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Gadd45, a p53-Responsive Stress Protein, Modifies DNA Accessibility on Damaged Chromatin

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This report demonstrates that Gadd45, a p53-responsive stress protein, can facilitate topoisomerase relaxing and cleavage activity in the presence of core histones. A correlation between reduced expression of Gadd45 and increased resistance to topoisomerase I and topoisomerase II inhibitors in a variety of human cell lines was also found. Gadd45 could potentially mediate this effect by destabilizing histone-DNA interactions since it was found to interact directly with the four core histones. To evaluate this possibility, we investigated the effect of Gadd45 on preassembled mononucleosomes. Our data indicate that Gadd45 directly associates with mononucleosomes that have been altered by histone acetylation or UV radiation. This interaction resulted in increased DNase I accessibility on hyperacetylated mononucleosomes and substantial reduction of T4 endonuclease V accessibility to cyclobutane pyrimidine dimers on UV-irradiated mononucleosomes but not on naked DNA. Both histone acetylation and UV radiation are thought to destabilize the nucleosomal structure. Hence, these results imply that Gadd45 can recognize an altered chromatin state and modulate DNA accessibility to cellular proteins.

The growth arrest and DNA damage-inducible (GADD) gene *GADD45* is a member of a group of genes inducible by multiple types of DNA-damaging agents and by certain stresses associated with growth arrest (15). GADD gene expression has been detected in many mammalian cells, and evidence for stress induction of *GADD45* has been observed in every human and other mammalian cell line examined to date (16). *GADD45* is the only member of the GADD group that is frequently inducible by ionizing radiation in many p53 wild-type (wt) cells (44). This induction is strictly dependent on the cell having functional wt p53 (31). In addition to playing an important role in cell cycle checkpoints, p53 is also involved in many other critical cellular events such as programmed cell death (apoptosis), gene regulation, DNA repair, and genomic stability (37). Loss or alteration of p53 function in tumor cells occurs in more than half of human malignancies and is associated with a variety of cellular aberrations such as genomic instability and aggressive tumor growth (35). A cooperative role for p53 has also been described for *GADD45* induction by other types of DNA-damaging agents such as the alkylating agent methyl methanesulfonate and UV radiation (82, 84, 85). Even though some aspects of Gadd45 regulation have been characterized, its function(s) remains ill defined. Gadd45 is a small acidic protein of 19 kDa, found in low abundance in the nucleus (10). Recent evidence (11) suggested that Gadd45 may have some functional similarity with the nucleosome assembly protein NAP-1 (18). The aim of the present study was therefore to determine whether Gadd45 could, like NAP-1, interact with the nucleosome assembly process or play some role(s) in chromatin structure.

Packaging of DNA into nucleosomal structures restricts accessibility to other proteins and is generally inhibitory to most cellular processes. In recent years, an increasing number of studies have indicated that DNA accessibility can be altered by several mechanisms including multiprotein complexes that can either stabilize or destabilize nucleosome structure (reviewed in references 34 and 70), histone modifications such as acetylation which weakens the histone-DNA interactions (7, 41), and the nucleosome unfolding which is likely to occur on actively transcribed genes (69). A number of proteins associated with nucleosome stability and chromatin structure are acidic or contain acidic domains. Some of them are involved in the transfer of histones from the acidic protein-histone complex to DNA. For example, the *Xenopus* egg and oocyte nucleoplasmin and N1/N2 proteins as well as yeast Spt6 and NAP-1 and human CAF-1 (6, 13, 18, 36, 61, 72) have been associated with a nucleosome assembly activity. Nucleoplasmin has a dual function since it is also associated with chromatin disassembly in the remodeling of sperm chromatin (48, 49). This effect is apparently mediated through the removal of the sperm-specific proteins X and Y and replacement with histones H2A and H2B. Sequence analysis have indicated that Gadd45 shares a stretch of 10-amino-acid identity with nucleophosmin (11), a member of the nucleoplasmin family (58). Other multiprotein complexes such as SWI/SNF and NURF are implicated in overcoming nucleosome-mediated repression, thereby facilitating transcriptional activation in yeast, *Drosophila*, and human cells (47, 67).

Several cellular mechanisms that need access to the DNA are also thought to take advantage of the looser chromatin structure encountered during transcription. For example, repair enzymes rely on components of the basal transcriptional machinery, such as TFIIH, and on posttranslational modification of histones to locally disrupt chromatin structure (reviewed in reference 79). The excision repair cross-complement protein ERCC3 (57) is identical to p89, the largest subunit of TFIIH, and thus has a dual function in DNA repair and transcription. Increased levels of histone acetylation, which weak-

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ens the histone-DNA interactions, have also been correlated with DNA repair efficiency (56). More than a decade ago, it was shown that UV radiation can also increase protein acetylation and particularly histone acetylation (55). It was suggested that histone acetylation occurring on long stretches of nucleosomes adjacent to those containing the UV-damaged lesions could facilitate the opening up of local chromatin to excision repair enzymes (55).

In this report, evidence is presented that Gadd45 facilitates topoisomerase cleavage and relaxing activity in the presence of core histones. This observation led us to investigate the possibility that Gadd45 may mediate this effect by changing chromatin structure and accessibility. Our data indicate that Gadd45 can associate with mononucleosomes that have been altered by histone acetylation or UV radiation and consequently modify DNA accessibility on these nucleosomes. These data suggest an attractive model whereby Gadd45 could modulate DNA accessibility on damaged chromatin.

MATERIALS AND METHODS

Protein purification. The full-length Gadd45 recombinant protein and the deletion mutants Δ72-166 and Δ125-166 were produced and purified as described elsewhere (10). After removal of the histidine tag, the protein was resolved on an ion-exchange column (Mono Q HR 5/5; Pharmacia) and eluted with a linear gradient of 0 to 1.0 M NaCl in 20 mM bis-Tris propane, pH 6.8 (buffer A). The purified protein was then dialyzed extensively against 50 mM Tris-HCl (pH 7.5)–20% glycerol–1 mM dithiothreitol (DTT)–1 mM EDTA and frozen in aliquots at −70°C. The Gadd45 protein used for mononucleosome interaction was purified without the use of a nickel column (10). The bacterial lysate was applied on a DEAE-cellulose column equilibrated and developed with 25 mM Tris-HCl (pH 7.4)–4 mM EDTA–10% glycerol–0.1 mM phenylmethylsulfonyl fluoride–14 mM β-mercaptoethanol. The bound protein was eluted with a linear NaCl gradient (0 to 1 M) in the same buffer. The protein was concentrated, and the buffer was changed to 20 mM Tris-HCl (pH 8.4)–150 mM NaCl–2.5 mM $CaCl_2$ (thrombin buffer). The histidine tag was removed enzymatically with thrombin, and the protein was separated on a Hiprep (26/200) S-200 column in thrombin buffer. The fractions containing Gadd45 were pooled and concentrated, and the buffer was changed to buffer A on a Sephadex 50 column. The Gadd45 protein was then resolved on a Mono Q column and processed as described above. Gadd45 baculovirus protein was produced as previously described (10). The protein was fractionated by ammonium sulfate precipitation. The 35% saturation pellet was resolved on a Mono Q column and eluted with a linear gradient of 0.1 to 1.0 M NaCl in 20 mM Tris-HCl (pH 7.5)–0.5 mM EDTA–1 mM DTT (buffer B). Fractions eluted from 0.51 to 0.55 M NaCl were pooled and dialyzed against buffer B containing 100 mM NaCl and refractionated on a Mono Q column with a shallower NaCl gradient. The active fractions, detected by Western blotting, were pooled and dialyzed against buffer A and resolved again on a Mono Q column preequilibrated with buffer A. The bound proteins were eluted with a linear gradient of 0 to 1.0 M NaCl in buffer A.

The HeLa core histones were purified by hydroxylapatite chromatography essentially as described previously (81) except that the core histones were either fractionated by steps with 0.9 M NaCl to elute H2A/H2B and with 2.5 M NaCl to elute H3/H4 or eluted together as a core with 2.5 M NaCl. The histones were stored frozen at −70°C in high salt and diluted in the assay buffer just before being added to the reactions. An aliquot of each storage buffer was also diluted similarly for the control reactions. The *Drosophila* core histones were purified on hydroxylapatite as described elsewhere (20), and the hyperacetylated histones were purified under the same conditions. Hyperacetylated histones were obtained by treatments of *Drosophila* SL-2 cells with sodium butyrate, a histone deacetylase inhibitor (5). The level of acetylation was determined by Western blot analysis using specific hyperacetylated histone antibodies (data not shown) and by Triton acid-urea gel analysis (20). The hyperacetylated histone H3 and H4 polyclonal antibodies were obtained from B. M. Turner (Birmingham, United Kingdom). The state of acetylation of histone H4 was about 25% of each mono-, di-, tri-, and tetraacetylated histone (20). The purity of the purified core histones was evaluated by Coomassie blue staining and was estimated to be at least 90%.

The yeast recombinant NAP-1 protein was purified as described previously (18) with the following modification. *Escherichia coli* BL21(DE3) transformed with pTN2 (NAP-1 expression plasmid) were lysed by sonication in presence of 0.1% Nonidet P-40. Plasmid pTN2 was provided by Akihilo Kikuchi, Tokyo, Japan.

Nucleosome assembly on relaxed plasmid. The nucleosome assembly assay was performed as described previously (18). Briefly, the reaction was performed in a final volume of 24 μl in an assay buffer containing 10 mM Tris-HCl (pH 8.0), 1 mM EDTA, 150 mM NaCl, and 100 μg of bovine serum albumin per ml. Two hundred nanograms of supercoiled plasmid DNA pBR322 was relaxed (4 μl) by preincubation with 10 U of topoisomerase I (Top1; Promega) for 10 min at 37°C. A mixture (20 μl) of core histones purified from HeLa cells (0.4 μg) and either Gadd45, ΔGadd45, or NAP-1 (0.5 to 1.0 μg) was also preincubated for 15 min at 37°C in the same buffer. The two reaction mixtures were then combined, and the reaction was allowed to proceed for 45 min at 37°C. Following the incubation, proteinase K (0.05 mg/ml) and sodium dodecyl sulfate (SDS; 0.2%) were added, and incubation was continued for an additional hour at 37°C. The DNA was then loaded on 1% agarose gel in Tris-acetate-EDTA in the absence or presence of chloroquine (40 μg/ml). After electrophoresis, the gel was stained with ethidium bromide and photographed.

Western blot analysis. Detection of topoisomerase in the histone preparations was performed by Western blotting. The proteins were separated on a 8% polyacrylamide gel and transferred onto nitrocellulose with a semidry blotting apparatus (Bio-Rad). The nonspecific sites were blocked by incubating the protein blot in 5% nonfat dry milk in 0.1% Tween–phosphate-buffered saline (PBS) for 1 h at room temperature. The blot was then incubated with human Top1 rabbit polyclonal antibody (Topogen) at a 1:2,500 dilution in 5% milk at 4°C overnight. The blot was then washed twice with Tween-PBS and incubated with a secondary antibody, goat anti-rabbit immunoglobulin G (Santa Cruz Biotechnology) conjugated to horseradish peroxidase, at a dilution of 1:5,000 in 5% milk for 1 h at room temperature. The blot was then washed with Tween-PBS and developed with an enhanced chemiluminescence kit (Amersham) according to the manufacturer's procedure.

Far-Western blotting. One-microgram aliquots of the different core histone preparations were separated on a 18% polyacrylamide gel. The protein were transferred to nitrocellulose, and the nonspecific sites were blocked as described above. Replicas of the same blots were then incubated with different solutions containing 5% milk in 0.1% Tween-PBS, 1 μg of recombinant Gadd45 protein per ml, and either 150 mM or 1.5 M NaCl for 2 h at room temperature. The blots were then washed twice with Tween-PBS and processed as described above for Western blotting except that the primary antibody was a rabbit polyclonal antibody against Gadd45 (2 μg/ml; Zymed).

