Dopamine receptor regulating factor, DRRF: A zinc finger transcription factor

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Dopamine receptor genes are under complex transcription control, determining their unique regional distribution in the brain. We describe here a zinc finger type transcription factor, designated dopamine receptor regulating factor (DRRF), which binds to GC and GT boxes in the D_{1A} and D_2 dopamine receptor promoters and effectively displaces Sp1 and Sp3 from these sequences. Consequently, DRRF can modulate the activity of these dopamine receptor promoters. Highest DRRF mRNA levels are found in brain with a specific regional distribution including olfactory bulb and tubercle, nucleus accumbens, striatum, hippocampus, amygdala, and frontal cortex. Many of these brain regions also express abundant levels of various dopamine receptors. *In vivo*, DRRF itself can be regulated by manipulations of dopaminergic transmission. Mice treated with drugs that increase extracellular striatal dopamine levels (cocaine), block dopamine receptors (haloperidol), or destroy dopamine terminals (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) show significant alterations in DRRF mRNA. The latter observations provide a basis for dopamine receptor regulation after these manipulations. We conclude that DRRF is important for modulating dopaminergic transmission in the brain.

Transcriptional regulation in eukaryotes is governed by the coordinated action of regulatory factors that bind to specific DNA elements. One class of these factors comprises zinc finger proteins of which Sp1 is a prototypical example, having three Cys-2–His-2 zinc finger motifs (1). Other family members, Sp2, Sp3, and Sp4, with similar structural and functional features also have been identified (2, 3). Sp1, Sp3, and Sp4 bind to the same recognition sequence (GC boxes) with similar affinities (3, 4). While Sp1 and Sp4 generally act as transcription activators, Sp3 can act as repressor or activator (5). Sp2, on the other hand, has a DNA-binding specificity different (2) from that of Sp1, Sp3, or Sp4. Several additional factors with the same zinc finger motif as Sp1 have been cloned and found to bind to the GC box sequence (6–8).

Central dopaminergic neurotransmission is crucial for normal brain function, and its aberrations are intricately involved in several neuropsychiatric disorders. The specific biological effects of dopamine are determined at least in part by the complex spatial and temporal regulation of genes encoding its receptors. To date, five different dopamine receptors have been identified and classified into two subtypes, D_1-like (D_{1A} and D_{1B} or D_5) and D_2-like (D_2, D_3 and D_4) (9). Analysis of transcription control mechanisms of D_{1A} and D_2 genes have revealed a delicate balance among several nuclear factors that tightly regulate expression of these genes (10–12). For example, the D_2 gene promoter is under strong negative control (13). One of its silencing elements (nucleotides −116 to −76), which consists of an Sp1 consensus sequence (GC box) and three TGGG repeats (GT box), interacts with Sp1, Sp3 (10), and an unidentified factor (13). In the present investigation, we characterized the nature and function of this nuclear protein, which regulates the expression of dopamine receptor genes.

Materials and Methods

Expression Cloning and 5′ Rapid Amplification of cDNA Ends. A λgt11 cDNA library constructed from murine NB41A3 cells was screened with a concatenated probe consisting of the Sp1(A) region of the rat D_2 gene by using the *in situ* filter detection method as described (7). Several clones were isolated including Sp1, Sp3, and a previously unidentified factor, designated here as dopamine receptor regulating factor (DRRF). To obtain the 5′ extent of the DRRF ORF, 5′ rapid amplification of cDNA ends (Life Technologies, Grand Island, NY) was used with mouse brain poly(A)$^+$RNA (CLONTECH), gene-specific primers 5′-CGATGCACCACGGCTCCCGA-3′ (corresponding to bases from +80 to +61 relative to the initiator codon), 5′-GGAGATGGCCATGAGCACGT-3′ (from +60 to +41), or 5′-CGGCGGCAAAGTAATCCACA-3′ (from +40 to +21). The resultant products were cloned in pCR2.1 (Invitrogen) and sequenced.

Construction of Plasmids. Full-length DRRF cDNA was constructed by ligating the original λgt11 clone with the longest 5′ rapid amplification of cDNA ends clone by using the unique *Eag*I site in DRRF. The 783-bp *Eco*RI–*Afl*III fragment representing the ORF was inserted into the *Eco*RI–*Sma*I sites of pUC19 yielding pUC-DRRF.

To construct the *Drosophila* expression vector pRm-DRRF, the 750-bp *Eco*RI–*Bam*HI fragment of pUC-DRRF was inserted into the same sites of pRmHa3 (a kind gift from C. Wu, National Cancer Institute, Bethesda, MD). The reporter plasmid BCAT-2, which has two Sp1-binding sites and a TATA box, was a kind gift from R. Tjian, (University of California, Berkeley) (14). The mammalian expression plasmid pc1-DRRF was constructed by inserting the 770-bp *Eco*RI–*Sph*I fragment of pUC-DRRF in the same sites of pcDNA1.1/amp (Invitrogen). To express tagged DRRF, the *Eco*RI–*Xba*I fragment of the coding region from pUC-DRRF was inserted into the same sites of pcDNA3.1/His C (Invitrogen), yielding pc3-DRRF. For riboprobe generation, pGEM-DRRF was constructed by subcloning the 390-bp *Nde*I–*Bam*HI fragment of the DRRF cDNA in the *Hin*dIII–*Bam*HI sites of pGEM3Zf(−). The integrity of all constructs was verified by restriction analysis and sequencing.

