Monoamine oxidase catalyzes the oxidative deamination of a number of neurotransmitters. A deficiency in monoamine oxidase A results in aggressive behavior in both humans and mice. Studies on the regulation of monoamine oxidase A gene expression have shown that the Sp1 family is important for monoamine oxidase A expression. To search for novel transcription factors, the sequences of three Sp1 sites in the monoamine oxidase A core promoter were used in the yeast one-hybrid system to screen a human cDNA library. A novel repressor, R1 (RAM2), has been cloned. The R1 cDNA encodes a protein with 454 amino acids and an open reading frame at the 5'-end. The transfection of R1 in a human neuroblastoma cell line, SK-N-BE (2)-C, inhibited the monoamine oxidase A promoter and enzymatic activity. The degree of inhibition of monoamine oxidase A by R1 correlated with the level of R1 protein expression. R1 was also found to repress monoamine oxidase A promoter activity within a natural chromatin environment. A gel-shift assay indicated that the endogenous R1 protein in SK-N-BE (2)-C cells interacted with the R1 binding sequence. R1 also bound directly to the natural monoamine oxidase A promoter in vivo as shown by chromatin immunoprecipitation assay. Immunochemical analysis showed that R1 was expressed in both cytosol and nucleus, which suggested a role for R1 in transcriptional regulation. Northern blot analysis revealed the presence of endogenous R1 mRNA in human brain and peripheral tissues. Taken together, this study shows that R1 is a novel repressor that inhibits monoamine oxidase A gene expression.

Mao1, a Novel Repressor of the Human Monoamine Oxidase A*

Kevin Chen‡, Xiao-Ming Ou‡, Gao Chen‡, Si Ho Choi‡, and Jean C. Shih§§

From the ‡Department of Molecular Pharmacology and Toxicology, School of Pharmacy and the §Department of Cell and Neurobiology, Keck School of Medicine, University of Southern California, Los Angeles, California 90033

Received for publication, September 1, 2004, and in revised form, January 6, 2005

Published, JBC Papers in Press, January 14, 2005, DOI 10.1074/jbc.M410033200

Monoamine oxidase (MAO) catalyzes the oxidative deamination of a number of biogenic and dietary amines in brain and peripheral tissues and produces hydrogen peroxide (1, 2). Two forms of MAO have been defined, MAO A (3) and MAO B (4). MAO A has higher affinity for the substrates serotonin and norepinephrine, whereas MAO B has higher affinity for phenylethylamine and benzylamine. MAO A and B are encoded by different genes (5); however both consist of 15 exons with identical exon-intron organization (6). They are closely linked, tail to tail, located on the X-chromosome (7). MAO A or B gene knock-out mice exhibit different alterations in neurotransmitter levels and behavior (8). Mice deficient in MAO A have shown elevated amounts of brain serotonin, norepinephrine, and dopamine (9), no change in phenylethylamine (8), and display a distinct behavioral syndrome that includes enhanced aggression in males (10). This is consistent with the impulsive aggression reported in men from a Dutch family with a MAO A deficiency (11). Mice deficient in MAO B show elevated levels of phenylethylamine but no changes in serotonin, norepinephrine, or dopamine. Phenylethylamine is implicated in modulating mood and affect (12). In addition, mice lacking MAO B are resistant to the neurodegenerative effects of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), a toxin that induces a Parkinson's disease-like condition (12). MAO A and B double knock-out mice display reduced body weight, with increases in anxiety and brain levels of serotonin, norepinephrine, dopamine, and phenylethylamine (13). The increased amounts of all four monoamines in MAO A/B double knock-out mice are significantly higher than in either MAO A or B single knock-out mice (13). MAO A/B double knock-out mice also show increased baroreceptor response (14) and abnormal heart rate dynamics (15). These studies suggest that MAO A and B regulate many important biochemical functions and behaviors. The identification of potential factors that regulate MAO A and B gene expression has great significance.

