Volume 317, number 1,2, 67–73 FEBS 12032 February 1993

Two 3′,5′-cyclic-adenosine monophosphate response elements in the promoter region of the human gastric inhibitory polypeptide gene

Yoshimichi Someya[a,b], Nobuya Inagaki[c], Toshio Maekawa[d], Yutaka Seino[b] and Shunsuke Ishii[d]

Second Division, [a]Department of Medicine and [b]Department of Metabolism and Clinical Nutrition, Kyoto University School of Medicine, Kyoto 606, Japan, [c]Division of Molecular Medicine, Center of Neurobiology and Molecular Immunology, Chiba University School of Medicine, Chiba 280, Japan and *[d]Laboratory of Molecular Genetics, Tsukuba Life Science Center, The Institute of Physical and Chemical Research (RIKEN), Tsukuba, Ibaraki 305, Japan*

Received 14 December 1992

Transfection of chimeric chloramphenicol acetyltransferase plasmids containing various deletions of the human gastric inhibitory polypeptide (GIP) promoter into hamster insulinoma (HIT T15) cells indicated that the region between −180 and +14 is sufficient for basal promoter activity. Two CRE-BP1 binding sites were identified in this promoter region by DNase I footprinting with the bacterially expressed cAMP response element (CRE) binding protein, CRE-BP1. Mutation analyses showed that these two CREs are required for the basal promoter activity, and furthermore that one site, at nucleotide-158, contributed mainly to the cAMP inducibility of the GIP promoter in HIT T15 cells. Interestingly, the GIP promoter activity was repressed by the c-*jun* proto-oncogene product, possibly through the CREs.

Gastric inhibitory protein; cAMP response element; Glucose; Jun

1. INTRODUCTION

Gastric inhibitory polypeptide (GIP) was first referred to as enterogastrone [1], since it was purified from porcine intestinal extracts on the basis of its ability to inhibit gastric acid secretion [2,3]. However, subsequent studies on GIPs biological role indicated that the major action of this peptide is to stimulate insulin secretion at elevated glucose levels [4,5], rather than to inhibit gastric acid secretion [6]. GIP consists of 42 amino acid residues [7], and is a member of the glucagon–secretin family, which also contains vasoactive intestinal peptide and growth hormone-releasing factor [8–10].

Analysis of the human GIP cDNA showed that GIP is derived by proteolytic processing of a 153 amino acid precursor [11]. The human gene coding for the GIP precursor contains six exons and was assigned to chromosome 17q21.3–q22 [12]. The promoter region of the human GIP gene contains potential binding sites for multiple transcription factors, including Sp1 and AP-1, but the roles of these sites are unknown [12].

GIP could act in maintaining glucose homeostasis by functioning as a glucose-dependent insulin-releasing peptide. In the regulation of glucose homeostasis, insulin has a major physiological role, and its own biosynthesis is regulated by blood glucose at the transcriptional [13,14] and translational [15] levels. Although the mechanism of glucose-induced insulin gene expression is poorly understood, the following facts support the possibility that cAMP may participate: glucose increases the cAMP concentration in pancreatic β cells [16], and analogues of cAMP augment insulin mRNA levels in isolated islets [17]. Since GIP could be also important for glucose homeostasis, it is interesting to know whether the GIP gene expression is also regulated by cAMP and glucose.

We have demonstrated that cAMP inducibility of the human insulin gene transcription is controlled by four cAMP response elements (CRE: TGACGTCA) [18]. CRE is an inducible enhancer of genes which can be transcribed in response to increased cAMP levels [19,20]. Four CREs contributed additively to the cAMP inducibility of the insulin promoter. Although multiple CRE binding proteins, including CREB [21,22], CRE-BP1 [23], and ATFs [24], all of which have the so-called 'B-ZIP' structure, were identified by cDNA cloning, only CREB was so far demonstrated to be involved in the cAMP-induced transcriptional activation. The *trans*-activating capacity of CREB is activated by phosphorylation of Ser-133 by cAMP-dependent protein kinase A [25]. CRE binding proteins bind to CRE as a homodimer or heterodimer with other protein, and CRE-BP1 can dimerize with the c-*jun* protooncogene product (c-Jun) [26,27]. Interestingly, c-Jun represses the cAMP-induced activity of the insulin promoter [18]. Since the CRE-BP1 homodimer, but not the CRE-BP1/

Correspondence address: S. Ishii, Laboratory of Molecular Genetics, Tsukuba Life Science Center, The Institute of Physical and Chemical Research (RIKEN), 3-1-1 Koyadai, Tsukuba, Ibaraki 305, Japan. Fax: (81) (298) 36 9030.

