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Human DJ-1-specific Transcriptional Activation of Tyrosine Hydroxylase Gene*[S]

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Loss-of-function mutation in the *DJ-1* gene causes a subset of familial Parkinson disease. The mechanism underlying DJ-1-related selective vulnerability in the dopaminergic pathway is, however, not known. DJ-1 has multiple functions, including transcriptional regulation, and one of transcriptional target genes for DJ-1 is the tyrosine hydroxylase (TH) gene, the product of which is a key enzyme for dopamine biosynthesis. It has been reported that DJ-1 is a neuroprotective transcriptional co-activator that sequesters a transcriptional co-repressor polypyrimidine tract-binding protein-associated splicing factor (PSF) from the *TH* gene promoter. In this study, we found that knockdown of human DJ-1 by small interference RNA in human dopaminergic cell lines attenuated *TH* gene expression and 4-dihydroxy-L-phenylalanine production but that knockdown or knock-out of mouse *DJ-1* in mouse cell lines or in mice did not affect such expression and TH activity. In reporter assays using the human *TH* gene promoter linked to the luciferase gene, stimulation of *TH* promoter activity was observed in human cells, but not mouse cells, that had been transfected with DJ-1. Although human DJ-1 and mouse DJ-1 were associated either with human or with mouse PSF, *TH* promoter activity inhibited by PSF was restored by human DJ-1 but not by mouse DJ-1. Chromatin immunoprecipitation assays revealed that the complex of PSF with DJ-1 bound to the human but not the mouse *TH* gene promoter. These results suggest a novel species-specific transcriptional regulation of the *TH* promoter by DJ-1 and one of the mechanisms for no reduction of TH in *DJ-1*-knock-out mice.

Parkinson disease (PD)[3] is the most common movement disorder caused by gradual loss of dopaminergic neurons in the substantia nigra pars compacta. Although most cases are sporadic, 5–10% of PD patients carry mutations with a Mendelian inheritance, and mutations in *parkin*, *DJ-1*, and *PINK1* genes have been linked to autosomal recessive forms of PD (1–3). Although a large number of studies have been carried out to determine whether inactivation of each of these genes in mice or fruit flies results in progressive and selective loss of dopaminergic neurons, almost all of the studies, including studies using mice with single or triple deficiency in *parkin*, *DJ-1*, and *PINK1 genes*, showed no loss of dopaminergic neurons in the substantia nigra pars compacta (4–12).

DJ-1 was first identified by our group as a novel oncogene that transformed mouse NIH3T3 cells in cooperation with activated H-*ras* (13). Deletion and point (L166P) mutations of DJ-1 have been shown to be responsible for the onset of familial Parkinson disease, PARK7 (2), and other homozygous and heterozygous mutations of DJ-1 have been identified in patients with familial or sporadic PD (14–16). DJ-1 is a multifunctional protein and plays roles in transcriptional regulation (17–25) and antioxidative stress function (26–31), and loss of its functions leads to the onset of Parkinson disease and cancer. Although DJ-1 does not directly bind to DNA, DJ-1 acts as a co-activator to activate various transcription factors, including the androgen receptor, p53, PSF, and Nrf2, by sequestering their inhibitory factors (17–21).

Dopamine is synthesized by two steps as follows. Tyrosine is converted to L-DOPA by tyrosine hydroxylase (TH), and L-DOPA is then converted to dopamine by L-DOPA decarboxylase. TH is, therefore, a key enzyme for dopamine biosynthesis and is used as a marker for dopaminergic neurons. It has been reported that PSF, a transcription co-repressor, binds to the promoter region of the *TH* gene to repress its expression and that human DJ-1 binds to PSF to sequester the PSF·co-repressor complex, leading to activation of *TH* gene expression in cultured human cells (20). In addition to transcriptional activation of the *TH* gene by DJ-1, we have reported that DJ-1 activated TH and L-DOPA decarboxylase through direct binding to TH and L-DOPA decarboxylase in an oxidative status of DJ-1-dependent manner (23). Although human DJ-1 activates *TH* gene expression in cultured human dopaminergic cells, the reason why knock-out of DJ-1 expression did not affect the dopamine level in mice is not known.

In this study, we compared the roles of human DJ-1 and mouse DJ-1 in expression of the *TH* gene, and we found that

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[3] The abbreviations used are: PD, Parkinson disease; TH, tyrosine hydroxylase; hTH, human TH; mTH, mouse TH; L-DOPA, 4-dihydroxy-L-phenylalanine; PSF, polypyrimidine tract-binding protein-associated splicing factor.

DJ-1 activates TH expression at the transcriptional level in human cells but not in mouse cells due to loss of PSF·DJ-1 binding to the mouse *TH* gene, suggesting different regulatory systems of the *TH* gene by DJ-1 in humans and mice.

EXPERIMENTAL PROCEDURES

Cell Culture and Knockdown of DJ-1—Human SK-N-SH cells (RCB0426 of Biological Resource) were provided by Riken BRC, which is participating in the National Bio-Resources Project of the MEXT, Japan. Human SH-SY5Y and SK-N-SH cells and mouse Neuro-2a cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% calf serum. The nucleotide sequences for siRNAs targeting DJ-1 and PSF were as follows: 5′-UGGAGACGGUCAUCCCU-GUdTdT-3′ (upper strand) and 3′-dTdTACCUCUGC-CAGUAGGGACA-5′ (lower strand) for human DJ-1, 5′-CCUUGCUAGUAGAAUAAACdTdT-3′ (upper strand) and 3′-dTdTGGAACGAUCAUCUUAUUUG-5′ (lower strand) for mouse DJ-1, 5′-UAUGGAGAACCAGGAGAAGTT-3′ (sense strand) and 5′-CUUCUCCUGGUUCUCCAUATT-3′ (antisense strand) for human PSF, and 5′-GGAUUUGUUUG-GCAUUAUATT-3′ (sense strand) and 5′-UAUAAUGC-CAAACAAAUCCTA-3′ (antisense strand) for mouse PSF. siRNA for the luciferase gene was purchased from Greiner (Frickenhausen, Germany). Twenty-five pmol of siRNA was transfected into cells using Lipofectamine 2000 (Invitrogen) according to the supplier's protocol.

Primary Neuronal Culture of Mouse Mesencephalon—DJ-1-knock-out mice were kindly provided by Dr. J. Shen (5). Primary neuronal cultures of the mouse mesencephalon from wild-type and DJ-1-knock-out mice were carried out as described previously (32). Briefly, mesencephalons were dissected from mouse embryos on the 16th day of gestation. Dissected regions included dopaminergic neurons from the substantia nigra. Neurons were mechanically dissected and plated out onto 0.1% polyethyleneimine-coated 24-well plates at a density of 2.5×10^6 cells/well. Cells were cultured for 2 days in DMEM with 10% fetal calf serum, and the medium was changed to DMEM containing 2% B-27 supplement (Invitrogen) and 2 mg/ml aphidicolin (Sigma) without fetal calf serum on third day. Mice were treated in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals.

RT-PCR and Real-time PCR—Nucleotide sequences of primers used for RT-PCR and real-time PCR were as follows: human DJ-1-sense (RT-PCR), 5′-GGTGCAGGCT-TGTAAACATATAAC-3′; human DJ-1-antisense (RT-PCR), 5′-CTCTAAGTGATCGTCGCAGTTCGC-3′; mouse DJ-1-sense (RT-PCR), 5′-GCTTCCAAAAGAGCT-CTGGTCA-3′; mouse DJ-1-antisense (RT-PCR), 5′-GCT-CTAGTCTTTGAGAACAAGC-3′; human TH-sense (RT-PCR), 5′-CGGGTCTCTAGATGGTGGATTTT-3′; human TH-antisense (RT-PCR), 5′-GCTGTGGCCTTT-GAGGAGAA-3′; mouse TH-sense (RT-PCR), 5′-GCC-AAGGACAAGCTCAGGAA-3′; mouse TH-antisense (RT-PCR), 5′-CTCAGTGCTTGGGTCAGGGT-3′; human PSF-sense (RT-PCR), 5′-ACCACCAGCAGCAT-CACC-3′; human PSF-antisense (RT-PCR), 5′-TCCC-AACAAACAACCGACA-3′; human PSF-sense (RT-PCR), 5′-GGCATGGGCCTCAATCAGAA-3′; human PSF-antisense (RT-PCR), 5′-CAGGCATTTTGCCGCCTTTG-3′; mouse PSF-sense (RT-PCR), 5′-GGCATGGGCCTCAACCA-GAA-3′; mouse PSF-antisense (RT-PCR), 5′-CGGGCATTT-TGCCGCCTTTT-3′; human β-actin-sense (RT-PCR), 5′-CCGACAGGATGCAGAAGGAG-3′; human β-actin-antisense (RT-PCR), 5′-GTGGGGTGGCTTTTAGGATG-3′, mouse β-actin-sense (RT-PCR), 5′-CCTAGGCACCAGGGT-GTGAT-3′; mouse β-actin-antisense (RT-PCR), 5′-GCTC-GAAGTCTAGAGCAACA-3′; human DJ-1-sense (real-time PCR), 5′-TTGTAGGCTGAGAAATCTCTGTG-3′; human DJ-1-antisense (real-time PCR), 5′-ATCCATTCTCACTGT-GTTCGC-3′; mouse DJ-1-sense (real-time PCR), 5′-GCAC-CGCTTGTTCTCAAAG-3′; mouse DJ-1-antisense (real-time PCR), 5′-TGGCAGGAGCTTGGTAAACT-3′; human TH-sense (real-time PCR), 5′-GCAGGCAGAGGCCATCATGT-3′; human TH-antisense (real-time PCR), 5′-GGCGATCT-CAGCAATCAGCT-3′; mouse TH-sense (real-time PCR), 5′-CGGTGTACTGGTTCACTGTG-3′; mouse TH-antisense (real-time PCR), 5′-CGCATGCAGTAGTAAGATGT-3′; human PSF-sense (real-time PCR), 5′-CGGCATGGGCCT-CAATCAGA-3′; human PSF-antisense (real-time PCR), 5′-CAGCAACGACGGGCTTGGAA-3′; mouse PSF-sense (real-time PCR), 5′-GGGCATGGGCCTCAACCAGA-3′; mouse PSF-antisense (real-time PCR), 5′-GACGACGGGCTTG-GATTCC-3′; human β-actin-sense (real-time PCR), 5′-CCC-TAAGGCCAACCGRGAAA-3′; human β-actin-antisense (real-time PCR), 5′-ACGACCAGAGGCATACAGGGA-3′; mouse β-actin-sense (real-time PCR), 5′-CCCTAAGGC-CAAGCGTGAAA-3′; and mouse β-actin-antisense (real-time PCR), 5′-ACGACCAGAGGCATAGAGGGA-3′. Forty-eight h after transfection of siRNA into cells, total RNAs were prepared and subjected to semiquantitative RT-PCR and quantitative RT-PCR (real-time PCR) analyses as described previously (33). Reverse images of black and white staining in semiquantitative RT-PCR are shown.

