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β-Cells at the crossroads: choosing between insulin granule production and proliferation

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Pancreatic β-cells are the sole source of insulin, the major hormonal regulator of glycaemia. In physiological and pathological conditions with increased insulin demand, β-cells adjust their insulin output either through increased insulin secretory granule (ISG) biogenesis and secretion, or hyperplasia. Failure of these compensatory mechanisms eventually results in hyperglycaemia and diabetes mellitus. Both of these major adaptive behaviours are positively regulated by several extrinsic factors, such as glucose, GLP-1, insulin and growth hormones (GH). Still unclear, however, it is how β-cells in response to these stimuli opt for one or the other strategy at a given time. Here we review recent advances concerning the factors and pathways that enhance ISG biogenesis and β-cell replication, and propose the existence of 'switch factors' that play a key role in regulating the shift between these two adaptive responses.
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Introduction

Pancreatic β-cells in the islet of Langerhans are the sole source of insulin, the main hormonal regulator of glycaemia. During embryonic development, β-cells are derived from pancreatic progenitor cells through a series of cellular processes that coordinate progenitor self-renewal with differentiation [1]. In the neonatal period, a marked increase in the proliferation rate of β-cells allows a massive expansion of their total mass [1]. Although neogenesis of β-cells, from precursor cells that are putatively found in the pancreatic ducts, may persist throughout life, it is now widely accepted that the majority of new adult β-cells originate from preexisting β-cells [2,3]. Replication of the differentiated β-cells slows down with time, maintaining the normal β-cell mass with a very low rate of β-cell turnover [1].

The β-cell mass may nevertheless undergo remarkable changes. For instance, in conditions associated with increased metabolic demands, such as pregnancy and obesity, β-cells upregulate their insulin synthesis and secretion as well as their replication rate. Failure of these compensatory mechanisms leads to hyperglycaemia and eventually the onset of diabetes mellitus. Thus, elucidating the molecular mechanisms involved in β-cell adaptation is critical for understanding the pathophysiology of this disease and improving its treatment. Concerning this latter point, an obvious approach is to attempt the expansion of β-cells from isolated adult islets *in vitro*. Genetic lineage-tracing studies in β-cells from transgenic mouse islets demonstrated that β-cells in culture could survive for several weeks, but dedifferentiate. Due to their low replication rate they are also overtaken by cells

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with faster replication rates, such as fibroblasts [4]. Lineage-tracing studies in primary human β-cells using lentivirus vectors, on the other hand, suggest that they can replicate to a significant degree *in vitro* [4]. Thus, a goal of future studies is the optimization of protocols that would allow β-cells to proliferate while retaining their differentiated characteristics. Elucidating how β-cells balance their activity between insulin secretory granule (ISG) production and replication may also foster the identification of treatments for promoting β-cell regeneration *in vivo*. Following this perspective, we briefly review here recent advances in our understandings of the factors and pathways that regulate ISG biogenesis and β-cell proliferation in the face of high insulin demands.

Regulation of ISG Biogenesis

Glucose

Glucose stimulation prompts β-cells to release insulin [5] while increasing production of insulin in order to replenish its stores. This adaptive response would not be effective, however, unless accompanied by the concomitant increased biosynthesis of other luminal and intrinsic membrane proteins that are necessary for the proper assembly and function of ISGs. Examples of luminal proteins regulated by glucose are the prohormone convertases PC1 and PC2, which convert pro-insulin into insulin, and the granins, which may facilitate the sorting and packaging of peptide hormones into ISGs. Most studies have focused their attention on the mechanisms that regulate the biosynthesis of insulin (figure 1), due to its physiological prominence, rather than on the whole ISG as a functional unit.

Glucose enhances insulin biosynthesis through post-transcriptional and transcriptional mechanisms. Upregulation of insulin production within 2 hours after glucose stimulation depends almost exclusively on post-transcriptional mechanisms, while transcriptional regulation becomes critical thereafter [6]. Post-transcriptional control includes enhanced stability and translocation of preproinsulin mRNA, its recruitment to the endoplasmic reticulum as well as its increased translation initiation and elongation [7]. The rapidly increased translation of the preproinsulin mRNA depends on both its 5′- and- 3′-untranslated region (UTRs) [8]. Relevant to this topic, our group has reported that glucose stimulation of rat pancreatic islets and insulinoma INS-1 cells promotes the nucleocytoplasmic translocation of heterogeneous nuclear ribonucleoprotein I, also known as polypyrimidine tract binding protein 1 (PTBP1) [9]. In the cytosol, PTBP1 binds to a degenerated pyrimidine-rich motif in the 3′-UTR of mRNAs of preproinsulin and other ISG components, thereby increasing their stability and translation. Conversely, knockdown of PTBP1 in INS-1 cells by RNA interference leads to the depletion of the ISG stores. To evaluate whether PTBP1 is critical for the biogenesis of ISGs *in vivo* the generation of a mouse, in which the gene is selectively removed from β-cells in an inducible fashion, is in progress.