The core promoter region of both human MAO A (16, 17) and B (18) consists of clusters of Sp1 sites. The human MAO B promoter contains two clusters of overlapping Sp1 sites separated by a CACCC element (19). Sp1 and Sp4 activate the MAO B core promoter via Sp1 overlapping sites, and its activation is repressed by Sp3. A Sp1-like transcription factor, TIEG2 (20), also activates MAO B promoter via Sp1 overlapping sites, although it represses the promoter activity via CACCC element (21).

The human MAO A core promoter region (−303 to −64 bp) contains four imperfect tandem repeats, each containing a Sp1 binding site in reverse orientation (17). A positive correlation has been found among cellular Sp1 concentration and MAO A promoter and catalytic activity (17), which indicates that Sp1 is an activator of MAO A gene expression. However other controlling factors involved in the regulation of the human MAO A gene are unknown. In this study, the Sp1 motif in the MAO A core promoter was used as bait, and a novel repressor R1 of the human MAO A was cloned and characterized.

MATERIALS AND METHODS

Yeast One-hybrid Screening—Because the human MAO A promoter contains a cluster of Sp1 motifs, a probe with three copies of Sp1 sequence 5′-cag gac gcc cag CCC CCG CCC GGC tcc cgg cag-3′ was used as bait in the yeast one hybrid system to search for additional transcription factors. The Matchmaker One-Hybrid System (Clontech) was used to screen the human cDNA library for protein-binding cDNA clones. After screening 105 colonies, four independent clones containing 1.9-kb inserts were found (GenBankTM BC009352, RAM2), which was named R1.
A Novel Repressor of MAO A

Plasmids—The coding region of R1 was amplified by PCR and cloned downstream to an expression vector driven by a CMV promoter (pcDNA3.1, KpnI/ApaI) for generating the R1-expression vector (pcDNA3.1-R1), which was used in stable cell line selection and transient transfection assay. The MAO A promoter-luciferase constructs were obtained as follows. The MAO A 2-kb promoter (Mlu I/Hind III) (−2072/−64 bp) was cloned into the polylinker site (Mlu I/Hind III) upstream of the luciferase gene in the pGL2-Basic vector (Promega) for generating the MAO A 2-kb-luc construct. The Sp1 sites deleted MAO A 2-kb-luc or the core promoter of MAO A (64 bp, 0.24-kb)-luciferase construct (MAO A 0.24-kb-luc) were generated by PCR, and the PCR product was subcloned into Zero Blunt TOPO vector (PCR cloning kit, Invitrogen) for verifying DNA sequencing. Subsequently, the PCR product was subcloned into pGL2-Basic luciferase reporter vector by self-ligation. All products were isolated into separate dishes after 6 days and cultured under continuous G418 selection. For generating MAO A 2-kb-luc stable cell lines, R1-expression vector or pcDNA3.1 vector were transiently transfected into SK-N-BE (2)-C cells. After 24 h, cells were replated into 5-cm dishes, and Geneticin (G418, 600 μg/ml) was added. Resistant clones were obtained by liquid scintillation spectroscopy (22).

MOA A Catalytic Activity Assay—SK-N-BE (2)-C cells stably expressing R1 protein or pcDNA3.1 empty vector (control group) was used for determining MAO A catalytic activity. One-hundred micrograms of total protein from ~1 × 10^6 cells were incubated with 100 μM [14C]serotonin in the assay buffer (50 mM sodium phosphate buffer, pH 7.4) at 37 °C for 20 min, and the reaction was terminated by the addition of 100 μl of HCl. The reaction products were then extracted with benzene/ethyl acetate and centrifuged at 4 °C for 10 min. The organic phase containing the reaction product was extracted, and its radioactivity was determined by liquid scintillation spectroscopy (22). For co-transfection experiments with pcDNA3.1-R1, the total amount of DNA for each transfection was kept constant by the addition of empty expression vector pcDNA3.1.

