Monoamine oxidases (MAO) A and B catalyze the oxidative deamination of many biogenic and dietary amines. Abnormal expression of MAO has been implicated in several psychiatric and neurodegenerative disorders. Human MAO B core promoter (−246 to −99 region) consists of CACCC element flanked by two clusters of overlapping Sp1 sites. Here, we show that cotransfection with transforming growth factor (TGF)-β-inducible early gene (TIEG2) increased MAO B gene expression at promoter, mRNA, protein, and catalytic activity levels in both SH-SY5Y and HepG2 cells. Mutation of the CACCC element increased the MAO B promoter activity, and cotransfection with TIEG2 further increased the promoter activity, suggesting that CACCC was a repressor element. This increase was reduced when the proximal Sp1 overlapping sites was mutated. Similar interactions were found with Sp3. These results showed that TIEG2 and Sp3 were repressors at the CACCC element but were activators at proximal Sp1 overlapping sites of MAO B. Gel-shift and chromatin immunoprecipitation assays showed that TIEG2 and Sp3 bound directly to CACCC element and the proximal Sp1 sites in both synthetic oligonucleotides and natural MAO B core promoter. TIEG2 had a higher affinity to Sp1 sites than CACCC element, whereas Sp3 had an equal affinity to both elements. Thus, TIEG2 was an activator, but Sp3 had no effect on MAO B gene expression. This study provides new insights into MAO B gene expression and illustrates the complexity of gene regulation.
mented with 10% (v/v) fetal bovine serum and antibiotics and grown at 25°C without CO₂. Monoclonal anti-TIEG2 antibody was purchased from Transduction Laboratories (BD Biosciences). Polyclonal antisera against Sp3 were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA).

Human MAO B Promoter-luciferase Reporter Constructs—The BamHI/BamHI MAO B promoter fragment (~2099 to ~99 bp) was cloned into the polylinker site (BglII) upstream of the luciferase gene (LUC) in the pGL2-basic vector (Promega, Madison, WI). Serial deletion constructs were generated by restriction enzyme digestion using the pGLB (~2099/~99LUC as a template, followed by Klenow fill-in and self-ligation. The following restriction enzymes were used to generate the deletion constructs: XhoI/ApaI (pGLB (~1313/~99)); XhoI/SpeI (pGLB (~1313/~99)); XhoI/AspI (pGLB (~868/~99)); XhoI/PstI (pGLB (~868/~99); XhoI/PstI (pGLB (~246/~99); and BglII/HindIII (pGLB (~2099/~261)). The restriction enzymes PstI and HindIII were used to select positive clones and to verify the correct orientation. One recombinant clone for each of the constructs was chosen, and the plasmid DNA was extracted and purified using the Qiagen Miniprep kit (Qiagen) following the manufacturer’s instructions.

Site-directed Mutagenesis of the Human MAO B Proximal Promoter (~246 to ~99 bp)—Site-directed mutagenesis was utilized to mutate

FIG. 1. TIEG2 increases the human MAO B promoter activity. A series of the 5’-deletion constructs of human MAO B promoter fused with pGL2-basic luciferase vector (1 µg) were cotransfected into SH-SY5Y or SL2 cells with TIEG2 expression vector (0.5 µg) together with 20 ng of pRL-TK (Renilla luciferase expression vector, for normalization of transfection efficiencies). Control group was cotransfected with pcDNA3.1 vector. After 48 h, cells were harvested and then assayed for luciferase activity. Data were the mean ± S.D. from three independent experiments, with triplicates for each experiment. Note that the TIEG2 response element was located within the core promoter region (~246 to ~99 bp) of the human MAO B. The same result was obtained in HepG2 cells. The A in the initiation site ATG is defined as +1.
TIEG2 and Sp3 Acted on CACCC and Sp1 Sites of MAO B Promoter