Luciferase Activity—The promoter regions of human and mouse *TH* genes were amplified by RT-PCR using specific primers and total RNA from human SH-SY5Y cells and mouse Neuro-2a cells as templates. Nucleotide sequences of oligonucleotides used for PCR primers were as follows: human *TH* promoter sense (5′-TCAGAACCTCAGTC-CTCGCATC-3′) and antisense (5′-GGAGATCTCAACAGG-GACTCAAACACCAGG-3′) and mouse *TH* promoter sense (5′- GGGGTACCATGATTGGTATGGCTGGGGTCC-3′) and antisense (5′-GGGGATCCAGAAGTTGCTCCAGAT-ACCCCT-3′). Amplified cDNAs containing 3416 and 3607 bp, corresponding to human and mouse *TH* genes, respectively, were digested with KpnI/BglII and KpnI/BamHI for human and mouse *TH* genes, and fragments obtained were inserted into KpnI/BglII sites of a pGL-3 basic vector (Promega, Madison, WI). These plasmids were named phTH-Luc and pmTH-Luc, respectively. To construct phTH-Luc and pmTH-Luc deleting the region spanning −2790 to −2829, an in-fusion system (Clontech) was used. Nucleotide sequences of oligonucleotides used for PCR primers in the in-fusion sys-

tem were as follows: human TH-del-Luc-sense1, 5′-CAGAACCTCAGTCCTCGCATC-3′; human TH-del-Luc-antisense1, 5′-GCTTAATCACCCAGATTAATC-3′; human TH-del-Luc-sense2, 5′-CTCGGGCTGAGAGGCTGTTGA-3′; human TH-del-Luc-antisense2, 5′-GGAGATCTCAACAGGGACTCAAACACCAGG-3′; mouse TH-del-Luc-sense1, 5′-GGGGTACCCTGACTTAGCATTTATCCTG-3′; mouse TH-del-Luc-antisense1, 5′-GGATTCAGGGCCCCCAGCTGC-3′; mouse TH-del-Luc-sense2, 5′-CACCCCTGTCTTCCTTGA-3′; and mouse TH-del-Luc-antisense2, 5′-GGGGATCCAGAAGTTGCTCCAGATAC-3′. PCR products were mixed and reacted according to the manufacturer's protocol. These plasmids were named phTH-del-Luc and pmTH-del-Luc, respectively. SH-SY5Y and Neuro-2a cells in 24-well dishes were transfected with 1.0 μg of pTH-Luc and various amounts (0–1.0 μg) of pcDNA3-HA-DJ-1, pcDNA3-FLAG-DJ-1, or pcDNA3-FLAG-PSF (provided by Dr. Watanabe at Nihon University, Tokyo, Japan) together with 1.0 μg of pSV2-β-gal by the calcium phosphate method (33). Two days after transfection, whole cell extract was prepared by the addition of Triton X-100-containing solution from the Pica gene kit (Wako Pure Chemicals, Osaka, Japan) to cells. About a one-fifth volume of the extract was used for β-galactosidase assay to normalize transfection efficiencies as described previously (34), and luciferase activity due to reporter plasmids was determined using a luminometer (Luminocounter Lumat LB 9507, EG&G Berthold, Bad Wildbad, Germany). Proteins in aliquots of the cell extract were analyzed by Western blotting with an anti-FLAG antibody (M2, Sigma) and visualized as described previously (23). The same experiments were repeated at least three times.

Tyrosine Hydroxylase Activity Assay—TH activity in human and mouse cells transfected with control (siRNA for the luciferase gene), DJ-1-specific siRNA, or PSF-specific siRNA was determined by measuring L-DOPA formation as described previously (35). Briefly, 2 days after siRNA transfection, human or mouse cells plated in a 24-well dish were rinsed twice with 1 ml of physiological medium and incubated for 2 h at 37 °C with 100 mM L-tyrosine hydrochloride (Sigma) and 500 μM *n*-hydroxybenzylhydrazine (Sigma), a selective L-DOPA decarboxylase inhibitor. After the incubation, the medium was discarded, and the cells were collected, resuspended with 0.1 N perchloric acid, and sonicated. A 10-μl aliquot was used to determine protein concentration, and the remainder was centrifuged at 12,000 rpm for 30 min at 4 °C. A 100-μl aliquot of the supernatant was injected into a CAPCELL PAK CR column (2.0-mm inner diameter × 250 mm, particle size of 5 μm, SHISEIDO, Tokyo, Japan), and TH activity was measured using HPLC (AKTA explorer 10 S/100, GE Healthcare). Mobile phase solution consisted of 10 mM $HCONH_4$ (pH 3.0)/CH_3CN.

Western Blotting and Antibodies—To examine expression levels of proteins in cells, proteins were extracted from cells with a buffer containing 150 mM NaCl, 1 mM EDTA, 20 mM Tris (pH 8.0), and 0.5% Nonidet P-40. Proteins were then separated on a 12.5% polyacrylamide gel and subjected to Western blotting with respective antibodies. Proteins on the membrane were reacted with an IRDye 800-conjugated (Rockland, Philadelphia, PA) or Alexa Fluor 680-conjugated secondary antibody (Molecular Probes, Inc., Eugene, OR) and visualized by using an infrared imaging system (Odyssey, LI-COR, Lincoln, NE). Antibodies used were anti-TH (1:1000, Chemicon, Temecula, CA), anti-PSF (1:1500, Sigma), anti-β-actin (1:4000, Chemicon), anti-DJ-1 (1:4000, 3E8, MBL, Nagoya Japan) and anti-SP1 (1:1000, Millipore, Billerica, MA).

In Vivo Co-immunoprecipitation Assay—Proteins were extracted from human and mouse cells by the procedure described previously (23). Proteins were immunoprecipitated with a rabbit anti-DJ-1 antibody (1:500) or normal IgG, and precipitates were analyzed by Western blotting with anti-PSF (1:1500) (Sigma) or mouse anti-DJ-1 antibody (1:1000) (3E8, MBL). The rabbit anti-DJ-1 antibody was prepared by us as described previously (13).

Pull-down Assay—^{35}S-Labeled p53 and PSF from human and mouse origins were synthesized *in vitro* using the reticulocyte lysate of the TNT transcription-translation-coupled system (Promega). Labeled proteins were mixed with GST or GST-DJ-1 expressed in and prepared from *Escherichia coli* at 4 °C for 60 min in a buffer containing 150 mM NaCl, 5 mM EDTA, 50 mM Tris (pH 7.5), 0.05% bovine serum albumin, and 0.1% Nonidet P-40 (Nonidet P-40). After washing with the same buffer, the bound proteins were separated in a 10% polyacrylamide gel containing SDS and visualized by fluorography.

Chromatin Immunoprecipitation (ChIP) Assay—ChIP assays were performed according to the protocol of the EZ ChIP kit (Millipore) with the following modifications. After proteins extracted from cells had been cross-linked with DNA, cell pellets were resuspended in an SDS-lysis buffer and sonicated on ice using a sonicator (Branson Ultrasonics Corp., Danbury, CT) with 16 sets of 12-s pulses each time at 80% maximum power. Genomic DNA was sheared to 300–1200 base pairs in length. Chromatin solution from 1×10^6 cells/dish was preincubated with protein A-agarose and incubated with species-matched IgG or with specific antibodies overnight at 4 °C. DNA fragments immunoprecipitated were then used as templates for PCR with hot start Ex taq (TaKaRa Bio, Kyoto, Japan) and reacted for the first 3 min at 95 °C; 34 cycles of 30 s at 95 °C, 30 s at 57 °C, and 30 s at 72 °C; and one cycle of 2 min at 72 °C. Sequences of oligonucleotides used for PCR primers were as follows: human TH-promoter-sense, 5′-GAGCCTTCCTGGTGTTTGTG-3′; human TH-promoter-antisense, 5′-CTCTCCGATTCCAGATGGTG-3′; mouse TH-promoter-sense, 5′-CCTCACTTCCACCAACTA-3′; mouse TH-promoter-antisense, 5′-CCAACCTCTCCATTTCAGAC-3′; human SP1 binding sequence-sense, 5′-GGTTAAATCCACATCCGGCA-3′; human binding sequence-antisense, 5′-CCTGGTGAAGCTCTGCAT-3′; mouse SP1 binding sequence-sense, 5′-GGCCAGTCTTAGATACAC-3′; mouse SP1 binding sequence-antisense, 5′-CCTCCGTCCCATTAGATCTA-3′; human TH-promoter-sense (negative control), 5′-CAATGGGAACTGAGGGACAG-3′; human TH-promoter-antisense (negative control), 5′-ATGTCTACTCCGCAGTTCTC-3′; mouse TH-promoter-sense (negative control), 5′-GGGTGGACTGGTCAAAGTGG-3′; and mouse TH-promoter-antisense (negative control), 5′-GAC-

TABLE 1

Nucleotide sequences of oligonucleotides used for probes

Sense and antisense oligonucleotides were annealed and their 3′-ends were labeled with Cy5.5 using Cy5.5-dCTP (GE Healthcare) and Klenow DNA fragment.

