Purification and Characterization of a DNA-binding Protein Activated by Ionizing Radiation

(Received for publication, January 16, 1992)

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Exposure of mammalian cells to a variety of agents leads to the activation of pre-existing proteins and the induction of specific genes. We have recently described the appearance of a specific DNA-binding protein in nuclei from cells exposed to ionizing radiation (Singh, S. P., and Lavin, M. F. (1990) Mol. Cell. Biol. 10, 5279–5285). This protein is present in the cytoplasm of unperturbed cells and is apparently translocated to the nucleus in response to radiation damage. We describe here the purification and characterization of this specific DNA-binding protein. Purification involved the use of affinity chromatography employing a multimeric form of the DNA-binding motif conjugated to cyanogen bromide-activated Sepharose. Three DNA-binding species were recognized by UV-cross-linking and South-Western analysis. The major species or that with the highest affinity was approximately 70 kDa in size. DNase-1 footprint analysis revealed a single binding site in the immunoglobulin gene enhancer and in a putative control sequence upstream from the c-myc gene. At salt concentrations as high as 1 M, up to 40% of the DNA-binding activity was maintained and the $K_d$ was calculated to be $1.205 \times 10^{-8}$ M$^{-1}$. Binding activity was found to be modulated by phosphorylation. Removal of phosphate groups from the protein resulted in a major loss of binding activity. It is not clear at this stage whether the factor(s) described here plays a role in transcription control or a more general DNA-processing role in response to radiation damage.

Genomic DNA is the most important target for cellular radiation damage, and a variety of lesions in DNA resulting from radiation exposure have been described (Hanawalt et al., 1979). These lesions can be repaired by a number of different DNA repair pathways (Sancar and Sancar, 1988). In Escherichia coli, a number of regulatory networks that include the adaptive response and the SOS response are induced after radiation damage (Walker, 1985). Evidence is also available for the presence of related networks in mammalian cells.

Inducible pathways have been described by which mammalian cells respond to several DNA-damaging agents (Herrlich et al., 1984; Lindahl et al., 1988; Fornace et al., 1989; Hanawalt, 1989). Several genes including collagenase, stromelysin, and metallothionein are induced by UV, mitomycin C, and 12-O-tetradecanoylphorbol-13-acetate (TPA)1 (Angel et al., 1986). Ionizing radiation has also been shown to induce the coordinated expression of a specific set of proteins, XIPs, in human cells (Boothman et al., 1989). Increased expression of EGRI and c-jun has been observed between 30 min and 3 h after exposure of serum-deprived human cells to x-ray irradiation (Kramer et al., 1990; Hallahan et al., 1991), the human $gadd$ 45 gene has been shown to be strongly and rapidly induced by x-rays (Papathanasiou et al., 1991).

Considerably less information is available on the activation of pre-existing proteins by DNA-damaging agents without any requirement for de novo protein synthesis. Glazer et al. (1989) described an increase in DNA-binding proteins in nuclear extracts from HeLa cells after exposure to UV light, and de novo synthesis of proteins was not required for induction of the binding activities. Induction of a group of genes by DNA-damaging agents and TPA is mediated by a rapid increase in AP-1 activity (Angel et al., 1986). It seems likely that this is achieved by post-translational modification in the form of a site-specific dephosphorylation of c-jun (Boyle et al., 1991). We have recently described the appearance of a specific DNA-binding protein in nuclei from human cells exposed to ionizing radiation (Singh and Lavin, 1990). This protein was not detected in nuclear extracts of unperturbed cells and was induced by a mechanism not requiring protein synthesis. The DNA-binding protein was present in the cytoplasm of untreated cells, apparently being translocated to the nucleus in response to radiation exposure. Activation was observed with ionizing radiation and radiomimetic agents but not after UV-irradiation or heat shock. This protein was originally identified by its ability to bind the 72-bp distal repeat of the SV40 enhancer. More recently, we have demonstrated that this protein is capable of identifying genomic target sequences (Hobson et al., 1991).