Potential transcription element, CACCC box, and two clusters of Sp1 sites in both the core promoter region (–246 to –99) and MAO B 2-kb promoter (–2099 to –99). Mutant promoter constructs were generated using pGLB (–246) construct or pGLB (–2099) as a template. Mutagenesis was carried out using the Amersham Mutagenesis kit (Amersham Biosciences) following the manufacturer’s instructions. The primers used for mutagenesis (with mutations in lower case) were 5′-GGCGGCTGGCCTGGGCTGCG-3′ (for CACCC element), 5′-GGCTGAGAGCTGGTGTTGGGCTGGCGGCTGGGTTGGGCGGG-3′ (for CACCC element), 5′-GGCTGCGAGAGCTGTTGGGCTGGCGGCGGCTGGC-3′ (for distal Sp1 sites), and 5′-GGCTGCGAGAGCTGTTGGGCTGGCGGCGGCTGGCGGCGGCG-3′ (for proximal Sp1 sites). The mutated nucleotide sequences of all mutant constructs were confirmed by DNA sequencing.

**Transient Transfection and Luciferase Assay**—Transfections in SH-SYSY, SL2, and HepG2 cells were performed using Superfect transfection reagent (Qiagen) following the manufacturer’s instructions. Exponentially growing cells were plated at a density of 5 × 10^5 cells/well in six-well plates (Costar, Cambridge, MA) with 2 ml of medium and 10% fetal bovine serum; cells were grown until 50% confluence (24–36 h). For promoter deletion and mutagenesis studies, 1 µg of MAO B promoter-luciferase construct was co-transfected into the cells with 20 ng of plasmid pRL-TK (the herpes simplex virus thymidine kinase promoter fused upstream to the Renilla luciferase gene which is used as an internal control; Promega), as described previously (16). Full-length human TIEG2 expression vector was a gift from Dr. Raul Urrutia (20), Mayo Clinic, Rochester, MN. The expression plasmid pCMV-Sp3 was kindly provided by Dr. P. Charnay. For co-transfection experiments, the total amount of DNA for each transfection was kept constant by the addition of empty vector pCMV3.1.

**Gel-shift Assays**—The DNA fragments (CACCC element, 5′-TAGGGCCGGGCCGGGCTGGG-3′, and the proximal Sp1 sites, 5′-CGCTGCGACCAGCCGCGGTGGG-3′) for the gel-shift assay were radio-labeled by Klenow fill-in. Labeled probes were purified by gel electrophoresis (5% polyacrylamide) and eluted in TE buffer (10 mM Tris/1 mM EDTA, pH 8.0). For DNA-protein binding, 15 µg of nuclear extracts were diluted in binding buffer (40 mM HEPES, pH 8.0, 50 mM KCl, 1 mM dithiothreitol, 0.1 mM EDTA, 10% glycerol, 10 µg/ml of poly(dI-dC); Sigma) with a total volume of 20 µl. Antibody against TIEG2 or Sp3 was added (when required), and the mixture was incubated for 20 min at room temperature. Labeled probe (0.2 ng) was added to the mixture and incubated for an additional 20 min at room temperature. The binding affinity of TIEG2 or Sp3 to the CACCC element or Sp1 sites was evaluated by electrophoretic mobility-shift assay using increasing concentrations of radiolabeled CACCC element or Sp1 sites, followed by quantification of bound and free oligonucleotide (21). For super-shift assays, 1 µl of antibody was included in the reaction mix along with the extract. The samples were then run on a 5% non-denaturing polyacrylamide gel in 1× Tris/borate/EDTA at 150 V for 3 h. Gels were dried and visualized by autoradiography.

**MAO B Catalytic Activity Assay**—SH-SYSY and HepG2 cells were grown to confluence, harvested, and washed with phosphate-buffered saline. One hundred micromolars of total proteins were incubated with 10 µM 14C-labeled PEA (Amersham Biosciences) in the assay buffer (50 mM sodium phosphate buffer, pH 7.4) at 37 °C for 20 min and terminated by the addition of 100 µl of 6 N HCl. The reaction products were then extracted with ethyl acetate:toluene (1:1) and centrifuged at 4 °C for 10 min. The organic phase containing the reaction product was extracted, and its radioactivity was obtained by liquid scintillation spectrometry (22).