MOA A Catalytic Activity Assay—SK-N-BE (2)-C cells stably expressing R1 protein or pcDNA3.1 empty vector (control group) was used for determining MAO A catalytic activity. One-hundred micrograms of total protein from ~1 × 10^6 cells were incubated with 100 μM [14C]serotonin in the assay buffer (50 mM sodium phosphate buffer, pH 7.4) at 37 °C for 20 min, and the reaction was terminated by the addition of 100 μl of HCl. The reaction products were then extracted with benzene/ethyl acetate and centrifuged at 4 °C for 10 min. The organic phase containing the reaction product was extracted, and its radioactivity was determined by liquid scintillation spectroscopy (22). For co-transfection experiments with pcDNA3.1-R1, the total amount of DNA for each transfection was kept constant by the addition of empty expression vector pcDNA3.1.

MOA A Catalytic Activity Assay—SK-N-BE (2)-C cells stably expressing R1 protein or pcDNA3.1 empty vector (control group) was used for determining MAO A catalytic activity. One-hundred micrograms of total protein from ~1 × 10^6 cells were incubated with 100 μM [14C]serotonin in the assay buffer (50 mM sodium phosphate buffer, pH 7.4) at 37 °C for 20 min, and the reaction was terminated by the addition of 100 μl of HCl. The reaction products were then extracted with benzene/ethyl acetate and centrifuged at 4 °C for 10 min. The organic phase containing the reaction product was extracted, and its radioactivity was determined by liquid scintillation spectroscopy (22). For co-transfection experiments with pcDNA3.1-R1, the total amount of DNA for each transfection was kept constant by the addition of empty expression vector pcDNA3.1.
coupled sulfolink resin (Pierce). IgG fractions were dialyzed, quantified, and stored.

Western Blot Analysis of R1—R1 or pcDNA3.1 stable or transient transfected cell lines were harvested and washed with phosphate-buffered saline. Nuclear proteins were extracted as described previously (24), and the protein concentration was determined by the Bradford assay (Bio-Rad). Forty micrograms of total protein were separated on 10% SDS-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) (24). The membranes were then incubated with rabbit anti-R1 antibody (1:4000) 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 DAB as substrate (Sigma). The DNA fragments within immunoprecipitates were isolated by heating at 65 °C for 1 h and stored.

Immunofluorescence—SK-N-BE (2)-C cells were plated on coverslips the day before experiment. After 24 h, the cells were fixed in 4% paraformaldehyde in phosphate-buffered saline for 20 min and then permeabilized in 0.2% Triton X-100 and 5% goat serum in phosphate-buffered saline for 15 min. Cells were incubated with rabbit anti-R1 antibody, followed by fluorescein-conjugated anti-rabbit secondary antibody (Vector Laboratories) for 1 h. The stained slides were mounted by Vectashield (Vector Laboratories) in the presence of 4',6-diamidino-2-phenylindole (for nuclear staining) and examined with a fluorescence microscope.

Gel-shift Assay—Nuclear proteins were extracted, and a gel-shift assay was performed as described previously (24). Nuclear extracts (20 μg/sample) of SK-N-BE (2)-C cells were preincubated with 2 μl of anti-R1 antibody in a 25-μl reaction containing DNA binding buffer (20 mM HEPES, 0.2 mM EDTA, 0.2 mM EGTA, 100 mM KCl, 5% glycerol, and 2 mM dithiothreitol, pH 7.9) and 2 mg of poly(dI-dC) at room temperature for 20 min. 32P-labeled Sp1-sites (5'-cgag cgc gag cgc CC CGC CCG CCC GCC GC CAC gcg cag-3') or cold Sp1-sites served as probes (60,000 cpm/sample) and were added and incubated for an additional 20 min (25). The reaction was separated on a 5% polyacrylamide gel. The gels were dried and then exposed to film overnight at −80 °C with an intensifying screen.

