

DNA-Binding Protein Activated by Gamma Radiation in Human Cells

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DNA damage-inducible responses in mammalian cells tend to lack specificity and can be activated by any one of a number of damaging agents. Although a number of different induced proteins have been described, their involvement in DNA processing and transcriptional control remains unresolved. We describe the appearance of a previously unreported, specific DNA-binding protein in nuclei from human cells exposed to ionizing radiation, which was not detected in nuclear extracts from unperturbed cells. The distal part of the simian virus 40 enhancer (without the AP-1 site) and oligonucleotide sequences derived from that sequence were used in binding studies. The appearance of this activity was dose dependent and transient, reaching a maximum at 1 h postirradiation and disappearing from nuclei by 9 h. This protein was induced in cells by a mechanism not requiring de novo protein synthesis, and the response was specific for ionizing radiation and radiomimetic agents; neither UV nor heat shock invoked a response. The DNA-binding protein was present in the cytoplasm of untreated cells, apparently being translocated to the nucleus only after radiation exposure. Southwestern (DNA-protein) analysis demonstrated that the nuclear and cytoplasmic proteins were approximately the same size, 43,000 daltons. The protected DNA-binding motif, using the distal fragment of the simian virus 40 enhancer as the substrate, was shown by DNase I footprint analysis to be ^{PTGTCAGTTAGGGT}ACAGTCAATCCCA_p. This was confirmed by dimethyl sulfate footprinting.

Exposure of mammalian cells to a variety of stress-causing agents gives rise to preferential transcription of certain genes (20) and activation of existing proteins (16, 37). This is best illustrated in the response of cells to elevated temperatures at which three major groups of heat shock proteins are synthesized to confer thermotolerance on the cells and protection against other forms of damage (29). A promoter element that acts as an inducible enhancer in mammalian cells has been demonstrated to be responsible for induction of heat shock genes (5, 22, 25, 36). A factor that is significantly increased after heat shock binds to this regulatory element and appears to be responsible for induction of proteins involved in the heat shock response (16). It seems likely that activation of a preexisting regulatory factor is involved, since cycloheximide does not interfere with its induction.

In addition to the heat shock response, other inducible pathways have been described by which mammalian cells respond to DNA-damaging agents (11, 12, 19, 23, 28). Induction of eight abundant proteins by UV, mitomycin C, or the tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA) has been found in human fibroblasts (30). Several genes, including cellular proto-oncogenes and collagenase, stromelysin, and metallothionein IIa genes, are induced by these agents (3). The promoter regions of these genes share a conserved sequence which is recognized by the transactivating factor AP-1 (2). The induction response and the increase in AP-1 activity are resistant to cycloheximide, suggesting a posttranslational modification mechanism. A recent report by Glazer et al. (10) described the induction of several DNA-binding proteins after exposure of HeLa cells to UV light. Metabolic inhibitors (dactinomycin and cycloheximide) failed to inhibit the response, revealing that de

novo protein synthesis was not required, but, rather, that an activation mechanism was needed.

Exposure of human lymphocytes to very low doses of ionizing radiation makes them less susceptible to genetic damage induced by subsequent exposure to higher doses of X rays (35). This adaptive response was accompanied by the expression of a number of proteins. Boothman et al. (6), using two-dimensional electrophoresis, demonstrated that X-irradiation of human cells induced the coordinate expression of a specific set of proteins (XIPs). On the basis of the kinetics of appearance of these proteins, it was suggested that they play a role in the restoration of chromosomal damage during potentially lethal damage repair. However, it was not possible to ascribe a function to any of these induced proteins, since they were identified only as spots on an electrophoresis gel. To investigate the response of human cells to ionizing radiation, we have attempted to identify DNA-binding proteins implicated in control of transcription or in the processing of DNA after radiation damage. We report here the presence of a specific DNA-binding protein in human cells activated by ionizing radiation.

MATERIALS AND METHODS

Cell culture. Epstein-Barr virus-transformed lymphoblastoid cells C3ABR and C5ABR were used in this study. The cells were maintained in RPMI 1640 medium containing 10% fetal calf serum at 37°C in a humidified atmosphere of 95% air and 5% CO₂.

