ABSTRACT  Hybridization subtraction at low ratios of RNA to cDNA was used to enrich for the cDNA of transcripts increased in Chinese hamster cells after UV irradiation. Forty-nine different cDNA clones were isolated. Most coded for nonabundant transcripts rapidly induced 2- to 10-fold after UV irradiation. Only 2 of the 20 cDNA clones sequenced matched known sequences (metallothionein I and II). The predicted amino acid sequence of one cDNA had two localized areas of homology with the rat hexasl-defestabilizing protein. These areas of homology were at the two DNA-binding sites of this nuclear acid single-strand-binding protein. The induced transcripts were separated into two general classes. Class I transcripts were induced by UV radiation and not by the alkylating agent methyl methanesulfonate. Class II transcripts were induced by UV radiation and by methyl methanesulfonate. Many class II transcripts were induced also by H2O2 and various alkylating agents but not by heat shock, phorbol 12-tetradecanoate 13-acetate, or DNA-damaging agents which do not produce high levels of base damage. Since many of the cDNA clones coded for transcripts which were induced rapidly and only by certain types of DNA-damaging agents, their induction is likely a specific response to such damage rather than a general response to cell injury.

In Escherichia coli, DNA damage caused by UV radiation or chemical agents induces approximately 20 genes of the SOS regulon; many of these genes code for proteins involved in DNA repair, mutagenesis, and recombination (1). Damage by alkylating agents or oxidative stress induces other well-characterized regulons in bacteria (1). In yeast, induction by UV radiation of the RAD2 excision-repair gene is similar to induction of the SOS response in bacteria: the transcript is of low abundance, and its induction is rapid and only 2- to 10-fold (2). The RAD2 gene is likely only one of many yeast DNA-damage-inducible (DDI) genes; Ruby and Szostak (3) have estimated there may be up to 80 such genes. In mammalian cells only a few DDI genes (4-6) have been identified; the cDNA clones of these genes were isolated by differential screening, which is effective for detecting the cDNA of only abundant transcripts [abundance > 0.1% of poly(A)+ RNA] (ref. 7, p. 226). These mammalian genes differed also from the RAD2 and SOS genes in that the former were induced slowly.

To determine if low-abundance DDI transcripts occur in mammalian cells, an approach is required to enrich for, and isolate, the cDNA of such transcripts. Standard hybridization subtraction, which employs a high ratio of RNA to cDNA, is an effective approach to enrich for the cDNA of low-abundance transcripts that are expressed at much higher levels in one cell type than in another (ref. 7, p. 228). We have previously shown that hybridization subtraction using low ratios of RNA to cDNA enriches for the cDNA of transcripts increased only a fewfold over control levels (8): when cDNA synthesized from the RNA of heat-shocked cells was hybridized with an equal amount of RNA from untreated cells, the cDNA of a transcript induced only 3-fold was shown to be enriched more than 30-fold. Such low-ratio hybridization subtraction (LRHS) should theoretically increase the sensitivity of screening cDNA clones compared to differential screening by at least 30-fold, making it possible to detect cDNA of transcripts as low as 0.003% abundance (<10 transcripts per cell). We have now used LRHS to enrich for the cDNA of transcripts induced in mammalian cells by UV radiation, and we have isolated numerous cDNA clones coding for nonabundant transcripts rapidly induced only 2- to 10-fold.

MATERIALS AND METHODS

Cell Treatment. Exponentially growing Chinese hamster cell lines, CHO-K1 and V79, and human fibroblast strains, AG1522 (Coriell Institute for Medical Research) and XP12BE (American Type Culture Collection), were maintained and irradiated with 254-nm UV radiation or treated with chemical agents as previously described (8). For cell survival determinations (8), CHO cells were treated in an identical manner as for the RNA studies (see below) and then replated at low density for colony survival immediately after treatment.