Reconstitution of nucleosomes by dialysis from high salt. Core histones from *Drosophila* embryos either normal or hyperacetylated or from HeLa cells were purified as described above. A 160-bp simian virus 40 fragment (positions 4881 to 5040) was amplified by PCR using a sense primer (5′-TTG CAT CAA CAC CAG GAT TTA AG-3′) labeled at the 5′ end. The labeled DNA and the core histone were mixed together in high salt and slowly dialyzed as described previously (67). Nucleosome formation was evaluated by gel electrophoresis and DNase I digestion.

Effects of Gadd45 on histone-DNA interactions. Increasing amounts of Gadd45 (0 to 100 ng) were incubated in the absence or presence of core histones (100 ng) for 15 min at 37°C. The reaction mixture contained 20 mM Tris-HCl (pH 8.0), 1 mM $MgCl_2$, 60 mM KCl, 4 mM ATP, 2 mM DTT, 0.2 mM EDTA, and 5% glycerol (buffer C). A labeled DNA oligomer (100-mer annealed to a 47-mer having 17-base homology) was then added to the mixture, and the reaction was allowed to proceed for an additional 30 min at 37°C. The reaction products were then loaded on a nondenaturing 18% polyacrylamide gel and exposed to X-ray film.

Band shift. Increasing amounts of Gadd45 (0 to 500 ng) were incubated with labeled mononucleosomes at 37°C for 30 min in 20 μl of buffer D (20 mM sodium phosphate [pH 6.5], 100 mM NaCl, 1 mM EDTA, 0.1 mM β-mercaptoethanol). The samples were then loaded on a 4.5% nondenaturing polyacrylamide gel in 0.5× Tris-borate-EDTA in the absence of loading dye. After running the samples, the gel was dried and exposed to X-ray film. Where indicated, the same experiment was repeated with UV-irradiated mononucleosomes or mononucleosomes assembled with hyperacetylated histones. The irradiation of the mononucleosomes was performed with a UV Stratalinker 1800 box (Stratagene) at a rate of 45 J m^{-2} s^{-1}. We estimated that five or six pyrimidine dimers can be formed on a 160-mer DNA fragment under these conditions. The mobility shift assay performed with Gadd45 monoclonal antibody was performed similarly except that 2 μl of Gadd45 hybridoma supernatant 30T-14 (32) was added to the reaction mixture prior to the addition of Gadd45. Densitometry analyses were performed with the GelExpert software and a Nucleo Tech video camera.

DNase I. Increasing amounts of Gadd45 were incubated with the mononucleosomes (10 ng) in buffer C for 30 min at 30°C. DNase I was then added, and the mixture was incubated for 1 min. The DNA was purified and run on a sequencing gel (67). The dried gel was scanned with a phosphorimager (Fuji), and the percentage of each digested band was calculated with respect to the total number of counts in each sample, using MacBAS version 2.0.

T4 Endo V. T4 endonuclease V (T4 Endo V) was obtained from Daniel Yarosh (AGI Dermatics Inc., Freeport, N.Y.). Naked or normal and hyperacetylated mononucleosomes were incubated in the absence and presence of Gadd45 (50 ng) and indicated amounts of T4 Endo V enzyme for 1 h at 37°C in buffer D. The samples were deproteinized, and the DNA was purified and run on an 8% sequencing gel. The gel was dried and exposed to X-ray film.

Top1 cleavage assay. The Top1 cleavage assay was performed as described before (51). Briefly, the reactions were performed at room temperature in 5 μl of reaction buffer (10 mM Tris-HCl [pH 7.5], 50 mM KCl, 5 mM $MgCl_2$, 0.1 mM EDTA, 15 μg of bovine serum albumin per ml, 0.2 mM DTT). Approximately 0.5 pmol of a labeled 36-mer double-stranded oligomer was incubated with Top1 in the presence of 10 μM camptothecin. Where indicated, 0.4 μg of core histones and 0.5 μg of Gadd45 were added to the reaction mixtures. The reactions were

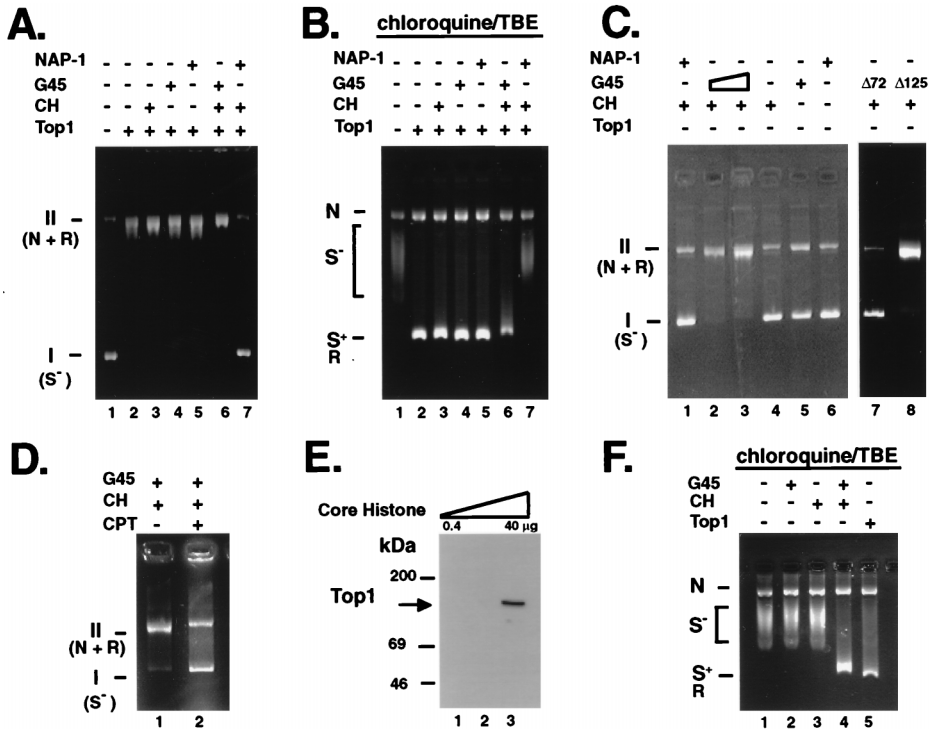

FIG. 1. Gadd45 facilitates topoisomerase relaxing activity in the presence of core histones. Reactions were performed as described in Materials and Methods ("Nucleosome assembly on relaxed plasmid"). (A) Supercoiled plasmid DNA (0.2 μg, pBR322) (lane 1) was relaxed by 10 U of Top1 (lane 2) and incubated in the presence (+) or absence (−) of core histones (CH), Gadd45 (G45), or NAP-1. After incubation, the samples were deproteinized and run on a 1% agarose gel in the absence of chloroquine. The gel was then stained with ethidium bromide. The positions of forms I (S^-, negatively supercoiled) and II (N, nicked circle; R, relaxed) of plasmid pBR322 are indicated. (B) Same as panel A but run in the presence of 40 μg of chloroquine per ml. (C) Plasmid relaxation assay as in panel A but performed in the absence of Top1. Amounts of Gadd45 were 0.5 and 1.0 μg (lanes 2 and 3); 0.5 μg of Gadd45 deletion mutants Δ72 (the resultant protein contains the first 71 amino acids) and Δ125 (mutant containing amino acids 1 to 124) were used (lanes 7 and 8). (D) Plasmid relaxation assay performed as for panel C in the absence (−) or presence (+) of 10 μM camptothecin, a Top1 inhibitor. (E) Increasing amounts (0.4 to 40 μg) of HeLa core histone were analyzed by Western blotting with human Top1 antibody. The proteins were blotted and hybridized as described in Materials and Methods. Positions of the protein markers and Top1 are indicated on the left. (F) Reactions similar to those in panel C, run in the presence of 40 μg of chloroquine per ml.

stopped by the addition of 1% SDS, 20 mM EDTA, and proteinase K. The DNA was ethanol precipitated and run on a sequencing gel.

Correlation analysis. Briefly, cellular sensitivity data were obtained from a 48-h cytotoxicity assay performed on a large number of potential cancer chemotherapy agents in the National Cancer Institute (NCI) anticancer drug screen, using a collection of human tumor lines (77). More than 150 molecular markers, such as p53 status, have been determined in these lines (77). The cytotoxicity data were processed by using COMPARE (45) and programs in the DISCOVERY program set (77). The data were correlated to the capacity to induce *GADD45* mRNA following exposure to ionizing radiation (2).

Cells and cell treatments. Human colon carcinoma (RKO) cells and RKO-AS45 cells were grown and maintained as previously described (2). Drug cytotoxicity was determined by clonogenic survival assays in the presence of different drug concentrations. Cells were plated at 5×10^2 per 100-mm-diameter petri dish and exposed for 4 h to the different drugs the following day. Cells were allowed to grow for 2 weeks, and the colonies were fixed with methanol-acetic acid (3:1), stained with crystal violet, and counted. Each point was done in triplicate, and experiments were performed at least twice. Camptothecin, etoposide (VP-16), and VM-26 were obtained from the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, NCI, Bethesda, Md.

DNA-protein cross-links. DNA-protein cross-links were measured by alkaline elution as described previously (38). The cells were labeled with 0.04 μCi of [^{14}C]thymidine per ml for 1.5 to 2 cell doublings at 37°C. The radioactive medium was removed, and the cells were then chased with fresh nonradioactive medium for at least 4 h before drug treatment. The drug was then added to the medium, and the cells were incubated for 1 h. Stock solutions of the drug were prepared in dimethyl sulfoxide at 10 mM and stored at −20°C.

RESULTS

Facilitation of Top1 DNA relaxation activity by Gadd45. Recently we found (11) that our Gadd45 polyclonal antibody cross-reacted with the human protein SET. Since no sequence homology was found between the two proteins, we reasoned that a potential structural or functional homology could exist between Gadd45 and SET. The SET protein has a long acidic tail at its C terminus and shows sequence identity and similarity with yeast NAP-1 (74). A function related to the nucleosome assembly protein has also been suggested for SET (74). Thus, we sought to determine whether Gadd45 could, like NAP-1, mediate nucleosome assembly in an in vitro assay. In the assay used for NAP-1 (18), a supercoiled plasmid DNA was first relaxed by incubation with Top1. In a separate tube, a mixture of core histones and acidic protein were also incubated, and then the two reaction mixtures were combined and incubated further. Removal of the nucleosomal proteins by digestion with proteinase K followed by DNA purification resulted in the appearance of negative superhelical turns corresponding to assembled nucleosomes in the closed circular DNA (21).

Using such an assay, we incubated Gadd45 or NAP-1 with the core histones and added the mixture to a relaxed plasmid. The samples were deproteinized and separated into two sets, one run in the absence (Fig. 1A) and the other run in the presence (Fig. 1B) of the intercalating agent chloroquine. This agent is used to distinguish positively from negatively supercoiled DNA and closed relaxed from nicked DNA. In the presence of chloroquine, negatively supercoiled DNA will be retarded while the electrophoretic migration of positively supercoiled or relaxed DNA will increase. The data presented on Fig. 1A indicate that, as expected, incubation of a supercoiled

plasmid DNA (lanes 1) with Top1 relaxed DNA (lanes 2). Independent addition of either core histones, Gadd45, or NAP-1 did not alter the Top1 relaxing activity (lanes 3 to 5). The concomitant addition of Gadd45 and core histone to the relaxed plasmid did not affect Top1 relaxing activity either, nor did it lead to significant reformation of a supercoiled DNA structure (Fig. 1B, lane 6). However, as reported previously (18), concomitant addition of NAP-1 and core histones led to the formation of negatively supercoiled DNA structure in the presence of Top1, which is an indication that nucleosomes were assembled (Fig. 1A and B, lanes 7); otherwise the DNA would have remained in a relaxed circular form. The addition of Gadd45 to the NAP-1 core histone mixture did not prevent the nucleosome assembly activity of NAP-1 (data not shown), suggesting that Gadd45 did not interfere with nucleosome assembly or the solubility of the reagents. Thus, despite the fact that several acidic proteins, including polyanions (62), have been reported to mediate the formation of supercoiled DNA structure in this assay, Gadd45, which is also an acidic protein (10), cannot significantly substitute for NAP-1, or antagonize NAP-1-induced nucleosome assembly activity, under those conditions.

