THE JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 274, No. 30, Issue of July 23, pp. 21095–21103, 1999
© 1999 by The American Society for Biochemistry and Molecular Biology, Inc.
Printed in U.S.A.

The Cyclic AMP Response Element Modulator Family Regulates the Insulin Gene Transcription by Interacting with Transcription Factor IID*

(Received for publication, March 30, 1999)

Akari Inada, Yoshimichi Someya, Yuichiro Yamada, Yu Ihara, Akira Kubota, Nobuhiro Ban‡, Rie Watanabe, Kinsuke Tsuda‡, and Yutaka Seino§

From the Department of Metabolism and Clinical Nutrition, Graduate School of Medicine, Kyoto University, Kyoto, Japan 606-8507 and the ‡Faculty of Integrated Human Studies, Kyoto University, Kyoto, Japan 606-8501

We analyzed a mechanism of transcriptional regulation of the human insulin gene by cyclic AMP response element modulator (CREM) through four cyclic AMP response elements (CREs). We isolated two novel CREM isoforms (CREMΔQ1 and CREMΔQ2), which lack one of the glutamine-rich domains, Q1 and Q2 respectively, and six known isoforms (CREMτα, CREMα, inducible cyclic AMP early repressor (ICER) I, ICER Iγ, CREM-17X, and CREM-17) from rat pancreatic islets and the RINm5F pancreatic β-cell line. CREM isoforms functioned as efficient transcriptional activators or repressors to modulate insulin promoter activity by binding to all of the insulin CREs. The binding activity of repressors is higher than that of activators and suppressed not only basal activity but also activator-induced activities. Furthermore, CREM activator interacted directly with the transcription factor IID components hTAF$_{II}$130 and TATA box-binding protein (TBP). These results suggest that the activation of the insulin gene transcription by CREM activator is mediated by not only direct binding to the CREs but also by recruiting transcription factor IID to the insulin promoter via its interaction with hTAF$_{II}$130 and TBP. On the other hand, the CREM repressor ICER competitively interrupts the binding of the activators to CREs and does not interact with either TBP or hTAF$_{II}$130; therefore, it might fail to stabilize the basal transcriptional machinery and repress transactivation.

Insulin gene transcription is regulated through *cis*-acting elements in response to stimulation, such as glucose concentration or increased cAMP level (1). One of the *cis*-acting elements, cAMP response element (CRE),[1] was first identified as an inducible enhancer of genes that can be transcribed in response to increased cAMP levels (2, 3).

In a previous study, we identified four functional CREs in the human insulin gene and demonstrated that each of these CREs has different sequences from the others and also from the consensus CRE motif (TGACGTCA) (4). Since a remarkable reduction of insulin gene transcription is observed when CREs are mutated (4), transcription factors that bind to this regulatory element might play an important role in the regulation of insulin gene transcription. However, which transcription factors are primarily involved in the regulation of insulin gene transcription through CREs is unclear.

Transcription factors such as CREB, CRE-BP1, and ATF1, which belong to the ATF/CREB family, bind to CRE (5–8). CRE modulator (CREM) is a member of this family, and the CREM gene generates both transcriptional activators (CREMτ, -τα, -τ1, and -τ2) and repressors (CREMα, CREMβ, CREMγ, S-CREM, CREM-17X, and CREM-17) by alternative splicing (9–14). In addition, use of an intronic promoter (P2) generates inducible cAMP early repressors (ICERs) (15, 16). This property is unique to CREM within this family of transcription factors. CREMτ and CREMτα contain the phosphorylation domain (P-box) and two glutamine-rich domains (Q1 and Q2), which may be essential for gene activation and function as transcriptional activators. The CREM repressors, CREMα, -β, and -γ, contain the P-box but lack both Q1 and Q2. Thus, by a shuffling of exons, various combinations of functional domains are generated, and a large number of functionally different CREM isoforms are produced (12). These isoforms have been well studied in the hypothalamic-pituitary-gonadal axis, where they play key physiological and developmental roles by regulating gene transcription through CREs (17–22). Since the human insulin gene also possesses CREs, CREM could be a candidate for promoting insulin gene transcription.

Recent studies have demonstrated direct interactions of site-specific transcriptional factors and TFIID (23–25), a multiprotein complex consisting of the TATA box-binding protein (TBP) and at least eight TBP-associated factors (TAFs) (26–28), and revealed that it enhances the rate of promoter binding and stabilization of TFIID-promoter complexes (29).

Here we have analyzed the mechanism of transcriptional regulation of the insulin gene by CREM. We identified two novel CREM isoforms, CREMΔQ1 and CREMΔQ2, and six known isoforms in pancreatic islets and the pancreatic β-cell line. We demonstrated that these isoforms regulated insulin gene transcription by binding to CREs and that CREM and its phosphorylation were involved in glucose-induced insulin pro-

* This study was supported in part by Grants-in-Aid for Scientific Research 09470219 and for Creative Basic Research NP10NPO201 from the Ministry of Education, Science, Sports and Culture, Japan; by a grant for Diabetic Research from Tsumura and Co., Japan; and by grants for the "Research for the Future" Program from the Japan Society for the Promotion of Science (JSPS-RFTF97I00201). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ To whom correspondence should be addressed: Dept. of Metabolism and Clinical Nutrition, Graduate School of Medicine, Kyoto University, 54 Shogoin-Kawahara-cho, Sakyo-ku, Kyoto 606-8507, Japan. Tel.: 81-75-751-3560; Fax: 81-75-771-6601; E-mail: inada@metab.kuhp.kyoto-u.ac.jp.

[1] The abbreviations used are: CRE, cyclic AMP response element; CREM, cyclic AMP response element modulator; CREB, cyclic AMP response element-binding protein; ICER, inducible cyclic AMP early repressor; nt, nucleotide(s); TBP, TATA box-binding protein; hTBP, human TBP; TAF, TBP-associated factor; hTAF, human TAF; GST, glutathione *S*-transferase; PAGE, polyacrylamide gel electrophoresis.