The regulation of insulin gene transcription in response to glucose is complex. In the first 2–6 hours, exposure to high concentrations of glucose stimulates insulin gene transcription. This stimulatory effect is lost after 12 hours of continuous exposure to high glucose, and by 24 hours glucose inhibits the activity of the insulin promoter [10]. These opposite effects of glucose on insulin gene transcription depend on extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) [10]. Briefly, glucose metabolism in β-cells increases the ATP/ADP ratio, which in turn leads to the closure of the ATP-dependent K^+ channels, and thus membrane depolarization and the opening of voltage-dependent Ca^{2+} channels. The increased levels of intracellular Ca^{2+} stimulate the exocytosis of ISGs and the activity of numerous kinases, including calmodulin kinase II and IV, protein kinase A (PKA), protein kinase C, phosphatidylinositol 3-kinase (PI3K) and ERK1/2. Activation of ERK1/2 also depends on the glucose-induced synthesis of cAMP [11] and the increased activity of calcineurin, a Ca^{2+}- and calmodulin-dependent phosphatase [12]. Activated ERK1/2, in turn, phosphorylate the transcription factors BETA2, PDX-1, MAFA, E2A and NFAT, thereby enhancing their nuclear translocation and/or DNA binding, and thus the transcription of insulin [10]. The synergic activity of BETA2, PDX-1 and MAFA, in particular, largely accounts for the β-cell-restricted expression of insulin, [10] and mutations of PDX-1 and BETA2 are responsible for maturity onset diabetes of the young (MODY) 4 and 6 respectively [13]. Glucose can stimulate the nuclear translocation of PDX-1 also through the PI3K pathway [14], whereas nuclear translocation of NFAT follows its dephosphorylation by calcineurin [15].

Negative regulators of insulin transcription, such as the CCAAT/enhancer-binding protein beta (C/EBP-β), have also been identified [12]. Chronic exposure (>24 h) of β-cells to 11 mM glucose induces the expression of C/EBP-β, which binds to NFAT on the insulin-gene promoter, thus replacing the NFAT-MAF complex. Induction of C/EBP-β is independent of ERK1/2, but its binding to DNA requires ERK1/2 activity. Thus, it

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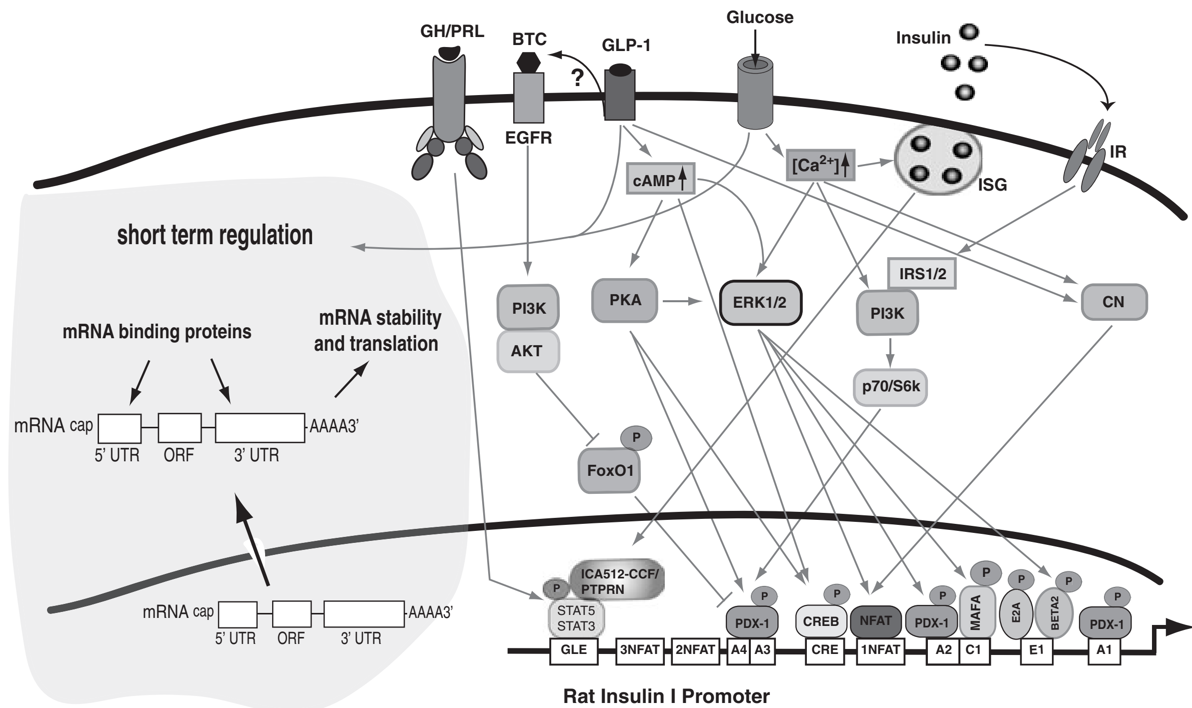

