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Rapid and Preferential Activation of the c-*jun* Gene during the Mammalian UV Response

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Exposure of mammalian cells to DNA-damaging agents leads to activation of a genetic response known as the UV response. Because several previously identified UV-inducible genes contain AP-1 binding sites within their promoters, we investigated the induction of AP-1 activity by DNA-damaging agents. We found that expression of both c-*jun* and c-*fos*, which encode proteins that participate in formation of the AP-1 complex, is rapidly induced by two different DNA-damaging agents: UV and H_2O_2. Interestingly, the c-*jun* gene is far more responsive to UV than any other immediate-early gene that was examined, including c-*fos*. Other *jun* and *fos* genes were only marginally affected by UV or H_2O_2. Furthermore, UV is a much more efficient inducer of c-*jun* than phorbol esters, the standard inducers of c-*jun* expression. This preferential response of the c-*jun* gene is mediated by its 5′ control region and requires the TPA response element, suggesting that this element also serves as an early target for the signal transduction pathway elicited by DNA damage. Both UV and H_2O_2 lead to a long-lasting increase in AP-1 binding activity, suggesting that AP-1 may mediate the induction of other damage-inducible genes such as human collagenase.

Exposure of mammalian cells to adverse environmental conditions triggers the onset of specific genetic responses, which are likely to have evolved as mechanisms that protect the cell against permanent damage and death. This occurs by the rapid induction of sets of genes whose products are supposed to exert a protective effect. The spectrum of genes that are turned on during these responses depends on the particular type of stress to which the cell is exposed. Thus, elevated temperatures cause the induction of heat shock proteins that are thought to allow cells to withstand such a condition, possibly by promoting the proper refolding of denatured proteins (28). Exposure to toxic heavy-metal ions, such as Hg and Cd, leads to induction of metallothioneins, which by their ability to chelate large amounts of these heavy-metal ions protect cells against their toxic effects (16, 20). Exposure to UV radiation and other DNA-damaging agents triggers a response known as the UV response, whose putative role is to protect cells against DNA damage (18, 35). While the first two responses have been extensively studied and the regulatory mechanisms involved in their elicitation are beginning to be unfolded (16, 28, 40), very little is known about the function of the mammalian UV response and the mechanisms by which it is triggered.

In *Escherichia coli*, DNA damage caused by UV radiation or chemical agents induces approximately 20 genes belonging to the SOS regulon. Many of these genes code for proteins involved in DNA repair, mutagenesis, and recombination (45). Damage by alkylating agents or oxidative stress induces other well-characterized regulons in bacteria (45). In yeast cells, induction of the *RAD2* excision repair gene by UV irradiation is analogous to the SOS response in bacteria (34). *RAD2* is likely to be one of many other yeast DNA-damage-inducible (DDI) genes. Ruby and Szostak have estimated there may be up to 80 such genes (36). A large number of DDI cDNA clones have been isolated from mammalian cells (5, 13, 14, 18, 19, 22, 23, 27). However, only a few of their products have been identified. These include metallothioneins (5), plasminogen activator (27), and collagenase (2). One common characteristic of these genes is their ability to be induced by phorbol esters such as 12-*O*-tetradecanoylphorbol-13-acetate (TPA) (2, 4, 5, 18, 22, 23, 27). The regulatory regions of these genes contain a specific DNA sequence motif that functions as a TPA-inducible *cis* element (TRE) recognized by transcription factor AP-1 (4). AP-1 itself is a complex composed of homodimers and heterodimers of the *jun* and *fos* gene products (1, 10, 21, 33, 44).

Since AP-1 has the ability to control the transcription of several mammalian DDI genes, we have initiated an investigation of the mechanisms by which DNA damage modulates AP-1 activity. The long-term goal of these studies is to elucidate the signal transduction mechanisms involved in the mammalian UV response. The findings reported here indicate that treatment of HeLa cells with two different DNA-damaging agents, UV and H_2O_2, leads to induction of c-*jun* and c-*fos*, two genes that encode the major components of the AP-1 complex. Interestingly, the c-*jun* gene is far more inducible by UV irradiation than by TPA, the standard inducer of c-*jun* expression (3). Furthermore, this high level of responsiveness to UV irradiation is specific to c-*jun* and was not observed with c-*fos*, *fosB*, *fra-1*, *junB*, *junD*, or five other mitogen- and TPA-inducible immediate-early genes. Transfection experiments indicate that the c-*jun* regulatory region is far more responsive to UV than to TPA. The rapid kinetics and its magnitude of induction suggest that the regulatory region of the c-*jun* gene is a preferential early target for the signaling cascade involved in the UV response.

MATERIALS AND METHODS

Cell culture. HeLa S3 cells were grown in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal calf serum

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(FCS), 100 U of penicillin per ml, and 100 μg of streptomycin per ml. All cells were kept at 37°C and 6% CO_2.