Immunofluorescence. The subcellular distribution of DRRF was studied by transfecting COS-7 cells with pc3-DRRF, using Lipofectamine (Life Technologies) and subjecting them to immunocytochemistry with an anti-Xpress mAb (Invitrogen) and a rhodamine-conjugated anti-mouse secondary antibody (Roche Molecular Biochemicals).

Cell Culture and Transfection. SL2, NB41A3, SH-SY5Y, and TE671 cells (all from American Type Culture Collection) as well as

Abbreviations: DRRF, dopamine receptor regulating factor; CAT, chloramphenicol acetyltransferase.

Data deposition: The sequence reported in this paper has been deposited in the GenBank database (accession no. AF283891).

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NS20Y cells (a kind gift from M. Nirenberg, National Heart, Lung, and Blood Institute, Bethesda, MD) were cultured and transfected as described (10, 15, 16), and chloramphenicol acetyl-transferase (CAT) protein was quantified by ELISA. After determining nonsaturating concentrations of reporter plasmids in the appropriate cell lines, the indicated amounts of test plasmids were used. The control vector pRmHa3 or pcDNA1.1/amp was added as appropriate to keep the total amount of plasmid DNA equal in all dishes.

Gel Mobility-Shift Assays. The following double-stranded oligonucleotides were used: D_2-TGGG, 5′-GG(AT)CCCTG(A)GGTGG(AA)GTGGG(AA)GCCTC-3′ having the GT box from the D_2 promoter (13); D_2-Sp1(A), 5′-TGTACAAGGGG(AA)CGG(AA)GGTTCCCG-3′ having a GC box from the D_2 promoter; and D_{1A}-AR1, 5′-AGGACCGCC(GG)CCCAGGGCAGGGGA-3′ having a GC box from the D_{1A} promoter (17). Underlined bases in the wild-type sequence were replaced with bases shown in parentheses in the mutant probes. *In vitro* transcription/translation (Life Technologies) was carried out with pc3-DRRF by using a 2,4,6-trinitrotoluene (TNT)-coupled reticulocyte lysate system (Promega). Double-stranded probe (20,000 cpm/binding reaction; 5 fmol), ^{32}P-end-labeled on one strand was used. In supershift assays, polyclonal antibodies to Sp1 and Sp3 (Santa Cruz Biotechnology) were coincubated with NB41A3 nuclear extract before adding the probe. The reaction mixture was electrophoresed in 4% polyacrylamide nondenaturing gel.

***In Situ* Hybridization.** To study the brain distribution of DRRF mRNA, 12-μm sections of an adult C57BL/6 mouse brain were subjected to *in situ* hybridization by using ^{35}S-UTP-labeled riboprobes according to a previously described procedure (18). pGEM-DRRF was linearized with *Hin*dIII to transcribe antisense probe from the T7 promoter and with *Bam*HI to transcribe sense probe from the SP6 promoter by using the SP6/T7 transcription kit (Roche Molecular Biochemicals). To examine the cellular colocalization of DRRF and D_2 dopamine receptor mRNAs, digoxigenin-UTP-labeled mouse DRRF and ^{35}S-UTP-labeled mouse D_2 riboprobes were used simultaneously. The DIG RNA-labeling kit (Sp6/T7) (Roche Molecular Biochemicals) was used to transcribe DRRF riboprobes from pGEM-DRRF, and the resultant products were alkaline hydrolyzed.^{35}S-UTP-labeled D_2 probes were generated as described (19). Double-label *in situ* hybridization was carried out on 12-μm sections of an adult Bl6SJL mouse brain as described (18) with stringent washing. To study the colocalization of DRRF and D_{1A} mRNAs, the two respective ^{35}S-labeled riboprobes were used on 4-μm thick adjacent coronal striatal sections from a Bl6SJL mouse brain. D_{1A} probes were generated as described (15).

Drug Treatments of Mice. Male C57BL/6 mice (25–30 g) were allowed to acclimate for at least 4 days before beginning treatment under standard conditions of 12 h light/day in a vivarium approved by the American Association for the Accreditation of Laboratory Animal Care. All experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee. In acute experiments, haloperidol (1 mg/kg; $n = 7$), cocaine (30 mg/kg; $n = 3$), caffeine (100 mg/kg; $n = 7$), or the control vehicle ($n = 10$, either 1 ml/kg 0.9% saline or 0.2 ml/kg DMSO) used to solubilize the drugs were administered i.p. 30–45 min before killing. For chronic experiments, the same doses of haloperidol ($n = 4$), cocaine ($n = 3$), caffeine ($n = 6$), or control vehicle ($n = 7$) were injected daily for 14 days before killing. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (10 mg/kg/i.p.; $n = 3$) was injected four times at 2-h intervals, and mice were killed 7 days later. After decapitation, brains were removed, frozen immediately, and sectioned coronally at 14-μm thickness. Sections including the striatum and nucleus accumbens were used for *in situ* hybridization and exposed to Biomax-MR film (Eastman Kodak) for 5 days. Optical density values were quantified by using National Institutes of Health IMAGE and corrected for background by subtracting the value in corpus callosum. Measurements obtained from 4–10 tissue sections for each brain region of an individual animal were averaged.

