ZIC2 and Sp3 Repress Sp1-induced Activation of the Human D1A Dopamine Receptor Gene*

Received for publication, August 29, 2000, and in revised form, September 11, 2000
Published, JBC Papers in Press, September 12, 2000, DOI 10.1074/jbc.M007906200

Young Yang‡, Cheol Kyu Hwang§, Eunsung Junn, Gwang Lee, and M. Maral Mouradian¶
From the Genetic Pharmacology Unit, Experimental Therapeutics Branch, NINDS, National Institutes of Health, Bethesda, Maryland 20042-1406

The human D1A dopamine receptor is transcribed from a tissue-specific regulated gene under the control of two promoters. An activator region (AR1) located between nucleotides −1154 and −1136 (relative to the first ATG) enhances transcription from the upstream promoter that is active in the brain. In this investigation, we sought to identify the nuclear factors that regulate the D1A gene through their binding to AR1 using yeast one-hybrid screening. Sp3 and Zic2 were among the positive clones isolated. Although Sp1 was not isolated from this screening and purified Sp1 alone does not bind to AR1 in gel shift experiments, this general transcription factor binds to AR1 in the presence of D1A expressing NS20Y nuclear extract and activates the D1A promoter. Thus, Sp1 appears to require an unknown factor(s) or post-translational modification to interact with AR1. On the other hand, Zic2 and Sp3 inhibit Sp1-induced activation of the D1A gene in an AR1-dependent manner. Zic2 and D1A genes have reciprocal brain regional distributions; Zic2 is expressed primarily in the cerebellum, and D1A is highly expressed in corpus striatum. These observations collectively suggest that one of the physiologic functions of Zic2 is repression of D1A gene transcription and that the intracellular balance among Sp1, Sp3 and Zic2 is important for regulating the tissue-specific expression of this dopamine receptor.

Sp1 and Sp3 are ubiquitous transcription factors that play major roles in the expression of many cellular genes including constitutive housekeeping and inducible genes (1). Sp3 shares extensive structural and sequence homology with Sp1 and can function as a synergist or antagonist of Sp1-mediated activation of target promoters (2–5). In addition, internally translated isoforms of Sp3 function as potent inhibitors of Sp1-mediated transcription in vivo since such truncated isoforms lack substantial portions of the Sp3 transactivation domain (6). Thus, the balance between Sp1 and Sp3 is an important regulator of target genes (7).

The murine zinc finger protein of the cerebellum (Zic) was cloned through a search for proteins involved in cerebellar development (8). Subsequently, Zic2 and Zic3 were cloned as members of the Zic gene family (9). Zic expression is highly restricted to the cerebellar granule cell lineage and in medulloblastoma cells (10). Furthermore, analysis of Zic knock-out mice confirms that this transcription factor is involved in cerebellar development (11). On the other hand, mutations in Zic2 have been associated with holoprosencephaly (12).

Dopamine plays important roles in several physiologic functions including locomotion (13, 14), learning and memory (15, 16), neuroendocrine modulation (17), control of renal sodium excretion (18), as well as in drug addiction (19, 20). Central dopaminergic effects are mediated by cell-surface receptors expressed in dopaminceptive cells that are found mainly in the striatum and prefrontal cortex. Dopamine receptors are a family of G protein-coupled receptors and are classified into two subtypes as follows: D1-like (D1A and D1B) and D2-like (D2A, D2B, and D2C) receptors based on their sequence homology and pharmacological criteria (21, 22). Both D1 and D2 dopamine receptors in the human brain have been found to decrease with aging, a finding that may relate to the decline in motor performance with advancing age (23, 24).

We had previously found that the human D1A receptor gene is transcribed in the brain from two promoters (25). A cis-acting element located between nucleotides −1154 and −1136 relative to the translation start site (termed activator region 1, AR1) mediates transactivation of the upstream promoter in neuronal cells (25). Although AR1 has a consensus sequence for AP2-binding site, a significant role for this factor in regulating D1A gene transcription has been excluded based on lack of functional AP2 in the striatum or in the D1A-expressing NS20Y cell line (26). In the present investigation employing the yeast one-hybrid screening method, we identified three transcription factors that interact with the AR1 sequence and regulate the human D1A gene promoter.