RNA preparation and analysis. Total cellular RNA was isolated by the guanidinium isothiocyanate extraction method (8). The RNA was electrophoretically separated on 1% formaldehyde-agarose gels and transferred to Duralose-U.T. membranes (Stratagene). Blots were sequentially hybridized with probes specific for c-*jun*, *junB*, *junD*, c-*fos*, *fosB*, *fra-1*, α-tubulin, Nur77, and Cyr61 as previously described (9, 17, 26, 30, 37). The levels of hybridization were quantitated by densitometry with a Zeineh Soft Laser scanner.

Transfection and analysis of gene expression. HeLa S3 cells were transfected with the various *jun*-chloramphenicol acetyltransferase (CAT) and other reporter plasmids (3) by the calcium phosphate coprecipitation method as previously described (4, 11). After transfection, the cells were incubated in DMEM plus 0.1% fetal calf serum for 12 h and exposed to UV (260 nm, 40 J/m^2), TPA (70 ng/ml), or mitomycin C (10 μg/ml). Protein extracts were prepared 24 to 48 h after these treatments, and CAT assays were performed as previously described (1, 3, 4). UV irradiation was done by using a Westinghouse germicidal 260-nm UV lamp at a fluence rate of 2 J $m^{-2} \cdot s^{-1}$, and the time of exposure was adjusted according to the described dose.

Mobility shift assays. HeLa S3 cells were grown on 100-mm plates and after 24 h of incubation in DMEM plus 0.1% FCS were treated with either TPA (100 ng/ml), UV (40 J/m^2), or H_2O_2 (250 μM). After the indicated time points, nuclear extracts were prepared and mobility shift assays were performed as described previously (32). In some experiments, 4-μg samples of nuclear extracts were incubated with 2 μl of anti-Fos monoclonal antibody 18-H-6, raised against a synthetic peptide corresponding to amino acids 4 to 17 in the amino terminus of c-Fos (12). This antibody was supplied by H. Leffert (University of California, San Diego).

Chemicals. Bryostatin was a gift from A. Kraft (University of Alabama). TPA was purchased from Sigma (St. Louis, Mo.).

RESULTS

c-*jun* and c-*fos* induction by UV irradiation. Earlier work indicated that expression of c-*fos* is induced in response to UV irradiation (7). To determine whether expression of the c-*jun* gene, which encodes another component of the AP-1 complex (1), is also induced by UV irradiation, HeLa S3 cells were exposed to a 40-J/m^2 dose of 260-nm UV, and total RNA was harvested at different time points after irradiation. For a reference, the response of c-*jun* and c-*fos* to UV was compared with their induction by TPA. As shown in Fig. 1, in response to UV irradiation, c-*jun* expression was rapidly induced by more than 55-fold. c-*jun* transcripts were already significantly elevated at the earliest time point examined (30 min) and peaked at 90 min, after which they slowly decreased. In comparison, TPA treatment led to a more modest (7- to 10-fold) induction of c-*jun*, in agreement with previous results (3). As expected, c-*fos* expression was also induced by UV irradiation, but in comparison with the extent of its induction by TPA, which was approximately 20-fold, the response of UV was of more modest magnitude (7-fold). Both for c-*jun* and c-*fos*, the response to UV was longer lasting than the response to TPA. Like c-*fos*, another member of the *jun* family, *junB*, was also more responsive to TPA than to UV. The response of *junB* to TPA lagged behind the induction of c-*jun* and actually corresponded to the decline in c-*jun* expression. These differential induction kinetics are consistent with a role for the JunB protein in repression of c-*jun* expression (9). The third member of the *jun* family, *junD*, was expressed at low levels that were not affected by TPA (not shown) or UV (Fig. 2).