We describe here the purification of this factor by affinity chromatography. It seems likely that more than one DNA-binding activity is present. The different forms may represent a family of proteins recognizing the binding motif. Other binding sites in the immunoglobulin enhancer and upstream of the c-myc gene are described. The binding characteristics of the protein(s) are also reported.

**EXPERIMENTAL PROCEDURES**

Cell Culture.—The Epstein-Barr virus-transformed lymphoblastoid cell line, C3ABR, was used in this study. Cells were routinely cultured in RPMI 1640 medium with 10% fetal bovine serum at 37° C under

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1 The abbreviations used are: TPA, 12-O-tetradecanoylphorbol-13-acetate; bp, base pair(s); kb, kilobase(s); SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; DTT, dithiothreitol; HEPES, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; EGTA, ethylenediaminetetraacetic acid.
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an atmosphere of 5% carbon dioxide, 95% air. For bulk cultures, 200 ml of cells (2 x 10^6 cells·ml⁻¹) were diluted to 1 liter in RPMI 1640 medium buffered with 100 mM HEPES (pH 8.0). Cultures were harvested at a density of 2 x 10⁶ cells·ml⁻¹. Regular flow cytometry was used to determine cell diploidy within cultures.

Preparation of Cellular Extracts—Cellular extracts were prepared from lymphoblastoid cells essentially as described by Dignam et al. (1983). Cells were pelleted in polypropylene tubes, washed in phosphate-buffered saline and resuspended. The cells were then resuspended in 2.5 ml (2 x 10⁶ cells) of ice-cold Buffer A (100 mM HEPES, pH 8.0, 50 mM NaCl, 500 mM sucrose, 1 mM EDTA, 0.25 mM EDTA, 0.6 mM spermidine, 0.5% (v/v) Triton X-100, 1 mM phenylmethylsulfonyl fluoride, 7 mM 2-mercaptoethanol) and treated with 30 strokes with a Dounce homogenizer yielding 90% cytolysis. The suspension was then transferred to 10 ml benchtop centrifuge tubes, the nuclei were pelleted at 1000 x g for 10 min, and a supernatant (cytoplasmic fraction) was collected and dialyzed against Buffer B (10 mM HEPES, pH 8.0, 100 mM NaCl, 10% glycerol, 0.1 mM EDTA, 0.1 mM EGTA, 0.6 mM spermidine, 1 mM PMSF, 7 mM 2-mercaptoethanol).

DNA-Protein Binding and Gel Electrophoresis—DNA-binding studies were performed using either a double-stranded oligonucleotide corresponding to the DNA-binding footprint on the 72-bp distal repeat of the SV40 enhancer, or a heptamer of this oligonucleotide (Singh and Lavin, 1990). The oligonucleotide was synthesized on an Applied Biosystems 380B model DNA synthesizer. End-labeling of the oligonucleotide was carried out with [³²P]dCTP using Klenow polymerase. The labeled DNA was purified using a Sephadex column, an appropriate amount of protein, and a corresponding amount of poly(dI-dC)·poly(dI-dC), 10 mM Tris-HCl (pH 7.5), 50 mM NaCl, and 1 mM EDTA. The incubations were performed at room temperature for 15 min, and a gel mobility shift assay was performed on a 5% polyacrylamide gel in TBE (50 mM Tris-HCl, pH 8.3, 50 mM borate, 1 mM EDTA). The gel was then dried and autoradiographed overnight.

DNase-1 Footprinting—DNase-1 footprinting was carried out as described previously (Singh and Lavin, 1990). A 215-bp fragment (pE2346) isolated from the c chain immunoglobulin enhancer (Max et al., 1981) was used for footprinting. A second sequence (HindIII-PstI), 210 bp in size and located 2 kb upstream from exon 1 of the human c-myc gene, was also used (Ariga et al., 1989).

