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AP-1 Repressor Protein JDP-2: Inhibition of UV-Mediated Apoptosis through p53 Down-Regulation

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Members of the AP-1 transcription factor family, especially c-Jun and c-Fos, have long been known to mediate critical steps in the cellular response to ultraviolet (UV) irradiation. We sought to examine whether two newly discovered members of the AP-1 family, JDP-1 and JDP-2, also participate in the mammalian UV response. Here we report that JDP-2, but not JDP-1, is transiently induced upon UV challenge and that elevated levels of JDP-2 increase cell survival following UV exposure. This protective function of JDP-2 appears to be mediated through repression of *p53* expression at the transcriptional level, via a conserved atypical AP-1 site in the *p53* promoter.

Repeated and prolonged exposure to sunlight and hence to UV radiation causes skin damage that may ultimately evolve into some form of skin cancer. In the United States alone, over a million people will develop such cancers, mostly basal cell carcinomas, this year. For example, a 2-h walk on a sunny afternoon at an altitude of 2,000 m exposes the skin surface to a dose equivalent to 40 J/m^2 of short-wavelength UV light (UV-C) (25). In vitro, such a dose may kill 95% of cells in a culture dish (22). Extensive investigation of the genomic response of mammalian cells to UV light has shown that, in addition to DNA damage, UV exposure results in induction of immediate early genes and activation of transcription factors such as NF-κB and c-Jun (5, 14, 15, 42). This response is similar to that induced by growth factors and cytokines, but since it is not associated with increased cell proliferation, it has been viewed as a pseudo-growth response (14). Indeed, c-Jun induction was recently shown to be required for the exit of UV-irradiated mouse fibroblasts from p53-imposed growth arrest and their return to the cell cycle (39a).

DNA damage inflicted by a variety of treatments including UV irradiation causes nuclear accumulation of the product of the tumor suppressor gene *p53*, resulting in enhanced transcription of target genes (25), including the one coding for the cell cycle inhibitor p21^{waf1}. DNA damage increases p53 stability either directly, through binding of p53 to single-stranded DNAs (35) and DNA lesions (32), or indirectly, through yet-to-be-elucidated signal transduction pathways (31). p53 protects cells and organisms from DNA damage in at least two ways. First, through induction of *p21^{waf1}*, p53 blocks DNA synthesis and cell cycle progression to provide sufficient time for repair of damaged DNA (22, 31). p53 may also be involved in the DNA repair process itself, since it has been reported that p53 binds directly to certain DNA repair proteins (45). Second, elevated p53 can trigger apoptosis (10) and thereby eliminate cells whose DNA has been damaged beyond repair to prevent their malignant transformation. Two genes that are regulated by p53 could influence the decision to commit to an apoptotic pathway: *bax* and *IGF-BP3* (6, 34). Indeed, Bax binds Bcl2 and antagonizes its ability to block apoptosis. Also, the insulin-like growth factor-binding protein 3 (IGF-BP3), by blocking the IGF cell survival pathway, could enhance apoptosis. However, p53 can also promote apoptosis in response to DNA damage by a mechanism that does not depend on transcriptional activation (9).

Although DNA damage is probably the primary signal leading to p53 accumulation, certain early steps in UV signaling can occur in enucleated cells (13). Rapidly activated membrane-associated protein kinases and signaling proteins were implicated in these early steps and shown to activate the pathways that lead to induction of AP-1 and NF-κB (13, 36). These early events converge to activate the mitogen- and stress-activated protein kinases JNK and p38 (37). JNK then specifically phosphorylates c-Jun (12, 23, 28, 40, 41) and ATF2 (20) and thereby enhances their transcription-promoting activities. The c-*jun* promoter itself contains two *cis*-acting elements which bind c-Jun–ATF2 heterodimers (14, 38). Another *cis* element in the c-*jun* promoter binds the transcription factor MEF2C, whose activity is stimulated in response to p38-mediated phosphorylation (21). Increased c-Jun synthesis and phosphorylation result in further induction of AP-1 target genes. Surprisingly, however, although c-Jun induction is a critical step required for cell cycle reentry upon p53-imposed growth arrest, this effect is mediated via gene repression rather than gene activation (39a). JNK and p38 also phosphorylate and stimulate the activity of ternary complex factors and thereby contribute to c-*fos* gene induction (11, 43, 46). Increased c-Fos or FosB synthesis contributes to induction of AP-1 activity.

We investigated whether two novel members of the AP-1 family, JDP-1 and JDP-2 (Jun dimerization partners 1 and 2, respectively) might also participate in the UV response. JDP-1 and JDP-2 are two recently identified c-Jun-interacting proteins (2). Preliminary studies suggested that JDP-2 might act as a repressor of gene activation mediated by c-Jun, possibly by

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competing for dimerization of c-Jun with c-Fos and also by introducing a repressor domain into the AP-1 complex (2). So far, no clear physiological role for JDP-1 or JDP-2 has been reported. We found that expression of JDP-2, but not JDP-1, is induced upon UV irradiation. In turn, JDP-2 down-regulates expression of *p53* and thereby protects cells from UV-mediated programmed cell death.

MATERIALS AND METHODS

Plasmids. Mammalian expression vectors for c-Jun, JDP-1, and JDP-2 have been described (2, 14, 15). The mouse *p53* promoter coupled to luciferase (p53-m0.7-Luc) was a kind gift from M. Oren (19). The *p53* promoter mutant lacking the AP-1 site (p53-m0.7 ΔPF-1-Luc) was previously described (39). Briefly, the atypical AP-1 site at positions −63 to −57 was mutated from TGA CTCT to TGAATTC, using the Quick Change kit (Stratagene). Successful mutagenesis was confirmed by restriction digest and sequence analysis. The JDP-2 dominant positive mutant (JDP-2mut) was generated by inserting in frame the transcriptional activation domain of c-Fos (amino acids 210 to 313) downstream of the JDP-2 open reading frame.

Cell culture. c-*jun*$^{+/+}$ or c-*jun*$^{-/-}$ fibroblasts were derived from E11.5 and E12.5 mouse embryos, and each cell line was immortalized using a 3T3 protocol starting with a primary culture. These cells were a kind gift from E. Wagner (24). *p53* null fibroblasts (16) and fibroblasts expressing elevated levels of JDP-1 or JDP-2 (2) have also been described. All cells were cultured in Dulbecco's modified Eagle's medium supplemented with glutamine and 10% fetal calf serum.

Transient-expression assays. Briefly, 50,000 cells were plated per 60-mm plate. The next day, cells were transfected according to manufacturer's recommendations with either Lipofectamine (Gibco BRL), Superfect, or Polyfect (Qiagen). DNA amounts were as indicated below. At various times, cells were harvested in 200 μl of lysis buffer (100 mM Tris-acetate, 10 mM Mg acetate, 1 mM EDTA, 1% Triton, 1 mM dithiothreitol) and luciferase activity was measured. Each transfection was performed in quadruplicate, and the results shown represent the means for four several independent experiments.

Proteins and Western blots. Cells were scraped off plates, spun down, and resuspended in 200 μl of lysis buffer (25 mM HEPES, 0.3 M NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.5% Triton, and a protease inhibitor cocktail). For coimmunoprecipitation experiments, Triton in the lysis buffer was replaced by octylglucopyrannoside. Whole-cell extracts (50 μg) were separated on denaturing polyacrylamide gels by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and the proteins were blotted onto Immobilon-P membranes. Western blotting was performed as described previously (2). Membranes were saturated with phosphate-buffered saline (PBS) plus 5% nonfat dry milk for an hour at room temperature, washed several times in PBS, and incubated for an hour with specific antibodies. After extensive washing, membranes were incubated with peroxidase-conjugated secondary antibody and developed using enhanced chemiluminescence (ECL) according to the manufacturer's instructions (Amersham).

DNA binding experiments. Electrophoretic mobility shift assay (EMSA) experiments were performed as described previously (39). Briefly, in vitro-translated proteins were generated using rabbit reticulocyte lysates (Promega), and the extracts were preincubated for 20 min on ice with poly(dI-dC) (0.17 mg/ml) and then with ^{32}P-radiolabeled probes for 10 min at room temperature. The reaction products were then resolved on a nondenaturing acrylamide gel.

Northern blots. Total cellular RNA was extracted using Trizol according to the manufacturer's instructions (Gibco BRL). Total RNA samples (10 μg) were separated on a denaturing formaldehyde-agarose gel and transferred onto a nylon-nitrocellulose membrane (Schleicher and Schuell). RNAs were then cross-linked to the membrane using a Stratagene UV cross-linker and hybridized in the presence of specific radiolabeled probes.

Flow cytometry. Trypsinized cells were resuspended in PBS at 10^6 cells/ml. Cells were then fixed by addition of ethanol to a final concentration of 70%. Ethanol-fixed cells were kept up to 1 week at −20°C before use. Cells were pelleted and resuspended in an isotonic buffered solution containing propidium iodide (1 mg/ml). Cell cycle distribution was determined by flow cytometry using a Becton Dickinson FACScan system. To gate on cells expressing JDP-1 or JDP-2, ethanol-fixed cells were incubated for an hour at room temperature with specific anti-JDP-1 or anti-JDP-2 antibodies (2). After several washes, the cells were incubated with a fluorescein isothiocyanate (FITC)-conjugated secondary antibody (Santa Cruz, Santa Cruz, Calif.). Finally cells were pelleted and resuspended in propidium iodide-containing isotonic buffer. Only cells labeled positively with FITC were included in the cell cycle analysis.

Pulse-chase experiments. Exponentially growing cells were pulse-labeled with [^{35}S]methionine (250 μCi/ml) for 45 min as described previously (39). Cells were then chased with an excess of cold methionine for the indicated times. Protein extracts were then made as described above, and p53 proteins were immunoprecipitated with a specific antibody (Santa Cruz). Immunoprecipitates were run on an SDS-PAGE gel, and the amount of p53-labeled proteins was quantified using a phosphorimager.

Apoptosis assays. Cells were harvested at various times, and the degree of apoptosis was quantified using colorimetric and fluorescent assays measuring the endogenous levels of caspase 3 and caspase 8 according to the manufacturer's instructions (Clontech).

RESULTS

We investigated whether two newly discovered AP-1 family members, JDP-1 and JDP-2, could be involved in the UV response. Protein extracts of established mouse embryo fibroblasts (c-*jun*$^{+/+}$) were prepared before and after exposure to UV irradiation. Western blot analysis showed that both JDP-1 and JDP-2 were expressed in exponentially growing cells (Fig. 1A). Following UV irradiation, JDP-1 expression was unaltered, while expression of JDP-2 had increased (Fig. 1A). Expression of both JDP-1 and JDP-2 was also analyzed in fibroblasts derived from c-*jun* null mouse embryos (24, 27). Basal JDP-1 expression was modestly decreased in c-*jun*$^{-/-}$ cells, whereas basal JDP-2 expression was considerably lower than in c-*jun*$^{+/+}$ cells (Fig. 1A). However, expression of JDP-2, but not JDP-1, was still induced upon UV irradiation of c-*jun*$^{-/-}$ cells. The increase in JDP-2 expression, however, was similar in the two cell lines (two- versus threefold). Although moderate, this induction was reproducible. These results suggest that JDP-2 and possibly JDP-1 expression and/or stability is influenced by the level of c-Jun expression. However, the increased accumulation of JDP-2 seen after UV irradiation appears to occur independently of c-Jun.