**Northern Blot Analysis of MAO B mRNA**—Total RNA was purified using TRIzol reagent (Invitrogen). Thirty micrograms of total RNA from
SH-SY5Y and HepG2 cells that were cotransfected with TIEG2 expression vector (0, 100, and 400 ng) and grown to confluence were loaded onto each gel lane. Electrophoresis, transfer onto BrightStar nylon membrane, and hybridization were carried out using NorthernMax according to the manufacturer's protocol (Ambion). The human MAO B probe (specific activity = 2 × 10^6 cpm/µg) was labeled by random-primer technique using the Multiprime kit (Amersham Biosciences) following the manufacturer's instructions. Membrane hybridized with the MAO B probe was autoradiographed for 48 h.

Western Blot Analysis of MAO B Protein—Cells were harvested and washed with phosphate-buffered saline. The protein concentration was determined by the Bradford protein assay (Bio-Rad). Forty micrograms of total protein was separated by SDS-10% polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. After the transfer, membranes were blocked at room temperature for 2 h with 5% bovine serum albumin in TTBS (10 mM Tris/HCl, pH 7.5, 150 mM NaCl, and 0.05% Tween 20). The membranes were then incubated with rabbit anti-MAO B antibody (1:1000) overnight at room temperature. After incubation with goat anti-rabbit IgG conjugated with horseradish peroxidase secondary antibody at room temperature for 2 h, the bands were visualized by horseradish peroxidase reaction using diaminobenzidine as substrate (Sigma).

Chromatin Immunoprecipitation (ChIP) Assays and Quantitative Real-time PCR—SH-SY5Y or HepG2 cells (7 × 10^6 cells/150-mm dish) were plated and grown in Dulbecco's modified Eagle's medium as described above for 2 days. Cells were cross-linked by incubating with formaldehyde (1% final concentration) at room temperature for 10 min. The dishes were rinsed twice with ice-cold PBS. Cells were then scraped in formaldehyde (1% final concentration) at room temperature for 10 min. The resulting cell lysates were prepared for immunoprecipitation after the methods described by Jia et al. (23). 35 mg of the supernatant was saved as input DNA. The nuclear protein-DNA complex was immunoprecipitated by incubating with anti-TIEG2 (with BioMag goat anti-mouse) or anti-Sp3 antibody (with BioMag anti-rabbit) overnight at 4 °C with rotation. They were recovered from the beads with an elution buffer (1% SDS and 0.1 M NaHCO3) and were analyzed by real-time PCR using an iCycler optical system (Bio-Rad). The PCR products were determined by SyBr Green reagent (2 × SyBr Green Supermix, Bio-Rad) following the manufacturer's instructions. The primers for MAO B core promoter (153 bp, from −287 bp to −134 bp) and irrelevant locus (160 bp, from −1940 to −1780 for negative control) were, for D5 forward, 5′-CTCTGCCAGGCAGCAGCCGCTCCG (bases −287 to −263); for D5 reverse, 5′-TGCGGGACGCCCTATAATTGCCC (bases −157 to −134); for 5′ irreverent locus forward, 5′-TTTGCTGCTCAGGCACCT- TATA-3′ (bases −1940 to −1717); and for 5′ irreverent locus reverse, 5′-ATGAAAGGAGGATCTGCTACG (bases −1802 to −1780).

The PCR reactions for input, TIEG2, Sp3-associated DNA, or 5′ irreverent locus were done in triplicate under the following conditions: 95 °C for 3 min, followed by 35 cycles of PCR consisting of 30 s at 94 °C, 30 s at 55 °C, and 30 s at 72 °C. The average threshold cycle (Ct) for the triplicate was used in all subsequent calculations. A ∆Ct value was calculated for each sample using the Ct value for the input DNA samples to normalize the CHIP assay results, as outlined in the iCycler optical systems protocol. The ∆Ct were converted to the fold induction required to reach the threshold amount of PCR by raising 2 to the ∆Ct power. The relative differences between input sample and TIEG2 or Sp3 ChIP assay or negative control were determined using the ∆Ct method and presented as the percent of input (taken as 100%). Data were the means ± S.D. from triplicate samples of three independent experiments.