Comparable assays were also performed in the absence of Top1, and samples were analyzed in the absence (Fig. 1C) or presence (Fig. 1F) of chloroquine. Early studies have shown that direct mixing of core histones at about 1:1 mass ratio with either 145 bp of DNA or simian virus 40 plasmid DNA under physiological ionic strength could form spontaneously nucleosomes complexes similar to those formed by salt gradient dialysis (62). At a 1:1 mass ratio about half of the DNA remains free in solution, while at a 2:1 histone/DNA ratio almost all the DNA is found in the core particles (65). Only when the core histones are present in large excess (3.1:1 and 4:1) do aggregations predominate (65). It is thus reasonable to assume that nucleosome-like interactions occur between the core histones and free DNA in this experiment where a 2:1 mass ratio was used. Under such conditions (Fig. 1C), concomitant addition of NAP-1 and core histone did not affect the DNA topology (lane 1). Unexpectedly, simultaneous addition of Gadd45 and core histones led to complete relaxation of the DNA (Fig. 1C, lanes 2 and 3; Fig. 1F, lane 4), while independent addition of either component did not affect the DNA topology (Fig. 1C, lanes 4 and 5; Fig. 1F, lanes 2 and 3). To determine the origin of this relaxing activity, we performed plasmid relaxation assays in the presence of Top1 and Top2 inhibitors. Figure 1D indicates that the relaxing activity observed in the presence of Gadd45 and the core histones was inhibited by 10 μM camptothecin, a Top1 inhibitor (23, 26). On the other hand, VM-26 and VP-16, two Top2 inhibitors (52), were ineffective (data not shown). Since the topoisomerases are associated with chromatin (42), we reasoned that trace amounts of topoisomerases could have coeluted with the core histones during purification. To test this possibility, we performed Western blot analysis with increasing amounts of core histones and found that with 100-fold more histones than what was used in the assay described above, Top1 could be detected by immunoblot (Fig. 1E). Consistent with our observation that the Top2 inhibitors were ineffective at blocking this effect, Top2 was not detected by immunoblotting (data not shown) in the histone preparation. Thus, these data indicate that traces of Top1 in the core histone preparation relaxed the DNA in the presence of Gadd45 but not NAP-1 (Fig. 1C, lanes 1 to 3). Gadd45 capacity to facilitate Top1 activity in the presence of core histone could conceivably be mediated by a direct interaction between Gadd45 and the core histones, resulting in increased Top1 accessibility to the supercoiled DNA. This possibility was investigated further.

The facilitation of circular DNA relaxation in the presence of core histones by Gadd45 was rather specific and not due simply to nonspecific charge interaction between Gadd45 and the core histone since, as mentioned above, the recombinant NAP-1 protein (Fig. 1C, lane 1), which has a similar charge (pI = 4.03, compared with 4.16 for Gadd45) and was purified from the same bacterial strain (*E. coli* BL21) under similar conditions as Gadd45, did not mediate the effect. Moreover, a Gadd45 deletion mutant (Δ72-166; the resultant protein contains the first 71 amino acids), also purified under the same conditions as the full-length Gadd45 protein, was ineffective in the relaxation assay (Fig. 1C, lane 7). To further address the issue of a possible contaminant, we also purified Gadd45 from a baculovirus system and found it to be as active as the protein purified from the bacterial system (data not shown), supporting the contention that the activity is specific to Gadd45. These results underscore the specificity for Gadd45 activity and stress the importance of the C-terminal end of Gadd45 for the relaxation effect. To narrow down the Gadd45 active domain, we evaluated another Gadd45 deletion mutant, Δ125-166, containing the first 124 amino acids. As indicated in Fig. 1C, lane 8, Δ125-166 was as effective as the full-length Gadd45 protein in this assay, indicating that Gadd45 activity probably depends on the Gadd45 region localized between amino acids 72 and 124. This domain has not been associated previously with any particular function or interaction but contains some overlap with regions apparently interacting with PCNA (24) and $p21^{Cip1/Waf1}$ (1).

There are at least two possible explanations for Gadd45 effect on facilitation of Top1 relaxing activity in the presence of core histones: (i) Gadd45 somehow activates the small amount of Top1 present in the assay, and (ii) Gadd45 interacts directly with the histones and facilitates Top1 accessibility to the DNA. We thus investigated the effects of Gadd45 in a plasmid relaxation assay in the absence of core histones and in the presence of increasing amounts of Top1. The data presented in Fig. 2A indicate that Gadd45 does not increase Top1 relaxing activity; if anything, it slightly inhibits it at lower concentration. Hence, it appears that Gadd45 does not have a direct stimulatory effect on Top1. The possibility that Gadd45 mediates its effect through direct interaction with the core histones was then investigated.

Gadd45 interacts with core histones. To determine whether Gadd45 could directly interact with the core histones, we performed a far-Western analysis with *Drosophila* core histones. Figure 2B indicates that under physiological salt concentration (NaCl at 150 mM), recombinant Gadd45 binds to all four core histones, either underacetylated or hyperacetylated. In the absence of recombinant Gadd45 during the preincubation step (Fig. 2B, No Gadd45), no binding to the core histones was detected in the Western blot analysis. The lack of binding was not due to poor reaction conditions since recombinant Gadd45 protein used as a positive control was detected. This finding indicates that Gadd45 antibody did not cross-react with the core histones. When the far-Western analysis was performed in high salt (NaCl at 1.5 M), binding to all four core histones was reduced but binding to histone H3 from both normal and hyperacetylated core histones was still clearly detectable, suggesting that Gadd45 might have a stronger affinity for histone H3. The stoichiometry of each respective core histone (H2A, H2B, H3, and H4) from either *Drosophila* or HeLa cells was estimated to be equimolar to the other, based on silver staining of SDS-polyacrylamide gels (20). Thus, the increased binding of Gadd45 to histone H3 is not due to a higher amount of H3

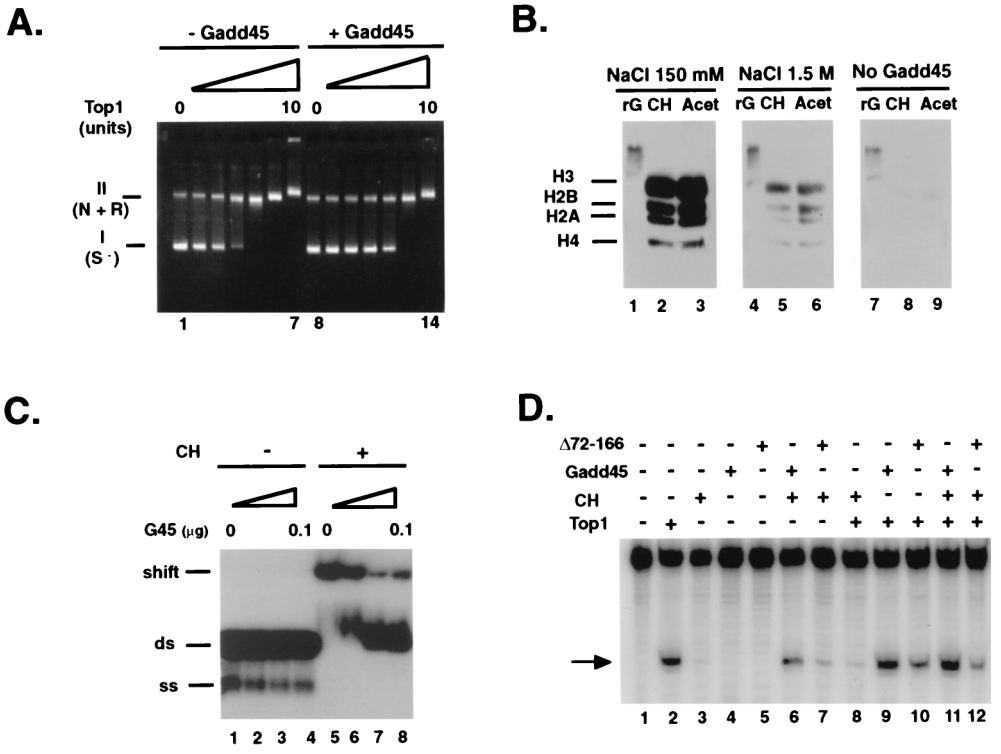

FIG. 2. Gadd45 interacts with core histones. (A) Plasmid relaxation assay performed as for Fig. 1A but in the absence of core histones. The assay was performed in the absence (lanes 1 to 7) or presence (lanes 8 to 14) of Gadd45 (1 μg). (B) Far-western analysis performed with 1 μg of normal (CH; lanes 2, 5, and 8) or hyperacetylated (Acet; lanes 3, 6, and 9) *Drosophila* core histones. Recombinant Gadd45 (rG; 15 ng) was used as a positive control (lanes 1, 4, and 7). The proteins were loaded on a 18% polyacrylamide gel, transferred to nitrocellulose, and processed as described in Materials and Methods. The blots were prehybridized in the absence (No Gadd45) or in the presence of 1 μg of recombinant Gadd45 protein per ml prior to Western blot analysis with Gadd45 polyclonal antibody. (C) Increasing amounts (0 to 100 ng) of Gadd45 were preincubated in the absence (−) or presence (+) of core histones (CH; 100 ng) before addition of a labeled oligonucleotide. The reaction products were run on a nondenaturing 4 to 20% polyacrylamide gel and exposed to X-ray film. The histone-DNA complex (shift), the double-stranded DNA probe (ds), and the residual single-stranded DNA (ss) are indicated. (D) The Top1 cleavage assay was performed in the presence of 10 μM camptothecin as described in Materials and Methods. A double-stranded DNA oligomer (30-mer) containing one Top1 site was labeled at the 3′ end of the sense strand and incubated in the presence (+) or absence (−) of core histones (CH; 0.4 μg), Gadd45 (0.5 μg), and Top1 (10 U). A 19-mer oligonucleotide fragment is released after cleavage by Top1 (lane 2, arrow).

in the histones preparation. Detection of protein interactions under high-salt conditions suggests that hydrophobic interactions might also be involved. Binding of Gadd45 to hyperacetylated histones appeared higher under both low- and high-salt conditions. This finding also suggests that the interaction between Gadd45 and the core histone is probably not limited to charge interactions between the basic histone tails and the acidic Gadd45 protein. Similar data were also obtained with normal (underacetylated) histones from HeLa cells (data not shown).

The consequence of a Gadd45 interaction with the core histones on chromatin stability was evaluated on complex formation of free histones with DNA and, as described below, on preassembled mononucleosomes. As shown in Fig. 2C, increasing amounts of Gadd45 were preincubated in the absence or presence of core histones, then a labeled oligonucleotide was added to the reaction mixture, and the mixture was incubated further. As expected, addition of core histone to the DNA in the absence of Gadd45 retarded the oligomer mobility (lane 5), indicating interaction between the core histones and the DNA. Addition of Gadd45 resulted in an increased mobility of the oligonucleotide comparable to free DNA (lanes 6 to 8). These data indicate that up to 100 ng of Gadd45 did not affect the DNA mobility (lanes 2 to 4) but interfered with histone-DNA interactions in a dose-dependent manner.