Downloaded from http://www.jbc.org/ by guest on October 4, 2017

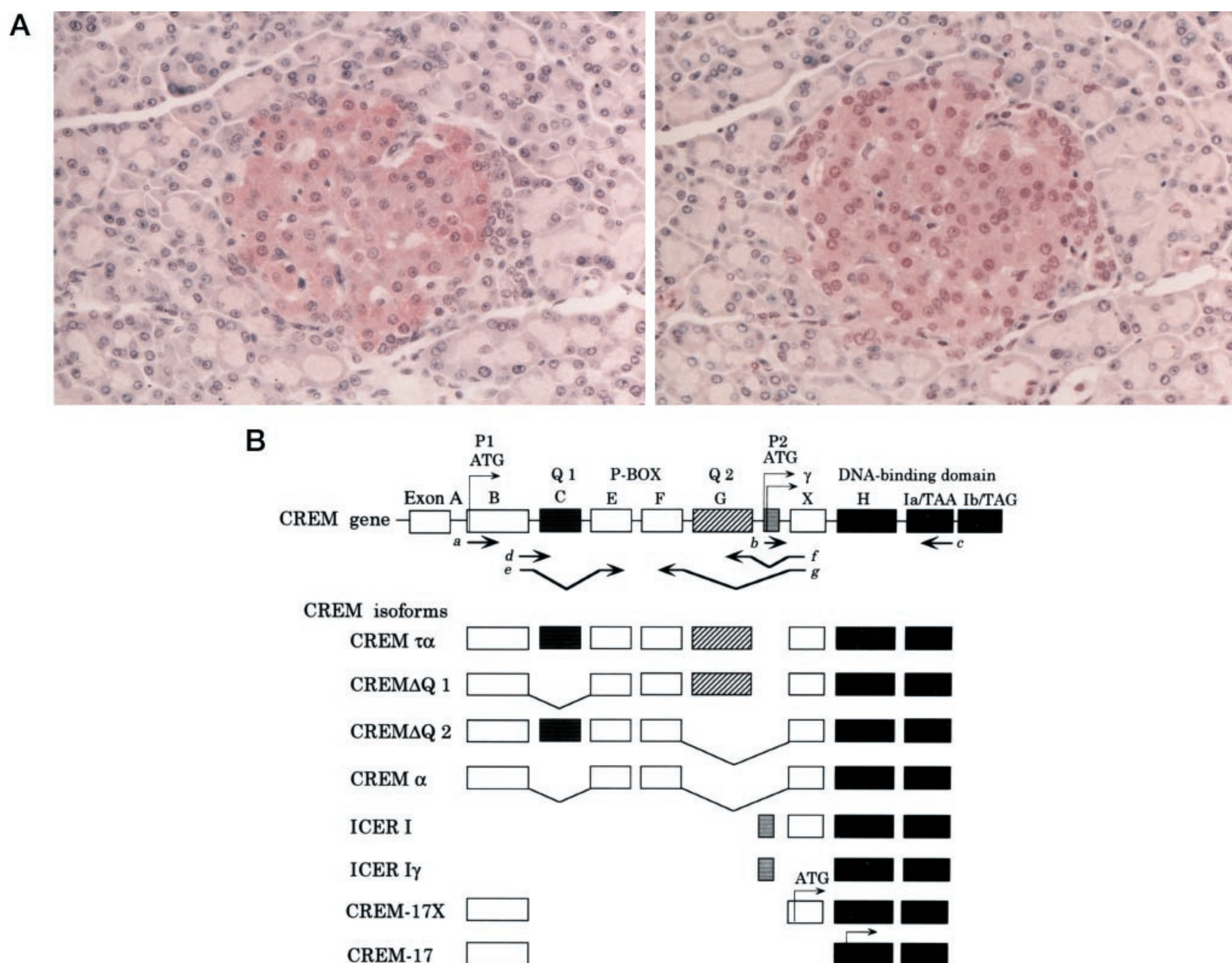

FIG. 1. **Expression of the CREM isoforms in rat pancreatic islets.** *A*, immunohistochemical localization of CREM protein in pancreatic islets of 8-week-old rats using anti-CREM antibody (dilted to 1:500). Immunocytochemical staining was performed by the avidin-biotin complex method. To ascertain the specificity of the CREM antibody, a testis section was used as a positive control. In agreement with previous data (9, 18, 41), the expression of CREM protein was found to be restricted to round spermatides (data are not shown). Two continuous rat pancreatic islet sections, one for the anti-insulin antibody (Fig. 1*A*, *left panel*) and the other for the anti-CREM antibody (Fig. 1*A*, *right panel*). *Left panel*, pancreatic β cells were stained red with the anti-insulin antibody (magnification, $\times$ 400). *Right panel*, pancreatic islets were stained red with the anti-CREM antibody. The CREM proteins were detected both in nuclei and cytoplasm of the pancreatic islets, mainly in the pancreatic β cells (magnification, $\times$ 400). *B*, schematic presentation of the structure of the CREM isoforms is shown. Functional domains are two glutamine-rich domains (Q1, Q2), the phosphorylation domain (P-BOX), and the DNA-binding domain (DBD I). Two novel isoforms, CREMΔQ1 and CREMΔQ2, lacked Q1 and Q2, respectively, and contained DBD I. Other isoforms are the known activator (CREM$\tau\alpha$) and the repressors (CREMα, ICER I, ICER Iγ, CREM-17X, and CREM-17) (9–14). ICER is transcribed from an alternative intronic promoter. The *arrows*, *a–g*, represent the positions of synthetic oligonucleotide primers used to detect isoforms.

moter activity. We showed the direct interaction between CREM isoforms and the TFIID component, hTAF$_{II}$130 or TBP, suggesting a mechanism by which CREM isoforms might regulate insulin gene transcription.

EXPERIMENTAL PROCEDURES

Immunohistochemistry—Wistar rat pancreas embedded in paraffin was cut into serial sections at 3.5 μm. For immunocytochemistry, the avidin-biotin complex method with alkaline-phosphatase was used as described previously (30) with a slight modification. Briefly, normal goat serum (diluted to 1:75; DAKO, Kyoto, Japan) for the inhibition of nonspecific binding of secondary antibody, the anti-CREM polyclonal antibody (diluted to 1:500; Upstate Biotechnology Inc., Lake Placid, NY), or the anti-insulin polyclonal antibody (diluted to 1:500; DAKO), biotin-labeled goat anti-rabbit IgG serum (diluted to 1:300; DAKO), and avidin-biotin-alkaline phosphatase complex (diluted to 1:100; DAKO) were sequentially applied. Staining was visualized by alkaline phosphatase substrate (red) (Vector Laboratories, Burlingame, CA). A section of rat testis was used as a positive control for CREM. Anti-CREM polyclonal antibody was raised against recombinant mouse CREMτ expressed in *Escherichia coli*.

Isolation of Pancreatic Islets and RNA—For each experiment, about 5000 islets were isolated from the pancreases of 12 Wistar rats by collagenase digestion (31), followed by purification on Ficoll gradients. Total RNA was isolated from freshly isolated islets by the guanidium thiocyanate-cesium chloride method (32) and were used as a template for cDNA synthesis (Superscript reverse transcriptase (Life Technologies, Inc.).

Reverse Transcriptase-Polymerase Chain Reaction Analysis and Plasmid Constructs—Rat pancreatic islet cDNA and the pancreatic β-cell line RINm5F (33) cDNA were amplified by the polymerase chain reaction using rat CREM-specific oligonucleotides. To exclude any amplification product derived from genomic DNA that could contaminate the RNA preparation, total RNA without reverse transcription was amplified as a negative control. The sequences of the 5′ primers for the full-length of CREM and ICER were as follows: primer a, 5′-CCGTATGACCATGGAAACAG-3′ (nt 5–24, Ref. 34); primer b, 5′-ATGGCTGTAACTGGAGATGA-3′ (nt 1–20, Ref. 15), respectively. The sequence of the 3′ primer (primer c) was 5′-CAGGTCCAAGTCAAACACAG-3′ (nt 1054–1035). Exon-specific primers were set to determine the expression of each isoform. The sequences of the isoform-specific 5′ primers for CREM were as follows: primer d, 5′-TCTAGCTCAGGTTTCTGTAG-3′

Downloaded from http://www.jbc.org/ by guest on October 4, 2017

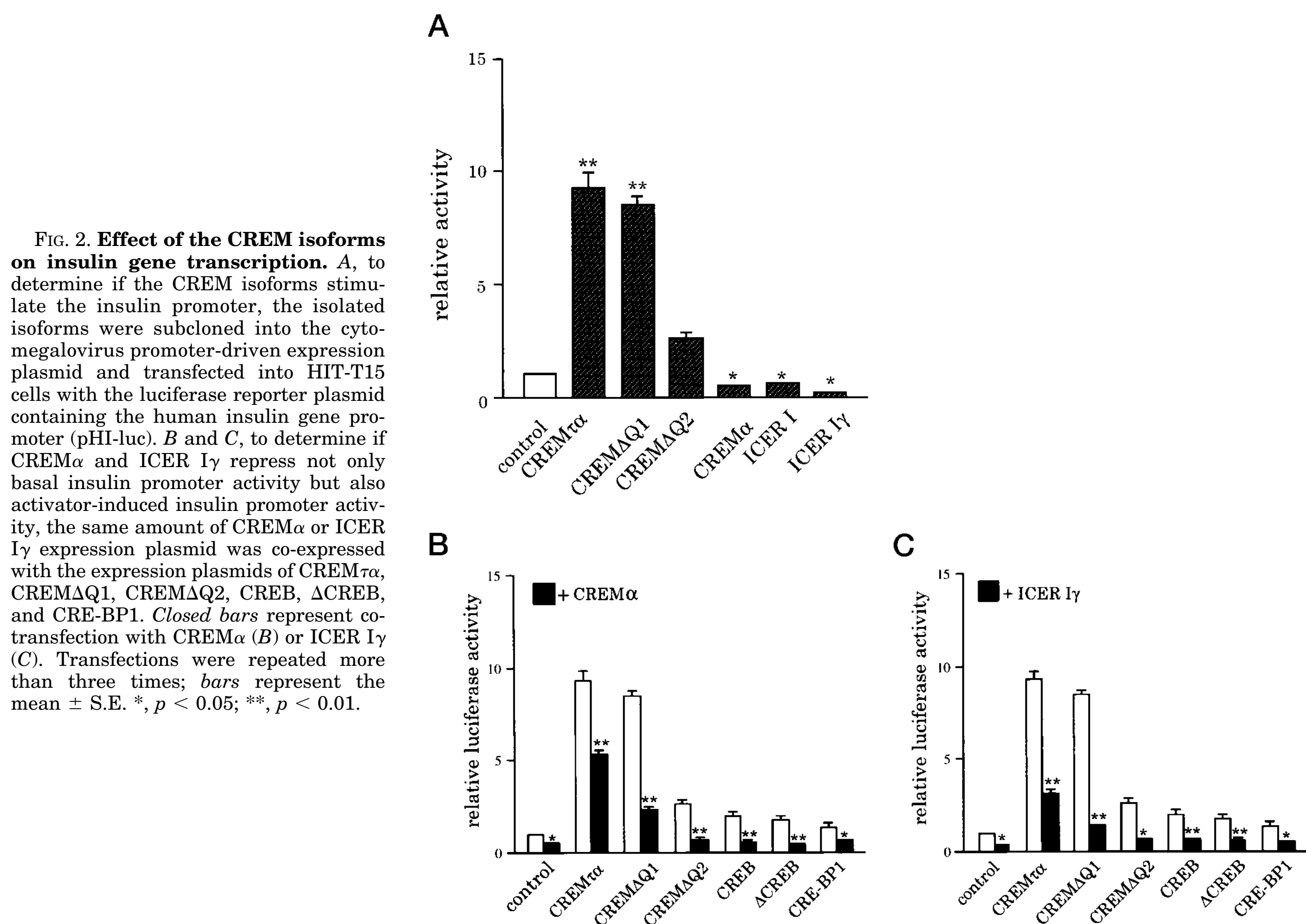

FIG. 2. **Effect of the CREM isoforms on insulin gene transcription.** *A*, to determine if the CREM isoforms stimulate the insulin promoter, the isolated isoforms were subcloned into the cytomegalovirus promoter-driven expression plasmid and transfected into HIT-T15 cells with the luciferase reporter plasmid containing the human insulin gene promoter (pHI-luc). *B* and *C*, to determine if CREMα and ICER Iγ repress not only basal insulin promoter activity but also activator-induced insulin promoter activity, the same amount of CREMα or ICER Iγ expression plasmid was co-expressed with the expression plasmids of CREMτα, CREMΔQ1, CREMΔQ2, CREB, ΔCREB, and CRE-BP1. *Closed bars* represent co-transfection with CREMα (*B*) or ICER Iγ (*C*). Transfections were repeated more than three times; *bars* represent the mean ± S.E. *, $p < 0.05$; **, $p < 0.01$.