Binding reactions were performed using 2 and 4 ng of purified protein, 1 µl of 10 x binding buffer (100 mM Tris-HCl, pH 7.5, 500 mM NaCl, 10 mM EDTA), 12,000-16,000 cpm of [³²P]end-labeled oligonucleotide, and up to 10 µl with Millipore water. The incubations were then placed on ice and 5 µl of 0.5 mM MgCl₂/CaCl₂ and 10 units of DNase-I were added. The reaction mix was incubated for 30 s, at 4 °C, and terminated with 5 µl of EDTA. Gel electrophoresis was carried out on a 12% polyacrylamide gel (7 x 20 cm) for 2 h at 37 °C, and 60 mA with a 50 W power supply. A gel mobility shift assay was performed on a 5% polyacrylamide gel in TBE (50 mM Tris-HCl, pH 8.3, 50 mM borate, 1 mM EDTA). The gel was then dried and autoradiographed overnight.

RESULTS

Preparation of Ionizing Radiation-activated DNA-binding Protein—We have previously described a site-specific DNA-binding protein activated by ionizing radiation (Singh and Lavin, 1990). This protein is present in an active form in the cytoplasm of untreated cells. Accordingly, cytoplasmic extracts from the lymphoblastoid cell line C3ABR were subjected to a 30-60% ammonium sulfate fractionation prior to application onto a Sepharose S-200 column for size fractionation. Specific DNA-binding activity eluted in a region that corresponded to 100 kDa in size. An oligonucleotide (17-mer) corresponding to the DNA-binding site with additional CTG sequences attached at the 5' ends, was phosphorylated with T4 polynucleotide kinase to obtain a sufficient quantity of the DNA-protein complex. Purified extract (1 µg) was separated on an SDS-PAGE gel for 16 h at 37 °C, followed by self-ligation in 20 µl containing ligase buffer (300 mM Tris-HCl, pH 7.5, 100 mM MgCl₂, 100 mM DTT, and 5 mM ATP) and T4 DNA ligase (10 units) at 14 °C for 16 h. This resulted in a 10-100-unit-length product that was subsequently coupled to CNBr-activated Sepharose 4B (Kadonaga and Tjian, 1986). Dialyzed extract (1-5 µg protein) in the presence of 3-5 µg.ml⁻¹ of poly(di-dC)·poly(di-dC) was applied to a Polyprep column (10 x 20 mm) equilibrated in Buffer C containing 100 mM NaCl at a flow rate of 10 ml·h⁻¹. The column was washed with 5 volumes of Buffer C containing 100 mM NaCl, followed by stepwise elution with 250 mM, 500 mM, and 1 M NaCl. Most of the binding activity was recovered in the 1 M NaCl fraction. The active fraction was dialyzed against Buffer C containing 100 mM NaCl, and the DNA affinity step was repeated. The purified protein was stored at 4 °C and was stable for several months.

UV Cross-linking—For UV cross-linking of the synthetic oligonucleotide probe was end-labeled using bromoethoxyuridine (50 µM) instead of thymidine. The binding reaction was performed as described above. DNA-binding proteins were cross-linked to [³²P]labeled oligonucleotide by exposure to 300-nm UV light for 20 min (a time course was initially performed to determine the optimum length of exposure). The gel slice containing cross-linked protein was placed in a microcentrifuge tube with 20 µl of SDS gel loading buffer (0.10% bromophenol blue, 6% SDS, 15% glycerol, 70 mM DTT) containing 1 M NaCl fraction. The active fraction was dialyzed against Buffer C containing 100 mM NaCl, and the DNA affinity step was repeated. The purified protein was stored at 4 °C and was stable for several months.

Alkaline Phosphatase Treatment—To determine the effect of dephosphorylation on the activity of the DNA-binding protein, in vitro, bovine pancreatic alkaline phosphatase was utilized. 1 ng sample of protein was treated with protein alkaline phosphatase (3 units), and the DNA-binding activity measured by Amicon filtration, was separated on an SDS-PAGE gel (Laemmli, 1970) and autoradiographed.