EXPERIMENTAL PROCEDURES

Yeast One-hybrid Screening for cDNAs Encoding AR1-binding Proteins—The MATCHMAKER One-hybrid System (CLONTECH) was used according to the supplier’s protocol. Three tandem repeats of the −1154 to −1136 bp sequence (AR1) from the human D1A gene were ligated into pHISi and pLacZ to generate pHISi-AR1 and pLacZ-AR1, respectively. These two bait constructs were then linearized with XhoI and NcoI, respectively, and integrated into the genome of YM4271 yeast strain. The resultant yeast cells with the integrated pHISi-AR1 were tested for growth on a medium lacking histidine (His−) in the presence of increasing concentrations of 3-amino-1,2,4-triazole (3-AT). Background growth was inhibited at 30 mM 3-AT, and this concentration was used.

* 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.

† The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AF193855.
‡ Present address: Korea Research Institute of Bioscience and Biotechnology, Eoul-dong 52, Yusong, Taejon 305–333, Korea.
§ Present address: Dept. of Pharmacology, 6-120 Jackson Hall, University of Minnesota, Minneapolis, MN 55455.
¶ To whom correspondence should be addressed: NINDS, National Institutes of Health, 10 Center Dr., MSC 1406, Bethesda, MD 20042-1406. Tel.: 301-496-7872; Fax: 301-496-6609; E-mail: MouradianM@ninds.nih.gov.

The abbreviations used are: AR1, activator region 1; PAGE, polyacrylamide gel electrophoresis; bp, base pair; 3-AT, 3-amino-1,2,4-triazole; PBS, phosphate-buffered saline; Pipes, 1,4-piperazinediethanesulfonic acid; kb, kilobase pairs; PCR, polymerase chain reaction; CAT, chloramphenicol acetyltransferase.
then used when yeast cells were transformed with a human brain cDNA library for one-hybrid screening. Seven positive transformants grown on His− plates were selected. To exclude false positive clones, plasmids recovered from these seven clones were used to transform yeast cells harboring the pLaCZ-AR1 construct. Positive transformants grown on His−/Leu− (an amino-acid-requiring [Leu−] medium containing 30% methionine) were streaked onto a nylon filter and incubated by placing the filter on the same medium at 30 °C for 24 days. The filter was then soaked in liquid nitrogen for 10 s and placed on a Whatman 3MM filter, which had been presoaked in 2% buffer (60 mM Na2HPO4, 60 mM NaH2PO4, 10 mM KCl, 1 mM MgSO4, 50 mM β-mercaptoethanol, pH 7.0), containing 0.01% 5-bromodeoxyuridine (5-BrdU) and 0.01% 5-iododeoxyuridine (5-IdU). The results were analyzed using the BLAST algorithm.

**Cloning Human Zic2 and in Vitro Translation**—The Zic2 cDNA isolated from the yeast one-hybrid screening was sequenced using Applied Biosystems ABI 7400 sequencer and cloned into pGEM-pZic2 that had been digested with SmaI and PstI (Promega), yielding pGEM-pZic2. Since this clone lacked the adenosine of the first ATG codon, full-length Zic2 cDNA was generated using adapter oligonucleotides containing the consensus Kozak sequence from murine Zic2 (9). These adapter sequences were as follows: upper strand, 5′-ATGCCCCAGGGCCAGGGGTCCGTCGCTCCGCCCCACGGGCAAGGGG-3′; lower strand, 5′-ATGGCCGGGAACTGCGGACCCGC-GTTCAGGAAGGATGCAGCCGG-3′. The annealed adapter was ligated into pGEM-pZic2 that had been digested with EcoRI and BglII. The result was designated pGEM-Zic2. Full-length Zic2 was then subcloned into pcDNA3.1 (Invitrogen) yielding pcDNA-Zic2. In *in vitro* transcription/translation (Life Technologies, Inc.), it was carried out with pcDNA-Zic2 in a reaction mixture containing [*S*]methionine (Amersham Pharmacia Biotech) using a TNT-coupled reticulocyte lysate system (Promega). The labeled protein was then electrophoresed in 10% SDS-PAGE to determine its size.