(nt 113–132); primer e, 5′-TCTAGCTCAGGTAGCAACAA-3′ (nt 113–122, nt 270–279). Sequences of the 3′ primers were as follows: primer f, 5′-GTCTCCTCATCTTGAACAAC-3′ (nt 706–687); primer g, 5′-GTCTCCTCATTGTATTGCCC-3′ (nt 706–697, nt 507–498). Primers a, b, c, d, e, f, and g correspond to Fig. 1*B*. Each DNA fragment was subcloned into the *Sma*I site of pBluescript II SK(+), and the sequences were confirmed by the dideoxynucleotide chain termination procedure. The full-length sequences of the CREM isoforms, CREMτα, CREMΔQ1, CREMΔQ2, CREMα, ICER I, and ICER Iγ, were subcloned into the cytomegalovirus promoter-driven expression plasmid. Mutation of CREMτα serine 117 to alanine, S117ACREMτα, was constructed by oligonucleotide-directed site mutagenesis using an *in vitro* mutagenesis system (Promega, Madison, WI) and the following nucleotide primer: 5′-TCACGAAGACCC**GCA**TATAGAAAA-3′ (boldface denotes the mutation). The sequences of the activation domains were as follows: primer h, 5′-GGTAGCAACAATTGCAGAGA-3′ (nt 270–289), primer i, 5′-GATTGCTATAGCCCAAGGTGG-3′ (nt 508–527); primer j, 5′- TTAGTATTGCCCCGTGCTAGTC-3′ (nt 507–488); primer k, 5′-TTATTGAACAACAACCTGGCT-3′ (nt 696–677). Primers h, i, j, and k correspond to Fig. 3. DNA fragments of P, Q2, PQ2, and S117APQ2 (mutation of CREMτα serine 117 to alanine) were subcloned in frame to the 3′-end of the GAL4 DNA-binding domain in plasmid pCG4. The control pCG4 was generated by introducing the coding sequence for the GAL4 DNA-binding domain (amino acids 1–147) from pGBT9 (CLONTECH, Palo Alto, CA), downstream of the cytomegalovirus promoter in plasmid pCMV6c.

Cell Culture and Transfection—Plasmids for the 5xGAL4-TATA-luciferase reporter gene were kindly provided by Dr. R. A. Maurer (Oregon Health Sciences University, Portland, OR). HIT-T15 cells (hamster pancreatic β-cell line; Ref. 35) were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum, 100 units/ml penicillin, and 100 μg/ml streptomycin in an atmosphere of 5% CO_2 at 37 °C. Transient transfections in HIT-T15 cells were performed by the calcium phosphate precipitation technique. Briefly, the media were switched to Dulbecco's modified Eagle's medium containing 10% fetal calf serum 4 h before transfection. The cells were transfected with a mixture of 12 μg of the luciferase reporter plasmid, pHI-luc, which contains human insulin gene promoter (nt −339 to +112; Ref. 4) or 5xGAL4-TATA-luciferase reporter plasmid, which contains 5xGAL4 DNA-binding sites, 0–4 μg of each full-length CREM expression plasmid or part-length CREM expression plasmid in which GAL4 DNA-binding domain fused, and 4 μg of internal control plasmid p-act-β-gal (36). For CREB and ΔCREB expression plasmid, human CREB and ΔCREB cDNA were amplified from human liver cDNA and were subcloned into the cytomegalovirus promoter-driven expression plasmid. The sequence of the 5′ primer was 5′-ATGACCATGGAATCTGGAGC-3′ (nt 144–163; Ref. 37), and the sequence of the 3′ primer was 5′-TTAATCTGATTTGTGGCAGT-3′ (nt 1169–1150). Expression plasmids of CREB, ΔCREB, and CRE-BP1 (7) were co-transfected. To analyze promoter activity stimulated by glucose, cells were treated with various concentration of glucose. Forty-eight hours after transfection, the cells were harvested, and cell extracts were prepared for luciferase assays and β-galactosidase assays. β-Galactosidase assays were performed for internal control. All transfection experiments were repeated more than three times.

Expression of Recombinant CREM Isoforms and Electrophoretic Mobility Shift Assays—Electrophoretic mobility shift assays were carried out to measure binding activity of CREMτα, CREMΔQ2, and ICER Iγ to the human insulin CREs with double-stranded oligonucleotide probes. For production of glutathione *S*-transferase (GST) fusion proteins, the DNA fragments of CREMτα, CREMΔQ2, and ICER Iγ were subcloned into pGEX4T-3 (Amersham Pharmacia Biotech) and transformed into *E. coli* strains M15. Following induction of protein expression with isopropyl thio-β-D-galactoside, the GST fusion proteins were purified according to the manufacturer's instructions. To check the size of products, the proteins were visualized by Coomassie Blue staining following fractionation by SDS-PAGE. Cy5-labeled DNA probes and 0–1.5 μg/μl proteins were incubated for 30 min at 25 °C. Electrophoreses were performed at 15 °C, at 38 milliamps constant current, and the binding activity was analyzed using an ALF DNA sequencer. Sense strands are shown (Table I): CRE1 (nt −220 to −191), 5′-TAAGACTCTAATGACCCGCTGGTCCTGAGG-3′; CRE2 (nt −188 to −164), 5′-GAGGTGCTGACGACAAGGAGATCT-3′; CRE3 (nt +13 to +32), 5′-CAGGCTGCAT-

Downloaded from http://www.jbc.org/ by guest on October 4, 2017

CAGAAGAGGC-3′; and CRE4 (nt +57 to +72), 5′-GGCCTTTGCGTC-AGGTGGGC-3′.

Western Blot Assays—HIT-T15 cells were harvested and were suspended in radioimmune precipitation buffer (50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 22 mM EDTA, 1% Trasilol). Cell lysis was sonicated in radioimmune precipitation buffer containing 0.5 mM phenylmethylsulfonyl fluoride. After removal of insoluble debris by centrifugation, supernatants were loaded on 12.5% SDS-PAGE and transferred to nitrocellulose membranes (Millipore Corp., Bedford, MA). Membranes were blocked with 5% skim milk in phosphate-buffered saline and then incubated with anti TBP-antibody (diluted to 1:250; Santa Cruz Biotechnology, Inc., Santa Cruz, CA) for 2 h at 37 °C. Membranes were incubated with horseradish peroxidase-linked anti-rabbit IgG antibody (Amersham Pharmacia Biotech) for 30 min at 37 °C. Each of these steps was followed by three washes for 10 min in 5% Tween 20/phosphate-buffered saline. Detection was performed as described in the ECL protocol (Amersham Pharmacia Biotech).

In Vitro Transcription and Translation—The plasmid encoding human $TAF_{II}130$ ($hTAF_{II}130$) cDNA was a generous gift of Dr. N. Tanese (New York University). Human TBP (hTBP) cDNA was amplified from human liver cDNA. The sequence of the 5′ primer was 5′-ATGGATCA-GAACAACAGCCT-3′ (nt 242–261; Ref. 38), and the sequence of the 3′ primer was 5′-TTACGTCGTCTTCCTGAATC-3′ (nt 1261–1242). hTBP and $hTAF_{II}130$ cDNA were subcloned into the *Sma*I site of pGEM 3Z and the *Eco*RI site of pBluescript II SK(+), respectively. hTBP and $hTAF_{II}130$ proteins were synthesized in a coupled *in vitro* transcription-translation system in the presence of [^{35}S]methionine (Promega). The radioactive products were visualized by separation on a 12.5% SDS-PAGE and autoradiography.

In Vitro Protein Interaction Assays—GST pull-down assays were performed as described previously (39, 40). Each assay contained GST fusion protein immobilized on glutathione-Sepharose beads (Amersham Pharmacia Biotech). Briefly, HIT-T15 cell extract or [^{35}S]methionine-labeled *in vitro* translated hTBP or $hTAF_{II}130$ was incubated with GST, GST-CREMτα, GST-CREMΔQ2, GST-CREMα, GST-ICER Iγ in binding buffer (20 mM Tris-HCl (pH 7.6), 0.1 mM EDTA, 20% glycerol, 300 mM KCl, 0.1% Nonidet P-40, 0.5 mM phenylmethylsulfonyl fluoride, 2 mM $MgCl_2$, 5 mM 2-mercaptoethanol) for 1 h at 4 °C. After incubation, the beads were washed five times with 1 ml of binding buffer. The bound proteins were resolved by 12.5% SDS-PAGE and visualized by autoradiography or by an anti-TBP antibody as described above.