In Fig. 1, we demonstrated that Gadd45 could facilitate Top1 relaxing activity in the presence of core histones. In an effort to determine whether Gadd45 could also facilitate Top1 cleavage activity in the presence of core histones, a classical Top1 cleavage assay (51) was performed on a small fragment of DNA (30 bp) in the presence of core histones (Fig. 2D). The Top1 inhibitor camptothecin was added to the reaction mixtures to facilitate the detection of the Top1-DNA cleavage intermediates. In this assay, a 19-mer DNA intermediate accumulates due to the inhibition of the Top1 religation step by camptothecin. As expected, Top1-induced DNA cleavage generated a 19-mer oligonucleotide from the 30-bp probe (Fig. 2D, arrow, lane 2) containing one Top1 site. Addition of core histone to the assay (lane 8) markedly reduced the capacity of Top1 to cleave the DNA. In the presence of Gadd45, the inhibitory effect observed with the core histones (Fig. 2C, lane 8) was relieved (lane 11) to levels comparable to those for the enzyme alone (lane 2). A similar effect was observed with the trace amounts of Top1 contained in the core histones preparation (lanes 3 and 6). A partial restoration of the Top1 activity was also observed with Gadd45Δ72-166 (lane 12), but to a lesser extent than with the intact protein. The effect of the Gadd45 deletion mutant thus appears to be slightly different from that seen in the plasmid relaxation assay (Fig. 1C), where no effect was detected. This could be due to the presence of camptothecin, which increases the sensitivity of the cleavage assay. Our data indicate that the Gadd45 full-length protein

not only facilitates Top1 relaxing activity in the presence of core histones (Fig. 1) but also facilitates Top1 cleavage activity under similar conditions.

Evidence that Gadd45 facilitates topoisomerase activity in vivo. The capacity of Gadd45 to facilitate topoisomerase activity in the presence of core histones may also play a role in DNA accessibility in vivo. In the presence of specific inhibitors, the topoisomerases enzymes can form stable covalent complexes with the DNA (75). Thus, a difference in the accessibility of cellular DNA to topoisomerases should be reflected in the cellular sensitivity to topoisomerase inhibitors. To determine whether Gadd45 could influence cellular sensitivity to topoisomerase inhibitors, we first compared the expression of *GADD45* to the cellular sensitivity to a variety of cytotoxic agents in a panel of human tumor lines. We had previously reported that a subset of the p53 wt lines lacked the normal *GADD45* responsiveness seen in other p53 wt lines (2); we considered the possibility that this attenuated expression after stress can affect chemosensitivity. An initial analysis of the NCI anticancer drug screen (76), using a set of 141 agents with well-defined mechanisms of action, indicated that the sensitivity to topoisomerase inhibitors was significantly less in this subset of 6 lines compared to 10 other lines among the 16 lines having a wt p53 genotype (data not shown). This initial study was expanded to encompass the approximately 50,000 compounds that had been tested in the NCI screen at the time of this analysis. The compounds were divided into three groups for comparison: one group composed of 421 Top2 inhibitors (identified by using the COMPARE computer program), another composed of 167 Top1 inhibitors (identified as camptothecin analogs), and the other composed of a very diverse group of remaining agents. Since these lines are not isogenic and many variables affect the actual sensitivity, Pearson correlation coefficients were calculated, using the levels of *GADD45* induction (2) as one variable and cytotoxicity as the other (data not shown). A Wilcoxon rank sum test showed that compounds in both of the topoisomerase inhibitor groups were more highly associated with *GADD45* induction than the bulk of compounds tested in the screen to date ($P < 0.0001$); this finding indicates that higher *GADD45* expression after stress correlated with enhanced sensitivity to Top1 and Top2 inhibitors. To more clearly show the difference in sensitivity for this subset of p53 wt lines, the Wilcoxon one-tailed *P* values are shown in Fig. 3A, where the sensitivity to specific agents was compared to whether or not the cell line was a member of this subset. For 400 Top2 inhibitor-type compounds the *P* values clustered near 1, while an essentially random distribution of *P* values was seen for 43,000 other compounds.

Since the above results are only a statistical correlation in a group of diverse cell lines, more direct evidence for a role for *GADD45* in affecting the cellular sensitivity to topoisomerase inhibitors was tested by using an antisense approach in the RKO human colon carcinoma line. This p53 wt line has been found to express an appreciable level of Gadd45 protein with further increase after stress (10). A subline, designated RKO-AS45, that stably expresses high levels of antisense *GADD45* RNA and has been shown to have markedly reduced expression of Gadd45 protein with no appreciable accumulation after stress has been established (59). An alternative approach would have been to determine cellular sensitivity to topoisomerase inhibitors in cell lines over expressing Gadd45. However, overexpression of Gadd45 causes growth arrest (83) and thus precludes the feasibility of such an experiment. The antisense approach was then investigated. While the RKO-AS45 line grew similarly to the parent line and showed similar sensitivity to ionizing radiation, the results shown in Fig. 3B and C demonstrate that this line was substantially less sensitive to both the Top1 inhibitor camptothecin and the Top2 inhibitor VP-16, as determined by clonagenic survival. In the case of camptothecin, the increased survival for RKO-AS45 was seen at every dose used and the biphasic shape of the survival curves is typical for results with this agent (23). With VP-16, cell killing was more pronounced, with a 3-log kill for RKO cells after the dose of 50 μM; in contrast, the survival at the same dose for RKO-AS45 was nearly 100 times greater. As mentioned above, topoisomerase inhibitors like VP-16 stabilize the covalent linkages formed between Top2 and DNA (52), and changes in cellular sensitivity should also be reflected by changes in the level of the linkages formed. As shown in Fig. 3D, this was the case for VP-16, where the level of linkages (DNA-protein cross-links formed) was measured by alkaline elution. At both 50 μM and a saturating dose of 100 μM, the level of cross-links was reduced by approximately half in the RKO-AS45 cells. These results are consistent with our hypothesis that Gadd45 facilitates chromatin accessibility to topoisomerases and that reduced levels of Gadd45 lead to the formation of fewer stabilized DNA-protein complexes in the presence of topoisomerase inhibitors.

Gadd45 interacts with altered mononucleosomes. Interaction of Gadd45 with the core histones (Fig. 2A) and destabilization of histone-DNA complex formation (Fig. 2B) indicated that Gadd45 could potentially affect chromatin structure. To evaluate this possibility, we analyzed the effect of Gadd45 on preassembled mononucleosomes. The mononucleosomes were reconstituted with either underacetylated or hyperacetylated core histones purified from *Drosophila* cells. The data presented in Fig. 4A indicate that increasing amounts (0 to 1 μg; lanes 1 to 4) of Gadd45 had no significant effect on the mobility of mononucleosomes assembled with underacetylated histone. Thus, even though Gadd45 could prevent histone-DNA interaction (Fig. 2C), it did not dissociate preassembled mononucleosomes.

When comparable experiments were repeated with mononucleosomes assembled with hyperacetylated histones (Fig. 4A, lanes 5 to 8), a shifted band clearly appeared in the presence of Gadd45. Densitometry analyses, performed in four different experiments, indicate a difference of approximately threefold between the normal and acetylated shifted complex in the presence of 1 μg of Gadd45 (data not shown). To determine whether the shift band resulted from Gadd45 association with free DNA or with nucleosomal DNA, excess amounts of Gadd45 were incubated with the hyperacetylated nucleosomes (lanes 9 to 11). The results obtained indicate that large amounts of Gadd45 can deplete the nucleosomal DNA from the nucleosomes and form a complex of slower mobility (shift). The presence of Gadd45 in the shifted band was confirmed by complete competition of the shifted band with Gadd45 antibody (data not shown; same as UV damage [see below]) and by Western blot analysis of the shifted band (data not shown). This result suggested that in contrast to the normal nucleosomes, hyperacetylated nucleosomes could interact with Gadd45, implying that structural modification of the mononucleosome could facilitate interaction with Gadd45. Other types of modifications that could potentially affect nucleosomal structure were therefore investigated.

UV-induced cyclobutane pyrimidine dimers and (6-4) photoproducts are likely to alter chromatin structure at the site of damage. In order for these dimers to form, an extensive rotation of the pyrimidines from the usual B-form DNA alignments must occur to allow the formation of dimer covalent bonds. In the vicinity of such dimers, the DNA is unwound and kinked by about 30° relative to the B-form DNA (27) and is

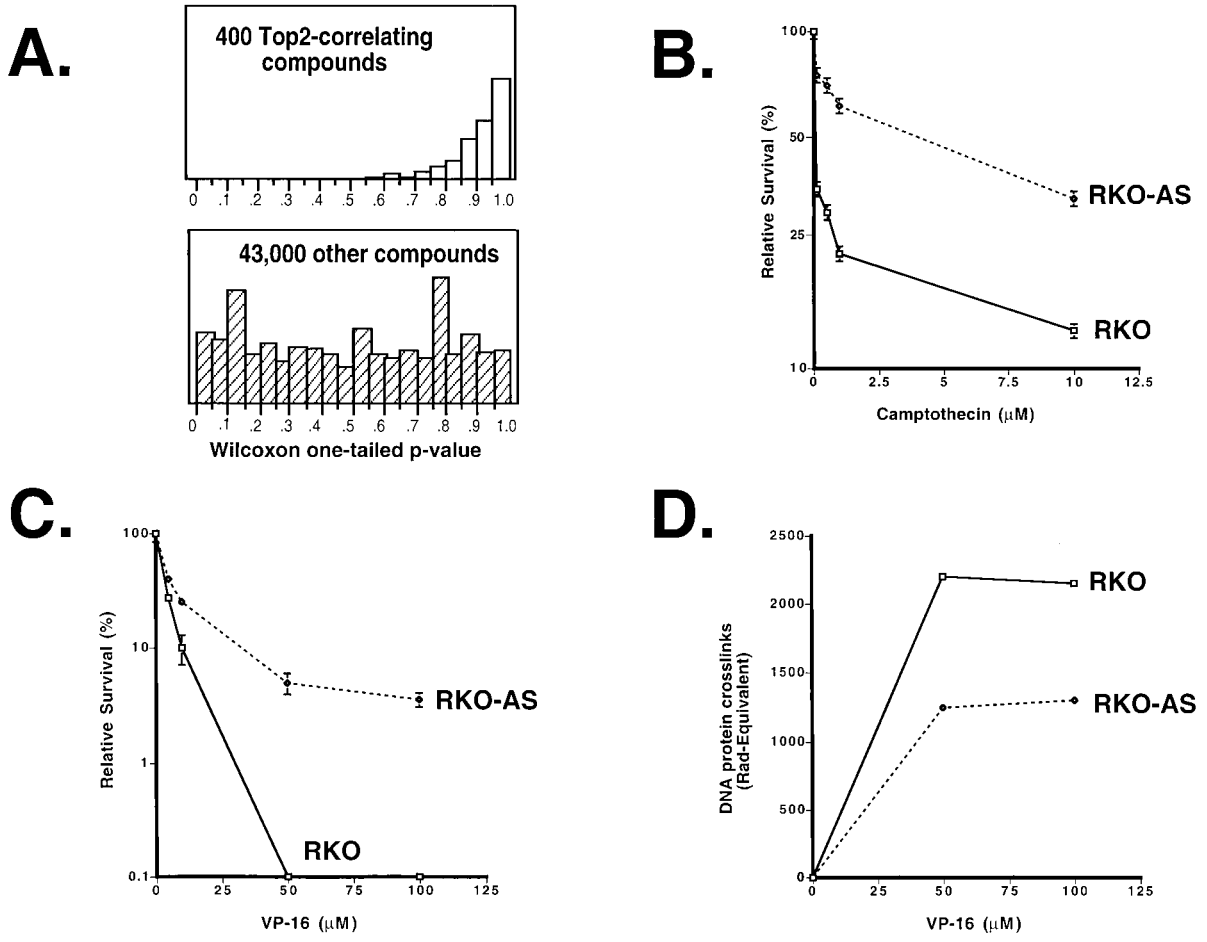

FIG. 3. Evidence that Gadd45 can modulate topoisomerase sensitivity in vivo. (A) Wilcoxon rank sum test was used to determine whether individual compounds tested in the NCI anticancer drug screen were more active in cell lines with competent *GADD45* induction ($P = 1$) or more active in cell lines with deficient *GADD45* induction ($P = 0$). Results for a selection of COMPARE-identified topoisomerase II inhibitors (top) and for the remainder of the database of tested compounds (bottom) are presented. (B) Clonagenic survival after treatment with the Top1 inhibitor camptothecin was determined in the human RKO colon carcinoma line and its derivative RKO-AS45, which were stably transfected with vector alone and with vector containing *GADD45* cDNA in the antisense orientation, respectively. Colonies were counted 2 weeks after treatment. Survival is expressed as the percentage of colonies obtained with untreated cells. (C) Survival analysis performed as in for panel B with VP-16, a Top2 inhibitor. (D) DNA-protein cross-links produced in RKO and RKO-AS45 cells after treatment with VP-16 as measured by alkaline elution.