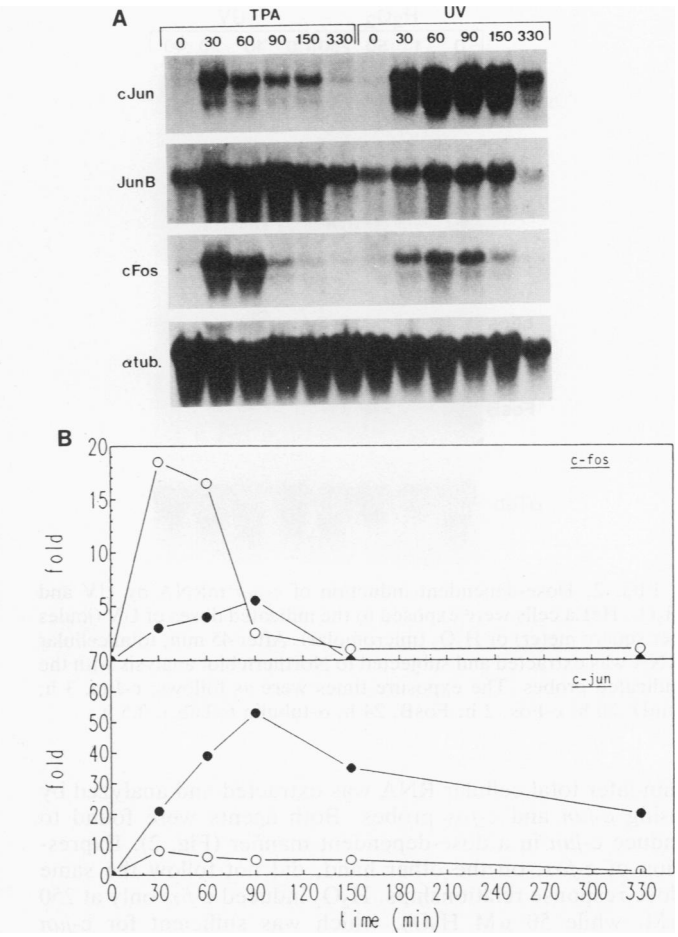

FIG. 1. Comparison of AP-1 and *fos* gene induction by TPA and UV. (A) Northern blot analysis of total cellular RNA isolated from HeLa cells exposed to TPA (100 ng/ml) or 260-nm UV (40 J/m^2). Cells were harvested at the indicated time points (in minutes), and total cellular RNA was extracted and analyzed on a 1% agarose gel. The blot was sequentially hybridized to c-*jun*, *junB*, c-*fos*, and α-tubulin cDNA probes. The exposure times for the different panels were as follows: c-Jun, 1 h; JunB, 19 h; c-Fos, 2 h; α-tubulin, 1 h. (B) Comparison of the induction kinetics of c-*jun* and c-*fos* by TPA (○) and UV (●). The autoradiogram shown in panel A was quantitated by laser densitometry, and the relative induction of c-*jun* and c-*fos* was determined relative to the level of the α-tubulin signal.

The differences in the responses of c-*jun* and c-*fos* to UV and TPA become quite clear after densitometric quantitation of the hybridization signals (Fig. 1B). While c-*jun* is preferentially induced by UV, c-*fos* is preferentially induced by TPA.

Induction by H_2O_2. To determine whether the induction of c-*jun* and c-*fos* by UV correlated with the known DNA-damaging activity of this agent (13), we examined the response of these genes to another DNA-damaging agent. We selected H_2O_2, which under the conditions used in this study is known to generate single-strand breaks (46). HeLa cells were exposed to increasing doses of H_2O_2 and UV, and 45

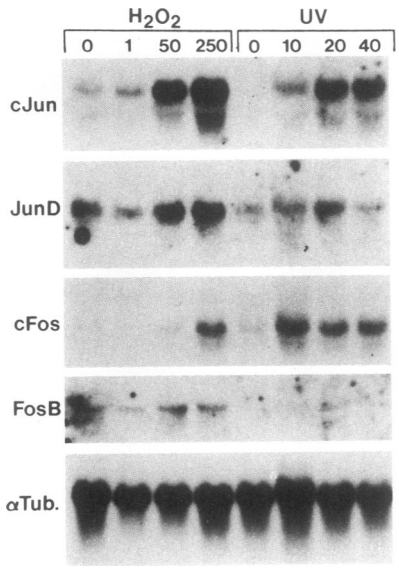

FIG. 2. Dose-dependent induction of c-*jun* mRNA by UV and H_2O_2. HeLa cells were exposed to the indicated doses of UV (joules per square meter) or H_2O_2 (micromolar). After 45 min, total cellular RNA was extracted and subjected to Northern blot analysis with the indicated probes. The exposure times were as follows: c-Jun, 3 h; JunD, 20 h; c-Fos, 2 h; FosB, 24 h; α-tubulin (αTub.), 0.5 h.

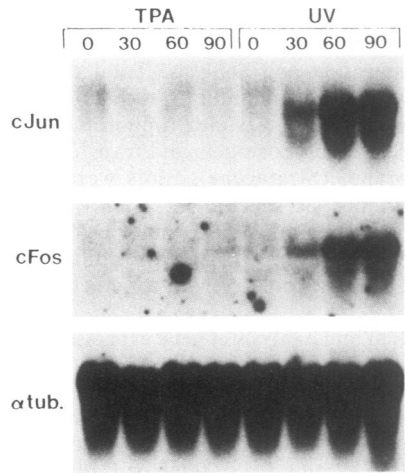

FIG. 3. Evidence that induction of c-*jun* and c-*fos* by UV is not entirely dependent on protein kinase C. HeLa cells were treated for 18 h with 4×10^{-7} M bryostatin, a potent down-regulator of protein kinase C. These cells were then exposed to either TPA (100 ng/ml) or 260-nm UV (40 J/m^2) and harvested at the indicated time points (in minutes). Total cellular RNA was analyzed by hybridization to c-*jun*, c-*fos*, and α-tubulin (αTub.) probes. Exposure times were as follows: c-Jun, 2 h; c-Fos, 4 h; α-tubulin, 1 h.