Fig. 1 Transcriptional and post-transcriptional regulation of insulin secretory granule (ISG) biogenesis. Right panel (in colour): signal transduction pathways and the promoter elements that regulate the transcription of the rat insulin gene 1. Stimulation of β-cells with glucose increases intracellular calcium levels, which leads to the phosphorylation of ERK1/2 and activation of the transcription factors PDX-1, MAFA, NFAT and BETA2. Calcium activates also PDX-1 and NFAT through PI3K and calcineurin respectively. In addition, calcium triggers ISG exocytosis, and thereby both insulin secretion and the calpain-mediated cleavage of islet cell autoantigen 512 (ICA512). Signalling by ICA512 converges with that of GH/PRL, which activates STAT3/5 to promote insulin gene transcription and ISG biogenesis in general. Stimulation of β-cells with GLP-1 activates adenyl cyclase, and thus the production of cAMP, which activates PKA. Co-stimulation of β-cells with glucose, and GLP-1 prompts also the activation of ERK1/2. The effect of GLP-1 on insulin transcription is mediated by CREB in a cAMP/PKA-dependent and -independent manner. GLP-1 can also activate PI3K through the transactivation of EGFR by cSrc-activated betacellulin, but the impact of this mechanism on insulin transcription is unknown. Upon phosphorylation by AKT, FoxO1 is retained in the cytoplasm, thus preventing its negative effect on PDX-1-mediated transcription of insulin. Albeit still debated, autocrine activation of IR may promote insulin transcription through IRS and PI3K, which stimulates PDX-1. Left panel (in grey): stimulation of β-cells with either glucose or GLP-1 leads to a rapid increase in the translation of insulin. This post-transcriptional pathway involves mRNA binding proteins, which bind to insulin mRNA and increase its stability and translation. AKT, serine/threonine kinase AKT; BETA2, neurogenic differentiation 1; BTC, betacellulin; CN, calcineurin; CRE, cAMP response element; CREB, cAMP-response element binding protein; EGFR, epidermal growth factor receptor; ERK1/2, extracellular signal-regulated protein kinases 1 and 2; FoxO1, forkhead box O1; GH, growth hormone; GLE, GH-responsive GAS-like element; GLP-1, glucagon-like peptide 1; ICA512-CCF, ICA512-CCF cytosolic fragment; IR, insulin receptor; IRS, insulin receptor substrate; MAFA, v-maf musculoaponeurotic fibrosarcoma oncogene homologue A; NFAT, nuclear factor of activated T-cells; p70/S6k, RPS6-p70-protein kinase; PDX-1, pancreatic and duodenal homeobox 1; PI3K, phosphatidylinositol 3-kinase; PKA, protein kinase A; PRL, prolactin; STAT, signal transducer and activator of transcription.

appears that ERK1/2 have opposite effects on insulin transcription depending on how long β-cells are exposed to high concentrations of glucose [12].