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in 0.1 M NaCl to heparin-agarose and eluted with a stepwise gradient of NaCl. Approximately 70% of the binding activity was recovered in the 1 M NaCl eluate. The final two steps of the purification involved the use of sequence-specific DNA affinity chromatography. A double-stranded oligonucleotide corresponding to the binding site was self-ligated (10–100 unit lengths) and coupled to cyanogen bromide-activated Sepharose. The active heparin-agarose fraction was diluted to 0.1 M NaCl in the extraction Buffer B without salt and applied to the affinity column. Most of the activity (80–85%) was recovered in the 1 M NaCl eluate (Fig. 1). The DNA affinity step was repeated in the presence of poly(dI-dC)-poly-(dI-dC) (1 μg·ml⁻¹), and again, approximately 80% of the specific binding activity was recovered (Table I). The first affinity step gave a greater than 20-fold purification, and the final step gave a greater than 7-fold increase largely due to the loss of nonspecific DNA-binding proteins. Activity is expressed as units, where 1 unit represents the amount of protein bound to 1 ng of oligonucleotide. Overall, approximately 16% of activity was recovered, and the purification factor was 5.3 × 10²-fold (Table I).

Identification of DNA Binding Activity—SDS-PAGE showed that there were four protein bands, approximately 70, 55, 47, and 31 kDa in size, present in the purified preparation. The 70 and 47 kDa bands represent the major proportion of the protein and are easily visible on SDS-PAGE (Fig. 2). South-Western analysis using a heptamer of the binding sequence as a probe revealed that two of the proteins had specific DNA binding activity (Fig. 3). The extent of binding was considerably greater with the 70-kDa species, and as observed previously, activity was only present in cytoplasm from unirradiated cells and in nuclei after irradiation (Singh and Lavin, 1990). This finding was confirmed using photoactivated cross-linking of the purified protein to ³²P-labeled oligonucleotide probe containing 5-bromodeoxyuridine residues. After DNA binding, the sample was UV-irradiated (400 J/cm²) and analyzed by SDS-PAGE and autoradiography. Three photoaffinity-labeled proteins were observed with apparent molecular masses corresponding to 70, 47, and 31 kDa (Fig. 4). In this case also, the 70-kDa species showed by far the greatest amount of DNA binding. The other major band of apparently higher molecular weight represents nonspecific binding, since it is not competed out with cold oligonucleotide during the cross-linking. The 70 kDa band is readily reduced by cold competition at the cross-linking stage (results not shown).

Further Characterization—A number of different properties of the protein were investigated. Increasing protein concentration over the range 0.5–5 ng led to a linear increase in binding activity that leveled off at 2.5–5 ng of protein (Fig. 5). The binding activity was shown to be heat-labile. Preheating the protein for 10 min at 37 °C had little or no effect on the binding activity, activity was reduced 5-fold when preheated at 42 °C, and activity was completely lost when preheated at 50 °C (Fig. 6).

Since the binding activity was eluted from the DNA affinity column at 1 M NaCl, it was expected that the binding complex would be resistant to salt. At 750 mM in the incubation mix, 68% of the binding activity was still retained, and that value dropped to 39% at 1 M (Fig. 7). The somewhat greater retention (39%) of binding activity in solution compared with that observed during affinity chromatography (20%) may be explained by the difference in the substrate used for binding in solution (oligonucleotide) versus that on the column (complex ligated form). Cold competition with the oligonucleotide corresponding to the binding site was used to determine the extent of dissociation of the complex, and consequently, a dissociation constant (Kₐ) using a double reciprocal plot based on the Langmuir isotherm (Freifelder, 1982). The Kₐ was calculated to be 1.205 × 10⁻⁶ M⁻¹ using a molecular mass of 70 kDa for the binding protein (Fig. 8).