Irradiation and other treatments. Cells were exposed to gamma rays by using a ¹³⁷Cs source (Atomic Energy of Canada) at a dose rate of 4 Gy/min. Experiments investigating the effect of increasing the dose, as well as time course experiments, were carried out. For UV irradiation, cells were centrifuged and suspended at 3 × 10⁶/ml in phosphate-buffered isotonic saline and irradiated with UV (254-nm) light at a dose rate of 5 J/m². Mock-irradiated cells were washed in phosphate-buffered saline. After irradiation, cells

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were immediately centrifuged and suspended in fresh medium containing 10% fetal calf serum and incubated at 37°C for the appropriate time. Cells were also exposed to streptonigrin (5 ng/ml) for 1 h prior to preparation of extracts. For experiments involving heat shock, cells were incubated at 42°C for 30 min and subsequently at 37°C for either 30 min or 1 h. To determine whether transcriptional induction or activation of preexisting protein was taking place, unirradiated and gamma-irradiated cells were incubated with either cycloheximide (50 µg/ml) or 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRFB; 125 µM), which was added to cells 10 min before irradiation, and incubation was carried out for 1 h prior to protein extraction.

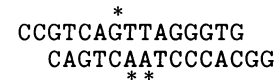
Preparation of extracts. Nuclear extracts were prepared from lymphoblastoid cells essentially by the method of Dignam et al. (9). Cells were centrifuged in Sorvall GSA tubes, and pellets were suspended in 1.5 ml of ice-cold buffer A [10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES; pH 8.0), 50 mM NaCl, 0.5 M sucrose, 1 mM EDTA, 0.25 mM ethylene glycol-bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA), 0.6 mM spermidine, 0.5% (vol/vol) Triton X-100, 1 mM phenylmethylsulfonyl fluoride, 7 mM 2-mercaptoethanol] and subjected to Dounce homogenization (10 to 15 strokes until 80% lysed). The suspension was then transferred to bench centrifuge tubes, and nuclei were recovered by centrifugation at 1,000 × *g* for 10 min. Nuclear pellets were washed twice in buffer A, suspended in buffer A containing 400 mM NaCl and 25% glycerol (buffer B), and stirred for 30 min. Nuclear debris were discarded after centrifugation at 2000 × *g* for 10 min. The supernatant was recovered after further centrifugation in a microcentrifuge for 15 min, adjusted to 55% with (NH₄)₂SO₄, and centrifuged at 12,000 × *g* for 15 min. The pellet was suspended in a minimum volume of buffer B, and dialysis was carried out with the same buffer.

Cytoplasmic extracts were prepared by collecting the supernatant from nuclear pellets prepared from cells homogenized in buffer A containing 0.5% (vol/vol) Triton X-100. In this case also a 55% (NH₄)₂SO₄ cut was carried out, followed by dialysis against buffer B. In some experiments cytosolic fractions were prepared from cells suspended in hypotonic solution and homogenized. Nuclear and cytoplasmic samples were stored at -20°C.

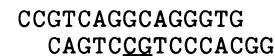
DNA-protein binding. DNA-binding studies were carried out with the 72-base-pair (bp) distal enhancer element (without the AP-1 site) of pSV2CAT. This fragment, subcloned into pUC19, was released by cleavage with *Hind*III and *Sma*I and end labeled with [α-³²P]dCTP and Klenow polymerase. Free fragment was separated from plasmid and recovered on 5% polyacrylamide gels. Incubations contained 1 ng of ³²P-labeled DNA fragment, 200 ng to 2 µg of nuclear or cytoplasmic extract, 3 µg of poly(dI-dC) · poly(dI-dC), 10 mM Tris hydrochloride (pH 7.5), 50 mM NaCl, and 1 mM EDTA. All incubations were carried out at 22°C for 15 min. Gel mobility shift experiments were carried out with 5% polyacrylamide gels in TBE (50 mM Tris hydrochloride [pH 8.3], 50 mM borate, 1 mM EDTA) to determine the extent of DNA-protein binding. A 365-bp *Hind*III-*Hin*FI fragment of the κ immunoglobulin chain gene, termed the matrix association region, was used as a non-specific binding sequence. The matrix association region fragment was isolated from a recombinant plasmid, pG19/45, containing a 2.85-kilobase κ gene fragment (7). Other sequences used in binding studies included a 515-bp *Hin*FI fragment of the κ enhancer also subcloned from pG19/45 (7) and a 300-bp *Pst*I-*Eco*RI fragment representing the heavy-chain mouse

immunoglobulin enhancer. DNA-protein binding was quantitated by densitometry on a Photo-Digitizer program (Jandel Instruments).

