THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 283, NO. 7, pp. 4022–4030, February 15, 2008
© 2008 by The American Society for Biochemistry and Molecular Biology, Inc. Printed in the U.S.A.

DJ-1 Decreases Bax Expression through Repressing p53 Transcriptional Activity*

Received for publication, August 27, 2007, and in revised form, November 26, 2007 Published, JBC Papers in Press, November 26, 2007, DOI 10.1074/jbc.M707176200

Jun Fan, Haigang Ren, Nali Jia, Erkang Fei, Tian Zhou, Peng Jiang, Mian Wu, and Guanghui Wang[1]

From the Laboratory of Molecular Neuropathology, Hefei National Laboratory for Physical Sciences at Microscale and School of Life Sciences, University of Science and Technology of China, Hefei, Anhui 230027, China

DJ-1, originally identified as an oncogene product, is a protein with various functions in cellular transformation, oxidative stress response, and transcriptional regulation. Although previous studies suggest that DJ-1 is cytoprotective, the mechanism by which DJ-1 exerts its survival functions remains largely unknown. Here we show that DJ-1 exerts its cytoprotection through inhibiting p53-Bax-caspase pathway. DJ-1 interacts with p53 *in vitro* and *in vivo*. Overexpression of DJ-1 decreases the expression of Bax and inhibits caspase activation, whereas knockdown of DJ-1 increases Bax protein levels and accelerates caspase-3 activation and cell death induced by UV exposure. Our data provide evidence that the protective effects of DJ-1 on apoptosis are associated with its ability of decreasing Bax level through inhibiting p53 transcriptional activity.

DJ-1, a ubiquitously expressed protein identified as an oncogene product, was first found to be involved in breast cancer, lung cancer, and prostate cancer (1–4). Meanwhile, DJ-1 was also named contraception associated protein 1 (CAP-1), a target of some toxicants that leads to male infertility (5). Although the exact function of DJ-1 remains unclear, increasing studies reveal that DJ-1 has multiple roles in various biological processes. Above all, DJ-1 scavenges oxidative stress by oxidizing itself to a more acidic form and/or by increasing glutathione synthesis through an increase of glutamate cysteine ligase (6, 7). Overexpression of DJ-1 in animals or cultured cells prevents cell death through its anti-oxidative stress activities, whereas knockdown or knock-out of DJ-1 increases the susceptibility to oxidative stress (6–9). DJ-1, structurally similar to Hsp31, has chaperone activity that prevents the aggregation of α-synuclein depending on DJ-1 oxidation state (7, 10, 11). Furthermore, studies implicate that DJ-1 plays an important role in anti-apoptosis (12–15). Overexpression of DJ-1 in prostatic benign hyperplasia (BPH-1) cell line causes its resistance to apoptosis induced by cytotoxic agents, whereas knockdown of DJ-1 enhances apoptosis of PC-3 (prostatic cancer) cells by the same toxic reagent (13). Overexpression of DJ-1 blocks neuronal apoptosis by inhibiting transcriptional silencing activity of the pyrimidine tract-binding protein-associated splicing factor (PSF) or by preventing the translocation of Daxx from nucleus to cytoplasm where Daxx activates apoptosis signal-regulating kinase 1 (ASK1) death pathway (12, 14, 15). Moreover, cells harboring DJ-1 are much more resistant to UV-induced apoptosis than DJ-1 knockdown cells (15). Besides those biological functions, DJ-1 was reported to function as an important transcriptional regulator in multiple pathways including restoring p53 transcriptional activity through its interaction with Topors/p53BP3 (14, 16–20). Recently, loss of DJ-1 function was found to be linked with autosomal recessive early onset Parkinson disease (PD)[2] (21–24). PD is a common neurodegenerative disorder caused by the progressive loss of dopaminergic neurons in the substantia nigra pars compacta 25). Although most cases of PD are sporadic, studies on the rare familial forms of PD due to inherited gene mutations have yielded crucial insights into the possible etiology of this disease. Many pathogenic mutations have been identified in DJ-1 including exonic deletion and missense mutations in populations of various ethnic origins (21–24).

Most recently, it was reported that knockdown of DJ-1 in zebrafish up-regulates Bax and enhances the cell death of dopaminergic neurons under oxidative stress, providing evidence that DJ-1 was able to regulate the Bax level *in vivo* (26). In our present study we show that DJ-1 interacts with p53 and down-regulates Bax in a p53-dependent manner. Our study implicates that the inhibition of p53 transcriptional activity by DJ-1 may play a role in control of p53-Bax-caspase apoptotic pathway.

EXPERIMENTAL PROCEDURES

Plasmid Constructs—Full-length DJ-1 cDNA was first amplified by reverse transcription (RT)-PCR using total RNA extracted from 293 cells with primers 5′-CGGGATCCCCATGGCTTCCAAAAGAAG-3′ and 5′-CGCTCGAGCTGCTGGAGTCTTTAAGAAC-3′. The PCR product was inserted into pGEX-5x-1 at BamHI/XhoI sites or pEGFP-N1 at BglII/SalI sites. pET-15b-DJ-1 was generated by subcloning the PCR product, amplified with primers 5′-CCCTCGAGATGGCTTCCAAAAGAGC-3′ and 5′-CGGGATCCGCTGCTCTAGTCTTTAAG-3′, into pET-15b vector. For creating a construct

* This work was supported in part by National Natural Sciences Foundation of China Grant 30770664, National High-tech Research and Development program of China 973-project 2006CB500703 and 863-project 2006AA02Z184, and the Chinese Academy of Sciences Knowledge Innovation Project (KSCX2-YW-R-138). 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.

[1] To whom correspondence should be addressed. Tel. and Fax: 86-551-3607058; E-mail: wghui@ustc.edu.cn.

[2] The abbreviations used are: PD, Parkinson disease; Bax, Bcl2-associated X protein; EGFP, enhanced green fluorescent (EGF) protein; GST, glutathione *S*-transferase; Erk1, extracellular signal-regulated kinases 1; ANOVA, analysis of variance; HRP, horseradish peroxidase; PI, propidium iodide; RT, reverse transcription; N2a cells, Neuro2a cells; CMV, cytomegalovirus; si-, small interfering-; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

This is an Open Access article under the CC BY license.

expressing FLAG-DJ-1, we cut out the full-length DJ-1 cDNA at XhoI/BamHI sites from pET-15b-DJ-1 and subcloned this fragment into p3×FLAG-Myc-CMV vector (Sigma). The human p53 cDNA was first obtained by PCR from a human fetal brain cDNA library (Clontech) with primers 5′-CGCGGATCCAAATGGAGGAGCCGCAG-3′ and 5′-CCGCTCGAGTGTTTGTCTGAGTCAGGC-3′ and then inserted into pGEX-5x-1 at BamHI/XhoI sites. pEGFP-N1-p53 was generated by excising p53 cDNA from pGEX-5x-1-p53 and inserting into pEGFP-N1. To create the construct expressing FLAG-p53, we inserted the PCR product, amplified with primers 5′-CCGCTCGAGATGGAGGAGCCGCAGTC-3′ and 5′-CGCGGATCCTCAGTCTGAGTCAGG-3′, into p3×FLAG-Myc-CMV vector (Sigma) at SalI/BamHI sites. The deletion mutants of p53 were generated by PCR with different primers. pGEX-5x-1-p53(NT) was created by subcloning the PCR product, amplified with primers 5′-CGCGGATCCAAATGGAGGAGCCGCAG-3′ and 5′-CCGCTCGAGCTTTTCTGGGAAGGGAC-3′ using pGEX-5x-1-p53 as the template, into pGEX-5x-1, pGEX-5x-1-p53(DBD), amplified with primers 5′-CGGGATCCAAATGTACCAGGGCAGC-3′ and 5′-CCGCTCGAGCTTTTCTTGCGGAGATT-3′, and pGEX-5x-1-p53(CT) amplified with primers 5′-CGGGATCCAAATGGAGCCTCACCAC-3′ and 5′-CCGCTCGAGTGTTTGTCTGAGTCAGGC-3′.

In Vitro Binding Assays—For GST pulldown assays, an aliquot containing 20 μg of protein from the soluble fraction of *Escherichia coli* cell lysates containing GST or GST-p53 was incubated with 20 μl of glutathione agarose beads (GE Healthcare) for 30 min on ice. After washing 3 times with 1× phosphate-buffered saline to remove unbound materials, the beads were further incubated with 50 μg of protein from the supernatants of *E. coli* crude extract containing recombinant DJ-1 expressed by pET-15b-DJ-1 in 0.25 ml of HNTG buffer (20 mM Hepes-KOH, pH 7.5, 100 mM NaCl, 0.1% Triton X-100, and 10% glycerol) for 1 h at 4 °C. After incubation, the beads were washed 5 times with 1 ml of 1× HNTG buffer. Bound proteins were eluted from the beads by boiling for 5 min in SDS sample buffer and visualized by immunoblot analysis.