It has previously been shown that the phosphorylation status of a number of DNA-binding proteins determines whether binding will occur (Prywes et al., 1988; Boyle et al., 1991). When the heptamer was used for binding, two retarded species were evident, as compared with a single one with the oligonucleotide (Fig. 9). Alkaline phosphatase was used to determine whether phosphorylation was important in the more complex binding pattern observed with the fragment containing several binding sites. Removal of the phosphate group led to a complete loss of the more retarded species and a major loss of the other species (Fig. 9).

Other DNA Sequences Recognized by the Binding Protein—We have previously shown that the affinity-purified DNA-binding protein interacts with a number of different unidentified human genomic DNA fragments (Hobson et al., 1991). Since the protein binds to the SV40 enhancer, other enhancer elements were also used to check for binding. Gel retardation demonstrated that binding occurred to a 215-bp fragment of the κ-immunoglobulin gene enhancer and also to a putative DNA-binding assay of DNA affinity-purified samples. Partially purified protein (heparin-agarose (HA load)) was applied to a DNA affinity column containing oligonucleotide-binding sequence, and washed in 100 mM NaCl in Buffer B. The column was then step-eluted with 200 mM, 500 mM, and 1 M NaCl in Buffer B, and the fractions were assayed by gel retardation with ³²P-end-labeled oligonucleotide.

**Fig. 1.** DNA-binding assay of DNA affinity-purified samples. Partially purified protein (heparin-agarose (HA load)) was applied to a DNA affinity column containing oligonucleotide-binding sequence, and washed in 100 mM NaCl in Buffer B. The column was then step-eluted with 200 mM, 500 mM, and 1 M NaCl in Buffer B, and the fractions were assayed by gel retardation with ³²P-end-labeled oligonucleotide.

**Table I**

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</table>

*Units represent the amount of protein that binds 1 ng of DNA.*
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FIG. 2. SDS-PAGE analysis of protein at different stages of purification. Protein samples were applied to an 8% (v/v) SDS-PAGE, and the resulting bands were analyzed by silver staining. The purified sample displayed the presence of two major bands at 70 (SpI) and 47 kDa (SpII), with two less defined bands at 55 and 31 kDa in size. Samples were taken at the various stages as outlined in Table I.

FIG. 3. South-Western analysis. Protein extracts from nuclei (lane 1) and cytoplasm (lane 2) from unirradiated cells and nuclei (lane 3) from irradiated (10 grays) cells were separated using an 8% (v/v) SDS-PAGE gel and transferred to nitrocellulose. The filter was then probed with the 32P-end-labeled heptamer probe and autoradiographed. Cytoplasmic and irradiated nuclear samples represent purified material, whereas unirradiated nuclear extracts are unfracti-ated.

FIG. 4. UV cross-linking. Purified cytoplasmic protein was incubated in the presence of 5-bromodeoxyuridine, 32P-end-labeled oligonucleotide and exposed to 300-nm ultraviolet light for 20 min (400 J/cm²). The resulting cross-linked DNA-protein complexes were then denatured and separated by SDS-PAGE. Lane 1, 20 ng of protein; lane 2, 50 ng of protein; lane 3, 100 ng of protein. Free refers to unbound oligonucleotide.

FIG. 5. Effect of increasing protein concentration of DNA binding. A gel retardation assay was performed with increasing quantities of purified DNA-binding protein over the range 0.05-5 ng. Incubation conditions were as described under “Experimental Procedures.”