Chromatin Immunoprecipitation (ChIP) Assays and Quantitative Real Time PCR—SK-N-BE (2)-C cells (7 × 10⁶ cells/150-mm dish) were plated and grown for 2 days. Cells were cross-linked by incubating with formaldehyde (1% final concentration) at room temperature for 10 min and then scraped into phosphate-buffered saline containing protease inhibitors (Sigma) and centrifuged for 3 min at 2000 rpm. Cells were resuspended in 350 μl of lysis buffer and processed for immunoprecipitation following the methods described previously (21). 35 μl of the supernatant was saved as input DNA. The nuclear protein-DNA complex was immunoprecipitated by incubating with anti-R1 (with BioMag Goat Anti-mouse) overnight at 4 °C with rotation. The DNA fragments within immunoprecipitates were isolated by heating at 65 °C for 4 h and recovered from the beads with an elution buffer (1% SDS and 0.1 M NaHCO₃) and analyzed by quantitative real time PCR using an iCycler optical system (Bio-Rad). The PCR product was determined by Sybr green reagent (2 × SYBR Green Supermix, Bio-Rad) following the manufacturer's instructions. The primers for the MAO A core promoter (342 bases, from −360 bp to −17 bp) and irrelevant locus were: core promoter forward, 5'-GTGCCCTGACACTCCGGGTT-3'; core promoter reverse, 5'-TCTCGGTGGTGTTGGCACCAGG-3'; irrelevant locus forward, 5'-ACAGTGGACCTGGAGGAGG-3' (bases −1377 to −1358); irrelevant locus reverse, 5'-GAAGCGGAGCTCATTGGAG-3' (bases −1043 to −1024).

The PCRs for input, R1-associated DNA or 5'-irrelevent locus were done in triplicate under the following conditions, 95 °C for 3 min, followed by 35 cycles of PCR consisting of 45 s at 94 °C, 45 s at 55 °C, and 45 s at 72 °C. The average threshold cycle (Cₚ) for the triplicate was used in all subsequent calculations. A ΔCₚ value was calculated for each sample using the Cₚ value for the input DNA samples to normalize the ChIP assay results as outlined in the iCycler optical systems protocol. The ΔCₚ values were converted to the -fold induction required to reach the threshold amount of PCR by raising 2 to the ΔCₚ power. The relative differences between input sample and R1 assay or negative control were determined using the ΔCₚ method (21) and presented as the percentage of input, which was taken as 100%. Data were the
RESULTS

A Novel Repressor R1 for Human MAO A Has Been Isolated by the Yeast One-hybrid System—The human MAO A core promoter contains a cluster of Sp1 motifs (17, 18). This DNA sequence (cgg gac ggc ccc CCG CCC CCG tac ggg cag)\textsubscript{3} (see Fig. 1A for location of Sp1 sites) was used as bait in the yeast one-hybrid system to search for additional transcription factors. After screening 1 \times 10^6 colonies, four independent clones containing 1.9-kb inserts were selected and sequenced. The sequences of the inserts were identical and matched with GenBankTM (accession number: BC009352, RAM2). The deduced protein sequence of 454 amino acids (molecular mass \approx 56 kDa) is shown in Fig. 1B. Several important protein domains were found in the R1 amino acid sequence (Fig. 1B).

The 77 amino acid residues (Fig. 1B, amino acids 349–425) in C-terminal show 87% identity with c-Myc target protein JPO1 (Fig. 2A), which ultimately leads to tumor genesis. There are 12 conserved cysteine residues within this sequence (Fig. 2A, numbered from 1 to 12). This region contains 4 CXCC zinc finger putative DNA binding domains (Fig. 2A, I, II, III, and IV). The interaction among cysteines in zinc finger domains is essential for providing proper protein conformation for the DNA binding. This region is highly conserved between human (HR1) and mouse R1 (Fig. 2B, e, MR1, 94% identity). This data suggests that there are DNA binding domains in R1. Thus R1 may be a transcription factor.