probably more distorted in the case of (6-4) dimers since the pyrimidine planes are almost perpendicular (17). The effect of Gadd45 on mononucleosomes altered by UV damage was therefore investigated. As indicated in Fig. 4B, incubation of Gadd45 with UV-irradiated mononucleosomes led to the formation of a shift complex in a Gadd45 dose-dependent manner. As was the case with the hyperacetylated nucleosome, the shift band was competed with Gadd45 antibody (lane 5). Incubation of a large excess of Gadd45 with UV-irradiated nucleosomes containing less than 5% free DNA (Fig. 4C) also resulted in the association of the nucleosomal DNA with Gadd45 complex. These data indicate that Gadd45 can recognize and associate with UV-damaged DNA on mononucleosomes. We have also investigated the effect of Gadd45 on irradiated mononucleosome assembled with hyperacetylated histones (data not shown). A shifted complex was also formed in the presence of Gadd45, but no increase in the binding intensity was noticed. Thus, the effect of histone acetylation and UV irradiation does not appear to be additive on individual nucleosomes. Binding of Gadd45 to UV-irradiated histone-free DNA was also investigated. Figure 4D indicates that Gadd45 binds preferentially to UV-irradiated DNA in a dose-dependent manner (lanes 7 to 12). Analyses of the percentage of retarded DNA indicate that Gadd45 binds approximately 1.5 times more efficiently to UV-irradiated DNA on mononucleosomes than to histone free UV-irradiated DNA.

Effect of Gadd45 on DNA accessibility. By interacting with mononucleosomes, Gadd45 could potentially modify DNA accessibility. To evaluate this possibility, we analyzed DNase I sensitivity on free and nucleosomal DNA in the presence of Gadd45 (Fig. 5). As indicated in Fig. 5A, increasing amounts (0 to 50 ng) of Gadd45 did not alter the DNase I cleavage pattern of free DNA (lanes 1 to 3). On mononucleosome assembled with underacetylated histones (Fig. 5A, Norm), an approximately 10-bp cleavage periodicity typical for a nucleosome (68) was obtained (lane 4). As was the case for naked DNA, addition of Gadd45 (25 and 50 ng) to the reconstituted mononucleosome (lanes 5 and 6) did not significantly alter the DNase I cleavage pattern. However, addition of Gadd45 to preassembled hyperacetylated mononucleosomes slightly, but consistently, increased DNase I sensitivity at several sites (Fig. 5A, lanes 8 and 9). Two representative sites are shown in Fig. 5B, where increased DNase I sensitivity was detected at Gadd45/histone ratios of 0.65:1.0 and 1.3:1.0 on hyperacety-

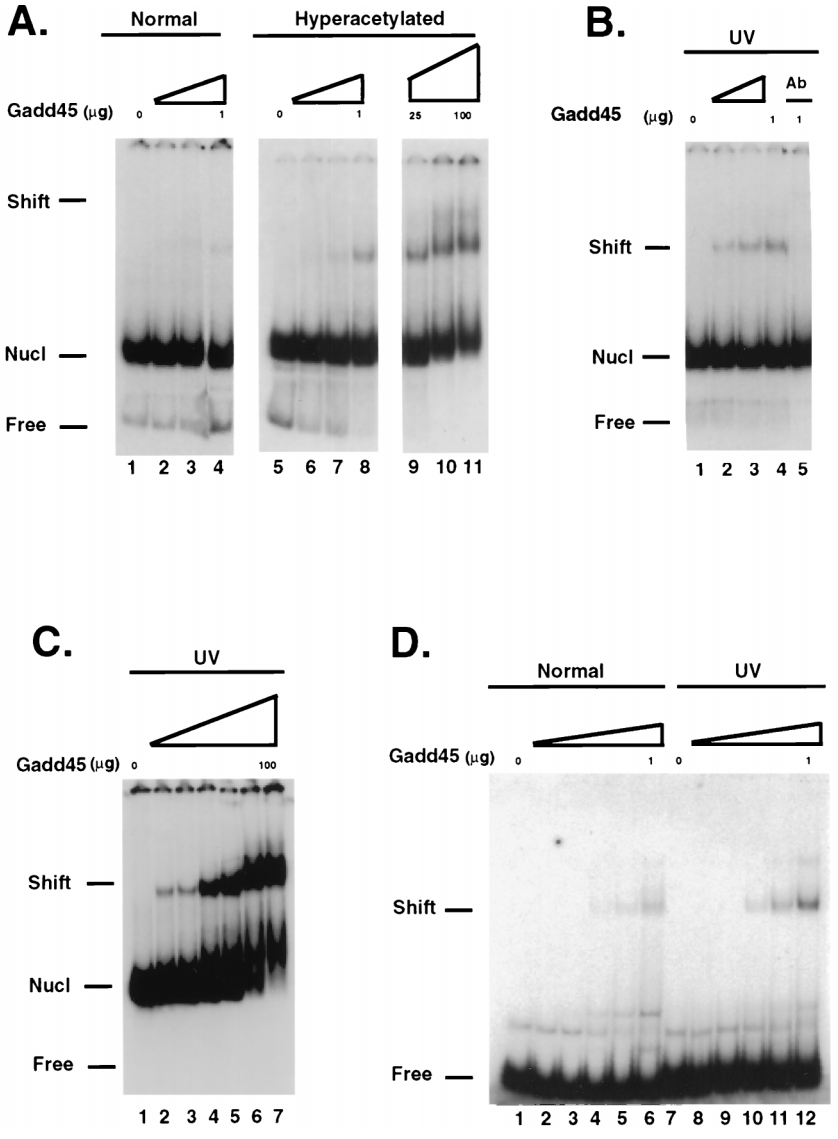

FIG. 4. Gadd45 interacts with altered mononucleosomes. (A) Mobility shift assay performed on underacetylated (normal) mononucleosomes (lanes 1 to 4) or hyperacetylated mononucleosomes (lanes 5 to 11) in the presence of increasing amount (0 to 1 μg [lanes 1 to 4 and 5 to 8] or 25 to 100 μg [lanes 9 to 11]) of Gadd45. The binding conditions were as described in Materials and Methods. (B) Mobility shift assay as in panel D except that UV (7,500 J m^{-2})-irradiated mononucleosomes were used in the reactions. In lane 5, Gadd45 monoclonal antibody 30T-14 (Ab) was added to the reaction. (C) Mobility shift assay as in panel D but performed with an excess amount (0 to 100 μg) of Gadd45 on UV-irradiated mononucleosomes. In panels A to C, positions of free DNA, nucleosomes (Nucl), and a shifted band are indicated. (D) Mobility shift assay as in panel B but on free DNA.

lated mononucleosomes only. To quantitate the effects of Gadd45, scanning analysis of representative bands was performed. A typical scanning analysis, shown in Fig. 5C, indicates that Gadd45 had no effect on the DNase I cleavage sites on normal nucleosomes but increased DNase I sensitivity in a dose-dependent manner on the hyperacetylated nucleosomes. An increase of at least 22% in DNase I sensitivity was invariably measured on several bands (Fig. 5A, bands A to E) of the hyperacetylated mononucleosomes in the presence of Gadd45 (50 ng). Given the small amount of complex shifted by Gadd45 on hyperacetylated mononucleosomes (Fig. 4A), the modest increase in DNase I is not unexpected.

To verify that the differences observed in the scanning analysis (Fig. 5C) were not due to loading differences, the intensity of each band was normalized to the total number of counts in each lane. An average of three typical DNase I experiments is represented in Fig. 5D, where five representative bands (Fig. 5A, bands A to E) were analyzed. On each band (A to E), DNase I sensitivity was consistently increased in the presence of 50 ng of Gadd45 on hyperacetylated mononucleosomes, while addition of Gadd45 had only a minimal effect on the DNase I sensitivity of the underacetylated mononucleosomes. We also evaluated DNase I sensitivity in the presence of Gadd45 of UV-irradiated hyperacetylated mononucleosomes (data not shown); even though a similar increased accessibility was noticed in the presence of Gadd45 (at least 20%), no additive effect was observed on mononucleosomes.

Gadd45 also interacted with UV-irradiated mononucleosomes (Fig. 4B and C). To determine the Gadd45 effect on local DNA accessibility at sites of DNA damage in the UV-irradiated nucleosomes, the cyclobutane dimer-specific enzyme T4 Endo V was used as a probe to demonstrate a change

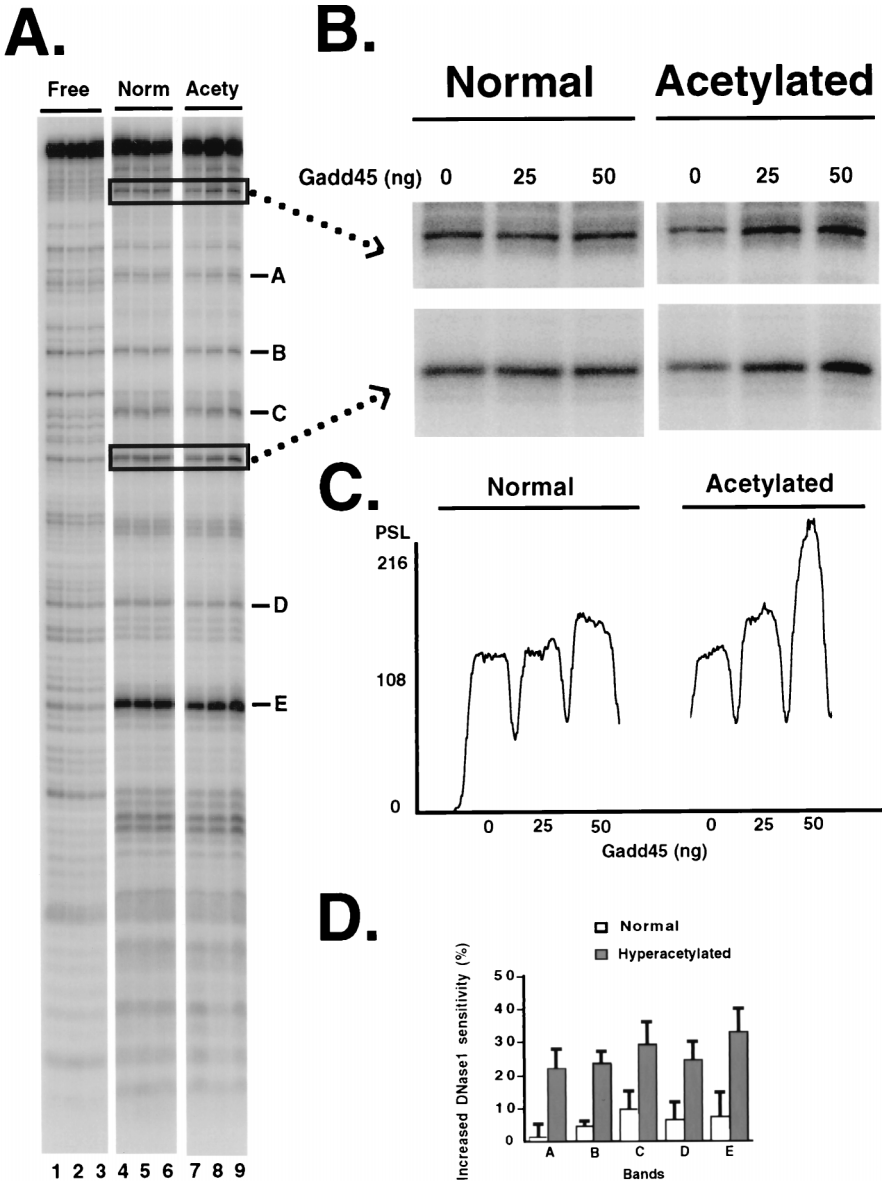

FIG. 5. Gadd45 increases DNase I sensitivity on hyperacetylated mononucleosomes. (A) DNase I digestion pattern of naked DNA (Free; lanes 1 to 3), underacetylated (Norm; lanes 4 to 6), and hyperacetylated mononucleosomes (Acety; lanes 7 to 9) in the presence of increasing amounts of Gadd45 (0 to 50 ng). Digestion was performed as described in Materials and Methods. (B) Magnification of two typical bands from the DNase I digestion pattern shown in panel A. (C) Scanning analysis of band B, shown in panel A, performed with the program MacBAS version 2.0. The intensity of each individual band is expressed in pixels (PSL). (D) Average of three typical DNase I experiments. Increased DNase I sensitivity was analyzed on all bands; analysis of representative bands A to E, as indicated in panel A, is shown. The intensity of bands A to E was normalized to the total number of counts in each lane. The normalized number obtained in the absence of Gadd45 was assigned a DNase I sensitivity value of 100% (0% increased sensitivity). The increased DNase I sensitivity is expressed as the differences between the percentage of DNase I sensitivity obtained in the absence of Gadd45 and in the presence of 50 ng of Gadd45.