RESULTS

Expression of the CREM Isoforms in Rat Pancreatic Islets—To determine if CREM is expressed in pancreatic islets, immunohistochemical studies of 8-week-old rat pancreatic islet sections were performed using anti-CREM antibody. To ascertain the specificity of the CREM antibody, a testis section was used as a positive control (data are not shown). In agreement with previous data (9, 18, 41), the expression of CREM protein was found to be restricted to round spermatides, mostly at stage VII–VIII of spermatogenesis, when the spermatozoa begin to be released into the lumen. We used two continuous rat pancreatic islet sections, one for the anti-insulin antibody (Fig. 1*A*, *left panel*) and the other for the anti-CREM antibody (Fig. 1*A*, *right panel*). Pancreatic β cells were stained red with the anti-insulin antibody. The CREM proteins were detected both in the nuclei and cytoplasm of the pancreatic islets, mainly in the pancreatic β cells. To identify which isoforms were present in the pancreatic islet cells and the pancreatic β-cell line, we performed reverse transcriptase-polymerase chain reaction with rat pancreatic islet cDNA and the RINm5F β-cell line cDNA using primers a–g (Fig. 1*B*). We isolated eight CREM isoforms, of which two are novel isoforms and the other six are known isoforms. A schematic presentation of two novel isoforms (CREMΔQ1 and CREMΔQ2), five repressors (CREMα, ICER I, ICER Iγ, CREM-17X, and CREM-17), and an activator (CREMτα) is shown in Fig. 1*B*. CREMΔQ1 and CREMΔQ2 lack one of the glutamine-rich domains (Q1 and Q2, respectively) and contain an exon Ia (DNA binding domain (DBD) I). These structures are similar to those of CREMτ2 and -τ1 (9, 12), which also lack one of the glutamine-rich domains (Q1 and Q2, respectively), and contain an exon Ib (DBD II).

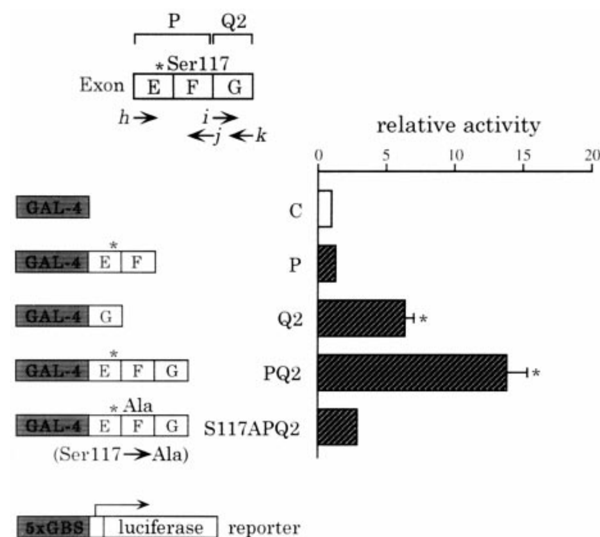

FIG. 3. **Transcriptional activity of the activation domain.** To investigate the function of the P domain and Q2 domain, P domain alone, Q2 domain alone, and the combination of P and Q2 domain (PQ2) were fused in-frame to the 3′-end of the GAL4 DNA-binding domain (*black box*) in plasmid pCG4. To further examine if the phosphorylation is involved, Ser^{117} was mutated by Ala (S117APQ2). The *arrows*, *h–k*, represent the positions of the synthetic oligonucleotide primers used. Exons E and F correspond to the phosphorylation (P) domain, and exon G corresponds to the glutamine-rich (Q) domain, Q2. The *asterisk* in exon E represents a serine residue at position 117 (*Ser117*), which is the target for the phosphorylation. HIT-T15 cells were transfected with the GAL4-fused expression plasmid and the luciferase reporter plasmid containing the 5xGAL4-binding sites (5xGAL4-TATA-luciferase reporter plasmid). *C*, control. Transfections were repeated more than three times; *bars* represent the mean ± S.E. *, $p < 0.05$; **, $p < 0.01$.

Effect of CREM Isoforms on Insulin Promoter Activity—Previously, we have reported that CREM-17X and CREM-17 functioned as strong repressors (13). To analyze the function of the other CREM isoforms in insulin gene transcription, the isolated isoforms were subcloned into the cytomegalovirus promoter-driven expression plasmid vector and were transfected into HIT-T15 cells with the luciferase reporter plasmid containing the human insulin promoter (pHI-luc). CREMτα, CREMΔQ1, and CREMΔQ2 activated insulin promoter 9-, 8-, and 2-fold, respectively (Fig. 2*A*). On the other hand, CREMα, ICER I, and ICER Iγ repressed the activity to about 30, 25, and 25% of controls, respectively. We then tested the abilities of the repressors, CREMα and ICER, to repress not only basal transcriptional activity but also activator-induced transcriptional activity. CREM activator induced transcriptional activity, whereas CREMα and ICER Iγ blocked this effect to about 30–50% of controls (Fig. 2, *B* and *C*). Moreover, CREMα and ICER Iγ also repressed CREB-, ΔCREB-, and CRE-BP1-induced transcriptional activity to about 30% of controls.

Transcriptional Activity of the Activation Domain—To investigate the function of the phosphorylation (P) and glutamine-rich (Q) domains in the transcriptional activity of CREM, the P domain alone, the Q2 domain alone, and the combination of P and Q2 domains (PQ2) were fused in frame to the 3′-end of the GAL4 DNA-binding domain in plasmid pCG4. To further examine if the phosphorylation is involved in CREM activation, we mutated the serine residue to alanine at position 117 in the P domain (S117APQ2), which is the target for the phosphorylation (9, 42, 43). Schematic presentation of GAL4-fused expression plasmids is shown (Fig. 3, *left*). HIT-T15 cells were transfected with the GAL4-fused expression plasmid, and the luciferase reporter plasmid containing 5xGAL4-binding sites (5xGAL4-TATA-luciferase reporter plasmid). Luciferase activ-

Downloaded from http://www.jbc.org/ by guest on October 4, 2017

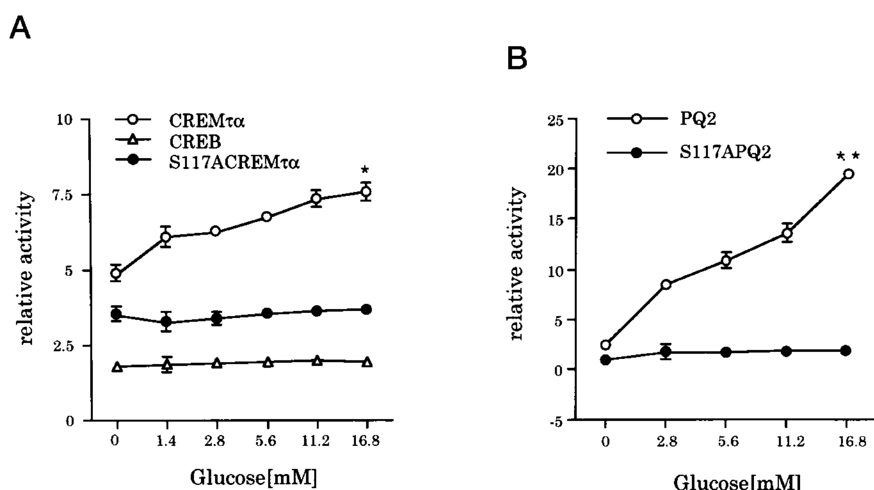

FIG. 4. **Effect of glucose stimulation on insulin promoter.** *A*, to determine if CREM and CREB are involved in glucose-induced insulin promoter activity, HIT-T15 cells were transfected with pHI-luc and the expression plasmid of CREM$\tau\alpha$, S117ACREM$\tau\alpha$ (Ser[117] was mutated by Ala) and CREB. Cells were treated with various concentrations of glucose. In the presence of CREM$\tau\alpha$, a significant increase in a dose-dependent manner in insulin promoter activity was observed by glucose stimulation ($p < 0.05$ *versus* 0 mM glucose). But in the presence of S117ACREM$\tau\alpha$ and CREB, no significant increase in insulin promoter activity was observed by glucose, suggesting that CREM but not CREB is involved in glucose-induced insulin promoter and that the increase is dependent on Ser[117], since mutating this residue to alanine leads to a strong decrease. *B*, to determine if glucose stimulation induces PQ2 domain activity, GAL4-fused PQ2 and S117APQ2 domain were transfected with 5xGAL4-TATA-luciferase reporter plasmid, and the cells were treated with various concentrations of glucose. In the presence of PQ2, a significant increase in luciferase activity was observed in a dose-dependent manner by glucose stimulation ($p < 0.01$ *versus* 0 mM glucose), but not in the presence of S117APQ2 domain. Transfections were repeated more than three times; *bars* represent the mean ± S.E. *, $p < 0.05$; **, $p < 0.01$.

ity increased about 6- and 13-fold in the presence of Q2 domain alone or PQ2 domains, respectively, but showed no change in the absence of Q2 domain (Fig. 3, *right*). The Ser to Ala mutation substantially decreased PQ2 domain activity.