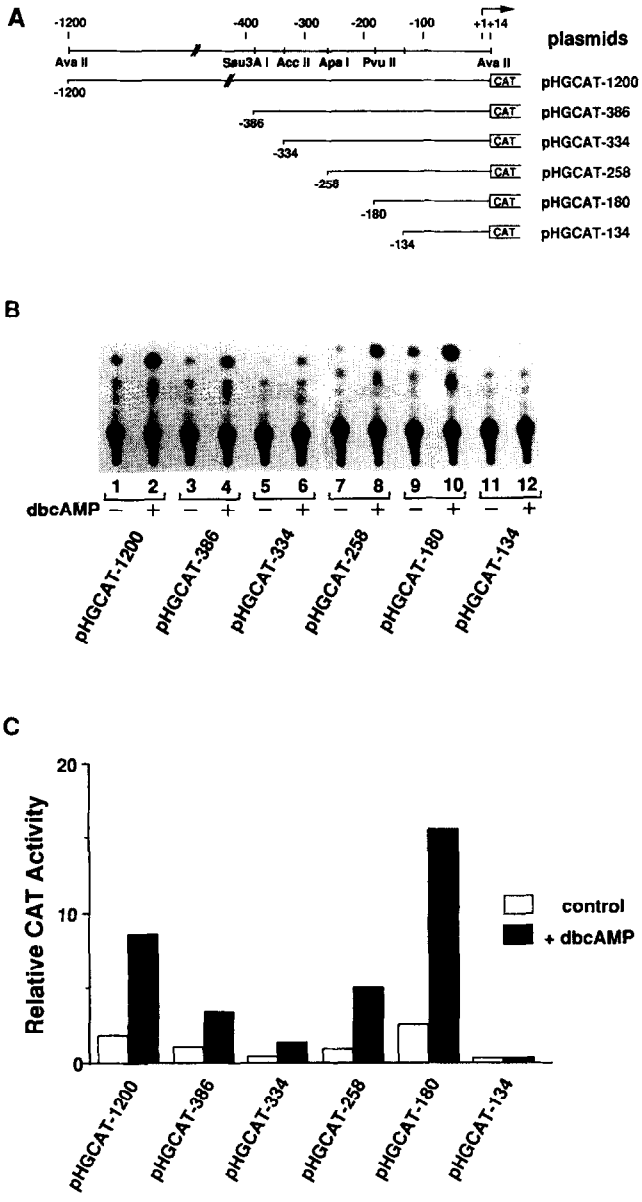


Fig. 1. Deletion analysis of the human GIP gene promoter. (A) Structure of the 5′ deletion mutants of the human GIP gene promoter. Deletions were generated using the restriction enzyme sites indicated above. (B) CAT activities of the 5′ deletion mutants. A mixture of 16 μg of each CAT plasmid and 4 μg of the internal control plasmid pact-β-gal was transfected into HIT cells, and CAT activity expressed in a transient assay was measured. Some cell samples were treated with Bt_2cAMP as indicated. (C) CAT activity obtained with each CAT plasmid is indicated relative to that with pHGCAT-386 without Bt_2cAMP treatment.

c-Jun heterodimer, binds to the CREs of the human insulin gene, a possibility is that c-Jun inhibits the binding of some CRE binding proteins, such as CRE-BP1, to the CREs of the insulin gene by formation of a heterodimer. These results, and another observation that the level of c-Jun is dramatically increased by glucose deprivation in hamster insulinoma (HIT T15) cells [18], suggest that glucose may regulate expression of the human insulin gene through CREs and c-Jun.

In this study, we have examined the regulation of GIP promoter activity by cAMP or c-Jun. We have identified two CRE-BP1-binding sites in the human GIP promoter and found that one of them is mainly responsible for cAMP inducibility. We have also found that the GIP promoter activity is also repressed by c-Jun like the human insulin promoter.

2. MATERIALS AND METHODS

2.1. *Plasmid construction*

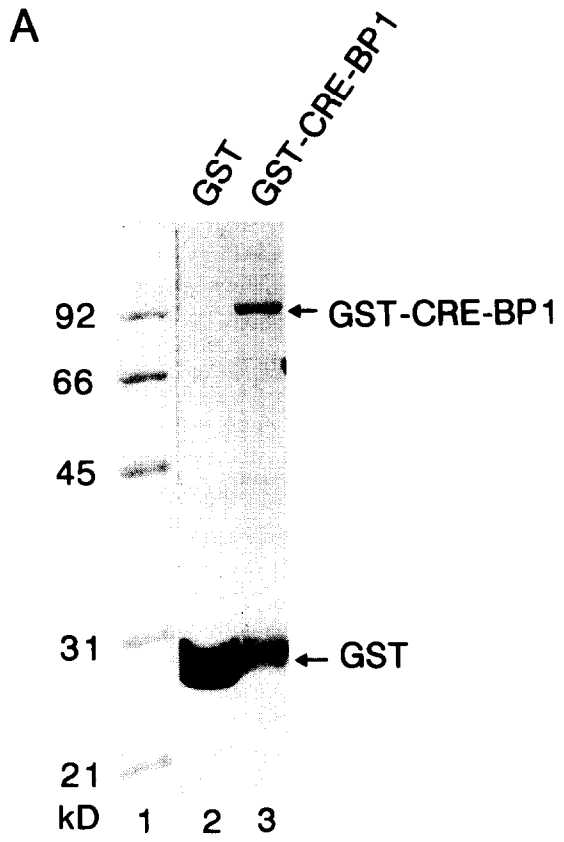
A series of chloramphenicol acetyltransferase (CAT) plasmids shown in Fig. 1A were generated by inserting various lengths of DNA fragments containing the human GIP promoter into the *Hin*dIII site of pSV00CAT [28] using a *Hin*dIII linker. To generate the plasmids containing the mutated CRE1 and CRE2 shown in Fig. 4A, oligonucleotide-directed mutagenesis was done as described by Sayers et al. [29]. The CRE-BP1 expression plasmid, pact-CRE-BP1 [30], and the c-Jun expression plasmid, pRSV-c-*jun* [31], were previously described. The plasmid, pGEX-CRE-BP1, to express the glutathione *S*-transferase (GST)-CRE-BP1 fusion protein in bacteria, was constructed as follows. A *Bam*HI site was introduced in front of the translational initiation codon of the CRE-BP1 gene. Then, the *Bam*HI–*Bgl*II DNA fragment containing the whole protein-coding region of CRE-BP1 gene was isolated and cloned into the *Bam*HI site of the pGEX-3X vector (Pharmacia).