Oligonucleotide binding. Three double-stranded oligonucleotides were used for binding studies. The first oligonucleotide (oligo 1),



was synthesized from the sequence of the DNase I footprint (see Fig. 7). Overhanging nucleotides (GG and CC) were included at the 5' ends to facilitate end labeling and ligation. The asterisks denote the nucleotides strongly protected in dimethyl sulfate (DMS) protection assays. Two other oligonucleotides, with altered nucleotides in the region where protection against methylation was observed, were synthesized for binding studies: oligo 2



and oligo 3



The two bases that were changed in these oligonucleotides are underlined. Binding was carried out as described above in the presence of 2 µg of extract, 0.1 ng of end-labeled oligonucleotide, and 1 µg of poly(dI-dC) · poly(dI-dC).

Footprint analysis. DNase I footprint (31) and DMS protection (1) analyses were used to determine the binding sequence. Binding reactions were as described above, except for a fivefold scale up with respect to extract, and the reaction volume was 25 µl. DNase I (10 U per incubation) digestion was carried out at 0°C for 30 s, and for DMS protection 1 µl of DMS was added to incubations for 2 min. Bound and free fragments were then electrophoretically separated on 5% polyacrylamide gels. Fragments were eluted overnight in 0.5 M ammonium acetate, extracted with phenol-chloroform-isoamyl alcohol, and precipitated with ethanol. Methylated DNA was then treated with 1 M piperidine at 90°C for 30 min and lyophilized twice with 50 µl of H₂O. DNase I- and DMS-treated samples were then resolved on 8% DNA sequencing gels (21).

Southwestern blotting. Southwestern (DNA-protein) blotting was performed by the method of Wendler and Grossbach (34) and Singh et al. (31). Up to 5 µg of partially purified extract from unirradiated and irradiated cells, prepared as described above, was electrophoresed on a sodium dodecyl sulfate (SDS)-8% polyacrylamide gel by the method of Laemmli (17). The separation gel was then soaked in 25 mM Tris-190 mM glycine (pH 8.3)-20% methanol for 30 min. Proteins were electrophoretically transferred onto a nitrocellulose membrane by using the same buffer with 0.1% SDS added (32). After transfer overnight at 4°C, the filters were blocked in Blotto (5% nonfat milk, 50 mM Tris [pH 7.5], 50 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol) with the protein surface turned up for 60 min. Filters were washed three times in 50 ml of TNE50 (10 mM Tris [pH 7.5], 50 mM NaCl, 1 mM EDTA) and sealed in bags. DNA binding was then carried out with TNE buffer, 10 µg of poly(dI-dC) · poly(dI-dC) per ml, and 10⁶ cpm of end-labeled oligonucleotide per ml. Concatamers of the oligonucleotide (phosphorylated with polynucleotide kinase) were prepared by self-ligation in 50 mM Tris (pH 7.4)-10 mM MgCl₂-20 mM

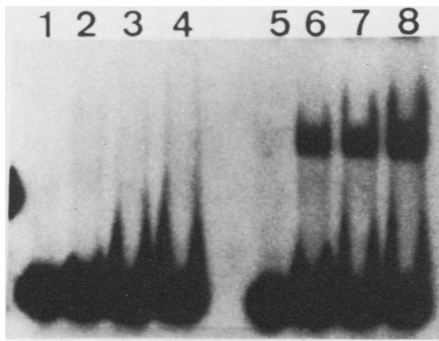


FIG. 1. Effect of gamma irradiation on the binding of nuclear extracts from the lymphoblastoid cell line C3ABR to the distal repeat sequence of the SV40 enhancer (nucleotides 200 to 270) by using the gel mobility shift assay with a ^{32}P -end-labeled fragment. Binding assays (as described in Materials and Methods) were carried out with C3ABR nuclear extracts. Lanes 1 to 4: 200, 400, 800, and 2,000 ng of extract from unirradiated cells, respectively; lanes 5 to 8: the same protein range from irradiated cells. In this figure and in others, free fragment migrates close to the gel front and retarded fragment represents protein-bound DNA.

dithiothreitol—1 mM ATP—5 μg of bovine serum albumin per ml—10 U of ligase for 6 h at 4°C . Products were end labeled with Klenow polymerase by using [α - ^{32}P]dCTP and size separated by polyacrylamide gel electrophoresis (8% polyacrylamide). Fragments corresponding to a heptamer were recovered and cloned into Bluescript. This fragment was used in the binding assays. Filters were washed for up to 30 min in TNE50 and blotted dry, and DNA-protein bands were visualized by autoradiography at -80°C with intensifying screens.