To understand the mechanism of JDP-2 activation by UV, analyses at the mRNA and protein levels were performed. Northern blot experiments indicated that, upon UV exposure, the levels of JDP-2 mRNA were not affected, arguing against a stimulation of JDP-2 transcription as the basis for its induction by UV (data not shown). Protein stabilization as a possible mechanism was investigated by preincubating c-*jun*$^{+/+}$ cells with or without MG132, a well-characterized proteasome inhibitor. In the absence of MG132, JDP-2 induction was transient, peaking around 6 h and returning to basal levels by approximately 24 h. In the presence of MG132, however, induction of JDP-2 expression was more sustained and its onset was accelerated (Fig. 1B). These results suggest that UV exposure causes stabilization of JDP-2. Furthermore, UV-induced JDP-2 accumulation may involve its phosphorylation at specific sites by JNK (A. Aronheim, unpublished results).

To explore the physiological role of JDP-2 in the UV response, we performed flow cytometric analysis of the above cell lines transiently transfected with JDP-2 or JDP-1 expression vectors 24 h prior to UV irradiation. Transfected cells were sorted on the basis of expression of either JDP-1 or JDP-2 and their cell cycle profiles were investigated. Following exposure to UV-C at 40 J/m^2, c-*jun*$^{-/-}$ cells accumulated mostly at the G_1 phase of the cell cycle, while a small fraction had undergone apoptosis or necrosis, as revealed by appearance of a sub-G_1 population (Fig. 2). By constrast, UV-irradiated c-*jun*$^{+/+}$ cells were only partially arrested in G_1, and the majority were dead,

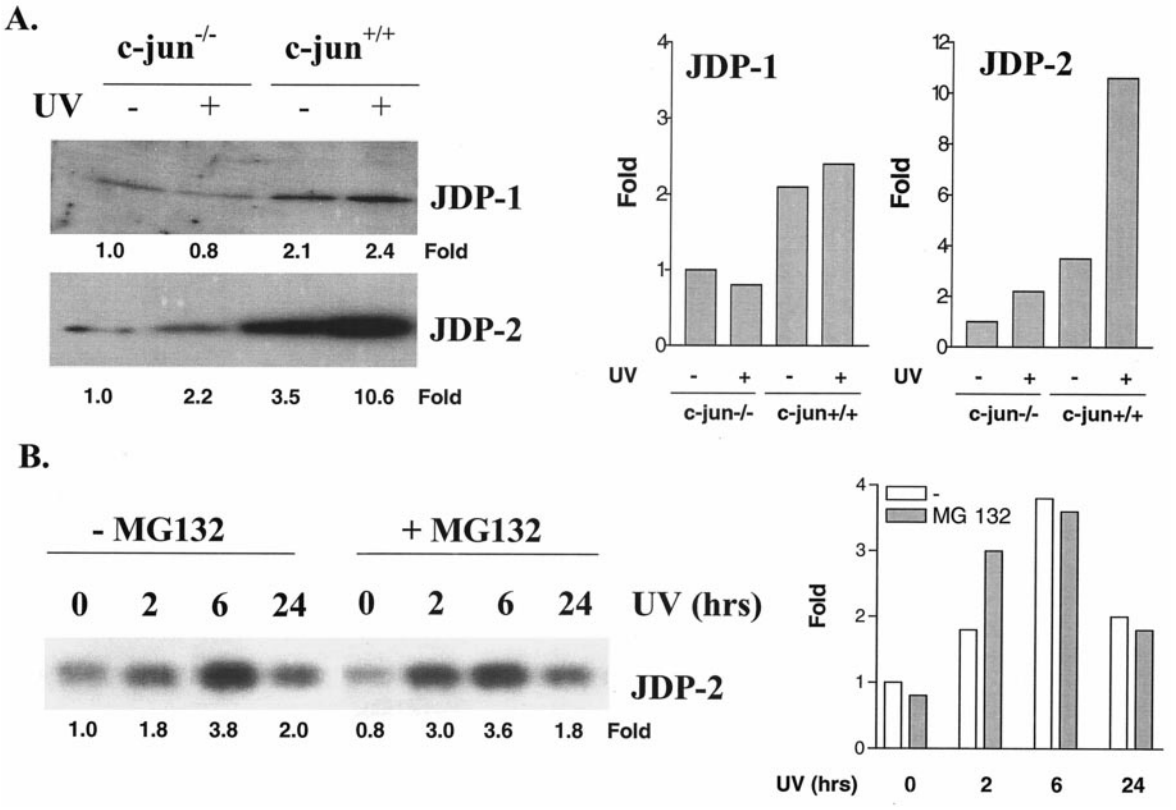


FIG. 1. Induction of JDP-2 following UV irradiation. (A) JDP-2, but not JDP-1, is induced upon UV irradiation. c-*jun*$^{+/+}$ or c-*jun*$^{-/-}$ fibroblasts were exposed to UV-C (40 J/m^2) or left unexposed and lysed after 2 h. After separation by SDS-PAGE, Western blots of lysate proteins were probed with specific antibodies against JDP-1 or JDP-2 and visualized by ECL. Responses were estimated by densitometry using a phosphorimager and plotted as a graph. (B) Time course of JDP-2 induction by UV. c-*jun*$^{+/+}$ fibroblasts were exposed to UV-C (40 J/m^2) or left unexposed and lysed at the indicated times. When indicated, cells were incubated for 2 h with MG132 (50 μM) prior to UV irradiation. After separation by SDS-PAGE, blots of lysate proteins were probed with specific antibodies against JDP-2 and visualized by ECL. Responses were estimated by densitometry using a phosphorimager and plotted as a graph.

based upon the appearance of a large sub-G_1 fraction. Transient expression of JDP-1 did not significantly alter UV-induced cell death in c-*jun*$^{-/-}$ cells and provided only marginal protection to c-*jun*$^{+/+}$ cells, possibly through G_1 arrest (Fig. 2). Thus, when transiently overexpressed, JDP-1 affects cell survival differently, either by marginally protecting against (c-*jun*$^{+/+}$ cells) or supporting (c-*jun*$^{-/-}$ cells) UV-induced cell death. On the other hand, expression of JDP-2 inhibited UV-induced cell death in both cell lines, independently of their c-Jun status. The inhibition of cell death by JDP-2 was much more dramatic in the c-*jun*$^{+/+}$ cells, because these cells are more susceptible to UV-induced cell death than c-*jun*$^{-/-}$ cells (39a). These findings support the notion that induction of JDP-2 following UV irradiation has a protective function. In addition, these results suggest that c-Jun expression may promote UV-induced cell death, confirming earlier findings (39a).

To better understand this newly discovered function of JDP-2, we established mouse NIH 3T3 cell lines that constitutively expressed higher-than-normal levels of either JDP-2 or JDP-1. Expression levels of exogenous JDP-1 and JDP-2 were similar in several clonal cell lines and an order of magnitude higher than the respective endogenous proteins (data not shown). Flow cytometry revealed that parental NIH 3T3 cells, when exposed to 40 J of UV-C/m^2, underwent considerable cell death within 48 h (Fig. 3A and B). After 72 h, about 60% of the cells were apoptotic based on their sub-G_1 DNA content. Constitutive expression of JDP-1 did not significantly alter this response, except for a small increase in the extent of G_1 arrest. Expression of JDP-2, on the other hand, delayed the appearance of sub-G_1 cells and decreased the extent of UV-induced death. At day 3, less than 20% of JDP-2-overexpressing cells were dead, while more than 60% of the parental cells or the JDP-1 overexpressors were dead at that point. To clearly define the nature of the observed cell death, we examined the cells by DAPI (4′,6′-diamidino-2-phenylindole) staining. The percentage of wild-type and JDP-1- and JDP-2-overexpressing cells, exposed to 40 J of UV-C/m^2, exhibiting fragmented nuclei after 24 h was similar to the percentage of sub-G_1 cells observed in Fig. 3A and B (data not shown), suggesting that the cell death is apoptotic. Thus, JDP-2, but not JDP-1, functions to reduce the extent of UV-mediated apoptosis.

We also assessed the effect of JDP-2 on clonogenic survival of irradiated cells. Even though JDP-2 may protect cells from entering apoptosis, it is not obvious whether such an action can enhance long-term cell survival, rather than causing an indefinite cell cycle arrest. In clonogenic survival assays, at least two parameters need to be taken into account: cell survival and cell proliferation. Stable overexpression of either JDP-1 or JDP-2