min later total cellular RNA was extracted and analyzed by using c-*jun* and c-*fos* probes. Both agents were found to induce c-*jun* in a dose-dependent manner (Fig. 2). Expression of c-*fos*, on the other hand, did not follow the same dose-response relationships. H_2O_2 induced c-*fos* only at 250 μM, while 50 μM H_2O_2, which was sufficient for c-*jun* induction, did not lead to c-*fos* induction. With UV, however, c-*fos* induction was already maximal at 10 J/m^2, while c-*jun* induction continued to increase with increasing doses. Expression of *junD* and *fosB*, on the other hand, was very low and was not significantly induced by UV. Both of these genes were slightly induced by H_2O_2. Expression of a third member of the *fos* family, *fra-1*, was not detected.

The dose-response relationship for c-*jun* induction by either UV or H_2O_2 showed a good correlation with previously published dose-response relationships for induction of DNA damage by these agents (15, 16).

UV induction of c-*jun* does not entirely rely on protein kinase C. The induction of c-*jun* and c-*fos* by TPA is likely to be mediated by protein kinase C, the known target for TPA action (29). Since induction by DNA-damaging agents is likely to be mediated by a mechanism different from that activated by TPA, we examined the induction of c-*jun* and c-*fos* by UV in cells in which protein kinase C was down-regulated by pretreatment with bryostatin, a compound different from phorbol esters, which are potent activators of protein kinase C and therefore capable of inducing its rapid degradation (25). After 19 h of incubation with 4×10^{-7} M bryostatin, HeLa cells were exposed to TPA or UV and analyzed for c-*jun* and c-*fos* induction (Fig. 3). While these cells were no longer responsive to TPA, both c-*jun* and c-*fos* transcripts were still inducible by UV irradiation. Examination of protein kinase C activity in extracts of bryostatin-treated HeLa cells with histone H1 as a substrate indicates at least a 60% decrease in this activity (12a). Thus the induction of both c-*jun* and c-*fos* by UV does not require the same level of functional protein kinase C as the response to TPA.

Induction of *jun*-CAT by DNA-damaging agents. A likely target for the signaling pathway involved in the UV response is the regulatory region of the c-*jun* gene. To test this possibility, HeLa cells were transfected with the −1.1/+740 *jun*-CAT reporter gene, which contains a large segment of the c-*jun* 5′-flanking region fused to the structural gene coding for CAT (3). As shown in Fig. 4A, CAT expression was highly stimulated after exposure of the transfected cells to increasing doses of UV. As observed for induction of c-*jun* transcripts, the induction of CAT activity by UV was also dose dependent. *jun*-CAT expression was also induced by the DNA-damaging agent mitomycin C (Fig. 4B). However, the response to this agent was slower (unpublished results), consistent with the dependence of the damaging effect of mitomycin C on DNA replication (31). Both UV and mitomycin C are more efficient inducers of *jun*-CAT expression than is TPA. While in these experiments the induction of CAT activity by TPA measured 24 h after the initial treatment was less than 2-fold, in previous experiments analyzed 6 h after the initial treatment the induction averaged 2.5-fold (3).

To determine which element of the c-*jun* promoter is required for the UV response, we compared the induction of the −1.1/+740*jun*-CAT construct with that of −132/+170*jun*-CAT and −132/+170ΔAP1*jun*-CAT (3). The latter construct has three point mutations that inactivate the AP-1 binding site (TRE) in the c-*jun* promoter (3). As shown in Fig. 5A, −132/+170*jun*-CAT was nearly as responsive as −1.1/+740*jun*-CAT, while −132/+170ΔAP1*jun*-CAT displayed only a marginal response to UV. Therefore the TRE, which was previously shown to be responsible for induction of c-*jun* by TPA (3), is also required for its UV responsiveness. We also compared the UV responsiveness of the c-*jun* promoter with that of the collagenase and thymidine kinase promoters. While −73col-CAT, which contains a fragment of the collagenase promoter with a functional TRE (2), was UV inducible, the pBLCAT2 construct, which contains the thymidine kinase promoter (4), was not.