RESULTS

The gel mobility shift assay was used to search for nuclear factors induced or activated by ionizing radiation. In initial experiments a number of DNA sequences, including the 341-bp simian virus 40 (SV40) enhancer-promoter-origin of replication fragment (14), were chosen to detect DNA-binding proteins. Binding was carried out under conditions of high ionic strength, electrophoretic separation was in $1\times$ TBE (compared with 0.25 to $0.5\times$ TBE in other studies), and the amount of nuclear protein per incubation (up to 2 μg of nuclear extract) was considerably smaller than previously reported in binding studies for major transcriptional factors. This had the effect of reducing binding to a minimum with extracts from unirradiated B-lymphoblastoid cells. Exposure of cells to ionizing radiation led to the appearance of a DNA-binding protein which bound specifically to the 72-bp distal repeat of the SV40 enhancer (Fig. 1, lanes 5 to 8). The extent of binding increased with increasing protein concentration, and no binding was observed with unirradiated extracts (Fig. 1, lanes 1 to 4). Cold competition experiments with different subfragments of the SV40 enhancer confirmed the localization of binding to the distal repeat sequence (Fig. 2, lanes 2 and 3). Other sequences including the immunoglobulin heavy-chain enhancer, the matrix-associated sequence of the κ immunoglobulin light chain, and the poly-linker region of pUC failed to compete for binding, but the κ immunoglobulin light-chain enhancer successfully competed (Fig. 2, lanes 4 and 5).

A dose-dependent increase in the amount of DNA-binding activity was observed, leveling off at a radiation dose of 10

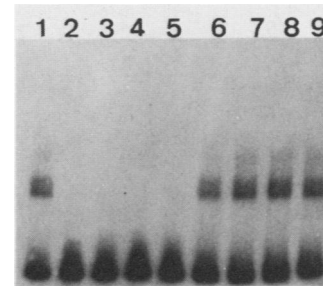


FIG. 2. Confirmation of the DNA-binding region by using cold competition analysis. Binding was carried out with 1,000 ng of nuclear extract and 1,500 ng of poly(dI-dC) · poly(dI-dC). Lanes: 1, no competitor; 2 and 3, 10- and 100-fold molar excess of distal repeat fragment; 4 and 5, 10- and 100-fold molar excess of the 515-bp *HindIII* fragment of the κ enhancer; 6 and 7, 10- and 100-fold molar excess of the 300-bp *PstI-EcoRI* fragment of the heavy-chain immunoglobulin enhancer; 8 and 9, 10- and 100-fold molar excess of the matrix association region from the mouse κ immunoglobulin gene.

Gy (Fig. 3). Desitometric analysis revealed that the amount of DNA in the bound fraction, assuming one binding site per molecule, would correspond to 10^7 protein molecules binding per μg of nuclear extract after a radiation dose of 10 Gy. This dose was selected to determine the time course of appearance of the binding activity. Low activity was first observed at 15 min postirradiation; the activity reached a maximum by 1 h and subsequently declined to negligible levels by 9 h after irradiation (Fig. 4).

The appearance of this protein in irradiated control cells was not due to de novo protein synthesis but rather to activation of a preexisting protein since neither DRFB (125 μM), an inhibitor of transcription, nor cycloheximide (50 $\mu\text{g}/\text{ml}$) interfered with the appearance of the activity (Fig. 5A, lanes 3 and 5). Since a number of insults to cells lead to the induction and/or activation of stress-related proteins, we examined the ability of agents other than gamma irradiation to cause activation of the DNA-binding protein. The radiomimetic agent streptonigrin caused the activation of a DNA-binding activity similar to that induced by gamma irradiation (Fig. 5B, lane 3). It is evident that this is a specific response to ionizing radiation or agents that generate oxygen radicals, since neither UV ($5 \text{ J}/\text{m}^2$) nor heat exposure led to the activation of the protein (Fig. 5B, lanes 4 to 6).

