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Regulation of insulin gene transcription

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Abstract

The mammalian insulin gene is exclusively expressed in the beta cells of the endocrine pancreas. Two decades of intensive physiological and biochemical studies have led to the identification of regulatory sequence motifs along the insulin promoter and to the isolation of transcription factors which interact to activate gene transcription. The majority of the islet-restricted (BETA2, PDX-1, RIP3b1-Act/C1) and ubiquitous (E2A, HEB) insulin-binding proteins have been characterized. Transcriptional regulation results not only from specific combinations of these activators through DNA-protein and protein-protein interactions, but also from their relative nuclear concentrations, generating a cooperativity and transcriptional synergism unique to the insulin gene. Their DNA binding activity and their transactivating potency can be modified in response to nutrients (glucose, NEFA) or hormonal stimuli (insulin, leptin, glucagon like peptide-1, growth hormone, prolactin) through kinase-dependent signalling pathways (PI3-K, p38MAPK, PKA, CaMK) modulating their affinities for DNA and/or for each other. From the overview of the research presented, it is clear that much more study is required to fully comprehend the mechanisms involved in the regulated-expression of the insulin gene in the beta cell to prevent its impairment in diabetes. [Diabetologia (2002) 45: 309–326]

Keywords Insulin gene, beta cell, transcription factor, promoter, PDX-1, glucose, diabetes, NEFA, leptin, GLP-1.

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Abbreviations: PDX-1, Pancreatic duodenal homeobox-1; RIPE3, rat insulin promoter element 3; NRE, negative regulatory element; IEF-1, insulin enhancer factor-1; IPF-1, insulin promoter factor-1; STF-1, somatostatin transcription factor-1; IDX-1, islet duodenum homeobox-1; GSF, glucose sensitive factor; IUF-1, insulin upstream factor-1; Isl-1, islet-1; Cdx-3, caudal homeobox-3; Imx-1, LIM homeobox-1; *HNF-1*α, hepatocyte nuclear factor-1α; HMGI(Y), high mobility group protein I (Y); bHLH, basic helix-loop-helix; USF, upstream stimulatory factor; GLUT 2, glucose transporter 2; IAPP, islet amyloid polypeptide; GK, Goto-Kakisaki; ZDF, Zucker diabetic fatty; GLP-1, glucagon like peptide-1; GH, growth hormone; PRL, prolactin; ILPR, insulin linked polymorphic region; VNTR, variable number of tandem repeats; HVR, hypervariable region; PI3-K, phosphatidylinositol 3-kinase; p38MAPK, p38 mitogen activated protein kinase; PKA, protein kinase A; CaMK, Ca/calmodulin-dependent protein kinase; PDE3B, phosphodiesterase 3B

Insulin, synthesized by the beta cells of pancreatic islets, is of major physiological importance in metabolic homeostasis. While mature insulin consists of two polypeptide chains joined by disulphide bridges, the gene encodes for a highly conserved single chain precursor, preproinsulin [1]. In most species preproinsulin exists as a single gene, whereas in the mouse and the rat two non-allelic insulin genes are present. The human insulin gene is located on the short arm of chromosome 11 (p15.5) [2], the rat insulin I and II genes are colocalized on chromosome 1 [3] and the mouse genes are positioned on two different chromosomes, insulin I on chromosome 19 [4] and insulin II on chromosome 7 [5]. In adult islets, the nonallelic genes appear to be coordinately expressed and regulated [6, 7]. The rodent insulin II and the human genes contain three exons and two introns, whilst insulin I lacks the second intron. The organisation and structure of the insulin gene has been reviewed in detail [8]. Insulin is regulat-

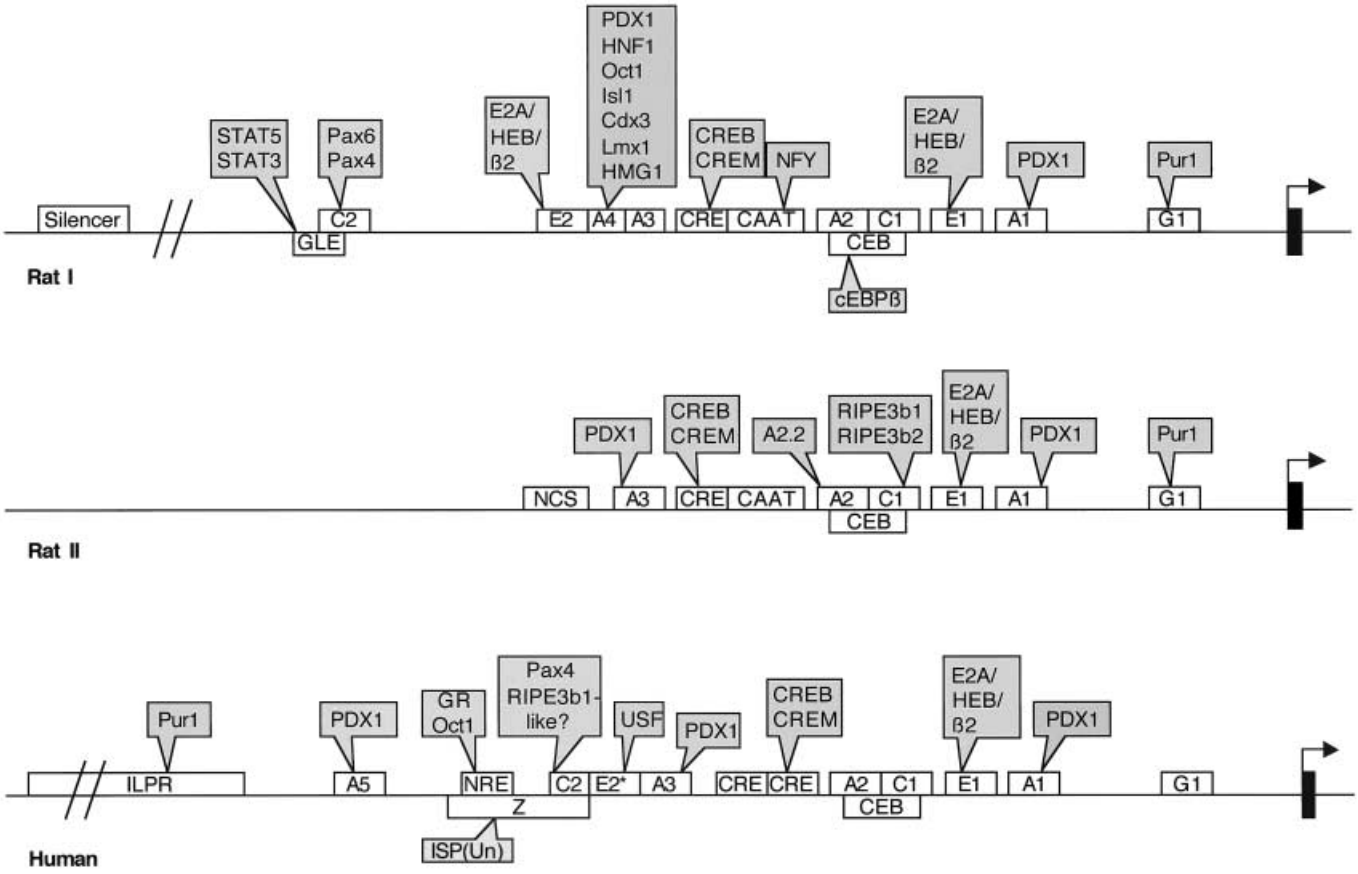

Fig. 1. Organization of human, rat I and rat II insulin enhancer and promoter regions. Cis-acting regulatory elements along the promoter regions of these genes are boxed. Proteins binding to these sequences are indicated above each box. A1-A5, E1-E2, C1-C2, and G1 elements are termed according to the nomenclature in [20]. E2* is an E2-like element; GR, glucocorticoid receptor; GLE, gamma-interferon-activated sequence (GAS)-like element; ISP(Un), islet-specific protein (unidentified); NRE, negative regulatory element; NCS, negative control sequences; Z, Z minienhancer

ed at several levels, from gene transcription to insulin secretion. This review focuses on the transcriptional regulation of the insulin gene.