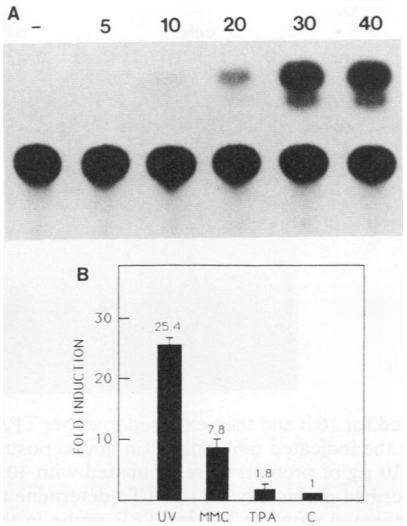

FIG. 4. Induction of *jun*-CAT expression by DNA-damaging agents. (A) HeLa cells were transfected with the −1.1/+740*jun*-CAT reporter plasmid (3). After 24 h, the cells treated with the indicated doses of 260-nm UV (joules per square meter); 24 h later, the cells were harvested and CAT activity was determined. (B) HeLa cells were transfected with the −1.1/+740*jun*-CAT reporter and 24 h later treated with either 260-nm UV (40 J/m^2), mitomycin C (MMC; 10 μg/ml), or TPA (100 ng/ml). Control, UV-treated, and TPA-treated cells were harvested after 24 h while mitomycin C-treated cells were harvested after 48 h, and the level of CAT activity was determined. The results represent the averages of two separate experiments.

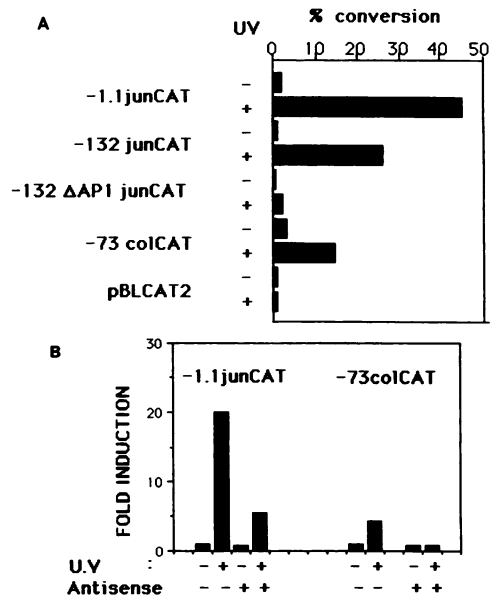

FIG. 5. Evidence that the UV response of c-*jun* requires a functional TRE and c-*jun* expression. (A) HeLa cells were transfected with the indicated reporter plasmids (1 μg each). After 24 h the cells were treated with 260-nm UV (40 J/m^2), and 24 h later they were harvested to measure CAT activity. The values shown represent the averages of two experiments. (B) HeLa cells were transfected with 1 μg of either −1.1/+740*jun*-CAT or −73col-CAT in the presence of 4 μg of either RSV-nuj or RSV-0 (an empty expression vector); 24 h later half of the transfected cultures were treated with 260-nm UV (40 J/m^2), as indicated, and after another 24 h the cells were harvested to measure CAT activity.

The requirement for a functional TRE suggests that c-*jun* induction by UV may be mediated by an autoregulatory mechanism as previously described for TPA (3). To explore the involvement of the c-*jun* gene product in the c-*jun* induction response, we made use of the RSV-nuj construct, which produces antisense c-*jun*-specific RNA (38). This construct was previously used to demonstrate the involvement of c-Jun in the activation of the transforming growth factor β1 gene (24). As indicated in Fig. 5B, the RSV-nuj vector was an effective inhibitor of both −1.1/+740*jun*-CAT and −73col-CAT induction by UV.

Induction of AP-1 binding activity by DNA-damaging agents. To test whether the induction of c-*jun* expression by UV and H_2O_2 results in increased AP-1 binding activity, nuclear extracts were prepared from HeLa cells treated for different lengths of time with UV or H_2O_2. Mobility shift assays were used to compare the level of AP-1 binding activity in these extracts to its level in nuclear extracts of untreated HeLa cells or TPA-treated HeLa cells. For this purpose, the nuclear extracts were incubated with a ^{32}P-labeled TRE probe corresponding in sequence to the AP-1 binding site of the collagenase gene, which had been extensively used to characterize AP-1 activity (1, 2, 4). Both UV and H_2O_2 treatments resulted in increased AP-1 binding activity (Fig. 6). Both the kinetics and extent of induction by UV and H_2O_2 were similar to the induction of AP-1 binding activity by TPA (Fig. 6A). The competition experiments shown in Fig. 6B indicate that the AP-1 binding activity induced in response to UV or H_2O_2 is specific. These AP-1 complexes contain the c-Fos protein, as indicated by the supershifts that appeared upon preincubation of the extracts with an anti-c-Fos monoclonal antibody (Fig. 6C). Very little c-Fos protein was present in the uninduced extract.