Name of oligonucleotide	Nucleotide sequence
hTH −2870/−2909 sense	5′-GGCTCTGACCCTGCCCCACGCGGGGAGTGGGCACCAGTCCCA-3′
hTH −2870/−2909 antisense	5′-GGTGGGACTGGTGCCCACTCCCCGCGTGGGGCAGGGTCAGAG-3′
hTH −2830/−2869 sense	5′-GGGGGCACAGACGTGCTGGGTGATTAATCTGGGTGATTAAGC-3′
hTH −2830/−2869 antisense	5′-GGGCTTAATCACCCAGATTAATCACCCAGCACGTCTGTGCCC-3′
hTH −2790/−2829 sense	5′-GGCTCGGGCTGAGAGGCTGTTGAGAGAGAACACGCTCCATTG-3′
hTH −2790/−2829 antisense	5′-GGCAATGGAGCGTGTTCTCTCTCAACAGCCTCTCAGCCCGAG-3′
hTH −2750/−2789 sense	5′-GGTGGAGCTGGCTCAGCATTCCTTACGGCCATGGTGGCAGGG-3′
hTH −2750/−2789 antisense	5′-GGCCTGCCACCATGGCCGTAAGGAATGCTGAGCCAGCTCCA-3′
hTH −2707/−2749 sense	5′-GGGCTGTAACCACAGGGACGGCGGAAGTGGTGGAGGGTGGTGGG-3′
hTH −2707/−2749 antisense	5′-GGCCCACCACCCTCCACCACTTCCGCCGTCCCTGTGGTTACAGC-3′
mTH −2870/−2909 sense	5′-GGTCTCTCCCCAACCCTGCCCTGACCTCAAGTGCACCTCTTC-3′
mTH −2870/−2909 antisense	5′-GGGAAGAGGTGCACTTGAGGTCAGGGCAGGGTTGGGGAGAGA-3′
mTH −2830/−2869 sense	5′-GGAAAGTCAGGTTTAGCAGCTGCAGCTGGGGGCCCTGAATCC-3′
mTH −2830/−2869 antisense	5′-GGGGATTCAGGGCCCCCAGCTGCAGCTGCTAAACCTGACTTT-3′
mTH −2790/−2829 sense	5′-GGCACCCCTGCTGTCTTCCTTGAAGACAGAAGTGTTGGGAGC-3′
mTH −2790/−2829 antisense	5′-GGGCTCCCAACACTTCTGTCTTCAAGGAAGACAGCAGGGGTG-3′
mTH −2750/−2789 sense	5′-GGTGAGGATCTGGGCTAGAGACTGGCTGTATGATCCAGAGAA-3′
mTH −2750/−2789 antisense	5′-GGTTCTCTGGATCATACAGCCAGTCTCTAGCCCAGATCCTCA-3′
mTH −2707/−2749 sense	5′-GGTAGTGTGCTTCTGGGCCTCAGATTTCCCTTCTGTAGAACAGG-3′
mTH −2707/−2749 antisense	5′-GGCCTGTTCTACAGAAGGGAAATCTGAGGCCCAGAAGCACACTA-3′

AGGCACATATACAGATG-3′. The PCR products were separated on a 2% agarose gel and stained with ethidium bromide. Reverse images of black and white staining are shown.

Gel Mobility Shift Assay—Nucleotide sequences of oligonucleotides used for probes are shown in Table 1. Oligonucleotides were annealed, and their 3′-ends were labeled with Cy5.5 using Cy5.5-dCTP (GE Bioscience) and Klenow DNA fragment. Nuclear extracts were prepared from SH-SY5Y and Neuro-2a cells, and gel mobility shift assays were carried out as described previously (17).

Statistical Analyses—Data are expressed as means ± S.E. Statistical analyses were performed using one-way analysis of variance followed by unpaired Student's *t* test.

RESULTS

Human-specific Reduction of TH Gene Expression in DJ-1-knockdown Cells—Human dopaminergic cell lines SH-SY5Y and SK-N-SH and mouse Neuro-2a cells were transfected with siRNAs targeting human *DJ-1*, mouse *DJ-1*, or luciferase as a negative control, and expression levels of *DJ-1* and *TH* mRNAs were examined by semiquantitative RT-PCR and quantitative RT-PCR (real-time PCR) (Fig. 1, *A–C* and *E–G*, respectively). β-Actin was used as a loading control. As reported previously (23), levels of *TH* gene expression both in SH-SY5Y and SK-N-SH cells were reduced after *DJ-1* gene expression was knocked down by siRNA to ~20% of the level of *DJ-1* in cells without siRNA or with siRNA targeting luciferase (Fig. 1, *A* and *B*). To confirm this, real-time PCR analyses were carried out, and similar results showing ~40 and 80% reductions of *TH* gene expression in SH-SY5Y and SK-N-SH cells, respectively, were obtained (Fig. 1, *E* and *F*). When the expression of *DJ-1* in Neuro-2a cells was knocked down to 10% compared with the level in untransfected cells, on the other hand, no reduction of *TH* gene expression was found by RT-PCR and real-time PCR analyses (Fig. 1, *C* and *G*, respectively). Furthermore, RT-PCR and real-time PCR were performed using primary neuronal cultures of mesencephalons from wild-type and *DJ-1*-knock-out mice, and no reduction of *TH* gene expression in mouse cells was confirmed (Fig. 1, *D* and *H*). It has been reported that PSF, a transcription co-repressor, binds to the promoter region (−2909 to −2707) of the *TH* gene to repress its expression and that human DJ-1 binds to PSF to sequester the PSF·co-repressor complex, leading to activation of *TH* gene expression in cultured human cells (20). To examine the effect of PSF on *TH* gene expression, cells were transfected with siRNA targeting *PSF*, and *TH* expression was analyzed (Fig. 1). The results showed ~160 and 150% increases of *TH* gene expression in SH-SY5Y and SK-N-SH cells, respectively, in which ~80% of *PSF* expression was observed (Fig. 1, *A*, *B*, *E*, and *F*). However, no change in *TH* gene expression was found in Neuro-2a cells transfected with siRNA targeting PSF (Fig. 1, *C* and *G*). These results suggest that expression of the *TH* gene is specifically regulated by DJ-1 and PSF in human cells.

Human-specific Reduction of TH Activity in DJ-1-knockdown Cells—We have reported that reduction of TH activity in *DJ-1*-knockdown human SH-SY5Y cells is caused by reduced levels of *TH* mRNA and of *DJ-1*, which binds to TH to stimulate enzymatic activity (23). To elucidate such effects of DJ-1 on TH activity in human and mouse cells, human SH-SY5Y, human SK-N-SH, and mouse Neuro-2a cells were transfected with siRNA targeting *DJ-1*. As shown in Fig. 2, *A–C*, TH activity was reduced in SH-SY5Y and SK-N-SH cells but not in Neuro-2a cells. Transfection of siRNA targeting luciferase did not affect TH activity in SH-SY5Y, SK-N-SH, and Neuro-2a cells. Furthermore, no reduction of TH activity was observed in primary neuronal cultures of mesencephalons from *DJ-1*-knock-out mice (Fig. 2*D*). To then examine the effect of *PSF* gene expression on TH activity, SH-SY5Y, SK-N-SH, and Neuro-2a cells were transfected with siRNA targeting *PSF*. The results showed that TH activity was increased in SH-SY5Y and SK-N-SH cells but not in Neuro-2a cells (Fig. 2, *E–G*). Because it has not been reported that PSF binds to TH, the increased level of TH activity by *PSF* knockdown is thought to be caused by an increased level of *TH* mRNA. When TH activity in the brains of *DJ-1*-knock-out mice was examined, similar levels of TH activity were found