Effect of Glucose Stimulation on the Insulin Gene Transcription—Glucose stimulation has been reported to increase insulin mRNA (44, 45). We investigated whether CREM and CREB are involved in glucose-induced insulin promoter activity. To determine the involvement of the phosphorylation in CREM, Ser[117] in the P domain of CREM$\tau\alpha$ was mutated by Ala (S117ACREM$\tau\alpha$). CREM$\tau\alpha$, CREB, and S117ACREM$\tau\alpha$ were transfected into HIT-T15 cells with pHI-luc, and the cells were treated with various concentrations of glucose. Interestingly, insulin promoter activity was increased remarkably by glucose stimulation in a dose-dependent manner in the presence of CREM$\tau\alpha$ (Fig. 4*A*). However, in the presence of S117ACREM$\tau\alpha$ and CREB, no significant increase in insulin promoter activity was observed by glucose stimulation, suggesting that CREM but not CREB is involved in glucose-induced insulin promoter and that the increase is dependent on Ser[117]. We next examined if glucose stimulation induces PQ2 domain activation; the PQ2 and S117APQ2 domains were transfected with 5xGAL4-TATA-luciferase reporter plasmid, and the cells were treated with various concentrations of glucose. The activity of the PQ2 domain increased in a dose-dependent manner, but the S117APQ2 activity did not (Fig. 4*B*). Again, this increase is dependent on Ser[117], suggesting that glucose might induce the activation of CREM by phosphorylation.

Binding Activity of the CREM Isoforms to Insulin Gene CREs—We have reported four CRE-like sequences in the human insulin gene that are somewhat different from each other and from the consensus CRE motif (4). To determine whether CREM isoforms bind to these CRE-like sequences of the human insulin gene, we performed electrophoretic mobility shift assays using bacterially produced CREM$\tau\alpha$, CREMΔQ2, and ICER Iγ proteins and Cy5-labeled human insulin gene CRE probes (Table I). The binding activity was analyzed using an ALF DNA sequencer. The binding of bacterially produced CREM$\tau\alpha$, CREMΔQ2, and ICER Iγ proteins to each CRE

TABLE I

Sequences of sense strands of the oligonucleotides synthesized and used in electrophoretic mobility shift assays (Fig. 5) that correspond to naturally occurring CRE sites

The eight-base CRE sequences are shown in boldface type. The consensus CRE sequence is TGACGTCA, but the sequences of CRE1 and CRE2 show the variation at the 3′-end, while CRE3 and CRE4 show it at the 5′-end.

Oligonucleotide	Coding sequence
Consensus CRE	TGACGTCA
Human insulin gene	
CRE1	TAAGACTCTAA**TGACCCGC**TGGTCCTGAGG
CRE2	GAGGTGC**TGACGACC**AGGAGATCT
CRE3	CAGG**CTGCATCA**GAAGAGGC
CRE4	GGCCT**TTGCGTCA**GGTGGGC

(CRE1 to 4) were detected as the peaks of fluorescence derived from Cy5-labeled probes (Fig. 5, *A*, *B*, and *C*). We next determined if ICER Iγ competes with CREM$\tau\alpha$ or CREMΔQ2 for CRE3. As shown in Fig. 5*D*, ICER Iγ competed with CREM$\tau\alpha$ in a dose-dependent manner to bind to CRE3 (*lanes 1–4*). Similar results were obtained using CREMΔQ2 (Fig. 5*E*). When the same amounts of ICER Iγ and CREM$\tau\alpha$ were added (*lane 5*), CRE3 of the insulin gene bound to ICER Iγ more than CREM$\tau\alpha$. On the other hand, the binding activity of ICER Iγ to CRE3 was the same as that of CREMΔQ2.

Interaction of CREM with TFIID in Vitro—To determine whether CREM might interact directly with the TFIID components TBP and $hTAF_{II}130$ we performed *in vitro* interaction assays. The GST-fused CREM isoforms GST-CREM$\tau\alpha$, GST-CREMΔQ2, GST-CREMα, and GST-ICER Iγ were bacterially produced. [^{35}S]methionine-labeled hTBP and $TAF_{II}130$ were synthesized in a coupled *in vitro* transcription-translation system using rabbit reticulocyte lysate in the presence of [^{35}S]methionine. Immobilized GST-CREM isoforms were incubated with [^{35}S]methionine-labeled hTBP or $hTAF_{II}130$. Specifically bound protein was resolved by SDS-PAGE and visualized by autoradiography. As shown in Fig. 6*A*, hTBP bound to CREM$\tau\alpha$, CREMΔQ2, and CREMα (*lanes 3–5*). However, hTBP did not bind only to ICER Iγ (*lane 6*). To confirm the interaction

Downloaded from http://www.jbc.org/ by guest on October 4, 2017

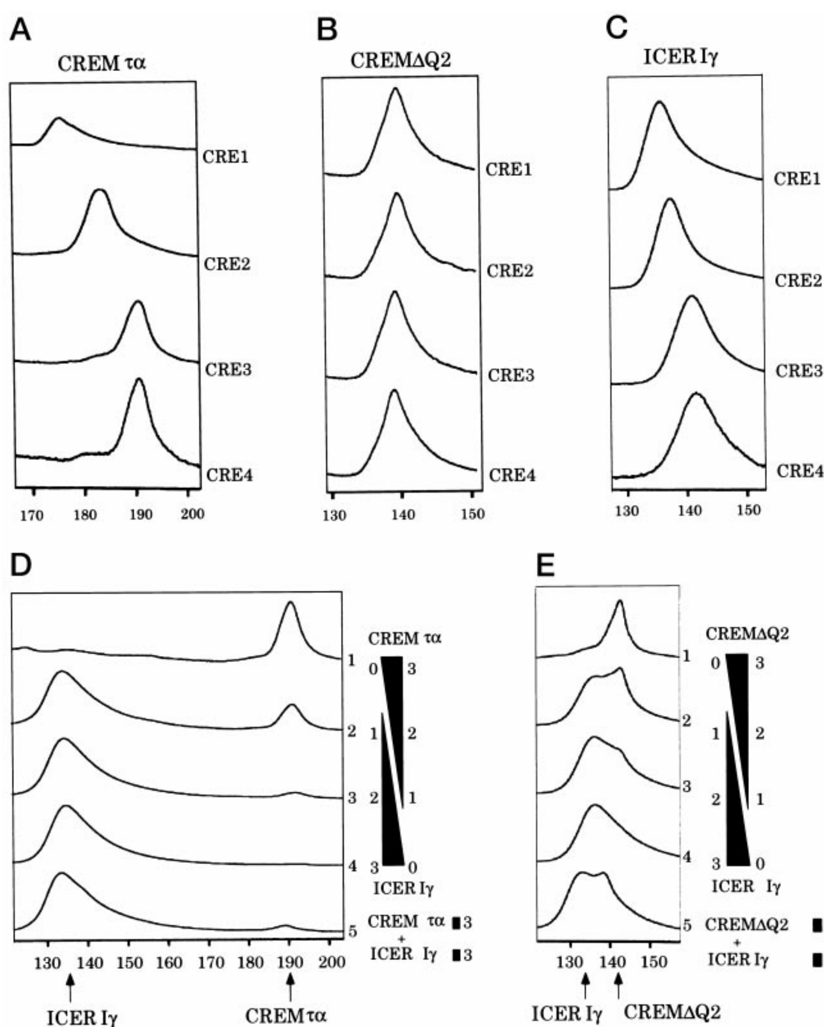

FIG. 5. **Binding activity of the CREM isoforms to human insulin gene CREs.** To determine if the CREM isoforms bind to the CRE-like sequences of the human insulin gene, electrophoretic mobility shift assays were performed using Cy5-labeled human insulin gene CRE probes and bacterially produced CREMτα, CREMΔQ2, and ICER Iγ proteins. The proteins were incubated with Cy5-labeled probes and electrophoresed. The binding activity was analyzed using an ALF DNA sequencer. The binding of CREMτα (*A*), CREMΔQ2 (*B*), or ICER Iγ (*C*) to each CRE (CRE1 to 4) was detected as the peaks of fluorescence derived from Cy5-labeled probes. The amounts of proteins are noted as arbitrary units. When the relative amounts of ICER Iγ were increased (*lanes 1–4*), ICER Iγ competed with CREMτα (*D*) or CREMΔQ2 (*E*) in a dose-dependent manner to bind to CRE3. The same amounts of ICER Iγ and CREMτα (*D*) or CREMΔQ2 (*E*) were added (*lane 5*).

between TBP and the CREM isoforms, we prepared cell extracts from HIT-T15 cells and examined the interaction of intracellular TBP and bacterially produced CREM isoforms. Specifically bound proteins were subjected to Western blotting with a polyclonal TBP antibody (Fig. 6*B*). hTBP bound to CREMτα and CREMΔQ2 (*lanes 3* and *4*) but did not bind to ICER Iγ (*lane 5*). We next investigated the interaction of $hTAF_{II}130$ and the CREM isoforms. $hTAF_{II}130$ bound only to CREMτα and CREMΔQ2 (*lanes 3* and *4*). The binding of $hTAF_{II}130$ to CREMΔQ2 was very weak. Moreover, $hTAF_{II}130$ did not bind to either CREMα or ICER Iγ (*lanes 5* and *6*) (Fig. 6*C*).