Based on studies in INS-1 cells, our group has recently proposed an additional Ca^{2+}-regulated pathway that directly couples the glucose-induced exocytosis of ISGs with their biogenesis [16,17]. This pathway involves the receptor tyrosine phosphatase-like protein ICA512/IA-2/PTPRN, which is enriched in the membrane of ISGs, and neurosecretory granules in general. When ISGs undergo exocytosis, ICA512 is transiently inserted in the plasma membrane and its intracellular part is cleaved by the Ca^{2+}-dependent cysteine protease calpain-1 [18]. This cleavage generates the

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ICA512 cleaved cytosolic fragment (ICA512-CCF), which includes the catalytically inactive tyrosine phosphatase domain. In addition to promoting insulin secretion through the mobilization of stored ISGs [19], ICA512-CCF moves into the nucleus [16], where it acts as a pseudophosphatase which binds tyrosine phosphorylated (PY) STAT5 and STAT3, and prevents their dephosphorylation [17]. By maintaining the PY-STAT levels induced by activation of GH/cytokine receptors, ICA512-CCF increases therefore the transcription of genes downstream of STATs, including insulin and other ISGs components [17]. To evaluate the relevance of this pathway *in vivo*, we are generating a knock-in mouse that overexpresses ICA512-CCF in an inducible and β-cell restricted fashion.

GLP-1

Glucagon-like peptide-1 (GLP-1) is an incretin hormone that is mainly produced by enteroendocrine L cells of the gut in response to nutrient ingestion. Binding of GLP-1 to its G-protein coupled receptor in β-cells leads to the activation of adenylate cyclase and production of cAMP, which in turn augments glucose-stimulated insulin secretion through PKA- dependent and independent pathways [20,21]. Additionally, GLP-1 increases insulin synthesis and β-cell proliferation. In this section, we discuss current knowledge concerning how GLP-1 enhances insulin biosynthesis (figure 1).

Overall, GLP-1 enhances insulin production through post-transcriptional and transcriptional mechanisms that overlap to a certain degree with those elicited by glucose. For instance, activation of PKA by GLP-1 induces the phosphorylation and nucleocytoplasmic translocation of PTBP1. As mentioned previously, cytosolic PTBP1 stabilizes insulin mRNA and enhances its translation [22]. In a recent proteomic study, we have shown that stimulation of INS-1 cells with the cAMP elevating agent IBMX prompts rapid post-transcriptional changes in seven other heterogeneous nuclear ribonucleoproteins [23]. To understand the impact of this complex post-transcriptional regulation on ISG biogenesis will require further studies. In the presence of stimulatory concentrations of glucose, GLP-1 activates also ERK1/2 through the concerted action of PKA and calmodulin kinases II [24]. Furthermore, GLP-1 triggers the calcineurin-dependent dephosphorylation and nuclear translocation of NFAT [25]. The roles of ERK1/2 and NFAT in insulin gene transcription have been discussed above.

Similarly to glucose, GLP-1 stimulates insulin gene transcription also through PDX-1. Specifically, GLP-1 upregulates in a PKA-dependent manner the expression of PDX-1, as well as its nuclear localization and binding to the A elements of rat insulin I promoter [26]. The GLP-1-induced nuclear localization of PDX-1 is partly mediated via the inactivation of FoxO1, which represses the activity of the PDX-1 promoter and fosters the nuclear exclusion of PDX-1. Transactivation of the EGF receptor by GLP-1 through betacellulin induces PI3K/AKT signalling. Upon phosphorylation by AKT, FoxO1 is exported from the nucleus, thus allowing the acute nuclear translocation of PDX-1 and its further synthesis [26]. GLP-1 also stimulates the insulin promoter activity through its cAMP-responsive elements [27]. However, the detail mechanisms involved in this process remain unclear.

Insulin

In addition to regulate glucose homeostasis, insulin has been proposed to modulate its own biosynthesis through the activation of its receptors on β-cells (figure 1) [28]. The two major pathways downstream of the insulin receptor (IR) involve PI3K and the ras-mitogen-activated protein kinase [28]. This autocrine feedback of insulin on its expression, however, remains controversial. Some reports showed no or just a negative role of insulin on its own production [29,30], while others supported a positive effect [31–34]. Overexpression of the IR in β-cells was found to increase both basal and glucose-induced insulin biosynthesis [32,34], which was instead diminished upon reduced expression of the receptor [31]. Selective deletion of the IR in the β-cells of βIRKO mice prevents insulin mRNA levels from increasing in response to stimulation with high glucose or insulin [35,36]. Further studies indicated that the positive effect of insulin on its own gene transcription was mediated by PI3K and p70s6k [32,36]. Glucose-induced nucleocytoplasmic translocation of FoxO1 was also attributed to the activation of PI3K signalling by insulin [37,38]. Exclusion of FoxO1 from nucleus leads to activation of PDX-1 and in turn insulin gene transcription [37,38]. In spite of these findings, it is nevertheless unclear how β-cells may generate a graded response to insulin or even remain sensitive to it, being conceivably exposed to far greater concentrations of the hormone than other cell types such as adipocytes or muscle cells. The identification of the autocrine insulin loop as a key mechanism for regulation of insulin production raises the question whether in each neuroendocrine cell type the coupling between exocytosis and biogenesis of secretory granules is mediated through a different mechanism.