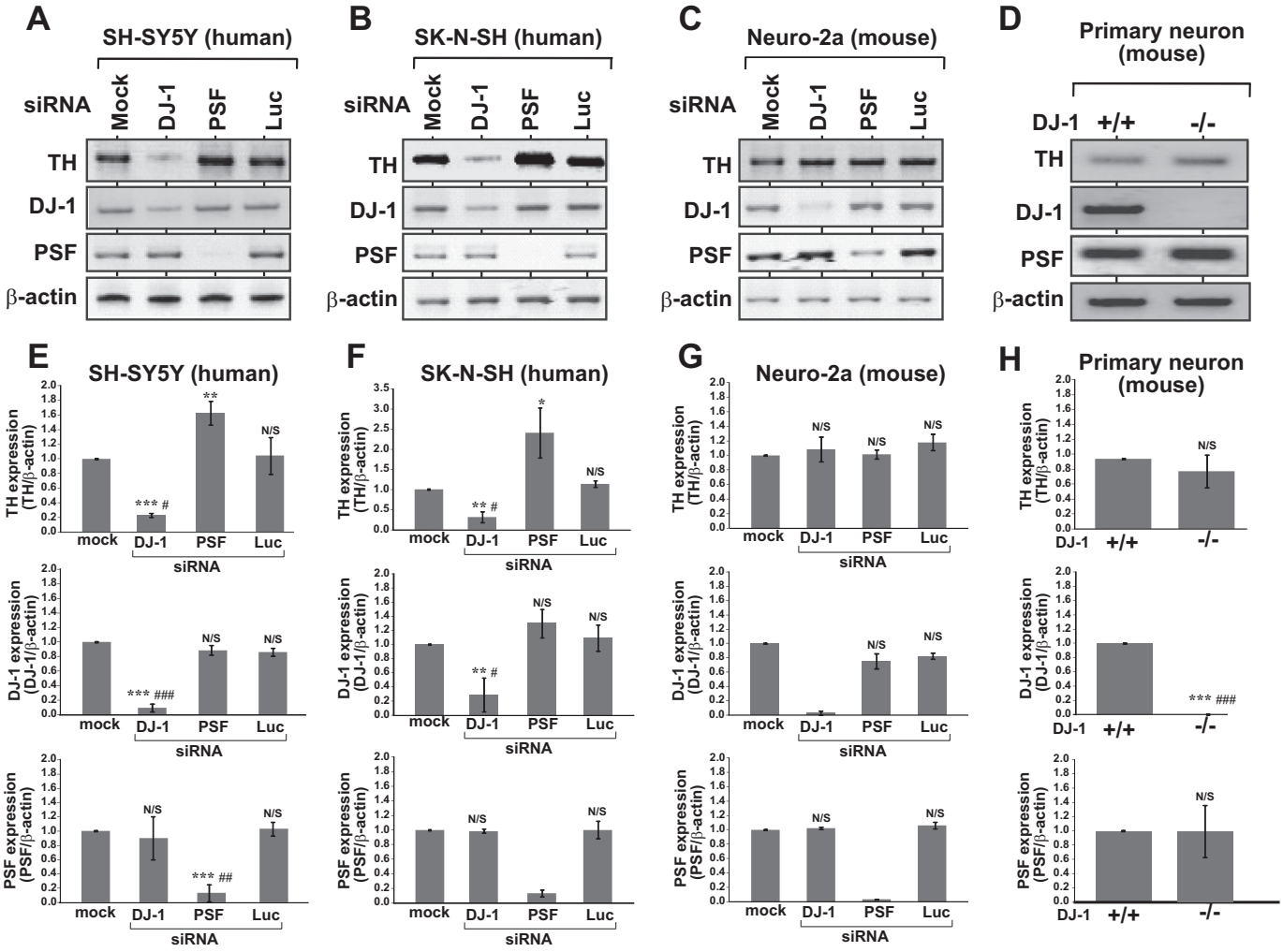

FIGURE 1. **Human-specific reduction of *TH* gene expression in *DJ-1*-knockdown cells.** *A* and *B*, human SH-SY5Y (*A*) and SK-N-SH (*B*) cells were transfected with siRNAs targeting human *DJ-1*, human *PSF*, and luciferase (*Luc*). Forty-eight h after transfection, mRNA levels of *TH*, *DJ-1*, and *PSF* were examined by semiquantitative RT-PCR (*RT-PCR*). β-Actin was used as a loading control. *C*, mouse Neuro-2a cells were transfected with siRNAs targeting mouse *DJ-1*, mouse *PSF*, and luciferase. Forty-eight h after transfection, mRNA levels of *TH*, *DJ-1*, and *PSF* were examined by RT-PCR. *D*, mRNA levels of *TH*, *DJ-1*, and *PSF* in primary neuronal cultures of mesencephalons from wild-type and *DJ-1*-knock-out mice were examined by RT-PCR. β-Actin was used as a loading control. *E* and *F*, mRNA levels of *TH*, *DJ-1*, and *PSF* in human SH-SY5Y (*E*) and SK-N-SH (*F*) cells that had been transfected with siRNA targeting human *DJ-1*, human *PSF*, and luciferase were examined by quantitative RT-PCR (*real-time PCR*). β-Actin was used as a loading control, and expression levels of *TH*, *DJ-1*, and *PSF* mRNA relative to that of β-actin are shown. *G*, mRNA levels of *TH*, *DJ-1*, and *PSF* in mouse Neuro-2a that had been transfected with siRNA targeting mouse *DJ-1*, mouse *PSF*, and luciferase were examined by real-time PCR. β-Actin was used as a loading control, and expression levels of *TH*, *DJ-1*, and *PSF* mRNA relative to that of β-actin are shown. *H*, mRNA levels of *TH*, *DJ-1*, and *PSF* in primary neuronal cultures of mesencephalons from wild-type and *DJ-1*-knock-out mice were examined by real-time PCR. β-Actin was used as a loading control, and expression levels of *TH*, *DJ-1*, and *PSF* mRNA relative to that of β-actin are shown. Values are means ± S.E. (*error bars*); $n = 3$ experiments. Significance was as follows. *, $p < 0.05$; **, $p < 0.01$; and ***, $p < 0.001$ compared with mock. #, $p < 0.05$; ##, $p < 0.01$; and ###, $p < 0.001$ compared with luciferase. *N/S*, no significance.

in *DJ-1*-knock-out and wild-type mice (Fig. 2*H*). These results suggest that expressions of *DJ-1* and *PSF* affect TH activity only in human dopaminergic cells.

Stimulation of Promoter Activity of the TH Gene by DJ-1—To assess the effect of DJ-1 on the expression of human and mouse *TH* genes, their promoter activities were examined. To do that, upstream regions up to approximately −3000 base pairs from the transcriptional start site in human and mouse *TH* genes were cloned from genomic DNAs in human SH-SY5Y cells and mouse Neuro-2a cells and inserted into a luciferase vector. Plasmids named phTH-Luc and pmTH-Luc were transfected into SH-SY5Y and Neuro-2a cells together with expression vectors for FLAG-tagged human DJ-1, mouse DJ-1, and human mutant DJ-1 (L166P), which was found in patients with Parkinson disease (2), and luciferase activities in transfected cells were measured at 48 h after transfection (Fig. 3, *A–D*). Expression levels of transfected FLAG-DJ-1 and endogenous DJ-1 in cells were examined by Western blotting (Fig. 3*E*). The results showed that luciferase activity derived from phTH-Luc in human SH-SY5Y cells was increased by human wild-type DJ-1 in a dose-dependent manner but not by mouse wild-type DJ-1 and by human L166P-DJ-1 (Fig. 3, *A* and *B*, respectively). Luciferase activity from pmTH-Luc in Neuro-2a cells was, on the other hand, increased by neither of the DJ-1s (Fig. 3, *C* and *D*). These results clearly indicate that human wild-type DJ-1 specifically activates the promoter activity of the human *TH* gene and that this situation is not the case in the mouse *TH* gene. To further examine whether DJ-1 activates the TH promoter in a hu-

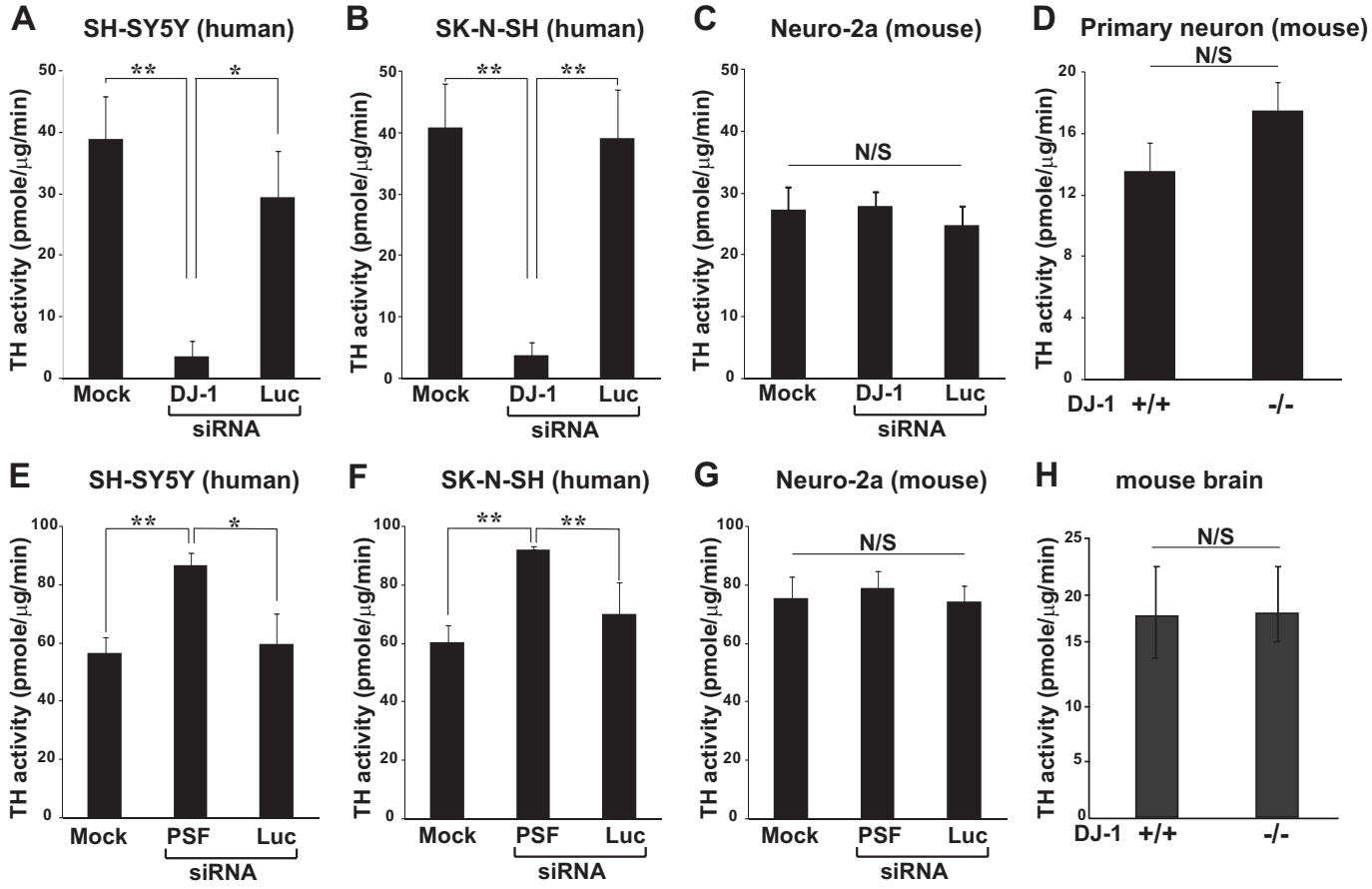

FIGURE 2. **Human-specific reduction of TH enzymatic activity in DJ-1-knockdown cells.** *A–C*, human SH-SY5Y (*A*), SK-N-SH (*B*), and mouse Neuro-2a (*C*) cells were transfected with siRNAs targeting human *DJ-1* and luciferase (*Luc*). Forty-eight h after transfection, TH activity of transfected cells was examined as described under "Experimental Procedures." *Mock*, non-transfected cells. *D*, TH activities in primary neuronal cultures of mesencephalons from wild-type and *DJ-1*-knock-out mice were examined as described under "Experimental Procedures." *E–G*, TH activities in human SH-SY5Y (*E*), SK-N-SH (*F*), and mouse Neuro-2a (*G*) cells that had been transfected with siRNA targeting human *PSF* or luciferase were examined as described in *A–C*. *H*, TH activities in brains of wild-type and *DJ-1*-knock-out mice were examined. Values are means ± S.E. (*error bars*); $n = 3$ experiments. Significance was as follows. *, $p < 0.05$; **, $p < 0.01$; and ** $p < 0.001$ compared with mock. *N/S*, no significance.

man dopaminergic cell-dependent manner, phTH-Luc and pmTH-Luc were transfected into human HeLa and mouse NIH3T3 cells together with expression vectors for human DJ-1, mouse DJ-1, and human L166P DJ-1, and luciferase activities in transfected cells were measured (Fig. 3, *A–D*). HeLa and NIH3T3 cells are not dopaminergic cells. The results showed that TH promoter activity in HeLa cells was one-one hundredth of that in SH-SY5Y cells, and no TH promoter activity in NIH3T3 cells was observed (supplemental Fig. 1), indicating that DJ-1 activates the TH promoter in a human dopaminergic cell-dependent manner.