LRHS. The hybridization subtraction procedure (8) is outlined in Fig. 1. cDNA was synthesized from poly(A)+ RNA of cells incubated for only 4 hr after UV irradiation to minimize both cell cycle effects and the long-term effects of a toxic dose of UV radiation [with a different damaging agent, heat shock, it was our experience that the level of induced transcripts was maximal by 4 hr (8)]. The cDNA was hybridized to a high Rp of an equal amount of poly(A)+ RNA from unirradiated cells. The cDNA, which remained single-stranded after the hybridization with the RNA of unirradiated cells, was then hybridized to the original poly(A)+ RNA isolated from the irradiated cells. The duplex cDNA-RNA was separated by hydroxylapatite chromatography from the remaining single-stranded cDNA, which contained A+T-rich cDNA and other nonhybridizing species (8).

cDNA Cloning. The single-stranded cDNA was used as template to synthesize second strand cDNA with random primers and DNA polymerase I: 100 ng of first-strand cDNA was incubated in 0.1 ml of 50 mM Hepes (pH 6.6)/5 mM Tris (pH 7.6)/6 mM MgCl2/5 mM ammonium acetate/100 mM KCl/0.1 mM dithiothreitol/deoxynucleotides at 40 µM each/ random 6-mer (P-L Biochemicals) at 250 µg/ml/bovine serum albumin at 50 µg/ml/15 units of DNA polymerase for 30 min at 15°C and then for 15 min at 22°C. Thereafter, 100

Abbreviations: DDI, DNA-damage-inducible; LRHS, low-ratio hybridization subtraction; MMS, methyl methanesulfonate; XP, xero-derma pigmentosum; AAAF, N-acetoxy-2-acetylaminofluorine; hnrNP, heterogeneous nuclear ribonucleoprotein; Gv, gray.

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UV-irradiate CHO cells with 14 J/m² and incubate for 4 hr

Isolate polyA RNA

80 µg

MMLV reverse transcriptase

50 µg cDNA

Hybridize to uncorrected blot of RNA with 80 µg polyA RNA from unirradiated cells

Hydroxylapatite chromatography

Duplex cDNA-RNA (90%)

Single-stranded cDNA (9.8%)

Hybridize to uncorrected blot of RNA with 80 µg polyA RNA from irradiated cells

Hydroxylapatite chromatography

Single-stranded cDNA (6.3%)

Duplex cDNA-RNA (3.5%)

Removal of RNA with NaOH treatment
cDNA enriched 29-fold

FIG. 1. LRHS scheme. MMLV, Moloney murine leukemia virus.

mM Tris-HCl (pH 7.6) was added with an additional 15 units of enzyme, and the incubation was continued for an additional 45 min at 25°C. With this protocol, approximately 60 ng of second-strand cDNA was synthesized. Double-stranded cDNA was size-fractionated with Sepharose CL-4B and inserted into a derivative of the plasmid vector pXF3 (9) by G-C-tailing (ref. 7, pp. 239–242), with a transformation efficiency of 10¹¹–10¹⁰ colonies per ng of cDNA (9); the average insert size was 420 base pairs (bp). cDNA colonies were replicate-plated and amplified with chloramphenicol prior to screening with labeled cDNA probes (ref. 7, p. 312). cDNA clones were sequenced as described elsewhere (10). Possible homology of our sequences with other published sequences was determined by using the seqlp program of Kanehisa et al. (11) (at a homology score of -15).

RNA Isolation and Analysis. Cells were lysed in situ with guanidine thiocyanate for RNA isolation, and poly(A)⁺ RNA was bound to nylon filters for dot blots or was size-separated in denaturing gels prior to transfer, as previously described (8). cDNA that had been excised from its plasmid vector was labeled (12) and used at 3–12 × 10⁶ dpm·ml⁻¹. Hybridization conditions were the same as previously described (8), except for human RNA, for which the hybridization was at 63°C and the final, most stringent, wash was at 63°C in 240 mM Na⁺. Relative hybridization was quantified as described previously (8), and a 50% increase in RNA relative abundance could consistently be distinguished from control.

RESULTS

Isolation of cDNA Clones Coding for UV-Radiation-Induced Transcripts. As outlined in Fig. 1 for CHO cells, theoretical enrichment was calculated to be 29-fold on the basis of the result that 3.5% of the starting cDNA remained after LRHS. This enriched cDNA was used to construct a cDNA library. Three thousand colonies were replicate-plated and hybridized with labeled (12) LRHS-enriched cDNA or cDNA not enriched by LRHS; 424 colonies preferentially hybridized with the enriched cDNA. To determine how many of these cDNA clones coded for transcripts which were induced—i.e., increased in abundance in UV-irradiated cells—dot blots of the poly(A)⁺ RNA used in LRHS from irradiated and unirradiated cells were hybridized with labeled individual cDNA clones. Eighty-nine (61%) of the 145 clones tested coded for transcripts induced in UV-irradiated CHO cells. These clones have been designated DDIA (DDI library A). A similar LRHS approach, using avian myeloblastosis virus reverse transcriptase in place of Moloney murine leukemia virus reverse transcriptase, was undertaken with Chinese hamster V79 lung fibroblasts; 45 (70%) of the 64 clones tested coded for transcripts more abundant in UV-irradiated cells and have been designated DDIU (DDI library U). (The DDI prefix has been deleted below for brevity.)