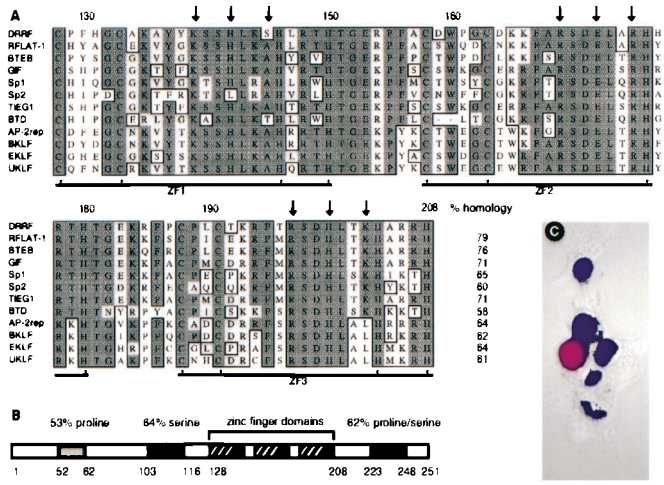

Fig. 1. (*A*) Alignment of the zinc finger domain of DRRF with the corresponding regions of other Sp1-like proteins: RFLAT-1 (AF132599; ref. 8), BTEB (Q01713; ref. 6), GIF (AF064088; ref. 7), Sp1 (A29635; ref. 1), Sp2 (A44489; ref. 2), TIEG1 (U21847; ref. 23), BTD (Q24266; ref. 45), AP-2rep (Y14295; ref. 46), BKLF (JC6100; ref. 30), EKLF (A48060; ref. 32), and UKLF (Q75840; ref. 31). Zinc finger motifs are underlined and the percentage of homology between DRRF and other proteins is indicated on the right. Nonhomologous residues are shown in boxes. Cysteine and histidine residues are marked with asterisks below the sequence. Identical amino acid residues are in dark gray shade and conservative substitutions are in light gray shade. Arrows point to amino acids that contact specific DNA bases (27, 28). (*B*) Schematic diagram of predicted DRRF protein domains. (*C*) Nuclear localization of DRRF in COS-7 cells transfected with an Xpress-tagged DRRF vector. Tagged protein (rhodamine, red) is visualized by fluorescent microscopy. Nontransfected cells show no rhodamine staining. Nuclei are counter stained dark blue with 4′,6-diamidino-2-phenylindole.

Results

Isolation and Characterization of DRRF. In our search for transcription factors that interact with the negative modulator of the D_2 dopamine receptor gene, we identified a zinc finger type protein and named it DRRF (Fig. 1) (GenBank accession no. AF283891) based on its function and expression profile described below. The screening probe consists of a TGGG repeat sequence (GT box) and an Sp1 consensus sequence (GC box), both of which bind to the same nuclear proteins (13).

The full-length DRRF cDNA has an ORF of 756 bp encoding a 251-aa polypeptide with a calculated molecular mass of 25,673 Da. The size of ^{35}S-methionine-labeled *in vitro*-translated DRRF band was consistent with this predication (data not shown). The deduced amino acid sequence of DRRF has three contiguous zinc fingers (Cys-X_{2-4}-Cys-X_3-Phe-X_5-Leu-X_2-His-X_3-His, where X represents any amino acid) located in its C terminus (Fig. 1 *A* and *B*) and identical to those found in Sp1 and other proteins in this family (Fig. 1*A*). The N-terminal portion of DRRF (amino acids 1–127) is notably rich in proline (17/127), serine (15/127), and alanine (30/127) residues, which constitute activation domains in a number of transcription factors (Fig. 1 *A* and *B*) (20). Consistent with the putative function of DRRF as a transcription factor, Xpress-tagged DRRF localized to the nucleus of COS-7 cells in transient transfection experiments

