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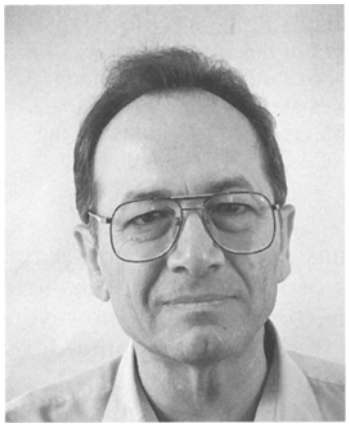
Erol Cerasi
Minkowski Award, 1974, Jerusalem



Born in 1935 in Istanbul, I studied medicine at the University of Istanbul (M.D. in 1960). Immediately thereafter I moved to Stockholm, Sweden to work with Dr. Rolf Luft. Sweden not recognizing my Turkish M.D., I wasted 3 years passing exams at the Karolinska Institute, where I got a second M.D. in 1964. Meanwhile, I started research in diabetes, and by 1967 obtained a Ph.D. from the Karolinska Institute, and completed specialties in medicine and endocrinology. During the years 1973–1975 I worked as Invited Professor at the Institut de Biochimie Clinique in Geneva. At this period I was asked by the Hebrew University of Jerusalem to create a Department of Endocrinology and Metabolism at the Hadassah Medical Center. Attracted by the genuine research Interest of the hospital management and staff, and by the charm of the city, I moved to Jerusalem in 1977, where I am Professor of Endocrinology and head of a clinical and laboratory unit. My research has been both clinical and basic on topics related to control of insulin release; pathogenesis of Type 2 diabetes; transcriptional regulation of insulin gene expression; regulation of muscle glucose transport and transcriptional control of GLUT-1; etc. I have been married for 35 years to a childhood friend. We are the proud parents of a musician, a philosopher and a physicist.

Transcription of the insulin gene: towards defining the glucose-sensitive *cis*-element and *trans*-acting factors

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Summary Previous work has shown that the sequence –196 to –247 of the rat insulin I gene mediates the stimulatory effect of glucose in fetal islets. We have used adult rat and human islets to delineate the glucose-sensitive *cis*-element to the sequence –193 to –227. In electrophoretic mobility shift assays, a 22 bp nucleotide corresponding to the sequence –206 to –227 bound all the nuclear proteins that could be bound by the entire minienhancer sequence –196 to –247. The rat insulin I sequence –206 to –227 formed three major complexes; in contrast, the corresponding human insulin sequence formed one single band with human and rat islet nuclear extracts, corresponding to the complex C1 of the rat insulin gene. Incubation of islets with varying glucose levels resulted in a dose-dependent increase in the intensity of the C1 band, while the other nuclear complexes formed with the insulin sequence, or the AP1 and SP1 binding activities used as control, were glucose insensitive. This is thus the first demonstration of a physiologic glucose-sensitive *trans*-acting factor for the insulin gene, whose further study may markedly enhance our understanding of the regulation of insulin biosynthesis in normal and diabetic beta cells. Furthermore, once cloned, the introduction of this glucose sensitive factor may enable the construction of truly physiologic artificial beta cells. [Diabetologia (1994) 37 [Suppl 2]: S3–S10]

Key words Beta cell, insulin gene, transcriptional regulation, glucose-sensitive *cis*-element, glucose-sensitive transactivator

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Abbreviations: bp, Base pair; NIDDM, non-insulin-dependent diabetes mellitus; CAT, chloramphenicol acetyl transferase; GSE, glucose sensitive element.

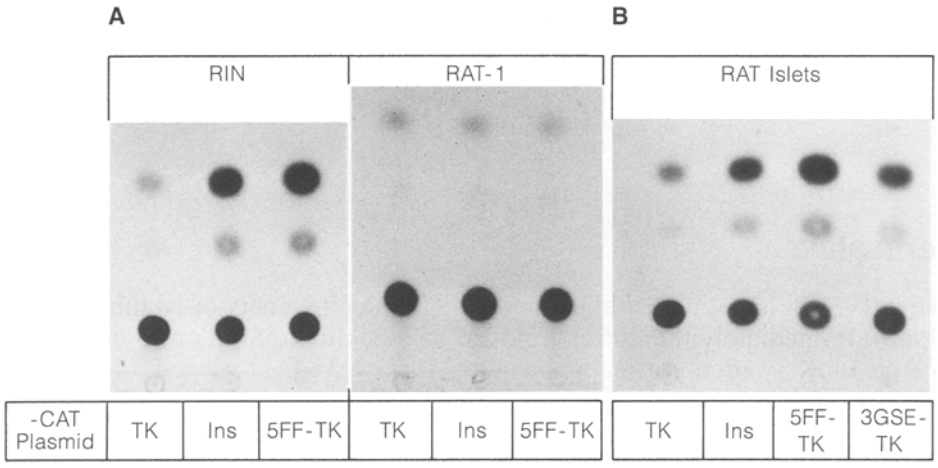


Fig. 1A, B. Activities of rat insulin I sequences in rat insulinoma (RIN) cells and Rat-1 fibroblasts (**A**) and isolated cultured islets (**B**) transiently transfected using the lipofectin method. TK is a plasmid containing the thymidine kinase promoter fused to the CAT gene. Ins contains the entire 5' flanking region of the rat insulin I gene from –410 to + 1, 5FF-TK carries 5 copies of the sequences from –196 to –247, and 3GSE-TK contains 3 copies of the sequence –193 to –227 (GSE) respectively linked to the TK-CAT chimeric gene