RESULTS

TIEG2 Was a Transcriptional Activator of the Human MAO B Promoter—To understand the function of TIEG2 in the human MAO B promoter and to identify the promoter regions which were responsible for TIEG2 function, the 5′-flanking sequence of MAO B promoter was systematically deleted, and each one was ligated to the luciferase reporter gene (pGL2-basic) and transfected into SH-SY5Y, SL2, and HepG2 cells. Full-length human TIEG2 expression vector was then cotransfected with each MAO B promoter-luciferase construct. As shown in Fig. 1 in SH-SY5Y cells, co-expression of TIEG2 increased the MAO B promoter activity up to 4-fold, when the deletion was up to −246 bp. However, the TIEG2 activation of MAO B promoter activity was abolished (Fig. 1, construct 5) when the −261 to −99 bp region was deleted. Thus, the TIEG2 response element was located in −246 to −99 bp, the core promoter region of MAO B (Fig. 1, constructs 1-4). Similar results were found in HepG2 cells (data not shown) and SL2 cells, which lack the Sp1 family (Fig. 1). Because the SL2 cell
TIEG2 and Sp3 Acted on CACCC and Sp1 Sites of MAO B Promoter

**A.**

![Translation start site](image)

-2099 -1940 -1780

5'-irrelevant locus

MAO B core promoter

**B.**

![PCR Base Line Subtracted CF RU](image)

Cycle

PCR Base Line Subtracted CF RU

5'-irrelevant locus

Input

TIEG2

**C.**

![Percentage Input](image)

5'-irrelevant locus

MAO B core promoter

Anti-TIEG2

Anti-Sp3

SH-SY5Y

HepG2


---

**Fig. 5.** TIEG2 and Sp3 bound to natural core promoter of MAO B directly. The occupation of TIEG2 and Sp3 on the MAO B core promoter or 5'-irrelevant region of MAO B was determined by ChIP assay combined with quantitative real-time PCR using SH-SY5Y or HepG2 cells. A, a schematic representation of the MAO B promoter. Real-time PCR-targeted regions containing core promoter (from −287 bp to −134 bp) and 5'-irrelevant locus (from −1940 to −1780 bp for negative control) were indicated. B, representative TIEG2 ChIP quantitative real-time PCR amplification plots (triplicates). The ChIP/quantitative real-time PCR amplification was performed for cross-linked inputs, TIEG2-associated MAO B core promoter (TIEG2), or 5'-irrelevant locus in SH-SY5Y cells. Nuclear protein-DNA complex was immunoprecipitated by anti-TIEG2 antibody and was quantitatively analyzed by real-time PCR. Cell lysates without immunoprecipitation were used as input sample (positive control). The x-axis represents the relative amount of PCR product determined by the change in the emission intensity of the reporter dye divided by the emission intensity of a passive reference dye after subtraction of the base line. The y-axis represents the relative amount of PCR product determined by the change in the emission intensity of the reporter dye divided by the emission intensity of a passive reference dye after subtraction of the base line. C, association of TIEG2 or Sp3 with MAO B core promoter or 5'-irrelevant locus analyzed in both SH-SY5Y and HepG2 cells. The relative differences between input sample and TIEG2 or Sp3 ChIP assay or negative control were determined by using the ΔCt method (see “Materials and Methods”). These values were presented as percent input in which a DNA cross-link input sample was taken as 100%. Data were the mean ± S.D. from triplicate samples of three independent experiments.

---

**TIEG2 and Sp3 Acted on CACCC and Sp1 Sites of MAO B Promoter**

The CACCC element and Sp1 sites in MAO B core promoter were mutated. The wild type or mutated constructs were cotransfected with TIEG2 into SH-SY5Y (Fig. 3A), HepG2 (data not shown), or SL2 cells, which lack Sp1 family (Fig. 3B). The result showed that TIEG2 activated the MAO B promoter (Fig. 3, A and B, control 1, TIEG2 versus basal) in both cell lines. Because Sp3 was found to repress the Sp1 activation on MAO B gene (16), we investigated whether Sp3 regulated MAO B gene expression by means of the CACCC element or Sp1 sites. Sp3 itself did not have an effect upon the MAO B promoter activity (Fig. 3, A and B, construct 1, Sp3 versus basal), which is consistent with our previous finding (16).