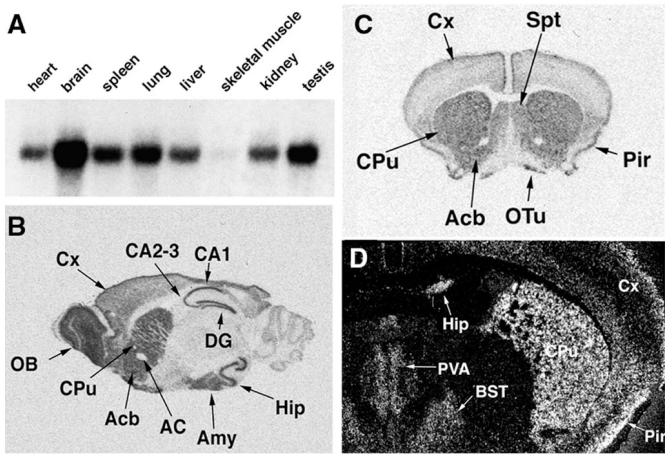

Fig. 2. (*A*) Northern blot analysis of a mouse multiple tissue blot (CLONTECH) with ^{32}P-labeled 390-bp *Nde*I–*Bam*HI fragment of the DRRF cDNA. Two micrograms poly(A)$^+$ RNA was loaded in each lane. (*B–D*) Distribution of DRRF mRNA in the adult mouse brain by *in situ* hybridization using a radiolabeled riboprobe. X-ray film autoradiogram of a sagittal (*B*) and coronal section (*C*) and dark-field photomicrograph from an emulsion autoradiogram of a coronal section (*D*). Hybridization with sense probes gave no signal. Amy, amygdala; CPu, caudate-putamen; Cx, cerebral cortex; Hip, hippocampus; Acb, accumbens; OB, olfactory bulb; PCx, pyriform cortex; DG, dentate gyrus; AC, anterior commissure; PVA, paraventricular thalamic nucleus; BST, bed nucleus striae terminalis; Pir, piriform cortex; OTu, olfactory tubercle; Spt, septum.

(Fig. 1*C*). The parental plasmid used as control gave no detectable signal (data not shown).

Distribution of DRRF mRNA. Northern blot analysis using a mouse multiple tissue blot and a DRRF probe revealed a 3.2-kb band in various tissues with highest expression in brain (Fig. 2*A*). *In situ* hybridization for DRRF mRNA on brain sections revealed abundant expression in olfactory tubercle, olfactory bulb, nucleus accumbens, and striatum (Fig. 2 *B–D*). In addition, the hippocampal CA1 region, cerebral cortex, dentate gyrus, and amygdala also express high levels of DRRF mRNA. Moderate expression is seen in CA2–3 regions of hippocampus, piriform cortex, septum, and distinct thalamic nuclei (e.g., habenula) whereas low expression is present in cerebellum.

Transcriptional Activity of DRRF. DRRF was expected to function as a transcription factor because it contains an Sp1-like zinc finger motif, has DNA-binding activity (see below), and is localized in the nucleus. To confirm this possibility, transient transfections were first carried out in *Drosophila* SL2 cells that do not express Sp family proteins (21), which bind to the same DNA sequences as DRRF, allowing analysis of DRRF function under controlled conditions. Cotransfection with a fixed nonsaturated amount of the reporter BCAT-2 and increasing amounts of the expression plasmid pRm-DRRF resulted in decreased CAT activity in a concentration-dependent manner (Fig. 3*A*). This repressive effect of DRRF on the promoter in BCAT-2 is contrary to the strong activation induced by Sp1 (10). Coexpression of these two proteins in SL2 cells revealed that DRRF inhibits Sp1-induced activation of the promoter in BCAT-2 (Fig. 3*B*).

DRRF was originally cloned by virtue of its binding to the D_2 promoter, and it is present in brain regions that have abundant levels of dopamine receptors. Thus, the transcriptional activity of DRRF on dopamine receptor genes was studied in appropriate mammalian cell lines after establishing a nonsaturated amount of each reporter construct individually. In the *D_2*-expressing NB41A3 cells, DRRF potently inhibited transcription from the