Beta cell-specific expression of the insulin gene

In the mouse embryo, insulin mRNA is first detected at stage E9 (20 somites) [9] in the foregut area from which the pancreas develops. In humans, insulin-positive cells are already present by the 8th week of embryonic development [10].

In post-natal life, insulin gene expression is restricted to the beta cell. Most studies on the regulated expression of insulin used human and rat genes. Tissue specificity has been shown to be controlled by 5' flanking regulatory regions of the gene [11]: a few hundred basepairs (bp) upstream of the transcriptional start site are sufficient to confer beta cell specific expression to an exogenous gene in transfected cells and transgenic mice. Thus, 660 bp of the rat insulin II promoter region (RIP) was shown to direct the expression of simian virus 40 T-antigen (*Tag*) to beta cells [12]. The expressed viral oncogene induced tumour formation, permitting the establishment of valuable beta cell lines (β-TCs). Moreover, correct initiation of insulin transcripts in mice together with cell-specific expression and physiological regulation of either the integrated human insulin gene [13, 14] or transgenes driven by the 5' flanking regions of insulin genes [12, 15, 16] implied functional conservation of regulatory sequences between different species. Deletion and mutational analyses of various insulin promoter regions, conducted in transfected cell lines and in transgenic mice, led to the identification of cis-acting regulatory sequences necessary for specific-gene expression. Regulated insulin gene transcription relies on the interaction of sequence motifs in the promoter with a number of ubiquitous and islet specific transcription factors (Fig. 1). These interactions determine the positive and negative regulation of insulin gene expression and its inducibility by physiological stimuli. The binding of proteins to regulatory elements has led to the identification and cloning of putative insulin transcription factors. The characterization of such factors (and their genes) contribut-

ed to our current understanding not only of insulin gene expression, but also of endocrine pancreas development and of beta cell differentiation.

Positive regulation

Regulatory cis-acting elements and trans-acting factors involved in insulin gene transcription. Insulin genes share a number of conserved DNA motifs in their 5' flanking region [11], implying that they might be regulated by similar trans-acting factors. Amongst these, E, A and C1/RIPE3 b elements seem to be major determinants of beta cell-specific expression of the insulin gene.

E boxes (E1 and E2). Systematic mutagenesis of the rat insulin I promoter and transfection studies led to the identification of two closely related repeats, found to play an important role in beta cell-specific expression [17]. These elements, located between –104 and –112 (IEB1/E1) and between –233 and –241 (IEB2/E2), contain the core sequence belonging to the class of regulatory motifs designated E boxes, sharing the consensus sequence CANNTG. Similarly, mutagenesis of the rat insulin II 5' flanking region also showed that the conserved E1 motif (-100 to –91) is essential for selective expression of the gene [18, 19]. As to the human insulin gene, a promoter fragment containing the E1 element (up to –170) showed low transcriptional activity in transfected fetal islet cells [20]. In contrast, a similar fragment significantly induced the expression of a reporter gene in transfected HIT-T15 cells [21]. Mutating the E1 box within the human insulin promoter would be necessary to resolve the conflict and evaluate its contribution to insulin gene expression.

E boxes bind proteins of the basic helix-loop-helix family (bHLH), which function as potent transcriptional activators of tissue-specific genes by forming heterodimers between ubiquitous (class A) and cell-restricted (class B) members. E1 binds the heterodimeric complex IEF1, which can include one of two alternatively spliced *E2A* gene transcripts, the widely distributed bHLH proteins E12/E47 and *HEB*-encoded proteins (reviewed in [22]). These, in turn, interact with the cell type-restricted bHLH protein, BETA2/NeuroD, expressed in pancreatic and neuroendocrine cells, which is an important regulator of both insulin gene expression [23] and pancreatic development [24]. The pancreas of mutant Beta2$^{-/-}$ mice contained fewer beta cells, failed to develop mature islets, and the animals became diabetic [24]. Similarly, mutations affecting the DNA binding or the transactivation properties of Neuro D predispose to diabetes [25]. However, disruption of the *E2A* gene had no effect on beta cell phenotype [26], indicating redundancy between members of the A class bHLH family. While the E47/BETA2 complex controls both insulin and glucagon gene transcription, it was shown to bind to the glucagon promoter with much less affinity [27]. Of interest, overexpression of E47 inhibited only E box-mediated glucagon gene expression whereas it activated insulin gene transcription, suggesting that the E47/BETA2 ratio is important for regulated gene expression in islet cells. Thus, unique cooperative interactions allow the insulin E box enhancer to discriminate between different bHLH factors for achieving tissue-specific activation of the gene.

In contrast to the rat I gene, rat II and human insulin 5' flanking regions do not contain the conserved E2 box, but a related sequence in the human gene binds the HLH protein USF (upstream stimulatory factor) [28].

A boxes (A1-A5). Additional prominent regulatory elements, containing AT rich sequences, are the A boxes (A1-A5) [11]. Except for A2 (GGAAAT), they all contain the core TAAT sequence which binds factors belonging to the homeodomain-containing protein family. Whereas the A1 and A3 boxes are the most conserved, mutated A3 had a more dramatic effect on transcription [29–31]. These promoter elements bind the pancreatic duodenal homeobox–1 (PDX-1) transcription factor, the mammalian homologue of the Xenopus laevis XlHbox8 [32], previously described as IPF-1 [33], STF-1 [34], IDX-1 [35], GSF [30, 36], and IUF-1 [37]. PDX-1 functions both in the early commitment of the primitive gut to pancreatic fate and in the maturation of beta cells (reviewed in [38]). Mainly expressed in beta cells, PDX-1 is a major transactivator of the insulin gene [29, 30, 33, 36, 39, 40] and of islet-specific genes such as *Glut 2* [41], glucokinase [42], *IAPP* [43] and somatostatin [34, 35]. Gene disruption experiments in mice showed that PDX-1 is imperative for the formation of the pancreas [44]. In experiments specifically carried out in beta cells using the Cre-loxP system, fewer insulin-positive cells and reduced insulin and *Glut 2* expression were observed, leading to diabetes in these mice [45]. Furthermore, a patient with pancreatic agenesis had a mutated *pdx-1* [46], whereas heterozygous mutants present MODY 4 [47]. Also, some cases of sporadic Type II (non-insulin-dependent) diabetes mellitus have been linked to *Pdx-1* mutations. However, these seem to be rare in the general diabetes population.

Although PDX-1 mostly stimulates islet-specific gene expression by binding as a monomer to its site, it was also shown to form a heterodimeric complex with PBX, the mammalian homologue of the *drosophila extradenticle*. This heterodimer bound the TAAT sequence of the somatostatin promoter but not that of the insulin promoter (A3 motif) [48]. PDX-1:PBX complexes have also been observed in exocrine cells, regulating elastase gene expression

[49]. Thus, such preferences could form the basis for target-site selection in developing islet cells, and the transcriptional activity of the gene might thus be highly context dependent. Indeed, the PBX interaction domain in PDX-1 was shown in vivo to be dispensable for the generation of pancreatic endocrine and exocrine cells, but to be required for expansion of these cell populations during development. The heterodimer can therefore induce the expression of genes involved in islet neogenesis [50].

