Pipeleers D & Schuit FC (2002). Critical role for cataplerosis via citrate in glucose-regulated insulin release. *Diabetes* **51**, 2018–2024.
- Garcia-Barrado MJ, Ravier MA, Rolland JF, Gilon P, Nenquin M & Henquin JC (2001). Inhibition of protein synthesis sequentially impairs distinct steps of stimulus-secretion coupling in pancreatic beta cells. *Endocrinology* **142**, 299–307.
- Gilon P & Henquin JC (2001). Mechanisms and physiological significance of the cholinergic control of pancreatic beta-cell function. *Endocr Rev* **22**, 565–604.
- Goode KA & Hutton JC (2000). Translational regulation of proinsulin biosynthesis and proinsulin conversion in the pancreatic beta-cell. *Semin Cell Dev Biol* **11**, 235–242.
- Gopel S, Zhang Q, Eliasson L, Ma XS, Galvanovskis J, Kanno T, Salehi A & Rorsman P (2004). Capacitance measurements of exocytosis in mouse pancreatic  $\alpha$ -,  $\beta$ - and  $\delta$ -cells studied in intact islets of Langerhans. *J Physiol* **556**, 711–726.
- Gromada J, Dissing S & Rorsman P (1996). Desensitization of glucagon-like peptide 1 receptors in insulin-secreting beta TC3 cells: role of PKA-independent mechanisms. *Br J Pharmacol* **118**, 769–775.
- Gromada J, Holst JJ & Rorsman P (1998). Cellular regulation of islet hormone secretion by the incretin hormone glucagon-like peptide 1. *Pflugers Arch* **435**, 583–594.
- Guest PC, Rhodes CJ & Hutton JC (1989). Regulation of the biosynthesis of insulin-secretory-granule proteins. Co-ordinate translational control is exerted on some, but not all, granule matrix constituents. *Biochem J* **257**, 431–437.
- Harding HP, Zeng H, Zhang Y, Jungries R, Chung P, Plesken H, Sabatini DD, Ron D *et al.* (2001). Diabetes mellitus and exocrine pancreatic dysfunction in *perk*<sup>-/-</sup> mice reveals a role for translational control in secretory cell survival. *Mol Cell* **7**, 1153–1163.
- Henquin JC (2000). Triggering and amplifying pathways of regulation of insulin secretion by glucose. *Diabetes* **49**, 1751–1760.
- Herchuelz A, Thonnart N, Sener A & Malaisse WJ (1980). Regulation of calcium fluxes in pancreatic islets: the role of membrane depolarization. *Endocrinology* **107**, 491–497.
- Hinke SA, Gelling RW, Pederson RA, Manhart S, Nian C, Demuth HU & McIntosh CHS (2002). Dipeptidyl peptidase IV-resistant [D-Ala<sup>2</sup>]glucose-dependent insulintropic polypeptide (GIP) improves glucose tolerance in normal and obese diabetic rats. *Diabetes* **51**, 652–661.

- Hinke SA, Pauly RP, Ehse J, Kerridge P, Demuth HU, McIntosh CHS & Pederson RA (2000). Role of glucose in chronic desensitization of isolated rat islets and mouse insulinoma (bTC-3) cells to glucose-dependent insulinotropic polypeptide. *J Endocrinol* **165**, 281–291.
- Holst JJ & Orskov C (2001). Incretin hormones – an update. *Scand J Clin Laboratory Invest* **234**, 75–85.
- Holz GG (2004). Epac: a new cAMP-binding protein in support of glucagon-like peptide-1 receptor-mediated signal transduction in the pancreatic beta-cell. *Diabetes* **53**, 5–13.
- Holz GG, Leech CA, Heller RS, Castonguay M & Habener JF (1999). cAMP-dependent mobilization of intracellular  $Ca^{2+}$  stores by activation of ryanodine receptors in pancreatic beta-cells. A  $Ca^{2+}$  signaling system stimulated by the insulinotropic hormone glucagon-like peptide-1-(7-37). *J Biol Chem* **274**, 14147–14156.