Cell Culture, Transfection, and Apoptosis Assay—N2a cells or 293 cells were cultured in Dulbecco's modified Eagle's medium (Invitrogen) containing 10% calf serum (Invitrogen), whereas A549 cells or H1299 cells were maintained in RPMI medium 1640 (Invitrogen) supplemented with 10% fetal bovine serum (Hyclone). Cells were transfected with expressing vectors using Lipofectamine 2000 reagent (Invitrogen) according to the manufacturer's instructions. Forty-eight hours after transfection cells were further incubated with 200 μM H_2O_2 for 6 h to induce apoptosis. Alternatively, cells were exposed to ultraviolet (UV)-B 48 h after transfection. Apoptosis were assessed using FACScalibur flow cytometer (BD Biosciences). Briefly, cells were harvested, rinsed with phosphate-buffered saline, and incubated with annexin V-fluorescein isothiocyanate and propidium iodide (PI) according to the manufacture's instructions (Biosea, Beijing, China). Data analysis was performed with CELLQuest software, and numbers of cells labeled with annexin V- fluorescein isothiocyanate or PI or combination were calculated.

DJ-1 siRNA Knockdown—Double-stranded oligonucleotides synthesized by Shanghai GenePharma (Shanghai, China) were designed against three separate regions starting from nucleotide 32 or nucleotide 328 or nucleotide 549 of mouse DJ-1 cDNA. The sequences of these duplexes are: sense 5′-CCAAAGGAGCAGAGGAGAUTT-3 and antisense 5′-AUCUCCUCUGCUCCUUUGGTT-3′ for si-DJ-1 32; sense 5′-GGCUCUGUUGGCUCACGAATT-3′ and antisense 5′-UUCGUGAGCCAACAGAGCCGT-3′ for si-DJ-1 328, sense 5′-CGCUUGUUCUCAAAGACUATT-3′ and antisense 5′-UAGUCUUUGAGAACAAGCGGT-3′ for si-DJ-1 549. Meanwhile, oligonucleotide targeting against GAPDH served as the positive control, whereas an irrelevant oligonucleotide served as the negative control. The transfection was performed with Oligofectamine (Invitrogen) according to its manufacture when cells grew to reach 40–50% confluence. Briefly, a mixture of Opti-MEM medium (Invitrogen) with Oligofectamine was incubated for 5 min and incubated with siRNA for a further 30 min at room temperature to allow the complex formation. At 12 h post-transfection, the culture medium was replaced by fresh complete medium. Cells were harvested 72 h after transfection followed by further analysis.

Isoelectric Focusing and Immunoblot Analysis—Proteins were separated by 12% SDS-PAGE or in isoelectric focusing gel of pH 5–8 ranges followed by transferring to polyvinylidene difluoride membrane (Millipore). The following primary antibodies were used. Monoclonal anti-Bax antibody and anti-p53 antibody were from Santa Cruz Biotechnology. Monoclonal anti-GFP antibody was from Santa Cruz Biotechnology or Roche Applied Science. Monoclonal anti-GAPDH antibody was from Chemicon. Monoclonal anti-FLAG antibody and anti-FLAG antibody conjugated with HRP were from Sigma. Polyclonal anti-GFP antibodies, anti-p53 antibodies, anti-Erk1 antibodies, anti-caspase-9 antibodies, and anti-caspase-3 antibodies were from Santa Cruz Biotechnology. Polyclonal anti-DJ-1 antibodies were from Chemicon. The secondary antibodies, sheep anti-mouse IgG-HRP antibodies, or anti-rabbit IgG-HRP antibodies were from Amersham Biosciences. The proteins were visualized using an ECL detection kit (Amersham Biosciences).

To reprobe the membrane for another primary antibody, the membrane was first incubated with Stripping buffer (50 mM Tris-HCl, pH 6.8, 0.1 mM β-mercaptoethanol, 20 mM SDS) for 30 min at 50 °C. Then the membrane was subjected to immunoblot analysis following the standard protocol.

Immunoprecipitation—293 cells co-transfected with FLAG-p53 along with EGFP or DJ-1-EGFP were collected 48 h after transfection and lysed in TSPI buffer containing 50 mM Tris-HCl pH 7.5, 150 mM sodium chloride, 1 mM EDTA, 1% Nonidet P-40 supplemented with complete mini protease inhibitor mixture (Roche Applied Science). Cellular debris was removed by centrifugation at 12,000 × *g* for 30 min at 4 °C. The supernatants were incubated with monoclonal anti-GFP antibody (Roche Applied Science) or anti-FLAG antibody for 1 h at 4 °C. After incubation, protein G-Sepharose was used for precipitation. The beads were washed with TSPI buffer four times and then eluted with SDS sample buffer for immunoblot analysis using anti-DJ-1 antibodies or anti-FLAG antibody. Alterna-

tively, 4 μg of monoclonal anti-p53 antibody or normal mouse IgG was incubated with protein G-Sepharose for 6 h at 4 °C, and then equal amounts of supernatant from A549 or H1299 cell lysates were added and incubated overnight at 4 °C. The resin was washed six times with TSPI buffer and resuspended in SDS sample buffer. Proteins were subjected to immunoblot analysis using anti-DJ-1 or anti-p53 antibodies.

Luciferase Reporter Gene Assay and RT-PCR—H1299 cells were co-transfected with pGL3-Bax Luciferase construct with or without a fixed amount of FLAG-p53 along with EGFP or DJ-1-EGFP. *Renilla*-expressing plasmid pRL-CMV was co-transfected to normalize the variations in transfection efficiency. The total amount of plasmid DNA was kept constant by the addition of empty plasmid. Cell lysates were prepared using Passive Lysis Buffer (Promega) 48 h after the transfection. Both firefly and *Renilla* activities were measured with Dual Luciferase Reporter Systems using Veritas Microplate luminometer according to the manufacturer's instructions. The absolute values of firefly luminescence were normalized to those of *Renilla*, and the ratios were presented as the mean ± S.E. with three transfection experiments. Meanwhile, total RNA from those cells was extracted using TRIzol reagent (Invitrogen) and then subjected to RT-PCR with primers 5′-TCAAAGAGGCGAACTGTGTG-3′ and 5′-GGTGTTGGAGCAAGATGGAT-3′ for luciferase.

RESULTS

DJ-1 Protects against Hydrogen Peroxide-induced Apoptosis by Down-regulating Bax in a p53-dependent Manner—To investigate whether DJ-1 protects against cell death in our cellular model, Neuro2a (N2a) cells, a mouse neuroblastoma cell line, were transiently transfected with DJ-1-EGFP or EGFP alone. Forty-eight hours after transfection cells were exposure to 200 μM hydrogen peroxide (H_2O_2) for 4 h. Then the cells were collected, and the cell lysates were subjected to immunoblot analysis. The cleaved form of caspase-9 (p35) was detected from cells transfected with EGFP using antibodies specific to cleaved caspase-9, whereas it was not detected from cells expressing DJ-1-EGFP (Fig. 1*A*). Furthermore, pro-caspase-3 was also processed into the active form (p17) in cells expressing EGFP but not in cells expressing DJ-1-EGFP (Fig. 1*A*). Based on the observation that DJ-1 inhibits caspase cleavage under oxidative stress, we next searched for the factors upstream of caspase activation. Bax, a proapoptotic member of bcl-2 family, is considered as an important molecule in apoptotic cell death. Therefore, we examined the level of Bax in the presence or absence of overexpressed DJ-1. The levels of Bax are markedly decreased in N2a cells expressing DJ-1-EGFP compared with cells expressing EGFP alone (Fig. 1*B*). Quantitative data are shown in Fig. 1*C*. The same results were obtained using human embryo kidney 293 cells (Fig. 1, *D* and *E*). However, the protein levels of p53 (Fig. 1*F*) as well as SOD1 (Fig. 1*G*) remain unchanged.

It is well known that p53 is important for Bax transcription. To further determine whether the down-regulation of Bax is dependent on p53, we transfected A549 ($p53^{+/+}$) cells and H1299 ($p53^{-/-}$) cells with DJ-1-EGFP or EGFP and treated the cells with H_2O_2 or not. In A549 cells overexpression of DJ-1