in chromatin accessibility near pyrimidine dimers. This bacterial phage enzyme, which has no equivalent in mammalian cells, cleaves DNA at the site of pyrimidine dimers via a combined action of a pyrimidine dimer-specific DNA glycosylase and an apyrimidinic-apurinic endonuclease action (22). The T4 Endo V enzyme does not recognize (6-4) photoproducts and is ineffective on undamaged DNA. The data presented on Fig. 6 indicate that incubation of T4 Endo V with the UV-irradiated mononucleosome (Fig. 6, lane 7) generated a cleavage pattern similar to the one observed on naked DNA, but the intensity of each site was substantially reduced. The differences between the naked and nucleosomal cleavage sensitivity probably reflects a difference in accessibility of T4 Endo V rather than a difference in the formation of the dimers since in contrast to (6-4) photoproducts, cyclobutane dimers occur randomly on chromatin (43). Addition of Gadd45 at a 1.3:1.0 ratio of histone (lane 8) prior to the incubation with T4 Endo V (10 ng) resulted in further protection of most of the nucleosomal sites. Since Gadd45 did not affect the T4 Endo V cleavage efficiency on naked DNA (lane 4), it is likely that the effect observed on the UV-damaged nucleosomes is mediated through a direct interaction of Gadd45 with damaged regions of the nucleosome. An effect of similar intensity was observed with mononucleosomes assembled with hyperacetylated his-

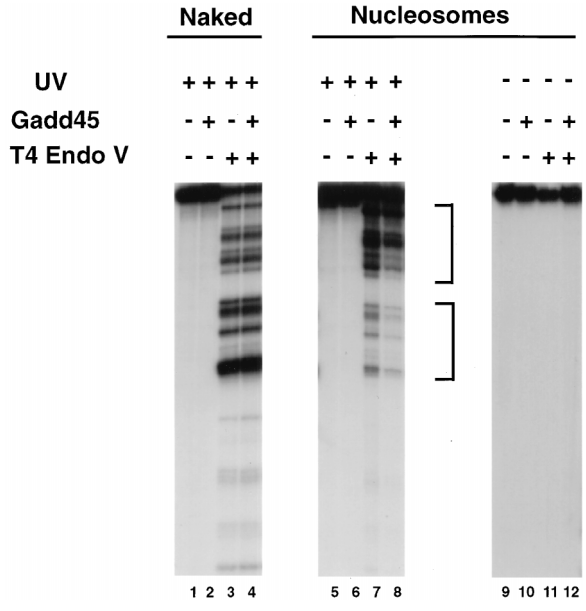

FIG. 6. Gadd45 protects UV-irradiated mononucleosomes from T4 Endo V digestion. Free DNA (Naked; lanes 1 to 4) and mononucleosomes (Nucleosomes; lanes 5 to 8) were UV (7,500 J m^{-2})-irradiated and incubated in the absence (−) or presence (+) of 50 ng of Gadd45 prior to the addition of T4 Endo V (10 ng). The reaction was performed as described in Materials and Methods. Bands protected by Gadd45 are indicated by brackets.

tones (data not shown). As expected, the enzyme was ineffective on undamaged probe (lanes 9 to 12).

The possibility that Gadd45 could act as a general facilitator of chromatin accessibility was investigated with different restriction enzymes. Digestions were performed on normal and altered nucleosomes with *Mnl*I, *Sfa*NI, and *Eco*NI. No increase accessibility was detected in the presence of Gadd45 (data not shown). Not all proteins affecting the chromatin structure increase accessibility to restriction enzyme. For example, the nucleosome remodeling factor NURF does not stimulate the sensitivity to restriction endonucleases (71).

DISCUSSION

Gadd45 is a stress response protein regulated in part by the tumor suppressor p53 (31). Our data indicate that Gadd45 can recognize damaged chromatin (Fig. 6) and modify chromatin accessibility. This report demonstrates for the first time a direct association between a cellular protein with damaged chromatin and suggests a mechanism by which p53 may potentially regulate some aspect(s) of chromatin accessibility following DNA damage.

The data presented in this report indicate that Gadd45 facilitates topoisomerase relaxing and cleavage activity in the presence of core histones (Fig. 1 and 2). This effect could be mediated through destabilization of histone-DNA interactions, since Gadd45 interacts directly with the core histone (Fig. 2B) and prevents the formation of histone-DNA oligomer complexes (Fig. 2C). The topoisomerase enzymes are relatively abundant in vivo, it has been estimated that Top1 is bound to active nucleosome at a molar ratio of 1:5 (78). In normal lymphocytes there are approximately 7×10^3 copies of Top2 enzyme per cell (53). The Gadd45 protein is of low abundance; as estimated by Western blot analysis, there are only a few femtomoles of Gadd45 per 10^6 cells (11). Gadd45 could affect topoisomerase activity by modifying chromatin accessibility locally, especially at sites altered by either histone acetylation or UV radiation. Our data suggest that Gadd45 could be recruited at these sites, which would increase its local concentration. Modification of chromatin accessibility by increased local concentration of an acidic protein has been proposed before (62).

Previous studies have shown that nucleosome configuration reduces the accessibility of Top2 to DNA, with resultant preferential cleavage in linker regions (9). Since the topoisomerase inhibitors form a ternary complex between the enzyme and the DNA, covalently trapping the enzyme on the DNA (12, 23, 75), in vivo sensitivity to topoisomerase inhibitors can reflect, at least to some extent, the level of DNA accessibility. Our data indicate that cell lines having reduced Gadd45 expression are more resistant to topoisomerase inhibitors (Fig. 3). Resistance to topoisomerase inhibitors has been associated with different parameters such as topoisomerase protein levels and reduction of cleavable complexes (23). The levels of topoisomerase proteins were the same in the two cell lines used here (19), and as shown in Fig. 3, cleavable complexes were reduced in the *GADD45* AS cells. Gadd45 could potentially contribute to cellular sensitivity to topoisomerase inhibitors by affecting aspects of chromatin compaction and thus DNA accessibility. Gadd45 binds to all four core histones (Fig. 2A) but also to histone H1 (data not shown). Previous reports (30) have indicated that addition of polyglutamic acid, which competes out histone H1, to nuclei resulted in unfolding of chromatin and consequently activation of Top2 cleavage. Gadd45 is a low-abundance acidic protein, but it has been reported that even a partial loss of histone H1 may be sufficient to induce unfolding (29). It seems therefore conceivable that Gadd45 could associate with a subpopulation of histone H1 and modify to some extent chromatin folding and thus sensitivity to topoisomerase inhibitors in vivo. Nucleolin, a protein containing long stretches of acidic residues, is also unable to act as a nucleosome assembly or disassembly factor in an assay similar to the one use in this study (14). However, nucleolin induces chromatin decondensation, possibly through interactions with histone H1 (14).

The importance of chromatin structure for topoisomerase activity is becoming more evident in light of new studies showing the effect of UV light-induced DNA distortion on topoisomerase activity. For example, damage produced by short-wave UV radiation interfered with the proper catalytic activity of Top1 (40), resulting in a stimulation of cleavage complexes in the absence of a Top1 poison. The UV-stimulated Top1 cleavage sites are apparently in close proximity to cyclopyrimidine clusters (64). These findings lead to the suggestion that Top1 can sense the helical distortions (40, 64) since it also responds to DNA mismatches and abasic sites (54). The possibility that Gadd45 can influence topoisomerase activity through chromatin modulation is appealing since in an in vivo situation where Gadd45 is induced by DNA-damaging agents, including UV radiation, DNA accessibility could be modulated locally (at damaged or hyperacetylated sites, for example) and facilitate Top1 activity. However, in contrast to DNase I and T4 Endo V, Gadd45 did not modify topoisomerase accessibility on mononucleosomes (data not shown). This might indicate that in vivo, Gadd45 interacts with other chromatin-associated proteins to mediate its effect or that long stretches of chromatin are required to detect modification of topoisomerase accessibility (55).

Protein complexes interacting with chromatin are usually large entities formed of several subunits. For example, the recently identified *Drosophila* chromatin remodeling complex CHRAC (71) has a mass of 670,000 Da and is formed of five

subunits. One of the CHRAC subunits has been identified as Top2, and two unidentified subunits have smaller masses of about 18 to 20 kDa. The masses of these small subunits are in the same range as for the core histone but also as for Gadd45. We have found no Gadd45-like proteins in *Drosophila*; however, in the event that CHRAC mammalian homologues are identified, it would be of interest to determine whether Gadd45 is part of such complex. Recent evidence has indicated that chromatin condensation can be regulated through phosphorylation of a protein complex named condensin (33). The phosphorylation and subsequent activation of condensin are mediated by the Cdc2/cyclin B1 kinase activity (33). It is thus expected that perturbation of the Cdc2/cyclin B1 complex could cause chromatin decondensation due to condensin hypophosphorylation (33). This could represent an indirect mechanism by which Gadd45 affects chromatin structure, since a most recent study has indicated that Gadd45 inhibits Cdc2/cyclin B1 kinase activity by dissociating this protein kinase complex (86).

The mechanism by which Gadd45 facilitated Top1 activity in the presence of core histone could be related to its highly acidic charge (10). Almost two decades ago, Stein et al. (62) suggested that acidic proteins could play a fundamental role in increasing accessibility of nucleosomal DNA in chromatin. It was proposed that at high local concentrations, the acidic proteins could displace histone octamers at low energy cost by simply transferring the octamers to the histone-binding proteins. Such a mechanism has been suggested for the small acidic protein nucleoplasmin in its role in nucleosome assembly (13). The binding of acidic proteins to the histones tails is thought to reduce the ionic affinity of the histones for DNA and allow an orderly transfer of histones to DNA, resulting in the formation of nucleosome cores (13). The direct interaction of Gadd45 with the core histones (Fig. 2B) suggests that Gadd45 could reduce locally the histone affinity for DNA binding on the chromatin, possibly by such a competing mechanism. Since addition of significant amounts of histones to DNA under physiological conditions often leads to precipitation in the absence of acidic components, Gadd45 being an acidic protein could have simply increase DNA solubility in the presence of core histone. However, this is unlikely since NAP-1, another acidic protein similar in charge to Gadd45, did not produce the effects observed with Gadd45 (Fig. 1C). Moreover, direct interaction of Gadd45 with preassembled mononucleosomes (Fig. 4A to C) and consequent modification of DNA accessibility (Fig. 5 and 6) supports the contention that Gadd45 activity is through perturbation of histone-DNA interactions and not the result of nonspecific aggregation. In addition, Gadd45 also interacted with hyperacetylated mononucleosomes (Fig. 4 and 5), where it is expected that neutralization of the histone tails would reduce the chances for nonspecific charge interactions (7, 41).