- Hoorens A, Van de Casteele M, Kloppel G & Pipeleers D (1996). Glucose promotes survival of rat pancreatic beta cells by activating synthesis of proteins which suppress a constitutive apoptotic program. *J Clin Invest* **98**, 1568–1574.
- Huypens P, Ling Z, Pipeleers D & Schuit F (2000). Glucagon receptors on human islet cells contribute to glucose competence of insulin release. *Diabetologia* **43**, 1012–1019.
- Jia X, Brown JC, Ma P, Pederson RA & McIntosh CH (1995). Effects of glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1-(7-36) on insulin secretion. *Am J Physiol* **268**, E645–E651.
- Jonas JC, Sharma A, Hasenkamp W, Ilkova H, Patane G, Laybutt R, Bonner-Weir S & Weir GC (1999). Chronic hyperglycemia triggers loss of pancreatic beta cell differentiation in an animal model of diabetes. *J Biol Chem* **274**, 14112–14121.
- Kashima Y, Miki T, Shibasaki T, Ozaki N, Miyazaki M, Yano H & Seino S (2001). Critical role of cAMP-GEFII-Rim2 complex in incretin-potentiated insulin secretion. *J Biol Chem* **276**, 46046–46053.
- Kaufman RJ (2002). Orchestrating the unfolded protein response in health and disease. *J Clin Invest* **110**, 1389–1398.
- Kieffer TJ, McIntosh CHS & Pederson RA (1995). Degradation of glucose-dependent insulinotropic polypeptide and truncated glucagon-like peptide 1 in vitro and in vivo by dipeptidyl peptidase IV. *Endocrinology* **136**, 3585–3596.
- Knoch KP, Bergert H, Borgonovo B, Saeger HD, Altkruger A, Verkade P & Solimena M (2004). Polypyrimidine tract-binding protein promotes insulin secretory granule biogenesis. *Nature Cell Biol* **3**, 207–214.
- Lawrence MC, Bhatt HS & Easom RA (2002). NFAT regulates insulin gene promoter activity in response to synergistic pathways induced by glucose and glucagon-like peptide-1. *Diabetes* **51**, 691–698.
- Lawrence MC, Bhatt HS, Watterson JM & Easom RA (2001). Regulation of insulin gene transcription by a  $Ca^{2+}$ -responsive pathway involving calcineurin and nuclear factor of activated T cells. *Mol Endocrinol* **15**, 1758–1767.
- Li Y, Hansotia T, Yusta B, Ris F, Halban PA & Drucker DJ (2003). Glucagon-like peptide-1 receptor signaling modulates beta cell apoptosis. *J Biol Chem* **278**, 471–478.
- Ling Z, Kiekens R, Mahler T, Schuit FC, Pipeleers-Marichal M, Sener A, Klöppel G, Malaisse WJ & Pipeleers DG (1996). Effects of chronically elevated glucose levels on the functional properties of rat pancreatic  $\beta$ -cells. *Diabetes* **45**, 1774–1782.
- Luo C, Burgeon E, Carew JA, McCaffrey PG, Badalian TM, Lane WS, Hogan PG & Rao A (1996). Recombinant NFAT1 (NFATp) is regulated by calcineurin in T cells and mediates transcription of several cytokine genes. *Mol Cell Biol* **16**, 3955–3966.
- Maechler P & Wollheim CB (2001). Mitochondrial signals in glucose-stimulated insulin secretion in the beta cell. *J Physiol* **529**, 49–56.
- Malaisse WJ, Hutton JC, Kawazu S & Sener A (1978). The stimulus-secretion coupling of glucose-induced insulin release. Metabolic effects of menadione in isolated islets. *Eur J Biochem* **87**, 121–130.
- Meier S, Hucking K, Ritzel R, Holst JJ, Schmiegel WH & Nauck MA (2003). Absence of a memory effect for the insulinotropic action of glucagon-like peptide 1 (GLP-1) in healthy volunteers. *Horm Metab Res* **35**, 551–556.