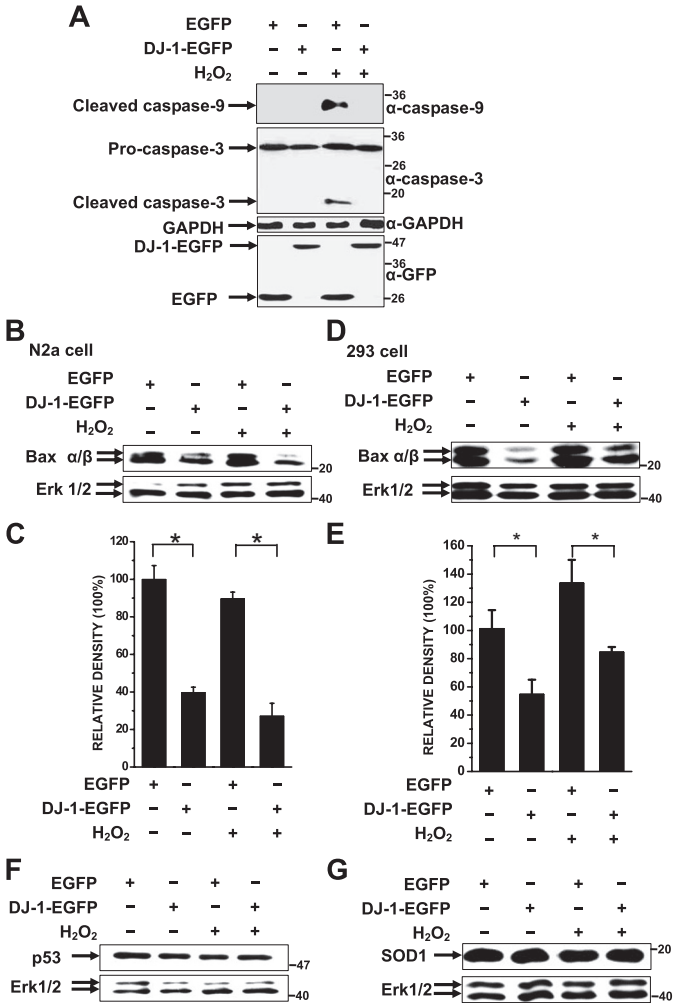

FIGURE 1. **DJ-1 protects against apoptosis induced by oxidative stress.** *A*, inhibition of the cleavage of caspase-9 and -3 induced by oxidative stress. Cells transfected with DJ-1-EGFP or EGFP were treated with or without 200 μM H_2O_2 for 4 h. Equivalent cell lysates were subjected to immunoblot analysis using anti-caspase-9 antibodies, anti-caspase-3 antibodies, and anti-GFP antibody or anti-GAPDH antibody. The molecular weight markers in kilodaltons are listed on each blot. *B*, DJ-1 down-regulates Bax in N2a cells. N2a cells expressing EGFP or DJ-1-EGFP were treated with or without H_2O_2 for 4 h, and the whole cell lysates were subjected to immunoblot analysis using anti-Bax antibody or anti-Erk1 antibodies. *C*, band density of Bax relative to that of Erk1 was analyzed by one way ANOVA with post hoc Tukey's test. Data are shown as the mean ± S.E. values from three independent transfection experiments. *, $p < 0.05$ *versus* groups expressing EGFP. *D*, DJ-1 down-regulates Bax in 293 cells. The same experiments in *A* were carried out using 293 cells. *E*, quantitative data from *D* are shown. Band density of Bax relative to that of Erk1 was analyzed by one way ANOVA with post hoc Tukey's test. *, $p < 0.05$ *versus* groups expressing EGFP. *F–G*, expression levels of p53 and SOD1 are not affected by DJ-1 overexpression. Total lysates from DJ-1-EGFP- or EGFP-expressing 293 cells treated with or without H_2O_2 were subjected to immunoblot analysis using anti-p53 antibody, anti-SOD1 antibody, or anti-Erk1 antibodies.

decreases the Bax levels in both groups that are treated with H_2O_2 or not (Fig. 2*A*). Quantitative data are shown in Fig. 2*B*. However, in p53-null H1299 cells, overexpression of DJ-1 does not affect the Bax levels (Fig. 2, *C* and *D*), suggesting that the down-regulation of Bax by DJ-1 depends on the presence of p53. To further determine that p53 is involved in the regulation of Bax by DJ-1, we co-transfected H1299 cells with or without DJ-1-EGFP in the presence of FLAG-p53. Expression of FLAG-p53 in H1299 cells increases the Bax level as compared with

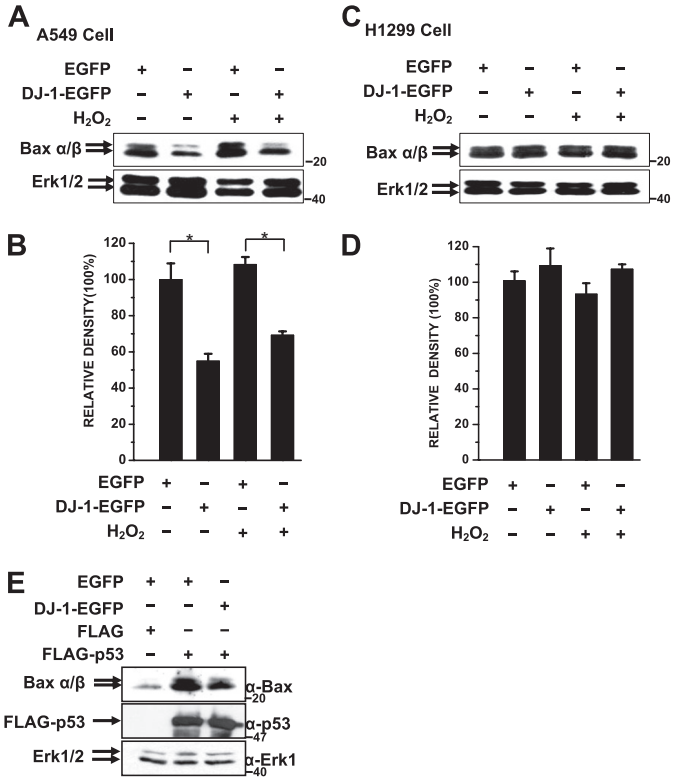

FIGURE 2. **DJ-1 down-regulates Bax in a p53-dependent manner.** *A*, DJ-1 down-regulates Bax in $p53^{+/+}$ cells. A549 ($p53^{+/+}$) cells were transfected with DJ-1-EGFP or EGFP. The cells were treated with or without H_2O_2 for 4 h, and then cells were collected for immunoblot analysis using anti-Bax antibody or anti-Erk1 antibodies. *B*, band density of Bax relative to that of Erk1 was analyzed by one way ANOVA with post hoc Tukey's test. Data represent the mean ± S.E. values from three independent experiments. *, $p < 0.05$ *versus* groups expressing EGFP. *C*, DJ-1 fails to down-regulate Bax in $p53^{-/-}$ cells. H1299 ($p53^{-/-}$) cells were transfected with DJ-1-EGFP or EGFP and treated with or without H_2O_2. The whole cell lysates were subjected to immunoblot analysis using anti-Bax antibody or anti-Erk1 antibodies. *D*, band density of Bax relative to that of Erk1 was analyzed by one way ANOVA with post hoc Tukey's test. Data are shown as the mean ± S.E. values from three independent transfection experiments. *, $p < 0.05$ *versus* groups expressing EGFP. *E*, DJ-1 attenuates Bax increase by p53 overexpression in H1299 cells. H1299 cells were co-transfected with EGFP or EGFP-DJ-1 along with FLAG-p53, whereas cells co-transfected with FLAG and EGFP work as controls. Cell lysates were collected and subjected to immunoblot analysis using anti-Bax antibody, anti-p53 antibody, or anti-Erk1 antibodies.

expression of FLAG-tag alone (Fig. 2*E*), suggesting that FLAG-p53 restores the function to up-regulate Bax. However, the Bax level in H1299 cells coexpressing FLAG-p53 and DJ-1-EGFP is significantly lower than that in cells coexpressing FLAG-p53 and EGFP (Fig. 2*E*), suggesting that the presence of p53 is indeed necessary for DJ-1 effects on Bax expression.

DJ-1 Protects against Apoptosis Induced by Ultraviolet Exposure—Although we observed that overexpression of DJ-1 down-regulates Bax with or without H_2O_2 stimulus, the Bax levels as well as p53 levels are not increased in the H_2O_2-treated cellular model. To further determine that the protective effect of DJ-1 is relevant to p53-dependent cell death, we exposed DJ-1-overexpressing cells to UV light, a widely accepted stimulus initiating p53-Bax-caspase cell death pathway (27). To confirm the toxic effect of UV in our cellular model, we exposed N2a cells to various doses of UV and recovered for 12 h followed by treatment with PI, which stains the nuclei of late apoptotic and dead cells. As shown in Fig. 3*A*, the PI positive staining cells are increased with the increase of UV exposure dose, indicating that UV exposure leads to cell death in a dose-dependent manner in N2a cells. Immunoblot analysis showed that UV exposure increases the Bax and p53 protein levels in a time-dependent manner (Fig. 3, *B* and *C*). In addition, a shift in pI of endogenous DJ-1 to a more acidic point was observed after UV exposure. Such a shift was also found in cells treated with hydrogen peroxide (Fig. 3*D*). Based on these observations, we next determine the role of DJ-1 in UV-induced cell death. N2a cells transiently transfected with FLAG-DJ-1 or FLAG were exposed to UV (20 mJ/cm^2) or not 2 days after transfection and then recovered for 6 h followed by annexin V/PI staining and flow cytometry analysis. In each dot-plot shown in Fig. 3*E*, the *lower right quadrant* indicates the population of cells labeled with annexin V, representing the early apoptotic cells, whereas the *upper right quadrant* indicates those with both annexin V and PI staining, representing late apoptotic and dead cells. The percentages of apoptotic cells are low in N2a cells expressing FLAG or FLAG-DJ-1 without UV exposure. However, after UV treatment, the percentages of annexin V-positive and annexin V/PI-positive cells increase to 17.43 and 10.93% in cells expressing FLAG, respectively. In contrast, they decrease significantly to 8.04 and 7.41% in those cells expressing FLAG-DJ-1, supporting the cytoprotective role of DJ-1 in UV-induced cell death (Fig. 3*E*). To further explore whether the protective effect is correlated with any change of Bax protein level, we transfected N2a cells with DJ-1-EGFP or EGFP and exposed the cells to UV. As shown in Fig. 3*F*, in N2a cells, overexpression of DJ-1 decreases Bax levels both in the absence and presence of UV exposure, although UV exposure increases Bax levels. Quantitative data are shown in Fig. 3*H*. Furthermore, procaspase-3 is also processed into the active form (p17) in cells expressing EGFP but not in cells expressing DJ-1-EGFP with the UV stimulus (Fig. 3*G*). Although p53 increases obviously after UV exposure, overexpression of DJ-1 has little effect on p53 protein levels (Fig. 3*G*). Quantitative data are shown in Fig. 3*I*.

