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GGAAAT motifs play a major role in transcriptional activity of the human insulin gene in a pancreatic islet beta-cell line MIN6

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Summary The insulin gene is specifically expressed in pancreatic islet beta cells. Various *cis*-acting DNA elements in the 5′-flanking region of the human insulin gene were examined for their contribution to the transcriptional activity using sensitive human growth hormone (hGH) reporter plasmids. The hGH constructs, having successively deleted human insulin promoter sequences, were transfected to a pancreatic islet beta-cell line MIN6. The deletion of two GGAAAT (GG) motifs, GG2 at –145 to –140 bp and GG1 at –134 to –129 bp, decreased the transcriptional activity to 6.5 % of that of the promoter sequence from –156 to + 1 bp. The selective mutations in both GG motifs also decreased the transcriptional activity to 5.5 %. One-base mutations of GG2 and GG1 decreased the transcriptional activity to 82 and 11 %, respectively. The two-base mutations between GG2 and GG1 affected the transcriptional activity more strongly than those just outside the GG motifs. A single set of GG motifs in the upstream of thymidine kinase promoter increased the transcriptional activity to 216 % compared to that of thymidine kinase promoter alone in MIN6 cells. With an electrophoretic mobility shift assay (EMSA), a nuclear factor in MIN6 cells was shown to bind the DNA fragments containing two GG motifs. This factor did not bind to another GGAAAT-like sequence at –313 to –305 bp in the human insulin gene. These results suggested that the GG motifs contributed to the cell-specific transcription of the human insulin gene in association with the binding of the sequence-specific nuclear factor. [Diabetologia (1996) 39: 1462–1468]

Keywords Insulin gene, GG motif, transcription, pancreatic islet, MIN6.

The insulin gene is specifically expressed in pancreatic islet beta cells. The role of *cis*-acting DNA elements involved in transcriptional regulation in islet beta cells has been more extensively studied for rat insulin I and II genes than the human insulin gene. Several transcriptional regulatory elements are located in the 5′-flanking region within 350 base pairs (bp) from the transcriptional start site of the insulin gene [1–4]. Two kinds of elements, TAAT motifs and E-box elements in the rat insulin I gene are known to play a crucial role in cell-specific transcriptional regulation. The TAAT motifs are located in A4/A3 at –215 to –200 bp and in A1 at –82 to –72 bp according to a simplified nomenclature for the *cis*-acting DNA elements in the insulin gene [5]. These elements were previously designated as the FLAT and P1 element, respectively. Insulin promoter factor 1 which is a homeodomain-containing protein, binds to these elements [6–8]. The E-box elements, E2 at –239 to –232 bp and E1 at –111 to –104 bp, previously designated as Far and Nir, respectively, contain the

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Abbreviations: hGH, human growth hormone; TK, thymidine kinase; SEAP, secreted-type of human placental alkaline phosphatase; RLU, relative light units; bp, base pair; EMSA, electrophoretic mobility shift assay; CAT, chloramphenicol acetyl-transferase; PCR, polymerase chain reaction; CMV, cytomegalovirus; FBS, fetal bovine serum.

Table 1. Nucleotide sequences of double-stranded probes and competitors

Name	5′ end	Nucleotide sequence	3′ end
– 156/GG	– 156	CCCAGCACCAG**GGAAAT**GGTCC**GGAAAT**TGCAGCC	– 122
– 148/GG	– 148	CAG**GGAAAT**GGTCC**GGAAAT**TGC	– 126
– 325/core	– 325	AGCTTGGTCTAATGTGGAAAGTGGCCCAGG	– 297
– 316/core	– 316	AATGTGGAAAGTGGCC	– 301

The number represents the nucleotide position from the transcriptional start site of the human insulin gene. Bold-faced letters denote the position of the GG2 and GG1. Underlined letters denote the position of the core enhancer sequence at – 313 to – 305 bp

consensus sequence of GC*CA*TC*TG* with underlined letters showing consensus, to which nuclear factors containing basic helix-loop-helix structures bind [9]. A4/A3 and/or E2 in the rat inulin I gene [10, 11], and C1, previously called as RIPE3b1, at –115 to –107 bp in the rat insulin II gene [12] were reported to play an important role in the glucose-responsive transcription.