DISCUSSION

The insulin gene has multiple *cis*-acting elements, and its transcription is thought to be regulated by the interaction of various positive-acting and negative-acting factors (1). In addition, multiple factors such as general polymerase II transcription factors are involved in transcription by interacting with each other and forming the initiation complex (19–25). In the present study, the mechanism of transcriptional regulation of the human insulin gene by CREM through CREs was examined.

First, we demonstrated by reverse transcriptase-polymerase chain reaction and by immunohistochemistry that CREM is expressed in pancreatic β-cells at the levels of mRNA and protein. We isolated two novel CREM isoforms (CREMΔQ1 and CREMΔQ2) and the six known isoforms (CREMτα, CREMα, ICER I, ICER Iγ, CREM-17X, and CREM-17) from rat pancreatic islets and the RINm5F pancreatic β-cell line. It has been reported that all positively acting isoforms contain at least one of the glutamine (Q)-rich domains, Q1 and Q2 (12). Considering that CREMΔQ1 and CREMΔQ2 also contain one Q-rich domain and that their structures are very similar to that of the CREMτ1 and τ2 activators (9), CREMΔQ1 and CREMΔQ2 were supposed to function as activators. As expected, CREMΔQ1 and CREMΔQ2 were found in the present study to function as activators of insulin promoter. In addition, we showed that CREMτα functions as an efficient activator, while CREMα, ICER I, and ICER Iγ act as strong repressors of insulin promoter. In the previous study, we have shown that CREM-17X and CREM-17 function as efficient repressors of insulin promoter (13).

We also found by immunohistochemistry that CREM proteins are located both in the nuclei and cytoplasm of pancreatic islets. A nine-amino acid sequence (RRKKKEYVK) defined as the nuclear translocation signal of CREB (46) is also conserved in the DBD of CREM isoforms (14). A previous study detected a translation product of the plasmid encoding CREB cDNA lacking the putative nuclear translocation signal in the cytoplasm when transfected in COS-1 cells (46), suggesting that the CREM isoforms containing nuclear translocation signals may occur in the nuclei and that the isoforms that lack the nuclear translocation signal occur in the cytoplasm.

In a previous study, we have identified four CRE-like sequences (CRE1 to 4) in the human insulin gene (4). These CREs are the characteristic sequences, because the consensus CRE

Downloaded from http://www.jbc.org/ by guest on October 4, 2017

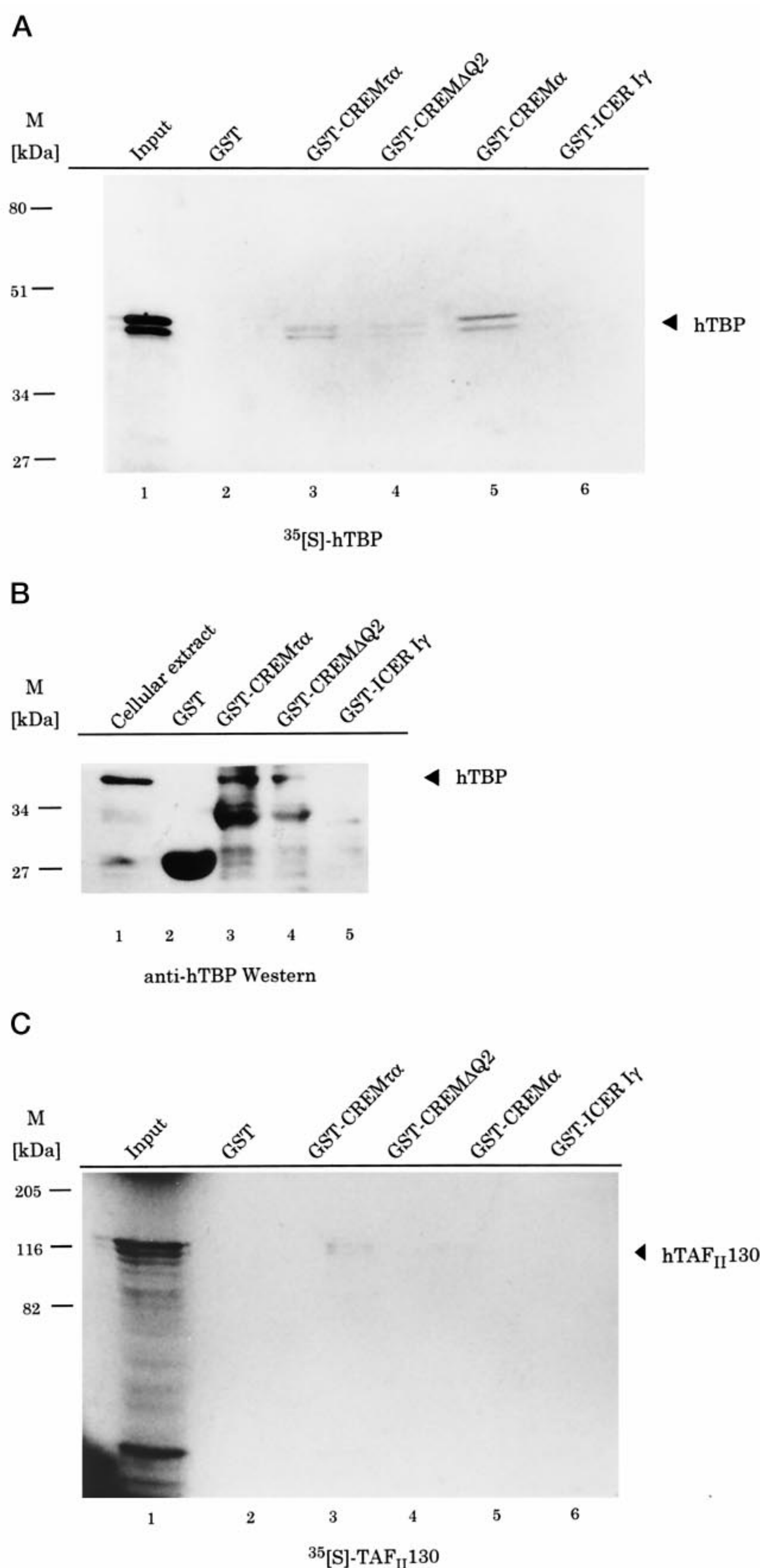

FIG. 6. **Interaction of CREM with TFIID detected *in vitro*.** To determine if CREM interacts directly with the TFIID components TBP and $TAF_{II}130$, *in vitro* interaction assays were performed. GST fusion CREM isoforms, GST-CREMτα, GST-CREMΔQ2, GST-CREMα, and GST-ICER Iγ, were bacterially produced. The plasmids containing the cDNA encoding human TBP (hTBP) and human $TAF_{II}130$ ($hTAF_{II}130$) were used to produce *in vitro* translated and [^{35}S]methionine-labeled proteins. *A* and *C*, the GST and GST-CREM isoform beads were incubated with [^{35}S]methionine-labeled proteins. After incubation, the beads were washed five times with the binding buffer. The bound protein was resolved by 12.5% SDS-PAGE and visualized by autoradiography. The *arrows* indicate the specific protein bands. The input samples contained one-sixth of the amounts used for incubation. *A*, hTBP bound to CREMτα, CREMΔQ2, and CREMα (*lanes 3–5*) but not to ICER Iγ (*lane 6*). The lack of binding to control GST demonstrates the specificity of the binding (*lane 2*). All of the isoforms that bound to hTBP commonly possess the phosphorylation domain, suggesting that CREM-hTBP interaction might be mediated by the phosphorylation domain. *B*, to confirm the interaction between TBP and CREM isoforms, cell extracts from HIT-T15 cells were prepared, and intracellular TBP and CREM isoforms were examined. Specifically bound proteins were subjected to Western blotting with a polyclonal anti-TBP antibody. hTBP bound to CREMτα and CREMΔQ2 (*lanes 3* and *4*) but not to ICER Iγ (*lane 5*). *C*, the interaction of $hTAF_{II}130$ and CREM isoforms was investigated. $hTAF_{II}130$ bound to CREMτα and very weakly to CREMΔQ2 (*lanes 3* and *4*) but not to CREMα or ICER Iγ (*lanes 5* and *6*), suggesting that CREM-$hTAF_{II}130$ interaction might be mediated by both of the glutamine-rich domains.