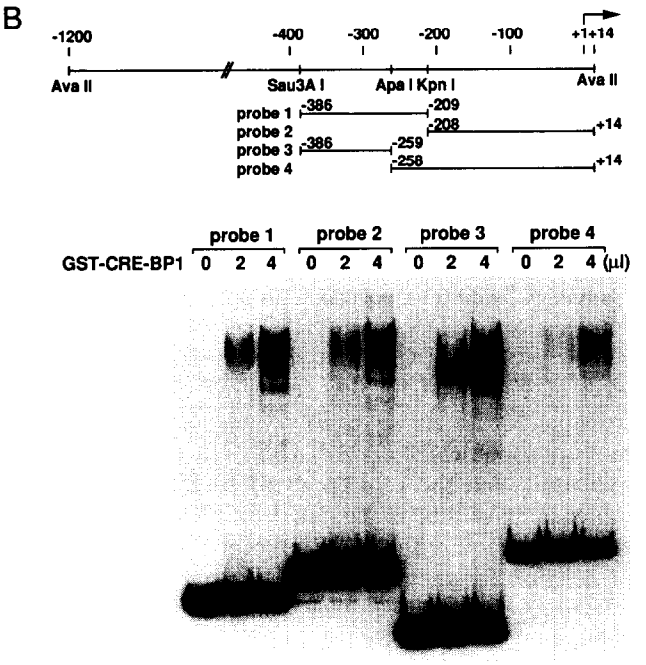
2.2. *DNA transfection and CAT assay*

CAT transfection experiments using HIT cells were done as described [18]. To examine the basal promoter activity and the cAMP inducibility, mixtures of 16 μg of each CAT plasmid DNA, 4 μg of the internal control plasmid, pact-β-gal [30], in which the 5′ regulatory region of the chicken β-actin gene was linked to the β-galactosidase gene, were transfected. To examine the effect of CRE-BP1 and c-Jun on the GIP promoter activity, two types of conditions were used. In the experiments shown in Fig. 5, mixtures of 6 μg of pHGCAT-386 DNA, various amounts of pact-CRE-BP1, pRSV-c-*jun*, or the control effector plasmid lacking the cDNA sequence to be expressed (total amount of the effector plasmid DNA was adjusted to 4 μg by varying the amount of the control effector plasmid), and 6 μg of the internal plasmid pras-β-gal were transfected. In pras-β-gal [32], the human c-Ha-*ras*-1 promoter [33] was linked to the β-galactosidase gene. In the experiments shown in Fig. 6, mixtures of 12 μg of each CAT plasmid DNA, 4 μg of pRSV-*c-jun*, and 4 μg of pact-β-gal were transfected. We had confirmed that the activity of the chicken β-actin promoter or the human c-Ha-*ras*-1 promoter was not affected by CRE-BP1 or c-Jun ([33], our unpublished results). 48 h after transfection, CAT activities were measured by the procedure of Gorman et al. [34]. The amount of cell extracts used for CAT assays was normalized with respect to β-galactosidase activity. The radioactivity of either [^{14}C]chloramphenicol or its acetylated form was measured by using a bioimage analyzer (Fuji). All CAT transfection experiments were repeated three or four times, and typical results are shown here. The differences between each set of experiments were within 20%.

2.3. *Preparation of GST-CRE-BP1 fusion protein*

GST-CRE-BP1 fusion protein expression and purification were essentially as described by Smith and Johnson [35]. *E. coli* SG12036 harboring pGEX-CRE-BP1 was grown at 37°C in 2× YT medium containing 50 μg/ml ampicillin. After this reached an absorption at 550 nm of 0.6, isopropyl-β-D-thiogalactopyranoside (IPTG) was added (final concentration 1 mM) and the bacterial growth was continued for 2 h at 37°C. The bacteria were lysed by mild sonication and the crude extracts (10 ml) prepared from 500 ml culture were rocked for