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Regulation of β-Cell Replication

Many factors and intracellular signalling pathways that regulate insulin biosynthesis stimulate also β-cell proliferation, conceivably as an alternative strategy to increase the overall insulin output. In several instances, the link between these factors and the cell cycle machinery remains unclear. In the following paragraphs, we briefly give an overview of several major factors that drive β-cell proliferation. More comprehensive overviews of β-cell mitogens are available in the literature [39,40].

Glucose

Glucose is a potent β-cell mitogen. For instance, it was recently shown that continuous infusion of mice with physiological levels of glucose for 4 days increased β-cell replication by fivefold [41]. However, because this treatment induced also hyperinsulinaemia, it cannot be excluded that the dominant mitogen was insulin (see later) rather than glucose. On the other hand, recent studies point to several other mechanisms that may link glucose to the β-cell proliferation machinery.

Evidence for the important role of glucose on β-cell replication was provided by studies on mice with targeted deletion of glucokinase (Gck) in β-cells [42]. Despite a similar degree of insulin resistance, $Gck^{+/-}$ mice fed with a high-fat diet displayed decreased β-cell replication and hyperplasia relative to their wild-type littermates [42]. The high-fat feeding increased also the β-cell expression of IR substrate 2 (Irs2) in wild-type, but not in $Gck^{+/-}$ mice [42]. Accordingly, overexpression of Irs2 in β-cells of high-fat diet-fed $Gck^{+/-}$ mice could partially prevent diabetes by increasing the β-cell mass [42]. Based on these findings, it was suggested that haploinsufficiency of Gck impairs intracellular Ca^{2+} responses to glucose and fails to trigger the phosphorylation of CREB, which upregulates the gene expression of Irs2 [42]. Another study showed that glucose induces the transcription of Irs2 in rat primary β-cells, even when glucose-stimulated insulin secretion is inhibited by somatostatin [43].

The glucose-Irs2 signalling conceivably propagates through PI3K and AKT. As mentioned, AKT phosphorylates FoxO1, leading to its nuclear export and inactivation, and thus fostering the activation of PDX-1 [37,38]. Overexpression of PDX-1 in $Irs2^{-/-}$ mice restores the β-cell mass through increased proliferation [44]. It is still unclear, however, how PDX-1 influences the cell cycle machinery. In some cell lines, FoxO1 was shown to downregulate cyclin D1 and D2, while it upregulates $p27^{kip1}$ [45]. Evidence that a similar pathway operates in β-cells is nevertheless missing. Clues in this direction are the nuclear exclusion of FoxO1 and the decreased expression of cyclin D2 in the $Gck^{+/-}$ mice [42].

The study of mice with a β-cell-specific deletion of the regulatory subunit of calcineurin Cnb1 indicated that calcineurin/NFAT signalling is involved in glucose-induced β-cell proliferation [46]. These mice, in particular, develop age-dependent diabetes with decreased β-cell replication and mass, consistent with the reduced expression of cyclin D1, D2 and Cdk4 in $Cnb1^{-/-}$β-cells. Both these deficits and diabetes, in turn, were rescued by the conditional expression of active NFATc1 in $Cnb1^{-/-}$β-cells. Because calcineurin is a Ca^{2+}-dependent phosphatase, this pathway is likely to operate not only in response to glucose, but also to any other mitogens and segretagogues that increase intracellular Ca^{2+} concentration.

Another Ca^{2+}-regulated pathway involves ICA512, which enhances β-cell proliferation by upregulating cyclin D levels through STATs [47]. Specifically, we found that knockdown of ICA512 decreased the levels of cyclin D1 and the proliferation of INS-1 cells, and that β-cell regeneration was impaired in partially pancreatectomized $ICA512^{-/-}$ mice. Conversely, overexpression of ICA512-CCF in INS-1 cells upregulated cyclin D1 and D2 levels and cell proliferation. Furthermore, this upregulation of cyclin D1 and D2 was reduced upon knockdown of STAT3 and STAT5 respectively, and it did not appear to depend on insulin signalling [47]. Taken together, these data suggest that Ca^{2+}/calpain-1-induced generation of ICA512-CCF upon granule exocytosis promotes not only insulin secretion and ISG biogenesis [16] but also β-cell proliferation by converging on STAT5 and STAT3 signalling [47].