Non-insulin-dependent diabetes mellitus is probably a heterogeneous disorder. Although insulin resistance appears to be an integral part of the pathophysiology of the disease, some degree of insulin deficiency seems to be a necessary condition for the establishment of hyperglycaemia. Over the past three decades most of the investigative effort has been directed towards understanding the beta-cell disorders that lead to disturbed stimulus-secretion coupling, the emphasis being on the kinetic aspects of the acute insulin response to glucose stimulation [for review, see 1]. However, also the maximal secretory capacity of the pancreas is markedly reduced in NIDDM patients [2], suggesting that total insulin production may be limited. Indeed, both autopsy material from NIDDM patients [3], and islet extractions in some animal models of insulin resistance and diabetes such as the sand rat (Gadot et al., unpublished observation) have demonstrated varying degrees of depletion in the insulin content of the pancreas. Finally, in Wistar fatty rats that develop diabetes, insulin mRNA levels failed to augment in adaptation to obesity [4]. All the above suggest that, in addition to secretory defects, insulin synthesis may also be deficient in NIDDM, and that effort should also be directed towards understanding the mechanisms that regulate this process.

Insulin production is the *raison d'être* of the pancreatic beta cell. In the adult organism, the insulin gene is expressed exclusively in beta cells, indicating that strict cell-specific control mechanisms operate under normal conditions. Cell-specific phenotypic expression appears to be the result of complex interactions between *cis*-acting DNA elements and several *trans*-acting factors [5–7]. Cell culture studies and experiments with transgenic mice have demonstrated that the transcription of the insulin gene is controlled by specific DNA sequences located within its 5' flanking region [8–19]. Binding of proteins to the putative regulatory sequences of the insulin gene has been extensively investigated using the gel mobility shift assay [20–27] and has provided the basis for identification and cloning of some insulin *trans*-acting factors [28–34]. It has to be stressed, however, that the interpretation of these results is quite difficult, partly because transcriptional regulation is an extremely complex process involving a multitude of ubiquitous and partially as well as fully cell-type specific factors, but also because different types of transformed beta-cell lines were used in these studies. Thus, both transcription activating and inhibiting factors have been described [for review, see 35–37]. In the following, some of the studies performed in our laboratory are described in this context, with emphasis on the use of non-transformed beta cells and on the investigation of the glucose effect on insulin gene expression.

Cis- and *trans*-acting factors that convey beta-cell specificity to the insulin gene

There is ample evidence that several DNA motifs in the 5' flanking region of the rat insulin I gene are implicated in the full quantitative expression of insulin in the beta cell [35, 36]. These bind several nuclear proteins which, in some cases, interact with each other. They include factors expressed in many cells, like IEF 1/Pan-1 [28, 29], the GAGA box binding protein Pur-1 [34] and the homeodomain protein cdx-3 [33]; some are shared with neural cells (isl-1) [38] or hepatocytes (HNF1α) [39, and unpublished results from our laboratory]; finally, some seem to be beta-cell specific (lmx) [33] or shared with other genes like glucokinase [40] or islet amyloid polypeptide [19].

DNA transfection into hamster insulinoma (HIT) cells has shown that beta-cell specific expression is controlled by sequences ~400 bp upstream from the transcription start site of the rat insulin I gene. In addition, a short region extending between –196 and –247 (FF) was identified as a tissue specific transcrip-