Activation of preexisting proteins by various stimuli involves posttranslational modification or unmasking prior to translocation into the nucleus (4, 8, 13, 26). To investigate

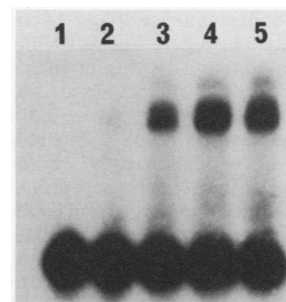


FIG. 3. Effect of radiation dose on the appearance of DNA binding activity. Lanes: 1, unirradiated extracts; 2, 2.5 Gy of gamma rays; 3, 5 Gy; 4, 10 Gy; 5, 20 Gy. A 1- μg quantity of protein was used for binding.

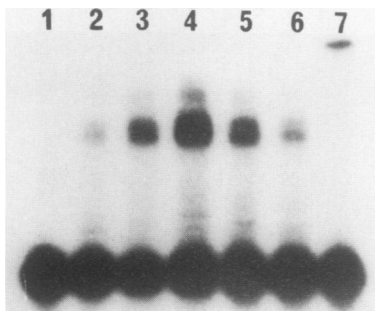


FIG. 4. Time course of appearance of DNA-binding activity in cells exposed to 10 Gy of gamma rays. Lanes: 1, zero time postirradiation; 2, 15 min postirradiation; 3, 30 min; 4, 1 h; 5, 3 h; 6, 5 h; 7, 9 h.

the mechanism of activation by radiation, we examined cytosolic and nuclear fractions for DNA-binding activity by using an oligonucleotide (oligo 1) corresponding to the binding site (Fig. 8). As expected, nuclear extracts from unirradiated cells did not possess activity (Fig. 6, lane 1), but it was present in cytosolic extracts from the same cells (lane 2). Exposure of cells to gamma irradiation caused DNA-binding activity to appear in nuclear extracts (lane 3), with evidence of only a small amount of activity still present in the cytoplasm (lane 4). The binding activity was detected in cytosolic extracts regardless of whether Triton or hypotonic conditions were used to separate nuclei from cytoplasm. Under these conditions there was no detectable, nonspecific distribution of proteins between the cytoplasm and the nucleus as evidenced by SDS-polyacrylamide gel electrophoresis (results not shown).

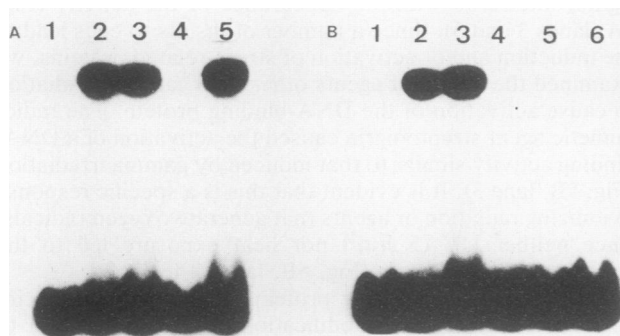


FIG. 5. (A) Effect of transcriptional and translational inhibitors on induction of the DNA-binding activity in irradiated C3ABR cells. Binding was carried out as described in Materials and Methods by using the oligonucleotide sequence (oligo 1) corresponding to the binding site as described in Fig. 8. End-labeled oligonucleotide (0.1 ng) was incubated for 15 min with 2 μ g of nuclear extract and 1 μ g of poly(dI-dC) · poly(dI-dC). Cycloheximide (50 μ g/ml) or DRFB (125 μ M) was added to cells as described. Lanes: 1, unirradiated extracts; 2, irradiated extracts (10 Gy); 3, irradiated extracts plus cycloheximide; 4, cycloheximide only; 5, irradiated extracts plus DRFB. (B) Specificity of the activating agent. DNA-binding studies were carried out with nuclear extracts from C3ABR cells pretreated with different agents by using the oligonucleotide sequence (oligo 1) as a probe (see the legend to Fig. 8). Lanes: 1, unirradiated extracts; lane 2, irradiated extracts (10 Gy); 3, cells incubated with streptomycin (5 ng/ml) for 1 h prior to preparation of extracts; 4, cells UV irradiated with 0.5 J of 254-nm light per m² and incubated for 1 h prior to preparation of extracts; 5 and 6, cells heated at 43°C for 5 min and subsequently incubated at 37°C for 30 min (lane 5) or 60 min (lane 6) prior to extraction.