Interaction of DJ-1 with PSF in Human and Mouse Dopaminergic Cells—Proteins extracted from SH-SY5Y and Neuro-2a cells were co-immunoprecipitated with an anti-DJ-1 antibody and nonspecific IgG, and precipitates were analyzed by Western blotting with an anti-PSF antibody (Fig. 4*A*). The results showed that PSF was co-immunoprecipitated with DJ-1 in both SH-SY5Y and Neuro-2a cells, indicating complex formation of DJ-1 with PSF in human and mouse cells. To further elucidate the binding relationship between DJ-1 and PSF of human and mouse origins, FLAG-tagged human DJ-1 or FLAG-tagged mouse DJ-1 was transfected into either SH-SY5Y or Neuro-2a cells. Forty-eight h after transfection, proteins extracted from transfected cells were immunoprecipitated with an anti-FLAG antibody or IgG, and precipitates were analyzed by Western blotting with the anti-PSF antibody. As shown in Fig. 4*B*, both FLAG-human DJ-1 and FLAG-mouse DJ-1 were associated with either endogenous human PSF or endogenous mouse PSF. Furthermore, FLAG-tagged L166P DJ-1 was found to be associated with endogenous human and mouse PSF, after FLAG-L166P DJ-1 was transfected into SH-SY5Y and Neuro-2a cells, respectively, and co-immunoprecipitation experiments were carried out (Fig. 4*C*).

To access the direct interaction of DJ-1 with PSF, pull-down experiments using purified GST-DJ-1 and ^{35}S-labeled human and mouse PSF, which were synthesized in reticulocyte lysates *in vitro*, were carried out. ^{35}S-Labeled human and mouse p53 were used as positive controls. As shown in Fig. 4*D*, both PSF and p53 from human and mouse origins bound to GST-DJ-1 but not to GST, indicating that DJ-1 directly binds to PSF. These results suggest that DJ-1 has a potential activity to bind to PSF of any mammalian species.

Transcriptional Regulation of Human TH Promoter by DJ-1 and PSF—To confirm transcriptional regulation of the *TH* gene by DJ-1 and PSF, competitive expression experiments of

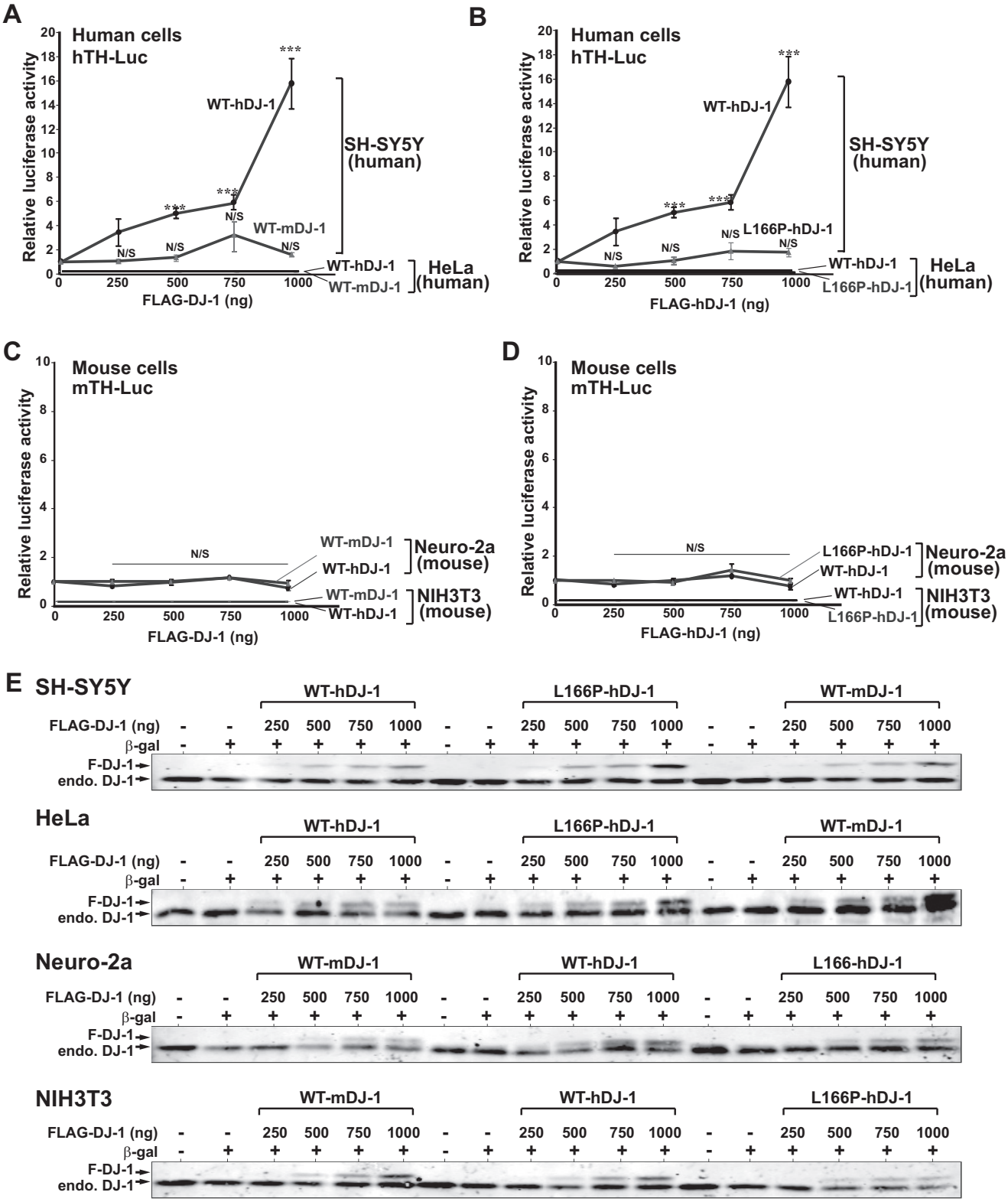

FIGURE 3. **Stimulation of promoter activity of *TH* gene by DJ-1.** *A* and *B*, phTH-Luc was transfected into SH-SY5Y and HeLa cells together with various amounts of expression vectors for human wild-type DJ-1 (*WT-hDJ-1*), mouse wild-type DJ-1 (*WT-mDJ-1*), or human L166P mutant of DJ-1 (*L166P-hDJ-1*). Forty-eight h after transfection, luciferase activities of transfected cells were examined as described under "Experimental Procedures." phTH-Luc is the human *TH* promoter linked to the luciferase gene. *C* and *D*, pmTH-Luc was transfected into Neuro-2a and NIH3T3 cells together with expression vectors for WT-hDJ-1, WT-mDJ-1, or L166P-hDJ-1. Forty-eight h after transfection, luciferase activities of transfected cells were examined. pmTH-Luc is the mouse *TH* promoter linked to the luciferase gene. Values are means ± S.E. (*error bars*); $n = 3$ experiments. Significance was as follows. ***, $p < 0.001$. *N/S*, no significance. *E*, expression levels of transfected FLAG-DJ-1 and endogenous DJ-1 in cells were examined by Western blotting with an anti-DJ-1 antibody.