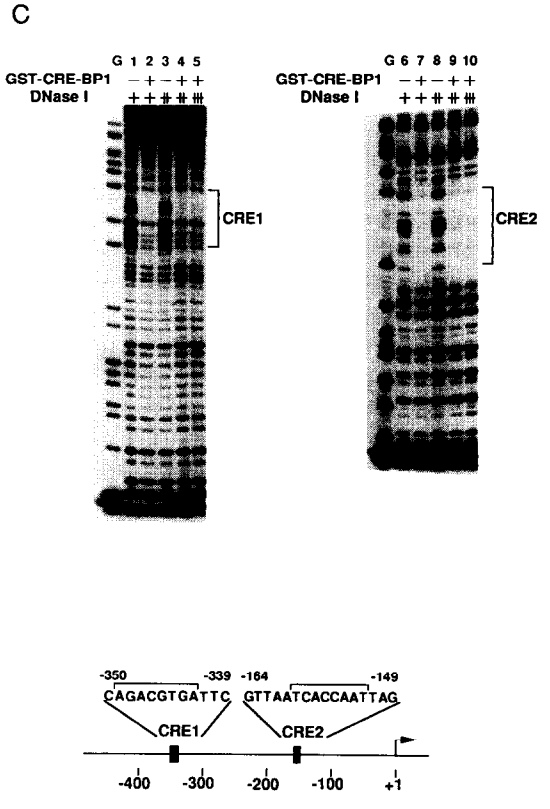


Fig. 2. Identification of CRE-BP1 binding sites in the promoter region of human GIP gene. (A) Purification of the bacterially expressed GST-CRE-BP1. The purified GST-CRE-BP1 fusion protein was separated on a 10% SDS-polyacrylamide gel and stained with Coomassie brilliant blue. (B) Gel mobility shift assay. Four DNA fragments, indicated above, were end-labeled and used as probes (probe 1–4). Each probe was incubated with various amounts of the purified GST-CRE-BP1 (30 ng/μl) as indicated, and electrophoresis was in 4% polyacrylamide gels of low ionic strength. (C) DNase I footprinting. The *Hin*dIII–*Kpn*I (nucleotides −386 to −209) fragment of pHGCAT-386 (lanes 1–5), or the *Bam*HI–*Hin*dIII (nucleotides −212 to +14) fragment of pHGCAT-180 (lanes 6–10) was ^{32}P-labeled, incubated with 8 μg of GST-CRE-BP1 (lanes marked +) or without protein (lanes marked −), and digested with 10 (lanes marked +), 20 (lanes marked ++), or 40 (lanes marked +++) μg/ml DNase I. Markers (lanes marked G) were obtained by the chemical cleavage of the same end-labeled DNA fragment. The protected regions are depicted on the right. Nucleotide sequences of two sites protected by GST-CRE-BP1 are shown below.

60 min at 4°C with 3 ml of glutathione–Sepharose beads in phosphate-buffered saline (PBS). The beads were washed with PBS, and the GST-CRE-BP1 fusion protein was then eluted with elution buffer (5 mM glutathione, 50 mM Tris-HCl, pH 8.0).

2.4. *Gel mobility shift assay and DNase I footprinting*

For gel mobility shift assay, variable amounts of purified GST-CRE-BP1 fusion proteins were incubated for 15 min at 25°C with 1 ng of ^{32}P-labeled DNA fragment in 15 μl of binding buffer (6.7 mM Tris-HCl, pH 7.5, 3.3 mM sodium acetate, 1 mM EDTA, 5% glycerol). The reaction mixture was then put onto a 4% polyacrylamide gel in 0.25× TBE (22.25 mM Trizma base, 22.25 mM boric acid, 0.5 mM EDTA) and electrophoresed. For DNase I footprint experiments, DNA binding reactions and DNase I digestions were done in a 60-μl volume with 3–5 ng (about 10–20 fmol) of an end-labeled DNA fragment and various amounts of purified GST-CRE-BP1, in the same buffer as that for the gel retardation assay. Reactions were incubated for 30 min at 25°C and then digested for 60 s at 20°C with 8 μl of freshly diluted 100–400 μg/ml solution of DNase I after addition of $MgCl_2$ to a final concentration of 10 mM.

3. RESULTS

3.1. *Deletion analysis of the human GIP gene promoter*

To examine the promoter activity of the region upstream from the RNA start site which was previously reported [12], the 1.2-kbp *Ava*II DNA fragment (nucleotides +14 to −1,200) was placed on the 5′ side of the bacterial CAT gene derived from the plasmid, pSV00CAT [28]. The ability of this construct (pHGCAT-1200) to express CAT activity was tested by transfection of DNA into HIT cells. After 48 h, the significant level of CAT activity was observed, and the level of CAT activity was increased about 5-fold by treatment with N^6,O^2-dibutyryladenosine 3′,5′-cyclic monophosphate (Bt_2cAMP) (Fig. 1B, lanes 1 and 2), indicating that the 1.2-kbp *Ava*II fragment has a promoter activity that is regulated by cAMP. To identify the region required for GIP promoter activity, a series of deletion fragments of the GIP gene promoter were ligated 5′ to the CAT gene and tested for promoter activity. The structures of the various deletions and their relative promoter activities in the presence or absence of Bt_2cAMP are shown in Fig. 1. Deletions from the 5′ end of the insert to nucleotides −386 (pHGCA-386) or −334 (pHGCAT-334) resulted in a 40% or 70% decrease in the basal promoter activity. Further deletions to nucleotides −258 (pHGCAT-258) or −180 (pHGCAT-180) increased the basal promoter activity to the level of 57% or 150% of that of pHGCAT-1200. These results suggest the presence of both positive and negative elements in the region between nucleotides −1,200 and −180. Furthermore, with these four deletion mutants, the cAMP inducibility was still observed. However, deletion to nucleotides −134 almost completely abolished both the basal promoter activity and the cAMP inducibility. These results indicate that the region between nucleotides −180 and −134 contains the *cis* element responsible for the cAMP-induced *trans*-activation and basal promoter activity.