- Meier JJ, Nauck MA, Kranz D, Holst JJ, Deacon CF, Gaeckler D, Schmidt WE & Gallwitz B (2004). Secretion, degradation, and elimination of glucagon-like peptide 1 and gastric inhibitory polypeptide in patients with chronic renal insufficiency and healthy control subjects. *Diabetes* **53**, 654–662.
- Moens K, Berger V, Ahn J-M, Chris Van Schravendijk C, Hruba VJ, Pipeleers D & Schuit F (2002). Assessment of the role of interstitial glucagon in the acute glucose secretory responsiveness of in situ pancreatic  $\beta$ -cells. *Diabetes* **51**, 669–675.
- Moens K, Heimberg H, Flamez D, Huypens P, Quartier E, Ling Z, Pipeleers D, Gremlich S, Thorens B & Schuit F (1996). Expression and functional activity of glucagon, glucagon-like peptide I, and glucose-dependent insulinotropic peptide receptors in rat pancreatic islet cells. *Diabetes* **45**, 257–261.
- Newgard CB & McGarry JD (1995). Metabolic coupling factors in pancreatic beta-cell signal transduction. *Annu Rev Biochem* **64**, 689–719.
- Nielsen LL, Young AA & Parkes DG (2004). Pharmacology of exenatide (synthetic exendin-4): a potential therapeutic for improved glycemic control of type 2 diabetes. *Regul Pept* **117**, 77–88.

- Odom DT, Zizlsperger N, Gordon DB, Bell GW, Rinaldi NJ, Murray HL, Volkert TL, Schreiber J, Rolfe PA, Gifford DK, Fraenkel E, Bell GI & Young RA (2004). Control of pancreas and liver gene expression by HNF transcription factors. *Science* **303**, 1378–1381.
- Ohneda K, Ee H & German M (2000). Regulation of insulin gene transcription. *Semin Cell Dev Biol* **11**, 227–233.
- Okamura H, Aramburu J, Garcia-Rodriguez C, Viola JP, Raghavan A, Tahiliani M, Zhang X, Qin J, Hogan PG & Rao A (2000). Concerted dephosphorylation of the transcription factor NFAT1 induces a conformational switch that regulates transcriptional activity. *Mol Cell* **6**, 539–550.
- Permutt MA (1974). Effect of glucose on initiation and elongation rates in isolated rat pancreatic islets. *J Biol Chem* **249**, 2738–2742.
- Pierce KL, Maudsley S, Daaka Y, Luttrell LM & Lefkowitz RJ (2000). Role of endocytosis in the activation of the extracellular signal-regulated kinase cascade by sequestering and nonsequestering G protein-coupled receptors. *Proc Natl Acad Sci U S A* **97**, 1489–1494.
- Rachman J, Barrow BA, Levy JC & Turner RC (1997). Near-normalisation of diurnal glucose concentrations by continuous administration of glucagon-like peptide-1 (GLP-1) in subjects with NIDDM. *Diabetologia* **40**, 205–211.
- Rao A, Luo C & Hogan PG (1997). Transcription factors of the NFAT family: regulation and function. *Annu Rev Immunol* **15**, 707–747.
- Renstrom E, Eliasson L & Rorsman P (1997). Protein kinase A-dependent and -independent stimulation of exocytosis by cAMP in mouse pancreatic B-cells. *J Physiol* **502**, 105–118.
- Roduit R, Morin J, Masse F, Segall L, Roche E, Newgard CB, Assimacopoulos-Jeannet F & Prentki M (2000). Glucose down-regulates the expression of the peroxisome proliferator-activated receptor- $\alpha$  gene in the pancreatic beta-cell. *J Biol Chem* **275**, 35799–35806.
- Ron D (2002). Translational control in the endoplasmic reticulum stress response. *J Clin Invest* **110**, 1383–1388.
- Rorsman P (1997). The pancreatic beta-cell as a fuel sensor: an electrophysiologist's viewpoint. *Diabetologia* **40**, 487–495.