Further analysis of the amino acid sequence of human R1 protein, the important functional domains and multiple phosphorylation sites in R1 protein (Fig. 2B) were identified; the N-terminal acidic region (amino acids 1–29) is highly conserved and has 91% identity between human and mouse R1 (Fig. 2B, a). A PEST region (Pro, Glu, Ser, Thr-rich region flanked by Arg and Lys; amino acids 30–54) known to be susceptible to proteasome degradation was identified using PEST finder website; therefore R1 is easily degraded (Fig. 2B, b). A nuclear targeting sequence between amino acids 301 and 318 has been found, which suggests that R1 may be translocated between nucleus and cytosol (Fig. 2B, d). This region is also highly conserved and has 85% identity between human and mouse R1. In addition, the multiple potential phosphorylation sites conserved in human and mouse R1, such as serine (S), threonine (T), tyrosine (Y) were found (Fig. 2B). It suggests that the cellular location of R1 may be dependent on the status of...
The degree of repression of R1 on MAO A promoter activity correlated with the level of R1 expression. Different amounts of R1 or pcDNA3.1 vector (0, 300, 600, 1200, and 2400 ng/dish) were cotransfected with MAO A 2-kb-luc into SK-N-BE (2)-C cells for 2 days and either the luciferase activity was determined (A), or nuclear proteins were extracted and analyzed by Western blot using anti-R1 antibody (B). The relative intensity of each R1 band was quantified by PhosphorImager (C). Values were normalized to actin levels on the corresponding reprobed filters and then expressed as the -fold of control in which the control was taken as 1. Data represented the mean ± S.E. of at least three independent experiments.

Repressor R1 Inhibits MAO A Catalytic Activity—The effect of R1 on the MAO A catalytic activity was studied (Fig. 3). After pcDNA3.1-R1 was transfected into SK-N-BE (2) cells and selected by G418, MAO A enzymatic activity was determined in five separated clones to evaluate the function of R1. The results showed that R1 inhibited MAO A catalytic activity by 50% compared with control when R1 was stably transfected into SK-N-BE (2)-C cell line (Fig. 3A). The level of R1 expression in stably transfected cell lines was analyzed by Western blot using anti-R1 antibody (Fig. 3B). The intensities of the bands were quantified by PhosphorImager (Fig. 3C). The level of stably expressed R1 was increased significantly compared with pcDNA3.1 alone stably expressed cell line (Fig. 3C). Data represented the mean ± S.E. of three independent experiments.

R1 Interacts with Sp1 Sites and Represses the MAO A Promoter Activity—After the R1 expression vector was cotransfected with MAO A 2-kb-luc into cells, R1 was found to repress MAO A at the transcriptional level. Luciferase activity was decreased 50% in MAO A 2-kb-luc activity (Fig. 4A). However, when R1 was cotransfected with Sp1 sites deleted MAO A 2-kb-luc, MAO A promoter activity was not repressed, indicating that the R1 effect is specific to the Sp1 sites (Fig. 4A). When R1 expression vector was cotransfected with MAO A 0.24-kb-luc containing 4 × Sp1 sites (Fig. 4C) into SK-N-BE (2)-C cells, ~60% decrease in MAO A core promoter activity (Fig. 4C) was observed, which confirmed that the R1 repression effect is through Sp1 sites.

The Degree of Repression of R1 on MAO A Promoter Activity Correlates with the Level of R1 Expression—For transiently transfected R1 expression, the different amounts of R1 or pcDNA3.1 vector (0, 300, 600, 1200, and 2400 ng/dish) were cotransfected with MAO A 2-kb-luc into SK-N-BE (2)-C cells for 2 days, and the luciferase activity was determined (Fig. 5B), or nuclear proteins were extracted and analyzed by Western blot using anti-R1 antibody. The relative intensity of each R1 band was quantified by PhosphorImager system. Values were normalized to actin levels on the corresponding reprobed filters and then expressed as the -fold of control in which the control was taken as 1. Data represented the mean ± S.E. of three independent experiments.
out R1, for sequence, see “Materials and Methods”). The R1-DNA complex was supershifted by anti-R1 antibody (Fig. 7, lane 4). This R1-DNA complex was competed by excess unla- beled R1 binding motif oligonucleotides (Fig. 7, lane 3), sug- gesting that R1 binds specifically to Sp1 sites. This indicates the presence of endogenous R1 in this cell line and that R1 interacts with R1 binding motif in MAO A core promoter directly.

\[ R1 \text{ interacted with the endogenous MAO A promoter directly in vivo as shown by ChIP assay.} \]