The human insulin gene has three TAAT motifs of A5, A3, and A1, which were previously called CT motifs of CT3, CT2, and CT1, respectively (Fig. 1). A3 was reported to play a major role in the cell-specific and glucose-responsive transcription using chloramphenicol acetyl-transferase (CAT) expression assays in isolated rat islet cells [8]. To further understand the role of *cis*-acting DNA elements of the human insulin gene in transcriptional activity, we carried out a sensitive transient expression assay with human growth hormone (hGH) reporter plasmids transfected to a murine islet beta-cell line MIN6. MIN6 cells secrete insulin in response to glucose concentrations in its physiologic range [13], and glucose metabolism in MIN6 cells including glucose transport, phosphorylation, and utilization was similar to that in isolated islets [14]. Due to these characteristics, MIN6 cells serve as a useful substitute for isolated islets to study both cell-specific and glucose-responsive insulin biosynthesis. In addition to E2 and A3, we investigated the role of other *cis*-acting DNA elements located in their downstream, including C1, E1, and A1, previously called RIPE3b1, IEB1, and CT1, respectively, and GGAAAT (GG) motifs in the present study.

Materials and methods

Plasmid constructs. The hGH reporter plasmids having various sizes of deleted 5′-flanking sequences of the human insulin gene, as shown in Figure 1, were constructed as follows. The fragments from –341, –248, –230, –208, –187, –156, –128, –117, –84, and –74 to + 1 bp of the transcriptional start site of the human insulin gene were amplified by polymerase chain reaction (PCR) using a plasmid, pgHI 12.5 [15] as a template, and inserted into the upstream of the promoterless hGH expression plasmid, p0GH (Nichols, San Juan Capistrano, Calif., USA) [16]. The PCR-amplified DNA sequences were confirmed by the dideoxy termination method. The hGH reporter plasmid driven by the herpes simplex virus thymidine kinase (TK) promoter was constructed by inserting the TK promoter of pBLCAT2 [17] into a *Hin*dIII and *Bam*HI site of p0GH, which was designated as pTK0GH. The double-stranded oligonucleotide including both GG2 and GG1 motifs was produced by annealing synthesized complementary oligonucleotides from – 148 to –126 bp, and it was inserted in the upstream of the TK promoter in pTK0GH, which was designated as pGGTK0GH. A plasmid containing a secreted-type of human placental alkaline phosphatase (SEAP) gene driven by a cytomegalovirus promoter, CMV-SEAP (Tropix, Bedford, Mass., USA) [18], was used as an internal control. Plasmids prepared on a large scale were purified using column chromatography (Qiagen, Chatsworth, Calif., USA) according to the manufacturer's instructions. Because the amount of plasmids necessary for detection and the portion of the sample necessary for assay are both significantly smaller in the hGH reporter system than the CAT assay system, the sensitivity of hGH reporter plasmids is at least 10 times more sensitive than that of the CAT plasmids [16].

Cell culture and DNA transfection. MIN6 cells were grown in Dulbecco's modified Eagle medium equilibrated with 5 % CO_2 and 95 % air at 37 °C. The medium was supplemented with 15 % fetal bovine serum (FBS), 50 μg/ml streptomycin, and 50 units/ml penicillin. MIN6 cells used in this study were harvested at passages of 20–23. The reporter constructs were transfected to the cells by a cationic liposome-mediated method. 5×10^6 cells were plated on a 90-mm dish, then 5 μg of the test plasmid and 2 μg of CMV-SEAP plasmid were mixed with Lipofectin (GIBCO/BRL, Gaithersburg, Md., USA) and added to the medium at 26.3 mmol/l glucose. NIH/3T3 cells, which were obtained from Japanese Cancer Research Resources Bank, Tokyo, Japan, were cultured under the same condition as MIN6 cells, except that the culture medium contained 10 % FBS and 6.8 mmol/l glucose. The culture medium for the hGH and SEAP assay was sampled 48 h later. The differences of the transfection efficiency among the experiments were corrected by the SEAP activity.