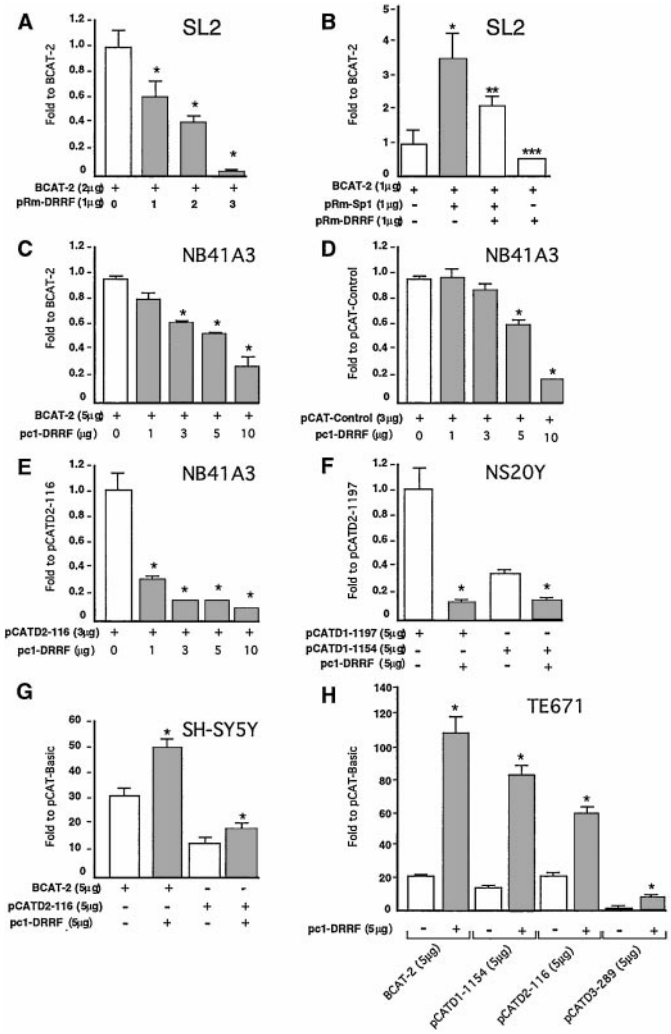

Fig. 3. Functional analysis of DRRF by cotransfection CAT assays. (*A*) SL2 cells cotransfected with a fixed amount of BCAT-2 and increasing amounts (μg) of a DRRF expression plasmid. (*B*) Competition between Sp1 and DRRF on BCAT-2 in SL2 cells. (*C–E*) NB41A3 cells cotransfected with rising amounts of pc1-DRRF and fixed amounts of BCAT-2 (*C*), pCAT-Control (*D*), or pCATD2–116 (*E*). (*F*) Effect of DRRF on the D_{1A} dopamine receptor promoter in pCATD1–1197 and pCATD1–1154 tested in NS20Y cells. (*G*) Effect of DRRF on BCAT-2 and on the D_2 promoter in SH-SY5Y. (*H*) Effect of DRRF on BCAT-2, D_{1A}, D_2, and D_3 promoters in TE671 cells. Data shown are means ± SEM for triplicates, and each experiment was repeated at least twice. (*A–G*) *, ANOVA, $P < 0.05$ compared to the open bar in the absence of DRRF in each respective experiment. In *B*, **, $P < 0.05$ compared to the second bar, and ***, $P < 0.05$ compared to the first bar.

synthetic promoter in BCAT-2, the simian virus 40 promoter in pCAT-Control (Promega), and the D_2 promoter in pCATD2–116 (13) (Fig. 3 *C–E*), suggesting that DRRF interacts and modulates Sp1-binding sites in these promoters. Similarly, DRRF repressed the D_{1A} receptor promoter in the D_{1A}-expressing NS20Y cells (Fig. 3*F*). Inhibition of pCATD1–1197 was 80% and that of pCATD1–1154 was 45% (17). On the other hand, DRRF activated the D_2 promoter in SH-SY5Y cells (Fig. 3*G*) and the D_{1A}, D_2, and D_3 promoters (16) in TE671 cells (Fig. 3*H*). Thus, DRRF regulates all three dopamine receptor promoters tested but has opposing effects depending on cellular context.

DNA-Binding Profile of DRRF. The specific binding of *in vitro*-translated DRRF to Sp1 consensus sequences in the *D_2* gene was