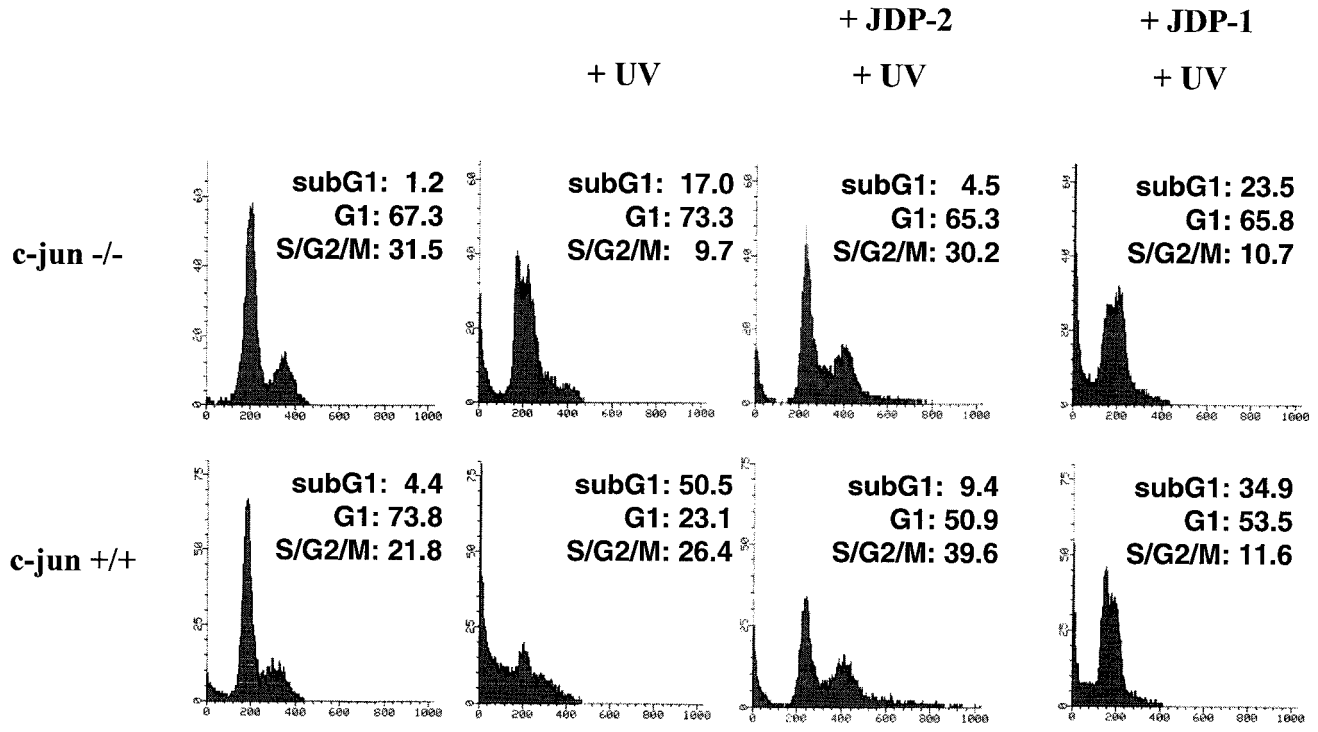
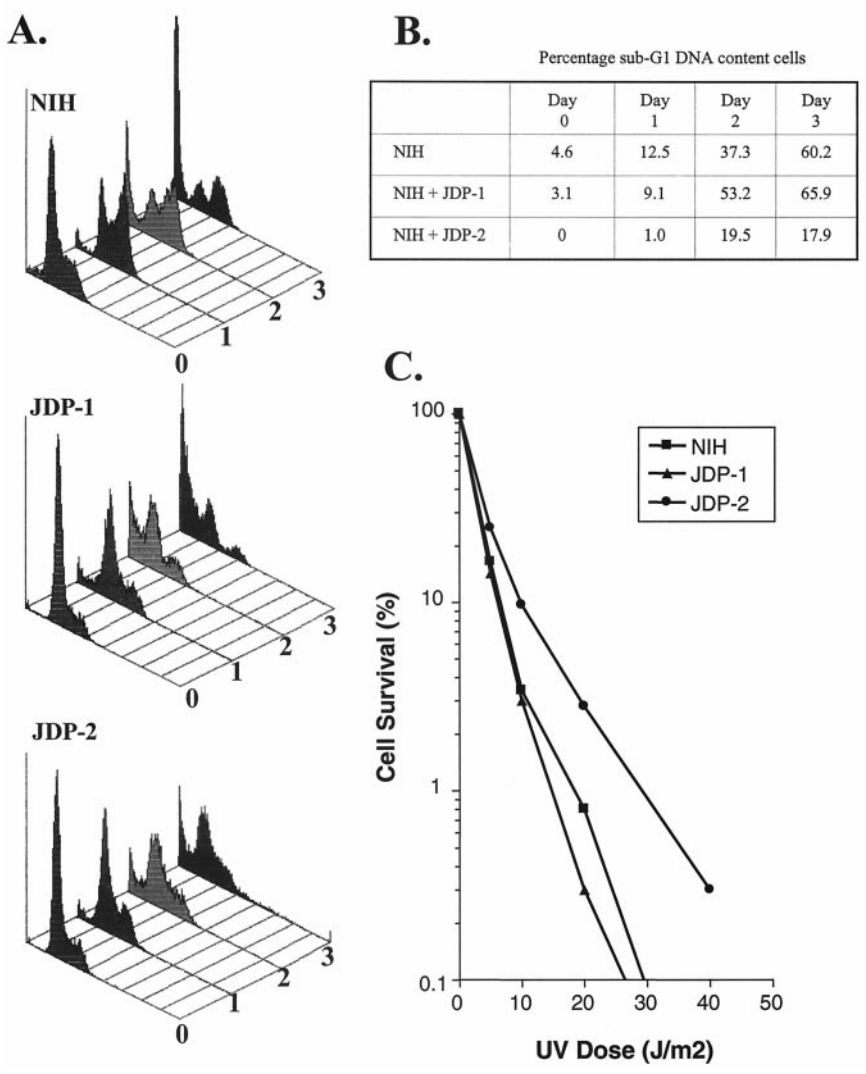


FIG. 2. Transient expression of JDP-2 inhibits UV-induced apoptosis. Cell cycle distribution was determined by flow cytometry. Spontaneously immortalized c-*jun*$^{+/+}$ and c-*jun*$^{-/-}$ mouse fibroblasts were transiently transfected with either JDP-1 or JDP-2 mammalian expression vectors or a control vector. Sixteen hours posttransfection, cells were UV irradiated (40 J/m^2) or left unirradiated and harvested 48 h later. Ethanol-fixed cells were incubated for 30 min in the presence of an antibody directed against either JDP-1 or JDP-2 or a control antibody, washed, and then incubated with an FITC-conjugated secondary antibody. Cells were stained with propidium iodide and examined by flow cytometry. In the case of JDP-1 and JDP-2-transfected cells, FITC-positive cells were sorted and further analyzed. The vertical and horizontal axes represent cell count and relative DNA content, respectively.

did not modify the kinetics of cell proliferation compared to parental NIH 3T3 cells (data not shown). Cultured fibroblasts with elevated JDP-1 or JDP-2 expression, as well as parental NIH 3T3 cells, were exposed to different doses of UV-C, and their survival was assessed by counting the number of viable colonies after 2 weeks (Fig. 3C). JDP-2-overexpressing cells were considerably more resistant to UV irradiation than the parental or JDP-1-overexpressing cells. Thus, by preventing apoptosis, JDP-2 increases the ability of cultured fibroblasts to survive exposure to relatively high doses of short-wavelength UV radiation.

The tumor suppressor gene *p53* has been widely described as a key mediator of apoptosis whose accumulation is induced by various genotoxic stresses, including UV irradiation (25, 29, 33). In cells exposed to low to moderate doses of UV, p53 through inhibition of DNA synthesis allows time for repair of damaged DNA (25). Only when DNA is damaged beyond repair, p53 commits cells to programmed cell death (3, 26). We therefore examined expression of p53 following UV exposure in parental cells and in cells overexpressing JDP-1 or JDP-2. In all three cell lines, the p53 polypeptide was barely detectable prior to UV irradiation (Fig. 4A). As expected, UV irradiation resulted in the fast accumulation of p53 polypeptide (within 2 h of irradiation), whose levels remained elevated for at least 24 h (Fig. 4A). Whereas JDP-1 did not significantly alter the extent of the kinetics of p53 induction, elevated JDP-2 expression both delayed and considerably reduced p53 induction (Fig. 4A). To investigate whether JDP-2 may affect p53 expression by altering its turnover rate, we performed pulse-chase experiments. Cells were UV-C (40 J/m^2) or mock irradiated 2 h prior to labeling with [^{35}S]methionine and then chased with an excess of cold methionine. p53 decay curves before and after UV treatment for all three cell lines are shown in Fig. 4B. In the absence of UV treatment, the calculated half-lives for the p53 polypeptide in NIH 3T3 and JDP-1- and JDP-2-overexpressing cell lines were comparable: 98, 94, and 106 min, respectively. Upon UV irradiation, the half-life of p53 increased significantly to 231, 219, and 243 min in NIH 3T3 and JDP-1- and JDP-2-overexpressing cells, respectively. Therefore, the inhibitory effect of JDP-2 on *p53* induction does not involve increased p53 turnover or decreased stabilization.

As a member of the AP-1 family, JDP-2 is likely to be a transcriptional regulator (2). Recent evidence suggests that c-Jun may act as a repressor of *p53* transcription (39). We therefore investigated whether JDP-2 might also regulate expression of *p53* at the transcriptional level. In vitro transient-transfection assays using the mouse *p53* promoter (19) were performed in c-*jun*$^{-/-}$ and c-*jun*$^{+/+}$ fibroblasts. Interestingly, we found that UV irradiation also stimulated *p53* promoter activity. Although basal *p53* promoter activity was higher in c-*jun* null cells as previously reported (39), the extent of UV-mediated enhancement was not considerably affected by the



	Day 0	Day 1	Day 2	Day 3
NIH	4.6	12.5	37.3	60.2
NIH + JDP-1	3.1	9.1	53.2	65.9
NIH + JDP-2	0	1.0	19.5	17.9

FIG. 3. Stable expression of JDP-2 delays and reduces UV-mediated cell death. (A) Constitutive JDP-2 expression reduces apoptosis after UV irradiation. NIH 3T3 cells expressing elevated levels of either JDP-1 or JDP-2 were generated (2). Cells were UV-C irradiated (40 J/m^2), and their cell cycle distribution was determined at 0, 1, 2, and 3 days postirradiation by flow cytometry. (B) Recapitulation of results from panel A. Values are indicative of apoptosis. (C) JDP-2 enhances clonogenic survival following UV irradiation. Clonogenic survival assays were performed on parental NIH-3T3 cells and cells that stably express JDP-1 and JDP-2, following exposure to the indicated doses of UV-C. After irradiation of a fixed number of cells, the number of surviving cells was determined by the number of colonies detected after 2 weeks.

c-Jun status (Fig. 5A). As previously described (39), transient overexpression of c-Jun repressed *p53* promoter activity. Overexpression of JDP-2 resulted in a similar repressive effect (20- to 50-fold), and coexpression of c-Jun and JDP-2 resulted in an even greater repressive effect (>100-fold) (Fig. 5A). Similarly, overexpression of JDP-1, alone or in combination with c-Jun, strongly repressed *p53* promoter activity. We also analyzed the kinetics of endogenous *p53* mRNA induction following UV exposure in cells overexpressing JDP-2. Endogenous levels of *p53* mRNAs were slightly lower in both the JDP-1 and JDP-2 cell lines than in parental cells (Fig. 4C), and this was reflected at the protein level (Fig. 4A). UV irradiation resulted in transient induction of *p53* mRNA in parental cells, but overexpression of JDP-2, unlike that of JDP-1, inhibited this induction (Fig. 4C). Thus, the repression of p53 induction by JDP-2 seen at the protein level (Fig. 4A) was also seen at the mRNA level. These results also indicate that UV has a dual effect on p53 expression, both inducing transient accumulation of *p53* mRNAs and stabilizing the protein. The inhibitory effect of JDP-2 on p53 induction appears to be mediated only at the mRNA level, and p53 protein stabilization occurs normally in cells overexpressing JDP-2.

The repressive effect of c-Jun is mediated via a conserved motif in the *p53* promoter, which differs from the consensus AP-1 site by a single base pair substitution (39). This motif, termed PF-1, also modulates *p53* promoter activity in response to serum growth factors and can confer transcriptional repression on a heterologous promoter (19). To determine whether this motif also mediates the repressive effect of JDP-2, an AP-1 family member, we used a mutant *p53* promoter whose PF-1 site was destroyed by site-directed mutagenesis (39). Gel retardation (EMSA) assays indicated that the mutated site was