Assay of secreted hGH or SEAP. The amount of secreted hGH in the culture medium was assayed using an immunoradiometric assay kit (Daiichi Radioisotope Laboratory, Tokyo, Japan). The activity of secreted SEAP in the culture medium was assayed using a chemiluminescence assay kit, Phospha-Light (Tropix, Bedford, Mass., USA) [19] and the transcriptional activity was standardized for 10 000 relative light units (RLU) of SEAP activity.

Electrophoretic mobility shift assay (EMSA). Nuclear extracts were prepared from MIN6, NIH/3T3, and COS-7 cells by the technique of Andrews et al. [20]. Synthesized oligonucleotides listed in Table 1 were annealed with synthesized complementary strands to make double stranded probes and competitors. Probes were end-labelled with [α-^{32}P]dCTP (3000 Ci/mmol, 10 mCi/ml)(Amersham, Amersham, Bucks., UK) using the Klenow fragment of DNA polymerase I. Binding reactions

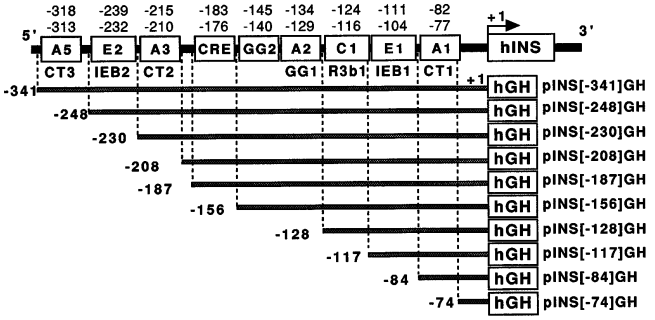

Fig. 1. The constructs of hGH reporter plasmids having successively deleted fragments of the human insulin promoter. The number in the figure represents the nucleotide position from the transcriptional start site of the human insulin gene. The upper panel shows the relative location of the *cis*-acting DNA elements and their nucleotide position. The names according to the simplified nomenclature are shown in boxes except GG2, with their previous names shown below

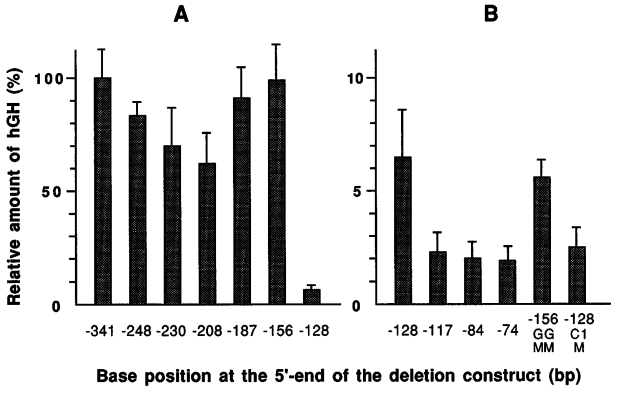

Fig. 2. Transcriptional activity of the human insulin promoter in a series of successive deletion constructs in MIN6 cells. MIN6 cells were transfected with 5 μg of the indicated deletion construct and 2 μg of a control plasmid, CMV-SEAP. These cells were grown for 48 h in the culture medium with 26.3 mmol/l glucose containing 15 % FBS. The amount of secreted hGH and the activity of SEAP in the culture medium were measured. The promoter activity of various deleted fragments of the human insulin gene was standardized by the relative amount of hGH to 10 000 RLU of SEAP activity. The results are presented as a percentage of the amount of secreted hGH from pINS[-341]GH. The number in the figure represents the nucleotide position from the transcriptional start site of the human insulin gene. Each bar represents mean ± SD of four independent transfections

contained 25 mmol/l 4-(2-hydroxyethyl)-1-perazine ethanesulfonic acid (pH 7.8), 60 mmol/l KCl, 5 mmol/l $MgCl_2$, 2 mmol/l dithiothreitol, 10 000 dpm of end-labelled double-stranded oligonucleotides, 5 μg nuclear extract, and 2 μg poly(dI-dC)/poly(dI-dC) (Pharmacia, Uppsala, Sweden). After the binding reaction, samples were loaded onto a 5 % non-denaturing polyacrylamide gel and run in high ionic strength buffer. After electrophoresis, the gels were dried and subjected to autoradiography.