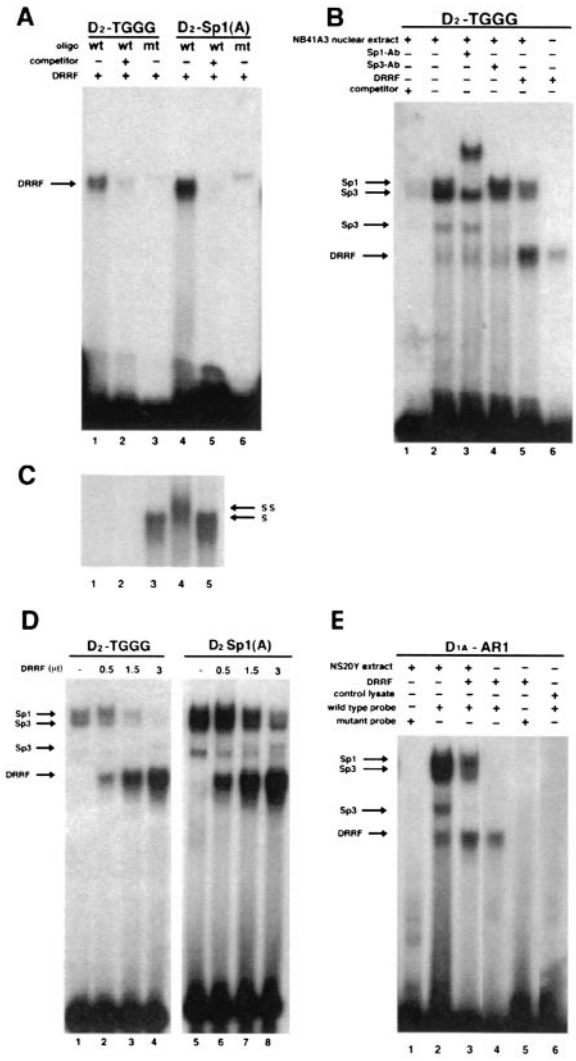

Fig. 4. Gel mobility-shift assays. (*A*) One microliter of *in vitro*-translated DRRF with TGGG repeat (GT box) and Sp1(A) (GC box) probes from the D_2 promoter, or their corresponding mutated probes, in the presence or absence of cold competitor. (*B*) Supershift assay with antibodies to Sp1 and Sp3 on the D_2-TGGG probe. Nuclear extract from NB41A3 cells was preincubated with antibodies against Sp1 or Sp3 (lanes 3 and 4, respectively). *In vitro*-translated DRRF was used in lanes 5 and 6. (*C*) Gel supershift (ss) of *in vitro*-translated Xpress-tagged DRRF and the Sp1(A) probe with anti-Xpress antibody (lane 4). Lane 1 is control reticulocyte lysate. Lane 2 is control vector pcDNA3.1. Lane 3 is DRRF expressed from pc3-DRRF with no antibody. Lane 5 is DRRF expressed from pc3-DRRF with the control antibody anti-myc, which had no effect. (*D*) Competitive displacement of Sp1 and Sp3 binding to TGGG repeat and Sp1(A) probes from the D_2 promoter by DRRF. Probes were incubated in the presence of a constant amount of NB41A3 extract (as source of endogenous Sp1 and Sp3) and increasing amounts of *in vitro*-translated DRRF. (*E*) Gel-shift assay of DRRF on the GC box in the D_{1A}-AR1 sequence. Control lysate is from *in vitro* transcription/translation kit without template DNA.

confirmed by gel-shift analysis (Fig. 4*A*). Mutant probes failed to bind to DRRF. The ability of DRRF to interact with Sp1-binding sites in D_{1A} and D_2 genes was studied next by using nuclear extracts from cells expressing the respective dopamine receptors. Using extract from the D_2-expressing NB41A3 cells, four major retarded bands were observed with the TGGG repeat (GT box) probe (Fig. 4*B*, lane 2). The slowest running band was supershifted with Sp1 antibody (lane 3), and the two bands with intermediate mobility were abrogated with Sp3 antibody (lane 4). These antibodies did not affect the fastest running band, which appeared to have a similar mobility to *in vitro*-translated DRRF (Fig. 4*B*, lane 6). The slightly slower mobility of the DRRF complex in lane 6 is due to the Xpress-epitope. As expected, the band retarded by *in vitro*-translated DRRF could be supershifted by anti-Xpress tag antibody (Fig. 4*C*). Coincubating NB41A3 nuclear extract with *in vitro*-translated DRRF significantly diminished the ability of Sp1 and Sp3 to bind to the probe (Fig. *4B*, lane 5). On the other hand, the binding affinity of recombinant DRRF was increased in the presence of extract compared to that of DRRF alone (lanes 5 and 6). The competition of DRRF for Sp1 and Sp3 binding was dose dependent (Fig. 4*D*). These gel retardation studies using limiting amounts of probe also revealed that recombinant DRRF does not affect the mobility of bands shifted by Sp1 or Sp3, suggesting that these factors bind to DNA competitively rather than simultaneously.

DRRF also binds to the Sp1 consensus sequence in the AR1 region of the D_{1A} promoter (-1154 to -1134) (17) (Fig. 4*E*) and competes with Sp1 and Sp3 (Fig. 4*E*, lane 2). Mutant probe failed to bind to DRRF (Fig. 4*E*, lane 5) and control lysate did not bind to the AR1 sequence (Fig. 4*E*, lane 6), demonstrating the DNA-binding specificity of DRRF.

Colocalization of *DRRF* with D_{1A} or D_2 Dopamine Receptor Messages in Striatal Neurons. Double-label *in situ* hybridization using digoxigenin-labeled DRRF and ^{35}S-labeled D_2 riboprobes demonstrated moderate overlap of the two signals in the striatum of the mouse brain (Fig. 5 *A* and *B*). Some cells express only DRRF mRNA and few neurons express only D_2 mRNA. The D_2 signal is strong and is present in discrete neurons whereas the DRRF signal is more diffuse, suggesting that it also may be present in other cells. Quantitative analysis revealed that ≈57% of DRRF-positive cells coexpress D_2 mRNA. *In situ* hybridization of DRRF and D_{1A} receptor mRNAs on thin adjacent striatal sections also revealed that these two genes are coexpressed in approximately one-third of DRRF-positive neurons (Fig. 5 *C* and *D*).

***In Vivo* Regulation of DRRF upon Perturbation of Dopaminergic Neurotransmission.** *DRRF* is highly expressed in brain regions that have abundant dopaminergic terminals and express high levels of dopamine receptors. We, therefore, sought to determine whether DRRF can be regulated by drugs that modulate dopaminergic neurotransmission (Fig. 6). Acute administration of cocaine significantly reduced DRRF mRNA levels in the core (19%, $P < 0.05$) and shell (24%, $P < 0.05$) regions of the mouse nucleus accumbens. Chronic administration of haloperidol decreased DRRF mRNA in the striatum (19%, $P < 0.05$) and nucleus accumbens core (23%, $P < 0.005$). In addition, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine caused up-regulation of DRRF mRNA in the striatum (24%, $P < 0.05$) and in nucleus accumbens core (23%, $P < 0.05$). No effect was seen after acute or chronic caffeine administration.