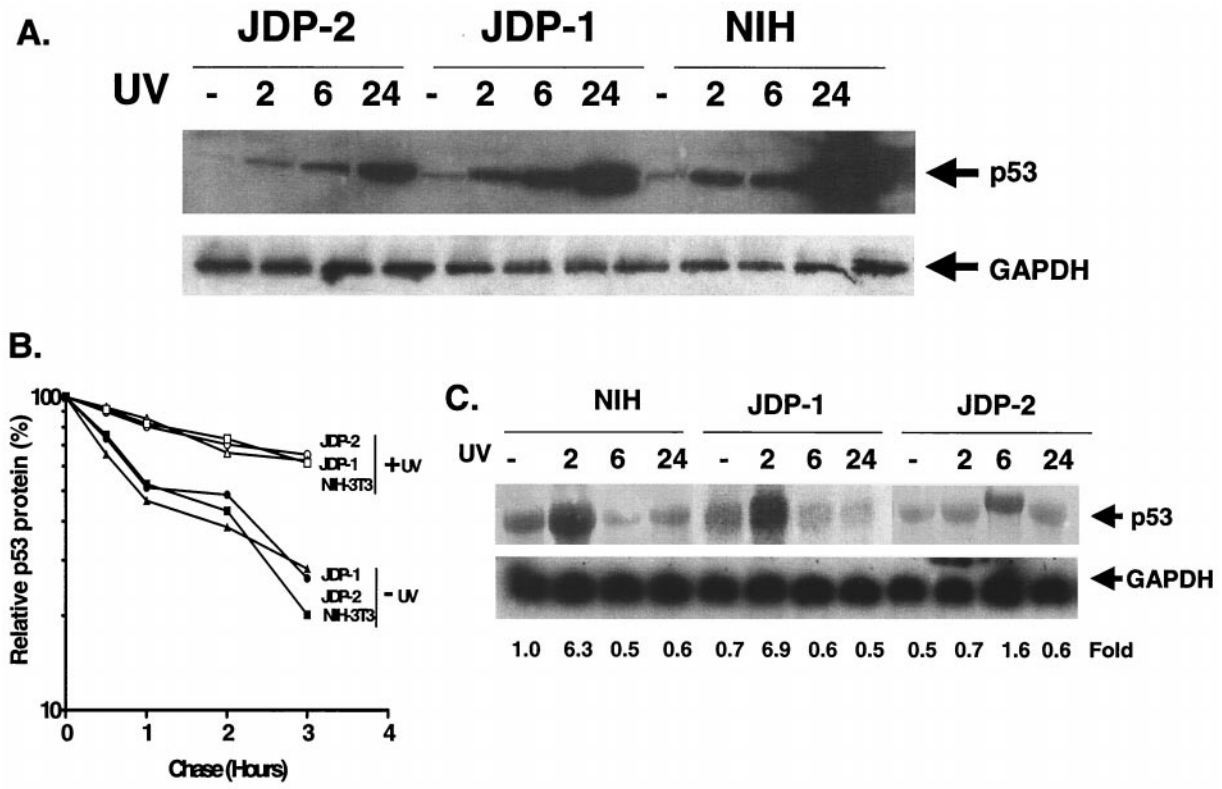


FIG. 4. p53 expression in JDP-2-overexpressing cells upon UV irradiation. (A) Induction of p53 is reduced and delayed in JDP-2-overexpressing cells. Parental NIH 3T3 cells and cells overexpressing JDP-1 or JDP-2 were exposed to UV-C (40 J/m^2). Protein lysates were prepared at the indicated times postirradiation (in hours) and analyzed by Western blotting for expression of p53. To detect low levels of endogeneous p53 proteins, 200 μg of cell lysates was loaded per lane. The amounts of protein loaded on the gel were compared by probing with anti-GAPDH (glyceraldehyde-3-phosphate dehydrogenase) antibodies. (B) Stability of p53 protein is not affected by JDP-2 upon UV. Parental NIH 3T3 cells and cells overexpressing JDP-1 or JDP-2 were subjected to UV-C irradiation (40 J/m^2). Two hours later, cells were briefly labeled (45 min) with [^{35}S]methionine and then chased with an excess of cold methionine for the indicated times. Protein lysates were immunoprecipitated with a specific antibody against p53 and then analyzed by SDS-PAGE. The relative amounts of p53-labeled proteins were quantified using a phosphorimager. Amounts were normalized for each cell line. (C) Accumulation of *p53* transcripts is reduced and delayed in JDP-2-overexpressing cells. NIH 3T3 and JDP-2-overexpressing cells were exposed to 40 J of UV-C/m^2. Total cellular RNA was harvested at the indicated times (in hours) and analyzed by Northern blotting for expression of *p53* mRNA. Adequate loading was ensured by probing with a control GAPDH probe. rRNA are indicated.

defective in binding of either JDP-2 or c-Jun and that the binding of JDP-2 to the wild-type PF-1 motif was specific (Fig. 5B). As shown in Fig. 5A, the mutant *p53* promoter lacking the PF-1 site was equally active in c-*jun*$^{+/+}$ and c-*jun*$^{-/-}$ cells, and it was no longer sensitive to the repressive effect of either c-Jun or JDP-2, alone or in combination. Similar results were also observed with JDP-1 (Fig. 5A). Thus, the PF-1 site is the primary target for c-Jun, JDP-1, and JDP-2, whose elevated expression represses *p53* transcription.

The transcriptional regulation of the *p53* promoter following UV exposure was also investigated in cell lines expressing elevated JDP-1 or JDP-2. Although basal *p53* promoter activity was relatively high in parental cells, UV irradiation further increased *p53* promoter activity (Fig. 6A). Elevated JDP-1 expression had a negligible effect on basal *p53* promoter activity and its induction response to UV irradiation. However, elevated JDP-2 expression strongly down-regulated basal *p53* promoter activity by a factor of about 5 to 10. Furthermore, JDP-2 also inhibited the induction of *p53* promoter activity by UV irradiation (Fig. 6A and data not shown). These effects on *p53* promoter activity were consistent with the effects on *p53* mRNA accumulation (Fig. 4C). A *p53* promoter lacking the PF-1 site was UV inducible in all of the cell lines and was no longer sensitive to expression of JDP-2 (Fig. 6B). Taken together, these results indicate that JDP-2 is a potent negative regulator of *p53* promoter activity and its induction upon UV irradiation. Although overexpression of JDP-1 also repressed *p53* promoter activity, this effect was seen only in transiently transfected cells. The transcriptional repression of *p53* by JDP-2 appears to be strictly dependent upon the presence of a functional PF-1 site within the *p53* promoter. This site, however, is not required for UV-mediated induction of the *p53* promoter.

To further understand the kinetics of *p53* repression by JDP-2, we characterized the composition of AP-1 dimers during the UV response. Gel retardation assays were performed, but under the conditions used no JDP-2 binding activity could be assessed (data not shown). The inability to detect binding of endogenous JDP-2 to DNA was also seen under different conditions (Aronheim, unpublished results). As a substitute, we examined the effects of UV irradiation on accumulation of c-Jun, c-Fos, and JDP-2 proteins. Western blot experiments

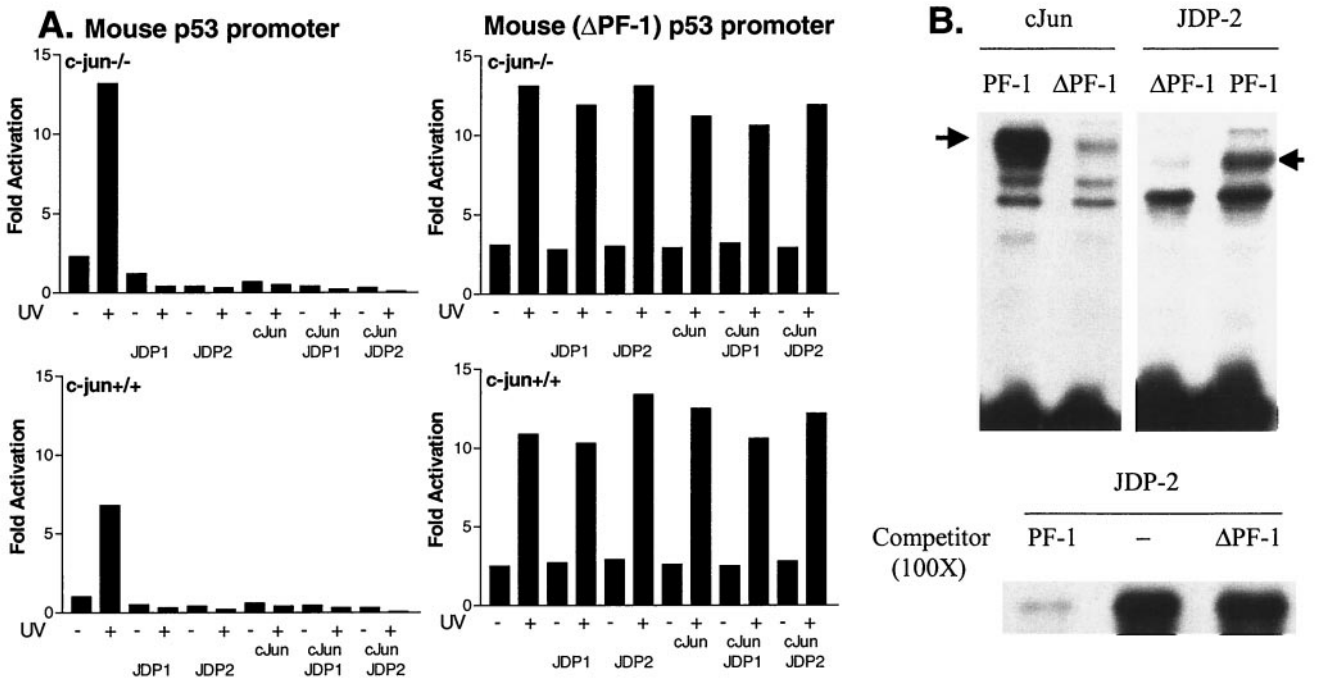


FIG. 5. Transcriptional regulation of *p53* by UV and AP-1 proteins. (A) c-*jun*$^{-/-}$ and c-*jun*$^{+/+}$ cells were transiently transfected with a wild-type or PF-1 deletion-containing *p53* promoter fused to a luciferase reporter and JDP-1, JDP-2, and c-Jun mammalian expression vectors. A β-galactosidase reporter driven by a β-actin promoter was included to normalize for transfection efficiency. Sixteen hours after transfection, cells were exposed to UV-C (40 J/m^2), and they were collected 9 h later to determine luciferase and β-galactosidase activities. The results are average relative luciferase induction levels, where the level expressed by untreated c-*jun*$^{+/+}$ cells transfected with wild-type *p53*-luciferase was given an arbitrary value of 1.0. (B) Specific binding of c-Jun and JDP-2 to an intact PF-1 site. Gel retardation (EMSA) experiments were performed using in vitro-translated c-Jun and JDP-2 against ^{32}P-radiolabeled PF-1 or ΔPF-1 primers. JDP-2 binding specificity to an intact PF-1 motif was confirmed through competition studies with a 100-fold excess of cold nonlabeled competitor (PF1 or ΔPF-1). Arrows indicate c-Jun–PF-1 and JDP-2–PF-1 DNA-protein complexes.

indicated that, as reported above, upon UV irradiation JDP-2 levels are increased about threefold within 2 h, reaching a maximum at 6 h and returning to basal levels by approximately 24 h (Fig. 7A). Expression of c-Jun, which was already quite substantial before UV irradiation, increased about threefold within 2 h of UV exposure and returned to higher-than-initial levels by 24 h. N-terminal phosphorylation of c-Jun, which correlates with its transcriptional activity, was maximal at 2 h postirradiation and remained for at least 24 h. Finally, c-Fos expression gradually increased, reaching a maximum at 24 h postirradiation (Fig. 7A), even though transcriptionally active c-Fos proteins are already present within 30 min postirradiation (8). These findings indicate that active c-Jun and c-Fos proteins are present at early times following UV irradiation and that JDP-2 polypeptides transiently accumulate at later points of the UV response. Most importantly, we characterized the dimerization partners of c-Jun and JDP-2 at various times during the UV response using coimmunoprecipitation experiments. As indicated in Fig. 7B, c-Jun coprecipitates with c-Fos prior to UV treatment, and the amount of coimmunoprecipitated c-Fos slightly decreased over time upon UV exposure. c-Jun–JDP-2 complexes were not detected prior to UV irradiation but appeared at 2 h postirradiation and peaked after 6 h. Similar results were obtained by using either c-Jun- or JDP-2-specific antibodies for the immunoprecipitation (Fig. 7B). Thus, it seems that at early times (i.e., within 2 h postirradiation) after UV exposure, c-Jun–c-Fos dimers are predominant and that c-Jun–JDP-2 dimers appear only after 2 h.