Silencing DJ-1 Increases Bax and Facilitates UV-induced Cell Death in a p53-dependent Manner—To further assess the effect of DJ-1 on Bax regulation, we investigated the impact of DJ-1 silencing in our cellular model. N2a cells were transfected with specific RNA oligonucleotides targeting against DJ-1 or control RNA oligonucleotides, and the whole cell lysates were collected 72 h after transfection and subjected to immunoblot analysis. Transfection with the two pairs of RNA oligonucleotides against two different regions of DJ-1 successfully suppresses the protein level of DJ-1 as compared with that in cells transfected with control siRNAs (Fig. 4*A*). Meanwhile, the protein levels of Bax increase synchronously along with the decrease of DJ-1 (Fig. 4*A*). Quantitative data are shown in Fig. 4*B*. Next, we went on to determine whether knockdown of DJ-1 affects the protective role of DJ-1 in UV-induced cell death. N2a cells transfected with siRNAs to DJ-1 or control siRNAs were exposed to UV (20 mJ/cm^2) 2 days after transfection and recovered for another 6 h followed by flow cytometry analysis. Although transfection with mock siRNA results in negligible cell death, transfection with siRNA against DJ-1 leads to a significant increase in cell death in the absence and presence of UV exposure (Fig. 4*C*).

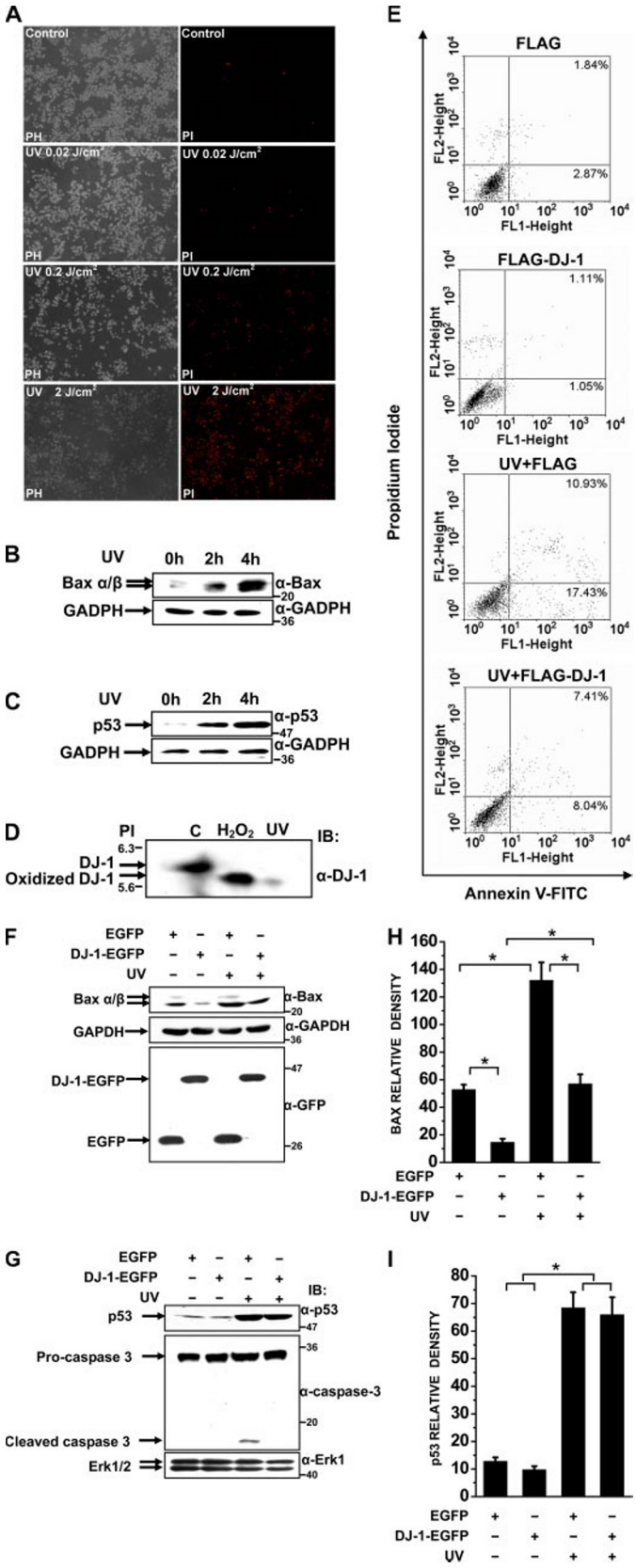

FIGURE 3. **DJ-1 protects against apoptosis induced by ultraviolet exposure.** *A*, UV radiation increases cell death in a dose-dependent manner. N2a cells were exposed to the indicated doses of UV and recovered for 12 h followed by PI staining and observation with an inverted system microscope. *B* and *C*, UV radiation enhances p53 and Bax expression. After UV exposure (20mJ/cm^2), the whole cell lysates were collected at the indicated times after the UV radiation. Immunoblot analysis was carried out using anti-p53 antibody, anti-Bax antibody, or anti-GAPDH antibody. *D*, pI shift in DJ-1 after UV or hydrogen peroxide treatment. Cells exposed to UV (20mJ/cm^2) or hydrogen peroxide (200 μM) or those without treatment (*C*) were collected and separated in isoelectric focusing gel of pH 5–8 ranges followed by immunoblot (*IB*) analysis using anti-DJ-1 antibodies. The markers on the *left* indicate approximate pI values. *E*, overexpression of FLAG-DJ-1 rescues N2a cells from UV-induced death. N2a cells transfected with FLAG-DJ-1 or FLAG were exposed to UV (20 mJ/cm^2) or not and recovered for 6 h followed by annexin V/PI staining and flow cytometry analysis. Annexin V staining is on the abscissa (*FL1-Height*), whereas PI staining is on the ordinate (*FL2-Height*). The percentage on the *lower right quadrant* indicates the population of cells labeled with annexin V, representing the early apoptotic cells, whereas that on the *upper right quadrant* indicates the population of cells with both annexin V and PI staining, representing late apoptotic and dead cells. *F* and *G*, DJ-1 down-regulates Bax and inhibits the cleavage of caspase-3. N2a cells were transiently transfected with DJ-1-EGFP or EGFP and treated with UV radiation or not. The whole cell lysates were collected and subjected to immunoblot analysis using anti-p53 antibody, anti-Bax antibody, anti-GFP antibody, anti-GAPDH antibody, anit-Caspase-3 antibodies, and anti-Erk1 antibodies. *H*, quantitative data from *F* are shown. Band density of Bax relative to that of GAPDH was analyzed by one way ANOVA with post hoc Tukey's test. Data are shown as the mean ± S.E. values from three independent transfection experiments. *, $p < 0.05$ *versus* groups expressing EGFP. *I*, quantitative data from G are shown. Band density of p53 relative to that of Erk1 was analyzed by one way ANOVA test with post hoc Tukey's test. Data are shown as the mean ± S.E. values from three independent transfection experiments. *, $p < 0.05$ *versus* groups without UV exposure.

Cleavage of caspase-3 was observed in cells transfected with siRNAs against DJ-1 but not in those with control siRNAs (Fig. 4*D*). These data suggest that knockdown of DJ-1 increases the protein level of Bax and makes N2a cells more susceptible to UV exposure. To further determine whether the increase of Bax protein level is related to the status of p53, we transfected both A549 (p53$^{+/+}$) cells and H1299 (p53$^{-/-}$) cells with control siRNAs and siRNAs against DJ-1. The total cell lysates were collected 72 h after transfection followed by immunoblot analysis and quantification (Fig. 4, *E–H*). Although the levels of DJ-1 protein decrease to a similar extent in both cell lines, the Bax protein level increases significantly in A549 cells (Fig. 4, *E* and *G*) but is unchanged in H1299 cells (Fig. 4, *F* and *H*), suggesting that the increase of Bax by knockdown of DJ-1 is dependent on the presence of p53. However, in A549 cells, no changes of the p53 protein levels were observed in cells transfected with siRNAs against DJ-1 and those with control siRNAs, implying that the p53 protein level is not regulated by DJ-1 (Fig. 4, *E* and *G*). Moreover, the dependence of Bax regulation by DJ-1 on p53 transcriptional activity is also determined by the experiments using pifithrin-α, an inhibitor of p53 transcriptional activity. A549 cells transfected with control siRNAs or siRNAs against DJ-1 were treated with 20 μM pifithrin-α (Sigma) or Me_2SO (as a control) for 12 h, and the total cell lysates were collected followed by immunoblot analysis and quantification. Although siRNA knockdown of DJ-1 results in a significant increase of Bax level in cells treated with Me_2SO, the Bax level only increases slightly in cells treated with pifithrin-α (Fig. 4*I*). Quantitative data are shown in Fig. 4*J*. These results suggest that regulation of Bax by DJ-1 is dependent on the presence of p53 transcriptional activity.