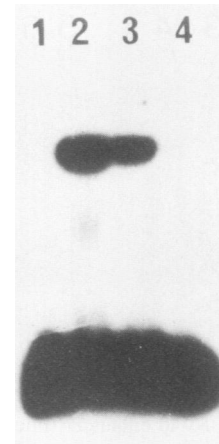


FIG. 6. Effect of gamma irradiation on localization of DNA-binding activity in C3ABR cells. Nuclear and cytosolic fractions were prepared as described in Materials and Methods. Lanes: 1, unirradiated nuclear extracts; 2, unirradiated cytosolic extracts; 3, irradiated nuclear extracts; 4, irradiated cytosolic extracts. In all cases 2 μ g of protein extract, 1 μ g of poly(dI-dC), and 0.1 ng of oligonucleotide probe were used for incubations.

To determine the exact binding site of the protein activated by irradiation, we carried out DNase I protection footprint analysis. The DNase I footprints revealed a protected region composed of 13 nucleotides with the sequence ACAGTCAATCCCA on the antisense strand (Fig. 7, lanes 2 and 3). This motif is located between nucleotides 247 and 259 in the 5'-flanking sequence of the distal repeat, between the AP-3 and AP-5 binding sites. A finer delineation of the protected region was achieved by using methylation protection, which showed that strong protection occurred for the AA sequence at nucleotides 253 and 254 (results not shown), which is within the DNase I-protected region. In addition, DMS protection with the sense strand revealed that the G nucleotide (position 255) immediately adjacent to the AA sequence was strongly protected and that the G nucleotide at position 259 was protected to a somewhat lesser extent (Fig. 8A). The binding site is depicted in Fig. 8B, showing the contact points determined by methylation protection. The CAA (GTT) sequence lies within a pentanucleotide motif (CAGTT sense strand) that is also found at two locations within the murine κ enhancer (23), which was the only other sequence shown to compete with the SV40 distal repeat for binding.

An oligonucleotide corresponding to the DNase I protection site in the SV40 sequence and two others, mutagenized at nucleotides shown to be strongly protected during DMS treatment, were used to study the binding in more detail. Nuclear extracts from irradiated cells gave rise to binding when the oligonucleotide corresponding to the protected region was used (Fig. 9A). The two mutagenized oligonucleotides showed no evidence of binding when 1 μ g of poly(dI-dC) · poly(dI-dC) was included in the incubation (Fig. 9A, lanes 2 and 3 and lanes 5 and 6). At lower concentrations of poly(dI-dC) · poly(dI-dC) it was possible to detect binding, with oligo 3 binding more strongly than oligo 2. This was expected since two strongly protected nucleotides (positions 256 and 257) were altered in the case of oligo 2, whereas only one strongly protected nucleotide at position 255 was substituted in oligo 3. In addition, 10-fold and 100-fold excesses of the oligonucleotide that showed binding (oligo 1) successfully competed with the SV40 distal repeat for binding

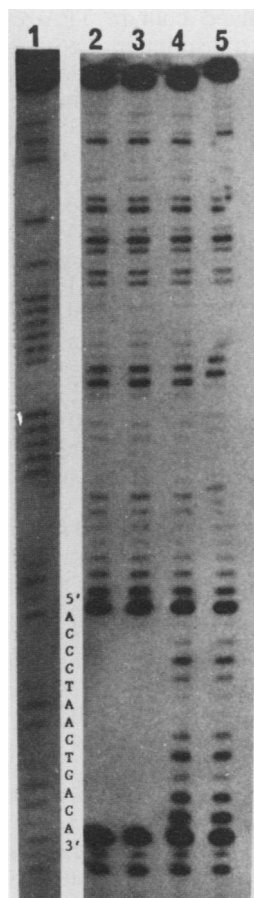


FIG. 7. DNase I footprint (31) analysis of DNA sequence that interacts with C3ABR and C5ABR (irradiated with 10 Gy) nuclear proteins. DNA probe (distal repeat as in Fig. 1) was 3' end labeled on the antisense strand. The binding reaction was as detailed in Materials and Methods, except that the reaction was scaled up fivefold with respect to extract and the reaction volume was 25 μ l. DNase I digestion was carried out for 30 s at 0°C. For chemical cleavage, the binding mix was treated with 1 M formic acid (Na^+ salt, pH 2) for 25 min at 25°C prior to cleavage with 1 M piperidine. DNA fragments were analyzed on an 8% sequencing gel. Lanes: 1, AG cleavage ladder of free fragment; 2 and 3, DNase I ladders of bound fragment eluted from C3ABR and C5ABR binding reactions; 4 and 5, DNase I ladders for the free fragment eluted from C3ABR and C5ABR binding reactions.