FIG. 6. Effect of preheating on DNA-binding activity. Purified protein samples (5 ng) were subjected to incubation at room temperature (control), 37, 42, 50, and 65 °C for 10 min, prior to DNA binding with 32P-end-labeled oligonucleotide for 5 min at room temperature followed by electrophoresis. With that of the SV40-binding site appear in Fig. 11B. Previous evidence from methylation protection and mutated oligonucleotides identified the central trinucleotide GTT as being critical for binding. This is present in all three sequences, and in addition, a pentameric sequence, CAGTT, is common to the k and SV40 fragments and is present in c-myc but has a C inserted. Some nucleotide similarity is also observed in regions flanking this core sequence, as depicted in Fig. 11B. Several clones isolated from human genomic DNA, based on their affinity for the DNA-binding activity (Hobson et al., 1991), also contained sequences closely related to the DNA-binding motif in the SV40 sequence (Fig. 11B).

Detection of DNA-binding Activity in Other Cell Types—DNA binding assays were also performed using extracts from human placenta and calf liver. As was the case with the lymphoblastoid cells, binding activity was shown to be present only in the cytoplasm of placental extracts (Fig. 12). With calf liver extracts, activity was detected in nuclei, but the majority was present in cytoplasm (Fig. 12).

DISCUSSION
Exposure of human lymphocytes to low level chronic radiation or low doses of x rays prior to exposure to a higher dose of ionizing radiation gives rise to a transient protection that is manifested as increased survival, a reduction in chromosomal damage, and reduced susceptibility to radiation-induced mutagenesis (Olivieri et al., 1984; Shadley and Wolff, 1987; Kelsey et al., 1991). This protective effect is blocked by inhibitors of RNA and protein synthesis, indicating that the process is mediated by the induction of particular genes (Youngblom et al., 1989). In recent years, genes coding from tumor necrosis factor α (Hallish et al., 1989), c-fos and c-
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Fig. 7. Effect of salt concentration on DNA-binding activity. A, gel retardation assay was performed using 5 ng of purified protein and 32P-end-labeled oligonucleotide with increasing NaCl concentration. As is evident, migration of free fragment was altered with increasing NaCl concentration. The resulting bands from the gel retardation assay were removed and analyzed by scintillation counting. Each reaction mix contained 53 ng of oligonucleotide, and the data represent the amount of bound and free at each salt concentration.

Fig. 8. Determination of dissociation constant (Kd). Gel retardation assays were performed using 32P-labeled oligonucleotide, constant protein, and increasing cold competitor DNA. The resulting bands were determined by scintillation counting, and the proportions of free and bound were calculated. A reciprocal plot of the Langmuir isotherm was used to determine a dissociation constant.

jun (Sherman et al., 1990), c-myc (Sullivan and Willis, 1989) and gadd 45 (Papathanasiou et al., 1991) have been shown to be induced by ionizing radiation. It is still not clear how the products of these genes are involved in cellular response to radiation. A recent report from this laboratory has described the appearance of a specific DNA-binding protein in nuclei from human cells exposed to ionizing radiation (Singh and Lavin, 1990). Ionizing radiation did not appear to cause gene induction but rather activation of a pre-existing protein. We have described here the purification and characteristics of this protein. Although it was previously demonstrated that the DNA-binding protein appeared in an active form in nuclei, postirradiation, it was also possible to detect active protein in the cytoplasm of unirradiated cells (Singh and Lavin, 1990).

Fig. 9. Effect of dephosphorylation on the DNA-binding activity. 1-ng protein samples were exposed to alkaline phosphatase at a concentration of 0.3 units·μl⁻¹ for 30 and 60 min at room temperature and analyzed by gel retardation using labeled heptamer. Lanes 1, free fragment; lanes 2 and 3, bound untreated; lanes 4 and 5, 30-min exposure to alkaline phosphatase; lanes 6 and 7, 60-min exposure to alkaline phosphatase; lanes 8 and 9, denatured alkaline phosphatase. Lanes 2, 4, 6, and 8, 5 ng of poly(dI-dC)·poly(dI-dC); lanes 3, 5, 7, and 9, 20 ng of poly(dI-dC)·poly(dI-dC).