3.2. *Identification of two CREs*

To identify the CRE within the human GIP promoter region, we next did a mobility shift assay and DNase I footprinting using the bacterially synthesized fusion proteins (GST-CRE-BP1) between glutathione S-transferase and the CRE binding protein, CRE-BP1. Four DNA fragments covering the region between nucleotides −386 and +14 of the human GIP gene were prepared and used as a DNA probe for a gel mobility shift assay. The GST-CRE-BP1 proteins bound to all four ^{32}P-labeled probe and generated protein–DNA complexes (Fig. 2A), indicating that multiple CRE-BP1-binding sites are localized in the GIP promoter region. In DNase I footprint assay, GST-CRE-BP1 protected two regions: CRE1 at nucleotides −350 to −339 and CRE2 at nucleotides −164 to −149 (Fig. 2B). We observed that these two regions were also protected in

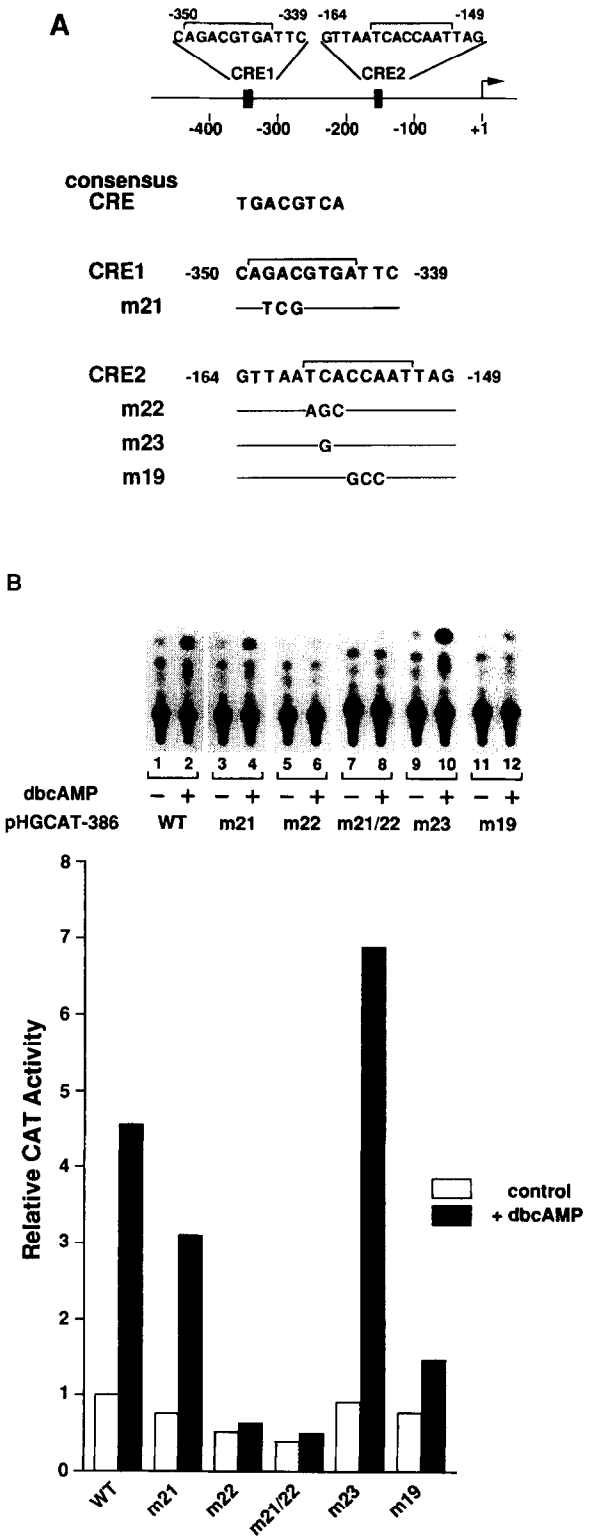


Fig. 3. Effects of mutation of the CRE-BP1-binding sites on the cAMP inducibility of GIP gene promoter. (A) Mutations introduced into the CRE-BP1-binding sites of the GIP promoter region. The wild-type sequences of two CRE-BP1-binding sites are shown at the top. The nucleotide sequences of the consensus CRE and each mutant are indicated below. (B) Transient expression of CAT activity. CAT co-transfection assays were done and the results are presented as in Fig. 1.