In the rat insulin I gene, the juxtaposed A3 and A4 boxes bind additional homeodomain-containing proteins; although not beta cell-specific, their expression is limited to few cell types. These include isl-1 [51], cdx-3, lmx-1 [52], HNF-1α [53], HMGI(Y) [54].

According to the new nomenclature [11] the GGAAAT element (or GG1) was renamed A2 motif in view of its A rich sequence. It is highly sensitive to mutations as shown in the human insulin gene (GG1 and the related GG2) [55]. The contribution to overall insulin gene expression of the 30000 M_r protein binding this element has not been solved.

RIPE3 b. The rat insulin II enhancer, RIPE3 (-126 to –86), was shown to mediate beta cell-specific activity in transfection experiments and to confer correct temporal and tissue-specific expression to a transgene in vivo [56]. Positive control of RIPE3 is mediated by the RIPE3 b and E1/ICE elements. RIPE3 b is composed of two overlapping sequences, the A2 (-126 to –113) and the C-rich C1 element (-118 to –107). A2 was recently reported to bind three specific complexes; the one designated A2.2 was only detected in insulin-producing cell lines [57]. C1 binds two complexes, the beta cell-specific RIPE3b1-Activator/C1, and the more generally distributed RIPE3b2 [58]. RIPE3b1 was also composed of several proteins and its binding activity depended on tyrosine-phosphorylation. The loss of binding activity in phosphatase-treated extracts was prevented by tyrosine phosphatase inhibitors [59, 60]. In addition, the ability to specifically immunoprecipate RIPE3b1 binding protein with anti-phosphotyrosine antibody suggests that tyrosine kinase and/or phosphatase signalling could be involved in the regulated expression of the insulin gene by acting upon RIPE3b1 [60]. Cloning of the genes coding for the RIPE3b1 complex of proteins will be of major importance to elucidate its role in insulin gene expression.

Cyclic AMP response element (CRE). The second messenger cyclic AMP (cAMP) regulates the transcription of genes carrying cAMP response elements (CREs), mainly through binding the transcription factor CREB (CRE binding protein) to this motif, a member of the basic region leucine zipper (bZIP) family. This occurs via a mechanism involving activation of protein kinase A. Thus, phosphorylated CREB interacts with the basal transcriptional machinery by binding the co-activator CREB binding protein (CBP) and activates transcription (reviewed in [61]). In the beta cell, glucose [62] and certain hormones including glucagon and the "incretin" GLP-1 [63, 64] increase intracellular cAMP. While cAMP, a potent enhancer of insulin secretion, stimulates gene transcription through CREs identified in the rat I [65] and II [18] insulin genes, it only has a modest effect on insulin mRNA levels [66–68].

In the human gene four sites were identified (two in the promoter region), all contributing additively to cAMP-inducibility [69]. The rat insulin I CRE confers basal activity, with relatively low cAMP-responsiveness. Although this correlates with poor binding of the cellular CREB to the insulin CRE, recombinant CREB showed higher binding affinity [70]. To clarify this paradox, additional CRE binding proteins acting as activators and repressors in islets and in beta cells have been identified [71–73].

The CRE modulator (CREM) gene generates a series of isoforms by alternative splicing which act as transcriptional activators and/or repressors [74]. CREM activators were shown to stimulate insulin transcription more efficiently than CREB, apparently by direct binding to components of the basal transcriptional machinery. CREM repressors, however, do not interact with these; they suppress transcription by competing with CREM activators or CREB on their binding sites [71]. Of interest, mRNA levels of a CREM repressor, which suppressed insulin transcription, were higher in islets of Goto-Kakisaki (GK) rats than in the control rats, suggesting a potential contribution to the decreased insulin gene transcription in this animal model of diabetes [72].

In the beta cell, activation of CREB also leads to the stimulated expression of the inducible cAMP early repressor (ICER). Plasma glucagon concentrations are sometimes increased in Type II diabetic patients; it is therefore of interest that glucagon induced the expression of ICER in beta cells, thus reducing insulin gene expression [73]. Whether such a mechanism contributes to decreased insulin production in Type II diabetes is not clear.

An alternative mechanism was suggested for rat insulin I where the CRE overlaps with a CAAT motif which binds the ubiquitous transcription factor NF-Y. This factor confers basal promoter activity and its binding attenuates the cAMP response [75].

Altogether, the fact that insulin CREs contain imperfect motifs, [TGACGTCA (consensus CRE); TGACGTCA (Rat I); TGttGTCc (Rat II); TGAtGTCc (Human)], and that differing sources of beta cells were used, could explain the disagreement regarding the mechanisms of cAMP-stimulation of the insulin gene.

C2 element. The C2 element, containing a direct CAGG repeat, was first described in the human insu-

lin gene from –253 to –244 [76]. Although no equivalent motif was detected in the rat promoters, a related CACC sequence was found between –329 and –307 of the insulin I gene. This C2 element binds the transcription factors containing a paired domain and a homeodomain, PAX6 which acts as a transactivator [77] and PAX4 as a transcriptional repressor [78–80]. When coexpressed with PAX6 in beta cells, PAX4 dose-dependently suppressed the PAX6-induced transactivation of a reporter gene driven by a PAX recognition sequence [78]. This was not due to competition for a common binding site because repression was maintained even when the PAX DNA binding domain was replaced by that of the heterologous GAL4 protein [79].

An additional factor, binding to the human C2 and which exhibits RIPE3b1-like properties, was characterized as D0. Both the oxidizing agent diamide and the alkylating agent N-ethylmaleimide abolished its binding, suggesting that a redox-dependent pathway involving a RIPE3b1-like factor could regulate insulin gene expression [76].

PAX6 and PAX4 are transcription factors that play an important role in pancreatic development (reviewed in [81]). Whilst PAX6 expression in islets is maintained until adulthood, PAX4 is only expressed in the early pancreas, being undetectable after birth. Hence, a direct effect on insulin gene transcription in adults can be excluded; rather, it could interact with other, as yet unidentified target genes involved in beta cell differentiation.

G1 or GAGA-box. Deletion analysis of the rat insulin I promoter led to the identification of the G/GAGA box as an important regulatory element [17, 31]. This is a purine-rich sequence located between –57 and –40 bp. The ubiquitous zinc finger Pur-1/MAZ binds to rat insulin I and II genes and stimulates promoter activity [82]. However, G1 contribution to overall human promoter activity is doubtful because it failed to bind the Pur-1 transcription factor and its mutation did not affect transcription [20].

ILPR (VNTR, HVR). Insulin-linked polymorphic region (*ILPR*), also referred to as variable number of tandem repeats (*VNTR*) or hypervariable region (*HVR*), is located at about –360 bp of the human gene. It has a variable number of repeats with the consensus sequence ACAGGGGT(G/C)(T/C)GGGG. *ILPR* is highly polymorphic due to varying numbers of repeats and minor nucleotide variations within each repeat and has been classified into three main groups. Class I alleles with an average of 40 repeats are the most common, class II with an average 85 repeats are the least frequent, and class III has an average of 157 repeats. The unexpected finding that the *IDDM2* locus of the susceptibility to Type I (insulin-dependent) diabetes mellitus was mapped to the *ILPR* region has generated great interest as to its possible involvement in affecting insulin gene transcription. Whilst the short class I *VNTR* alleles have been reported to predispose to Type I diabetes (reviewed in [83]), class III alleles have been implicated in Type II diabetes susceptibility [84]. The correlation between these classes of *ILPR* alleles and insulin gene expression is not clear. Most of the evidence suggests that class III *ILPR* is associated with low levels of insulin mRNA [83, 85]. In contrast, using transfected beta cells, class I ILPR alleles were found to be transcriptionally less active than class III alleles [86]. Furthermore, variations within each repeat at *IDDM2* also correlated with insulin mRNA levels in heterozygous individuals [87] as well as with transcriptional activity in transfection experiments [88].