Recently, a different mechanism for how ICA512 and its paralogue phogrin/IA-2β/PTPRN2 regulate glucose-stimulated β-cell proliferation has been proposed [48]. In particular, it was shown that knockdown of either protein decreased the proliferation of MIN6 and INS-1E cells, accompanied by the reduction of Irs2 levels. Conversely, downregulation of phogrin did not affect the proliferation of a β-cell line derived from the βIRKO mouse. Both phogrin and ICA512 interacted with the IR, and in the case of phogrin this interaction was promoted by glucose stimulation and correlated with the stabilization of Irs2. Thus, it was proposed that phogrin and ICA512 are key regulators of the autocrine insulin action in β-cells. Further studies in mouse animal models will help to clarify how ICA512 and phogrin support glucose-induced replication of β-cells.

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GLP-1

In addition to enhance insulin secretion and biosynthesis, GLP-1 restores glucose competence in glucose-resistant β-cells and expands the β-cell mass [26]. These properties led to the approval of long-acting GLP-1 analogs, such as exendin-4, as therapeutic agents for type 2 diabetes. Administration of GLP-1/exendin-4, in particular, improves glucose tolerance and increases β-cell mass in rodents with surgically or chemically induced diabetes by stimulating β-cell proliferation and islet neogenesis, while preventing β-cell apoptosis [26]. Importantly, these beneficial effects were maintained long after the termination of GLP-1 or exendin-4 infusion in diabetic subjects [26].

GLP-1 appears to promote β-cell proliferation through multiple signalling pathways. The increment of cAMP levels in response to GLP-1 activates PKA, which further regulates PDX-1 and CREB [26]. Accordingly, exendin-4 failed to increase β-cell proliferation in mice with β-cell restricted deletion of PDX-1 [49]. CREB, in turn, stimulates the transcription of Irs2, which is upstream of PI3K in insulin signalling [26]. PI3K was also stimulated in response to GLP-1-induced transactivation of the EGF receptor by betacellulin, which fosters β-cell replication [26]. PI3K, in turn, activates AKT and PKCζ, which are both involved in β-cell proliferation. The inactivation of FoxO1 by AKT is required for the proliferative action of GLP-1, as indicated by the inability of exendin-4 to increase the replication of β-cells expressing a constitutively nuclear FoxO1 transgene [50]. Unexpectedly, GLP-1 may also induce the expression of several negative regulators of cAMP/CREB and ERK1/2 signalings, such as cAMP-response element modulator-α and DUSP14. Knockdown of these factors enhanced GLP-1-induced β-cell proliferation [51]. Additional studies showed that GLP-1 analogues can increase the expression of cyclin D1 through various pathways, including the cAMP/CREB, PI3K and ERK1/2 pathways [52,53].

Insulin

The role of insulin and insulin-like growth factor-1 (IGF-1) in β-cell proliferation was extensively studied in β-cell specific IR (βIRKO) and IGF-1 receptor (IGF-R; βIGFRKO) knockout mice. βIRKO, but not βIGFRKO mice, showed an age-dependent reduction of β-cell mass and increased susceptibility to develop diabetes [35,54], pointing to insulin/IR having a more prominent role in β-cell proliferation. $Irs1^{-/-}$ and $Irs2^{-/-}$ mice displayed systemic insulin resistance. However, in $Irs1^{-/-}$ mice the β-cell mass showed a compensatory increase [45], whereas in $Irs2^{-/-}$ mice the mass and proliferation of β-cells progressively decreased, while apoptosis was increased [55]. These data indicate that Irs2 is a key mediator of insulin-regulated β-cell proliferation.

Irs2-mediated recruitment of PI3K to the IR leads to the activation of 3-phosphoinositide-dependent protein kinase 1 (PDK1), which in turn activates AKT. Overexpression of AKT was shown to promote islet growth [56,57], while β-cell specific deletion of PDK1 reduced β-cell mass, causing insulin deficiency [58]. Conversely, β-cell specific deletion of phosphatase with tensin homology (PTEN), which negatively regulates PI3K signalling, increased the islet cell mass [59]. Further studies have shown that AKT phosphorylates $p27^{kip1}$, an inhibitor of cell cycle at G1 phase, leading to its nuclear exclusion [60]. AKT may also downregulate $p27^{kip1}$ function through the inactivation of FoxO1, which enhances both $p27^{kip1}$ transcription and stability [61]. Deletion of the gene encoding $p27^{kip1}$ or FoxO1 rescues the diabetic phenotype of $Irs2^{-/-}$ mice [37,60]. Moreover, β-cell specific overexpression of constitutively active AKT increased the levels of cyclin D1 and D2, and thus β-cell proliferation, albeit the expression of p21 was also increased [62].