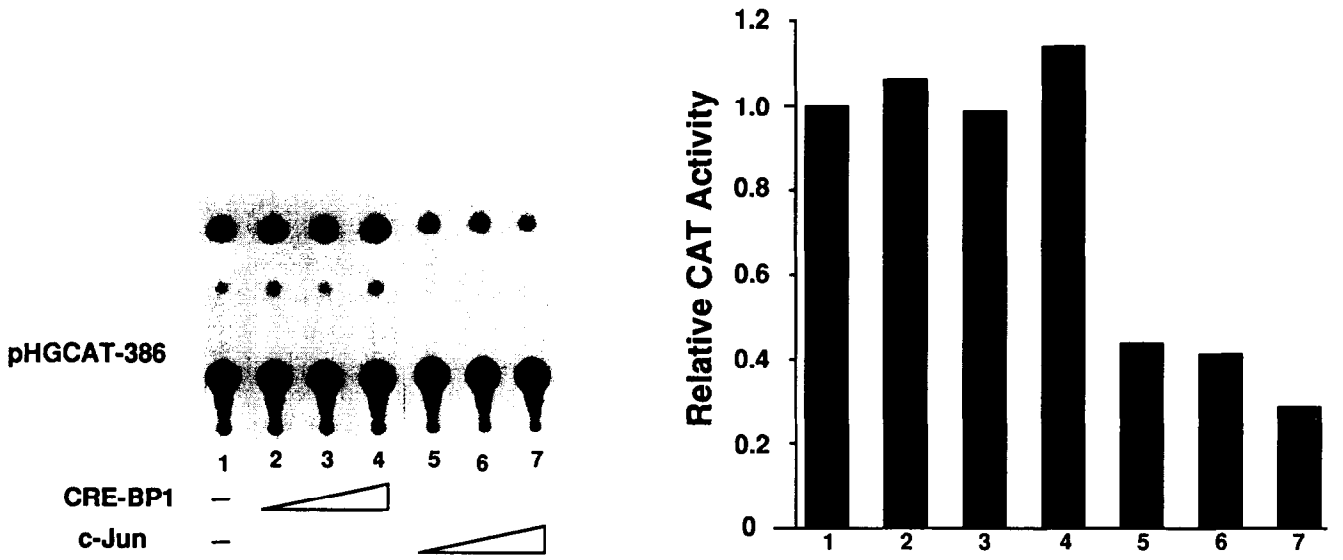


Fig. 4. Repression of promoter activity of the human GIP gene by c-Jun. pHGCAT-386 was transfected with 1, 2, or 4 μg of the CRE-BP1 expression plasmid (lanes 2–4), with 1, 2, or 4 μg of the c-Jun expression plasmid (lanes 5–7), or with the control effector plasmid (lane 1) into HIT cells, and CAT activities were measured. Relative CAT activities are indicated by a bar graph on the right.

DNase I footprinting using HIT cells nuclear extract (data not shown). Within the core of CRE1-protected region, the CRE-like sequence is localized (AGACGTGA: the underlined residues are different from the consensus CRE). However, the nucleotide sequence in the CRE2-protected region had no striking homology with the consensus CRE sequence, and this region contains the CCAAT box.

3.3. *Role of CREs in the GIP gene transcription*

To examine whether the two CRE-BP1 binding sites confer the cAMP inducibility on the human GIP gene, mutant pHGCAT plasmids containing mutated CREs were constructed and used for CAT transfection assays. The CAT activities expressed in HIT cells by the wild-type and mutant plasmids are shown in Fig. 3B. The level of CAT activity expressed from the control plasmid, pHGCAT-386, was increased about 4.5-fold by treatment with Bt_2cAMP. When CRE1 was disrupted (m21), the degree of induction by Bt_2cAMP was slightly reduced to a 3-fold increase. Although the CRE-like sequence was not found in the CRE2 site, the sequence TCAC in the 5′ half of the center of the protected region is similar to the sequence TGAC of the half sequence of consensus CRE (TGACGTCA). To examine whether this TCAC sequence is important for the cAMP-induced activation of the GIP promoter, this TCAC sequence was changed to AGCC (m22). This mutation almost completely abolished the cAMP inducibility of the GIP promoter. To confirm further that the presence of a half sequence (TGAC) of the consensus CRE at this position is sufficient for cAMP inducibility, a one-base mutation was introduced to generate the sequence TGAC (m23). As expected, a generation of the sequence TGAC increased the cAMP inducibility to 7-fold. Introduction of the 3-base mutation (m19) in the center of the protected region also dramatically reduced the cAMP inducibility to 2-fold. Disruption of both the CRE1 and CRE2 sites (m21/22 produced by combination of m21 and m22) gave a result similar to that obtained with m22. These results indicate that among two CRE-BP1-binding sites, the CRE2 site is mainly responsible for the cAMP-induced activation of the GIP promoter. Furthermore, a combination of the half sequence of consensus CRE and the CCAAT box sequence gives rise to a functional CRE.

In the CAT assays described above, smaller amount of extracts prepared from the transfected cells were used for CAT assays to measure the degree of cAMP inducibility as precisely as possible. Under those conditions, however, an accurate comparison of the basal promoter activities between the wild-type and mutant promoters was difficult. To examine whether mutations of the CRE-BP1-binding sites affect the basal promoter activity, CAT activities obtained by more of the cell extracts were compared (see the data obtained without a c-Jun expression plasmid in Fig. 5). Disruption of CRE1 (m21) and CRE2 (m22 and m19) decreased the basal promoter activity to about half and to 1/9 to 1/7 of the wild-type promoter, respectively. Combination of CRE1 and CRE2 mutations (m21/22) suppressed it to about 1/15. These results show that two CRE-BP1 binding sites also contributed to the basal promoter activity.

3.4. *c-Jun-induced repression of the GIP promoter activity*

Previously we observed that c-Jun can repress the human insulin promoter activity [18]. Since the level of

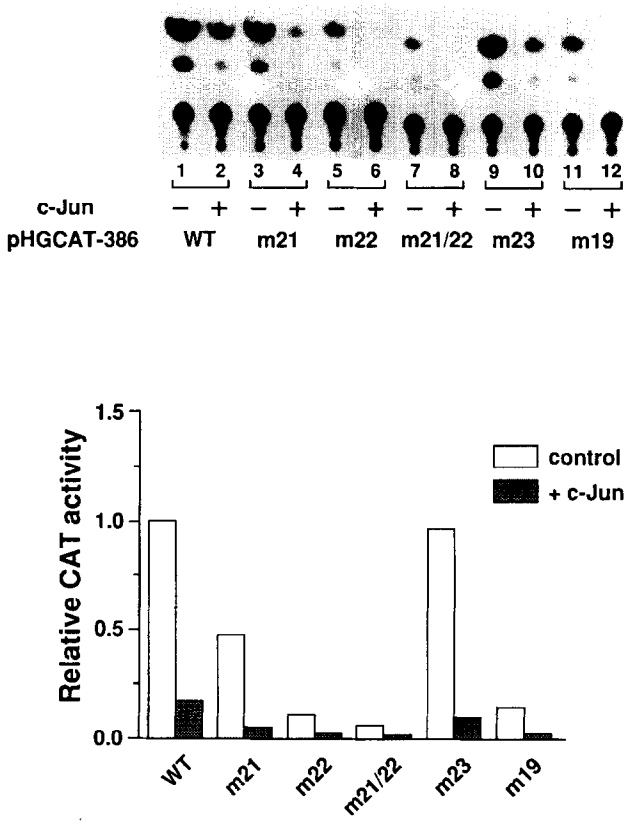


Fig. 5. Effects of mutation of CRE-BP1-binding sites on the c-Jun-induced repression. A series of CAT plasmids indicated in Fig. 3, were co-transfected with the c-Jun expression plasmid or the control effector plasmid into HIT cells, and CAT activities were assayed. Relative CAT activities are shown by a bar graph below.