This polymorphic G-rich minisatellite has the characteristic of forming unusual tetramer DNA structures in vitro, presumably through formation of G-quartets [89]. *ILPR* contains high-affinity binding sites for the transcription factor Pur-1, which binds the purine-rich G1 (GAGA) element in the insulin promoter [82]. Since for most but not all repeats activation by Pur-1 correlated with Pur-1 binding activity in vitro, other factors that recognize the G-quartet structure are likely to be involved in transcriptional regulation of the gene [88]. Indeed, single or double mutations that destabilise the loop-loop and loop-tetrad interactions dramatically affected insulin promoter activity [90].

Whilst the upstream *ILPR*, and more specifically the allelic variation within the repeat, seems to play a role in insulin gene expression, the relation to susceptibility to both types of diabetes is as yet difficult to assess.

Synergism. It has become evident that the insulin gene is regulated through the action of multiple activators, thereby providing a mechanism for combinatorial control. In fact, the arrangement of multiple activators in a single complex provides the capacity to integrate multiple regulatory inputs into a single output.

The most studied cooperative interactions are those between the proteins binding to the juxtaposed E and A elements. The rat insulin I promoter and more specifically its mini enhancer, previously named the Far-Flat region (FF), composed of an E2 motif and of the unique AT-rich A3/A4 sequence elements, has been the regulatory region of choice for this type of analysis. Homeodomain-containing factors binding to A3/A4 elements functionally interact with the E2A-encoded proteins in transfected islet beta cells. The better studied PDX-1, acts synergistically with E47, a member of the bHLH family of transcription factors, to activate transcription [39, 40, 91]. Synergism requires the DNA binding and activation do-

mains of both PDX-1 and bHLH proteins. It was further shown that the synergistic transactivation results not only from the coexpression of both E2A and PDX-1 but also of BETA2 involving its C-terminal activation domain and a region within the N-terminus [92]. The role of PDX-1 was extended to recruitment of the transcription factors E47, BETA2/NeuroD and the high mobility group protein I, HMGI(Y), to form an activation complex on the FF mini enhancer [54]. The HMGI(Y) itself bound directly to the A3/A4 elements to increase PDX-1 and bHLH transcriptional synergism [54]. Another A3/A4-binding homeoprotein, lmx1.1, acted synergistically via its LIM domain with E47 to activate transcription [52].

Like the rat I mini enhancer, the RIPE3 enhancer of the rat II promoter contains an E1 (ICE) box which cooperates with the RIPE3b1 element [58, 93]. In this context, E47:BETA2 heterodimers require the presence of the beta cell-specific RIPE3b1-Act/C1 complex to activate insulin gene transcription [23, 94]. In the human gene as well, synergism was reported between E1 and A1 or E1 and A2C1 [20].

The mechanisms responsible for efficient activation of the insulin promoter are not fully clarified. Conceivably, E2A, BETA2 and PDX-1 display cooperative binding not only through direct but also indirect protein-protein interactions, i.e. with the participation of coactivators. Although no beta cell-specific coactivator has yet been characterized, recently a new E2A-interacting protein, Bridge-1, which contains a PDZ-like domain has been identified [95]. It is highly expressed in islets where it functioned as a co-activator enhancing E12-mediated or E47-mediated transcription of the rat I gene. Inactivation of endogenous Bridge-1 in INS-1 cells using antisense RNA led to reduced insulin I promoter activity.

The role of the ubiquitous coactivator p300 and its close homologue CBP has been investigated extensively. These are histone aceyltransferases, which stimulate transcription of specific genes by interacting either directly or through co-factors with a number of transcription factors and with the basal transcriptional machinery (reviewed in [96]). The adenoviral E1A oncoprotein, which binds a specific domain in the p300/CBP proteins, was able to inhibit insulin promoter activity [97], probably by sequestering the coactivator. Cooperativity between p300 and bHLH proteins in activating E box-mediated transcription has been described [98]. Interactions of p300 with the bHLH members, E2A and BETA2 involved in the beta cell-specific IEF1 complex were shown to stimulate insulin gene transcription [99, 100]. The functional interacting domains are in p300 (aa 1945–2377) and BETA2 (aa 156–355). In E47 the activation domains spanning the aa sequences 1–99 and 325–432 were associated with the region aa 1–1257 in p300.

Microinjection of p300/CBP antibodies in muscle precursor cells blocked the transcriptional activity of myogenic bHLH and myotube formation [98]. It will therefore be important to assess the role of these co-activators in islet development and beta cell differentiation, especially in view of the ability of CBP, through its E1A binding domain, to interact with the activation domain of the key beta cell regulator, PDX-1 [101].

Thus, tissue-specific expression of insulin seems to depend not only on specific combinations but perhaps more importantly on the concentration of transcription factors, only some of which are restricted to the beta cell. Complete understanding of the molecular mechanisms underlying this expression will require detailed analysis of the interactions between the various transcription factors and coactivators.

Negative regulation

A silencer which decreases the activity of a heterologous promoter has been located between –4.0 and –2.0 kb in the repetitive sequences of the rat I gene [102], whilst in the rat II gene a region between –217 and –197 was identified as the site of negative control [103]. In the human insulin gene, a negative regulatory element (NRE) was mapped between –280 and –260 [21, 104]. NRE was shown to contain overlapping putative sequences binding several nuclear proteins, among them the glucocorticoid receptor [105] and the ubiquitous factor Oct1 [104]. In fact, in HIT-T15 cells, dexamethasone inhibited the activity of an NRE-containing plasmid [105]. However, primary beta cells transfected with the human insulin promoter lacking this element showed reduced activity, indicating the presence of a positive rather than negative element in this region [20]. These observations suggest that NRE activity might be limited to transformed cells, again stressing the importance of using primary beta cells to confirm results obtained in cell lines.

Since cooperativity between transcription factors seems essential for strong expression of the insulin gene, proteins interfering with these positive interactions could provide the mechanism for down-regulating insulin expression.

E47-mediated inhibition of insulin gene expression. The inhibitory effect on transcription of Id proteins of the HLH family (lacking the basic DNA-binding domain) is a result of nonfunctional heterodimeric complex formation with lower DNA binding ability. Of interest, beta cells exposed to high glucose showed increased expression of *Id-1* and *Id-3* genes [106]. Their concomitant induction with that of insulin gene transcription and insulin secretion suggest that physiological concentrations of Ids do not inhibit in-

sulin gene expression [106], but when overexpressed, Id protein was able to inhibit the rat II insulin promoter activity [107].

A stronger candidate as negative regulator of E box-mediated insulin gene expression is BETA3. Although a member of the B class of specific bHLHs and highly homologous to BETA2/NEUROD, it is not able to bind the insulin E box despite its intact basic region. BETA3 inhibited the binding of both E47 homodimer and E47/BETA2 heterodimer to DNA and consequently repressed E47/BETA2-mediated transactivation of the insulin enhancer [108].

Inhibition of insulin transcription by c-Jun was also shown to act through the E1 element; the repressed transcriptional activation implicated the basic leucine zipper region of c-Jun and the conserved activation domain AD2 of the E2A proteins, E12 and E47 [109].