The association of R1 with the endogenous MAO A core promoter (containing 4 × Sp1 sites) compared with a 5′-irrelevant region of MAO A was determined by ChIP assay combined with quantitative real time PCR using SK-N-BE (2)-C cells (Fig. 8). The nuclear protein-DNA complex was immunoprecipitated by anti-R1 antibody and quantitatively analyzed by real time PCR. The core promoter sequence and 5′-irrelevant region (negative control) were used for occupancy analysis (Fig. 8A). The representative R1 ChIP/quantitative real time PCR amplification plots (triplicate) are shown in Fig. 8B. The average \( C_T \) for input sample, TIEG2 cross-linked DNA, and 5′-irrelevant region were 18.06 ± 0.98, 24.44 ± 1.09, and 31.12 ± 0.92, respectively (Fig. 8B). The relative differences between input sample and R1 ChIP assay or negative control are summarized in Fig. 8C. The analysis of 5′-irrele vant region yielded negligible values (less than 0.05% input DNA, Fig. 8C). This result indicated that R1 bound to the endogenous core promoter of MAO A directly.

\[ R1 \text{ protein was located in both nucleus and cytosol as shown by immunocytochemistry.} \]

To examine cellular localization of R1 protein, the immunofluorescent staining of cells in culture was performed (Fig. 9) by using rabbit anti-R1 antibody.

![Fig. 7. The novel repressor protein R1 was present in nuclear extracts of SK-N-BE (2)-C cells and was bound to R1 binding sequence. The gel-shift assay was performed with 20 μg of nuclear extracts from SK-N-BE (2)-C cells (lanes 2, 3, and 4) and the 32P-labeled R1 binding sequence (Sp1 sites), which was used in the yeast one-hybrid system to fish out R1. Excess cold R1 binding motif probe (lane 3) or anti-R1 antibody (lane 4) was added as indicated. An arrow indicates the R1-DNA complex. The super-shifted complex is also indicated.](http://wilke.fhcrc.org)

![Fig. 8. R1 interacted with the endogenous MAO A promoter directly in vivo. The occupation of R1 on the MAO A core promoter or 5′-irrelevant region was determined by ChIP assay combined with quantitative real time PCR using SK-N-BE (2)-C cells. A, a schematic representation of the MAO A promoter. Real time PCR-targeted regions containing core promoter and Sp1 sites (from -360 to -17 bp) and 5′-irrelevant loci (from -1377 to -1024 bp for negative control) were indicated. B, representative R1 ChIP/quantitative real time PCR amplification plots (triplicates). The ChIP/quantitative real time PCR amplification was performed for cross-linked inputs, R1-associated MAO A core promoter, or 5′-irrelevant loci in SK-N-BE (2)-C cells. The nuclear protein-DNA complex was immunoprecipitated by anti-R1 antibody and was quantitatively analyzed by real time PCR. C, analysis of association of R1 with MAO A core promoter or 5′-irrelevant locus. The relative differences between input sample and R1 or negative control were determined using the ΔC_T method (see “Materials and Methods”). These values were presented as percent input in which the DNA cross-linked input sample was taken as 100%. Data were the mean ± S.D. from triplicate samples of three independent experiments.](http://wilke.fhcrc.org)
Repressor R1 was located in both nucleus and cytosol of human neuroblastoma SK-N-BE (2)-C cells. Immunofluorescence was performed using anti-R1 antibody. SK-N-BE (2)-C cells were plated on coverslips the day before experiment. Then the cells were fixed and incubated with rabbit anti-R1 antibody, followed by fluorescein-conjugated anti-rabbit secondary antibody (A, green). The stained slides were mounted in the presence of 4,6-diamino-2-phenylindole (DAPI) for nuclear staining (B, blue) and examined under fluorescence microscope. C, the merge of A and B.

**Fig. 10.** The presence of endogenous R1 mRNA (3 kb) in human brain, peripheral tissues, and cell lines as shown by Northern blot analysis. A, 10 μg of total RNA from SK-N-BE (2)-C (neuroblastoma), 1242-MG (astrocytoma), LNCaP (prostate cancer), and human brain samples. B, 2 μg of poly(A)+ RNA from human peripheral tissues as indicated were loaded on each gel and then transferred to membranes. A 500-bp EcoRI fragment R1-specific probe was labeled with 32P and hybridized to the membranes overnight at 42 °C. Blots were exposed for 16 h. Bottom blots, the same blots were hybridized with glyceraldehyde-3-phosphate dehydrogenase positive control probe and hybridized and washed under the same condition. The film was exposed for 4 h to demonstrate that the same amount of RNA was loaded on each lane.