The 89 CHO cDNA clones which coded for UV-radiation-induced transcripts were divided into 37 groups which did not cross-hybridize with each other by colony hybridization. Members of the same group had identical Northern-blot patterns (data not shown). Most of these 37 groups coded for transcripts of different-size RNA [0.4 to >4 kilobases (kb)], indicating they were derived from different transcripts. Representative cDNA clones are shown in Fig. 2. Most transcripts were induced 2- to 6-fold, as determined by RNA dot blots or by densitometry measurements of Northern blots. Equal amounts of poly(A)⁺ RNA from unirradiated and irradiated cells were used; the abundance of β-actin RNA, which is not UV inducible, was not significantly different. Hybridization with the β-actin probe was much greater than with the DDIA clones in Fig. 2 at the same autoradiography exposure times. As a crude measure of RNA abundance, the relative hybridization of DDI clones was compared to the hybridization of a probe of similar length which coded for B2 RNA, which is of 1–5% abundance in poly(A)⁺ RNA (8). Hybridization with the B2 probe was 10⁻⁶⁻¹⁰⁶ times greater. These results indicate that most of our clones code for nonabundant transcripts.

Eight of the 15 CHO cDNA clones tested hybridized with human RNA. An example with A45 is shown in Fig. 2B; this CHO cDNA clone hybridized with a 1-kb transcript from cells of a normal individual and an excision-deficient XP patient. This transcript was strongly induced in both the normal cells and XP cells after 14 J/m². With the lower dose (lane d), which induced cytotoxicity in XP cells similar to the higher dose in normal cells, there was a smaller increase. These results demonstrate that both the sequence of A45 RNA and its inducibility by UV radiation have been conserved in human cells.

The V79 cDNA clones were divided into 12 different groups that did not cross-hybridize with each other or the CHO clones. The results with the V79 cell library were limited, since many were found to code for metallothionein (see below), and only the cDNAs of short transcripts were isolated by LRHS when avian myeloblastosis virus reverse

FIG. 2. Northern blots of RNA from unirradiated and UV-irradiated CHO cells (A) and human fibroblasts (B). In A, equal amounts of poly(A)⁺ RNA (from the same preparation used in the hybridization subtraction of Fig. 1) from untreated (left lane of pair) and irradiated (right lane) CHO cells were size-separated and hybridized with labeled cDNA probes (DDIA prefix has been omitted). In B, poly(A)⁺ RNA from normal human fibroblasts (lanes a and b) or xeroderma pigmentosum (XP) fibroblasts (XP12BE) (lanes c, d, and e) were analyzed. Cells were incubated for 4 hr after 14 J/m² (lanes b and e) or 1 J/m² (lane d). Size markers, designated M, consisted of labeled single-stranded DNA (8).
transcriptase was used. In the case of the CHO library, metallothionein clones were not isolated, for these genes are not expressed in CHO cells (13).

**Sequence Analysis of DDI cDNA Clones.** Twenty cDNA clones were sequenced, A7, A9, A13, A18, A20, A29, A31, A33, A45, A50, A77, A88, A94, A99, A153, U1, U2, U4, and U5. All 20 sequences were compared at low homology scores to all sequences in the GenBank data base on Jan. 15, 1988. U1 and U2 corresponded to the published sequences for metallothionein II and I (14). None of the other cDNA clones corresponded to known sequences. The predicted amino acid sequence of A18 had two short areas of high homology with the rat helix-stabilizing protein, A1 heterogeneous nuclear ribonucleoprotein (hnRNP) (15) (Fig. 3). This protein shared with other RNA-binding proteins the RNP consensus sequence (Fig. 3), which was also present in the predicted amino acid of A18. Recently, Merrill et al. (16) found that Phe-58 in this consensus sequence and also Phe-16 were at or near the protein’s major binding sites for single-stranded DNA. These phenylalanine residues were in the only two areas of homology of this protein to A18.