(results not shown), confirming that the same site was involved. Southwestern analysis (31), using a concatamer of the oligonucleotide (oligo 1) sequence as a probe, revealed that the protein from irradiated nuclear extracts was 43,000 daltons (Fig. 9B, lanes 2 and 3). It is clear that no binding activity is present in unirradiated nuclear extracts (lane 1) but is present in the cytosolic fraction of these cells (lane 4) and in a second control cell line (lane 5). In addition, irradiation did not change the size of the protein in cytosolic extracts from irradiated cells. Cytosolic activity in unirradiated cells was the same as observed with nuclear extracts. These results demonstrate that radiation causes the translocation of the binding activity from the cytoplasm to the nucleus in control cells and that this does not involve an appreciable change in molecular size. However, it is also possible that the protein is normally a nuclear protein that diffuses into the cytoplasm during extraction of unirradiated

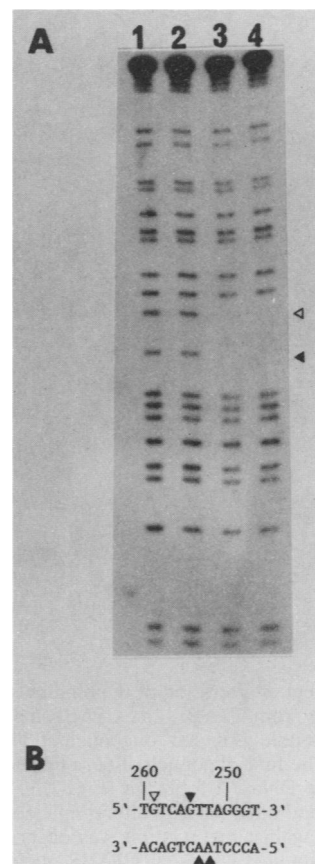


FIG. 8. (A) Methylation protection footprints of the radiation-activated factor bound to the distal repeat of the SV40 enhancer. The footprints are on the 3'-end-labeled sense strand. Lanes: 1 and 2, free (unbound) DNA methylation ladders from binding reactions with C3ABR and C5ABR; 3 and 4, bound-fragment methylation protection footprint for C3ABR and C5ABR extracts. (B) DNA-protein-binding motif including contact points for both the sense and antisense strands.

cells but after irradiation binds to DNA and is retained in the nucleus.

DISCUSSION

We have described the activation of a previously undescribed DNA-binding protein from a preexisting protein in human cells exposed to gamma irradiation. This is the first description of an ionizing radiation-activated protein in human cells for which a function can be envisaged. The appearance of the protein in the nucleus of irradiated cells is dose dependent and transient. By 5 h postirradiation most of the activity had disappeared from the nucleus. This period corresponds to the time taken to repair a large proportion of radiation-induced lesions in DNA and for the recovery of DNA synthesis to occur (24). A recent report by Lamb et al. (18) provides evidence that radiation-induced inhibition of DNA replication is mediated by a *trans*-acting factor. This may be related to the protein described here. Alternatively, since this factor binds to the κ enhancer, it may function as a negative regulatory factor which reduces or eliminates transcription of a limited number of genes in response to radiation damage to chromatin. A negative regulation or reducer element has recently been described within a mem-