The transfection experiments indicated that the TRE in the c-*jun* promoter is required for its response to UV. To further investigate the involvement of AP-1 activity in the UV induction of c-*jun*, we examined the effect of TPA and UV on AP-1 binding to the collagenase and c-*jun* TREs at early time points. As seen in Fig. 7, both UV and TPA led to a considerable increase in AP-1 binding activity within 15 min of exposure, measured by either the collagenase or the c-*jun* TRE probe. These inductions were not blocked by pretreating the cells with 50 μg of cycloheximide per ml, a protein synthesis inhibitor. With use of the anti-c-Fos antibody, very little c-Fos-reactive material was present at these early time points, in contrast to its presence after several hours of induction (data not shown).

Effect of UV on expression of other early-response genes. Both c-*jun* and c-*fos* are members of a class of genes known as early-response or immediate-early genes because they are rapidly induced in response to cell stimulation with growth factors and phorbol ester tumor promoters (21). Since the results shown in Fig. 1 suggested that the c-*jun* gene was preferentially induced by UV in comparison to c-*fos* and *junB*, we wished to extent this analysis to other members of this group. The blot used in Fig. 1 was reprobed with specific cDNA probes to five immediate-early genes: Nur77, Cyr61, 3CH92, 3CH96, and 3CH134 (17, 26, 30). While Fig. 8 shows only the results obtained with the Nur77 and Cyr61 probes, the responses of all five genes were essentially identical: they were efficiently induced by TPA but exhibited a very low level of responsiveness to UV (no more than twofold induction). Therefore, of the 11 immediate-early genes tested in this study, only c-*jun* is preferentially induced by UV.

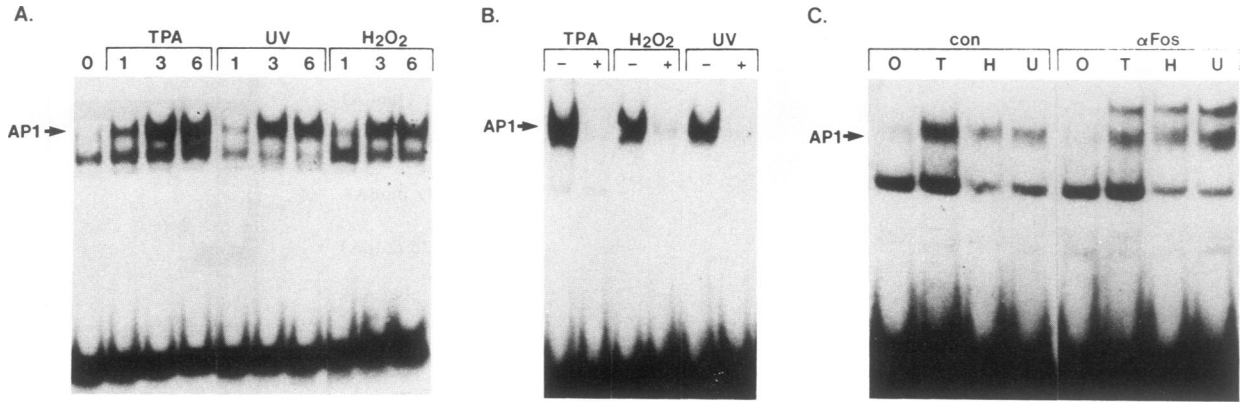

FIG. 6. Induction of AP-1 DNA binding activity. (A) HeLa cells were serum starved for 16 h and then exposed to either TPA (100 ng/ml), UV (40 J/m^2), or H_2O_2 (250 μM) or kept in DMEM plus 0.1% FCS (0 time point). At the indicated time points (in hours) posttreatment, the cells were harvested and nuclear extracts were prepared. Samples of each extract (10 μg of protein) were incubated with 10,000 cpm of a ^{32}P-labeled TRE probe. Electrophoretic mobility shift assays were performed as described previously (31). (B) To determine the specificity of the induced TRE binding activity, the extracts from the 6-h time points were incubated with the labeled TRE probe in the absence or presence of a 10-fold molar excess of cold TRE competitor. (C) To examine the presence of c-Fos in the different AP-1 complexes, 10-μg samples of the 6-h TPA (T)-, UV (U)-, and H_2O_2 (H)-induced cell extracts and the uninduced cell extract (0) were incubated with 2 μl of either phosphate-buffered saline or anti-c-Fos monoclonal antibody (12) for 45 min before incubation with the ^{32}P-labeled TRE probe and mobility shift assay.