Discussion

Precise transcriptional regulation of dopamine receptor genes in the brain is crucial for normal neurobehavioral function. Several classes of nuclear proteins are intricately involved in controlling expression of these genes. The D_2 gene, which is regulated by many antiparkinsonian and antipsychotic drugs, is under tight inhibitory control operating at an element that has consensus Sp1-binding sites (GC and GT boxes) (10, 13). Three nuclear factors, Sp1, Sp3, and DRRF, bind to this negative regulatory element. Sp1 activates the D_2 promoter, Sp3 does not modulate it in transiently transfected cells (10), whereas DRRF silences it in certain neuronal populations. The present report further demonstrates that DRRF regulates not only the D_2 receptor gene but the D_{1A} and D_3 promoters as well.

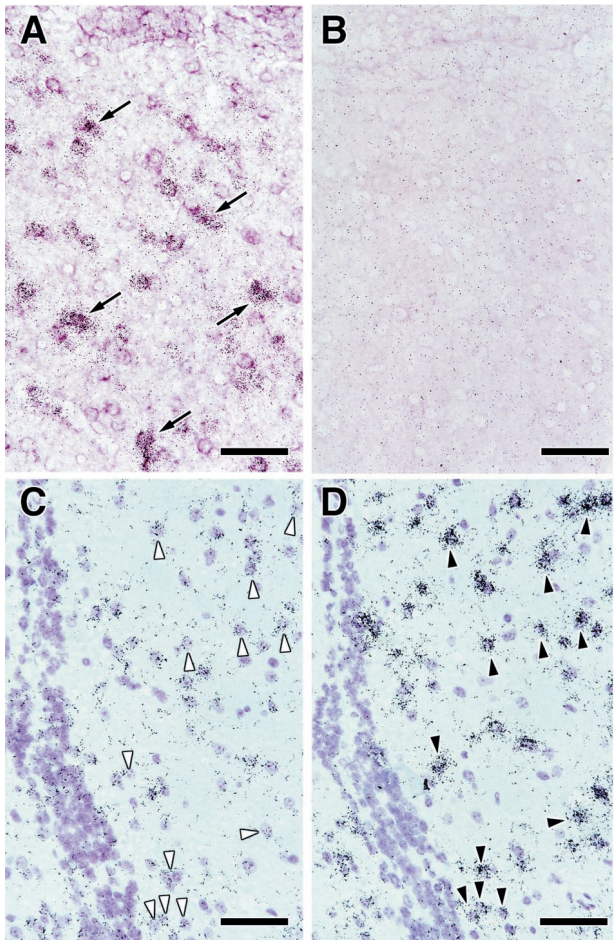

Fig. 5. Colocalization of DRRF with D_2 and D_{1A} dopamine receptor messages in the adult mouse striatum by *in situ* hybridization. (*A*) Antisense DIG-labeled DRRF (purple color) and ^{35}S-labeled D_2 (silver grains) riboprobes were hybridized simultaneously. Arrows point to cells that coexpress both signals. (*B*) Corresponding sense probes labeled similarly to *A* indicate specificity of both signals. (*C* and *D*) Hybridization with ^{35}S-labeled DRRF and D_{1A} antisense riboprobes, respectively, on 4-μm adjacent mouse brain sections. Arrowheads point to cells that coexpress both messages. (Scale bars = 50 μm.)

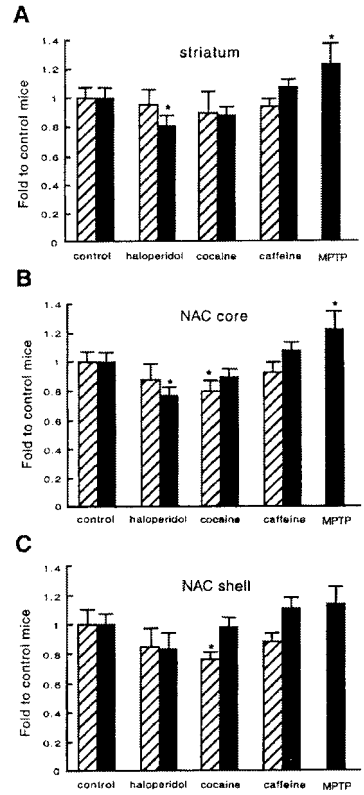

Fig. 6. Regulation of DRRF message *in vivo*. DRRF mRNA levels in the brains of mice treated acutely (hatched bars) or chronically (black bars) relative to control vehicle injected animals. Values are means ± SEM. *, ANOVA, $P < 0.05$ compared to vehicle-treated control group. NAC, nucleus accumbens.