**Dose Response and Kinetics of Induction.** Fig. 4 shows the dose response and time course for induction (measured by relative abundance in irradiated samples compared to untreated samples prepared on the same day). The dose-response experiments were done on a different day than the time-course experiments. When the abundance of a particular transcript in poly(A)* RNA from unirradiated samples prepared on different days was compared, variability was less than 2-fold (data not shown). No induction of β-actin was found at any dose or at any time. To permit a ready comparison of the variability in induction of a given transcript in experiments conducted on different days, samples treated with the same dose (14 J m⁻²) and postirradiation incubation time (4 hr) are represented by solid symbols; variability between experiments was less than 2-fold. For the dose-response experiments, maximal induction was usually at less than the highest dose; e.g., maximal induction with A9 was at 14 J m⁻². Maximal induction with A18 was achieved at the lowest dose, 7 J m⁻², with no further increase at higher doses; at 7 J m⁻² cell survival was >50%. In the time-course experiments with 17 different CHO DDI probes (A8, A9, A15, A18, A26, A29, A31, A45, A77, A94, A99, A109, A115, A143, A148, A153), the patterns of induction varied, but induction was usually rapid and frequently maximal within 2 hr of irradiation. Induction was slow after UV irradiation only for A15. When transcription was blocked with actinomycin D (Δ) no induction was seen.

**Induction by Various Agents.** Results are summarized in Table 1. The 254-nm UV radiation and the AAAF have been grouped together, since DNA damage by both these agents is repaired by nucleotide-excision repair (17). The dose of UV radiation used (14 J m⁻²) reduced survival to 4–10% of that of untreated cells, while the dose of AAAF used (20 μM) would be expected to reduce cell survival to 1–10% of untreated cells (18). Both MMS and H₂O₂ produce predominantly nonbulky base damage in DNA which is repaired by base-excision repair mechanisms (17). In contrast to induction by UV radiation, maximal induction by MMS of DDI transcripts was found at a dose (100 μg/ml) which reduced cell survival to 0.003%; no increase in induction was found with a higher dose (data not shown). The dose of H₂O₂ used (400 μM) reduced cell survival to 1%. The heat-shock treatment (45.5°C for 9 min) produced maximal induction of HSP70 RNA (see Table 1) and other heat-shock-induced RNA (8).

The results indicated that there were two major classes of induced transcripts. Class I transcripts were induced (i.e., increased by ≥2.0-fold) by UV radiation, but not by MMS. They were not induced by H₂O₂ or heat shock. Induction by AAAF was determined for three class I transcripts, and all were found to be induced. For example, after irradiation, A18 RNA increased 3.1-fold over the unirradiated sample, it increased 3.0-fold after treatment with AAAF, while its relative abundance in MMS-treated cells was only 1.1. Class II transcripts were induced by both UV radiation and MMS but, with the exception of A15, not by heat shock. Many of the class IIa transcripts were induced also by AAAF.

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**Fig. 3.** Comparison of DDIA18 protein with a single-stranded nucleic acid-binding protein. Amino acid residues are symbolized by the standard single-letter code. The predicted amino acid sequence of the partial length cDNA clone, DDIA18, and rat helix-stabilizing protein (A1 hnRNP) (15) with the RNP consensus sequences (15, 16) underlined are shown. The first 20 residues of DDIA18 have no homology and are omitted. Colonics indicate identity and asterisks indicate DNA-binding sites (16).
DISCUSSION

In this investigation LRHS was found to be an effective approach to enrich for, and isolate, the cDNA of UV-radiation-induced transcripts. While only a third of the UV-radiation-inducible candidates were examined from the CHO library, 37 different cDNA clones were isolated. By screening the cDNA library with cDNA enriched by LRHS, clones coding for nonabundant transcripts were identified which would probably not have been identified by standard differential screening. Another advantage of LRHS over differential screening was that our cDNA libraries were constructed with enriched cDNA, so that screening only 3 x 10^3 colonies for the cDNA of induced transcripts would be equivalent to screening nearly 10^5 colonies from a library made from cDNA without LRHS.