Similarly, c/EBPß, a member of the leucine zipper family of transcription factors, specifically repressed insulin gene transcription in beta cells. In this case, the repression involved the direct interaction of the heptad leucine zipper of c/EBPß with the AD2 of E47, hence inhibiting the E47-mediated transactivation of the insulin enhancer [110]. Of interest, c/EBPß is upregulated in beta cells chronically exposed to high glucose, and in the rat diabetes models of Zucker (*fa/fa*) and 90 % pancreatectomy, contributing to the downregulation of insulin expression [111].

The E box-mediated inhibition by either Id, BETA3, c-Jun or c/EBPß is probably due to their interference with E47 homo-dimer or hetero-dimer formation and consequently with the synergism involving A element-binding proteins.

Nutrient and hormonal effects on insulin gene expression

Glucose. Mammalian glucose homeostasis requires tight regulation of insulin production and release. Although the first evidence of glucose effect on insulin gene was reported about 30 years ago [112], we are only beginning to understand the complexity of the molecular mechanisms by which the sugar elicits this effect. The pioneering work of Permutt and Kipnis [113] revealed that glucose increases insulin synthesis by stimulating gene expression. It was suggested that glucose controls the steady-state insulin mRNA levels by enhancing both transcription and mRNA stability (reviewed in [114]).

The beta cell responds to the increase of blood glucose by increasing its rate of glucose metabolism, as well as stimulating insulin release and biosynthesis. The key elements of glucose sensing are the non-rate-limiting glucose transporter 2 [115] and the high Km hexokinase, the beta cell-specific glucokinase [116]. Glucose-induced insulin secretion in beta cells is initiated by closure of ATP-regulated potassium channels, causing plasma membrane depolarization with subsequent opening of the voltage-dependent calcium channels. The ensuing rise in cytosolic calcium triggers the exocytosis process. Several lines of evidence indicate that glucose metabolism is essential for beta cell-specific induced gene expression. This was evidenced by using metabolisable sugars such as mannose [117, 118] and glyceraldehyde [118], whereby the effect is blocked by mannuoheptulose, an inhibitor of glucokinase [117, 118]. Moreover, glucose metabolism is also necessary for the stimulatory effect on insulin promoter activity [117]. As for insulin secretion, glucose-induced transcriptional activity is mediated by the end-products of mitochondrial oxidation, as shown by co-transfecting beta cells with cDNAs encoding enzymes of glucose metabolism [119].

Inducible transcription seems to depend on the very same sequences in the promoter and/or enhancer region that control the cell-type specificity of the insulin gene. Thus, glucose-responsiveness is mediated by cis-acting elements within the 5' flanking region of the gene [29, 30, 36, 94, 120]. In beta cells it seems that a combination of several elements within the insulin promoter can produce the full glucose response and that more than one transcription factor is regulated by the hexose. The most studied elements in this context are A3, C1 and E1. Factors binding to these sites were suggested to be modulated by glucose and mutation of the elements led to impaired glucose-regulated transcription [20, 29–31, 121]. Numerous studies have shown that extracellular glucose concentrations regulate the binding of PDX-1 and RIPE3b1-Act/C1 to the A3 and C1 elements, respectively. However, little [31] or no increase [94] in the binding of the IEF1 complex to E1 has been observed. An additional glucose-responsive element in the human insulin gene (-292/-243, also called Z mini enhancer) was described using cultured primary fetal islet cells, and the sequence between –292 and –263 interacted with a yet unidentified islet-specific glucose-modulated complex [122].

As described previously, the RIPE3 region contains the E1/ICE and RIPE3b1/C1 elements. Whilst RIPE3b1-Act/C1 binding activity was selectively stimulated by high glucose concentrations, binding to E1/ICE and RIPE3a2 elements were not affected [94]. Additional metabolic effectors such as mannose and pyruvate were shown to modulate RIPE3b1-Act/C1 in beta cells, suggesting that a common signal during glucose metabolism mediates both insulin transcription and secretion [121]. As mentioned earlier, tyrosine kinase/phosphatase was recently shown to influence RIPE3b1 binding to the insulin C1 element. Since phosphorylation seems to be required for the binding, it will be of great interest to assess whether RIPE3b1 activity in glucose-treated beta

cells is regulated by this process. The proteins involved in the RIPE3b1-Act/C1 complex are being purified [59] and it is hoped that the gene encoding them will soon be isolated to determine its role in the cell-specific and nutrient-regulated expression of the insulin gene.

Of the factors associated with glucose-regulated expression of the insulin gene, the PDX-1 protein has been the most studied. The importance of the FF mini enhancer element of the rat I gene (around –200 to –250) in conferring glucose-responsiveness to a reporter gene was first shown in transfected fetal rat islets [120]. Using normal adult rat and human islets we have further mapped the glucose-sensitive element to the sequence containing the conserved A3 motif [36]. Furthermore, we identified an islet-specific glucose sensitive factor (GSF) whose DNA binding to the A3 motif is modulated by extracellular glucose concentrations [36]. The sequence of the purified protein was shown to correspond to PDX-1 [30], thus providing direct evidence for the involvement of this homeodomain protein in glucose-responsiveness. A point mutation in the A3 element in the human insulin promoter abolished the stimulatory effect of glucose in adult islets, supporting the suggestion that PDX-1 plays a major physiological role in glucose-regulated expression of the insulin gene [29, 30]. Furthermore, expression of PDX-1 in NES2Y cells lacking this protein [123], as well as in islets of the Type I diabetes model *Psammomys obesus*, also devoid of the conserved form of PDX-1, restored their ability to regulate insulin mRNA levels in response to glucose [124]. Thus, in addition to its important roles in the development and differentiation of pancreatic islets and in beta cell-specific gene expression, this protein functions as an essential mediator of the glucose effect on insulin gene transcription in differentiated beta cells [29, 30, 36, 37].

The transcriptional mechanisms involved in this process are not fully understood; to that end, the mapping of the functional domains of PDX-1 is required. The activation domain of PDX-1 is located within the N-terminus of the protein [91, 125, 126] and the regions essential for its transactivation have been delineated [126, 127]. Of interest, the transactivating potency of PDX-1 is also modulated by glucose [126, 128] and the metabolisable hexose mannose, but not by 2-deoxy-glucose [128]. We found that the subdomains in the human PDX-1 protein of importance for glucose-mediated transactivation span the amino acid sequences 20–50 and 97–120. Thus, glucose modulates the DNA binding and the transactivation activities of PDX-1 to fully regulate insulin gene transcription in differentiated beta cells.

The signalling pathways responsible for glucose induction of insulin gene transcription are under investigation. The finding that insulin gene transcription is inhibited by calcium channel blockers [120, 129, 130] and activated after beta cell membrane depolarization in HIT-T15 and islet cells [130], suggests that the stimulatory effect is in part mediated by increased intracellular calcium, analogous with the regulation of glucose-induced insulin secretion. However, this concept has been contested in beta cell lines [117, 131, 132], in islets [131], and the human beta cell line NES-PDX-1 which lacks voltage-gated calcium channel activity [123]. To date, while glucose metabolism is indisputably required for the transmission of the glucose stimulus for beta cell-specific gene expression, the exact nature of the intracellular signalling pathway has not been solved. The variability of the beta cell systems and testing conditions used hampers firm conclusions; nevertheless, the results indicate that the insulin secretion and transcription pathways differ in some aspects and that these two processes can occur independently.