**Cell Culture**—The murine neuroblastoma cell line NS20Y was a kind gift from Dr. Marshall Nirenberg (NHLBI, National Institutes of Health, Bethesda). Cells were grown in Dulbecco's modified Eagle's medium (Mediatech) supplemented with 10% fetal bovine serum (Bio-Whittaker) at 37 °C in a humidified atmosphere of 10% CO2. The human monocytic leukemia cell line THP-1 (ATCC) was cultured in RPMI 1640 medium (Life Technologies, Inc.) supplemented with 10% fetal bovine serum (Life Technologies, Inc.) and the samples were placed at 45 °C for 4 h to reverse the formaldehyde-induced cross-linking. Digestion buffer was added (10 mM of 2-mer Tris, pH 8.5, 10 μg/ml of EDTA, 2 μl of proteinase K (20 μg/ml)), and the samples were placed at 45 °C for 2 h. Chromatin DNA was extracted with phenol/CHCl3 followed by ethanol precipitation. DNA was resuspended in 50 μl of sterile H2O, and 5 μl was used in PCR analysis. Primers for amplifying the 152-bp fragment encompassing the AR1 region of the D1A receptor promoter were designed as follows: 5D1ch-5′-CGCAACTCTGGCTCTGTAAG-3′ and 3D1ch-5′-CTTCACGCAGGCGTTGGC-3′. Following 30 cycles of amplification, PCR products were electrophoresed in a 1.5% agarose gel and visualized by ethidium bromide staining.

**Gel Mobility Shift Assays**—AR1 probe was synthesized using an *in vitro* transcription kit (T7 polymerase, 5′-ACAGGCGGGCTCGGG-3′; lower strand, 5′-TCCCTCGCCTGGGGGGCCTGCT-3′.) The upper strand was labeled with [γ-32P]ATP and annealed with the cold lower strand. Double-stranded, end-labeled DNA probe (20,000 cpm/binding reaction; 5 fmol) was incubated with 2 μl of in vitro translated Zic2 protein or control lysate or with NS20Y nuclear extract in a final volume of 20 μl at room temperature for 30 min in binding buffer (20 mM Tris, pH 7.6, 2 mM MgCl2, and 0.5 mM dithiothreitol). NS20Y nuclear extracts were used. After 16 h incubation, Sp1 or Sp3 expression was induced with 0.7 mM CuSO4, and the cells were harvested 24 h after induction and lysed. CAT protein was quantified by the CAT enzyme-linked immunosorbent assay kit (Roche Molecular Biochemicals). Each experiment was carried out in triplicate.

**Transfections and Transcription Assay**—Transfection of SL2 cells was carried out using Lipofectin (Life Technologies, Inc.) with HyQ-CMC 3 serum-free medium (HyClone Laboratories) in 60-mm dishes. Two μg of pCAT11-1154 (26) with 1 μg of pRMSp1, pRMSp3 (5), or both were used. After 16 h incubation, Sp1 or Sp3 expression was induced with 0.7 mM CuSO4, and the cells were washed twice with 1 ml of wash buffer (0.1% [v/v] Triton X-100, 20 mM Tris, pH 8.0, 150 mM NaCl, 2 mM EDTA) and eluted by three successive 5-min incubations with 150 μl of elution buffer (1% [w/v] SDS, 50 mM NaHCO3). The eluates were pooled, and NaCl was added at a final concentration of 0.3 M, and the samples were incubated at 65 °C for 4 h to reverse the formaldehyde-induced cross-linking. Digestion buffer was added (10 μl of 2-mer Tris, pH 6.5, 10 μg/ml of EDTA, 2 μl of proteinase K (20 μg/ml)), and the samples were placed at 45 °C for 2 h. Chromatin DNA was extracted with phenol/CHCl3 followed by ethanol precipitation. DNA was resuspended in 50 μl of sterile H2O, and 5 μl was used in PCR analysis. Primers for amplifying the 152-bp fragment encompassing the AR1 region of the D1A receptor promoter were designed as follows: 5D1ch-5′-CGCAACTCTGGCTCTGTAAG-3′ and 3D1ch-5′-CTTCACGCAGGCGTTGGC-3′. Following 30 cycles of amplification, PCR products were electrophoresed in a 1.5% agarose gel and visualized by ethidium bromide staining.