Physical Interaction of DJ-1 with p53—Because DJ-1 down-regulates Bax in a p53-dependent manner, we therefore evaluated the binding of p53 with DJ-1 using GST pulldown assays. GST-p53, which is coupled to glutathione-agarose beads, pulls down DJ-1 expressed by pET-15b-DJ-1 in *E. coli*; however, GST

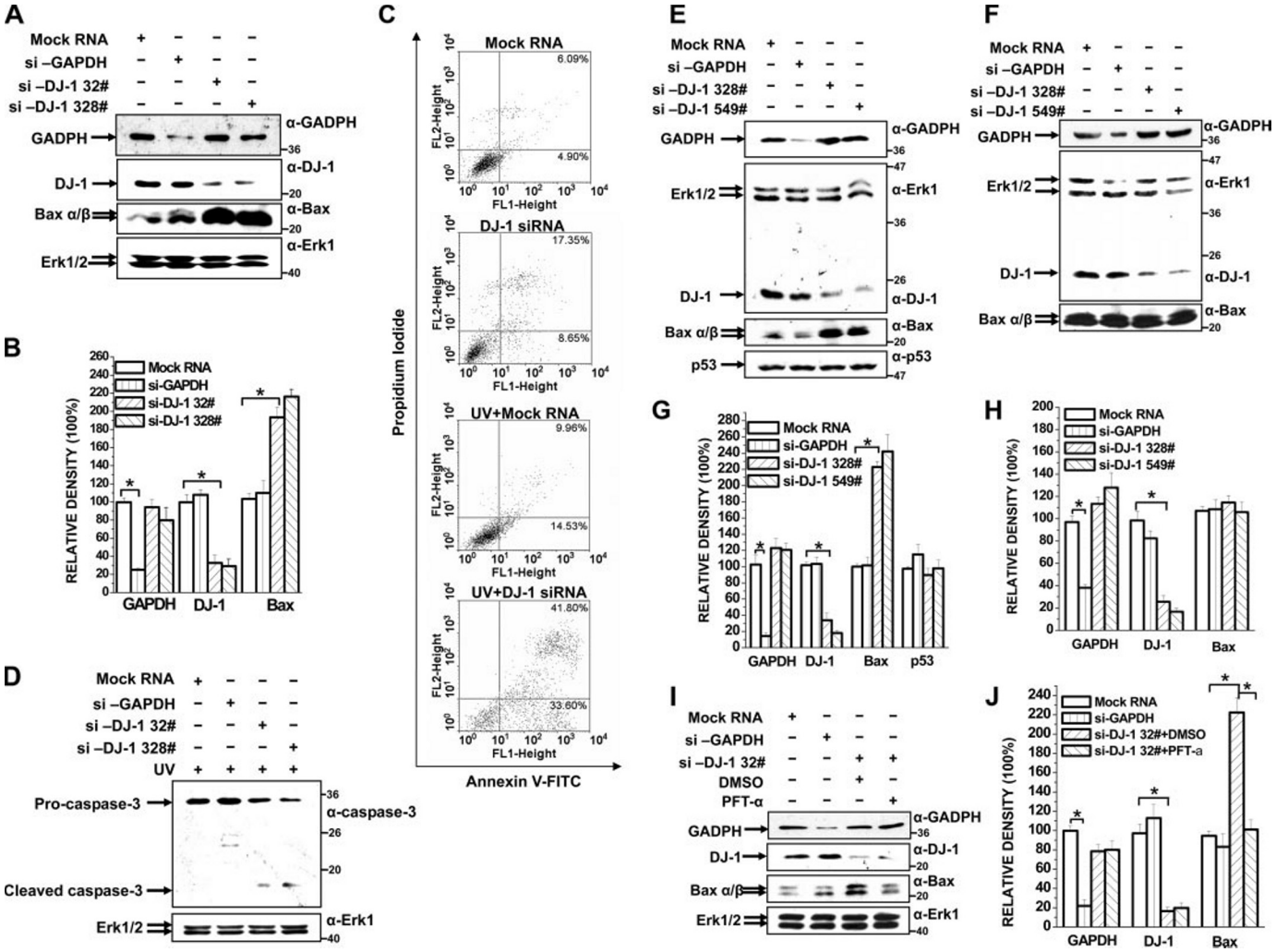

FIGURE 4. **Knockdown of DJ-1 enhances UV-induced cell death in a p53-dependent manner.** *A*, transfection of siRNAs against DJ-1 increases Bax protein expression. N2a cells were transfected with two oligonucleotides targeting against respective regions of DJ-1 mRNA and an oligonucleotide targeting against GAPDH, serving as positive control. Negative control was also set by transfection of an irrelevant oligonucleotide. The whole cell lysates were collected 72 h after the transfection followed by the immunoblot analysis using anti-GAPDH antibody, anti-Bax antibody, anti-DJ-1 antibodies, and anti-Erk1 antibodies. *B*, quantitative data from *A* are shown. Band density of GAPDH, DJ-1, and Bax relative to that of Erk1 was analyzed by one way ANOVA with post hoc Tukey's test. Data are shown as the mean ± S.E. values from three independent transfection experiments. *, $p < 0.05$ *versus* groups transfected with mock RNA. *C*, knockdown of DJ-1 enhances UV-induced N2a cell death. N2a cells transfected with siRNA against DJ-1 and control siRNA were exposed to 20mJ/cm^2 followed by annexin V/PI staining and flow cytometry analysis. Numbers within the *upper* or *lower right quadrants* represent the percentages of dead or apoptotic cells. *D*, knockdown of DJ-1 increases UV-induced caspase-3 activation in N2a cells. N2a cells transfected with siRNAs against DJ-1 and control siRNAs were exposed to 10mJ/cm^2 and recovered for 2 h, and the whole cell lysates were harvested and subjected to immunoblot analysis with anti-caspase-3 antibodies and anti-Erk1 antibodies. *E* and *F*, knockdown of DJ-1 increases the Bax level in A549 cells but not in H1299 cell. A549 cells and H1299 cells were transfected with siRNAs against DJ-1 or that against GAPDH or the irrelevant oligonucleotide. The total cell lysates were subjected to immunoblot analysis by using anti-GAPDH antibody, anti-Bax antibody, anti-DJ-1 antibodies, and anti-Erk1 antibodies. *G*, quantitative data from E are shown. Band density of GAPDH, DJ-1, Bax, and p53 relative to that of Erk1 was analyzed by one way ANOVA with post hoc Tukey's test. Data are shown as the mean ± S.E. values from three independent transfection experiments. *, $p < 0.05$ *versus* groups transfected with mock RNA. *H*, quantitative data from *F* are shown. Band density of GAPDH, DJ-1, and Bax relative to that of Erk1 was analyzed by one way ANOVA with post hoc Tukey's test. Data are shown as mean ± S.E. values from three independent transfection experiments. *, $p < 0.05$ *versus* groups transfected with mock RNA. *I*, knockdown of DJ-1 fails to increase the Bax level in the presence of pifithrin-α. N2a cells were transiently transfected with siRNA against DJ-1 or that against GAPDH or the irrelevant oligonucleotide. Sixty hours after the transfection, cells transfected with siRNA against DJ-1 were treated with either pifithrin-α (20 μM) or Me_2SO (as control) and were cultured for additional 12 h. The total cell lysates were subjected to immunoblot analysis by using anti-GAPDH antibody, anti-Bax antibody, anti-DJ-1 antibodies, and anti-Erk1 antibodies. *J*, quantitative data from I are shown. Band density of GAPDH, DJ-1, and Bax relative to that of Erk1 was analyzed by one way ANOVA with post hoc Tukey's test. Data are shown as the mean ± S.E. values from three independent transfection experiments. *, $p < 0.05$ *versus* groups transfected with mock RNA or *versus* groups treated with pifithrin-α.