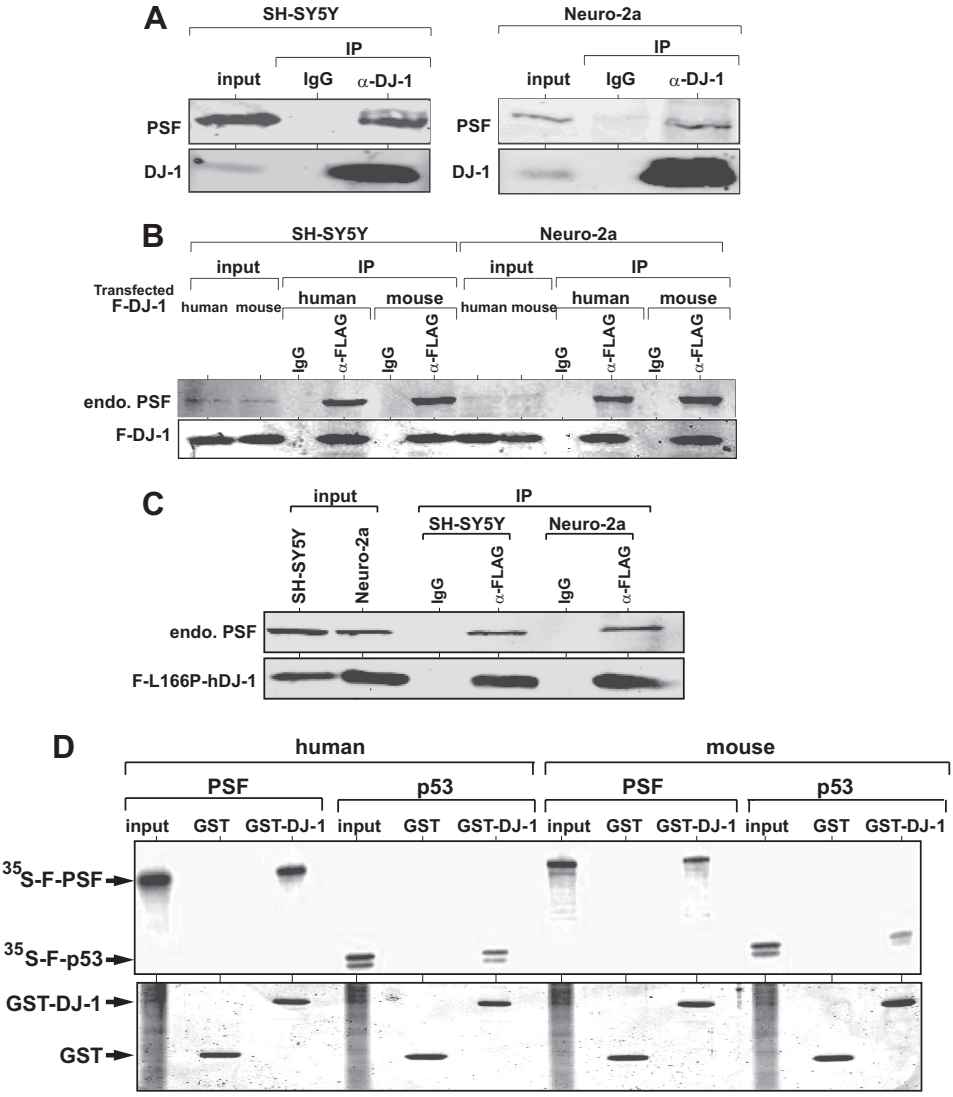

FIGURE 4. **Interaction of DJ-1 with PSF.** *A*, proteins extracted from human SH-SY5Y (*left*) and mouse Neuro-2a (*right*) cells were co-immunoprecipitated (*IP*) with a rabbit anti-DJ-1 polyclonal antibody (α-DJ-1) or IgG (IgG), and precipitates were analyzed by Western blotting with anti-PSF (Sigma) and mouse anti-DJ-1 monoclonal antibodies (3E8, MBL). *B*, FLAG-tagged human DJ-1 or FLAG-tagged mouse DJ-1 was transfected into either SH-SY5Y or Neuro-2a cells. Forty-eight h after transfection, proteins extracted from transfected cells were co-immunoprecipitated with an anti-FLAG antibody (*α-FLAG*) or IgG (*IgG*), and precipitates were analyzed by Western blotting with anti-PSF (*endo. PSF*) and anti-FLAG antibodies. *C*, FLAG-tagged human L166P mutant of DJ-1 (*F-L166P-hDJ-1*) was transfected into either SH-SY5Y or Neuro-2a cells, and co-immunoprecipitation experiments were carried out as described in *B*. *D*, GST or GST-DJ-1 was expressed in and prepared from *E. coli* and reacted with ^{35}S-labeled FLAG-PSF or FLAG-p53 that had been synthesized *in vitro* using a coupled transcription-translation system (Promega). After the reaction, the mixture was subjected to pull-down assays as described under "Experimental Procedures."

DJ-1 and PSF in reporter assays for the human TH promoter were carried out. When SH-SY5Y cells were transfected with a constant amount of human DJ-1 together with various amounts of human PSF, luciferase activity stimulated by human DJ-1 was found to be decreased by human PSF in a dose-dependent manner (Fig. 5*A*). As a reverse combination, when SH-SY5Y cells were transfected with a constant amount of human PSF together with various amounts of human DJ-1, luciferase activity was found to be increased by human DJ-1 in a dose-dependent manner (Fig. 5*B*). Human L166P DJ-1 had no stimulatory effect on the *TH* promoter (Fig. 5, *A* and *B*). In Neuro-2a cells, on the other hand, no combination between mouse DJ-1 and mouse PSF caused changes in luciferase activity (Fig. 5, *C* and *D*), indicating that competitive regulation of the *TH* gene occurs only in human cells.

To assess the different regulation of *TH* gene expression by DJ-1 and PSF in human and mouse cells, chromatin immunoprecipitation (ChIP) assays were carried out. Chromatin solutions from SH-SY5Y and Neuro-2a cells were immunoprecipitated with an anti-DJ-1 or anti-PSF antibody. DNA segments spanning −4000 to −3800 and −2909 to −2707 in the human *TH* gene and those spanning −4070 to −3730 and −2909 to −2707 in the mouse *TH* gene were amplified by PCR (Fig. 6). The latter regions contain PSF-binding regions, and DNA segments spanning −4000 to −3800 were used as a negative control (Fig. 6*C*). An anti-SP1 antibody was used as a positive control and negative control. The anti-SP1 antibody did not precipitate the two segments spanning −4000 to −3800 and −2909 to −2707 in SH-SY5Y cells and precipitated each segment spanning −1157

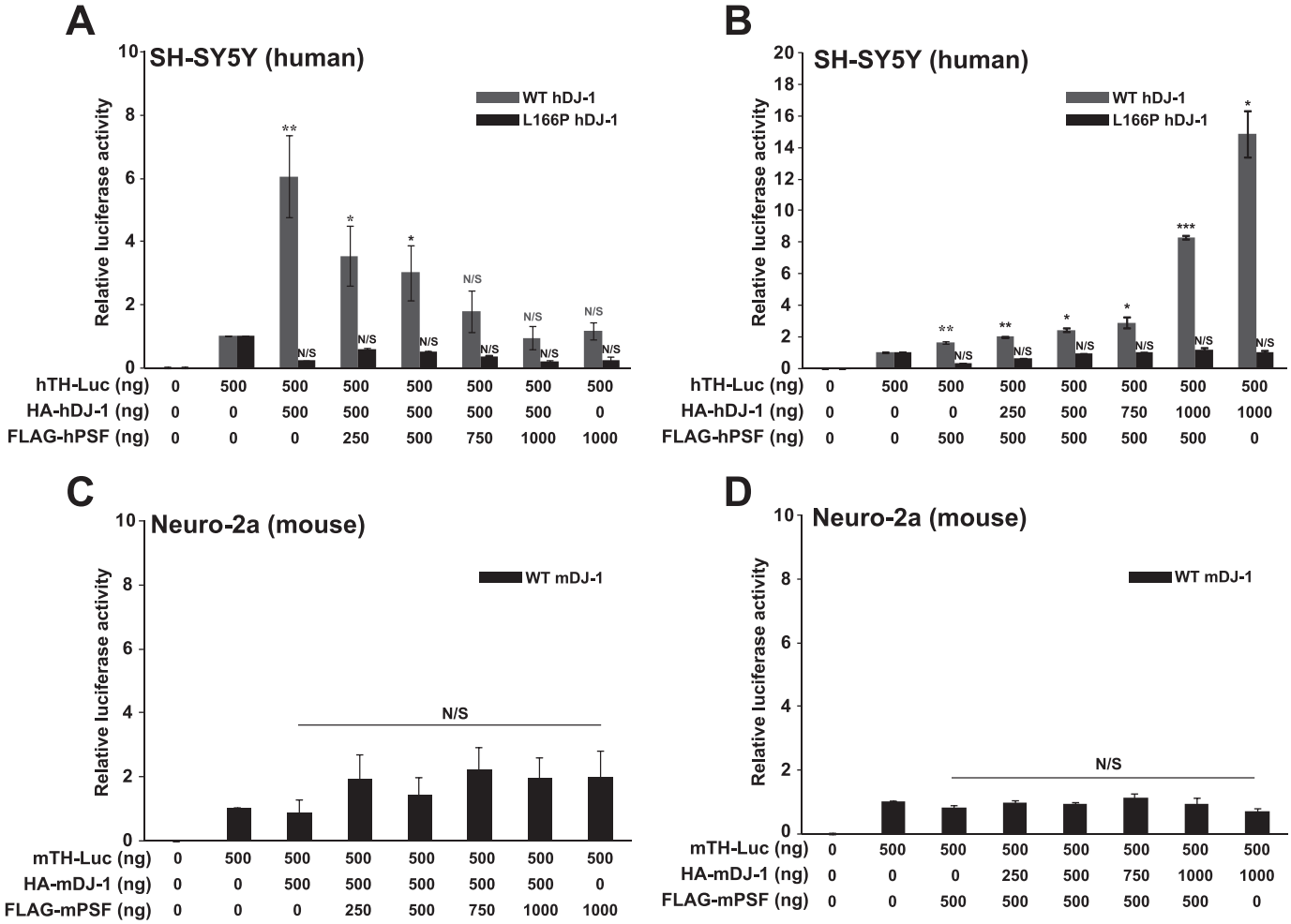

FIGURE 5. **Effects of DJ-1 and PSF on promoter activity of the *TH* gene.** *A*, SH-SY5Y cells were transfected with a constant amount of human phTH-Luc and human DJ-1 together with various amounts of human PSF. Forty-eight h after transfection, luciferase activities in transfected cells were measured as described under "Experimental Procedures." phTH-Luc and pmTH-Luc are human and mouse *TH* promoters linked to the luciferase gene, respectively. *B*, SH-SY5Y cells were transfected with a constant amount of phTH-Luc and PSF together with various amounts of human DJ-1. Forty-eight h after transfection, luciferase activities in transfected cells were examined. *C*, Neuro-2a cells were transfected with a constant amount of pmTH-Luc and mouse DJ-1 together with various amounts of mouse PSF. Forty-eight h after transfection, luciferase activities in transfected cells were examined. *D*, Neuro-2a cells were transfected with a constant amount of pmTH-Luc and PSF together with various amounts of mouse DJ-1. Forty-eight h after transfection, luciferase activities in transfected cells were examined. Values are means ± S.E. $n = 3$ experiments. Significance was as follows. *, $p < 0.05$; **, $p < 0.01$; and ***, $p < 0.001$. *N/S*, no significance.

to −932 and −612 to −187, which contain SP1-binding sites, in SH-SY5Y and Neuro-2a cells, respectively (Fig. 6, *A* and *B*). The results clearly showed that anti-DJ-1 and anti-PSF antibodies immunoprecipitated the segment possessing the PSF-binding region in human cells but not in mouse cells (Fig. 6, *A* and *B*), indicating that the complex of PSF with DJ-1 binds to the human *TH* promoter but not to the mouse *TH* promoter (Fig. 6*C*).