Statistical analysis. Statistical analysis was performed by Student's unpaired *t*-test. A value of p less than 0.05 was considered statistically significant.

Results

Transcriptional activity of the human insulin promoter in a series of successive deletion constructs. The transcriptional activity of the successively deleted human insulin promoter (Fig. 1) was estimated with the amount of hGH secreted for 48 h after transfection (Fig. 2). In the same culture medium, the SEAP activity which was calculated from a standard curve obtained in each experiment, fell into a narrow range of 16 % of standard deviation of the mean in each experiment, suggesting that the differences of transfection efficiency among these deletion constructs were relatively small. The amount of hGH from pINS[-341]GH, which was standardized for SEAP activity, was maximum among these deletion constructs. The removal of the fragment from –341 to –249, –231, or –209 bp gradually decreased the transcriptional activity to 83, 70, or 62 % (Fig. 2A). In these regions, there are positive regulatory elements including A5 at –318 to –313 bp, E2 at –239 to –232 bp, and A3 at –215 to –210 bp. Further removal of the fragment from –208 to –188, or –157 bp resumed the transcriptional activity to the same degree as that of pINS[–341]GH.

The most drastic effect on the transcriptional activity was observed when the fragment from –156 to –129 bp was deleted (Fig. 2A). The amount of secreted hGH from pINS[–128]GH was 6.5 % of that from pINS[–156]GH ($p < 0.001$; $n = 4$). In this region, there are two GGAAAT sequences, designated as GG motifs, including GG2 at –145 to –140 bp and GG1 at –134 to –129 bp [1]. Although the GG1 was newly designated as A2 by the simplified nomenclature, no new name was assigned to the GG2. For this reason, we have used the terms of GG motifs, GG1, and GG2 in this paper. To examine the effect of the removal of the sequences of the GG motifs on the transcriptional activity, we made another hGH construct, pINS[–156GGMM]GH, in which the GGAAAT sequences of the GG2 and the GG1 were selectively mutated to *CTGC*A*G* and *CTCG*A*G*, respectively, with underlined letters showing mutated bases in the fragment from –156 to + 1 bp. The amount of secreted hGH from this construct was only 5.5 % of that from pINS[–156]GH in MIN6 cells ($p < 0.001$; $n = 4$) (Fig. 2B). In NIH/3T3 cells, pINS[–156GGMM]GH did not significantly change the transcriptional activity when compared to that of pINS[–156]GH (data not shown).

The transcriptional activity of the deleted fragments shorter than that of pINS[–128]GH was further examined (Fig. 2B). The removal of the fragment from –128 to –118 bp significantly decreased the transcriptional activity from 6.5 to 2.2 % of that from pINS[-156]GH ($p < 0.02$; $n = 4$). A possible *cis*-acting DNA element located at –124 to –116 bp in this region corresponds to C1 in the rat insulin II gene [2,

Table 2. Nucleotide sequences of the human insulin promoters in the indicated hGH constructs