- Rutter GA (2001). Nutrient-secretion coupling in the pancreatic islet beta-cell: recent advances. *Mol Aspects Med* **22**, 247–284.
- Scheuner D, Song B, McEwen E, Lui C, Laybutt R, Gillespie P, Saunders T, Bonner-Weir S & Kaufman RJ (2001). Translational control is required for the unfolded protein response and *in vivo* glucose homeostasis. *Mol Cell* **7**, 1165–1176.
- Schuit F, De Vos A, Farfari S, Moens K, Pipeleers D, Brun T & Prentki M (1997). Metabolic fate of glucose in purified islet cells. Glucose-regulated anaplerosis in beta cells. *J Biol Chem* **272**, 18572–18579.
- Schuit F, De Vos A, Flamez D & Pipeleers D (2002). Glucose-regulated gene expression maintaining the glucose-responsive state of beta cells. *Diabetes* **51**, S326–S332.
- Schuit FC, Huypens P, Heimberg H & Pipeleers DG (2001). Glucose sensing in pancreatic beta-cells: a model for the study of other glucose-regulated cells in gut, pancreas, and hypothalamus. *Diabetes* **50**, 1–11.
- Schuit FC, In't Veld PA & Pipeleers DG (1988). Glucose stimulates proinsulin biosynthesis by a dose-dependent recruitment of pancreatic beta cells. *Proc Natl Acad Sci U S A* **85**, 3865–3869.
- Schuit FC, Kiekens R & Pipeleers DG (1991). Measuring the balance between insulin synthesis and insulin release. *Biochem Biophys Res Commun* **178**, 1182–1187.
- Seufert J, Weir GC & Habener JF (1998). Differential expression of the insulin gene transcriptional repressor CCAAT/enhancer-binding protein beta and transactivator islet duodenum homeobox-1 in rat pancreatic beta cells during the development of diabetes mellitus. *J Clin Invest* **101**, 2528–2539.
- Shalev A, Pise-Masison CA, Radonovich M, Hoffmann SC, Hirshberg B, Brady JN & Harlan DM (2002). Oligonucleotide microarray analysis of intact human pancreatic islets: identification of glucose-responsive genes and a highly regulated TGF $\beta$  signaling pathway. *Endocrinology* **143**, 3695–3698.
- Suga S, Kanno T, Dobashi Y & Wakui M (1997a). GLP-1 (7–36) amide activates L-type Ca<sup>2+</sup> channels of pancreatic B-cells through c-AMP signaling. *Jpn J Physiol* **47**, S13–S14.
- Suga S, Kanno T, Nakano K, Takeo T, Dobashi Y & Wakui M (1997b). GLP-1 (7–36) amide augments Ba<sup>2+</sup> current through L-type Ca<sup>2+</sup> channel of rat pancreatic beta-cell in a cAMP-dependent manner. *Diabetes* **46**, 1755–1760.
- Takahashi N, Kadowaki T, Yazaki Y, Ellis-Davies GC, Miyashita Y & Kasai H (1999). Post-priming actions of ATP on Ca<sup>2+</sup>-dependent exocytosis in pancreatic beta cells. *Proc Natl Acad Sci U S A* **96**, 760–765.
- Tillmar L, Carlsson C & Welsh N (2002). Control of insulin mRNA stability in rat pancreatic islets. Regulatory role of a-3'-untranslated region pyrimidine-rich sequence. *J Biol Chem* **277**, 1099–1106.
- Tseng CC & Zhang XY (1998). The cysteine of the cytoplasmic tail of glucose-dependent insulinotropic peptide receptor mediates its chronic desensitization and down-regulation. *Mol Cell Endocrinol* **139**, 179–186.
- Tseng CC & Zhang XY (2000). Role of G protein-coupled receptor kinases in glucose-dependent insulinotropic polypeptide receptor signaling. *Endocrinology* **141**, 947–952.