Because *TH* promoter activity that had been inhibited by human PSF was restored by human DJ-1 in a dose-dependent manner (Fig. 5), it is thought that binding of PSF to the *TH* promoter is inhibited by DJ-1. To examine this possibility, SH-SY5Y cells were transfected with a constant amount of FLAG-PSF together with various amounts of HA-DJ-1 and subjected to ChIP assays (Fig. 7). Because the human L166P-DJ-1 mutant had no stimulatory effect on the *TH* promoter (Fig. 3), L166P-DJ-1 was also transfected into SH-SY5Y cells as a negative control. After chromatin from cells transfected with FLAG-PSF was immunoprecipitated with an anti-FLAG antibody, the region spanning −2909 to −2707 but not that spanning −4000 to −3800 was amplified by PCR (Fig. 7*A*). After cells had been transfected with various amounts of HA-DJ-1, the levels of precipitated DNA corresponding to the region spanning −2909 to −2707 were decreased in a dose-dependent manner, and no precipitated DNA appeared by transfection of 10,000 ng of an expression vector for HA-DJ-1. Under the same condition as above, an anti-SP1 antibody precipitated the region spanning −1157 to −932, which contains an SP1-binding sequence, but the levels of precipitated DNA were not changed (Fig. 7*B*). When various amounts of HA-L166P-DJ-1 and a constant amount of FLAG-PSF were transfected into SY-SY5Y cells, the levels of DNA that had been precipitated with the anti-FLAG or anti-SP-1 antibody were not changed (Fig. 7, *C* and *D*, respectively), indicating that L166P-DJ-1 does not affect binding of PSF and SP1 to DNA. These results clearly showed that PSF is sequestered from the

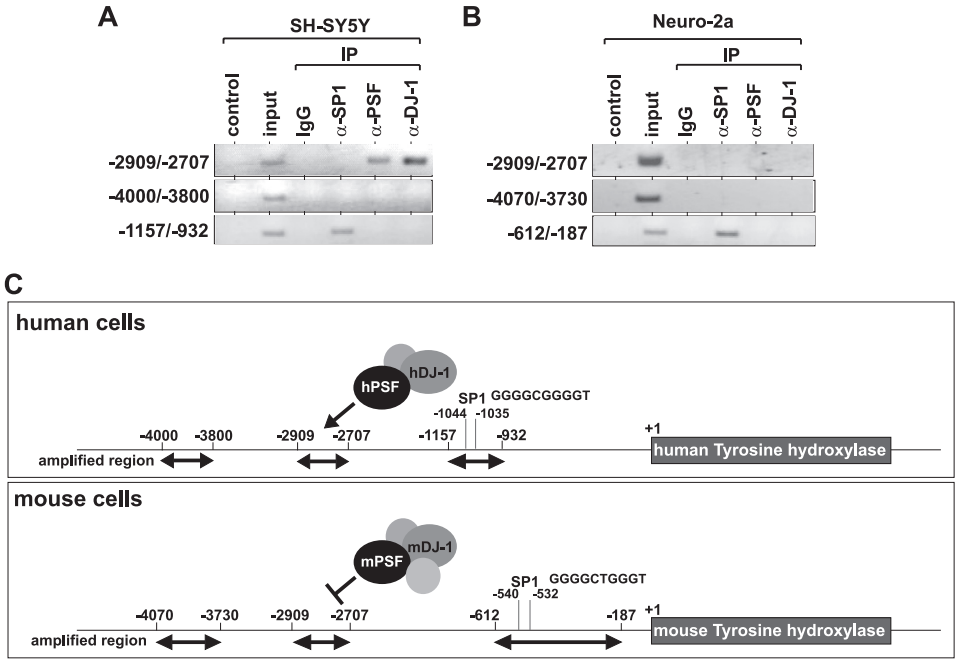

FIGURE 6. **Association of DJ-1 and PSF with the *TH* promoter.** *A* and *B*, chromatin solutions from SH-SY5Y (*A*) and Neuro-2a (*B*) cells were co-immunoprecipitated (*IP*) with anti-DJ-1 (α-*DJ-1*), anti-PSF (α-*PSF*), and anti-SP1 (α-*SP1*) antibodies. Segments in DNA extracted from precipitated chromatin were amplified by PCR using specific primers as described under "Experimental Procedures." *C*, regions to be amplified by PCR are schematically drawn.

TH promoter by DJ-1, resulting in activation of *TH* gene expression.

To further determine the PSF·DJ-1-binding region in DNA segments spanning −2909 to −2707, gel mobility shift assays were carried out using 40 base pairs each of DNA fragments as labeled probes and nuclear extracts from SH-SY5Y and Neuro-2a cells. Specific DNA·protein complexes were first identified, and then supershift assays were carried out using an anti-DJ-1 antibody, anti-PSF antibody, and nonspecific IgG. As shown in the *left panels* of Fig. 8, *A* and *B*, the DNA·protein complex on a DNA fragment spanning −2790 to −2829 from SH-SY5Y cells but not from Neuro-2a cells was supershifted with anti-DJ-1 and anti-PSF antibodies but not with IgG, indicating that the PSF and DJ-1 complex bound to the region spanning −2790 to −2829 of the human TH promoter. To assess whether this region is responsible for activation of TH promoter activity by DJ-1, human and mouse TH promoters deleting the region spanning −2790 to −2829 were constructed and linked to the luciferase gene (termed TH-del-Luc). SH-SY5Y and Neuro-2a cells were then co-transfected with human TH-del-Luc and mouse TH-del-Luc together with FLAG-human DJ-1 and FLAG-mouse DJ-1, respectively, and their luciferase activities were measured. Human and mouse TH-Luc without deletions were also transfected to cells as positive controls. The results showed that luciferase activity of deleted human TH promoter was reduced compared with that of undeleted promoter after activation of the TH promoter by human DJ-1 in SH-SY5Y cells but that no change of mouse TH promoters was observed in Neuro-2a cells (*left panels* of Fig. 8, *A* and *B*). The results also showed that luciferase activity of hTH-del-Luc was stimulated by DJ-1, suggesting that another transcription factor(s) participating in the expression of the human TH gene is regulated by DJ-1. Indeed, the recognition site of p53, which is a DJ-1-regulating transcription factor (19, 22), is present in the region spanning −1103 to −1094 in the human TH promoter. These results clearly indicate that the region spanning −2790 to −2829 in the human TH promoter is a target of DJ-1 and PSF.

DISCUSSION

In this study, we found that expression of the *TH* gene and TH activity were reduced in *DJ-1*-knockdown human cells but not in *DJ-1*-knockdown or *DJ-1*-knock-out mouse cells and that this occurred at the transcriptional level, where PSF, a transcription co-repressor, was sequestered from the promoter region by DJ-1 in human cells. Although mouse DJ-1 was associated with mouse PSF, ChIP assays showed that the recognition sequence was absent in the mouse *TH* promoter, meaning that there was no repression of *TH* gene expression by PSF in mouse cells. These findings indicate a species-specific regulation of *TH* gene expression by DJ-1 and PSF. It has been reported that PSF binds to the promoter region of the *TH* gene to repress its expression and that human DJ-1 binds to PSF to sequester the PSF·co-repressor complex, leading to activation of *TH* gene expression in cultured human cells (36). In *DJ-1*-knock-out mice, however, no severe phenotype, including loss of dopamine, has been reported (13, 37, 38). Our study, therefore, shows one reason for no loss of dopamine in *DJ-1*-knock-out mice.

In reporter assays using luciferase gene-linked promoters from human and mouse *TH* genes, stimulation of *TH* promoter activity by DJ-1 was observed in the homologous combination between the human *TH* promoter, human DJ-1, and human cells but not between human *TH* promoter, human DJ-1, and mouse cells or between mouse *TH* promoter,

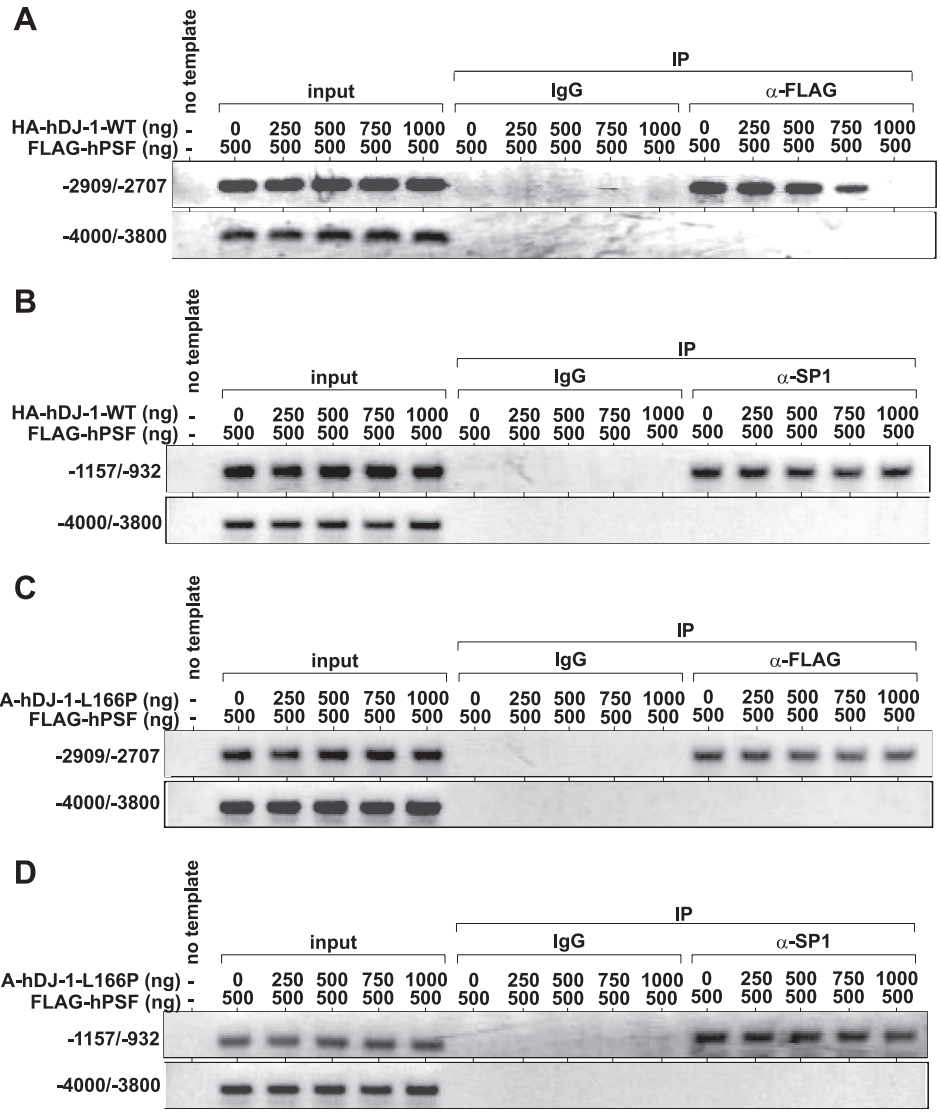

FIGURE 7. **Sequestration of PSF from DNA by DJ-1.** *A*, SH-SY5Y cells were transfected with a constant amount of FLAG-human PSF and various amounts of HA-human DJ-1. Forty-eight h after transfection, DNA extracted from chromatin was immunoprecipitated (*IP*) with an anti-FLAG antibody or IgG, and regions spanning −2909 to −2707 and −4000 to −3800 were amplified by PCR using specific primers. *B*, SH-SY5Y cells were transfected under the same conditions as described for *A*. After chromatin had been immunoprecipitated with an anti-SP1 antibody or IgG, regions spanning −1157 to −932 and −4000 to −3800 were amplified by PCR using specific primers. *C*, SH-SY5Y cells were transfected with a constant amount of FLAG-human PSF and various amounts of HA-human L166P DJ-1 and subjected to ChIP assays as described in *A*. *D*, SH-SY5Y cells were transfected with a constant amount of FLAG-human PSF and various amounts of HA-human L166P DJ-1 and subjected to ChIP assays as described in *B*.