Growth Hormones

Increasing evidence indicates that GH, prolactin (PRL) and placental lactogen (PL) are potent growth factors for pancreatic β-cells [39]. Their effects are especially pronounced in conditions of increased metabolic demand such as pregnancy, in which expression of their receptors on β-cells is increased, [39,63] and a marked hyperplasia of β-cells is observed both in rodents and man [64]. Accordingly, β-cell mass and proliferation are increased in the transgenic mice with misexpression of PL in β-cells [65], while being reduced in mice lacking the PRL/PL receptor (PRLR) or GH receptor (GHR) [66,67]. GHR and PRLR belong to the cytokine receptor superfamily, which activate STAT proteins through their tyrosine phosphorylation by receptor-associated Janus kinases (JAKs). PY-STATs dimerize and translocate to the nucleus where they bind to specific DNA elements and activate the transcription of various genes such as insulin [68] and cyclin D2 [69], thus accounting for their role as potent mediators of mitogenic signals [70]. However, glucose alone cannot activate JAK2/STAT5. On the other hand, GH cannot induce β-cell proliferation unless glucose is present within a physiological concentration range of 6–18 mM [71], suggesting that glucose either through its metabolism

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or through some other mechanism is essential for GH-mediated INS-1 cell proliferation [71]. As discussed previously, ICA512-CCF is a candidate molecule for mediating the convergence of glucose with the STAT pathway elicited by GHs in order to stimulate β-cell proliferation [47].

Recently it has been proposed that PRL promotes adaptive β-cell proliferation through the inhibition of menin [72]. Menin is an endocrine tumour suppressor, which is encoded by the Men1 gene and whose mutation causes multiple endocrine neoplasia type 1. $Men1^{+/-}$ mice display many neuroendocrine tumours and a higher β-cell proliferation rate [45]. Further studies have shown that menin promotes the histone methyltransferase activity of a nuclear complex, which prevents β-cell proliferation by supporting the expression of $p18^{Ink4c}$ and $p27^{kip1}$ [73]. Recently it was shown that Men1 was repressed by Bcl6, whose expression was upregulated by PRL through STAT5 [72].

How do β-Cells Switch Between LSG Production And Proliferation: Searching For 'Switch Factors'

Increasing the production of ISGs or the proliferating rate of β-cells are the two major adaptive strategies for enhancing insulin output in conditions of increased metabolic needs, albeit increased β-cell survival and neogenesis may also contribute to this effect. These two main adaptive behaviours are complementary and are often observed to occur together. This is not surprising, as several regulatory factors and signalling pathways, as we have illustrated above, regulate both processes (table 1). For instance, PDX-1 is critical for β-cell function, including insulin biosynthesis, as well as β-cell proliferation, although how it regulates the latter is still not clear. A key unanswered question, however, is how β-cells choose between production of ISGs and proliferation in response to a given signal. Clearly ISG biogenesis cannot occur while a β-cell is replicating, due to the profound changes in the transcriptional–translational program, the cytoskeleton and the secretory apparatus during cell division. A likely explanation is that the type of adaptive response depends on the duration of the inductive signal, most prominently hyperglycaemia, and its combinatorial association with other factors that concomitantly affect β-cell function.

Increased ISGs production is a quick and short-term adaptation, which exploit both post-transcriptional and transcriptional mechanisms and which should occur first in response to increased insulin demand. Recent findings from our group indicate that PTBP1, while

Table 1 Extrinsic factors and regulation of insulin secretory granule (ISG) production and β-cell proliferation