To further confirm the critical role of transcriptional repression of *p53* by JDP-2, we generated a JDP-2 mutant construct (JDP-2mut) in which the transcriptional activation domain of c-Fos was inserted in frame downstream of JDP-2. Characterization of the transcriptional properties of the JDP-2mut construct is shown in Fig. 8A. Unlike JDP-2, which repressed the activity of a reporter gene containing three consensus AP-1 sites (3×TRE-luciferase), JDP-2mut did not decrease its basal expression. Moreover, while c-Jun-mediated activation of the 3×TRE-luciferase construct is inhibited by JDP-2, the activation by c-Jun is unaffected by expression of JDP-2mut. Thus, although fusion of the activation domain of c-Fos to JDP-2 is not sufficient to generate a potent transcriptional activator similar to c-Fos, it is sufficient to neutralize the repressor activity of JDP-2 (2). Correspondingly, JDP-2mut no longer interfered with the transcriptional induction of *p53* promoter activity by UV exposure (Fig. 8B). Consistent with these results, transient expression of JDP-2mut affected neither the normal cell cycle distribution nor the apoptotic response of c-*jun*$^{+/+}$ and c-*jun*$^{-/-}$ cells that were either UV irradiated or left untreated (Fig. 8B). These results support the hypothesis

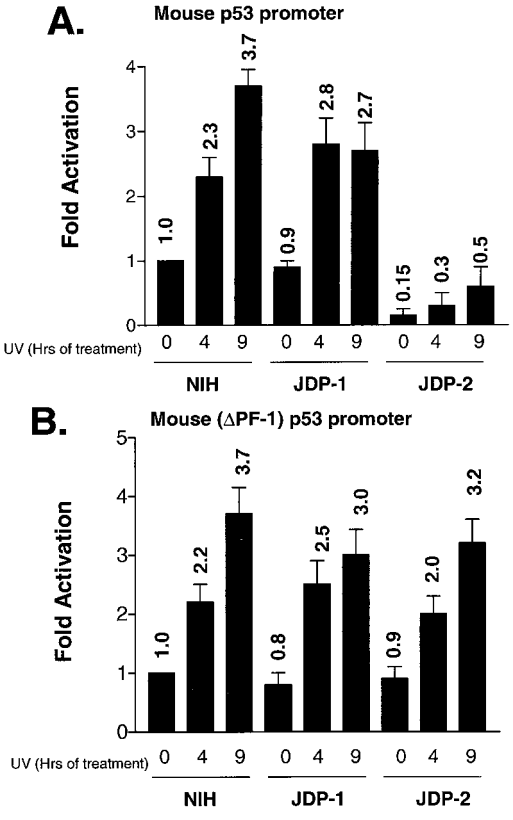


FIG. 6. JDP-2 represses *p53* induction following UV in vivo. Parental NIH 3T3 cells and JDP-1- or JDP-2-overexpressing cells were transiently transfected with either wild-type (A) or a PF-1 site deletion-containing (ΔPF-1) (B) *p53* promoter-luciferase reporter. An actin–β-galactosidase reporter was included to normalize for transfection efficiency. Sixteen hours after transfection the cells were UV-C irradiated (40 J/m^2), and luciferase and β-galactosidase activities were determined at the indicated times postirradiation. The normalized levels of luciferase expression in NIH 3T3 cells transfected with the wild-type *p53*-luciferase reporter were given an arbitrary value of 1.0. All other values are expressed relative to that value and are averages for three separate experiments.

that the antiapoptotic function of JDP-2 primarily resides in its ability to transcriptionally repress expression of *p53*.

To ascertain the biological relevance of the transcriptional repression of *p53* by JDP-2, we investigated whether transient expression of JDP-2 would affect UV-mediated apoptosis of *p53* null cells (16). Flow cytometry analyses indicated that a moderate but significant proportion (about 20%) of *p53* null cells accumulated at the sub-G_1 peak, suggestive of apoptosis, within 48 h of UV exposure (Fig. 9A). Cells transiently transfected with JDP-1 or JDP-2 displayed an overall similar cell cycle profile before and after UV irradiation, except for a notably smaller sub-G_1 population in nontreated, overexpressing JDP-1 and JDP-2 cells (3.1 and 3.9%, respectively, compared to 13.5% for the control). Thus, JDP-1 and JDP-2 reduce the low rate of spontaneous cell death occurring in these cells through an unknown mechanism. Besides, unlike its effect on $p53^{+/+}$ cells (Fig. 2), transient expression of JDP-2 in *p53* null cells did not inhibit the accumulation of sub-G_1 cells (Fig. 9A). The amount of apoptosis observed in UV-irradiated *p53* null cells was significantly lower than the amount seen in UV-irradiated parental NIH 3T3 cells (about 40 to 50% after 48 h), in agreement with the crucial function of p53 in induction of apoptosis. Therefore, in UV-irradiated fibroblasts, both p53-dependent and p53-independent mechanisms contribute to apoptosis, even though p53-dependent pathways are largely predominant. JDP-2 expression, however, affects only p53-dependent UV-induced apoptosis, as seen in $p53^{+/+}$ and $p53^{-/-}$ cells expressing comparable levels of JDP-2 (Fig. 9B and C). Taken together, these data strongly suggest that the antiapoptotic function of JDP-2 is primarily dependent on the presence of *p53*.

Finally, we investigated whether the inhibition of p53-mediated apoptosis is a general function of JDP-2. Cell cycle distribution profiles of human cell lines (*p53* wild type and *p53* null) expressing either JDP-2 or JDP-2mut following UV-C treatment are presented in Fig. 10A and B. Human $p53^{+/+}$ (HEK293) and $p53^{-/-}$ (Saos-2) cells were transiently transfected with expression vectors for JDP-2 or JDP2-mut or a control vector. Sixteen hours posttransfection cells were mock or UV-C irradiated (40 J/m^2), and their cell cycle distribution was analyzed 48 h postirradiation. Nontreated cells expressing JDP-2 (Fig. 10A and B) or JDP-1 (data not shown) displayed sub-G_1 populations similar to those of parental cells. Thus, unlike what was observed in mouse $p53^{-/-}$ cells (Fig. 9A), no reduction of spontaneous cell death by JDP-2 or JDP-1 was evident in human $p53^{+/+}$ and $p53^{-/-}$ cells. Therefore, this property is unlikely to be a general feature of either JDP-1 or JDP-2 and appears so far to be solely restricted to a particular *p53* null mouse fibroblast cell line. Upon UV irradiation, a large amount of human $p53^{+/+}$ (HEK293) cells displayed a sub-G_1 population (40 to 45%) suggestive of apoptosis (Fig. 10A and B). Expression of JDP-2, but not JDP-2mut, significantly inhibited UV-mediated cell death. In contrast, human $p53^{-/-}$ (Saos-2) cells were less sensitive to UV treatment, as only about 20 to 25% of cells had a sub-G_1 DNA content after UV irradiation, suggesting that p53-independent mechanisms contribute to a weak UV-induced cell death phenomenon. Moreover, neither JDP-2 nor JDP-2mut affected UV-induced apoptosis in these cells. Apoptosis assays quantifying the amount of caspase 3 and caspase 8 activities present in each cell conditions confirmed these findings (Fig. 10C); thus, JDP-2 is a general antiapoptotic factor acting via the repression of *p53* expression.

DISCUSSION

Our results provide strong evidence that a recently identified member of the AP-1 family, JDP-2, can protect cells against UV-induced apoptosis. In contrast, the related JDP-1 protein does not appear to have a similar function. First, unlike JDP-2, JDP-1 expression is not induced by UV. Second, in vivo experiments showed that p53 expression before or after UV exposure is not affected in cells that modestly overexpress JDP-1, nor are these cells any different from the parental cells in their UV sensitivity. In JDP-2-overexpressing cells, however, p53 expression is down-regulated. Only during transient overexpression experiments can JDP-1, like JDP-2, down-regulate p53 expression through a variant AP-1 site in the *p53* promoter (compare Fig. 4A and C with Fig. 5A). In this context, JDP-1 can display a marginal protective function against UV irradi-

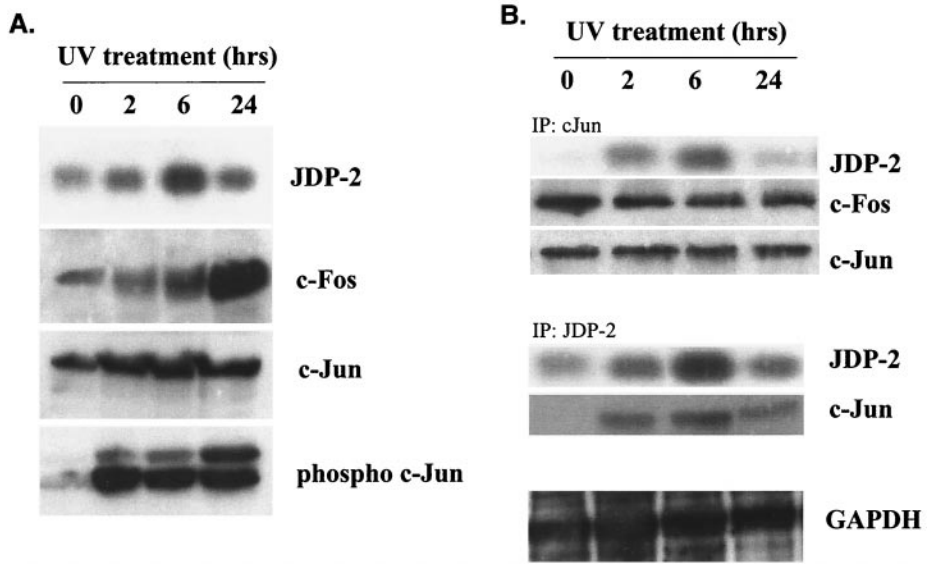


FIG. 7. Characterization of the AP-1 dimers timely involved in the UV response. (A) JDP-2, c-Jun, and c-Fos have different expression profiles upon UV irradiation. Exponentially growing c-*jun*$^{+/+}$ cells were UV-C irradiated (40 J/m^2), and protein extracts were prepared at the indicated times. Expression and phosphorylation of JDP-2, c-Jun, and c-Fos were analyzed by Western blotting using specific antibodies. (B) c-Jun–c-Fos and c-Jun–JDP-2 heterodimers accumulate with different kinetics upon UV irradiation. Exponentially growing c-*jun*$^{+/+}$ cells were irradiated with UV-C (40 J/m^2). Protein extracts were harvested at the indicated times and immunoprecipitated (IP) with specific c-Jun or JDP-2 antibodies. The various immune complexes were then analyzed by Western blotting with antibodies directed against c-Jun, c-Fos, or JDP-2 and against c-Jun or JDP-2, respectively. The amount of proteins in the extracts (quantified by the Bradford method) was further confirmed by Western blotting with a specific anti-GAPDH antibody.

ation which depends on the cell's c-Jun status (Fig. 2). Most likely, expression of copious amounts of JDP-1 results in loss of specificity, allowing JDP-1 to exhibit a JDP-2-like *trans*-repressive activity on targets that it does not affect physiologically. DNA titration experiments revealed that much higher levels of JDP-1 are required for repression of *p53* transcription than JDP-2 (data not shown).