Name of hGH constructs	5′ end	Nucleotide sequence
pINS[– 156]GH	– 156	CCCAGCACCAG**GGAAAT**GGTCC**GGAAAT**TGC
pINS[– 156GGMM]GH	– 156	CCCAGCACCAG**CTCGAG**GGTCC**CTGCAG**TGC
pINS[– 156GG2M]GH	– 156	CCCAGCACCAG**GGACAT**GGTCC**GGAAAT**TGC
pINS[– 156GG1M]GH	– 156	CCCAGCACCAG**GGAAAT**GGTCC**GGACAT**TGC
pINS[– 156GG2M5′]GM	– 156	CCCAGCACCGA**GGAAAT**GGTCC**GGAAAT**TGC
pINS[– 156GG2M3′]GH	– 156	CCCAGCACCAG**GGAAAT**AATCC**GGAAAT**TGC
pINS[– 156GG1M5′]GH	– 156	CCCAGCACCAG**GGAAAT**GGTAA**GGAAAT**TGC
pINS[– 156GG1M3′]GH	– 156	CCCAGCACCAG**GGAAAT**GGTCC**GGAAAT**AAC

Nucleotide sequences from the 5′ ends to – 126 bp are shown. All constructs contain the same 3′ sequence up to + 1 bp. Underlined letters denote the mutated sequences. Bold-faced letters denote the position of the GG2 at – 145 to – 140 bp and the GG1 at – 134 to – 129 bp

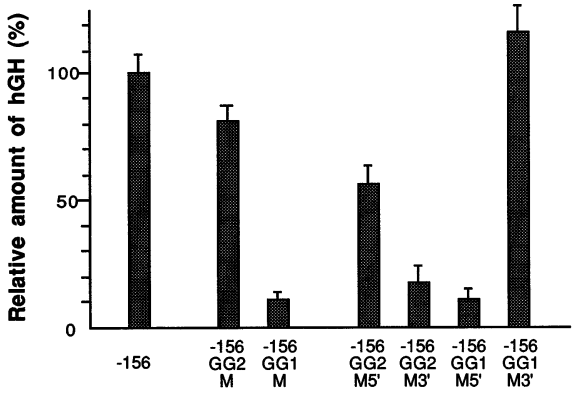

Fig. 3. Effects of small mutations in and outside the GG motifs on transcriptional activity of the human insulin promoter in MIN6 cells. The data presentation and statistical analysis are the same as in Figure 2 except that the results are presented as a percentage of the amount of hGH concentration for pINS[-156]GH. The names of the hGH constructs and their nucleotide sequences of the human insulin promoters are described in Table 2. Each bar represents mean ± SD of six independent transfections

12, 22]. The activity of C1 of the rat insulin II gene was reported to be abolished by the substitution of two nucleotides of TC with CG [12, 22]. To examine the effect of this mutation in the human insulin gene on the transcriptional activity, we made another hGH construct, pINS[-128C1M]GH. pINS[-128 C1M]GH had the fragment from –128 to + 1 bp in which C1 sequence of GCC*TC*AGCC was mutated to GCC*CG*AGCC. The introduction of this mutation decreased the transcriptional activity from 6.5 to 2.5 % ($p < 0.02$; $n = 4$) (Fig. 2B). Further deletions of the fragment from –117 to –85 and –75 bp which contained E1 at –111 to –104 bp and A1 at –82 to –77 bp, respectively, gradually decreased the transcriptional activity to 2.0 and 1.9 %, respectively (Fig. 2B). The amount of secreted hGH from the promoterless p0GH, which was well above the detection limit of hGH at 10 pg/ml in our experimental system, was less than 0.8 % of that from pINS[-341]GH and pINS[-156]GH (data not shown).

Small mutations in and outside the GG motifs affected the transcriptional activity. The effects of one-base mutation from GGA*A*AT to GGA*C*AT in the GG2 or the GG1 were examined. The constructs of pINS[-156GG2M]GH and pINS[-156GG1M]GH had the above-mentioned one-base substitutions in the GG2 and the GG1, respectively (Table 2). Although both constructs significantly decreased the transcriptional activity ($p < 0.001$; $n = 6$), pINS[-156 GG1M]GH decreased more strongly to 11 % than that of 82 % for pINS[-156GG2M]GH (Fig. 3), suggesting that the more important role of GG1 than GG2. As shown in Table 2, four hGH constructs with mutations of two bases in the 5′- or 3′-flanking regions of the GG motifs were made, and the effects of these mutations on the transcriptional activity were examined. As shown in Figure 3, pINS[-156 GG2M5′]GH, pINS[-156GG2M3′]GH, pINS[-156 GG1M5′]GH, and pINS[-156GG1M3′]GH significantly changed the transcriptional activity to 56, 17, 10, and 117 %, respectively ($p < 0.001$, < 0.001, < 0.001, and < 0.01, respectively; $n = 6$). These results suggested that the sequences between the GG2 and GG1 were affected more strongly than those just outside the GG motifs in the transcriptional activity.