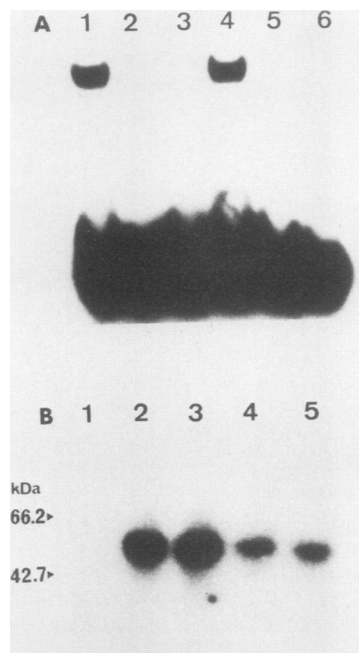


FIG. 9. (A) Effect of alteration of the binding site on the activity of nuclear extracts from C3ABR and C5ABR cells (irradiated with 10 Gy). Three double-stranded oligonucleotides were used for binding studies. The first oligonucleotide, oligo 1, was synthesized on the basis of the DNase I footprint (Fig. 7). Two other oligonucleotides (oligo 2 and oligo 3), with altered nucleotides in the region where protection against methylation was observed, were synthesized for binding studies. Lanes: 1, C3ABR nuclear extract (irradiated) with oligo 1; 2, same extract with oligo 2; 3, same extract with oligo 3; 4, C5ABR nuclear extract (irradiated) with oligo 1; 5, same extract with oligo 2; 6, same extract with oligo 3. (B) Southwestern analysis of C3ABR and C5ABR nuclear and cytosolic protein binding to a concatamer of oligo 1 corresponding to the binding site. Nuclear and cytosolic proteins (20 μ g) were resolved by SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose, and filter probed (31) with end-labeled oligonucleotide sequence (10^6 cpm/ml). Lanes: 1, C3ABR (unirradiated) nuclear extract; 2, C3ABR (irradiated) nuclear extract; 3, C5ABR (irradiated) nuclear extract; 4, C3ABR (unirradiated) cytosolic extract; 5, C5ABR (unirradiated) cytosolic extract. The positions of molecular mass standards (Bio-Rad) are indicated in kilodaltons (kDa).

ber of the African green monkey *Alu* family of interspersed repeats (27). This element decreased transcription in both directions from a cellular SV40-like bidirectional promoter. The reducer element was not promoter specific and showed several characteristics of enhancer sequences. Although there is no obvious sequence relatedness between the 38-bp reducer element and the binding site described in the present study, it is possible that the radiation-activated factor acts through a similar mechanism controlling the transcription of several genes simultaneously.

Unlike in previous studies, a general stress-related phenomenon is not involved since neither UV nor heat exposure causes activation of the protein. A recent report by Boothman et al. (6) identified a pleiotropic protein expression response activated by X-irradiation in confluence-arrested human cells. No functional activity was described for these proteins, and, contrary to our observations, the response was dependent on de novo protein synthesis. UV light, mitomycin C, and TPA cause transcriptional activation of several proteins in human fibroblasts. The promoter regions

of the genes involved contain TPA-responsive elements recognized by the transcription factor AP-1 (2). The TPA-responsive elements in collagenase (ATGAGTCAGA), stromelysin (ATGAGTCAGA), interleukin-2 (TTCAGTCAGT), and nucleotide region 119 to 128 of the SV40 enhancer (ATTAGTCAGC) have some overlap with the binding site described in this study (GTCAGTTAGGGTG) (2). However, since the protein described here failed to bind to nucleotides 119 to 128 of the SV40 enhancer, as determined by cold competition and direct binding (results not shown), it does not appear to be AP-1.

UV induction of DNA-binding proteins by a mechanism involving modification of preexisting proteins has been reported, but no binding-site data were provided (10). A heat-inducible human factor that binds to the hsp70 promoter is also activated from a preexisting factor (16), but the consensus sequence differs considerably from that described here. A number of different mechanisms have been described to account for activation of preexisting proteins; these include posttranslational modification of proteins (26), release from a cytoplasmic inhibitor, and translocation to the nucleus (4, 8, 13). Activation of the pleiotropic transcription regulator NF- κ B by translocation into the nucleus represents a rapid response to extracellular signals (4). In the present case a rapid response appears to be achieved in the same way by translocation, but there is no evidence for the presence of an inhibitor such as I κ B, which binds to and masks the activity of NF- κ B. After radiation exposure the protein described here is present in both the nucleus and cytoplasm but is subsequently lost from the nucleus. A distribution between the nucleus and cytoplasm has also been observed for hsp70 during heat shock (33). In the recovery period hsp70 leaves the nucleus and is found only in the cytoplasm. Single-amino-acid alterations in the sequence Lys-129-Arg-130-Lys-131 of the SV40 large T antigen also cause the protein to be distributed between the nucleus and cytoplasm (15).

The description of this DNA-binding protein represents an important advance in the study of the molecular basis of the response of human cells to ionizing-radiation damage. Elucidation of the mechanism of activation and identification of the binding site in the human genome will help to outline its role in the processing of DNA and regulation of gene expression postirradiation.

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