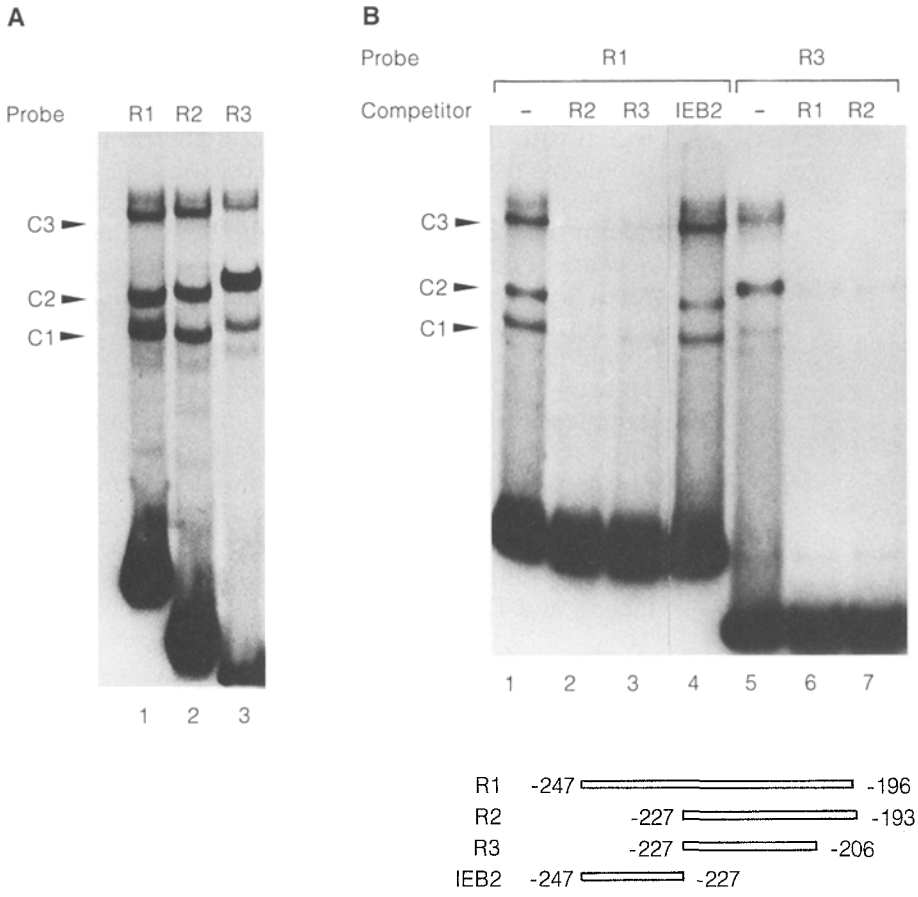


Fig. 2 A, B. Binding of islet nuclear factors to sequences in the mini-enhancer element FF of the rat insulin I gene. **A** Binding of islet nuclear extracts to the rat insulin I sequences corresponding to the following probes: R1, from –196 to –247 (FF); R2, from –193 to –227 (GSE); and R3, from –206 to –227. **B** Competition analysis of the mini-enhancer FF sequence in islet nuclear extracts. Fifty-fold excess of unlabelled R1, R2, R3 or IEB2 motif/Far box competitors were simultaneously added with R1 or R3 probes to the binding reactions as indicated. The IEB2 motif/Far box corresponds to the insulin regulatory region between –227 and –247. The binding of proteins was tested by gel mobility shift assay. From [45], with permission

tional activator when linked to a heterologous promoter [14, 19]. This is also illustrated in Figure 1A where rat insulinoma (RIN) cells were transfected with chloramphenicol acetyl transferase (CAT) plasmids containing either the entire 400 bp promoter region of the rat insulin I gene linked to the reporter CAT gene (Ins-CAT), or multimers of the FF region cloned upstream of the thymidine kinase promoter (5FF-TK-CAT). High levels of CAT activity were obtained only in insulin producing cells, while the rat-1 fibroblasts failed to activate the chimeric genes containing insulin sequences (Fig. 1A). The chimeric gene containing the FF element was highly expressed in transfected fetal islets cultured in high glucose [41].

Transformed beta cells deviate markedly from normal islet cells, and fetal islets are different from mature islets in their insulin secretory activity, responding poorly to nutrients [42, 43]. These considerations motivated us to focus on normal islets of Langerhans, exploiting a novel monolayer culture system for adult rat islets developed in our laboratory [44]. Figure 1B demonstrates that we were successful in transfecting adult islets by the lipofectin method: high CAT levels were obtained in islets transfected with the insulin containing constructs, while levels were low in those transfected with the parental TK-CAT vector. However, even the full FF is not necessary to direct beta-cell specific gene expression. Thus, deletion of 20 bp from the upstream portion of FF (denominated Far element in [14 and 19]) did not reduce significantly the intensity of the CAT signal when introduced into adult rat islet cells through the construct 3GSE-TK (Fig. 1B).

The FF minienhancer element of the rat insulin I gene (-196 to –247) was tested for its ability to bind islet nuclear factors in gel electrophoresis mobility shift assays [45]. As shown in Figure 2A, three major complexes (C1-C3) were detected. By competition with fragments containing mutations along the minienhancer sequence, the 35 bp-long subfragment extending from –193 to –227 (R2) was found sufficient to bind these complexes; it also competed efficiently for the binding of proteins to the original 52 nucleotide-long mini-enhancer (Fig. 2B). To further characterize the specificity of binding of islet nuclear factors, a shorter sequence extending from –206 to –227 in the rat insulin I gene (designated R3) was synthesized. In gel retardation assays this sequence produced similar complexes (C1-C3) which were efficiently displaced by the longer DNA fragments (Fig. 2).

The human insulin gene is expressed in transgenic mice in a cell-type specific manner [10, 11], implying that similar *trans*-activating factors regulate the rodent and human insulin genes. Cross-species comparisons show that the region between –206 and –227 is