mouse DJ-1, and mouse cells. Furthermore, stimulation of human TH promoter activity by human DJ-1 was specific to dopaminergic cells (Fig. 3). Luciferase activity in mouse Neuro-2a cells was higher than that in human SH-SY5Y cells (supplemental Fig. 1). The expression levels of DJ-1 in Neuro-2a and SH-SY5Y cells are similar. Because transfection efficiency of plasmid DNA in Neuro-2a cells is higher than that in SH-SY5Y cells, it is thought that high luciferase activity in Neuro-2a cells was obtained due to different transfection efficiency. Expression levels of endogenous DJ-1 in all of the cells are at a similar level (Fig. 3*E*), and luciferase activity corresponding to the human TH promoter was stimulated by transfected FLAG-human DJ-1 in a dose-dependent manner (Fig. 3, *A* and *B*). Because two plasmid DNAs, expression vectors for luciferase and FLAG-DJ-1, are generally transfected into the same cell at high frequency, luciferase activities obtained are thought to be responses to transfected FLAG-DJ-1 but not to endogenous DJ-1. Although it has been reported that PSF binds to the region spanning −2909 to −2707 upstream of the transcriptional start site to repress expression of the human *TH* gene, the DNA-binding sequence of PSF has not yet been determined. Because the identity of amino acid sequences between human and mouse PSFs is 93.51% and because we showed that both human DJ-1 and mouse DJ-1 bind to human PSF and that human and mouse PSF bind to each other (Fig. 4), human and mouse DJ-1s have a potential activity to bind to PSF of any mammalian species. Since we identified DJ-1 in 1997, we have been examining the DNA binding activity of DJ-1. No binding activity of DJ-1 was observed until now, and this study showed that DJ-1 directly binds to PSF, suggesting that DJ-1 binds to DNA via PSF. The results also show that mouse DJ-1 possessing binding activity

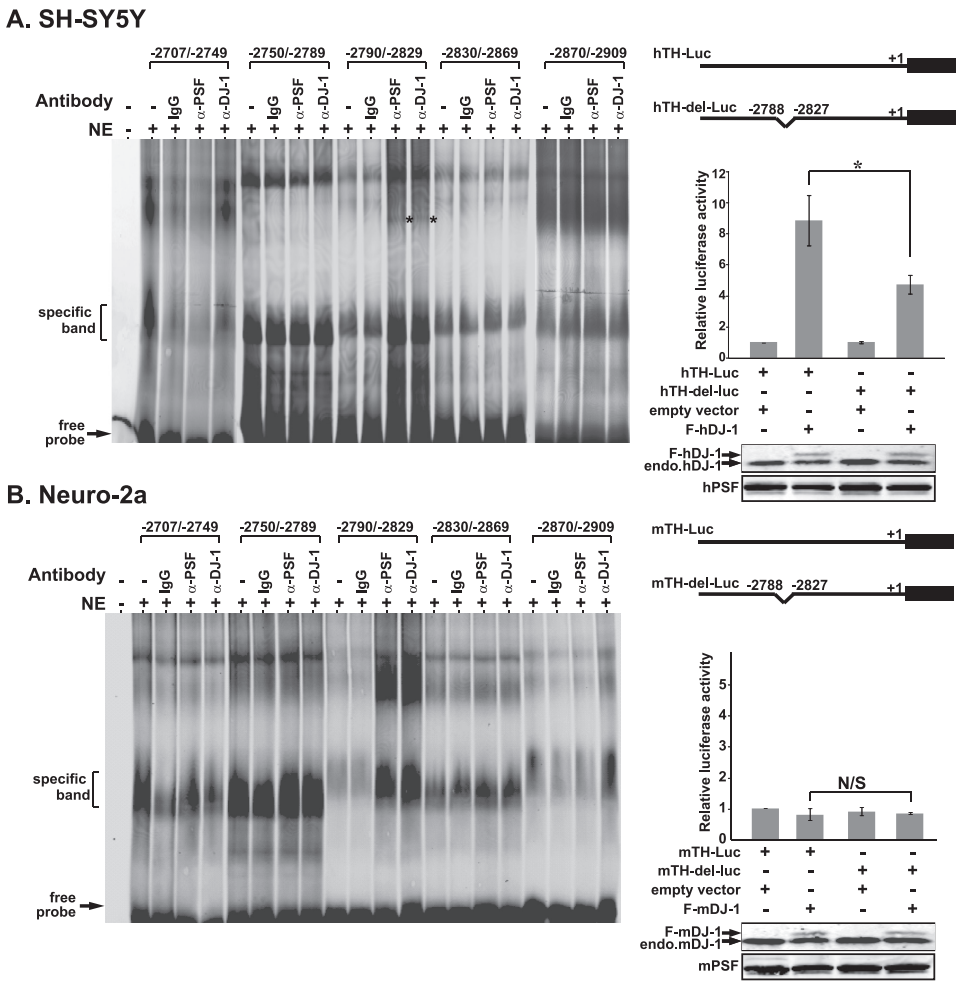

FIGURE 8. **Identification of the PSF·DJ-1 target region in the PSF promoter.** *Left*, nuclear extracts (*NE*) from SH-SY5Y cells (*A*) and Neuro-2a cells (*B*) were first reacted with 1 μg each of an anti-PSF antibody, anti-DJ-1 antibody, or IgG for 10 min at room temperature. Mixtures were then reacted with 40 pairs of Cy5.5-labeled oligonucleotides corresponding to each segment in the region spanning −2909 to −2707 and subjected to gel mobility shift assays as described under "Experimental Procedures." *, supershifted bands. *Right*, *A*, SH-SY5Y cells were transfected with 1 μg of phTH-Luc or phTH-del-Luc together with 1.0 μg of pcDNA3-FLAG-hDJ-1 or pcDNA3-FLAG. *B*, Neuro-2a cells were transfected with 1.0 μg of pmTH-Luc or pmTH-del-Luc together with 1.0 μg of pcDNA3-FLAG-mDJ-1 or pcDNA3-FLAG. Forty-eight h after transfection, luciferase activities in transfected cells were measured as described under "Experimental Procedures." Expression levels of transfected F-DJ-1, endogenous DJ-1 (*endo.DJ-1*), and endogenous PSF were examined by Western blotting.

to human PSF did not activate the human TH promoter in human SH-SY5Y cells. If sequestration of human PSF from the human TH gene promoter by DJ-1 is critical for TH gene expression, mouse DJ-1 seems to have some effect on TH gene expression. We do not have a clear answer to this point at present. Because the identity of amino acid sequences between human DJ-1 and mouse DJ-1 is 97%, there seem to be some structural/conformational differences between the two proteins. Because transcriptional activation or repression requires a proper complex comprised of multiple proteins, some structural/conformational differences may affect the regulation of gene expression. The identity of nucleotide sequences of the region corresponding to −2909 to −2707 between human and mouse *TH* genes is 44.29%. Furthermore, we found that DJ-1·PSF complex bound to the region spanning −2829 to −2790 in the human *TH* promoter (Fig. 8). Although the PSF-DNA binding sequence has not been determined, knockdown and knock-out of PSF and DJ-1 expression in mouse cells and in primary neuron culture, respectively, did not affect mouse TH gene expression, and no binding of DJ-1·PSF complex in this region was found in mouse cells (Fig. 6). Furthermore, competitive stimulation of human but not mouse *TH* promoter activity that had been inhibited by PSF was restored by DJ-1 (Fig. 5). DNA binding activity of PSF was attenuated by DJ-1 in a dose-dependent manner (Fig. 7). DJ-1-stimulated activity of human TH promoter with the region spanning −2829 to −2790 deleted was lower than that of human TH promoter without the deletion (Fig. 8). These results suggest that the regulation system of *TH* gene expression by DJ-1·PSF is present in human cells but not in mouse cells. Our study, therefore, shows one reason for no loss of dopamine in *DJ-1*-knock-out mice.

Because DJ-1 has multiple functions to inhibit cell death (25–27, 29, 39, 40) and the loss of DJ-1 functions causes early onset Parkinson disease (2, 16), it is surprising that *DJ-1*-knock-out mice appear normal without histological abnormalities although exhibiting minor motor deficits (14, 18, 41). Although genetically engineered mice are valuable tools for understanding neurodegenerative diseases, they often do not reproduce all of the symptoms and pathological hallmarks of

human diseases, probably due to the sum of multiple factors, including compensatory response, short life span, and difference in biological systems. Although we showed one possibility, that regulation of *TH* gene expression by DJ-1 differs in humans and mice, a better animal model of *DJ-1* deficiency is needed to fully understand the function of DJ-1. It has very recently been reported that mice with double knock-out of *DJ-1* and *Ret*, a receptor for glial cell line-derived neurotrophic factor, displayed trophically impaired dopaminergic neurons, suggesting that degeneration of dopaminergic neurons by DJ-1 requires an additional factor(s) (42).

Several groups have established *Drosophila* models of *DJ-1* deficiency (11, 38, 43). Different strategies to inactivate DJ-1 have, however, led to distinct phenotypes (41). Interestingly, only a study using siRNA to inactivate the *Drosophila DJ-1* gene demonstrated an age-dependent decrease in the number of TH-positive neurons and total brain dopamine content that resembles the neuropathology in PD patients (43). Although DJ-1 siRNA-induced apoptosis certainly contributes to this observation, it would be of interest to examine whether DJ-1 inactivation leads to transcriptional down-regulation of *TH* gene expression in *Drosophila* as well.

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