When the CACCC element was mutated into CttCC in the MAO B core promoter (−246 to −99 bp; Fig. 3, A and B, construct 2), the basal promoter activity was increased by 8-fold compared with the wild type in SH-SY5Y cell line (Fig. 3A, compare basal activity in construct 2 versus construct 1). This result suggested that the CACCC element was a repressor element. The mutation of the CACCC element destroyed the binding of TIEG2 and Sp3 to CACCC element by gel-shift assay (data not shown); thus, the increased basal MAO B promoter activity may be mediated by another cis-element, such as Sp1 element. In contrast to SH-SY5Y cell line, when CACCC was mutated, the basal promoter activity in the SL2 cell line (no Sp1 family) was not increased, compared with the wild type (Fig. 3B, construct 2 basal versus construct 1 basal). This result suggested that the Sp1 family and Sp1 sites were responsible for the increased promoter activity seen in CACCC mutated promoter construct.

Cotransfecting TIEG2 or Sp3 with CACCC-mutated MAO B core promoter further activated the luciferase activity in SH-SY5Y (Fig. 3A, construct 2, compare TIEG2 or Sp3 to basal promoter activity), suggesting that TIEG2 and Sp3 were repressors at CACCC element, and the activation function of these factors might occur by means of Sp1 sites. When the CACCC-mutated construct was cotransfected with TIEG2 or
Sp3 in SL2 cells, similar activation of MAO B promoter (Fig. 3B, construct 2, compare TIEG2 or Sp3 to basal) was observed.

To confirm that TIEG2 or Sp3 repressed MAO B promoter activity through CACCC element, the Sp1 sites were mutated on MAO B core promoter (Fig. 3, C and D) to further study the repressor function of CACCC element. Cotransfecting TIEG2 or Sp3 with proximal Sp1 site-mutated MAO B core promoter, the luciferase activity was reduced slightly in both cell lines (Fig. 3, C and D, compare TIEG2 or Sp3 to basal). Cotransfecting TIEG2 or Sp3 with both Sp1 sites mutated MAO B core promoter, the luciferase activity was decreased significantly in both cell lines (Fig. 3, C and D, construct 4, compare TIEG2 or Sp3 to basal). These results provided further evidence that the CACCC element was a repressor element for both TIEG2 and Sp3.

**TIEG2 and Sp3 Are Activators at the Proximal Sp1 Overlapping Sites of MAO B Promoter**—When both CACCC element and distal overlapping Sp1 sites were mutated, the function of TIEG2 and Sp3 were reduced slightly in both cell lines (Fig. 3, A and B, construct 3 versus construct 2), suggesting that TIEG2 and Sp3 bind weakly to the distal Sp1 sites. However the mutation of the proximal overlapping Sp1 sites together with CACCC element occurred, the activation of TIEG2 and Sp3 were blocked (Fig. 3, A and B, construct 4 versus construct 2) in both cell lines, suggesting that the proximal Sp1 sites are important for TIEG2 and Sp3 activation.

To confirm that the proximal Sp1 sites are responsible for TIEG2 and Sp3 activation, either distal Sp1 sites or proximal Sp1 sites were mutated in MAO B core promoter. Mutation of the distal Sp1 sites had slight effect on TIEG2 activation (Fig. 3, C and D, compare construct 2 to construct 1, TIEG2 activation) in SH-SY5Y and SL2 cells. On the other hand, the mutation of the proximal Sp1 sites abolished the activation of TIEG2 on MAO B promoter (Fig. 3, C and D, compare construct 3 to construct 1, TIEG2 cotransfected) in both cell lines, indicating that the proximal Sp1 sites are important for the activation of MAO B gene expression.

The effects of TIEG2 and Sp3 on the human MAO B 2-kb promoter were also studied by transient transfection assay using different deletion mutants of MAO B 2-kb promoter. The effects of TIEG2 and Sp3 on the MAO B 2-kb promoter (data not shown) are similar to the effect on −246 to −99 bp MAO B core promoter (Fig. 3).

All of the transient transfection experiments in SH-SY5Y cell line described above were also performed in HepG2 cells. The results were the same as SH-SY5Y cells (data not shown).