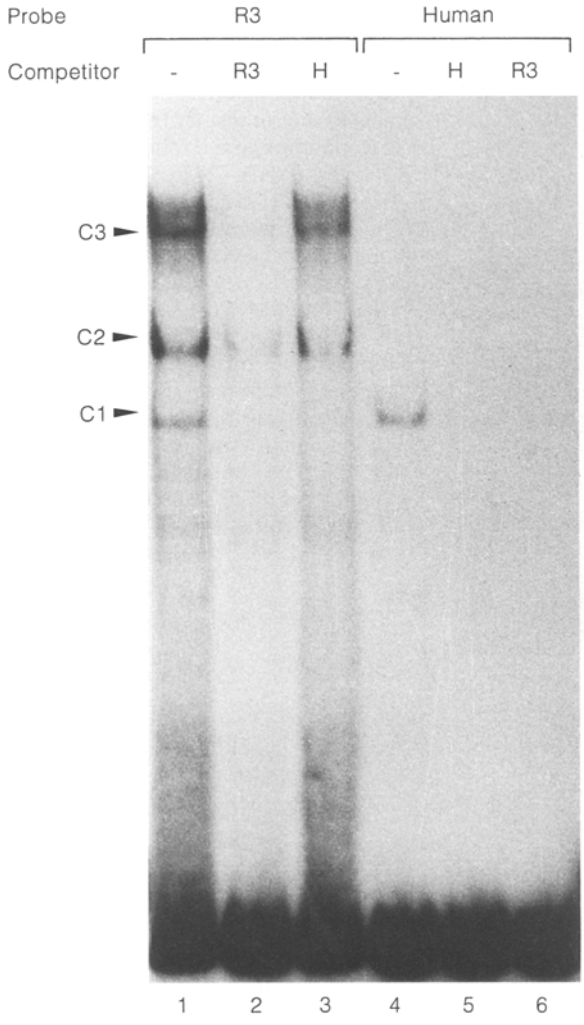


Fig. 3. Binding of rat islet nuclear proteins to the rat I and human insulin DNA sequences. Gel retardation assays were performed using the rat (R3) and the human insulin probes with rat islet nuclear extracts. Competition was carried out using a 50-fold excess of unlabelled double stranded oligonucleotides of the rat sequence (R3) in lanes 2 and 6, or the human sequence (H) in lanes 3 and 5. From [45], with permission

highly conserved [46]. A human DNA sequence homologous to that of the –206 to –227 region of the rat insulin I gene (R3) was synthesized and tested in gel mobility shift assays. Figure 3 shows that using the human probe a single complex was detected, which co-migrated with the rat C1 complex (lane 4). In competition experiments, both the human and the rat R3 sequences displaced efficiently the complex formed with the human probe (Fig. 3, lanes 5 and 6), while the human probe displaced only the C1 band from the rat sequences (Fig. 3, lane 3). These results strongly suggest that a similar nuclear factor interacts in a sequence-specific manner with a conserved element in the human and rat insulin genes. By testing nuclear extracts from a variety of tissues and cell lines with both probes we ascertained that the C1 complex is detected only in insulin-producing cells; furthermore, no other binding activity could be detected with the human sequence in any insulin non-producing tissue or cell line tested.

The specific binding of the C1 complex by the human insulin enhancer probe suggests that this factor is expressed also in human islets. This was tested directly by preparing nuclear extracts from human islets for binding to the rat and the human sequences spanning the region from –206 to –227. As shown in Figure 4 lane 1, the rat probe formed the expected complexes with rat islet extracts, the lowest binding intensity being for C1. In contrast, only two complexes were detectable in human islet extracts, the predominant one co-migrating with the C1 complex (Fig. 4, lane 3). Again, the human sequence identified only the C1 complex in both species (Fig. 4, lanes 2 and 4).

Stimulation of insulin gene expression by glucose

In pancreatic beta cells, glucose is the major regulator of insulin gene expression, of translational biosynthesis, and of release. Glucose controls the production of insulin at different levels, giving rise to a ~20-fold increase in its biosynthesis in response to high hexose concentrations. Studies in rat and human islets, as well as in beta cell lines, have indicated that long-term control of insulin gene expression by glucose is effectuated by simultaneous enhancement of the transcription rate and inhibition of the degradation of proinsulin mRNA [47–54]. Also in vivo, a close relationship was found between changes in blood glucose level and islet content of proinsulin mRNA [55, 56]. More recently, using the sensitive nuclear run-on analysis for measuring nascent insulin transcripts, Efrat et al. [57] could detect a 3-fold increase in the transcription of both the endogenous mouse insulin genes and the transgene in βTC3 cells as early as 10 min after incubating islets at high glucose. Thus, it seems justified to presume that stimulation of the transcription rate is an early event in the action of glucose on insulin gene expression.

Little is known about the molecular mechanisms by which metabolic signals regulate gene expression in eukaryotes. So far, it has not been possible to demonstrate the presence of an insulin gene *trans*-acting factor whose DNA binding is regulated by glucose in any insulin-producing cell. Transient transfection experiments with cultured fetal rat islets revealed that the glucose effect on transcription rate is mediated by the FF *cis*-acting element located within the enhancer region of the rat insulin I gene [41]. We tested whether also in adult islets such a glucose effect could be demonstrated. In cultured adult rat and

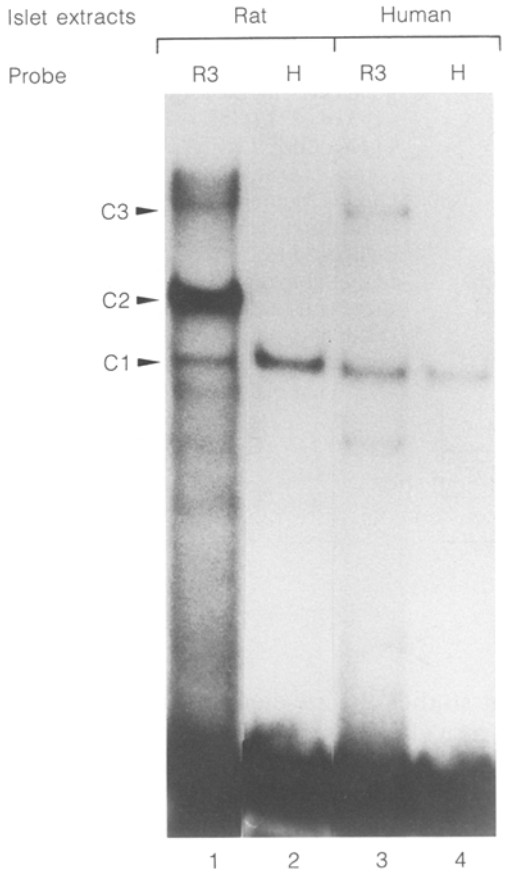


Fig. 4. Binding of nuclear factors from human islets to the DNA sequences of the rat and the human insulin genes. Probes corresponding to the rat insulin I gene between –206 and –227 (R3): lanes 1 and 3, and the corresponding human homologous region (H): lanes 2 and 4, were incubated with nuclear extracts from isolated rat (lanes 1 and 2) and human islets (lanes 3 and 4). From [45], with permission