**RESULTS**

**Isolation of Transcription Factors That Interact with the Activator Region AR1 in the D1A Promoter**—The yeast one-hybrid screen was performed to find transcriptional regulators that bind to the AR1 region (Fig. 1) of the human D1A dopamine receptor promoter. A double-stranded oligonucleotide having the following sequence was synthesized as a yeast one-hybrid screening probe.

**Fig. 1. Nucleotide sequence of the AR1 region in the human D1A gene.** Three tandem repeats of this sequence were used for yeast one-hybrid screening. Underlined nucleotides indicate the AP2 consensus sequence and the boxed nucleotides denote the Sp1 consensus site.

**Protease Inhibitors**—To reduce nonspecific binding, samples were incubated with 80 μl of salmon sperm DNA/protein A-agarose slurry (Upstate Biotechnology, Lake Placid, NY) at 4 °C for 1 h on a rotating wheel, and beads were collected by centrifugation at 500 × g for 1 min. Preclotted chromatin solutions were incubated with antibody to Sp1 (10 μg) or to Sp3 (10 μg) (Santa Cruz Biotechnology) or with no antibody and rotated at 4 °C for 12 h. Immune complexes were collected by adding 80 μl of salmon sperm DNA/protein A-agarose slurry for 4 h with rotation. Samples were subsequently washed four times with 1 ml of wash buffer (0.1% [v/v] Triton X-100, 20 mM Tris, pH 8.0, 150 mM NaCl) and eluted by three successive 5-min incubations with 150 μl of elution buffer (1% [w/v] SDS, 50 mM NaHCO3). The eluates were pooled, and NaCl was added at a final concentration of 0.3 M, and the samples were incubated at 65 °C for 4 h to reverse the formaldehyde-induced cross-linking. Digestion buffer was added (10 μl of 2-mer Tris, pH 6.5, 10 μg/ml of EDTA, 2 μl of proteinase K (20 μg/ml)), and the samples were placed at 45 °C for 2 h. Chromatin DNA was extracted with phenol/CHCl3 followed by ethanol precipitation. DNA was resuspended in 50 μl of sterile H2O, and 5 μl was used in PCR analysis. Primers for amplifying the 152-bp fragment encompassing the AR1 region of the D1A receptor promoter were designed as follows: 5D1ch-5′-CGCAACTCTGGCTCTGTAAG-3′ and 3D1ch-5′-CTTCACGCAGGCGTTGGC-3′. Following 30 cycles of amplification, PCR products were electrophoresed in a 1.5% agarose gel and visualized by ethidium bromide staining.
three tandem repeats of the AR1 sequence was subcloned into pHISi and introduced into yeast cells. The resultant strain was transformed with a human brain cDNA library. Plasmids prepared from clones grown on the selection medium were transformed into yeast strain containing pLacZ-AR1. Plasmids from 15 blue clones selected from the β-galactosidase assay were sequenced. These included Sp3, Zic2, AP2a, and AP2b. Although AR1 has an Sp1 consensus sequence (Fig. 1), the yeast one-hybrid screen did not isolate an Sp1 clone.