DISCUSSION

Exposure of mammalian cells to DNA-damaging agents elicits a specific genetic response known as the UV response, which has been postulated to protect the exposed cells against permanent damage to their DNA (18, 35). This genetic response is analogous to the SOS response in bacteria (45) and involves the induction of specific gene products, activation of endogenous viruses, and gene amplication (18, 35). While several of the bacterial genes that are induced during the SOS response are directly involved in the repair of damaged DNA, none of the mammalian UV- and damage-inducible genes identified thus far appears to play an obvious role in either repair of damaged DNA or protection against further DNA damage or mutagenesis. However, the existence of such a specialized genetic response to DNA damage suggests that it is likely to play a protective role. Up to now, most of the work on the mammalian UV response had concentrated on identification of the set of genes activated

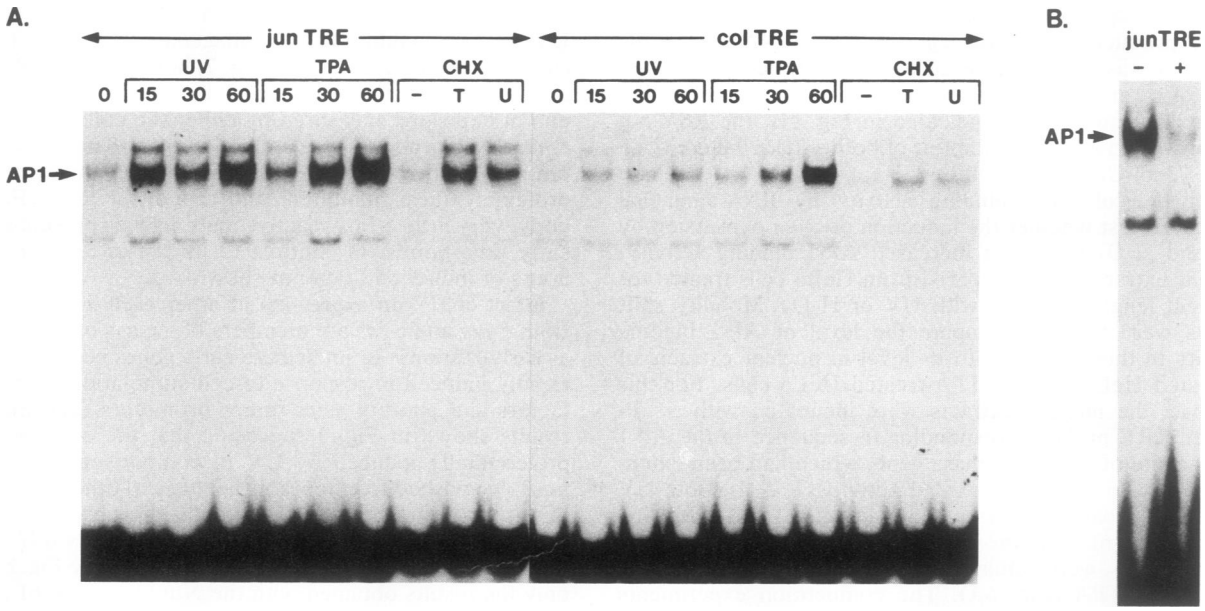

FIG. 7. Rapid induction of TRE binding activity. (A) HeLa cell cultures were exposed to 260-nm UV (40 J/m^2) or TPA (100 ng/ml) and at the indicated time points (in minutes) were harvested for preparation of nuclear extracts. Where indicated, the cells were pretreated with cycloheximide (CHX; 50 μg/ml) prior to exposure to UV (U) or TPA (T) for 30 min. Then 4-μg samples of each extract were incubated with 10,000 cpm of ^{32}P-labeled oligonucleotide probes corresponding to the TREs of the human collagenase and c-*jun* genes and analyzed by the mobility shift assay. (B) To examine the specificity of binding to the *jun* TRE, 10 μg of nuclear extract from UV-treated cells (60-min time point) was incubated with the labeled *jun* TRE probe in the absence (−) or presence (+) of a 10-fold molar excess of unlabeled *jun* TRE.

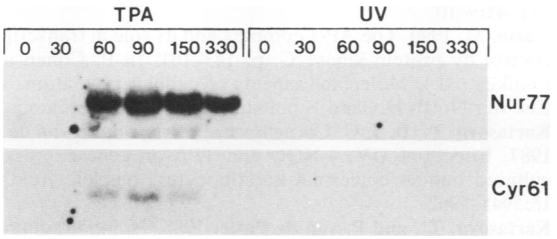

FIG. 8. Induction of other early-response genes. The blot shown in Fig. 1A was reprobed with cDNA probes specific for Nur77 and Cyr61.

during this response. While early work suggested that many of the genes induced by UV and other DNA-damaging agents are also activated by the phorbol ester TPA (5, 18, 22, 23, 27), more recent work led to identification of several other mammalian DDI genes which are not induced by TPA (13, 14). Thus, mammalian cells appear to contain at least two classes of DDI genes, one of which is activated by both phorbol esters and DNA damage and the other of which responds only to DNA damage. These two classes are analogous to the distinct but also partially overlapping bacterial regulons activated in response to different types of DNA damage (45). While DNA-damaging agents such as UV and H_2O_2 act rapidly, the induction of all of the mammalian DDI genes identified prior to this work, with the exception of c-*fos* (7), occurs with rather slow kinetics (5, 13, 14, 18, 22, 23, 27). In addition, none of these genes except c-*fos* appear to encode putative regulatory proteins that could be responsible for the activation of downstream genes. Since at least part of the mammalian DDI genes are also induced by phorbol esters by a mechanism that involves the action of transcription factor AP-1 (21), we wished to investigate whether AP-1 activity is also increased in response to DNA damage and if so start to analyze the mechanisms responsible for this increase.