The rapidity by which PDX-1 alters its binding implies a post-translational modification rather than de novo protein synthesis. A variety of signalling pathways are activated by beta cell glucose metabolism, several of which are presumed to regulate insulin gene transcription. Cyclic AMP is one, as it has been shown to augment insulin mRNA levels [67, 120]. However forskolin, which raises intracellular cAMP concentrations, had no effect on PDX-1 binding apart from the glucose concentration [37]. Another mechanism proposed to control gene expression in response to extracellular stimuli is the activation of specific protein kinases and/or phosphatases that uniquely modify the phosphorylation status of sequence-specific transcription factors. In beta cells, several signalling pathways have been described which involve kinase activation including protein kinase A (PKA), protein kinase C (PKC), members of the calcium calmodulin-dependent kinases (CaMK) and of the mitogen activated protein kinases (MAPK) [133].

It was reported that in human islets, or in MIN6 beta cells, a specific inhibitor of stress-activated p38MAPK, SB103580, inhibits glucose-stimulated binding of PDX-1 to DNA as well as PDX-1-dependent gene transcription [37, 134]. Moreover, overexpression of p38 kinase mimicked the glucose effect on PDX-1 binding and stimulated transcription of the human insulin promoter. Furthermore, the transfer of islets from low to high glucose concentrations was associated with the translocation and the rapid conversion of a cytoplasmic 31 000 M_r PDX-1 protein to a nuclear 46 000 M_r form [135]. Both findings have been challenged. Using the same specific inhibitor as well as overexpressing the p38 MAPK/SAPK2 or the upstream kinase MKK6, no effect on insulin promoter activity was observed [136]. However, overexpression of a constitutively active form of phosphatidylinositol 3-kinase (PI3-K) mimicked the glucose effect on the promoter activity, which was blocked by LY294002, a PI3-K inhibitor, as well as by the expres-

sion of a dominant negative form of the p85 subunit. No change in the apparent molecular weight of PDX-1 was observed in extracts of cells incubated at low or high glucose concentrations [136]. Most importantly, the endogenous PDX-1 seems to reside predominantly within the nucleoplasm in cells maintained in either glucose concentration [136] with only a small proportion residing in the cytoplasm at low glucose.

Mitogen-activated protein kinases play a major role in the regulation of a number of transcription factors in response to extracellular stimuli. However, in glucose-induced insulin gene transcription the situation is far from clear. MAPKs are believed by some authors to participate in the regulation of insulin secretion (reviewed in [133]). It was suggested that in beta cells p38MAPK/SAPK2 is activated via the PI3-kinase pathway [134]. It is conceivable that p38MAPK/SAPK2 activity is sensitive to the slight osmotic stress produced by high glucose concentrations similarly to that obtained with arsenite, which triggered the activation of MAPKAP kinase-2, a downstream target of p38MAPK, in a PI3-kinase independent manner.

In conclusion, PI3-kinase could be a central regulator of PDX-1 and of glucose-induced insulin gene transcription. However, it is essential to elucidate the link between glucose metabolism and kinase activation. This process has been suggested in part to be the result of a feedforward mechanism involving the binding of the secreted insulin to its receptor on the beta cell surface (Fig. 2). The degree of complexity of the signalling pathways that link glucose to insulin transcription is such that it will take some time to elucidate all the mechanisms involved. It is therefore of importance to map the specific phosphorylation sites not only of PDX-1 but of other transcription factors modified by change in extracellular glucose concentration and consequently to study the kinases potentially involved in glucose-regulated insulin gene expression.

Effects of chronic exposure to high glucose. It is controversial as to whether in Type II diabetes insulin biosynthesis is compromised to the same extent as is insulin secretion. While the maximal insulin responsiveness to stimuli is markedly reduced, upon autopsy of Type II diabetic subjects most islets showed only limited reduction in proinsulin mRNA [137]. Chronic blood glucose increase in humans and in experimental animals reduces insulin secretion. This has led to the concept of "glucose toxicity", whose effect on insulin gene expression has attracted the attention of several laboratories. In HIT-T15 beta cells cultured at high glucose concentrations, a reduced binding of both PDX-1 and RIPE3b1-Act/C1 to their sites was reported [138]. However, only RIPE3b1-Act/C1 binding was affected in ßTC6 cells cultured in high glucose [139]. This indicates that the role of insulin transcription factors in overall insulin gene expression varies with the specific beta cell line investigated. We found that glucose-induced insulin release and islet insulin content were markedly reduced in cultured human islets chronically exposed to high glucose concentrations. This was accompanied by a major reduction in insulin mRNA levels and in human insulin promoter transcriptional activity [140]. The loss of promoter activity was associated with marked reduction in PDX-1 binding to the human insulin A3 element. RIPE3b1-Act/C1 binding was also reduced, albeit to a much lesser extent. In contrast, IEF1 binding to the E1 element was not affected. The decline in PDX-1 binding was largely associated with a reduction in *pdx-1* mRNA; correction of the "hyperglycaemic milieu" reversed these effects to a certain degree [140]. Decreased insulin gene transcription was also associated with upregulation of the transcription factor c/EBP-ß in beta cells exposed to high glucose concentrations [110], as well as in the Zucker fatty (*fa/fa*) rat and in 90 % pancreatectomized rats [111].

Although the molecular mechanisms that lead to glucose toxicity are not known, glycation and generation of reactive oxygen species have been proposed as mediators [141]. The decreased *pdx-1* mRNA could result from deleterious effects of supraphysiological glucose concentrations on the transcription factors controlling its expression. It is therefore important to elucidate the regulatory elements and transcription factors involved in the controlled expression of the *pdx-1* gene in beta cells, and as such, we have recently shown that *pdx-1* gene transcription is controlled in part by the PDX-1 protein itself [142]. Additional factors regulating *pdx-1* gene transcription have been identified [142–145] and the damaging effect of high glucose on their activity needs to be addressed.

An additional correlation between *pdx-1* and diabetes came from genetic studies of MODY families where few mutations have been described in the gene, one with a frameshift mutation showing a dominant negative effect in vitro [146]. Three additional missense mutations were identified in subjects with Type II diabetes; these showed variable reduction to the A3 site of the human insulin gene, and decreased promoter activity [147]. Since they were not highly penetrant MODY mutations, it was postulated that they might predispose to Type II diabetes, with phenotype depending on the severity of the mutation.

Ultimately, to determine the molecular mechanisms responsible for the deleterious effects of high glucose concentrations on insulin gene transcription, further studies in primary islet cells will be necessary. Whatever the mechanisms involved, the experimental work cited here strongly suggests that uncontrolled hyperglycaemia can lead to reduced insulin gene expression, loss of beta cell insulin reserves,

and therefore, aggravation of the insulin deficiency and of the metabolic dysfunction.

Insulin. Evidence for a negative feedback of insulin secretion [148, 149] and gene expression [150] by insulin has been reported. More recent studies indicate that insulin exerts stimulatory effects on insulin production (reviewed in [151]). General disruption of the insulin receptor substrate 2 (*IRS-2*) in mice caused important beta cell defects [152]. A functional role of the insulin receptor in insulin-producing cells was directly demonstrated by beta cell-targeted inactivation of the receptor in mice. This resulted in a selective loss of first-phase secretion in response to glucose and a progressive impairment of glucose tolerance [153] which are typical characteristics of Type II diabetes. Insulin might also be acting on other beta cell-specific gene products such as GLUT 2 and glucokinase and a defect in insulin signalling pathway could lead to impaired glucose sensing. This is supported by the recent finding that insulin regulates glucokinase gene transcription in beta cells [154].