The results obtained in yeast cells prompted us to investigate the nature of transcription factors that bind to the AR1 sequence in the D1A-expressing NS20Y neuroblastoma cells. Gel mobility shift analysis using nuclear extract from these cells showed four major bands shifted with the AR1 probe (Fig. 2A). Because of the presence of Sp1 and AP2 consensus sequences in AR1 and because Sp3 was one of the clones isolated from the yeast one-hybrid screen using AR1 as bait, we first sought to determine if these general transcription factors bind to AR1. Antibodies to the respective factors were used in gel supershift assays. Preincubation with an AP2 antibody did not affect any of the bands retarded by NS20Y nuclear extract (Fig. 2A), although purified AP2 alone shifted the AR1 probe (Fig. 2B), suggesting that NS20Y cells do not have functional AP2 capable of binding to AR1, consistent with our previous observation (26). To confirm this fact, reverse transcriptase-PCR analysis was carried out with RNA from NS20Y and THP-1 cells using AP2 primer pairs. An AP2-specific band was amplified in THP-1 cells but not in NS20Y cells (data not shown). In contrast to the ineffectiveness of the AP2 antibody, an Sp1 antibody supershifted band I, and an Sp3 antibody abrogated bands II–IV. Thus, Sp3 was identified as an AR1 binding factor both by yeast one-hybrid screening and by gel supershift assay. Although Sp1 found in NS20Y cells could bind to AR1 (Fig. 2A), purified Sp1 alone failed to retard the AR1 probe (Fig. 2B), and the yeast one-hybrid screen did not isolate an Sp1 clone. The latter observations raised the possibility that Sp1 might require a cofactor(s) to bind to the AR1 sequence. To address this hypothesis, NS20Y nuclear extract was subjected to repeated cycles of freezing and thawing to remove endogenous Sp1 binding activity and then spiked with purified Sp1. Whereas nonspiked freeze/thawed extract showed very weak binding to the AR1 probe (Fig. 2C, lane 1), compared with the strong binding of non-manipulated extract (Fig. 2A, lane 1), purified Sp1 in the presence of nuclear extract shifted the probe, and this band was supershifted with an anti-Sp1 antibody. These data indicate that Sp1 requires a cofactor(s) present in NS20Y nuclei, and not affected by freeze/thaw cycles, to bind to the AR1 sequence.
In Vivo Interaction of Sp1 and Sp3 with the AR1 Sequence—
The ability of Sp1 and Sp3 to bind specifically to the AR1 region of the D1A promoter in vivo was evaluated using the chromatin immunoprecipitation method. NS20Y cells were treated with formaldehyde to induce cross-linking between transcription factors and chromatin. Prior to immunoprecipitation with antibodies against Sp1 or Sp3, cross-linked chromatin was sonicated to an average length of below 2 kb (Fig. 3). Subsequently, successful immunoprecipitation of Sp1 and Sp3 was confirmed by Western blot analysis using the same respective antibodies (Fig. 3B). Immunoprecipitated DNA-protein complexes were then dissociated, and DNA was amplified by PCR using primers specific for the AR1 sequence. The correct size band was amplified from DNA precipitated by Sp1 or Sp3 antibody but not from a similarly handled sample in the absence of antibody (Fig. 3C). These observations confirmed that Sp1 and Sp3 interact with the AR1 sequence in vivo in NS20Y cells.

Sp1 and Sp3 Activity in SL2 and NS20Y Cells—To test the functional activity of Sp1 and Sp3 on the D1A promoter, transient cotransfection assays were performed. Schneider’s Drosophila SL2 cells were chosen first because they do not express Sp family proteins to allow interpretation of quantitative results (28, 29). Expression plasmids for Sp1 and Sp3 were used along with pCATD1-1154 reporter plasmid which includes the AR1 sequence and the D1A core promoter (26). Sp1 indeed increased CAT gene expression from pCATD1-1154, whereas Sp3 suppressed Sp1-induced transactivation (Fig. 4A) indicating that Sp1 functions as an activator of the D1A promoter, whereas Sp3 functions as a repressor.

The transcriptional activity of Sp1 and Sp3 was also tested in the D1A receptor expressing NS20Y cells (Fig. 4B). The results obtained were consistent with our observations in SL2 cells. Sp1 alone activated the D1A promoter in pCATD1-1154 by about 2-fold, similar in magnitude to the induction of the apolipoprotein AI promoter by Sp1 in HepG2 cells (30). Sp3 alone was ineffective on the D1A promoter in NS20Y cells, but the combination of both factors neutralized this promoter. Thus, the presence of Sp3 inhibited the activity of Sp1.

Construction of Full-length Zic2 cDNA—One of the clones isolated from the yeast one-hybrid screen was Zic2. Full-length human Zic2 cDNA was generated and the entire 2.6 kb sequenced (GenBank TM accession number AF193855). Human Zic2 encodes a 532-amino acid polypeptide, compared with 530 amino acids in murine Zic2. The homology in the zinc finger domains of human and murine Zic2 is 100%, but their C-terminal regions (253–415 amino acids) are only 59% identical. Comparison among murine Zic1, Zic2, and Zic3 sequences revealed that the region with the least homology is C-terminal to the zinc finger domain suggesting that this less conserved sequence could be important for the functional differentiation among Zic family proteins.

Brain Regional Distribution of Human Zic2 Expression—To identify the major tissues that express Zic2, Northern hybridization was done with a multiple tissue blot. The only tissue with detectable Zic2 mRNA was the brain (Fig. 5A), suggesting a unique role for this protein. Within the brain, Zic2 is expressed as two bands of approximately 3.2 and 3.5 kb predominantly in the cerebellum and as very faint bands in other
regions but not in caudate, putamen, or substantia nigra (Fig. 5).