Gadd45 preferentially interacted with mononucleosomes that have been modified by histone acetylation or altered by UV radiation (Fig. 4A to C). Gadd45 caused a modest increase in DNase I sensitivity on hyperacetylated mononucleosomes (Fig. 5) and substantially reduced T4 Endo V accessibility to cyclobutane pyrimidine dimers on mononucleosomes but not on naked DNA (Fig. 6). The bacterial phage repair enzyme T4 Endo V was used as a probe to demonstrate a change in chromatin accessibility near pyrimidine dimers in the presence of Gadd45. The change in cleavage with Gadd45 is consistent with association of this protein with local regions containing pyrimidine dimers. The apparently different effects of Gadd45 on DNA accessibility on mononucleosomes, simply indicate that Gadd45 favors interaction with altered chromatin structure. The level of histone acetylation is usually an indication of the chromatin transcriptional state since hyperacetylation of histones correlates with transcriptionally active or poised genes while underacetylation is associated with transcriptionally repressed chromatin (7, 41, 80). So far, we have found no evidence indicating that Gadd45 can play a role in transcription. Nonetheless, increased levels of histone posttranslational modification are not restricted to transcriptional competence. In general, these modifications are thought to locally disrupt chromatin structure and facilitate overall DNA accessibility. For example, increased levels of histone acetylation (56) and ADP-ribosylation (3) have been shown to correlate with greater DNA repair efficiency. Histone acetylation has also been shown to be induced by UV irradiation, and it has been suggested that long stretches of acetylated nucleosomes adjacent to UV lesions can facilitate the local opening of chromatin at the sites of lesion (55). Formation of UV-induced cyclobutane pyrimidine dimers is also likely to distort chromatin structure. In order for those dimers to form, extensive rotation of the pyrimidines must occur, causing DNA to unwind and kink by about 30° in the vicinity of the dimers (27). Our experimental approach using mainly individual nucleosomes did not detect an additive effect of histone acetylation and UV damage. However, it remains possible that in vivo, Gadd45 contributes to locally stabilize an open state of chromatin and modulate DNA accessibility to cellular proteins activated by DNA damage. While the role of Gadd45 in cellular DNA repair remains to be elucidated, recent evidence indicates that aspects of nucleotide excision repair are reduced in *gadd45*$^{-/-}$ mouse embryo fibroblasts compared to matched control cells (60).

Since, as mentioned before, Gadd45 is a low-abundance protein, we do not expect it to play a general role in chromatin accessibility. The preferential interaction of Gadd45 with altered nucleosomes (Fig. 4A to 6) suggests a possible recruitment and therefore concentration of Gadd45 at sites where the nucleosomes have been weakened by either histone posttranslational modifications or DNA damage. While binding of sequence specific transcription factors is generally inhibited on UV-damaged templates (66, 73), it is apparently increased for proteins recognizing altered DNA structure (8, 25, 39, 45, 63). For example, the high-mobility-group protein HMG1 has recently been shown to bind preferentially to UV-damaged DNA (45). This chromosomal protein has also been shown to bind preferentially to other distorted DNA structures such as synthetic cruciform (4) and lesions formed by the anticancer drug cisplatin (50). HMG1 is also apparently involved in the activation of p53 (28). These recent studies and the one presented here indicate that there is increasing evidence for a connection between chromatin modulation and maintenance of genomic stability.

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REFERENCES

1. **Antinore, M. J.** Unpublished data.
2. **Bae, I., M. L. Smith, M. S. Sheikh, Q. Zhan, D. A. Scudiero, S. H. Friend, P. M. O'Connor, and A. J. Fornace, Jr.** 1996. An abnormality in the p53 pathway following γ-irradiation in many wild-type p53 human melanoma lines. Cancer Res. **56:**840–847.
3. **Bhatia, K., Y. Pommier, C. Giri, A. J. Fornace, Jr., M. Imaizumi, T. R. Breitman, B. W. Cherney, and M. E. Smulson.** 1990. Expression of the poly(ADP-ribose) polymerase gene following natural and induced DNA strand breakage and effect of hyperexpression on DNA repair. Carcinogenesis **11:**123–128.

4. **Bianchi, M. E., M. Beltrame, and G. Paonessa.** 1989. Specific recognition of cruciform DNA by nuclear-protein HMG1. Science **243:**1056–1059.
5. **Boffa, L. C., G. Vidali, R. S. Mann, and V. G. Allfrey.** 1978. Suppression of histone deacetylation in vivo and in vitro by sodium butyrate. J. Biol. Chem. **253:**3364–3366.
6. **Bortvin, A., and F. Winston.** 1996. Evidence that Spt6p controls chromatin structure by a direct interaction with histones. Science **272:**1474–1476.
7. **Bradbury, E. M.** 1992. Reversible histone modifications and the chromosome cell cycle. Bioessays **14:**9–16.
8. **Burns, J. L., S. N. Guzder, P. Sung, S. Prakash, and L. Prakash.** 1996. An affinity of human replication protein A for ultraviolet-damaged DNA. J. Biol. Chem. **271:**11607–11610.
9. **Capranico, G., C. Jaxel, M. Roberge, K. W. Kohn, and Y. Pommier.** 1990. Nucleosome positioning as a critical determinant for the DNA cleavage sites of mammalian DNA topoisomerase II in reconstituted Simian virus 40 chromatin. Nucleic Acids Res. **18:**4553–4559.
10. **Carrier, F., M. L. Smith, I. Bae, K. E. Kilpatrick, T. J. Lansing, C.-Y. Chen, M. Engelstein, S. H. Friend, H. D. Henner, T. M. Gilmer, M. B. Kastan, and A. J. Fornace, Jr.** 1994. Characterization of human Gadd45, a p53-regulated protein. J. Biol. Chem. **269:**32672–32677.
11. **Carrier, F.** Unpublished data.
12. **Chen, A. Y., and L. F. Liu.** 1994. DNA topoisomerases: essential enzymes and lethal targets. Annu. Rev. Pharmacol. Toxicol. **94:**194–218.
13. **Earnshaw, W. C., B. H. Honda, R. A. Laskey, and J. O. Thomas.** 1980. Assembly of nucleosomes: the reaction involving X. laevis nucleoplasmin. Cell **21:**373–383.
14. **Erard, M. S., P. Belenguer, M. Caizergues-Ferrer, A. Pantaloni, and F. Amalric.** 1988. A major nucleolar protein, nucleolin, induces chromatin decondensation by binding to histone-H1. Eur. J. Biochem. **175:**525–530.
15. **Fornace, A. J., Jr., I. J. Alamo, and M. C. Hollander.** 1988. DNA damage-inducible transcripts in mammalian cells. Proc. Natl. Acad. Sci. USA **85:** 8800–8804.
16. **Fornace, A. J., Jr.** 1992. Mammalian genes induced by radiation; activation of genes associated with growth control. Annu. Rev. Genet. **26:**507–526.
17. **Franklin, W. A., P. W. Doetsch, and W. A. Haseltine.** 1985. Structural determination of the ultraviolet light induced thymine-cytosine pyrimidine-pyrimidone (6-4) photoproduct. Nucleic Acids Res. **13:**5317–5325.
18. **Fujii-Nakata, T., Y. Ishimi, A. Okuda, and A. Kikuchi.** 1992. Functional analysis of nucleosome assembly protein, NAP-1. J. Biol. Chem. **267:**20980–20986.
19. **Gariboldi, M.** Unpublished data.
20. **Georgel, P. T., T. Tsukiyama, and C. Wu.** 1997. Role for histones tails in nucleosome remodeling by Drosophila NURF. EMBO J. **16:**4717–4726.
21. **Germond, J. E., B. Hirt, P. Oudet, M. Gros-Bellard, and P. Chambon.** 1975. Folding the DNA double helix into chromatin-like structures from simian virus 40. Proc. Natl. Acad. Sci. USA **72:**1843–1847.
22. **Gordon, L. K., and W. A. Haseltine.** 1980. Comparison of the cleavage of pyrimidine dimers by the bacteriophage T4 and Micrococcus luteus UV-specific endonucleases. J. Biol. Chem. **255:**12047–12050.
23. **Gupta, M., A. Fujimori, and Y. Pommier.** 1995. Eukaryotic DNA topoisomerases 1, review Biochim. Biophys. Acta **1261:**1–14.
24. **Hall, P. A., J. M. Kearsey, P. J. Coates, D. G. Norman, E. Warbrick, and L. S. Cox.** 1995. Characterization of the interaction between PCNA and Gadd45. Oncogene **10:**2427–2433.
25. **Hirschfeld, S., A. S. Levine, K. Ozato, and M. Protic.** 1990. A constitutive damage-specific DNA-binding protein is synthesized at higher levels in UV-irradiated primate cells. Mol. Cell. Biol. **10:**2041–2048.
26. **Hsiang, Y. H., R. Hertzberg, S. Hecht, and L. F. Liu.** 1985. Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerase I. J. Biol. Chem. **260:**14873–14878.
27. **Husain, I., J. Griffith, and A. Sancar.** 1988. Thymidine dimers bend DNA. Proc. Natl. Acad. Sci. USA **85:**2558–2562.
28. **Jayaraman, L., N. C. Moorthy, K. G. K. Murthy, J. L. Manley, M. Bustin, and C. Prives.** 1998. High mobility group protein-1 (HMG-1) is a unique activator of p53. Genes Dev. **12:**462–472.
29. **Kamakaka, R. T., and J. O. Thomas.** 1990. Chromatin structure of transcriptionally competent and repressed genes. EMBO J. **9:**3997–4006.
30. **Kas, E., L. Poljak, Y. Adachi, and U. K. Laemmli.** 1993. A model for chromatin openings: stimulation of topoisomerase II and restriction enzyme cleavage of chromatin by distamycin. EMBO J. **12:**115–126.
31. **Kastan, M. B., Q. Zhan, W. S. El-Deiry, F. Carrier, T. Jacks, W. V. Walsh, B. S. Plunkett, B. Vogelstein, and A. J. Fornace, Jr.** 1992. A mammalian cell cycle checkpoint utilizing p53 and *GADD45* is defective in ataxia telangiectasia. Cell **71:**587–597.
32. **Kilpatrick, K. E., F. Carrier, M. L. Smith, C.-Y. Chen, A. J. Lee, D. W. Rusnak, M. B. Kastan, A. J. Fornace, Jr., B. R. Champion, T. M. Gilmer, and J.-L. Su.** 1995. The production and characterization of murine monoclonal antibodies to human Gadd45 raised against a recombinant protein. Hybridoma **14:**355–359.
33. **Kimura, K., M. Hirano, R. Kobayashi, and Y. Hirano.** 1998. Phosphorylation and activation of 13S condensin by Cdc2 in vitro. Science **282:**487–490.
34. **Kingston, R. E., C. A. Bunker, and A. N. Imbalzano.** 1996. Repression and activation by multiprotein complexes that alter chromatin structure. Genes Dev. **10:**905–920.
35. **Kinzler, K. W., and B. Vogelstein.** 1996. Life (and death) in a malignant tumour. Nature **379:**19–20.
36. **Kleinschmidt, J. A., C. Dingwall, G. Maier, and W. W. Franke.** 1986. Molecular characterization of a karyophilic histone-binding protein: cDNA cloning, amino acid sequence and expression of nuclear protein N1/N2 of *Xenopus laevis*. EMBO J. **5:**3547–3552.
37. **Ko, L. J., and C. Prives.** 1996. p53: puzzle and paradigm. Genes Dev. **10:**1054–1072.
38. **Kohn, K. W., R. A. G. Ewig, L. C. Erickson, and L. A. Zwelling.** 1981. Measurement of strand breaks and cross-links by alkaline elution, p. 379–401. *In* E. C. Friendberg and P. C. Hanawalt (ed.), DNA repair: a laboratory manual of research procedures. Marcel Dekker Inc., New York, N.Y.
39. **Kuraoka, I., E. H. Morita, M. Saijo, T. Matsuda, K. Morikawa, M. Shirakawa, and K. Tanaka.** 1996. Identification of a damaged-DNA binding domain of the XPA protein. Mutat. Res. **362:**87–95.
40. **Lanza, A., S. Tornaletti, C. Rodolfo, M. C. Scanavini, and A. M. Pedrini.** 1996. Human DNA topoisomerase I-mediated cleavages stimulated by ultraviolet light-induced DNA damage. J. Biol. Chem. **271:**6978–6986.
41. **Lee, D. Y., J. J. Hayes, D. Pruss, and A. P. Wolffe.** 1993. A positive role for histone acetylation in transcription factor binding to nucleosomal DNA. Cell **72:**73–84.
42. **Meller, V. H., P. A. Fisher, and M. Berrios.** 1995. Intranuclear distribution of DNA topoisomerase II and chromatin. Chromosome Res. **4:**255–260.
43. **Mitchell, D. L., T. D. Nguyen, and J. E. Cleaver.** 1990. Nonrandom induction of pyrimidine-pyrimidone (6-4) photoproducts in ultraviolet-irradiated human chromatin. J. Biol. Chem. **265:**5353–5356.
44. **Papathanasiou, M. A., N. C. Kerr, J. H. Robbins, O. W. McBride, I. J. Alamo, S. F. Barrett, I. D. Hickson, and A. J. Fornace, Jr.** 1991. Induction by ionizing radiation of the *gadd45* gene in cultured human cells: lack of mediation by protein kinase C. Mol. Cell. Biol. **11:**1009–1016.
45. **Pasheva, E. A., I. G. Pashev, and A. Favre.** 1998. Preferential binding of high mobility group 1 protein to UV-damaged DNA. J. Biol. Chem. **273:**24730–24736.
46. **Paull, K. D., E. Hamel, and L. Malspeis.** 1995. Prediction of biochemical mechanism of action from the in vitro antitumor screen of the National Cancer Institute, p. 9–45. *In* W. O. Foye (ed.), Cancer chemotherapeutic agents. American Chemical Society, Washington, D.C.
47. **Peterson, C. L., and J. W. Tamkun.** 1995. The SWI/SNF complex: a chromatin remodeling machine? Trends Biochem. Sci. **20:**143–146.
48. **Philpott, A., G. H. Leno, and R. A. Laskey.** 1991. Sperm decondensation in Xenopus egg cytoplasm is mediated by nucleoplasmin. Cell **65:**569–578.
49. **Philpott, A., and G. H. Leno.** 1992. Nucleoplasmin remodels sperm chromatin in Xenopus egg extracts. Cell **69:**759–767.
50. **Pil, P. M., and S. J. Lippard.** 1992. Specific binding of chromosomal protein-HMG1 to DNA damaged by the anticancer drug cisplatin. Science **256:**234–237.
51. **Pommier, Y., G. Kohlhagen, K. Kohn, F. Leteurtre, M. C. Wani, and M. E. Wall.** 1995. Interaction of an alkylating camptothecin derivative with a DNA base at topoisomerase I-DNA cleavage sites. Proc. Natl. Acad. Sci. USA **92:**8861–8865.
52. **Pommier, Y.** 1997. DNA topoisomerase II inhibitors, p. 153–174. *In* B. A. Teicher (ed.), Cancer therapeutics: experimental and clinical agents. Humana Press, Totowa, N.J.
53. **Potmesil, M., Y. H. Hsiang, L. F. Liu, B. Bank, H. Grossberg, S. Kirschebaum, T. J. Forlenza, A. Penziner, D. Kanganis, and T. J. Forlenzar.** 1988. Resistance of human leukemic and normal lymphocytes to drug-induced DNA cleavage and low levels of DNA topoisomerase II. Cancer Res. **48:** 3537–3543.
54. **Pourquier, P., L. Ueng, G. Kohlhagen, A. Mazumder, M. Gupta, K. Kohn, and Y. Pommier.** 1997. Effects of uracil incorporation, DNA mismatches and abasic sites on cleavage and religation activities of mammalian topoisomerase I. J. Biol. Chem. **272:**7792–7796.
55. **Ramanathan, B., and M. J. Smerdon.** 1986. Changes in nuclear protein acetylation in u.v.-damaged human cells. Carcinogenesis **7:**1087–1094.
56. **Ramanathan, B., and M. J. Smerdon.** 1989. Enhanced DNA repair synthesis in hyperacetylated nucleosomes. J. Biol. Chem. **264:**11026–11034.
57. **Schaeffer, L., R. Roy, S. Humbert, V. Moucollin, W. Vermenlin, J. H. J. Hoeijmakers, P. Chambon, and J. M. Egly.** 1993. DNA repair helicase: a component of BTF2 (TFIIH) basic transcription factor. Science **260:**58–63.
58. **Schmidt-Zachmann, M., and W. W. Franke.** 1988. DNA cloning and amino acid sequence determination of a major constituent protein of mammalian nucleoli. Correspondence of the nucleoplasmin-related protein NO38 to mammalian protein B23. Chromosoma **96:**417–426.
59. **Smith, M. L., H. U. Kontny, Q. Zhan, A. Sreenath, P. M. O'Connor, and A. J. Fornace, Jr.** 1996. Antisense GADD45 expression results in decreased DNA repair and sensitizes cells to UV-irradiation or cisplatin. Oncogene **13:**2255–2263.
60. **Smith, M. L.** Personal communication.
61. **Smith, S., and B. Stillman.** 1989. Purification and characterization of CAF-I,