c-jun mRNA was increased by glucose deprivation in HIT cells [18], this observation suggest that glucose may regulate the human insulin promoter activity through c-Jun. To examine whether c-Jun also regulated transcription of the human GIP gene, which is also important for glucose homeostasis, like the insulin gene, the CAT co-transfection assays were done. Co-transfection of pHGCAT-386 and various amounts of the c-Jun expression plasmid into HIT cells showed that c-Jun represses the basal activity of the GIP promoter to one-third to one-fifth (Figs. 4 and 5). In contrast, co-transfection of the CRE-BP1 expression plasmid did not affect the basal promoter activity (Fig. 4), suggesting that the level of endogenous level of CRE-binding proteins in HIT cells is sufficient to maintain the activity of the GIP promoter. To investigate whether the two CREs mediate the c-Jun-induced repression, the mutants of CREs were used for a co-transfection experiment (Fig. 5). The mutation of CRE1 (m21) or CRE2 (m22) did not affect the repression by c-Jun, but disruption of both CRE1 and CRE2 (m21/22) partially relieved the c-Jun-induced repression. These results indicate that c-Jun represses the basal promoter activity of the human GIP gene partly through two CREs.

4. DISCUSSION

These studies demonstrate that the human GIP gene transcription can be induced by an increased level of cAMP. DNase I footprinting with the recombinant CRE binding proteins and mutagenesis analysis show that two CRE-BP1 binding sites are in the human GIP promoter region and that one of them, at nucleotide −155, is mainly responsible for the cAMP inducibility of the human GIP promoter. In addition, these two sites also contribute to the basal activity of the GIP promoter. Surprisingly, the nucleotide sequence of the CRE-BP1 binding site at nucleotide −155 does not have a high homology with the consensus CRE sequence. The 5′ half of this site contains the sequence, TCAC, which is similar to the 5′ half sequence of the consensus CRE (TGACGTCA), and the 3′ half of this site has the CCAAT box. The importance of this 5′ half has been demonstrated by experiments in which a generation of TGAC in this region by introducing a point mutation increases the cAMP inducibility (Fig. 3, m23). These results suggest that only one molecule of CRE-BP1 homodimer, which is formed through the leucine zipper, may recognize the TCAC sequence of the 5′ half in a sequence-specific manner. It is not clear whether another molecule of CRE-BP1 homodimer can specifically recognize the CCAAT sequence. Among multiple proteins binding to a CCAAT box, C/EBP has the B-ZIP structure consisting of the basic amino acid cluster and a leucine zipper [36], and was recently demonstrated to be able to bind to a CRE sequence [37]. These results raise the possibility that a homodimer of CRE binding proteins or C/EBP, or a heterodimer of some CRE binding protein and C/EBP, binds to the CRE2 site of the human GIP gene promoter in vivo, although a heterodimer formation between these two kinds of proteins has not been demonstrated. An increase of the cAMP level has been shown to stimulate a nuclear localization and the transactivation capacity of NFIL-6, one member of the C/EBP family [38]. Therefore, it might be possible that the NFIL-6-like protein may contribute to the cAMP inducibility of the human GIP promoter, if some member of C/EBP family binds to the CRE2 site. It is quite interesting to examine what kind of transcription factor binds to CRE2 in the GIP promoter and regulates its activity.

We previously reported that c-Jun represses the promoter activity of the human insulin gene through four CREs [18]. Our results in this study indicate that the human GIP promoter activity can also be repressed by c-Jun, at least partly, through two CREs in HIT cells. Since the c-*jun* mRNA level increases upon glucose deprivation in HIT cells [18], glucose may induce the GIP gene transcription by reducing the level of c-*jun* mRNA. At present, it is not clear whether the GIP gene expression is regulated by glucose, because HIT cells have a weak responsiveness to glucose at physiological concen-

trations. A study using primary β-cells that can respond to glucose will be necessary to clarify this point. Although the mechanism of c-Jun-induced transcriptional repression of the insulin and GIP genes is not yet clear, the fact that expression of both of two genes, which are important for glucose homeostasis, are similarly regulated, suggests a physiological importance of this type of regulation.

Acknowledgements: We are very grateful to Drs. K. Umesono and S. Hirai for providing the plasmids pras-β-gal and pRSV-c-*jun*.