One set of GG motifs enhanced the transcriptional activity of the TK promoter in MIN6 cells. To examine the effect of one set of GG motifs on the transcriptional activity in MIN6 or NIH/3T3 cells, the transcriptional activities of the pTK0GH and pGGTK0GH were examined (Fig. 4). pGGTK0GH had the fragment from –148 to –126 bp of the human insulin gene including two GG motifs, but without the elements of C1, E1, or A1, in the upstream of the TK promoter (Fig. 4A). pGGTK0GH increased the transcriptional activity to 216 % compared to that of pTK0GH in MIN6 cells ($p < 0.001$; $n = 6$) (Fig. 4B, left). In NIH/3T3 cells, however, pGGTK0GH did not significantly change the transcriptional activity compared to that of pTK0GH (Fig. 4B, right).

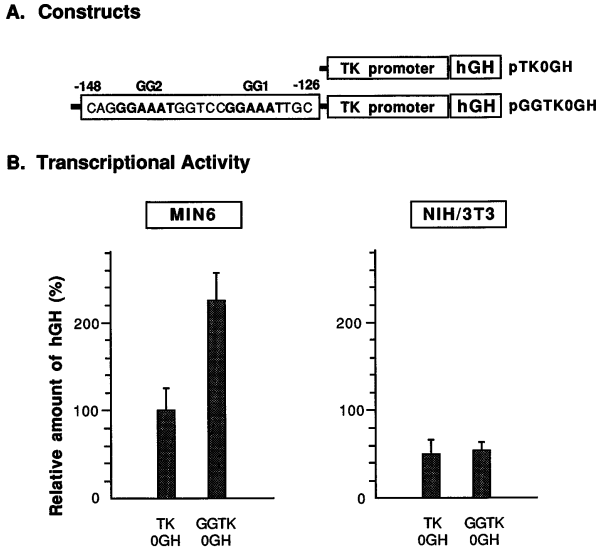

Fig. 4. A, B Effects of the isolated GG motifs on the transcriptional activity of the TK promoter in MIN6 and NIH/3T3 cells. **A** The hGH constructs with or without the GG motifs in the upstream of the TK promoter. **Bold-faced** letters in the nucleotide sequences denote the consensus sequence of the two GG motifs. **B** The transcriptional activities of these constructs in MIN6 and NIH/3T3 cells are shown. The data presentation and statistical analysis are the same as in Figure 2 except that the results are presented as a percentage of the amount of hGH concentration for pTK0GH in MIN6 cells. Each bar represents mean ± SD of six independent transfections