**TIEG2 and Sp3 Interacted with CACCC Directly, as Shown by Gel-shift Assay**—To confirm the presence of TIEG2 and Sp3 in the binding to MAO B core promoter region, a gel-shift assay was performed using a 23-bp DNA fragment spanning the region −222 to −201 containing the CACCC element. The radio-labeled DNA probe was incubated with nuclear extracts isolated from SH-SY5Y and HepG2 cells. Two to three major DNA-protein complexes were observed in both cell lines (Fig. 4A, lane 2 and 6). Excess of cold oligonucleotides reduced all bindings (Fig. 4A, lane 3 and 7), suggesting that these DNA-protein complexes were specific. The lower complex was completely supershifted by incubation with anti-TIEG2 antibody (Fig. 4A, lane 4 and 8), suggesting that this DNA-protein complex contained TIEG2. The upper complex was completely supershifted by incubating with anti-Sp3 antibody (Fig. 4A, lane 5 and 9), suggesting that the upper DNA-protein complex contained Sp3. These results indicated that transcriptional factors TIEG2 and Sp3 bound to CACCC element in both SH-SY5Y and HepG2 cells.

To ensure that TIEG2 and Sp3 were indeed bound to CACCC element, the CACCC element in the 23-bp DNA fragment was
mutated to CttCC and radiolabeled for gel-shift assay. DNA-protein complex was not found in either cell line (data not shown).

TIEG2 and Sp3 Bound to the Natural Core Promoter of MAO B—To study whether these transcription factors interacted with the natural human MAO B promoter in vivo, the occupancy of endogenous TIEG2 and Sp3 to the native core promoter of MAO B was determined by ChIP assay combined with quantitative real-time PCR. The core promoter sequence and 5′-irrelevant region (negative control) were used for occupancy analysis (Fig. 5A). The representative TIEG2 ChIP/quantitative real-time PCR amplification plots (triplicate) were shown in Fig. 5B. The average Ct value for the input sample, TIEG2 cross-linked DNA and 5′-irrelevant region, were 17.02 ± 1.02, 22.84 ± 0.69, and 29.14 ± 0.92, respectively, in SH-SY5Y cells (Fig. 5B). A ΔCt value was calculated for each sample using the Ct value for the input DNA sample by subtracting the Ct value of input control and then converted to fold differences (see "Materials and Methods"). The relative differences between input sample and TIEG2 or Sp3 ChIP assay or negative control were summarized in Fig. 5C and presented as the percent of input (which was taken as 100%). The relative differences of TIEG2- or Sp3-conjugated DNA was 1.68 or 1.44%, respectively, compared with the input DNA (Fig. 5C) for SH-SY5Y cells, and 1.51 or 1.25% for HepG2 cells (Fig. 5C). Quantitative PCR analysis of 5′-irrelevant region yielded negligible values (less than 0.03% input DNA, Fig. 5C). This result indicated that anti-TIEG2 or anti-Sp3 antibody is able to immunoprecipitate the native TIEG2-DNA or Sp3-DNA complex and thus demonstrated that TIEG2 and Sp3 indeed bound to the natural core promoter of MAO B.

The Binding Affinity of TIEG2 for the Proximal Sp1 Sites Was Higher than CACCC Element, but the Affinity of Sp3 Was Similar between the Proximal Sp1 Sites and CACCC Element—To understand why TIEG2 activated overall MAO B gene expression but Sp3 had no effect on gene expression (Fig. 3A, construct 1), the affinity of TIEG2 for CACCC element and the proximal Sp1 sites was evaluated by gel-shift experiments (Fig. 6). TIEG2 protein was ectopically expressed in SL2 cells. The binding affinity was evaluated by using increasing concentrations of radiolabeled Sp1 sites or CACCC DNA probe containing the proximal Sp1 sites, CACCC element was determined by Scatchard analysis. A Scatchard plot is displayed together with the Kd values calculated from three independent experiments.

**Fig. 7.** Binding affinity of Sp3 factor to CACCC and proximal Sp1 sites in the human MAO B core promoter. Nuclear extracts from Sp3-over-expressed SL2 cells were incubated with increasing concentrations of 32P-labeled 23-bp DNA probe containing CACCC element (−221 bp to −201 bp) (A) or 32P-labeled 25-bp DNA probe containing the proximal Sp1 sites of MAO B (−198 bp to 177 bp) (B). Free and bound probes were resolved by non-denaturing PAGE and visualized by autoradiography. Arrows, positions of Sp3 protein-DNA complexes. C, bound and free radiolabeled Sp1 sites and CACCC element in panels A and B were quantitated by phosphorimager analysis, and the Kd of Sp3 binding to radiolabeled Sp1 sites and CACCC element was determined by Scatchard analysis. A Scatchard plot is displayed together with the Kd values calculated from three independent experiments.
The same affinity analysis was performed using Sp3 protein, which was ectopically expressed in SL2 cells. As shown in Fig. 7, the binding affinity of Sp3 to the CACC element (Fig. 7A, \( K_d = 0.32 \times 10^{-9} \) M) and proximal Sp1 sites (Fig. 7B, \( K_d = 0.30 \times 10^{-8} \) M) were similar (Fig. 7C).

