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/m² 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. Response of mammalian cells to UV irradiation causes nuclear accumulation of the product of the tumor suppressor gene p53 (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 p21waf1. 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 p21waf1, 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 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...
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 CTG to TGAATTG, 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). E12.5 mouse 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.

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 polyacrylamide 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. Trypsinated cells were resuspended in PBS at 106 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 0.5% 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 antibodies. After extensive washing, membranes were incubated with32P-radiolabeled probes for 10 min at room temperature. The reaction products were then resolved on a nondenaturing acrylamide gel.

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, JDP-2 induction was 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/m2, c-jun−/− cells accumulated mostly at the G1 phase of the cell cycle, while a small fraction had undergone apoptosis or necrosis, as revealed by appearance of a sub-G1 population (Fig. 2). By contrast, UV-irradiated c-jun−/− cells were only partially arrested in G1, and the majority were dead,
based upon the appearance of a large sub-G₁ 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 G₁ 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/m², 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-G₁ DNA content. Constitutive expression of JDP-1 did not significantly alter this response, except for a small increase in the extent of G₁ arrest. Expression of JDP-2, on the other hand, delayed the appearance of sub-G₁ 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/m², exhibiting fragmented nuclei after 24 h was similar to the percentage of sub-G₁ 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
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/m²) or mock irradiated 2 h prior to labeling with [35S]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
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...
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...
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 already present within 2 h postirradiation 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
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-G1 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-G1 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-G1 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 JDP-2mut or a control vector. Sixteen hours posttransfection cells were mock or UV-C irradiated (40 J/m²), 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-G1 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-G1 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-G1 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 irrad-
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, 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.

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-
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 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. 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 3xTRE, 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/m²) 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.
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 \( p21waf1 \).

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-
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 mechanism 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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