Transcripts of many of the cDNA clones isolated by LRHS had several similarities with the RNA of yeast and bacterial DD1 genes: low abundance, rapid induction, and induction of 2- to 10-fold. Induction was blocked by actinomycin D, which indicates that the increase in RNA after UV irradiation was due to increased transcription of UV-radiation-inducible genes. A possible alternative explanation for the increase in the relative abundance of the RNA of the DD1 genes might be the preferential repair of these genes compared to those coding for the bulk of poly(A)^+ RNA (19). If the damage in certain preferentially repaired genes is repaired more rapidly (permitting the recovery of normal transcription rates), then the relative abundance of these transcripts would increase, since the transcription of other genes would remain depressed. This explanation is unlikely because induction was seen in excision-deficient CHO cells (data not shown) and XP cells (e.g., A45 in Fig. 2), which do not repair UV-radiation-type damage. Preferential repair is an unlikely explanation also because substantial induction of many of our sequences was seen within 2 hr of irradiation; to see a relative increase in abundance due to preferential repair of a gene would require that a substantial fraction of the total poly(A)^+ RNA present before irradiation had turned over within 2 hr. This was not the case in any of our experiments: the yield of poly(A)^+ RNA per 10^5 unirradiated cells was similar to that in 10^5 irradiated cells in all the samples of Fig. 4 whose RNA was isolated within 4 hr of irradiation at ≤14 J m^{-2}, including the 2-hr sample treated with actinomycin D. Therefore, the turnover of poly(A)^+ RNA in irradiated cells was too slow to account for any appreciable relative increase in specific transcripts caused by preferential repair.

Induction of most of our transcripts differed in several respects from that of previously described UV-radiation-induced transcripts in mammalian cells. In human cells RNA of several genes, such as those for collagenase (4), metallothionein (4), and plasminogen activator (5), were induced by UV radiation or phorbol 12-tetradecanoate 13-acetate. These human transcripts, in contrast to ours, were induced slowly and had optimal induction at doses (25-50 J m^{-2}) that were higher than those used in our study. Induction was mediated by a secreted protein factor detected in the conditioned medium of treated cells (4, 5); none of our DD1 transcripts tested (A7, A8, A9, A15, A18, A29, A31, A33, A34, A45, A77, A94, A99, A109, A115, A148, and A153) except A153. In a dose range of 20-2000 ng ml^{-1}, a 4-hr exposure to phorbol 12-tetradecanoate 13-acetate produced no induction except for A31 and A99, which were induced 2- to 3-fold; when the phorbol ester exposure at 20 ng ml^{-1} was increased to 8 hr [the minimum time used by Angel et al. (4)], no induction was observed except for A31 and A99 (data not shown). As in Fig. 4, the level of β-actin RNA did not increase in the treated samples.

In Table 1, the induction of DD1 transcripts by various agents was examined. The UV radiation (254 nm) dose was 14 J m^{-2}. Treatment time was 4 hr with 20 µM N-acetoxy-2-acetylaminofluorene (AAAF), 4 hr with 100 µg ml^{-1} methyl methanesulfonate (MMS), and 1 hr with 400 µM H2O2. For heat shock, cells were heated to 45.5°C for 9 min and then returned to 37°C. CHO cells were harvested 4 hr after the start of treatment; relative abundance of transcripts was determined by RNA dot-blot hybridization: <, <2-fold increase in a particular RNA compared to untreated cells isolated at the same time; +, 2- to 10-fold increase; ++, 10- to 20-fold increase; three increases are given more precisely.

Table 1. Induction of DD1 transcripts by various agents

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The UV radiation (254 nm) dose was 14 J m^{-2}. Treatment time was 4 hr with 20 µM N-acetoxy-2-acetylaminofluorene (AAAF), 4 hr with 100 µg ml^{-1} methyl methanesulfonate (MMS), and 1 hr with 400 µM H2O2. For heat shock, cells were heated to 45.5°C for 9 min and then returned to 37°C. CHO cells were harvested 4 hr after the start of treatment; relative abundance of transcripts was determined by RNA dot-blot hybridization: <, <2-fold increase in a particular RNA compared to untreated cells isolated at the same time; +, 2- to 10-fold increase; ++, 10- to 20-fold increase; three increases are given more precisely.

and H2O2. Class Ib transcripts were separated from class Ila on the basis of a higher induction by MMS (19- to 35-fold). Induction by other base-damaging agents such as N-methyl- N-nitro-N-nitrosoguanidine or nitrogen mustard was found for many class II transcripts but for none of class I (data not shown). X-rays (5 or 40 grays (Gy)), bleomycin (50 µg ml^{-1}), and adriamycin (0.4 µg ml^{-1}) were ineffective inducing agents for most of the class II members and all of the class I members tested (data not shown). Transcript levels were also measured in nonirradiated plateau-phase cells which were put in clonal phase by medium depletion (2 days at high density without fresh medium); compared to growing cells, no increase was observed for any tested (A8, A18, A29, A77, A94, A99, A109, A115, A148, and A153) except A153. In a dose range of 20-2000 ng ml^{-1}, a 4-hr exposure to phorbol 12-tetradecanoate 13-acetate produced no induction except for A31 and A99, which were induced 2- to 3-fold; when the phorbol ester exposure at 20 ng ml^{-1} was increased to 8 hr [the minimum time used by Angel et al. (4)], no induction was observed except for A31 and A99 (data not shown). As in Fig. 4, the level of β-actin RNA did not increase in the treated samples.