Extrinsic factors	Pathways/molecules involved in ISG biogenesis	Pathways/molecules involved in β-cell proliferation
Glucose	PTBP1	PTBP1
	Ca^{2+}-ERK1/2-BETA2, PDX-1, MAFA, NFAT	Gck-Ca^{2+}-CREB-IRS2-PI3k-AKT-FoxO1-PDX-1
	Ca^{2+}-Calcineurin-NFAT	Ca^{2+}-Calcineurin-NFAT-cyclin D2, cyclin D1, Cdk4
	PI3K-PDX-1	ICA512-STATs-cyclin D1, cyclin D2
	ICA512-STATs	Phogrin/ICA512-IR-IRS2
GLP-1	cAMP-PTBP1	cAMP-PTBP1
	PKA- and CaMKII-ERK1/2	cAMP-PKA-CREB- IRS2-PI3k-AKT-FoxO1-PDX-1
	Ca^{2+}-Calcineurin-NFAT	Ca^{2+}-Calcineurin-NFAT-cyclin D2, cyclin D1, Cdk4
	PKA-PDX-1	cAMP-PKA-PDX-1
	Betacellulin-EGFR-PI3K-AKT-FoxO1-PDX-1	Betacellulin-EGFR-PI3K-AKT-FoxO1-PDX-1
		cAMP-PKA-CREB-cyclin D1
Insulin	IR-PI3K-p70s6k	IR-IRS2-PI3K-PDK1-AKT-$p27^{kip1}$
	IR-PI3K-FoxO1-PDX-1	IR-PI3K-FoxO1-$p27^{kip1}$
		AKT-cyclin D1, cyclin D2, p21-Cdk4
GH, PRL, PL	JAK-STATs	JAK-STATs-cyclin D1 and D2
		JAK-STAT5-Bcl6-Men1-$p18^{Ink4c}$, $p27^{kip1}$

promoting ISG biogenesis, prevents β-cell proliferation by inhibiting cell cycle (Knoch, K. P. and Solimena, M. in prep.). As long as the PTBP1-mediated inhibition on cell cycle persists, other signals may foster ISG biogenesis, but not β-cell replication. Similar to PTBP1, other glucose-regulated factors may also play a key role in stirring β-cells towards ISG production rather than replication. The activity of these 'switch factors', on the other hand, may transiently be attenuated by persistent exposure of β-cells to high glucose, thus allowing β-cell replication. This process would eventually be reverted upon restoration of euglycaemia as a consequence of increased ISG biogenesis and β-cell mass. The glucose-regulated cycling of such 'switch factors' could therefore be critical for the proper function of β-cells (figure 2). Notably, *in vivo,* but not *in vitro,* β-cells can replicate while retaining the differentiated phenotype. An intriguing possibility is that cycling of the 'switch factors' is progressively lost as a consequence of the static environment applied to cultured β-cells. Thus, in the future it will be interesting to test whether dynamic

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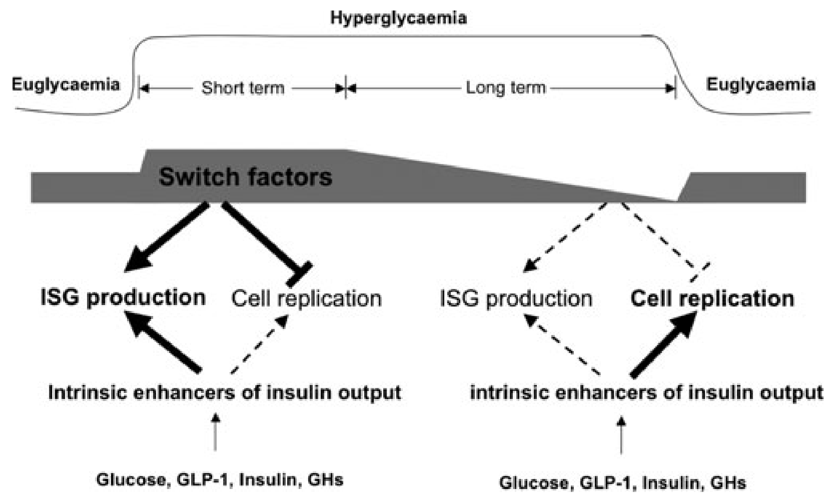

Fig. 2 Model for how switch factors may regulate insulin secretory granule (ISG) biogenesis and β-cell proliferation. Switch factors activated by short-term hyperglycaemia stimulate ISG production but inhibit cell replication. As long as this inhibition persists, other intrinsic enhancers of insulin output activated by extrinsic factors such as glucose, GLP-1, insulin and growth hormones (GHs), can promote ISG biogenesis, but not cell replication. Inactivation of switch factors by long-term hyperglycaemia, however, allows intrinsic enhancers of insulin output to induce β-cell replication, while ISG production is temporarily halted.

changes in the culture conditions would provide an environment more suitable for the *in vitro* production of properly functioning β-cells.

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Conflict of Interest

The authors declare no conflicts of interest.

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