JDP-1 and JDP-2 were initially isolated by their ability to specifically interact with the AP-1 transcription factor c-Jun (2). Even though no apparent function has been ascribed so far to JDP-1, JDP-2 as a heterodimer with c-Jun was found to form a repressive form of AP-1 in nonstimulated cells (2). We found that, following UV irradiation, expression of JDP-2, but not JDP-1, is up-regulated. JDP-2 induction appears to involve phosphorylation at a specific threonine residue (Aronheim, unpublished results) which probably causes its stabilization. Unlike with c-Jun (14), no UV-induced changes in JDP-2 transcription were detected. Constitutive elevated expression of JDP-2 affected expression of the tumor suppressor *p53* gene at the transcriptional level in two different ways. First, JDP-2 lowered the basal activity of the *p53* promoter. Second, upon UV irradiation, JDP-2 delayed and reduced the extent of induction of *p53* expression. This effect was seen consistently (Fig. 6) even though the extent of the inhibition was variable. In control cells the induction of *p53* mRNA expression upon UV treatment was consistent and roughly 4-fold, whereas in JDP-2-expressing cells this induction varied from 1.5- to 3-fold. This repression of *p53* transcription is an unusual mode of p53 regulation, as it has been assumed that most genotoxic stimuli, including UV irradiation, regulate p53 expression at the post-transcriptional level, most likely by modulating the turnover of p53 protein (29). However, pulse-chase experiments indicated that JDP-2 inhibition of *p53* expression did not take place at the posttranslational level, and the effect of JDP-2 was traced to repression of *p53* promoter activity. As a major portion of UV-induced cell death depends on p53 expression, up-regulation of JDP-2 and down-regulation of *p53* transcription could provide considerable protection against UV-induced cell death. Indeed, JDP-2-overexpressing cells exhibit both lower levels of UV-induced apoptosis, detectable in short-term assays, and higher levels of clonogenic survival, detectable in long-term assays.

Most published reports emphasize translational and post-translational events as being the major regulatory steps in p53 expression and function (18, 29, 31, 44). Except during embryonic development (30), transcriptional regulation of *p53* has not been widely documented. Recently however, it was shown that c-*jun* null cells express elevated levels of *p53* mRNA and protein and that reintroduction of a constitutive c-*jun* allele into such cells repressed *p53* transcription (39). Unexpectedly, we found that upon UV irradiation, *p53* mRNA and promoter activity are induced four- to fivefold within 9 h of treatment. However, p53 protein levels are increased by a factor of 10 to 20 and with faster kinetics following UV irradiation. Thus, increased *p53* transcription makes a secondary but significant contribution to p53 accumulation, consistent with previous reports attributing a major role to protein stabilization in this induction process (18, 44). Nevertheless, delayed induction of *p53* transcription by a yet-to-be-identified mechanism is likely to contribute to the prolonged elevation of p53 expression seen in UV-irradiated cells.

Although the variant AP-1 binding site within the *p53* pro-

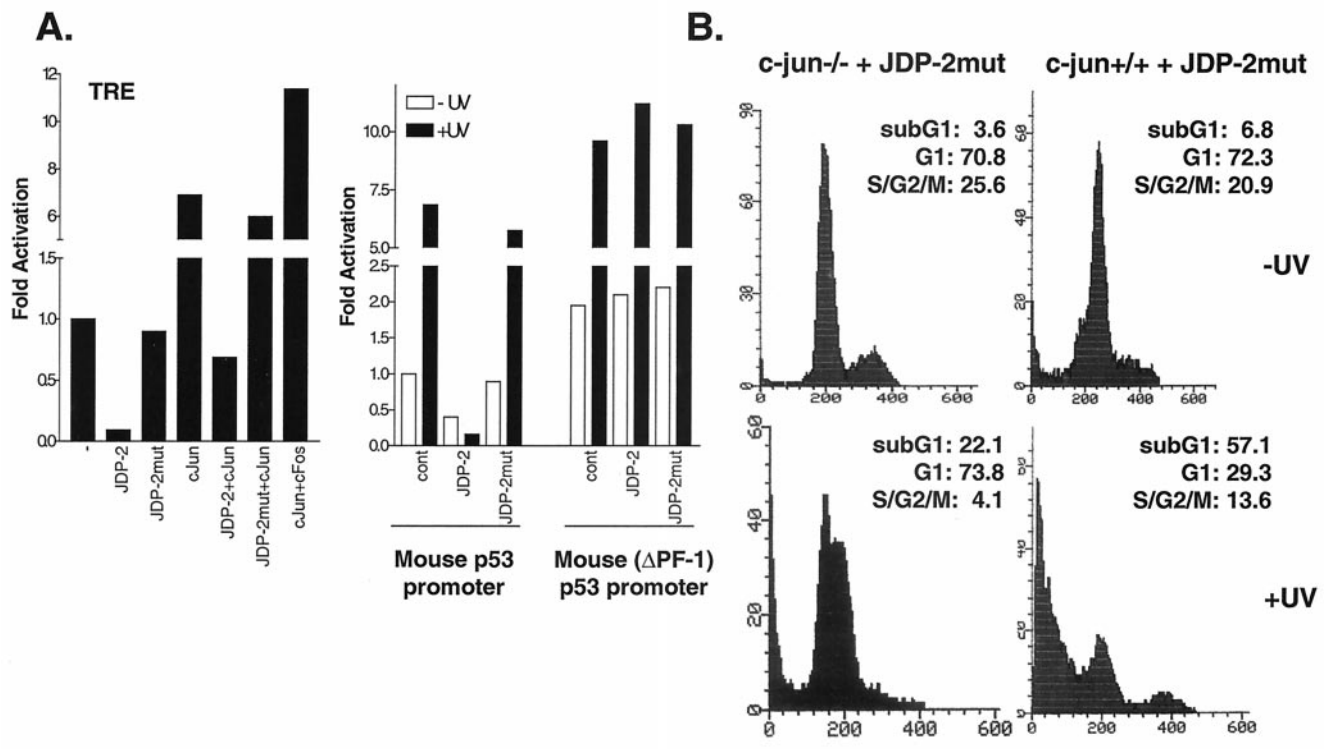


FIG. 8. A dominant positive JDP-2 mutant (JDP-2mut) lacks the ability to inhibit UV-induced *p53* transcriptional activation and apoptosis. (A) Characterization of the dominant positive JDP-2 mutant transcriptional properties. NIH 3T3 cells were transiently transfected with luciferase reporter genes containing either 3×TRE, mouse wild-type *p53* promoter, or *p53* (ΔPF-1) promoter, with various combinations of expression vectors for c-Jun, c-Fos, JDP-2, and JDP-2mut. The JDP-2 mutant was constructed by fusing in frame with JDP-2 the transcriptional activation domain of c-Fos. An actin–β-galactosidase reporter was included to normalize for transfection efficiency. Cells transfected with the *p53* promoter (wild-type and ΔPF-1) reporter constructs were UV irradiated (40 J of UV-C/m^2) or left unirradiated, and extracts were analyzed 9 h postirradiation. Data are averages for two independent experiments done in triplicate. (B) JDP-2mut expression does not affect UV-induced apoptosis. Cell cycle distribution was determined by flow cytometry as described for Fig. 2. Spontaneously immortalized c-*jun*$^{+/+}$ and c-*jun*$^{-/-}$ mouse fibroblasts were transiently transfected with JDP-2mut mammalian expression vectors. JDP-2 FITC-positive cells were sorted and further analyzed. The vertical and horizontal axes represent cell count and relative DNA content, respectively.

moter (PF-1) is not required for its induction by UV, this site is absolutely required for repression of *p53* transcription by c-Jun (39) and JDP-2 (Fig. 6). Interestingly, this variant AP-1 site is conserved throughout evolution and is present in all vertebrate *p53* promoters (19), suggesting an important regulatory function. The PF-1 site differs by a single base pair substitution from the canonical AP-1 site (1) and was reported to mediate transcriptional modulation by growth factors and cytokines, as well as to confer transcriptional repression to a heterologous promoter (19). Recently, the PF-1 site was found to be an important element in cell cycle and proliferation control by c-Jun (39). The present findings demonstrate that the PF-1 site is also required for transcriptional repression of the *p53* promoter by JDP-2 and most likely plays a central role in the protective effect of JDP-2, further extending the importance of this element in p53 biology. Furthermore, studies with a mutant form of JDP-2 that has lost its repressive activity due to fusion of the c-Fos transcriptional activation domain indicate that the role of JDP-2 in *p53* regulation and protection from UV-induced apoptosis relies on its ability to act as a transcriptional repressor. The results with this mutant also suggest that JDP-2 contains an active transcriptional repression domain rather than being devoid of an activation domain (2).

The ability of the PF-1 site to specifically modulate *p53* transcription in response to certain AP-1 proteins raises the question of which AP-1 dimers are physiologically involved in the regulation of *p53* expression. In vitro, various members of the AP-1 family were found to bind to the PF-1 site (19, 39) (Fig. 5A). In vivo, however, overexpression of c-Jun (39) and JDP-2, but not JDP-1, results in decreased *p53* mRNA expression. As both c-Jun (14) and JDP-2 are expressed at higher levels following UV irradiation and can form stable heterodimers (2), it is likely that JDP-2–c-Jun heterodimers may play a critical role in attenuating *p53* transcription in UV-irradiated cells. Furthermore, JDP-2 can compete with c-Fos for binding to c-Jun and and thereby convert activating c-Jun–c-Fos dimers to repressing JDP-2–c-Jun dimers (2). Moreover, c-Jun–c-Fos dimers do not bind very well to the PF-1 site (39). However, JDP-2 appears to have lower affinity for c-Jun than c-Fos does, and therefore formation of JDP-2–c-Jun heterodimers may occur only when substantial amounts of JDP-2 and c-Jun are present, such as after UV exposure. Indeed, at early phases of the UV response, c-Jun is mostly associated