REFERENCES

[1] Brown, J.C., Pederson, R.A., Jorpes, J.E. and Mutt, V. (1969) Can. J. Physiol. Pharmacol. 47, 113–114.
[2] Brown, J.C., Mutt, V. and Pederson, R.A. (1970) J. Physiol. 209, 57–64.
[3] Pederson, R.A. and Brown, J.C. (1972) Gastro-enterology 62, 393–400.
[4] Dupre, J., Ross, S.A., Watson, D. and Brown, J.C. (1973) J. Clin. Endocrinol. Metab. 37, 826–828.
[5] Anderson, D.K., Elahi, D., Brown, J.C., Tobin, J.D. and Andres, R. (1978) J. Clin. Invest. 62, 152–161.
[6] Maxwell, V., Shulkes, A., Brown, J.C., Solomon, T.E., Walsh, J.H. and Grossman, M.I. (1980) Dig. Dis. Sci. 25, 113–116.
[7] Moody, A.J., Thim, L. and Valverde, I. (1984) FEBS Lett. 172, 142–148.
[8] Bell, G.I. (1986) Peptides 7, 27–36.
[9] Gubler, U., Monahan, J.J., Lomedico, P.T., Bhatt, R.S., Collier, K.J., Hoffman, B.J., Bohlen, P., Esch, F., Ling, N., Zeytin, F., Brazeau, P., Poonian, M.S. and Gage, L.P. (1983) Proc. Natl. Acad. Sci. USA 80, 4311–4314.
[10] Mayo, K.E., Vale, W., Rivier, J., Rosenfeld, M.G. and Evans, R.M. (1983) Nature 306, 86–88.
[11] Takeda, J., Seino, Y., Tanaka, K., Fukumoto, H., Kayano, T., Takahashi, H., Mitani, T., Kurono, M., Suzuki, T., Tobe, T. and Imura, H. (1987) Proc. Natl. Acad. Sci. USA 84, 7005–7008.
[12] Inagaki, N., Seino, Y., Takeda, J., Yano, H., Yamada, Y., Bell, G.I., Eddy, R.L., Fukushima, Y., Byers, M.G., Shows, T.B. and Imura, H. (1989) Mol. Endocrinol. 3, 1014–1021.
[13] Neilson, D.A., Welsh, M., Casadaban, M.J. and Steiner, D.F. (1985) J. Biol. Chem. 260, 13585–13589.
[14] Welsh, M., Nielsen, D.A., MacKrell, A.J. and Steiner, D.F. (1985) J. Biol. Chem. 260, 13590–13594.
[15] Itoh, N. and Okamoto, H. (1980) Nature 283, 100–102.
[16] Charles, M.A., Lawecki, J., Pictet, R. and Grodsky, G.M. (1975) J. Biol. Chem. 250, 6134–6140.
[17] Hammonds, P., Schofield, P.N., Ashcroft, S.J.H., Sutton, R. and Gray, D.W.R. (1987) FEBS Lett. 223, 131–137.
[18] Inagaki, N., Maekawa, T., Sudo, T., Ishii, S., Seino, Y. and Imura, H. (1992) Proc. Natl. Acad. Sci. USA 89, 1045–1049.
[19] Comb, M., Birnberg, N.C., Seasholtz, Herbert, E. and Goodman, H.M. (1986) Nature 323, 353–356.
[20] Montiminy, M.R., Sevarino, K.A., Wagner, J.A., Mandel, G. and Goodman, R.H. (1986) Proc. Natl. Acad. Sci. USA 83, 6682–6686.
[21] Hoeffler, J.P., Myer, T.E., Yun, Y., Jameson, J.L. and Habener, J.F. (1988) Science 242, 1430–1433.
[22] Gonzalez, G.A., Yamamoto, K.K., Fischer, W.H., Karr, D., Menzel, P., Biggs III, W.B., Vale, W.W. and Montminy, M.R. (1989) Nature 337, 749–752.
[23] Maekawa, T., Sakura, H., Kanei-Ishii, C., Sudo, T., Yoshimura, T., Fujisawa, J., Yoshida, M. and Ishii, S. (1989) EMBO J. 8, 2023–2028.
[24] Hai, T., Liu, F., Coukos, W.J. and Green, M.R. (1989) Genes Dev. 3, 2083–2090.
[25] Gonzalez, G.A. and Montminy, M.R. (1989) Cell 59, 675–680.
[26] Macgregor, P.F., Abate, C. and Curran, T. (1990) Oncogene 5, 451–458.
[27] Ivashkiv, L., Liou, H., Kara, C., Lamph, W., Verma, I. and Glimcher, L.H. (1990) Mol. Cell. Biol. 10, 1609–1621.
[28] Araki, E., Shimada, F., Shichiri, M., Mori, M. and Ebina, Y. (1988) Nucleic Acids Res. 16, 1627.
[29] Sayers, J.R., Schemidt, W. and Eckstein, F. (1988) Nucleic Acids Res. 16, 791–802.
[30] Maekawa, T., Matsuda, S., Fujisawa, J.-I., Yoshida, M. and Ishii, S. (1991) Oncogene 6, 627–632.
[31] Hirai, S., Bourachot, B. and Yaniv, M. (1990) Oncogene 5, 39–46.
[32] Umesono, K. and Evans, R.M. (1989) Cell 57, 1139–1146.
[33] Ishii, S., Merlino, G.T. and Pastan, I. (1985) Science 230, 1378–1381.
[34] Gorman, C.M., Moffat, L.F. and Howard, B.H. (1982) Mol. Cell. Biol. 2, 1044–1051.
[35] Smith, D.B. and Johnson, K.S. (1988) Gene 67, 31–40.
[36] Landshulz, W.H., Jonson, P.F. and McKnight, S.L. (1988) Science 240, 1759–1764.
[37] Bakker, O. and Parker, M.G. (1991) Nucleic Acids Res. 19, 1213–1217.
[38] Metz, R. and Ziff, E. (1991) Genes Dev. 5, 1754–1766.