human islets transfected with a CAT construct containing the rat insulin I mini-enhancer from –196 to –247, glucose stimulated markedly the transcription rate [45], the extent of stimulation being similar to that previously observed in fetal islets [41]. This indicates that despite major differences in the physiological responses of beta cells at different stages of development, glucose-induced insulin gene transcription is conserved in adult islets. We also verified the ability of the rat insulin I 35 bp subfragment (R2) to confer glucose responsiveness in transfected islets. As seen in Figure 5, the construct with three copies of R2 linked 5' to the TK-CAT chimeric gene responded as efficiently to glucose as the one containing 5 copies of FF. These findings indicate that the subfragment of 35 nucleotides (R2) contains the glucose sensitive element (GSE) of the rat insulin I gene. Glucose usually induced 6- to 7-fold and 2- to 3-fold increase in CAT activity of rat and human islets, respectively, the lower response in human islets being probably due to the prolonged storage of the islets during transport.

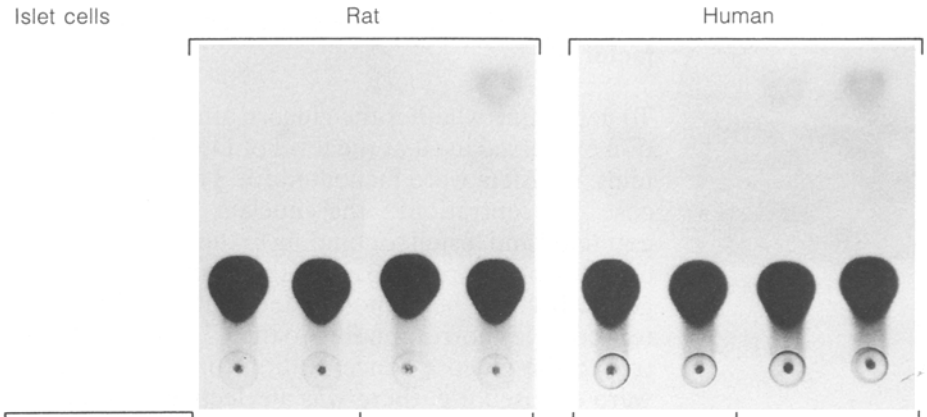
Identification of an islet specific glucose sensitive factor

To determine whether the glucose effect on transcription expressed itself at the level of DNA-binding proteins, rat islets were incubated for 3 h at various glucose concentrations; the nuclear proteins were extracted and tested for binding to the –206 to –227 sequences of the rat (R3) and the human (H) insulin genes. It could be demonstrated that the DNA-protein complex corresponding to the C1 band was sensitive to the glucose concentration to which the islets were exposed, i.e. there was a selective dose-dependent modulation of the C1 signal by extracellular glucose [45]. This is also illustrated in Figure 6 using the human insulin sequence as probe, which shows that the glucose effect on the C1 complex was already detected in islets incubated for 1 h in high glucose as compared to those maintained in low glucose levels (lanes 1–3). In summary, the overall intensity of the C1 band was 4- to 6-fold higher in extracts from islets incubated for 1–3 h at 20 mmol/l glucose than those maintained at 2 mmol/l, whether the rat or the human DNA sequences were used to form the complex. Whereas the tissue-specific C1 complex was sensitive to glucose, neither the AP1 (Fig. 6, lanes 4–6) nor the SP1 binding proteins (data not shown) were affected by the glucose concentration of the islet incubate.

Mode of action of glucose on the transcriptional activation of insulin

It is generally accepted that all actions of glucose on beta cell functions require the uptake and metabolism of the sugar [for review, see 58]. Two recent studies support the idea that insulin gene expression is controlled by the glucose metabolism of the islet. Thus, Goodison et al. [59] found that in HIT cells transfected with a CAT construct containing the sequence –345 to + 1 of the rat insulin I gene the glucose effect on transcription could be entirely blocked by mannoheptulose, an inhibitor of glucose phosphorylation. While the islet substrate mannose was as effective as glucose, galactose, which is not metabolized in beta cells, did not stimulate the CAT activity. Supporting these findings, German [60] demonstrated in fetal rat islets transfected with a similar CAT construct that neither fructose nor the non-metabolized analogue 2-deoxyglucose could substitute for glucose. Interestingly, pyruvate was inactive (as it is regarding insulin release). Thus, these results strongly suggest that early steps in beta cell glycolysis mediate the effect of glucose on the transcriptional activation of the insulin gene.