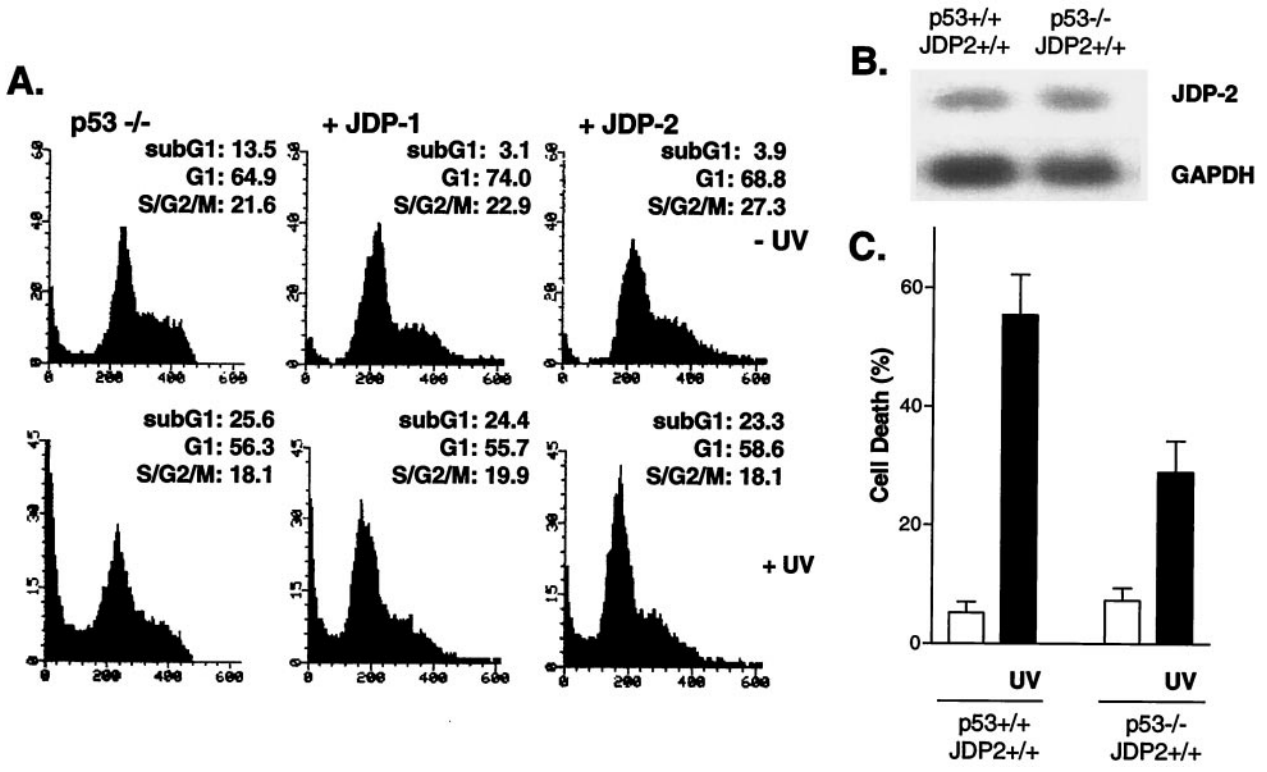


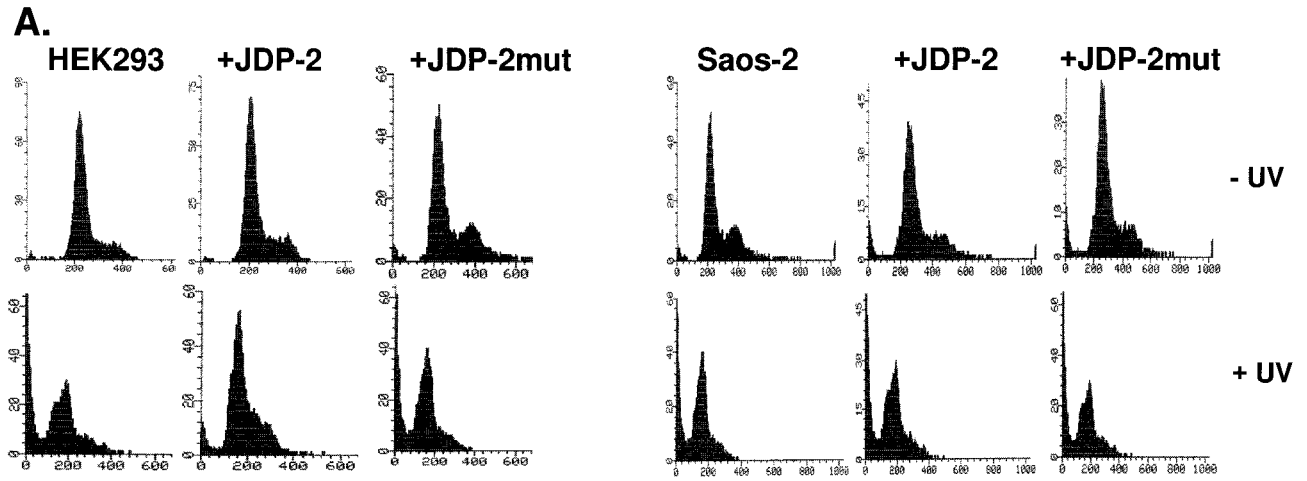
FIG. 9. JDP-2 does not affect UV-induced apoptosis in cells lacking *p53*. (A) Cell cycle analysis of *p53* null cells transiently expressing JDP-1 or JDP-2. *p53* null cells were either mock or transiently transfected with JDP-1 or JDP-2 expression vectors. Sixteen hours after transfection, cells were UV irradiated (40 J of UV-C/m^2) and harvested 48 h later. Ethanol-fixed cells were then stained with propidium iodide and analyzed by flow cytometry. Cells expressing transiently transfected JDP-1 or JDP-2 were sorted for FITC staining as described above (Fig. 2). (B) Expression levels of JDP-2 in *p53* wild-type and null cells. Exponentially growing $p53^{+/+}$ $JDP\text{-}2^{+/+}$ and $p53^{-/-}$ $JDP\text{-}2^{+/+}$ cells were harvested, and whole-cell protein extracts were made and analyzed by SDS-PAGE. Western blots were probed with specific antibodies against JDP-2 and GAPDH and revealed using ECL. (C) JDP-2 requires p53 to inhibit UV-induced apoptosis. $p53^{+/+}$ $JDP\text{-}2^{+/+}$ and $p53^{-/-}$ $JDP\text{-}2^{+/+}$ cells were mock or UV treated (40 J of UV-C/m^2), and apoptosis was evaluated 48 h postirradiation. Apoptosis was quantified by measuring levels of caspase 3 and caspase 8 present in extracts using a colorimetric assay.

with c-Fos, and only at later time points do c-Jun–JDP-2 heterodimers appear. Even though c-Jun–JDP-2 heterodimers may not be as abundant as c-Jun–c-Fos heterodimers, their preferential binding to the PF-1 site may be sufficient for repression of *p53* promoter activity several hours after UV irradiation, when their concentration peaks. Such a mechanism would result in delayed accumulation of p53 and delayed cell cycle arrest via induction of p53 target genes such as $p21^{waf1}$. As sustained high levels of p53 expression can trigger apoptosis (4, 7), this repressive effect of JDP-2–c-Jun heterodimers should have a protective function as observed in JDP-2 overexpressing cells.

Surprisingly, the antiapoptotic effect of JDP-2 in the mammalian UV response was observed in both c-$jun^{+/+}$ and c-$jun^{-/-}$ cells. These results suggest that JDP-2 may also heterodimerize with other Jun proteins (JunB or JunD) that are still expressed in c-$jun^{-/-}$ cells. In fact, recent results confirm earlier findings and indicate that JunB and JunD may function as negative regulators of c-Jun target genes and that therefore, their heterodimers with JDP-2 may even be stronger repressors than c-Jun–JDP-2 heterodimers. It is also possible that JDP-2 associates with other AP-1 transcription factors, such as ATF-2. ATF-2 both plays an active part in the UV response and binds to atypical AP-1 sites such as the PF-1 motif (38). However, it is unlikely that JDP-2 could act in conjunction with c-Fos family members, since in vitro cotranslated JDP-2 and c-Fos do not bind to TRE elements, as observed in gel retardation assays (Aronheim, unpublished results).

Recent results from our group indicate that c-Jun induction in response to UV irradiation is essential for exit of cells from p53-imposed cell cycle arrest (39a). This effect is mediated through the ability of c-Jun to repress *p53*-mediated transactivation. The present results indicate that the cross talk between AP-1 family members and p53 is not limited to c-Jun and that JDP-2 may be involved in such interactions as well. However, unlike with c-Jun, the major effect of JDP-2 on p53 is exerted, as discussed above, at the level of the *p53* promoter, resulting in down-regulation of p53 expression that is much more substantial than the one observed in cells that express c-Jun constitutively (39, 39a).

Findings that *p53* null cells (of both human and mouse origin) can undergo moderate apoptosis upon UV irradiation indicate that there exist p53-independent mechanisms of cell death. Such pathways have been already described (3, 4, 7). They potentially could involve p53-like mechanisms involving the p53-related proteins p73 and p63 and their different isoforms (33a). Alternatively, irradiation induced-apoptosis in *p53* null cells was shown to involve activation of the Fas path-



B.

	Sub G1	G1	S/G2/M
HEK293	2.1	71.6	26.3
HEK293 +UV	38.3	54.6	7.1
HEK293 + JDP-2	1.8	65.6	32.6
HEK293 + JDP-2 +UV	9.3	67.3	23.4
HEK293 +JDP-2mut	2.6	63.9	33.5
HEK293 + JDP-2mut +UV	35.5	55.6	8.9
Saos-2	2.1	72.7	25.2
Saos-2 +UV	21.0	65.2	13.8
Saos-2 + JDP-2	3.0	73.5	23.5
Saos-2 + JDP-2 +UV	26.7	65.6	7.7
Saos-2 +JDP-2mut	3.0	75.6	21.4
Saos-2 + JDP-2mut +UV	25.4	65.2	9.4

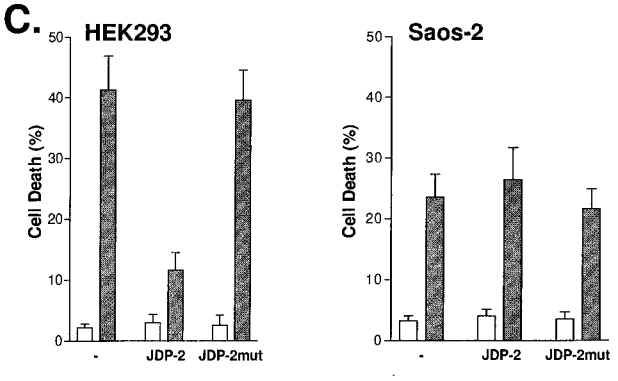


FIG. 10. JDP-2 ability to inhibit UV mediated apoptosis is a general phenomenon and is dependent upon p53. (A) Cell cycle distribution of UV irradiated human cell lines expressing JDP-2. Human HEK293 ($p53^{+/+}$) and Saos-2 ($p53^{-/-}$) cells were UV irradiated (40 J/m^2), and their cell cycle distribution was examined 24 h postirradiation by fluorescence-activated cell sorting. Cells transiently expressing JDP-2 or JDP-2mut were sorted and treated as described above (Fig. 2). (B) Recapitulation of results from panel A. (C) Apoptosis of $p53^{-/-}$ and $p53^{+/+}$ human cell lines expressing JDP-2 and JDP-2mut. HEK293 ($p53^{+/+}$) and Saos-2 ($p53^{-/-}$) cells were UV irradiated (40 J of UV-C/m^2), and their respective degree of apoptosis were quantified after 24 h by measuring endogeneous levels of caspase 3 and caspase 8 using a fluorescent substrate.

way (35a). Such mechanisms could also be responsible for the low rate of spontaneous apoptosis observed in mouse *p53* null cells. How JDP-1 and JDP-2 could antagonize such pathways and/or others to reduce the extent of spontaneous apoptosis in these cells is unclear. However, and more importantly, upon UV irradiation of $p53^{-/-}$ cells, expression of JDP-2, or JDP-1 for that matter, does not provide protection from UV-induced apoptosis, indicating that the mechansim of JDP-2 action is closely linked to p53.