DISCUSSION

This is the first study to demonstrate that there is a novel repressor R1 that regulates the human MAO A gene expression. R1 binds to Sp1 sites in MAO A core promoter (Figs. 7 and 8) and inhibits MAO A promoter and catalytic activities (Figs. 3–6). The human R1 has an 83% similarity with the mouse (Fig. 2). The presence of a mouse R1 homolog suggests that R1 may be functionally significant.

Similar to MAO A (27), R1 is expressed widely in the human brain and peripheral tissues. The presence of a nuclear targeting sequence (amino acids 301–318) in R1 and the subcellular distribution of R1 in nucleus (Fig. 9) are consistent with its functional role in the transcriptional regulation of MAO A.

The C-terminal of R1 contains 77 amino acid residues (amino acids 349–425), which have 87% identity with the c-Myc target protein JPO1, which leads to tumor genesis (26). Therefore R1 may also be a target gene of c-Myc and may play important role in tumor genesis.

It has been shown that MAO A has been a pro-apoptotic gene (28). MAO A expression was increased in cells during nerve growth factor withdrawal-induced apoptosis, which inhibited neuronal proliferation and accelerated cell apoptosis through the p38 mitogen-activated protein kinase pathway (28). The inhibition of MAO A by clorgyline prevented the cells from undergoing apoptosis (28). In addition, MAO A is a key enzyme for catalyzing the oxidative deamination of a variety of amine neurotransmitters. The byproduct of MAO A activity is hydrogen peroxide (H2O2), which has cytotoxic effects and causes apoptosis or cell death (29). R1 is a potential repressor of MAO A, thus, increased R1 may decrease MAO A gene expression and therefore may prevent cells from undergoing apoptosis. The role of R1 in apoptosis remains to be studied.

Further, multiple phosphorylation sites have been identified on human R1 protein (Fig. 2B), which are conserved in human and mouse R1. This suggests that the cellular location of R1 may depend on the status of phosphorylation and the presence or absence of phosphatases and kinases.

This study shows that R1 regulates MAO A gene expression via Sp1 sites. The core promoter region of MAO A contains four Sp1 binding sites. The Sp3 and Sp4 may interact with these sites in addition to Sp1 (19). Therefore it is possible that R1 may coordinate with other Sp1 family or/and other unknown factors to regulate MAO A gene expression.

Because R1 recognizes Sp1 sites, other promoters that contain Sp1 sites may also be regulated by R1. This novel R1 may regulate a group of genes that bear similar promoter sequences as MAO A. These interesting features need to be investigated.

In summary, this study identified the function of protein R1. R1 is the first novel transcription factor demonstrated to neg-
atively regulate MAO A gene expression. R1 was found both in the nucleus and cytosol. It was further shown to be expressed in brain and peripheral tissues. The identification of R1 has provided new information concerning the molecular mechanism of MAO A gene transcriptional regulation. The R1 regulation of MAO A gene expression may provide insight on the new function of MAO A in cell growth and apoptosis.