sequence is TGACGTCA, but the sequences of CRE1 and CRE2 show the variation at the 3′-end, while CRE3 and CRE4 show it at the 5′-end. Therefore, the examination for the binding of CREM to these CRE-like sequences was required. In the current study, we demonstrated that the CREM activators, CREMτα and CREMΔQ2, bind to each CRE. This result suggested that CREM activator modulates insulin promoter activity by binding directly to CREs. In addition, we demonstrated that the CREM repressor, ICER Iγ, also binds to each CRE and that the binding activity of repressor to insulin CRE is higher

Downloaded from http://www.jbc.org/ by guest on October 4, 2017

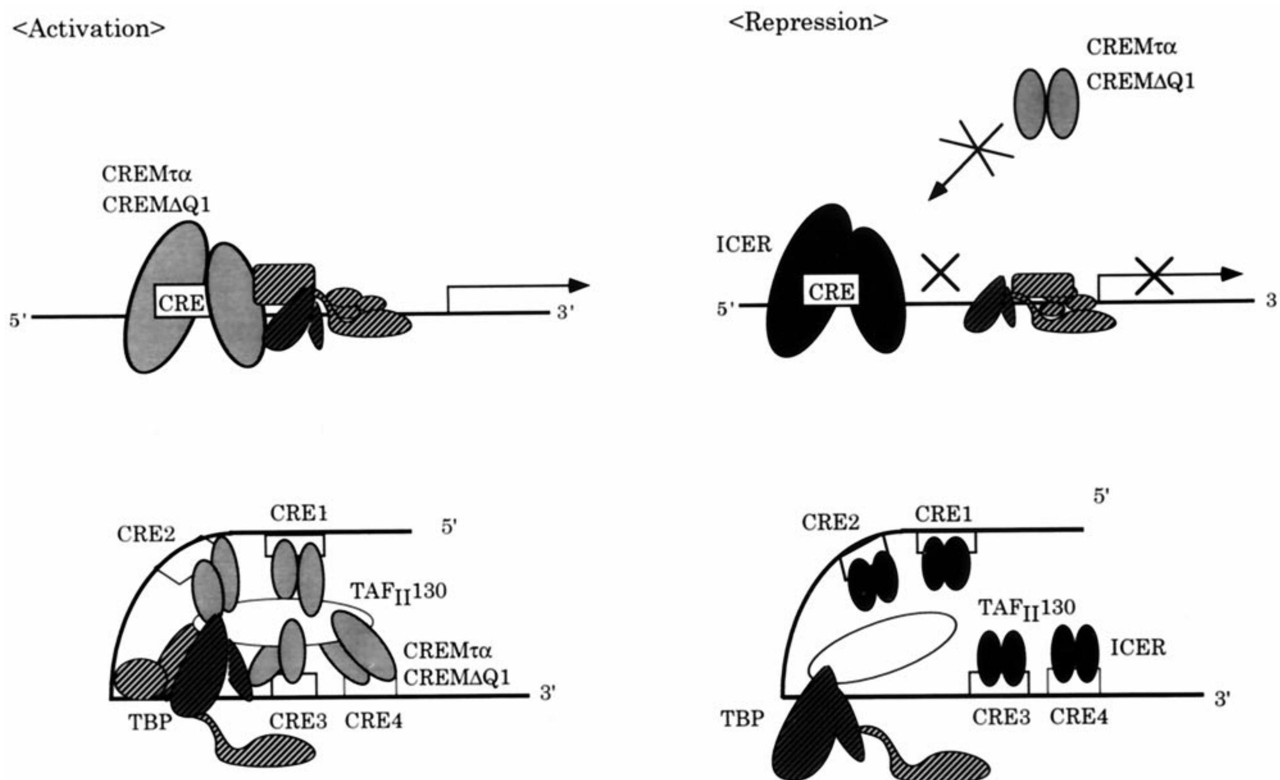

FIG. 7. **Activation and repression of the human insulin gene transcription by CREM$\tau\alpha$ and ICER.** *Left*, CREM$\tau\alpha$ activates insulin promoter not only by direct binding to CREs but also by recruiting TFIID to the insulin promoter via its interaction with hTBP and hTAF$_{II}$130. *Right*, ICER competitively interrupts the binding of the CREM activators to CREs and also does not interact with either TBP or hTAF$_{II}$130, which might fail to stabilize the basal transcriptional machinery and thus repress *trans*-activation.

than that of activators, suggesting that CREM repressor suppresses insulin promoter activity by competitively interrupting the binding of the activators to CREs.

We compared the ability of the members of the CREB/ATF family to activate the insulin promoter. We found in the present study that CREB could not highly activate insulin promoter, compared with CREM. Therefore, it is possible that among the members of the CREB/ATF family, CREM plays a pivotal role in modulating insulin promoter activity in pancreatic β cells. We further compared the ability of CREM and CREB to activate insulin promoter in the presence of glucose. It is well known that glucose increases insulin promoter activity, but the mechanism is unclear (44, 45). Here we showed for the first time that CREM but not CREB is involved in glucose-induced insulin promoter activity. It is interesting that CREM and CREB show a difference in the ability to promote insulin promoter not only in the absence but also in the presence of glucose, despite their extensive homology. This result suggests that CREM plays a role different from that of CREB in promoting insulin promoter in pancreatic β cells. Since Ser^{117} of CREMτ has been shown to be the target for phosphorylation by protein kinase A, protein kinase C, calmodulin kinase, and p34^{cdc2} and such phosphorylation increases CREM activity (9, 42, 43), we mutated Ser^{117} to analyze the involvement of this residue in glucose-induced insulin promoter activity. We found by mutation analysis that the phosphorylation of CREM at serine 117 is necessary for glucose-induced insulin promoter activity. Taken together, it is suggested that glucose stimulation might activate an intracellular signal transduction to enhance the phosphorylation of Ser^{117}, which increases CREM *trans*-regulatory function to promote insulin promoter activity.

It has been reported that TFIID components, TAFs, interact with each other (39, 47) and that certain TAFs can function as co-activators by direct interaction with site-specific transcription factors (24, 26, 48, 49); therefore, it was of interest to determine if CREM directly interacts with TFIID. In this study, we found that CREM activators interact with both TBP and hTAF$_{II}$130. Previously, hTAF$_{II}$130 and its *Drosophila* homologues dTAF$_{II}$100 and dTAF$_{II}$110 have been shown to intreract with CREB or Sp1 directly through the glutamine-rich domain (Q2) of CREB or Sp1 (24, 25, 48–51). Considering that all CREM activators possess one or two glutamine-rich domains (Q1, Q2) (9), Q1 and Q2 seem to be required for CREM to interact with hTAF$_{II}$130 and to function as an activator; conversely, the interaction of Q1 and Q2 with hTAF$_{II}$130 may enable CREM to function as an activator. Our results suggested that CREM-hTAF$_{II}$130 interaction and CREM-TBP interaction might be mediated by the glutamine-rich domains (Q1, Q2) and by the phosphorylation domain of CREM, respectively. Since it has been reported that the interaction of TFIID with site-specific transcriptional factors enhances the rate of binding of TFIID to the promoter or stabilizes TFIID-promoter complexes (29), it is possible that an interaction of CREM with TBP and hTAF$_{II}$130 may enhance and stabilize the binding of TFIID to the insulin promoter. In other words, CREM activators could increase the levels of insulin gene transcription by both binding directly to the CREs and recruiting TFIID to the insulin promoter via its interaction with hTBP and hTAF$_{II}$130 (Fig. 7). On the other hand, there were no CREM repressors that interact with both hTAF$_{II}$130 and TBP, suggesting that CREM repressor might fail to stabilize TFIID-promoter complexes. Therefore, it is suggested that CREM repressor might suppress insulin promoter activity by competitively interrupting the binding of the activators to CREs and, further, by failing to stabilize the basal transcriptional machinery. Since the only interactions of CREM with hTBP and hTAF$_{II}$130 were examined in this study, further studies of the interactions between CREM and the individual components of the TFIID

Downloaded from http://www.jbc.org/ by guest on October 4, 2017

complex such as $hTAF_{II}250$ (52), $hTAF_{II}100$ (25, 39), and $hTAF_{II}80$ (47) will be required to reveal the molecular mechanisms of the the insulin gene transcription in more detail.

In conclusion, we found in this study that CREM activators and repressors are expressed in pancreatic β cells and that they play important roles in modulating the insulin gene transcription in response to glucose. An important point from our study is that the activation of insulin gene transcription by CREM activator is mediated by not only direct binding to the CREs but also by recruiting TFIID to the insulin promoter via its interaction with hTBP and $hTAF_{II}130$. Furthermore, the CREM repressor competitively interrupts the binding of the activators to CREs and does not interact with both TBP and $hTAF_{II}130$; thereby, it might fail to stabilize the basal transcriptional machinery and repress transactivation.