alone does not (Fig. 5*B*). To further determine which domain(s) of protein p53 is responsible for the interaction with DJ-1, we generated different deletion mutants of p53 (Fig. 5*A*) and examined their interactions with DJ-1 using GST pulldown assays. As shown in Fig. 5*C*, the DBD (DNA binding domain) and the C terminus (*CT*) of p53 pull down DJ-1, whereas the N terminus (*NT*) of p53 and GST alone do not. To further identify the interactions between p53 and DJ-1, we carried out the coimmunoprecipitation experiments. We co-transfected 293 cells with DJ-1-EGFP along with FLAG-p53 or FLAG-BAP (as a control). When DJ-1-EGFP is immunoprecipitated from the cell supernatants using anti-GFP antibody, FLAG-p53, but not FLAG-BAP, is co-immunoprecipitated (Fig. 5*D*). However, no FLAG-p53 is co-immunoprecipitated using anti-GFP antibody from 293 cells co-transfected with FLAG-p53 and EGFP (Fig. 5*D*). Moreover, in 293 cells transfected with FLAG-p53 (but not

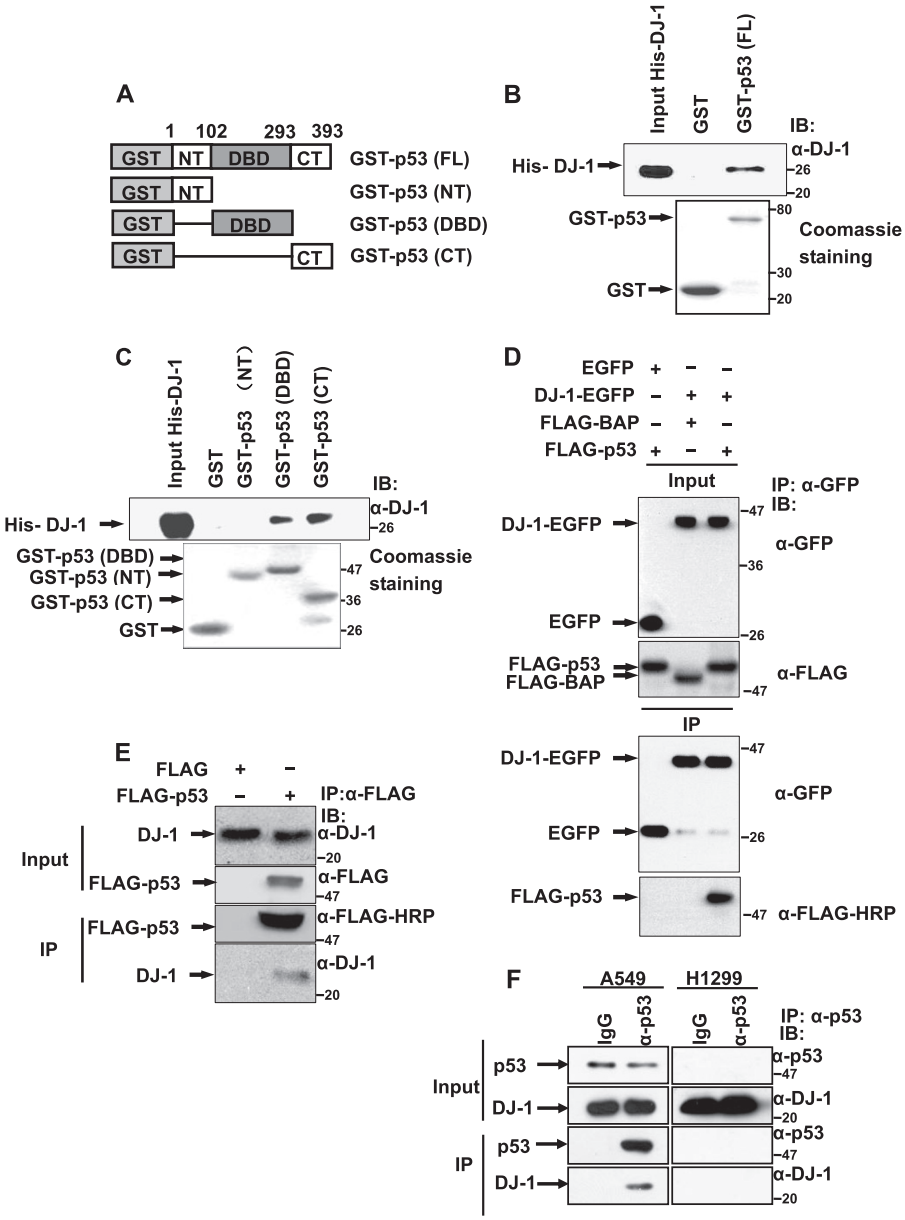

FIGURE 5. **Interaction of DJ-1 with p53.** *A*, schematic diagram of p53 deletion mutants fused to GST. *FL*, full-length; *NT*, N terminus; *DBD*, DNA binding domain; *CT*, C terminus. *B* and *C*, GST-p53 interacts with His-DJ-1 *in vitro*. The supernatant of *E. coli* crude extract containing recombinant His-DJ-1 expressed by pET-15b-DJ-1 was incubated with glutathione-agarose beads bound GST or GST-p53 (*FL*) (*B*) or with indicated p53 deletion mutants (*C*). *IB*, immunoblot. After incubation the beads were washed with HNTG buffer, and the bound proteins were detected using anti-DJ-1 antibodies by immunoblot analysis. *Input* represents 10% of DJ-1 incubated with GST or GST-p53. The *lower panels* in *B* and *C* show the inputs of GST, GST-p53, and indicated p53 deletion mutants. *D*, FLAG-p53 is co-immunoprecipitated (*IP*) with DJ-1-EGFP. 293 cells were co-transfected with DJ-1-EGFP or EGFP along with FLAG-p53 or FLAG-BAP. The supernatants of cell lysates were immunoprecipitated using anti-EGFP antibody. The immunoprecipitants were subjected to immunoblot analysis using anti-FLAG-HRP antibody or anti-GFP antibody. *Input* represents 10% of cell lysates used in the co-immunoprecipitation experiment. *E*, endogenous DJ-1 is co-immunoprecipitated with FLAG-p53. The supernatants of 293 cells transfected with FLAG-p53 or FLAG were subjected to immunoprecipitation using anti-FLAG antibody, and the immunoprecipitants were subjected to immunoblot analysis using anti-DJ-1 antibodies or anti-FLAG-HRP antibody. *Input* represents 10% of cell lysates used in the co-immunoprecipitation experiment. *F*, interaction of endogenous DJ-1 with p53 exists in A549 cells. The supernatant of A549 or H1299 cell lysates were incubated with anti-p53 antibody or normal mouse serum coupled to protein G-Sepharose followed by immunoblot analysis using DJ-1 antibodies or anti-p53 antibodies. *Input* represents 10% of cell lysates used in the co-immunoprecipitation experiment.

FLAG tag only), endogenous DJ-1 is also able to be co-immunoprecipitated when FLAG-p53 is immunoprecipitated using anti-FLAG antibody (Fig. 5*E*). To further ascertain specific interactions of endogenous DJ-1 and p53, we performed co-immunoprecipitation experiments using A549 and H1299 cell lysates. The supernatants of both cell lysates were incubated with protein G-agarose beads that have been preincubated with anti-p53 antibody. The immunoprecipitants were subsequently separated by SDS-PAGE and immunoblotted using anti-DJ-1 antibodies or anti-p53 antibodies. As shown in Fig. 5*F*, DJ-1 is co-immunoprecipitated from A549 cell lysates when p53 is immunoprecipitated by anti-p53 antibody but not by nonspecific mouse IgG. Meanwhile, in H1299 cells, neither p53 is immunoprecipitated nor DJ-1 is co-immunoprecipitated (Fig. 5*F*). These results suggest the physical interaction of DJ-1 with p53.

DJ-1 Inhibits p53 Transcriptional Activity on Bax Promoter—To further determine whether the down-regulation of endogenous Bax by DJ-1 is mediated by the inhibition of p53 transcriptional activity, we performed the reporter gene assays. As shown in Fig. 6*A*, expression of FLAG-p53 significantly activates the transcription of the luciferase reporter gene. However, DJ-1-EGFP greatly decreases FLAG-p53 transcriptional activity. These results support that the down-regulation of Bax by DJ-1 is mediated by the inhibition of p53 transcriptional activation to *Bax* promoter. To exclude the possibility that RNA stability and translational effects affect on luciferase activity, we assessed the levels of luciferase RNA using RT-PCR. Consistent with our data in luciferase assay, the RNA level of luciferase is much higher in cells transfected with FLAG-p53 than those only transfected with FLAG tag. Meanwhile, expression of DJ-1-EGFP sharply decreases the RNA level of luciferase (Fig. 6*B*).