**DISCUSSION**

Our Results Show that TIEG2 Was an Activator for the Human MAO B Gene, although It Was a Repressor for Most Other Genes—TIEG2, also called FKLF (26), is ubiquitously expressed in human tissues, with enrichment in pancreas and muscle. TIEG2 shares 91% homology with TIEG1 within the zinc finger region and 44% homology in the N terminus. Based upon the primary structure, TIEG2 is a nuclear protein which can specifically bind to an Sp1-like DNA sequence and can repress promoters containing Sp1-like binding sites in transfected Chinese hamster ovary epithelial cells (20). Previous study indicated that three independent transcriptional repression domains were found within TIEG proteins. Interestingly, in contrast to several other members of the Sp1-like family, no transcriptional activation domain was found in this transcription factor so far (28). Therefore, TIEG2 is a repressor at Sp1 sites for most of the genes (18). Only one exception showed that TIEG2 activated promoter mediated by CACC element, which is the human embryonic gamma globulin (29). However, the molecular mechanism of activation function of TIEG2 (FKLF) is unknown. The present study is the first time that TIEG2 has been found to activate human MAO B gene expression through Sp1 overlapping sites. TIEG2 is a TGF-\( \beta \)-inducible early response gene (18). TGF-\( \beta \)-mediated signaling pathways inhibit epithelial cell growth (30). Because TIEG2 activated MAO B, this may suggest that MAO B might have anti-proliferating functions. On the other hand, TIEG2 was found to be a repressor at CACC element of MAO B promoter, although the overall function of TIEG2 is as an activator for MAO B. This is the first study to show that TIEG2 is a bi-functional regulator for the same gene expression.

**Sp1-like Proteins Play Key Roles in the Regulation of MAO B**—Our results indicate that Sp1-like proteins play important roles in the regulation of MAO B gene expression, because the mutation of CACC element resulted in a dramatic increase in the basal MAO B promoter activity in SH-SY5Y cells (Fig. 3A, construct 2 basal versus construct 1 basal), but not in SL2 cells. This result suggests that the increase in SH-SY5Y cells was due to the mutated CACC element and the Sp1 protein-activated Sp1 sites, because no increase was observed when there were no Sp1 proteins in SL2 cells.

**Sp3 Is a Bi-functional Regulator for the Same Gene Expression**—This is the first study to demonstrate that Sp3 is a bi-functional regulator for the same gene. Sp3 is a ubiquitously expressed transcription factor, closely related to Sp1 but, unlike Sp1, often functioning as a transcriptional repressor by means of interacting with GC-rich Sp1-like sites (31, 32). It has also been shown that Sp3 acts as a bi-functional regulator whose activating or repressing activity is dependent upon both the promoter and the cellular context (27, 33). Sp3 contains independent modular repressor and activator domains. The activation potential of Sp3 is distributed over an extensive glutamine-rich N-terminal region. The negative regulatory function has been mapped to 5’ of the zinc finger region. Sp3 was found to repress transcription of promoters bearing multiple GAL4 DNA-binding sites, whereas it could activate reporters containing a single GAL4-binding site (33). However, it has not been reported that Sp3 functions as both repressor and activator in the same gene regulation. Our study is the first time to provide the evidence that repressor Sp3 for MAO B also acts as an activator via overlapping Sp1 sites, which displayed the bi-functional regulator in the same gene expression.