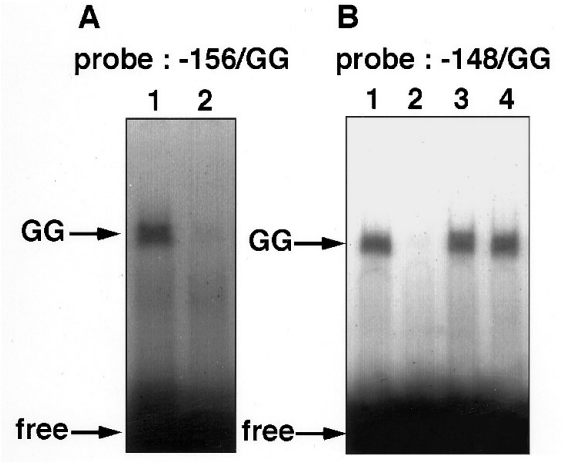

Fig. 5. Binding of the oligonucleotides containing the GG2 and the GG1 to the nuclear extract from MIN6 cells. The binding specificity of nuclear protein to probes and competitors (Table 1) was tested by the EMSA. Nuclear extracts were added to the ^{32}P-labelled probes and the DNA-protein complexes were analysed on a 5 % non-denaturing polyacrylamide gel in high ionic strength buffer. The binding specificity was examined by adding a 50-fold excess of the non-labelled oligonucleotides as competitors. *Lanes 1* and *2* in panel **A** denote no competitor and a competitor of –148/GG, respectively. *Lanes 1* through *4* in panel **B** denote no competitor, a competitor of –148/GG, –325/core, and –316/core, respectively. The *upper* and *lower arrows* show the specific GG-binding activity and the non-specific reaction plus the labelled free probe, respectively

Binding of a nuclear factor to the DNA fragment containing both the GG2 and the GG1. *Trans*-acting factors to the GG motifs in the human insulin gene were studied using EMSA. As shown in Figure 5A, the EMSA, using the labelled oligonucleotide of –156/GG containing the sequences from –156 to –122 bp as a probe (Table 1) and the nuclear extract from MIN6 cells, showed a single retarded band of the DNA-protein complex. This signal of the DNA-protein complex disappeared by adding the excess of non-labelled oligonucleotide of –148/GG from –148 to –126 bp as a competitor (Fig. 5A). The opposite experiment using the labelled –148/GG as a probe also formed a single retarded band (Fig. 5B) which disappeared by the excess of non-labelled –148/GG in lane 2 and also –156/GG (data not shown), indicating that the common oligonucleotides containing the two GG motifs are responsible for their binding activity to the nuclear factor in MIN6 cells. There is another GGAAAT-like motif in the human insulin gene, which was GTGGAAAGT at –313 to –305 bp designated as a core enhancer sequence [1]. As shown in Figure 5B, the excess of oligonucleotides of –325/core from –325 to –297 bp and –316/core from –316 to –301 bp (Table 1) did not compete with the GG-binding activity of the labelled –148/GG. These results showed that the GG-binding factor did not bind to the core enhancer sequence of the human insulin gene, and suggested that nuclear factors bind to the GG motifs in a sequence-specific manner.

The GG-binding factor in MIN6 cells was not present in NIH/3T3 cells. Tissue distribution of this GG-binding factor was examined. As shown in Figure 6, similar GG-binding activity was observed weakly in the nuclear extract from NIH/3T3 cells using labelled –148/GG as a probe. In spite of the apparent similarity, this binding activity was competed with the excess of the non-labelled oligonucleotide sequence including not only –148/GG, but also –325/core and –316/core. These results showed that the GG-binding factor in MIN6 was different from that in NIH/3T3. The same property of the GG-binding as observed in the extract from NIH/3T3 cells was also recognized in the extract from COS-7 cells with the EMSA (data not shown). These results suggested that the GG-binding factor observed in MIN6 cells was specific to this cell line and may also be specific to pancreatic islet beta cells.

Discussion

Various *cis*-acting DNA elements of the human insulin gene were examined for their contribution to the transcriptional activity. The constructs having the

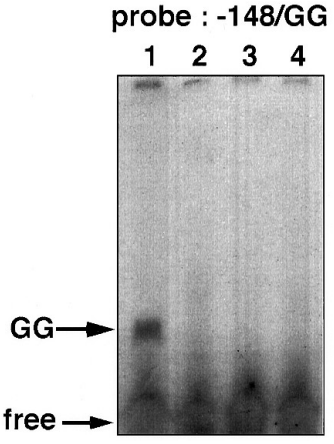

Fig. 6. Binding of the oligonucleotides containing the GG2 and the GG1 to the nuclear extracts from NIH/3T3 cells. The data presentation is the same as in Figure 5. *Lanes 1* through *4* denote no competitor, a competitor of –148/GG, –325/core, and –316/core, respectively. The *upper* and *lower arrows* show the specific GG-binding activity and the non-specific reaction plus the labelled free probe, respectively

deleted human insulin promoter, as shown in Figure 2A, showed mild changes in the transcriptional activity ranging from 62 to 100 % among deletion constructs from –341 to –156 bp. Boam et al. [1] previously showed that the existence of the negative regulatory element called NRE at –279 to –258 bp resulted in a 25-fold reduction of the transcriptional activity in a hamster insulinoma cell line HIT, but we did not observe such strong negative effects on the overall transcriptional activity in MIN6 cells. It is presumably due to the presence of the positive element located within the region from –341 to –279 bp as reported by Clark et al. [21].