Cytoplasmic extracts were used as the source of protein in the purification, since large volumes of cell suspension were involved that would have made radiation treatment unfeasible. It is not evident at this stage why the protein is present in an active form in the cytoplasm. This might be explained by the presence of apparently different sized forms of the protein, 70, 47, and 31 kDa. SDS-PAGE, South-Western analysis, and photoaffinity labeling provide evidence for more than one species. The greatest amount of DNA binding was observed with the 70-kDa protein. This contrasts with South-Western data with crude nuclear extracts from irradiated cells where the dominant band was 49–47 kDa (Singh and Lavin, 1990). This difference might be explained by conversion of a precursor form (70 kDa) found in the cytoplasm to the 47-kDa species in response to irradiation or alternatively to some form of interference with DNA binding by the 70-kDa species in crude nuclear extracts.

Other proteins, such as the transcription regulators NF-κB, E factor, and the glucocorticoid receptor are present in the cytoplasm in an inactive form bound by an inhibitor (Baerwal and Baltimore, 1988; Howard and Distelhorst, 1988; Dale et al., 1989). The presence of these specific binding species of different sizes, even in the affinity-purified form, could account for changes in subcellular location. Purification of the binding activities from nuclear extracts of irradiated cells may reveal differences in the relative proportions of the three species after SDS-PAGE, UV cross-linking, or South-Western analyses.

The protein described here was initially detected by its ability to bind to a sequence between nucleotides 247 and 259 in the 5'-flanking region of the distal 72-bp repeat of the SV40 enhancer. Methylation protection and mutagenesis studies point to the importance of a core sequence, CAGTT.
The murine κ enhancer was previously shown to compete with the SV40 sequence for protein binding and four CAGTT sites were observed in that sequence (Max et al., 1981). One of these sequences (5'-ACAGTTGACCTGTTCT-3') was found to be present in a well defined DNase-1 footprint (Fig. 11A) after binding of the protein to the κ enhancer. A closely related sequence, CACGTT, was detected in the footprint obtained after binding with a sequence upstream of c-myc exon 1 (Ariga et al., 1989). The pentanucleotide sequence is also present in unidentified human DNA sequences that were cloned due to their ability to bind to the protein described here (Hobson et al., 1991; see also Fig. 11B). The pentanucleotide sequence also appears to be related to the TPA-responsive element TTCAGTCAGT found upstream of the interleukin-2 gene (Angel et al., 1987). However, purified AP-1, which binds this sequence, failed to bind to the SV40 sequence that was used to isolate the protein described here (results not shown).

We have demonstrated that phosphorylation influences the pattern of DNA-protein binding (Fig. 9). Since more than one species is present in the purified extracts, it is possible that the two retarded fragments represent monomeric/dimeric binding or dimeric binding only involving homo- or heterodimers. Dephosphorylation leads to a complete loss of the more retarded band, indicating that phosphorylation may play a role in dimer formation or stability of interaction with DNA since the less retarded band is also reduced to a large extent. Previous studies have shown that phosphorylation both enhances and inhibits the DNA-binding activity of different factors. Increased AP-1 binding activity has been observed to coincide with dephosphorylation of c-jun in response to protein kinase C activation (Boyle et al., 1991). On the other hand, phosphorylation is required for the DNA-binding activity of serum response factor (Prywes et al., 1988). In the case of AP-1, at least some of the c-jun protein is present in resting...
cells in a latent form and is recruited for DNA binding after dephosphorylation (Boyle et al., 1991). After irradiation, a change in phosphorylation could change the specificity of association of the DNA-binding protein described here with another protein and thus activate it so that it binds to DNA as a positive or negative regulator of transcription.

The factor is not a lymphoid-specific activity since we have shown that it is also present in fibroblasts, liver, and placenta. These results point to a general role in response to radiation damage.

Acknowledgments—We thank the National Health and Medical Research Council of Australia and the Queensland Cancer Fund for support. We are grateful to Priscilla Le Poidevin, Heather Beamish, and Aine Farrell for growing cells and to Hazel Dunn for typing this manuscript.

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