The cellular functions for many of the DD1 sequences are unknown, but a role for the A18 protein in nucleic acid metabolism is likely, on the basis of its homology with rat helix-distabilizing protein, A1 hnRNP. A1 hnRNP was isolated on the basis of binding to single-stranded DNA or RNA with much higher affinity than binding to double-stranded DNA; it depresses the melting temperature of double-stranded DNA and stimulates the activity of purified DNA polymerase α (20). The protein sequences of A18 and
A1 hnRNP were dissimilar, except for the two regions of the protein which have recently been reported to bind to single-stranded DNA (16). A1 hnRNP CDNA did not hybridize to A18 size RNA but hybridized to a more abundant transcript which was not induced by UV radiation or a variety of other DNA-damaging agents (data not shown). Whether A18 protein binds to DNA or RNA in the cell remains to be determined, but, considering its induction only by UV-mimetic DNA-damaging agents, a role in DNA metabolism is possible.

The results in Table 1 can be used to address the question of the specificity of the inducing signal. For example, is induction related to changes in viability or cell cycle, or is it a general response to cell injury? Induction was not caused simply by cell killing; e.g., 40 Gy of x-rays essentially killed all the cells but was an ineffective inducing agent. In addition, maximal induction by UV radiation (Fig. 4) often occurred at the lower rather than at the higher, more toxic, doses. Heat shock, which may damage cells by thermal denaturation of proteins, induced only one (A15) of the many DDI transcripts (Table 1). Both heat shock and the DNA-damaging agents delay progression through the cell cycle, but only DNA-damaging agents induced our DDI transcripts, and no increase in DDI transcripts occurred in unirradiated G0 cells, with one exception (A153). These results indicate that the induction of most of our DDI transcripts is not a general response to cellular injury or cell cycle perturbation.

Many of the DDI clones coded for transcripts which were induced by only certain types of DNA-damaging agents. Class I transcripts were induced by UV radiation and AAAF but not by the other DNA-damaging agents tested (Table 1). UV radiation, which produces predominantly pyrimidine-dimer base damage, and AAAF, which produces bulky base adducts, are similar in that their DNA damage results in substantial helical distortion and is repaired by the same nucleotide-excision repair pathway. It is therefore possible that class I DDI transcripts are induced by the helical distortion or by a step or steps in the nucleotide-excision repair pathway.

Class II transcripts were induced by both UV radiation and MMS (Table 1). In addition, many of them were induced also by AAAF, H2O2, N-methyl-N'-nitrosoguanidine, and other alkylating agents. All of these agents produce high levels of base damage in DNA (e.g., pyrimidine dimers by UV radiation, adducts formed with AAAF, or methylated bases by MMS). While chemical base-damaging agents produce substantial damage to cellular constituents in addition to DNA, the UV radiation used has very little effect on non-DNA targets. Thus, it is possible that the signal for induction of most class II transcripts results from the base damage itself. The fact that class II transcripts were not induced by x-rays supports this possibility; even at the highest dose used (40 Gy), the frequency (21) of x-ray-induced base damaged would be <10% of that of agents which induced the transcripts. However, because the one transcript, A15, was induced also by heat shock and because induction of A15 was not rapid after UV irradiation, its induction may represent a general response to stress rather than a specific response to DNA damage.

Our work demonstrates that there is induction of numerous transcripts in the response of mammalian cells to DNA-damaging agents. Of particular interest is the finding that the class II DDI transcripts were induced not only by our 254-nm UV radiation and by AAAF, whose DNA damage is repaired by the nucleotide-excision repair pathway, but also by other base-damaging agents (e.g., MMS and H2O2), whose damage is repaired by the base-excision repair pathways. This response may have functional significance in nature; while the radiation source used in our study was a germicidal lamp emitting predominantly 254-nm light, the UV radiation in sunlight that reaches the surface of the earth consists of the UV B (280–320 nm) and UV A (320–400 nm) spectra. This sunlight causes not only pyrimidine dimers but also types of DNA damage that are repaired by base-excision mechanisms. For example, some of the damage in sunlight has been shown to be mediated by free radicals and peroxides (discussed in ref. 22). Thus, the induction of our class II DDI transcripts may represent a cellular response to some of the DNA damage produced by sunlight.

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