REFERENCES
1. Shih, J. C. (1991) *Neuropsychopharmacology* **4**, 1–7
2. Thorpe, L. W., Westlund, K. N., Kohersperger, L. M., Abell, C. W., and Denney, R. M. (1987) *J. Histochem. Cytochem.* **35**, 23–32
3. Johnston, J. P. (1968) *Biochem. Pharmacol.* **17**, 1285–1297
4. Knoll, J., and Magyar, K. (1972) *Adv. Biochem. Psychopharmacol.* **5**, 393–408
5. 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
6. Grimsby, J., Chen, K., Wang, L. J., Lan, N. C., and Shih, J. C. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88**, 3637–3641
7. Lan, N. C., Heinzmann, C., Gal, A., Klosak, I., Orth, U., Lai, E., Grimsby, J., Sparkes, R. S., Mohandas, T., and Shih, J. C. (1989) *Genomics* **4**, 552–559
8. Shih, J. C., and Chen, K. (1999) *Neurobiology* **7**, 235–246
9. Cases, O., Seif, I., Grimsby, J., Gaspar, P., Chen, K., Pourrin, S., Muller, U., Auge, M., Babinet, C., and Shih, J. C. (1995) *Science* **269**, 1763–1766
10. Shih, J. C., Chen, K., and Ridd, M. J. (1999) *Annu. Rev. Neurosci.* **22**, 197–217
11. Brunner, H. G., Nelen, M., Breakefield, X. O., Ropers, H. H., and van Oost, B. A. (1999) *Science* **282**, 578–580
12. Grimsby, J., Toth, M., Chen, K., Kumaizawa, T., Klabiman, L., Adams, J. D., Karoum, F., Gal, J., and Shih, J. C. (1997) *Nat. Genet.* **17**, 206–210
13. Chen, K., Holschneider, D. P., Wu, W., Rebrin, I., and Shih, J. C. (2004) *J. Biol. Chem.* **279**, 21021–21028
14. Holschneider, D. P., Serremin, O. U., Roos, K. P., Chialvo, D. R., Chen, K., and Shih, J. C. (2002) *Am. J. Physiol.* **282**, H964–H972
15. Holschneider, D. P., Serremin, O. U., Chialvo, D. R., Chen, K., and Shih, J. C. (2002) *Am. J. Physiol.* **282**, H1751–H1759
16. Zhu, Q. S., Grimsby, J., Chen, K., and Shih, J. C. (1992) *J. Neurosci.* **12**, 4437–4446
17. Zhu, Q. S., Chen, K., and Shih, J. C. (1994) *J. Neurosci.* **14**, 7393–7403
18. Shih, J. C., Grimsby, J., Chen, K., and Zhu, Q. S. (1993) *J. Psychiatry Neurosci.* **18**, 25–32
19. Wong, W. K., Chen, K., and Shih, J. C. (2001) *Mol. Pharmacol.* **59**, 852–859
20. Zhang, J. S., Moncrieffe, M. C., Kaczynski, J., Ellenrieder, V., Prendergast, F. G., and Urrutia, R. (2001) *Mol. Cell. Biol.* **21**, 5041–5049
21. Ou, X. M., Chen, K., and Shih, J. C. (2004) *J. Biol. Chem.* **279**, 21021–21028
22. Wong, W. K., Ou, X. M., Chen, K., and Shih, J. C. (2002) *J. Biol. Chem.* **277**, 22222–22230
23. Geha, R. M., Rebrin, I., Chen, K., and Shih, J. C. (2001) *J. Biol. Chem.* **276**, 9877–9882
24. Ou, X. M., Storring, J. M., Kushwaha, N., and Albert, P. R. (2001) *J. Biol. Chem.* **276**, 14299–14307
25. Ou, X. M., Jafar-Nejad, H., Storring, J. M., Meng, J. H., Lemende, S., and Albert, P. R. (2000) *J. Biol. Chem.* **275**, 8161–8168
26. Prescott, J. E., Osthuis, R. C., Lee, L. A., Lewis, B. C., Shim, H., Barrett, J. F., Guo, Q., Hawkins, A. L., Griffin, C. A., and Dang, C. V. (2001) *J. Biol. Chem.* **276**, 48276–48284
27. Saura, J., Bleuel, Z., Ulrich, J., Mendelowitsch, A., Chen, K., Shih, J. C., Malherbe, P., Da Prada, M., and Richards, J. G. (1996) *Neuroscience* **70**, 735–774
28. De Zutter, G. S., and Davis, R. J. (2001) *Proc. Natl. Acad. Sci. U. S. A.* **98**, 6168–6173
29. Girgin Sagin, F., Sozmen, E. Y., Ersoz, B., and Mentes, G. (2004) *Neurotoxicology* **25**, 91–99