In summary, our results indicate that TIEG2 increased the human MAO B gene expression. Both TIEG2 and Sp3 exhibited a repressor function at CACC element, but both also serve as an activator by interacting with the proximal Sp1 overlapping sites in SH-SY5Y, HepG2, and SL2 cells. This work provides the first evidence that TIEG2 activates MAO B gene through the proximal Sp1 overlapping sites. This is also the first study to show that TIEG2 and Sp3 are bi-functional regulators for the same gene. These findings may provide new insight into the regulation of not only the human MAO B genes but also to the genes bearing CACC elements and Sp1 sites.

**Acknowledgments**—We thank Dr. Raul Urrutia for TIEG2 vector, Dr. F. Charney for pCMV-Sp3 plasmid, and Dr. Wai Wong for some of the MAO B promoter constructs.

**REFERENCES**

1. Johnston, J. P. (1968) Biochem. Pharmacol. 17, 1285–1297
2. Knoll, J., and Magyar, K. (1972) Adv. Biochem. Psychopharmacol. 8, 383–408
3. Shih, J. C., Chen, K., and Ried, M. J. (1989) Ann. Rev. Neurosci. 12, 197–217
4. Weyler, W., and Salach, J. I. (1985) J. Biol. Chem. 260, 13199–13207
5. Bond, P. A., and Cundall, R. L. (1977) Clin. Chim. Acta 80, 317–326
6. Donnelly, C. H., and Murphy, D. L. (1977) Biochem. Pharmacol. 26, 853–858
7. Fowler, J. S., MacGregor, G. R., Wolf, A. P., Arnett, C. D., Dewey, S. L., Schlyer, D., Christman, D., Logan, J., Smith, M., and Sachs, H. (1987) Science 235, 481–485
8. Thorpe, L. W., Westlund, K. N., Kochergerger, L. M., Abell, C. W., Denney, R. M., Thorpe, E., and Ford, E. J. (1987) J. Histochem. Cytochem. 35, 23–22
9. Ariy, Y., and Kinemuchi, H. (1988) J. Neurotransm. 79, 99–105
10. Bach, A. W., Lan, N. C., Johnson, D. L., Abell, C. W., Bembenek, M. E., Kwan, S. W., Seeburg, P. H., and Shih, J. C. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 4934–4938
11. Lan, N. C., Heinzmann, C., Gal, A., Khisak, I. Orth, U., Lai, E., Grimson, J., Sparkes, R. S., Mohandas, T., and Shih, J. C. (1989) Genomics 4, 552–559
12. Grimson, J., Chen, K., Wang, L. J., Lan, N. C., and Shih, J. C. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 3657–3661
13. Zhu, Q. S., Chen, K., and Shih, J. C. (1994) J. Neurosci. 14, 7393–7403
14. Sabel, S. H., Zs, H., and Hamer, D. (1998) Hum. Genet. 101, 273–279
15. Zhu, Q. S., Grimson, J., Chen, K., and Shih, J. C. (1992) J. Neurosci. 12, 4437–4446
16. Wong, W. K., Chen, K., and Shih, J. C. (2001) Mol. Pharmacol. 59, 852–859
17. Tachibana, I., Imoto, M., Adji, P. N., Gores, G. J., Subramanium, M., Spelsberg, T. C., and Urrutia, R. (1997) J. Clin. Invest. 99, 2365–2374
18. Cook, T., Gebelein, B., Belal, M., Mesa, K., and Urrutia, R. (1999) J. Biol. Chem. 274, 29505–29504
19. Zhang, S. J., Moncrieffe, M. C., Kaczynski, J., Ellerieder, V., Prendergast, F. G., and Urrutia, R. (2001) Mol. Cell. Biol. 21, 5041–5049
20. Cook, T., Gebelein, B., Mesa, K., and Urrutia, R. (1998) J. Biol. Chem. 273, 29292–29296
Dual Functions of Transcription Factors, Transforming Growth Factor-β-inducible Early Gene (TIEG)2 and Sp3, Are Mediated by CACCC Element and Sp1 Sites of Human Monoamine Oxidase (MAO) B Gene

Xiao-Ming Ou, Kevin Chen and Jean C. Shih

J. Biol. Chem. 2004, 279:21021-21028.
doi: 10.1074/jbc.M312638200 originally published online March 15, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M312638200

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 33 references, 18 of which can be accessed free at http://www.jbc.org/content/279/20/21021.full.html#ref-list-1