DISCUSSION

Our study demonstrates that DJ-1 exerts its cytoprotection function through inhibiting caspase activation and decreasing Bax expression by repressing p53 transcriptional activity. Several studies showed the involvement of caspases in the pathogenesis of PD by the presence of activated caspases in various cellular or animal models and, more impor-

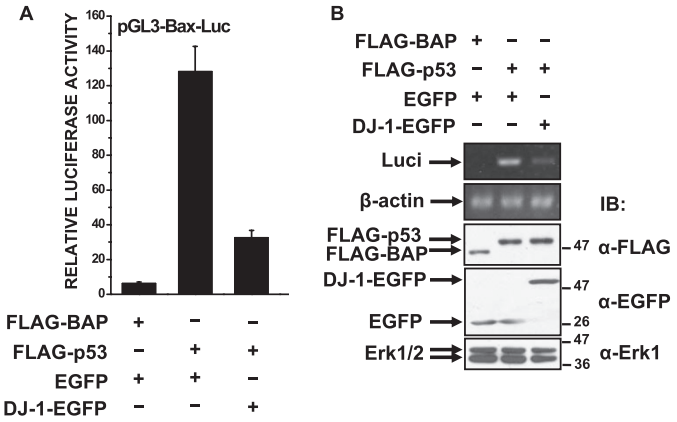

FIGURE 6. **DJ-1 inhibits p53 transcriptional activity on *Bax* promoter.** *A*, H1299 cells were co-transfected with pGL3-Bax luciferase construct with or without a fixed amount of FLAG-p53 along with EGFP or DJ-1-EGFP. The total amount of plasmid DNA was kept constant by the addition of empty plasmid. *Renilla* expressing vector pRL-CMV was used as a transfection internal control. Quantification of luciferase activities and calculations of relative ratios were performed. Data are shown as the mean ± S.E. values from three independent transfection experiments. *B*, total RNA from those cells above was extracted and subjected to RT-PCR using specific primers against luciferase. Amplification for β-actin is shown as loading control. Total cell lysates from those cells were prepared and subjected to immunoblot (*IB*) using anti-FLAG antibody, anti-GFP antibody, and anti-Erk1 antibodies showing similar loading amounts in each lane.

tantly, in postmortem PD brains (28–30). Also in PD brains, the percentage of DA neurons positive for Bax in the substantia nigra pars compacta is higher than that in controls (31). The mRNA and protein levels of Bax were found to be up-regulated in the substantia nigra pars compacta in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated mice, a widely used animal model for PD (32). Furthermore, Bax-deficient mice are resistant to MPTP toxicity as compared with their wild type littermates (32). In the present study we show that overexpression of DJ-1 down-regulates Bax in N2a, 293, and A549 cells, which harbor p53, but not in H1299 cells, the p53-null cells. In contract to overexpression, knockdown of DJ-1 up-regulates the Bax expression in N2a and A549 cells but not in H1299 cells. These data suggest that the regulation of Bax by DJ-1 depends on the presence of p53. Our results are also consistent with the findings by other investigators who reported that in zebrafish, knockdown of DJ-1 up-regulates Bax level in the presence or absence of the toxin exposure and increases the susceptibility of dopaminergic neurons to oxidative stress (26). Taken together, we propose that the regulation of Bax by DJ-1, which is dependent on p53, may play an important role in PD pathogenesis. In our present study neither the expression of p53 nor that of Bax increases in cells treated with hydrogen peroxide. Because the protein p53, as well as Bax, increases within a specific time window after H_2O_2 treatment, we might miss the appropriate time point to detect such changes (33). Instead, we applied UV exposure, a genotoxic stress that induces an increase of p53 as well as Bax to investigate the effect of DJ-1 on p53-dependent cell death. In this way we found that p53 and Bax increase in response to UV exposure; meanwhile, overexpression of DJ-1 inhibits the increases of Bax and the cleavage of caspase-3 induced by UV. Interestingly, a shift in the pI of endogenous DJ-1 to more acidic point was observed both after UV exposure and after H_2O_2 treatment, implying that another common pathway independent of p53 status might be involved in after both treatments.

Increased levels of p53 and phosphorylated p53 were found in the substantia nigra and striatum of the postmortem PD brains (34, 35); DJ-1 is also selectively expressed within these two regions critically involved in PD pathogenesis (36, 37). Interestingly, our study demonstrates that the protective effects of DJ-1 are not caused by the change of p53 levels but by inhibiting p53 transcriptional activities. Evidence for the critical role of p53, with emphasis on its transcriptional activity, has been provided in neuronal death in PD by the studies using *in vitro* and *in vivo* models (38–42). Pifithrin-α, an inhibitor for p53 transcriptional activity, was reported to preserve dopamine neurons in PD cellular model (41). Besides this chemical agent, several other proteins or peptides are able to repress p53 activity directly or indirectly through different mechanisms. For example, Mdm2, Pirh2, and COP1, identified as E3 ubiquitin ligases, target p53 to the proteasome for degradation (43–45). Parc interacts with and sequesters p53 in the cytoplasm and, therefore, prevents its translocation to the nucleus (46). Histone deacetylase 1 (HDAC-1) down-regulates p53-dependent gene activation by reducing the acetylation level of p53, whereas nerve growth factor promotes the p53 deacetylation by increasing HDAC-1 activity (47, 48). SV 40 large antigen and Plk1 interact with the DNA binding domain and attenuate the binding of p53 to promoter, thus repressing p53 transcriptional activity (49, 50). Some other proteins may repress p53 transcriptional activity in rather indirect ways including facilitating Hdm2-p53 interaction or recruiting co-repressor such as histone deacetylase 1 to p53 (51, 52). Here we demonstrate that DJ-1 interacts with p53 through the DNA binding domain and the C terminus of p53. We suppose that DJ-1, probably like SV40 large T antigen or Plk1, interferes with the binding of p53 to the *Bax* promoter, thus regulating Bax expression, although we have not yet addressed this possibility in our present study. Because p53 is acetylated on the C terminus by p300/CBP, which enhanced the DNA binding ability of p53 (53, 54), it is supposed that this acetylation-dependent enhancement of DNA binding ability might be reduced due to the mask of acetylation sites by the interaction of DJ-1 and the C terminus of p53, which therefore represses p53 transcriptional activity. It is also possible that DJ-1 acts as the WRN protein, the product of *WRN* gene that is responsible for Werner's syndrome, which binds to the C terminus of p53 and attenuates p53-mediated apoptotic response, although the mechanism remains to be elucidated (55).

In contrast to our present observation, DJ-1 has been reported previously to support p53 transcriptional activity by interacting with the Topors/p53BP3 (17). The discrepancy above all may result from the overexpression of Topors/p53BP3, which binds to DJ-1 and potentially interferes with the direct interaction of DJ-1 and p53. Another possible explanation for this discrepancy is that our reporter construct contains a natural human *Bax* promoter, whereas the reporter construct used by those authors harbors an array of 13 p53 binding sites. It was reported that the minimal *Bax* response element capable of mediating p53-dependent transcriptional activation consists of two p53 half-sites plus an adjacent six base pairs (56).

In summary, we demonstrate here that DJ-1 exerts its cytoprotection through a p53-Bax-caspase pathway. DJ-1 decreases the Bax protein level and, therefore, blocks the caspase activation by possibly repressing p53 transcriptional activity. Because loss of DJ-1 function is responsible for the onset of PD, the findings here will be of help to expand our knowledge of DJ-1 functions as well as its possible role in PD.