We now describe that exposure to UV irradiation or H_2O_2, two different DNA-damaging agents, leads to very rapid induction of both c-*jun* and c-*fos* expression in HeLa cells. Interestingly, the c-*jun* gene is much more responsive to UV than c-*fos* or any of nine other early-response genes that were examined in this study. Not only do UV and H_2O_2 lead to increased expression of c-*jun* and c-*fos* transcripts, but they also elevate the level of AP-1 DNA binding activity in HeLa nuclear extracts. A similar increase in AP-1 binding activity in response to UV was previously described by Stein et al. (42), who also demonstrated increased transcription of one of the AP-1 target genes coding for collagenase. From the experiments described above, it appears that c-*jun* is the most rapidly and highly inducible DDI gene identified thus far. While this report was in preparation, it was reported that c-*jun* expression was also induced after exposure of HL60 cells to ionizing radiation, although in this case the response was slower than the one reported here (39).

The unique responsiveness of the c-*jun* gene to DNA-damaging agents appears to be mediated via its regulatory region. A *jun*-CAT reporter plasmid containing only 132 bp of the c-*jun* 5'-flanking sequence is preferentially induced by UV in comparison with TPA. The differential response displayed by this construct is very similar to that of the endogenous c-*jun* gene. Mutational inactivation of the TRE in the c-*jun* promoter abolished its induction by UV, as was previously reported for its TPA responsiveness (3). While the mechanism of signal transduction elicited by DNA damage is still unknown, it is biochemically distinct from the mechanism of action of phorbol esters. As expected, down-regulation of protein kinase C prevents the induction of c-*jun* and c-*fos* by TPA. However, this treatment does not prevent the induction of these genes by UV. In addition, the response to UV is of higher magnitude than the response to TPA. Further work is required to determine the basis for these differences, but one possibility is that UV leads to some additional posttranslational modifications of AP-1 not caused by TPA which convert it to a more efficient activator of the c-*jun* promoter. While the induction of c-*jun* by UV is mediated via the TRE, induction of c-*fos* by UV is mediated via the SRE, a *cis* element that is recognized by a different *trans*-acting factor (7). At this point, the promoters of the other early response genes surveyed in this study have not been analyzed. However, because they are only marginally induced by UV, these genes are unlikely to be regulated by AP-1.

Recent work indicates that TPA treatment leads to dephosphorylation of preexisting c-Jun homodimers, thereby increasing their DNA binding activity (6). At this point, the effect of UV on the phosphorylation state of c-Jun is not yet known, but the experiment shown in Fig. 7 indicates that UV treatment leads to a rapid increase in AP-1 binding activity in a manner that is independent of de novo protein synthesis. TPA was also shown to induce AP-1 binding activity by a mechanism independent of de novo protein synthesis (4) that is thought to be mediated by c-Jun dephosphorylation (6). During the first 60 min of UV or TPA induction, very little c-Fos protein is present within the AP-1 complex, in agreement with previous suggestions that the targets for the initial signal that leads to AP-1 induction in HeLa cells are c-Jun homodimers (3, 6).

Exposure of HeLa cells to either UV or TPA results in elevated AP-1 activity. Given the relative ratios of the c-*jun* and c-*fos* transcripts induced by these agents, it was conceivable that the AP-1 activity induced by TPA contains more c-Jun–c-Fos heterodimers than the activity induced by UV or H_2O_2. However, the use of Fos antibodies (Fig. 6) indicates that after several hours, the AP-1 complexes induced by TPA, UV, and H_2O_2 contain similar amounts of c-Fos protein. Therefore, it is unlikely that the AP-1 complexes induced by the different agents control different subsets of genes. In fact, at least two AP-1 target genes, collagenase and metallothionein II_A, are induced to similar extents by TPA and UV (5). Transcription factors that activate those genes which are exclusively induced by DNA damage remain to be identified.

Regardless of these open questions, this work identifies AP-1 as a likely regulator of gene activation during the mammalian UV response. The mechanism of AP-1 induction by DNA-damaging agents is different from the mechanism of its activation by phorbol esters. Since c-*jun* is so far the earliest identified nuclear target for the signal transduction pathway elicited by DNA damage, analysis of the mechanism responsible for its activation should shed more light on this unique signaling pathway.

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