**Zic2 Binds to the AR1 Sequence**—Zic2 was isolated from the yeast one-hybrid screen by virtue of its ability to interact with the AR1 sequence. To confirm whether Zic2 can bind to this sequence, gel mobility shift assay was carried out using *in vitro* translated Zic2. Protein electrophoresis revealed that Zic2 migrates slower than expected from its calculated molecular weight likely due to the abundance of prolines (Fig. 6A). In gel retardation experiments, Zic2 was able to shift the target AR1 probe, and cold AR1 oligonucleotide competed off the Zic2-DNA complex (Fig. 6B) indicating that Zic2 can specifically bind to the AR1 sequence.

**Zic2 Represses the D1A Promoter through AR1**—The transcriptional activity of Zic2 on the D1A gene was tested in NS20Y cells, which have no detectable endogenous Zic2 mRNA by Northern analysis (data not shown). Cotransfection with a mammalian Zic2 expression construct along with pCATD1-1154 or pCATD1-1102 (26) was carried out. Since the AR1 region is an important activator of the D1A promoter, Zic2 was expected to enhance the activity of pCATD1-1154. Surprisingly, Zic2 markedly suppressed transcription of pCATD1-1154 but not that of pCATD1-1102. Data shown are means ± S.E. for triplicate samples. *p < 0.0006 compared with no Zic2. B, Zic2 suppresses endogenous D1A gene expression. NS20Y cells were transfected with pcDNA-Zic2 or control vector, and total RNA was prepared 48 h later for D1A Northern analysis. C, Zic2 competes with Sp1 and Sp3 binding to AR1. Three μg of NS20Y nuclear extract was incubated with 2 μl of *in vitro* translated Zic2 or control lysate and subjected to gel shift assay. Lanes 6–8 represent longer exposure of lanes 3–5, respectively. The Zic2 band appeared after long exposure (lane 8).

**DISCUSSION**

The present investigation employing several different approaches indicated that the human D1A dopamine receptor gene is regulated by Sp1, Sp3, and by Zic2 through their inter-
action with the activator AR1 sequence. The yeast one-hybrid screening with the AR1 sequence as bait isolated Sp3, Zic2, AP2α, and AP2β. Among these factors, AP2 family members were ruled out to play a functional role in D1A gene regulation, based on lack of specific AP2 binding activity in the striatum or in the D1A-expressing NS20Y cell line (26) and based on absent AP2 mRNA in these cells. Although AR1 has a consensus sequence for Sp1 binding, Sp1 was not cloned from this screen. Furthermore, purified Sp1 could not bind to the AR1 sequence in gel mobility shift assays despite the fact that Sp1 expressed in NS20Y nuclear extract does. These observations together suggest that Sp1 might require another factor(s) expressed in NS20Y cells to bind to AR1. Such a requirement could explain the inability of the yeast one-hybrid screen to detect Sp1. This possibility was confirmed by co-inubating purified Sp1 with NS20Y nuclear extract that had been stripped of its endogenous Sp1 and demonstrating specific binding of exogenous Sp1 to the AR1 sequence. Alternatively, modification of Sp1 by a factor(s) present in NS20Y nuclear extract was entertained since phosphorylation by the protein kinase A pathway has been reported to enhance the interaction of Sp1 with target DNA (31). However, the DNA binding activity of purified Sp1 to the AR1 sequence was not improved in the presence of 40 units of the catalytic subunit of protein kinase A (Promega) (data not shown). Post-translational modifications not involving protein kinase A cannot be excluded, and the nature of cofactor(s) required for Sp1 binding to AR1 remains to be investigated.

Although Sp1 is a ubiquitously expressed general transcription factor, considerable evidence indicates that Sp1 participates in cell type-specific gene expression as well (32, 33). Sp3, on the other hand, can function either as an activator or repressor depending on promoter context (34–37). Both Sp1 and Sp3 are expressed in most brain regions (5), and our present results indicate that Sp1 activates D1A gene transcription, whereas Sp3 represses this Sp1 effect. Thus, the ratio between Sp1 and Sp3 appears to be a regulatory mechanism by which the general activity of Sp1 is modulated in specific cells. A similar scenario has also been described for a number of other promoters (7, 29, 38). Although Sp3 can directly compete with Sp1 for the same DNA-binding site, it also appears to contain a functional repressor domain (36, 39). In addition, Sp3 isoforms are expressed in most brain regions (5), and our present results indicate that Sp1 activates D1A gene transcription, whereas abundant presence of Zic2 in the cerebellum could be an essential repressor of D1A gene transcription.