The deduced amino acid sequence of DRRF reveals three contiguous Sp1-like zinc fingers near the C terminus and places DRRF in the multigene Sp1 family (1). Based on structural similarities among their zinc finger domains, members of this family are classified into three subgroups (22): (*i*) the four Sp transcription factors, Sp1, Sp2, Sp3, and Sp4 (2), (*ii*) RFLAT-1, BTEB1, mGIF, and TIEGs (6–8, 23), and (*iii*) the Kruppel-like factors XKLFs (24). Phylogenetic analysis of zinc fingers reveal that DRRF belongs to the second subgroup.

Unlike its similarity to Sp family proteins in the zinc finger domain, DRRF lacks a highly conserved glutamine-rich transactivation domain or serine/threonine stretches in its N-terminal region (21). Instead, DRRF has proline- and serine-rich domains in its N terminus and a proline/serine-rich domain in its C terminus. Proline-rich domains may contain discrete activation and repression subdomains (25) and also can mediate protein–protein interactions (26).

Studies of the DNA-binding characteristics of zinc finger proteins have suggested that residues KHA within the first, RER within the second, and RHK within the third zinc finger motifs contact specific nucleotides (27, 28). These critical amino acids are conserved in all Sp family members except in Sp2 and XKLFs. Consistently, Sp1, Sp3, and Sp4 recognize classical Sp1-binding sites with identical affinities (3, 29). Sp2, on the other hand, which has a leucine in place of the critical histidine in the first zinc finger, binds to a GT-rich element and not to the GC box (2). Similarly, EKLF, UKLF, and BKLF, in which the lysine in the third zinc finger is replaced by leucine, have a binding preference for GT rather than the classical GC box (30–32). DRRF is the only member of this family with a serine instead of the critical alanine in the first zinc finger. This substitution of a hydrophobic with a hydrophilic amino acid could determine the DNA-binding preferences of DRRF. Our present data show that DRRF binds to both GC and GT boxes and can regulate several types of promoters.

DRRF recognizes the same DNA sequences as Sp1 and Sp3 and competes with them effectively for the same sites. Unlike Sp1 and Sp3, which bind to their target sequences simultaneously in the D_{1A} and D_2 dopamine receptor genes (Fig. 4 *B–D*), DRRF displaces them. Another zinc finger protein Zic2 also can compete with Sp1/Sp3 binding to their consensus sequence in the D_{1A} gene and represses Sp1-induced activation of this promoter (12). Furthermore, DRRF represses the D_{1A} promoter in pCATD1–1197 to a greater extent than the shorter variant in pCATD1–1154. The cell-specific regulatory element present immediately upstream of the Sp1 consensus sites in pCATD1–1154, which is activated by meis2 and repressed by TGIF (15), appears to influence the function of DRRF. The recognition of specific DNA elements by more than one nuclear protein and the competition among these proteins appears to be a common mechanism to maintain a homeostatic balance of dopamine receptors in the brain (10, 15).

DRRF represses or activates transcription from several different promoters depending on cellular context similar to a

number of other eukaryotic dual function regulators (6, 33–35). Although the molecular determinants underlying such actions remain to be fully characterized, the coexpression of other proteins and their abundance level are likely important variables. Furthermore, the presence of factors such as DRRF and Zic2 with unique brain regional distributions confers relative cell specificity to certain ubiquitous factors like Sp1.

Consistent with the regulation of dopamine receptor genes by DRRF in cultured cells, DRRF mRNA can be regulated *in vivo* by measures that alter dopamine receptor expression. Chronic dopamine receptor blockade, which is known to cause up-regulation of D_{1A}, D_2, and D_3 dopamine receptors (36–38), resulted in decreased DRRF mRNA levels in striatum and nucleus accumbens core. Considering the ability of DRRF to repress transcription in cultured cells, the down-regulation of DRRF message *in vivo* could underlie the haloperidol-induced up-regulation of dopamine receptor message due to derepression.

The psychostimulant cocaine given acutely resulted in decreased DRRF mRNA levels in both core and shell regions of nucleus accumbens. Cocaine is known to cause a large increase in extracellular dopamine in striatum and nucleus accumbens, which could conceivably mediate the postsynaptic decrease in DRRF. This hypothesis is supported by the fact that caffeine, which produces a much smaller increase in extracellular striatal dopamine relative to cocaine (39), did not alter DRRF mRNA. In keeping with the suggested role of DRRF as a regulator of dopamine receptor gene transcription, the cocaine-induced decrease in DRRF mRNA might contribute to alterations in dopamine receptors seen after acute cocaine administration (40, 41).

Finally, destruction of dopamine nerve terminals in striatum and nucleus accumbens by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine resulted in increased DRRF mRNA levels in both regions. It is possible that the decrease in striatal dopamine (42) mediates the increase in DRRF mRNA, which leads to alterations in dopamine receptor expression seen after chronic 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine treatment (43, 44). Taken together, these *in vivo* data provide evidence that alterations in dopaminergic transmission result in regulation of DRRF, which in turn could mediate altered expression of dopamine receptors.

In conclusion, the unique expression pattern of *DRRF* in the brain as well as its regulation by pharmacological agents that modulate dopaminergic neurotransmission suggests an important homeostatic role for DRRF in neurobehavioral functions.

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