The mechanism by which insulin exerts stimulatory effects on its own transcription was suggested to involve the IRS2/phosphatidylinositol 3-kinase (PI3-K) and p70 S6 kinase, as well as the calcium calmodulin kinase pathways, but not the stress-activated protein SAPK2 [130]. Stimulation of insulin gene expression by insulin receptor signalling has been reported to be glucose dependent, also involving PI3-K in this process [155]. While data from mutational analysis of the rat insulin I promoter [130] indicated that the A3/4 elements are involved in the insulin effect, the E boxes could be the main mediators in islet and HIT-*15T* cells. However, the effect of insulin on the homeodomains binding to A3/4 (including PDX-1) and on IEF binding to the E elements needs to be tested. Similar experiments were conducted using the human insulin promoter where it was shown that insulin stimulates both the DNA-binding activity of PDX-1 to its cognate A3 sequence and the insulin promoter activity in MIN6 cells [156]. It was further shown that these effects are mediated not only by PI3-K but also by SAPK2. Here again discrepancies can originate from the use of insulin promoters and beta cells from different origins. Taken together, however, the data indicates that glucose and insulin (involving PI3-K) activate insulin gene transcription and that the effects can be additive in the beta cell, as suggested recently [157]. Defects in insulin signalling in the beta cell could contribute to the pathology of Type II diabetes, thus forming a link between peripheral insulin resistance and deficient beta cell function. Nevertheless, it is our impression that, in quantitative terms, glucose is the dominating regulator of insulin gene expression. Further studies involving several sources of beta cells and various diabetes models will be needed to evaluate the exact role of the insulin effect on insulin biosynthesis.

Non-esterified fatty acids (NEFA). Fatty acids represent important physiological fuels for beta cells, where they stimulate glucose-induced insulin secretion in the short-term, but after prolonged exposure exert inhibitory effects (reviewed in [158]). Although differences in fatty acid effects on insulin gene transcription have been reported, high concentrations of the saturated fatty acid palmitate represses insulin gene expression in the presence of high glucose [159–162]. This inhibition was shown on insulin transcription and mRNA stability [160]. Whilst decreased insulin transcription was shown to be associated with reduced activity of the rat I [160] or the human [159] insulin promoters, the mechanism underlying this effect is controversial. A study [161] showed dose-dependent decrease in *pdx-1* mRNA and protein in islets exposed to palmitate, which correlated with impaired expression of islet-specific genes such as insulin, *Glut 2*, glucokinase, and somatostatin, all regulated by PDX-1. In contrast, other investigators [160] failed to observe any effect of palmitate on DNA binding of PDX-1. This and other discrepancies, such as the adverse effects of the monosaturated fatty acid oleate [160], could be explained in part by differences in glucose concentration as well as in the origin of the beta cells used. The inhibitory effect of palmitic acid on insulin gene expression does not seem to require mitochondrial oxidation, since it was reproduced by the non-oxidizable fatty acid analogue bromopalmitate [160, 162]. It was also suggested to be associated with glucose-dependent increase in esterification of fatty acids [162]. This resembles the situation described in Zucker diabetic fatty (ZDF) rats, carrying a defective leptin receptor, which have increased esterification and high intracellular triglyceride concentrations, believed to contribute to beta cell dysfunction, apoptosis, and diabetes.

Glucagon-like peptide-1 (GLP-1). GLP-1, a physiological incretin hormone, is derived from post-translational processing of proglucagon and secreted from enteroendocrine L-cells of the intestine. GLP-1 binds to a G protein-coupled receptor on beta cells to stimulate glucose-induced insulin secretion, biosynthesis and cell growth. These stimulatory effects on beta cell functions have partly been attributed to increased intracellular cAMP concentrations with concomitant activation of PKA (reviewed in [163]).

GLP-1 increases the preproinsulin mRNA by stimulating insulin gene transcription [63, 164, 165] and by increasing mRNA stability [165]. The effect of GLP-1 on insulin gene expression seems to be mainly mediated via the CRE in the promoter region [63, 164]. Indeed, mutated CRE introduced into the rat insulin I promoter attenuated the GLP-1 effect

and a dominant negative form of CREB inhibited the action of GLP-1 on the wild type promoter [64]. Furthermore, in this study there was evidence that activation of CRE occurs independent of the cAMP-mediated activation of PKA [64]. The nature of such an alternative pathway remains to be determined. It was recently suggested that an additional effect of GLP-1 on rat I promoter activity is mediated by the glucose-sensitive sequences (E2-A3/A4) [166]. This is likely to be caused in part by the activation of *pdx-1* expression (via its own CRE) by GLP-1 [64, 66, 167] resulting in increased PDX-1. GLP-1 also stimulated the PDX-1 transactivating domain potency in the presence of low and high glucose [166]. The mechanism, as well as the PDX-1 subdomains involved in this process, have yet to be determined. It would be of interest to test whether this effect is mediated by phosphatidyl 3-kinase, shown to be activated by GLP-1 [66] and to be important for glucose regulation of the insulin gene [136].

Leptin exhibits opposing actions to GLP-1 in beta cells, suppressing insulin secretion and gene expression. It is interesting to note that responsiveness of the rat I promoter to both factors was impaired when the sequence from –410 to –307 was deleted. This region contains the STAT5 recognition site, shown to be involved in growth hormone responsiveness [168]. How these three signalling pathways converge on this sequence to modulate insulin gene expression is intriguing.

Leptin. The *ob* gene, its product leptin and its cell surface receptor (OB-R) are important factors in the regulation of food intake and energy balance. Although rare in mice and humans, leptin deficiency causes severe obesity suggesting that the hormone plays a similar role in both species. However, the increased leptin concentrations observed in other obese rodents and humans point towards leptin resistance rather than leptin deficiency as contributor to obesity. Obesity is associated with several pathological conditions including Type II diabetes, thought to result from the inability of the beta cell to increase insulin production in compensation for insulin resistance [169]. OB-R mRNA was detected in great abundance in islets, mainly in beta and δ-cells but not α-cells. Thus, the beta cell is a target for leptin action. Apart from the variability depending on the experimental system used, it appears that leptin inhibits insulin secretion in the presence of high glucose concentrations, mainly when potentiators that increase intracellular cAMP are added [169]. Leptin causes dependent the activation of the PI3-K-cyclic nucleotide phosphodiesterase 3B (PDE3B), with subsequent reduction of cAMP concentrations; consequently, it exhibits opposing actions to GLP-1 in beta cells [170].

Leptin has been shown to repress insulin expression in *ob/ob* islets as well as in beta cell lines [171]. In contrast, other studies showed that leptin had a stimulatory [172] or no [173] effect on insulin biosynthesis. The leptin and growth hormone receptors-associated janus kinase (JAK) converts the latent monomeric STAT molecule (member of the signal transducers and activators of transcription family) to an active dimeric form through tyrosine phosphorylation. The dimers bind to specific DNA response elements and induce transcription. The leptin-sensitive inhibitory sequences, located between –410 and –307 of the insulin gene, bind multiple protein complexes activated in extracts from *ob/ob* islets treated with leptin. One of these includes STAT5b and is formed on a consensus STAT binding site in the rat insulin I promoter [171]. Although STAT5b has been shown to activate the rat I promoter through the same STAT5 motif in response to growth hormone, it is likely that leptin mediates the repression through multiple additional complexes detected in this region. Leptin activated the STAT3 pathway by increasing the phosphorylation of STAT3 and MAP Kinase pathway in RINm5F cells [174]. Thus, the importance of JAK/STAT and possibly MAP Kinase signalling pathways in the regulation of insulin gene expression by leptin is not clear. Nevertheless, if the inhibitory effect of leptin on insulin gene expression is verified in human islets, it could point towards one additional mechanism that would favour the development of Type II diabetes in obese individuals.