Glucose stimulation of beta cells is accompanied by a rapid increase in intracellular calcium. The glucose induction of insulin transcription was inhibited



Glucose mmol/l	2	20	2	20	2	20	2	20
Plasmid	TK-CAT		GSE-TK-CAT		TK-CAT		GSE-TK-CAT	
Fold of induction	1.3		8.6		1.1		2.6	

Fig. 5. Effect of glucose on CAT activity of rat and human islet cultures transiently transfected with a hybrid gene carrying the sequence from –193 to –227 (GSE) of the rat insulin I gene. GSE-TK-CAT contains 3 copies of GSE linked to the TK-CAT chimeric gene. The relative fold of induction in response to 20 mmol/l compared to 2 mmol/l glucose shown in the table represents the mean from three (rat) and two (human) independent experiments. From [45], with permission

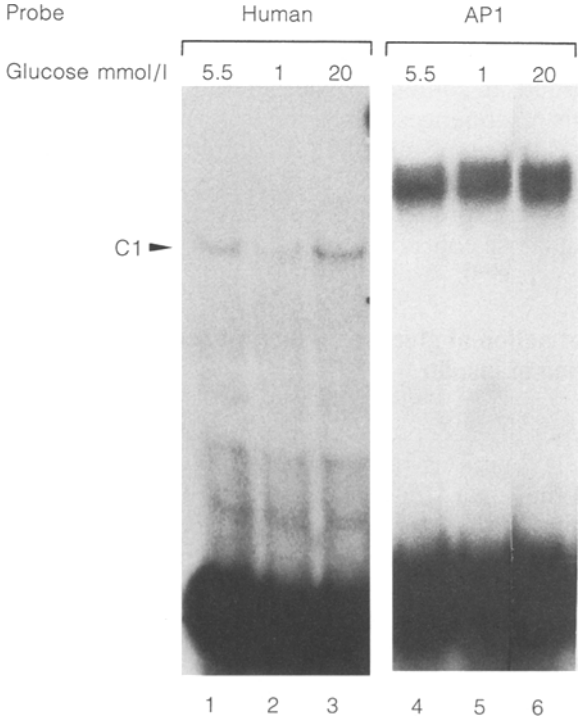


Fig. 6. Glucose sensitive factor in rat islet cells binds to the –206 to –227 sequence of the human insulin gene. Nuclear extracts from rat islets exposed to the indicated glucose concentrations for 1 h were incubated with the labelled human probe, as well as with the AP1 binding site, and tested using the gel mobility shift assay

by calcium channel blockers in some [41, 57] but not all [59] studies. Thus, while it is attractive to postulate that glucose might utilize the beta cell calcium pathway as a common means for stimulating both the synthesis and the release of insulin, further work is necessary to clarify the mechanism by which the observed glucose effects on insulin gene expression are mediated.

Conclusions

In the present studies the *cis*-element of the insulin gene that acts as a glucose sensitive element has been delineated with greater precision in rat as well as human islets, and a putative nuclear factor for the glucose effect described. It is clear from these results that treatment of islets with high glucose did not cause the formation of a new DNA binding complex, but rather elicited a rapid change in the binding intensity of the C1 complex. These changes in binding correspond to the overall increase in insulin mRNA level following rise in extracellular glucose in different systems [22, 30–32], as well as to the increase in CAT activity in transfected islet cells as shown here (Fig. 5) and elsewhere [41]. We suggest the term of glucose sensitive factor (GSF) for the protein(s) involved in the C1 complex. This is the first demonstration of a physiologic glucose-sensitive *trans*-acting factor for the insulin gene, and we expect that further studies on the mode of action of glucose sensitive factor may greatly enhance our understanding of the regulation of insulin biosynthesis in normal and diabetic islets.

The long-term goal for diabetes therapy is development of a genetically engineered "beta cell" capable of secreting insulin in response to glucose under physiological conditions. The genetic engineering of the AtT20 pituitary cell by Hughes et al. [61] has helped to move forward towards such a goal, although their "artificial beta cell" secretes insulin in response to dbcAMP but not to glucose. We believe that the introduction of the glucose sensitive factor described here to such a system could allow the regulation of insulin production by glucose.