The deletion of the sequence from –156 to –129 bp drastically decreased the transcriptional activity to about 6 %, which was not recovered by the further deletion constructs. These results suggested the presence of a strong *cis*-acting DNA element between –156 and –128 bp. Because the selective mutations of two GG motifs in this region decreased the transcriptional activity in MIN6 cells to about 6 %, the GG motifs were regarded to be mainly responsible for the transcriptional activity of the human insulin gene. Only one-base mutation in GG1 decreased the transcriptional activity to as low as 11 % of that of pINS[-156]GH, while one-base mutation in GG2 decreased it to 82 %. Although these data suggested the more important role of GG1 than GG2 in the transcriptional activity, further study is necessary to fully elucidate the distinctive roles of the GG motifs.

The removal of the fragment from –128 to –118 bp and the introduction of mutation from TC to CG in this region significantly decreased the transcriptional activity from 6.5 to 2.2 and 2.5 % of that of pINS [-156] GH, respectively, which corresponded to 34 and 38 % of that of pINS[-128]GH, respectively. This region contains the sequence corresponding to C1 in the rat insulin II gene. These results suggested that C1 in the human insulin gene played a role in the transcriptional activity.

The effects of the introduced isolated GG motifs on the beta-cell-specific transcriptional activity were examined using the hGH construct having the fragment from –148 to –126 bp in the upstream of the TK promoter (Fig. 4). Introduction of the isolated GG motifs resulting in pGGTK0GH increased the transcriptional activity in MIN6 cells to 216 % compared to that of the TK promoter alone in pTK0GH. In NIH/3T3 cells, the introduction of the isolated GG motifs did not significantly change the transcriptional activity, suggesting that the GG motifs function in a beta-cell-specific manner.

EMSA showed the presence of a nuclear factor in MIN6 cells that bound to the DNA fragment containing the GG motifs in a sequence-specific manner. This nuclear factor was not present in NIH/3T3 or COS-7 cells, suggesting that this factor is specific to pancreatic islet beta cells. Further characterization of this factor regarding the distinctive roles of the GG2 and the GG1 in its binding is underway in our laboratory.

The GG motifs in the human insulin gene were first designated by Boam et al. [1]. They found that the region between –153 and –127 bp containing two GGAAAT sequences was protected by a nuclear protein of a pancreatic beta-cell line of HIT M2.2.2 or HIT.T15-G using the DNase footprint analysis. In addition, they showed that the ubiquitous factor bound to the GG1, and that the islet beta cell-specific factor bound to the GG2. However, the functional role of the GG motifs was not studied. Shieh et al. [22] showed that the region between –127 and –89 bp of the rat insulin II gene played an important role in the islet beta cell-specific transcription. This region was designated as RIPE3, which contains GG1, C1, and E1. Although the roles of C1 and E1 in the rat insulin II gene were examined, the role of the GG1 has not been further evaluated.

In this study, the GG motifs in the human insulin promoter were shown to significantly contribute to the transcriptional activity in a murine islet beta-cell line of MIN6. Furthermore, the new nuclear factor was shown to specifically bind to the GG motifs by the EMSA for the first time. These data suggested that the GG motifs contributed to the transcriptional regulation of the human insulin gene by mediating the binding of the specific nuclear factor. Further study on the characteristics of this nuclear factor, including its molecular cloning, and elucidation of the controlling mechanism of the function of this factor is necessary to understand the whole mechanisms of the transcriptional regulation of the human insulin gene.

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