In summary, our results have uncovered a new level of regulation of *p53* expression in the context of the mammalian UV response. UV irradiation results in *p53* promoter activation in addition to its established effects on p53 protein turnover. As *p53* mRNA induction is more transient than the accumulation of p53 protein, it must also be under negative control. While the conserved AP-1 site in the *p53* promoter (PF-1) and c-Jun are not required for UV-mediated induction of *p53* transcription, both the AP-1 site and c-Jun as well as JDP-2 are involved in down-regulation of *p53* transcription. The transcriptional repression of *p53* by JDP-2 appears to be a critical event, conserved in different mammalian species, that mediates the protective effect of JDP-2, a repressing AP-1 protein (2), on UV-irradiated cells.

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REFERENCES

1. **Angel, P., and M. Karin.** 1991. The role of Jun, Fos and the AP-1 complex in cell-proliferation and transformation. Biochim. Biophys. Acta **1072:**129–157.
2. **Aronheim, A., E. Zandi, H. Hennemann, S. Elledge, and M. Karin.** 1997. Isolation of an AP-1 repressor by a novel method for detecting protein-protein interactions. Mol. Cell. Biol. **17:**3094–3102.
3. **Bates, S., and K. H. Vousden.** 1996. p53 in signaling checkpoint arrest or apoptosis. Curr. Opin. Genet. Dev. **6:**12–18.
4. **Bennett, M. R.** 1999. Mechanisms of p53-induced apoptosis. Biochem. Pharmacol. **58:**1089–1095.
5. **Blattner, C., P. Kannouche, M. Litfin, K. Bender, H. J. Rahmsdorf, J. F.**

Angulo, and P. Herrlich. 2000. UV-induced stabilization of c-*fos* and other short-lived mRNAs. Mol. Cell. Biol. **20:**3616–3625.
6. **Buckbinder, L., R. Talbott, S. Valesco-Miguel, I. Takenaka, B. Faha, B. Seizinger, and N. Kley.** 1995. Induction of the growth inhibitor IGF-binding protein 3 by p53. Nature **377:**646–649.
7. **Burns, T. F., and W. S. El-Deiry.** 1999. The p53 pathway and apoptosis. J. Cell. Physiol. **181:**231–239.
8. **Buscher, M., H. Rahmsdorf, M. Liftin, M. Karin, and P. Herrlich.** 1988. Activation of the c-fos gene by UV and phorbol ester: different signal transduction pathways converge to the same enhancer element. Oncogene **3:**301–311.
9. **Caelles, C., A. Helmberg, and M. Karin.** 1994. p53-dependent apoptosis in the absence of transcriptional activation of p53-target genes. Nature **370:** 220–223.
10. **Canman, C., and M. Kastan.** 1995. Induction of apoptosis by tumor suppressor genes and oncogenes. Sem. Cancer Biol. **6:**17–25.
11. **Cavigelli, M., W. W. Li, A. Lin, B. Su, K. Yoshioka, and M. Karin.** 1996. The tumor promoter arsenite stimulates AP-1 activity by inhibiting a JNK phosphatase. EMBO J. **15:**6269–6279.
12. **Derijard, B., M. Hibi, I. Wu, T. Barrett, B. Su, T. Deng, M. Karin, and R. Davis.** 1994. JNK1: a protein kinase stimulated by UV light and Ha-Ras that binds and phosphorylates c-jun activation domain. Cell **76:**1025–1035.
13. **Devary, Y., R. Gottlieb, T. Smeal, and M. Karin.** 1992. The mammalian ultraviolet response is triggered by activation of Src tyrosine kinases. Cell **71:**1081–1091.
14. **Devary, Y., R. A. Gottlieb, L. F. Lau, and M. Karin.** 1991. Rapid and preferential activation of the c-jun gene during the mammalian UV response. Mol. Cell. Biol. **11:**2804–2811.
15. **Devary, Y., C. Rosette, J. DiDonato, and M. Karin.** 1993. NF-kappa B activation by ultraviolet light not dependent on a nuclear signal. Science **261:**1442–1445.
16. **Donehower, L., M. Harvey, B. Slagle, M. McArthur, C. J. Montgomery, J. Butel, and A. Bradley.** 1992. Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. Nature **356:**215–221.
17. **Engelberg, D., E. Zandi, C. S. Parker, and M. Karin.** 1994. The yeast and mammalian Ras pathways control transcription of heat shock genes independently of heat shock transcription factor. Mol. Cell. Biol. **14:**4929–4937.
18. **Ewen, M., and S. Miller.** 1996. p53 and translational control. Biochim. Biophys. Acta **1242:**181–184.
19. **Ginsberg, D., M. Oren, M. Yaniv, and J. Piette.** 1990. Protein-binding elements in the promoter region of the mouse p53 gene. Oncogene **5:**1285–1290.
20. **Gupta, S., D. Campbell, B. Derijard, and R. J. Davis.** 1995. Transcription factor ATF2 regulation by the JNK signal transduction pathway. Science **267:**389–393.
21. **Han, J., Y. Jiang, Z. Li, V. V. Kravchenko, and R. J. Ulevitch.** 1997. Activation of the transcription factor MEF2C by the MAP kinase p38 in inflammation. Nature **386:**296–299.
22. **Herrlich, P., C. Sachsenmaier, A. Radler-Pohl, S. Gebel, C. Blattner, and H. Rahmsdorf.** 1994. The mammalian UV response: mechanism of DNA damage induced gene expression. Adv. Enzyme Regul. **34:**381–395.
23. **Hibi, M., A. Lin, T. Smeal, A. Minden, and M. Karin.** 1993. Identification of an oncoprotein- and UV-responsive protein kinase that binds and potentiates the c-Jun activation domain. Genes Dev. **7:**2135–2148.
24. **Hilberg, F., A. Aguzzi, N. Howells, and E. F. Wagner.** 1993. c-jun is essential for normal mouse development and hepatogenesis. Nature **365:**179–181. (Erratum, **366:**368.)
25. **Holbrook, N., Y. Liu, and A. J. Fornace.** 1996. Signaling events controlling the molecular response to genotoxic stress. EXS **77:**273–288.
26. **Jacks, T., and R. A. Weinberg.** 1996. Cell-cycle control and its watchman. Nature **381:**643–644.
27. **Johnson, R. S., B. van Lingen, V. E. Papaioannou, and B. M. Spiegelman.** 1993. A null mutation at the c-jun locus causes embryonic lethality and retarded cell growth in culture. Genes Dev. **7:**1309–1317.
28. **Kallunki, T., T. Deng, M. Hibi, and M. Karin.** 1996. c-Jun can recruit JNK to phosphorylate dimerization partners via specific docking interactions. Cell **87:**929–939.
29. **Kastan, M., O. Onyekwere, D. Sidransky, B. Vogelstein, and R. Craig.** 1991. Participation of p53 protein in the cellular response to DNA damage. Cancer Res. **51:**6304–6311.
30. **Komarova, E., M. Chernov, R. Franks, K. Wang, G. Armin, C. Zelnick, D. Chin, S. Bacus, G. Stark, and A. Gudkov.** 1997. Transgenic mice with p53-responsive LacZ: p53 activity varies dramatically during normal development and determines radiation and drug sensitivity in vivo. EMBO J. **16:**1391–1400.
31. **Lane, D. P.** 1992. Cancer. p53, guardian of the genome. Nature **358:**15–16.
32. **Lee, S., B. Elenbaas, A. Levine, and J. Griffith.** 1995. p53 and its 14 kDa C-terminal domain recognize primary DNA damage in the form of insertion/deletion mismatches. Cell **81:**1013–1020.
33. **Levine, A.** 1997. p53, the cellular gatekeeper for growth and division. Cell **88:**323–331.
33a. **Lohrum, M. A., and K. H. Vousden.** 2000. Regulation and function of the p53-related proteins: same family, different rules. Trends Cell Biol. **10:**197–202.
34. **Miyashita, T., and J. Reed.** 1995. Tumor suppressor p53 is a direct transcriptional activator of the human bax gene. Nature **378:**203–206.
35. **Oberosler, P., P. Hloch, U. Ramsperger, and H. Stahl.** 1993. p53-catalyzed annealing of complementary single-stranded nucleic acids. EMBO J. **12:** 2389–2396.
35a. **O'Connor, L., A. W. Harris, and A. Strasser.** 2000. CD95 (Fas/APO-1) and p53 signal apoptosis independently in diverse cell types. Cancer Res. **60:** 1217–1220.
36. **Radler-Pohl, A., C. Sachsenmaier, S. Gebel, H. Auer, J. Bruder, U. Rapp, P. Angel, H. Rahmsdorf, and P. Herrlich.** 1993. UV-induced activation of AP-1 involves obligatory extranuclear steps including Raf-1 kinase. Cell **12:**105–112.
37. **Rosette, C., and M. Karin.** 1996. Ultraviolet light and osmotic stress: activation of the JNK cascade through multiple growth factor and cytokine receptors. Science **274:**1197–1197.
38. **Rozek, D., and G. Pfeifer.** 1993. In vivo protein-DNA interactions at the c-jun promoter: preformed complexes mediate the UV response. Mol. Cell. Biol. **13:**5490–5499.
39. **Schreiber, M., A. Kolbus, F. Piu, J. Tian, U. Mohle-Steinlein, M. Karin, P. Angel, and E. Wagner.** 1999. Control of cell cycle progression by c-Jun is p53 dependent. Genes Dev. **13:**607–619.
39a. **Shaulian, E., M. Schreiber, F. Piu, M. Beeche, E. F. Wagner, and M. Karin.** 2000. The mammalian UV response: c-Jun induction is required for exit from p53-imposed growth. Cell **103:**897–907.
40. **Smeal, T., B. Binetruy, D. Mercola, A. Grover-Bardwick, G. Heidecker, U. R. Rapp, and M. Karin.** 1992. Oncoprotein-mediated signalling cascade stimulates c-Jun activity by phosphorylation of serines 63 and 73. Mol. Cell. Biol. **12:**3507–3513.
41. **Smeal, T., B. Binetruy, D. A. Mercola, M. Birrer, and M. Karin.** 1991. Oncogenic and transcriptional cooperation with Ha-Ras requires phosphorylation of c-Jun on serines 63 and 73. Nature **354:**494–496.
42. **Stein, B., H. J. Rahmsdorf, A. Steffen, M. Litfin, and P. Herrlich.** 1989. UV-induced DNA damage is an intermediate step in UV-induced expression of human immunodeficiency virus type 1, collagenase, c-*fos*, and metallothionein. Mol. Cell. Biol. **9:**5169–5181.
43. **Treisman, R.** 1995. Journey to the surface of the cell: Fos regulation and the SRE. EMBO J. **14:**4905–4913.
44. **Tyrell, R.** 1996. UV activation of mammalian stress proteins. EXS **77:**255–271.
45. **Wang, X., H. Yeh, L. Schaeffer, R. Roy, V. Moncollin, J. Egly, Z. Wang, E. Friedberg, M. Evans, V. Bohr, G. Weeda, J. Hoeijmakers, K. Forrester, and C. Harris.** 1995. A p53 modulation of TFIIH-associated for nucleotide excision repair activity. Nature **10:**188–195.
46. **Whitmarsh, A. J., P. Shore, A. D. Sharrocks, and R. J. Davis.** 1995. Integration of MAP kinase signal transduction pathways at the serum response element. Science **269:**403–407.