Acknowledgments—We thank Dr. N. Tanese (New York University) for kindly providing human $TAF_{II}130$ plasmid and Dr. R. A. Maurer (Oregon Health Sciences University, Portland, OR) for 5xGAL4-TATA-luciferase reporter plasmid. We acknowledge Dr. S. Ishii (Tsukuba Life Science Center, RIKEN, Tsukuba, Japan) for critical reading of the manuscript and Dr. S. Toyokuni (Department of Pathology and Biology of Diseases, Kyoto University, Kyoto, Japan) for comments on immunohistochemistry.

REFERENCES

1. Sander, M., and German, M. S. (1997) *J. Mol. Med.* **75,** 327–340
2. Comb, M., Birnberg, C., Seasholtz, A., Herbert, E., and Goodman, H. M. (1986) *Nature* **323,** 353–356
3. Montminy, M. R., Sevarino, K. A., Wangner, J. A., Mandel, G., and Goodman, R. H (1986) *Proc. Natl. Acad. Sci. U. S. A.* **83,** 6682–6686
4. Inagaki, N., Maekawa, T., Sudo, T., Ishii, S., Seino, Y., and Imura, H. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89,** 1045–1049
5. Hoeffler, J. P., Meyer, T. E., Yun, Y., Jameson, J. L., and Habener, J. F. (1988) *Science* **242,** 1430–1433
6. Gonzalez, G. A., Yamamoto, K. K., Fischer, W. H., Karr, K., Menzel, P., Biggs, W., III, Vale, W. W., and Montminy, M. R. (1989) *Nature* **337,** 749–752
7. Maekawa, T., Sakura, H., Kanei-Ishii, C., Sudo, T., Yoshimura, T., Fujisawa, J., Yoshida, M., and Ishii, S. (1989) *EMBO J.* **8,** 2023–2028
8. Hai, T., Liu, F., Coukos, W. J., and Green, M. R. (1989) *Genes Dev.* **3,** 2083–2090
9. Sassone-Corsi, P. (1994) *EMBO J.* **13,** 4717–4728
10. Foulkes, N. S., and Sassone-Corsi, P. (1992) *Cell* **68,** 411–414
11. Foulkes, N. S., Borrelli, E., and Sassone-Corsi, P. (1991) *Cell* **64,** 739–749
12. Laoide, B. M., Foulkes, N. S., Schlotter, F., and Sassone-Corsi, P. (1993) *EMBO J.* **12,** 1179–1191
13. Inada, A., Yamada, Y., Someya, Y., Kubota, A., Yasuda, K., Ihara, Y., Kagimoto, S., Kuroe, A., Tsuda, K., and Seino, Y. (1998) *Biochem. Biophys. Res. Commun.* **253,** 712–718
14. Gellersen, B., Kempf, R., and Telgmann, R. (1997) *Mol. Endocrinol.* **11,** 97–113
15. Stehle, J. H., Foulkes, N. S., Molina, C. A., Simonneaux, V., Pévet, P., Sassone-and Corsi, P. (1993) *Nature* **365,** 314–320
16. Molina, C. A., Foulkes, N. S., Lalli, E., and Sassone-Corsi, P. (1993) *Cell* **75,** 875–886
17. Foulkes, N. S., Mellström, B., Benusiglio, E., and Sassone-Corsi, P. (1992) *Nature* **355,** 80–84
18. Delmas, V., Van Der Hoon, F., Mellström, B., Jégou, B., and Sassone-Corsi, P. (1993) *Mol. Endocrinol.* **7,** 1502–1514
19. Foulkes, N. S., Borjigin, J., Snyder, S. H., and Sassone-Corsi, P. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93,** 14140–14145
20. Fitzgerald, L. R., Li, Z., Machida, C. A., Fishman, P. H., and Duman, R. S. (1996) *J. Neurochem.* **67,** 490–497
21. Monaco, L., Foulkes, N. S., and Sassone-Corsi, P. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92,** 10673–10677
22. Deadouets, C., Matesic, G., Molina, C. A., Foulkes, N. S., Sassone-Corsi, P., Brechot, C., and Sobczak-Thepot, J. (1995) *Mol. Cell. Biol.* **15,** 3301–3309
23. Chiang, C-M., and Roeder, R. G. (1995) *Science* **267,** 531–536
24. Tanese, N., Pugh, B. F., and Tjian, R. (1991) *Genes Dev.* **5,** 2212–2224
25. Tanese, N., Saluja, D., Vassallo, M., Chen, J-L., and Admon, A. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93,** 13611–13616
26. Tjian, R. (1995) *Sci. Am.* **272,** 54–61
27. Goodrich, J. A., Cutler, G., and Tjian, R. (1996) *Cell* **84,** 825–830
28. Burley, S. K., and Roeder, R. G. (1996) *Annu. Rev. Biol.* **65,** 769–799
29. Struhl, K. (1996) *Cell* **84,** 179–182
30. Hsu, S. M., Raine, N., and Fanger, H. (1981) *Am. J. Clin. Pathol.* **75,** 734–738
31. Gotoh, M., Maki, T., Kiyoizumi, T., Satomi, S., and Monaco, A. P. (1985) *Transplantation* **40,** 437–438
32. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J., and Rutter, W. J. (1979) *Biochemistry* **18,** 5294–5299
33. Oie, H. K., Gazdar, A. F., Minna, B., Wier, G. C., and Baylin, S. F. (1983) *Endocrinology* **112,** 1070–1075
34. Meyer, T. E., Habener, J. F. (1992) *Nucleic Acids Res.* **20,** 6106
35. Ashcroft, S. J. H., Hammonds, P., and Harrison, D. E. (1986) *Diabetologia* **29,** 727–733
36. Graham, F., and Van Der Eb, A. (1973) *Virology* **52,** 456–467
37. Yoshimura, T., Fujisawa, J., and Yoshida, M. (1990) *EMBO J.* **9,** 2537–2542
38. Peterson, M. G., Tanese, N., Pugh, B. F., and Tjian, R. (1990) *Science* **248,** 1625–1630
39. Tao, Y., Guermah, M., Martinez, E., Oelgeschläger, T., Hasegawa, S., Takada, R., Yamamoto, T., Horikoshi, M., and Roeder, R. G. (1997) *J. Biol. Chem.* **272,** 6714–6721
40. Klemm, R. D., Goodrich J. A., Zhou, S., and Tjian, R. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92,** 5788–5792
41. Nantel, F., Monaco, L., Foulkes, N. S., Masquilier, D., LeMeur, M., Henriksén, K., Dierich, A., Parvinen, M., and Sassone-Corsi, P. (1996) *Nature* **380,** 159–162
42. deGroot, R. P., Derua, R., Goris, J., and Sassone-Corsi, P. (1993) *Mol. Endocrinol.* **17,** 1495–1501
43. deGroot, R. P., den Hertog Vandenheede, J. R., Goris, J., and Sassone-Corsi, P. (1993) *EMBO J.* **12,** 3903–3911
44. Hammonds, P., Schofield, P. N., Ashcroft, S. J. H., Sutton, R., and Gray, D. W. R. (1987) *FEBS Lett.* **223,** 131–137
45. Hammonds, P., Schofield, P. N., and Ashcroft, S. J. H. (1987) *FEBS Lett.* **213,** 149–154
46. Waeber, G., and Habener, J. F. (1991) *Mol. Endocrinol.* **5,** 1431–1438
47. Hisatake, K., Ohta, T., Takada, R., Guermah, M., Horikoshi, M., Nakatani, Y., and Roeder, R. G. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92,** 8195–8199
48. Chen, J. L., Attardi, L. D., Verrijzer, C. P., Yokomori, K., and Tjian, R. (1994) *Cell* **79,** 93–105
49. Hoey, T., Weinzierl, R. O. J., Gill, G., Chen, J-L., Dynlacht, B. D., and Tjian, R. (1993) *Cell* **72,** 247–260
50. Ferreri, K., Gill, G., and Montminy, M., (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91,** 1210–1213
51. Nakajima, T., Uchida, C., Anderson, S. F., Parvin, J. D., and Montminy, M. (1997) *Genes Dev.* **11,** 738–747
52. Hisatake, K., Hasegawa, S., Takada, R., Nakatani, Y., Horikoshi, M., and Roeder, R. G. (1993) *Nature* **362,** 179–181

Downloaded from http://www.jbc.org/ by guest on October 4, 2017

The Cyclic AMP Response Element Modulator Family Regulates the Insulin Gene Transcription by Interacting with Transcription Factor IID

Akari Inada, Yoshimichi Someya, Yuichiro Yamada, Yu Ihara, Akira Kubota, Nobuhiro Ban, Rie Watanabe, Kinsuke Tsuda and Yutaka Seino

J. Biol. Chem. 1999, 274:21095-21103.
doi: 10.1074/jbc.274.30.21095

Access the most updated version of this article at http://www.jbc.org/content/274/30/21095

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 52 references, 16 of which can be accessed free at http://www.jbc.org/content/274/30/21095.full.html#ref-list-1

Downloaded from http://www.jbc.org/ by guest on October 4, 2017