The forgoing observations taken collectively indicate that an activating cis-acting element can be used to regulate target gene transcription both by transcriptional activators and repressors like Sp1, Sp3 (7), and Zic2. We also conclude that the tissue-specific and brain regional specific expression of the D1A gene is determined by a delicate balance among several transcription factor(s), some of which recognize and interact with the same target DNA sequence. This complex mode of regulation appears to be a common mechanism for regulating D1A gene expression (42, 43).

REFERENCES
1. Courey, A. J., and Tjian, R. (1993) Transcriptional Regulation, pp. 743–771, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
2. Ding, H., Benetmanne, A. M., Suske, G., Colen, D., and Belayew, A. (1999) J. Biol. Chem. 274, 19573–19580
3. Tsai, C. N., Lee, C. M., Chien, C. K., Kuo, S. C., and Chang, Y. S. (1999) J. Biol. Chem. 274, 298–304
4. Muller, S., Maas, A., Islam, T. C., Sideras, P., Suske, G., Philippsen, S., Xanthopoulos, K. G., Hendriks, R. W., and Smith, C. I. (1999) Biochem. Cell Biol. 77, 364–369
5. Yamina, S., Lee, S. H., Minowa, T., and Mouradian, M. M. (1998) DNA Cell Biol. 17, 471–479
6. Kennett, S. B., Ulvadia, A. J., and Horowitz, J. M. (1997) Nucleic Acids Res. 25, 3110–3117
7. Hata, Y., Duh, E., Zhang, K., Robinson, G. S., and Aiiello, L. P. (1998) J. Biol. Chem. 273, 19284–19303
8. Aruga, J., Yokota, N., Hashimoto, M., Furuschi, T., Fukuda, M., and Mikoshiba, K. (1994) J. Neurochem. 63, 1880–1890
9. Aruga, J., Nagai, T., Takai, S., Yajima, S., and Mouradian, M. M. (1998) J. Biol. Chem. 271, 1034–1047
10. Yajima, S., Aruga, J., Takai, S., Yajima, S., Kusada, K., Hanamaki, M., Iwasa, T., Sugimura, H., and Mikoshiba, K. (1996) Cancer Res. 56, 377–383
11. Aruga, J., Minowa, O., Yajimuna, H., Kuno, J., Nagai, T., Noda, T., and Mikoshiba, K. (1998) J. Neurosci. 18, 284–293
12. Brown, S. A., Warburton, D., Brown, L. Y., Yu, C. Y., Roeder, E. R., Stengel-Rutkowski, S., Hennekam, R. C., and Muenke, M. (1998) Nat. Genet. 20, 180–183
13. Waddington, J. L., Molloy, A. G., O’Boyle, K. M., and Mashurano, M. (1986) Clin. Pharmacol. Ther. 9, Suppl. 4, 20–22
14. Waddington, J. L., and O’Boyle, K. M. (1998) Pharmacol. Ther. 43, 1–52
15. White, N. M., and Vaud, M. (1991) Behav. Neural Biol. 55, 255–269
16. White, N. M., Packard, M. G., and Seams, J. (1993) Behav. Neural Biol. 59, 230–241
17. Vanderhure, L. J., Schmidt, E. D., Veers, T. J., Van Moore, C. A., Tilders, F. J., and Schuitemaker, A. N. (1999) J. Neurosci. 19, 9579–9586
18. Hussain, T., and Lokhandwala, M. F. (1998) Hypertension 32, 187–197
19. Self, D. W., Barnhart, W. J., Lehman, D. A., and Nester, E. J. (1996) Science 271, 1386–1389
20. Phillips, G. D., Robbins, T. W., and Everitt, B. J. (1994) Psychopharmacology 114, 477–485
21. Decarli, A. Gingrich, J. A., Fallerade, P., Fremeau, R. T., Jr., Bates, M. D., and Caron, M. G. (1990) Nature 347, 72–76