Growth hormone (GH) and prolactin (PRL). Growth hormone (GH), prolactin (PRL) and placenta lactogen (PL) are primary regulators of pancreatic beta cell proliferation. In addition, these hormones stimulate insulin production, in part by stimulating insulin gene transcription ([168, and reviewed in 175]). Expression of GH and PRL receptors was found in rat islets and various beta cell lines. Upon binding, these hormones activate the JAK/STAT pathway resulting in the nuclear translocation of STAT proteins which then bind γ-interferon-activated sequence (GAS)-like elements, thus activating transcription of target genes [176]. In fact, a STAT5 binding element in the rat insulin I gene was identified in the sequence between –330 and –322 and shown to confer GH and PRL responsiveness to a heterologous promoter [168]. Furthermore, GH and PRL activated DNA binding of STAT-5a and STAT–5b in beta cells [168, 177]; this correlates with the GH-induced and PRL-induced nuclear translocation of STAT-5a and STAT-5b in these cells [178]. Activation of STAT proteins, which are able to form homo dimeric and heterodimeric DNA binding complexes, could be an important determinant of gene-specific effects in beta cells. Such induction of insulin gene expression seems to require the stimulation of calcium uptake, since the calcium channel blocker verapamil prevented GH-induced insulin gene transcription in beta

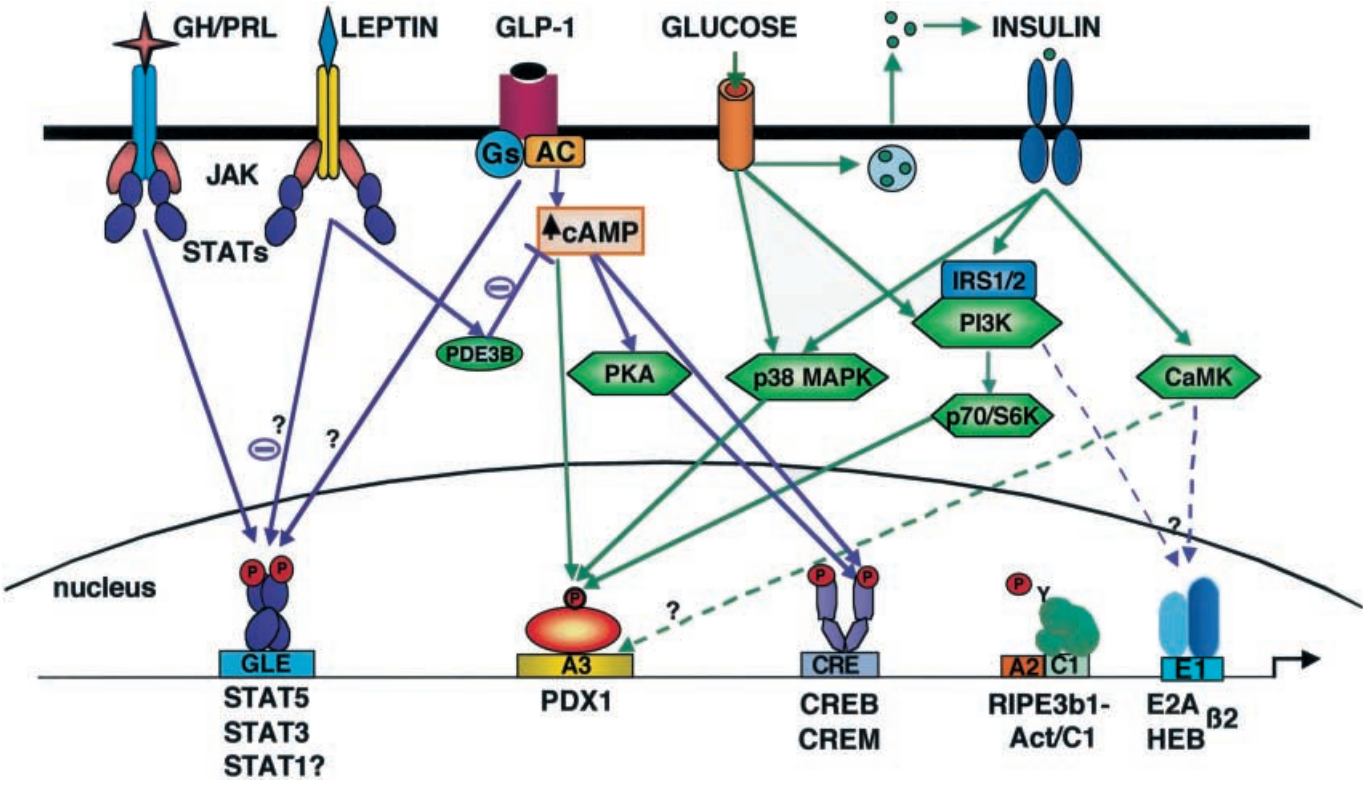

Fig. 2. Scheme illustrating possible signal transduction pathways and target transcription factors which regulate insulin gene transcription. The names of binding proteins are indicated underneath their corresponding sites. Green arrows converge to the PDX-1 protein. GH, growth hormone; PRL, prolactin; GLP1, Glucagon-like peptide 1; AC, adenylate cyclase; JAK, Janus tyrosine kinase; PKA, protein kinase A; PI3-K, phophatidylinositol 3-kinase; MAPK, mitogen activated protein kinase; CaMK, Ca/calmodulin-dependent protein kinase; IRS, insulin receptor substrate; PDE3B, phosphodiesterase 3B

cells [179]. Investigating the role of glucose in PRL-activated islet gene expression showed that PRL could increase insulin mRNA in a glucose-independent manner, but when in combination, glucose and PRL acted synergistically to induce rat I promoter activity [180]. Insulin acts at the level of the pituitary to induce PRL gene transcription, which in turn activates insulin transcription, suggesting a possible mechanism for the regulation of carbohydrate metabolism during pregnancy.

Effects of GH (which also binds the PRL receptor) on beta cell proliferation and gene expression seem to involve different domains of the receptor. Activation of JAK2 directly phoshorylates the STAT1 and STAT3 proteins, which then bind and activate the c-Fos gene, hence promoting mitosis [175]. Thus GH and PRL seem to activate different signalling pathways affecting distinct functions in the beta cell.

Conclusions

This review presents the complexity of the regulation of insulin gene expression, which is in accordance with the principal role this hormone plays in the coordination of metabolic processes. A considerable body of evidence has shown that beta cell-specific expression of the insulin gene is ascertained by a unique combination of transcription factors, which synergistically activate transcription. This mechanism also allows the beta cell to differentially respond to external stimuli by regulating the transcriptional response through more than one pathway. It is becoming apparent that positive and negative cross-talk between the signalling pathways (via second messengers and protein kinases) converge on the insulin promoter by modulating the appropriate transcription factors involved. The homeodomain-containing transcription factor PDX-1 emerges as a key player whose activity is modulated by several external stimuli (glucose, GLP-1, insulin, palmitate), i.e. via the activation of the PI3-K and MAPK pathways, thereby regulating insulin gene transcription (Fig. 2). A thorough understanding of the intracellular signalling events in response to external stimuli should clarify essential pathways for the normal functioning of beta cells. Ultimately, this will provide the basis for novel therapies for Type II diabetes.

Sources. The review is based on the relevant literature in the English language during the period 1967–2001. The sources available to the authors

were integrated with sources identified through PubMed. The search was based on the following keywords: insulin, pancreas, islet, beta cells, transcription, VNTR, PDX-1/IPF-1, helix loop helix, homeobox, CRE, coactivators, glucose, glucocorticoid, GLP-1, FFAs, leptin, growth hormone, prolactin, STAT, kinases, glucotoxicity, diabetes.

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