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References

1. Cerasi E (1992) Aetiology of type II diabetes. In: Ashcroft FM, Ashcroft SJH (eds) Insulin: molecular biology to pathology: a practical approach. Oxford University Press, Oxford, pp 347–392
2. Nesher R, Della Casa L, Litvin Y et al. (1987) Insulin deficiency and insulin resistance in type 2 (non-insulin-dependent) diabetes. Quantitative contributions of pancreatic and peripheral responses to glucose homeostasis. Eur J Clin Invest 17: 266–274
3. Rahier J (1988) The diabetic pancreas: a pathologist's view. In: Lefèbvre PJ, Pipeleers DG (eds) The pathology of the endocrine pancreas in diabetes. Springer-Verlag, Berlin, pp 17–40
4. Seino Y, Yamamoto T, Koh G (1992) Insulin and glucose transporter gene expression in obesity and diabetes. Proc Soc Exp Biol Med 200: 210–213
5. Jones NC, Rigby PWJ, Ziff EB (1988) Trans-acting protein factors and the regulation of eukaryotic transcription: lessons from studies on DNA tumour viruses. Genes Dev 2: 267–281
6. Ptashne M (1988) How eukaryotic transcriptional activators work. Nature 333: 638–639
7. Johnson PF, McKnight SL (1989) Eukaryotic transcriptional regulatory proteins. Annu Rev Biochem 58: 799–839
8. Walker MD, Edlund T, Boulet AM, Rutter WJ (1983) Cell-specific expression controlled by the 5' flanking region of insulin and chymotrypsin genes. Nature 306: 557–561
9. Edlund T, Walker MD, Barr PJ, Rutter WJ (1985) Cell specific expression of the rat insulin gene: evidence for the role of two distinct 5' flanking sequences. Science 230: 912–916
10. Hanahan D (1985) Hereditable formation of pancreatic β cell tumours in transgenic mice expressing recombinant insulin/simian virus 40 oncogenes. Nature 315: 115–122
11. Selden RF, Skoskiewicz MJ, Howie KB, Russell PS, Goodman H M (1986) Regulation of human insulin gene expression in transgenic mice. Nature 320: 525–528
12. Fromont-Racine M, Bucchini D, Madsen O et al. (1990) Effect of 5'-flanking sequence deletions on expression of the human insulin gene in transgenic mice. Mol Endocrinol 4: 669–677
13. Karlsson O, Edlund T, Moss LB, Rutter WJ, Walker MD (1987) A mutational analysis of the insulin gene transcription control region: expression in beta cells is dependent on two related sequences within the enhancer. Proc Natl Acad Sci USA 84: 8819–8823
14. Karlsson O, Walker MD, Rutter WJ, Edlund T (1989) Individual protein-binding domains of the insulin gene enhancer positively activate β cell specific transcription. Mol Cell Biol 9: 823–827
15. Crowe DT, Tsai M (1989) Mutagenesis of the rat insulin II 5'-flanking region defines sequences important for expression in HIT cells. Mol Cell Biol 9: 1784–1789
16. Hwung Y-P, Gu YZ, Tsai MJ (1990) Cooperativity of sequence elements mediates tissue specificity of the rat insulin II gene. Mol Cell Biol 10: 1784–1788
17. Whelan J, Poon D, Weil PA, Stein R (1989) Pancreatic β-cell-specific expression of the rat insulin II gene is controlled by positive and negative cellular transcriptional elements. Mol Cell Biol 9: 3253–3259
18. Boam DSW, Clark AR, Docherty K (1990) Positive and negative regulation of the human insulin gene by multiple trans-acting factors. J Biol Chem 265: 8285–8296
19. German MS, Moss LG, Wang J, Rutter WJ (1992) The insulin and islet amyloid polypeptide genes contain similar cell-specific promoter elements that bind identical β-cell nuclear complexes. Mol Cell Biol 12: 1777–1788
20. Ohlsson H, Edlund T (1986) Sequence-specific interactions of nuclear factors with the insulin gene enhancer. Cell 45: 35–44
21. Ohlsson H, Karlsson O, Edlund T (1988) A beta cell specific protein binds to two major regulatory sequences of the insulin gene enhancer. Proc Natl Acad Sci USA 85: 4228–4231
22. Moss LG, Moss JB, Rutter WJ (1988) Systematic binding analysis of the insulin gene transcription control region: insulin and immunoglobulin enhancers utilize similar transactivators. Mol Cell Biol 8: 2620–2627
23. Ohlsson H, Thor S, Edlund T (1991). Novel insulin promoter- and enhancer-binding proteins that discriminate between pancreatic α- and β-cells. Mol Endocrinol 7: 897–904
24. Hwung YP, Crowe DT, Wang LH, Tsai SY, Tsai M-J (1988) The COUP transcription factor binds to an upstream promoter element of the rat insulin II gene. Mol Cell Biol 8: 2070–2077
25. Whelan J, Cordle SR, Henderson E, Weil PA, Stein R (1990) Identification of a pancreatic β-cell insulin gene transcription factor that appears to activate cell-type-specific expression: its possible relationship to other cellular factors that bind to a common insulin gene sequence. Mol Cell Biol 10: 1564–1572
26. Boam DSW, Docherty K (1989) A tissue specific nuclear factor binds to multiple sites in the human insulin gene enhancer. Biochem J 264: 233–239
27. Scott V, Clark AR, Hutton JC, Docherty K (1991) Two proteins act as the IUF1 insulin gene enhancer binding factor. FEBS Lett 290: 27–30
28. Walker MD, Park CW, Rosen A, Aronheim A (1990) A cDNA from a mouse pancreatic β cell encoding a putative transcription factor of the insulin gene. Nucl Acids Res 18: 1159–1166
29. German MS, Blanar MA, Nelson C, Moss LG, Rutter WJ (1991) Two related helix-loop-helix proteins participate in separate cell specific complexes that bind the insulin enhancer. Mol Endocrinol 5: 292–299
30. Cordle SR, Henderson E, Masuoka H, Weil PA, Stein R (1991) Pancreatic β-cell-type-specific transcription of the insulin gene is mediated by basic helix-loop-helix DNA-binding proteins. Mol Cell Biol 11: 1718–1734
31. Shibasaki Y, Sakura H, Takaku F, Kasuga M (1990) Insulin enhancer binding protein has helix-loop-helix structure. Biophys Res Commun 170: 2214–2221
32. Karlsson O, Thor S, Norberg T, Ohlsson H, Edlund T (1990) Insulin gene enhancer binding protein Isl-1 is a member of a novel class of proteins containing both a homeo- and Cys-His domain. Nature 344: 8799–8802
33. German MS, Wang J, Chadwick RB, Rutter WJ (1992) Synergistic activation of the insulin gene by a LIM-homeo domain protein and a basic helix-loop-helix protein: building a functional insulin minienhancer complex. Genes Dev 6: 2165–2176
34. Kennedy GC, Rutter WJ (1992) Pur-1, a zinc-finger protein that binds to purine-rich sequences, transactivates an insulin promoter in heterologous cells. Proc Natl Acad Sci USA 89: 11498–11502
35. Steiner DF, James DE (1992) Cellular and molecular biology of the beta cell. Diabetologia 35 [Suppl 2]: S41-S48