- Vahl TP, Paty BW, Fuller BD, Prigeon RL & D'Alessio DA (2003). Effects of GLP-1-(7–36)NH<sub>2</sub>, GLP-1-(7–37), and GLP-1-(9–36)NH<sub>2</sub> on intravenous glucose tolerance and glucose-induced insulin secretion in healthy humans. *J Clin Endocrinol Metab* **88**, 1772–1779.

- Wang H, Maechler P, Antinozzi PA, Herrero L, Hagenfeldt-Johansson KA, Bjorklund A & Wollheim CB (2003). The transcription factor SREBP-1c is instrumental in the development of beta-cell dysfunction. *J Biol Chem* **278**, 16622–16629.
- Webb GC, Akbar MS, Zhao C & Steiner DF (2000). Expression profiling of pancreatic beta cells: glucose regulation of secretory and metabolic pathway genes. *Proc Natl Acad Sci U S A* **97**, 5773–5778.
- Welsh M, Nielsen DA, MacKrell AJ & Steiner DF (1985). Control of insulin gene expression in pancreatic beta-cells and in an insulin-producing cell line, RIN-5F cells. II. Regulation of insulin mRNA stability. *J Biol Chem* **260**, 13590–13594.
- Welsh M, Scherberg N, Gilmore R & Steiner DF (1986). Translational control of insulin biosynthesis. Evidence for regulation of elongation, initiation and signal-recognition-particle-mediated translational arrest by glucose. *Biochem J* **235**, 459–467.
- Wheeler MB, Gelling RW, Hinke SA, Tu B, Pederson RA, Lynn F, Ehses J & McIntosh CHS (1999). Characterization of the carboxyl-terminal domain of the rat glucose-dependent insulinotropic polypeptide (GIP) receptor. A role for serines 426 and 427 in regulating the rate of internalization. *J Biol Chem* **274**, 24593–24601.
- Widmann C, Dolci W & Thorens B (1996). Desensitization and phosphorylation of the glucagon-like peptide-1 (GLP-1) receptor by GLP-1 and 4-phorbol 12-myristate 13-acetate. *Mol Endocrinol* **10**, 62–75.
- Widmann C, Dolci W & Thorens B (1997). Internalization and homologous desensitization of the GLP-1 receptor depend on phosphorylation of the receptor carboxyl tail at the same three sites. *Mol Endocrinol* **11**, 1094–1102.
- Wobser H, Dussmann H, Kogel D, Wang H, Reimertz C, Wollheim CB, Byrne MM & Prehn JH (2002). Dominant-negative suppression of HNF-1 alpha results in mitochondrial dysfunction, INS-1 cell apoptosis, and increased sensitivity to ceramide-, but not to high glucose-induced cell death. *J Biol Chem* **277**, 6413–6421.
- Xie J, Lee JA, Kress TL, Mowry KL & Black DL (2003). Protein kinase A phosphorylation modulates transport of the polypyrimidine tract-binding protein. *Proc Natl Acad Sci U S A* **100**, 8776–8781.
- Xu G, Marshall CA, Lin TA, Kwon G, Munivenkatappa RB, Hill JR, Lawrence JC & McDaniel ML (1998). Insulin mediates glucose-stimulated phosphorylation of PHAS-I by pancreatic beta cells. An insulin-receptor mechanism for autoregulation of protein synthesis by translation. *J Biol Chem* **273**, 4485–4491.
- Zawalich WS, Diaz VA & Zawalich KC (1988). Role of phosphoinositide metabolism in induction of memory in isolated perfused rat islets. *Am J Physiol* **254**, E609–E616.
- Zawalich WS & Zawalich KC (2000). Glucose-induced insulin secretion from islets of fasted rats: modulation by alternate fuel and neurohumoral agonists. *J Endocrinol* **166**, 111–120.

### Acknowledgements

S.A.H. is Visiting Postdoctoral Fellow at the FWO Vlaanderen; current research of F.C.S. is supported by grants from the Juvenile Diabetes Research Foundation (1- 2002-801) and from the KU-Leuven (GOA/2004/11).