22. Gingrich, J. A., and Caron, M. G. (1993) Annu. Rev. Neurosci. 16, 299–321
23. Wang, Y., Chan, G. L., Holden, J. E., Dobko, T., Mak, E., Schulzer, M., Huser, V. M., and Mikoshiba, K. (1996) J. Neurosci. 16, 255–269
24. Morris, E. D., Chefer, S. I., Lane, M. A., Muzic, R. F., Jr., Wong, D. F., Dannals, R. F., Matochik, J. A., Bonab, A. A., Villemonte, V. L., Grant, S. J.,ingram, D. K., Roth, G. S., and London, E. D. (1999) J. Cereb. Blood Flow Metab. 19, 218–229
25. Lee, S. H., Minowa, T., and Mouradian, M. M. (1996) J. Biol. Chem. 271, 25292–25299
26. Minowa, T., Minowa, T., and Mouradian, M. M. (1993) J. Biol. Chem. 268, 23544–23551
27. Lee, S. H., Yajima, S., and Mouradian, M. M. (1999) Neurosci. Res. 34, 225–234
28. Courey, A. J., and Tjian, R. (1988) Cell 55, 887–896
29. Hagen, O., Muller, S., Beato, M., and Suske, G. (1994) EMBO J. 13, 3843–3851
30. Zheng, X. L., Matsubara, S., Diao, C., Hallenberg, M. D., and Wong, N. C. (2000) *J. Biol. Chem.* **275**, 31747–31754
31. Rohlf, C., Ahmad, S., Borellini, F., Lei, J., and Glazer, R. I. (1997) *J. Biol. Chem.* **272**, 21137–21141
32. D’Angelo, D. D., Oliver, B. G., Davis, M. G., McCluskey, T. S., and Dorn, G. W., III (1996) *J. Biol. Chem.* **271**, 19696–19704
33. Baker, D. L., Dave, V., Reed, T., and Periasamy, M. (1996) *J. Biol. Chem.* **271**, 5921–5928
34. Birnbaum, M. J., van Wijnen, A. J., Odgren, P. R., Last, T. J., Suske, G., Stein, G. S., and Stein, J. L. (1995) *Biochemistry* **34**, 16503–16508
35. Liang, Y., Robinson, D. F., Dennig, J., Suske, G., and Fahl, W. E. (1996) *J. Biol. Chem.* **271**, 11792–11797
36. Majello, B., De Luca, P., Hagen, G., Suske, G., and Lania, L. (1994) *Nucleic Acids Res.* **22**, 4914–4921
37. Prowse, D. M., Bolgan, L., Molnar, A., and Dotto, G. P. (1997) *J. Biol. Chem.* **272**, 1308–1314
38. Ritchie, S., Boyd, F. M., Wong, J., and Bonham, K. (2000) *J. Biol. Chem.* **275**, 847–854
39. Majello, B., De Luca, P., and Lania, L. (1997) *J. Biol. Chem.* **272**, 4021–4026
40. Dennig, J., Hagen, G., Beato, M., and Suske, G. (1995) *J. Biol. Chem.* **270**, 12737–12744
41. Jung, A. R., and Bennett, J. P., Jr. (1996) *Brain Res. Dev. Brain Res.* **94**, 109–120
42. Okazawa, H., Imafuku, I., Minowa, M. T., Kanazawa, I., Hamada, H., and Mouradian, M. M. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93**, 11933–11938
43. Imafuku, I., Kamei, M., Kanazawa, I., Mouradian, M. M., and Okazawa, H. (1996) *Biochem. Cell Biol.* **74**, 736–741
ZIC2 and Sp3 Repress Sp1-induced Activation of the Human $D_{IA}$ Dopamine Receptor Gene
Young Yang, Cheol Kyu Hwang, Eunsung Junn, Gwang Lee and M. Maral Mouradian

*J. Biol. Chem.*, 2000, 275:38863-38869. doi: 10.1074/jbc.M007906200 originally published online September 12, 2000

Access the most updated version of this article at doi: 10.1074/jbc.M007906200

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 42 references, 19 of which can be accessed free at http://www.jbc.org/content/275/49/38863.full.html#ref-list-1