36. Stein R (1993) Regulation of insulin gene transcription. Trends Endocr Metab 4: 96–101
37. Clark AR, Docherty K (1992) The insulin gene. In: Ashcroft FM, Ashcroft SJH (eds) Insulin: molecular biology to pathology: a practical approach. Oxford University Press, Oxford, pp 37–63
38. Ericson J, Thor S, Edlund T, Jessell TM, Yamada T (1992) Early stages of motor neuron differentiation revealed by expression of homeobox gene *islet-1*. Science 256: 1555–1560
39. Emens LA, Landers DW, Moss LG (1992) Hepatocyte nuclear factor 1α is expressed in a hamster insulinoma line and transactivates the rat insulin I gene. Proc Natl Acad Sci USA 89: 7300–7304
40. Shelton KD, Franklin AJ, Khoor A, Beechem J, Magnuson MA (1992) Multiple elements in the upstream glucokinase promoter contribute to transcription in insulinoma cells. Mol Cell Biol 12: 4578–4589
41. German MS, Moss LG, Rutter WJ (1990) Regulation of insulin gene expression by glucose and calcium in transfected primary islet cultures. J Biol Chem 265: 22063–22066
42. Grill V, Asplund K, Hellerström C, Cerasi E (1975) Decreased cyclic AMP and insulin response to glucose in isolated islets of neonatal rats. Diabetes 24: 746–752
43. Sodoyez-Goffaux F, Sodoyez J-C, Foa PP (1971) Effects of gestational age, birth and feeding on the insulinogenic response to glucose and tolbutamide by fetal and newborn rat pancreas. Diabetes 20: 586–591
44. Kaiser N, Corcos AP, Tur-Sinai A, Ariav Y, Cerasi E (1988) Monolayer culture of adult rat pancreatic islets on extracellular matrix: long term maintenance of differentiated B-cell function. Endocrinology 123: 834–840
45. Melloul D, Ben-Neriah Y, Cerasi E (1993) Glucose modulates the binding of an islet-specific factor to a conserved sequence within the rat I and the human insulin promoters. Proc Natl Acad Sci USA 90: 3865–3869
46. Steiner DF, Chan SJ, Welsh JM, Kwok SCM (1985) Structure and evolution of the insulin gene. Annu Rev Genet 19: 363–384
47. Jarrett RJ, Keen H, Track N (1967) Glucose and RNA synthesis in mammalian islets of Langerhans. Nature 213: 634–635
48. Permutt MA, Kipnis DM (1972) Insulin biosynthesis. On the mechanism of glucose stimulation. J Biol Chem 247: 1194–1199
49. Itoh N, Sei T, Nose K, Okamoto H (1978) Glucose stimulation of the proinsulin synthesis in isolated pancreatic islets without increasing amounts of proinsulin mRNA. FEBS Lett 93: 343–347
50. Nielsen DA, Welsh M, Casadaban MJ, Steiner DF (1985) Control of insulin gene expression in pancreatic β-cells and in an insulin-producing cell line, RIN-5F cells. I. Effects of glucose and cyclic AMP on the transcription of insulin mRNA. J Biol Chem 260: 13585–13589
51. Welsh M, Nielsen DA, MacKrell AJ, Steiner DF (1985) Control of insulin gene expression in pancreatic β-cells and in an insulin-producing cell line, RIN-5F cells. II. Regulation of insulin mRNA stability. J Biol Chem 260: 13590–13594
52. Hammonds P, Schofield PN, Ashcroft SJH (1987) Glucose regulates preproinsulin messenger RNA levels in a clonal cell line of simian virus 40-transformed B cells. FEBS Lett 213: 149–154
53. Hammonds P, Schofield PN, Ashcroft SJH, Sutton R, Gray DWR (1987) Regulation and specificity of glucose-stimulated insulin gene expression in human islets of Langerhans. FEBS Lett 223: 1371–1377
54. Nagamatsu S, Grodsky GM (1988) Glucose-regulated proinsulin processing in isolated islets from rat pancreas. Diabetes 37: 1426–1430
55. Giddings SJ, Chirgwin J, Permutt MA (1981) The effects of fasting and feeding on preproinsulin messenger RNA in rats. J Clin Invest 67: 952–960
56. Giddings SJ, Chirgwin J, Permutt MA (1982) Effects of glucose on proinsulin messenger RNA in rats in vivo. Diabetes 31: 624–629
57. Efrat S, Surana M, Fleischer N (1991) Glucose induces insulin gene transcription in a murine pancreatic β-cell line. J Biol Chem 266: 11141–11143
58. Ashcroft FM, Ashcroft SJH (1992) Mechanism of insulin secretion. In: Ashcroft FM, Ashcroft SJH (eds) Insulin: molecular biology to pathology: a practical approach. Oxford University Press, Oxford, pp 97–150
59. Goodison S, Kenna S, Ashcroft SJH (1992) Control of insulin gene expression by glucose. Biochem J 285: 563–568
60. German MS (1993) Glucose sensing in pancreatic beta cells: the key role of glucokinase and the glycolytic intermediates. Proc Natl Acad Sci USA 90: 1781–1785
61. Hughes SD, Johnson JH, Quade C, Newgard CB (1992) Engineering of glucose-stimulated insulin secretion and biosynthesis in non-islet cells. Proc Natl Acad Sci USA 89: 688–689