a human cell factor required for chromatin assembly during DNA replication in vitro. Cell **58:**15–25.
62. **Stein, A., J. P. Whitlock, and M. Bina.** 1979. Acidic polypeptides can assemble both histones and chromatin in vitro at physiological ionic strength. Proc. Natl. Acad. Sci. USA **76:**5000–5004.
63. **Sung, P., J. F. Watkins, L. Prakash, and S. Prakash.** 1994. Negative superhelicity promotes ATP-dependent binding of yeast RAD3 protein to ultraviolet-damaged DNA. J. Biol. Chem. **269:**8303–8308.
64. **Subramanian, D., B. S. Rosenstein, and M. T. Muller.** 1998. Ultraviolet-induced DNA damage stimulates topoisomerase 1-DNA complex formation in vivo: possible relationship with DNA repair. Cancer Res. **58:**976–984.
65. **Tatchell, K., and K. E. van Holde.** 1977. Reconstitution of chromatin core particles. Biochemistry. **16:**5295–52303.
66. **Tommasi, S., P. M. Swiderski, Y. Tu, B. Kaplan, and G. P. Pfeifer.** 1996. Inhibition of transcription factor binding by ultraviolet-induced pyrimidine dimers. Biochemistry **35:**15693–15703.
67. **Tsukiyama, T., and C. Wu.** 1995. Purification and properties of an ATP-dependent nucleosome remodeling factor. Cell **83:**1011–1020.
68. **van Holde, K. E.** 1989. Chromatin. Springer-Verlag, New York, N.Y.
69. **van Holde, K. E., D. E. Lohr, and C. H. Robert.** 1992. What happens to nucleosomes during transcription? J. Biol. Chem. **267:**2837–2840.
70. **Varga-Weisz, P. D., and P. B. Becker.** 1995. Transcription factor-mediated chromatin remodeling: mechanisms and models. FEBS Lett. **369:**118–121.
71. **Varga-Weisz, P. D., M. Wilm, E. Bonte, K. Dumas, M. Mann, and P. B. Becker.** 1997. Chromatin-remodeling factor CHRAC contains the ATPases ISWI and topoisomerase II. Nature **388:**598–602.
72. **Verreault, A., P. D. Kaufman, R. Kobayashi, and B. Stillman.** 1996. Nucleosome assembly by a complex of CAF-1 and acetylated histones H3/H4. Cell **87:**95–104.
73. **Vichi, P., F. Coin, J. P. Renaud, W. Vermeulen, J. H. J. Hoeijmakers, D. Moras, and J. M. Egly.** 1997. Cisplatin- and UV-damaged DNA lure the basal transcription factor TFIID/TBP. EMBO J. **16:**7444–7456.
74. **von Lindern, M., S. van Baal, J. Wiegant, A. Raap, A. Hagemeijer, and G. Grosveld.** 1992. *can*, a putative oncogene associated with myeloid leukemogenesis, may be activated by fusion of its 3′ half to different genes: characterization of the *set* gene. Mol. Cell. Biol. **12:**3346–3355.
75. **Wang, J. C.** 1996. DNA topoisomerases. Annu. Rev. Biochem. **65:**635–692.
76. **Weinstein, J. N., K. W. Kohn, M. R. Greever, V. N. Viswanadhan, L. V. Rubinstein, A. P. Monks, D. A. Scudiero, L. Welch, A. D. Koutsoukos, A. J. Chiausa, and K. D. Paul.** 1992. Neural computing in cancer drug development: predicting mechanism of action. Science **258:**447–451.
77. **Weinstein, J. N., T. G. Myers, P. M. O'Conner, S. H. Friend, A. J. Fornace, K. W. Kohn, T. Foho, S. E. Bates, L. V. Rubenstein, N. L. Anderson, J. K. Buolamwini, W. W. van Osdol, A. P. Monks, D. A. Scudiero, E. A. Sausville, D. W. Zaharevitz, B. Bunow, G. S. Johnson, R. E. Wittes, and K. D. Paull.** 1997. An information-intensive approach to the molecular pharmacology of cancer. Science **275:**343–349.
78. **Weisbrod, S.** 1982. Properties of active nucleosomes as revealed by HMG 14 and 17 chromatography. Nucleic Acids Res. **10:**2017–2042.
79. **Wolffe, A.** 1995. Chromatin; structure and function, 2nd ed. Academic Press Inc., San Diego, Calif.
80. **Wolffe, A. P.** 1996. Histones deacetylase: a regulator of transcription. Science **272:**371–372.
81. **Workman, J. L., I. C. A. Taylor, and R. E. Kingston.** 1991. Control of class II gene transcription during in vitro nucleosome assembly. Methods Cell Biol. **35:**419–447.
82. **Zhan, Q., F. Carrier, and A. J. Fornace, Jr.** 1993. Induction of cellular p53 activity by DNA-damaging agents and growth arrest. Mol. Cell. Biol. **13:**4242–4250.
83. **Zhan, Q., K. A. Lord, I. Alamo, Jr., M. C. Hollander, F. Carrier, D. Ron, K. W. Kohn, B. Hoffman, D. Liebermann, and A. J. Fornace, Jr.** 1994. The *gadd* and *MyD* genes define a novel set of mammalian genes encoding acidic proteins that synergistically suppress cell growth. Mol. Cell. Biol. **14:**2361–2371.
84. **Zhan, Q. M., S. J. Fan, M. L. Smith, I. Bae, K. Yu, I. Alamo, P. M. O'Connor, and A. J. Fornace.** 1996. Abrogation of p53 function affects gadd gene response to DNA base-damaging agents and starvation. DNA Cell Biol. **15:**805–815.
85. **Zhan, Q. M., I. T. Chen, M. J. Antinore, and A. J. Fornace, Jr.** 1998. Tumor suppressor p53 can participate in transcriptional induction of the *GADD45* promoter in the absence of direct DNA binding. Mol. Cell. Biol. **18:**2768–2778.
86. **Zhan, Q. M., M. J. Antinore, X. W. Wang, F. Carrier, M. L. Smith, C. C. Harris, and A. J. Fornace, Jr.** Association with Cdc2 and inhibition of Cdc2/cyclin B1 kinase activity by the p53-regulated protein Gadd45. Oncogene, in press.