REFERENCES

1. Nagakubo, D., Taira, T., Kitaura, H., Ikeda, M., Tamai, K., Iguchi-Ariga, S. M., and Ariga, H. (1997) *Biochem. Biophys. Res. Commun.* **231,** 509–513
2. Le Naour, F., Misek, D. E., Krause, M. C., Deneux, L., Giordano, T. J., Scholl, S., and Hanash, S. M. (2001) *Clin. Cancer Res.* **7,** 3328–3335
3. Miura, K., Bowman, E. D., Simon, R., Peng, A. C., Robles, A. I., Jones, R. T., Katagiri, T., He, P., Mizukami, H., Charboneau, L., Kikuchi, T., Liotta, L. A., Nakamura, Y., and Harris, C. C. (2002) *Cancer Res.* **62,** 3244–3250
4. Grzmil, M., Voigt, S., Thelen, P., Hemmerlein, B., Helmke, K., and Burfeind, P. (2004) *Int. J. Oncol.* **24,** 97–105
5. Wagenfeld, A., Gromoll, J., and Cooper, T. G. (1998) *Biochem. Biophys. Res. Commun.* **251,** 545–549
6. Taira, T., Saito, Y., Niki, T., Iguchi-Ariga, S. M., Takahashi, K., and Ariga, H. (2004) *EMBO Rep.* **5,** 213–218
7. Zhou, W., and Freed, C. R. (2005) *J. Biol. Chem.* **280,** 43150–43158
8. Martinat, C., Shendelman, S., Jonason, A., Leete, T., Beal, M. F., Yang, L., Floss, T., and Abeliovich, A. (2004) *PLoS Biol.* **2,** e327
9. Yokota, T., Sugawara, K., Ito, K., Takahashi, R., Ariga, H., and Mizusawa, H. (2003) *Biochem. Biophys. Res. Commun.* **312,** 1342–1348
10. Shendelman, S., Jonason, A., Martinat, C., Leete, T., and Abeliovich, A. (2004) *PLoS Biol.* **2,** e362
11. Zhou, W., Zhu, M., Wilson, M. A., Petsko, G. A., and Fink, A. L. (2006) *J. Mol. Biol.* **356,** 1036–1048
12. Junn, E., Taniguchi, H., Jeong, B. S., Zhao, X., Ichijo, H., and Mouradian, M. M. (2005) *Proc. Natl. Acad. Sci. U. S. A.* **102,** 9691–9696
13. Hod, Y. (2004) *J. Cell. Biochem.* **92,** 1221–1233
14. Xu, J., Zhong, N., Wang, H., Elias, J. E., Kim, C. Y., Woldman, I., Pifl, C., Gygi, S. P., Geula, C., and Yankner, B. A. (2005) *Hum. Mol. Genet.* **14,** 1231–1241
15. Shinbo, Y., Niki, T., Taira, T., Ooe, H., Takahashi-Niki, K., Maita, C., Seino, C., Iguchi-Ariga, S. M., and Ariga, H. (2006) *Cell Death Differ.* **13,** 96–108
16. Clements, C. M., McNally, R. S., Conti, B. J., Mak, T. W., and Ting, J. P. (2006) *Proc. Natl. Acad. Sci. U. S. A.* **103,** 15091–15096
17. Shinbo, Y., Taira, T., Niki, T., Iguchi-Ariga, S. M., and Ariga, H. (2005) *Int. J. Oncol.* **26,** 641–648
18. Zhong, N., Kim, C. Y., Rizzu, P., Geula, C., Porter, D. R., Pothos, E. N., Squitieri, F., Heutink, P., and Xu, J. (2006) *J. Biol. Chem.* **281,** 20940–20948
19. Jeong, H., Kim, M. S., Kwon, J., Kim, K. S., and Seol, W. (2006) *Neurosci. Lett.* **396,** 57–61
20. Niki, T., Takahashi-Niki, K., Taira, T., Iguchi-Ariga, S. M., and Ariga, H. (2003) *Mol. Cancer Res.* **1,** 247–261
21. Bonifati, V., Rizzu, P., van Baren, M. J., Schaap, O., Breedveld, G. J., Krieger, E., Dekker, M. C., Squitieri, F., Ibanez, P., Joosse, M., van Dongen, J. W., Vanacore, N., van Swieten, J. C., Brice, A., Meco, G., van Duijn, C. M., Oostra, B. A., and Heutink, P. (2003) *Science* **299,** 256–259
22. Bonifati, V., Rizzu, P., Squitieri, F., Krieger, E., Vanacore, N., van Swieten, J. C., Brice, A., van Duijn, C. M., Oostra, B., Meco, G., and Heutink, P. (2003) *Neurol. Sci.* **24,** 159–160
23. Hague, S., Rogaeva, E., Hernandez, D., Gulick, C., Singleton, A., Hanson, M., Johnson, J., Weiser, R., Gallardo, M., Ravina, B., Gwinn-Hardy, K., Crawley, A., St. George-Hyslop, P. H., Lang, A. E., Heutink, P., Bonifati, V., Hardy, J., and Singleton, A. (2003) *Ann. Neurol.* **54,** 271–274
24. Tang, B., Xiong, H., Sun, P., Zhang, Y., Wang, D., Hu, Z., Zhu, Z., Ma, H., Pan, Q., Xia, J. H., Xia, K., and Zhang, Z. (2006) *Hum. Mol. Genet.* **15,** 1816–1825
25. Cookson, M. R. (2005) *Annu. Rev. Biochem.* **74,** 29–52
26. Bretaud, S., Allen, C., Ingham, P. W., and Bandmann, O. (2007) *J. Neurochem.* **100,** 1626–1635
27. Latonen, L., and Laiho, M. (2005) *Biochim. Biophys. Acta* **1755,** 71–89
28. Yamada, M., Iwatsubo, T., Mizuno, Y., and Mochizuki, H. (2004) *J. Neurochem.* **91,** 451–461
29. Viswanath, V., Wu, Y., Boonplueang, R., Chen, S., Stevenson, F. F., Yantiri, F., Yang, L., Beal, M. F., and Andersen, J. K. (2001) *J. Neurosci.* **21,** 9519–9528
30. Hartmann, A., Hunot, S., Michel, P. P., Muriel, M. P., Vyas, S., Faucheux, B. A., Mouatt-Prigent, A., Turmel, H., Srinivasan, A., Ruberg, M., Evan, G. I., Agid, Y., and Hirsch, E. C. (2000) *Proc. Natl. Acad. Sci. U. S. A.* **97,** 2875–2880
31. Tatton, N. A. (2000) *Exp. Neurol.* **166,** 29–43
32. Vila, M., Jackson-Lewis, V., Vukosavic, S., Djaldetti, R., Liberatore, G., Offen, D., Korsmeyer, S. J., and Przedborski, S. (2001) *Proc. Natl. Acad. Sci. U. S. A.* **98,** 2837–2842
33. McNeill-Blue, C., Wetmore, B. A., Sanchez, J. F., Freed, W. J., and Merrick, B. A. (2006) *Brain Res.* **1112,** 1–15
34. de la Monte, S. M., Sohn, Y. K., Ganju, N., and Wands, J. R. (1998) *Lab. Investig.* **78,** 401–411
35. Nair, V. D., McNaught, K. S., Gonzalez-Maeso, J., Sealfon, S. C., and Olanow, C. W. (2006) *J. Biol. Chem.* **281,** 39550–39560
36. Olzmann, J. A., Bordelon, J. R., Muly, E. C., Rees, H. D., Levey, A. I., Li, L., and Chin, L. S. (2007) *J. Comp. Neurol.* **500,** 585–599
37. Bandopadhyay, R., Kingsbury, A. E., Cookson, M. R., Reid, A. R., Evans, I. M., Hope, A. D., Pittman, A. M., Lashley, T., Canet-Aviles, R., Miller, D. W., McLendon, C., Strand, C., Leonard, A. J., Abou-Sleiman, P. M., Healy, D. G., Ariga, H., Wood, N. W., de Silva, R., Revesz, T., Hardy, J. A., and Lees, A. J. (2004) *Brain* **127,** 420–430
38. Alves Da Costa, C., Paitel, E., Vincent, B., and Checler, F. (2002) *J. Biol. Chem.* **277,** 50980–50984
39. Culmsee, C., and Mattson, M. P. (2005) *Biochem. Biophys. Res. Commun.* **331,** 761–777
40. Eberhardt, O., and Schulz, J. B. (2003) *Toxicol. Lett.* **139,** 135–151
41. Duan, W., Zhu, X., Ladenheim, B., Yu, Q. S., Guo, Z., Oyler, J., Cutler, R. G., Cadet, J. L., Greig, N. H., and Mattson, M. P. (2002) *Ann. Neurol.* **52,** 597–606
42. Morrison, R. S., Kinoshita, Y., Johnson, M. D., Guo, W., and Garden, G. A. (2003) *Neurochem. Res.* **28,** 15–27
43. Haupt, Y., Maya, R., Kazaz, A., and Oren, M. (1997) *Nature* **387,** 296–299
44. Leng, R. P., Lin, Y., Ma, W., Wu, H., Lemmers, B., Chung, S., Parant, J. M., Lozano, G., Hakem, R., and Benchimol, S. (2003) *Cell* **112,** 779–791
45. Dornan, D., Bheddah, S., Newton, K., Ince, W., Frantz, G. D., Dowd, P., Koeppen, H., Dixit, V. M., and French, D. M. (2004) *Cancer Res.* **64,** 7226–7230
46. Nikolaev, A. Y., Li, M., Puskas, N., Qin, J., and Gu, W. (2003) *Cell* **112,** 29–40
47. Juan, L. J., Shia, W. J., Chen, M. H., Yang, W. M., Seto, E., Lin, Y. S., and Wu, C. W. (2000) *J. Biol. Chem.* **275,** 20436–20443
48. Vaghefi, H., and Neet, K. E. (2004) *Oncogene* **23,** 8078–8087
49. Tan, T. H., Wallis, J., and Levine, A. J. (1986) *J. Virol.* **59,** 574–583
50. Ando, K., Ozaki, T., Yamamoto, H., Furuya, K., Hosoda, M., Hayashi, S., Fukuzawa, M., and Nakagawara, A. (2004) *J. Biol. Chem.* **279,** 25549–25561
51. Sui, G., Affar el, B., Shi, Y., Brignone, C., Wall, N. R., Yin, P., Donohoe, M., Luke, M. P., Calvo, D., Grossman, S. R., and Shi, Y. (2004) *Cell* **117,** 859–872
52. Luo, J., Su, F., Chen, D., Shiloh, A., and Gu, W. (2000) *Nature* **408,** 377–381
53. Gu, W., and Roeder, R. G. (1997) *Cell* **90,** 595–606
54. Luo, J., Li, M., Tang, Y., Laszkowska, M., Roeder, R. G., and Gu, W. (2004) *Proc. Natl. Acad. Sci. U. S. A.* **101,** 2259–2264
55. Spillare, E. A., Robles, A. I., Wang, X. W., Shen, J. C., Yu, C. E., Schellenberg, G. D., and Harris, C. C. (1999) *Genes Dev.* **13,** 1355–1360
56. Thornborrow, E. C., and Manfredi, J. J. (2001) *J. Biol. Chem.* **276,** 15598–15608