Nucleic Acids Research, 1999, Vol. 27, No. 14 **2905–2911**

Plasmid linking number change induced by topoisomerase I-mediated DNA damage

Pu Duann, Mei Sun, Chin-Tai Lin, Hui Zhang[1] and Leroy F. Liu*

Department of Pharmacology, UMDNJ-Robert Wood Johnson Medical School, 675 Hoes Lane, Piscataway, NJ 08854, USA and [1]Department of Genetics, Yale University, New Haven, CT 06520, USA

Received March 15, 1999; Revised and Accepted June 2, 1999

ABSTRACT

The state of cellular chromatin in response to DNA damage has been examined by monitoring the change in the linking number of circular episomes. COS cells transfected with an SV40-based vector were treated with camptothecin (CPT), a eukaryotic DNA topoisomerase I (TOP1) poison which induces TOP1-mediated DNA damage. Within minutes, a large increase in the linking number (over 10 linking number) of a small fraction (5–15%) of the episomal DNA was observed. A similar CPT-induced increase in plasmid DNA linking number was observed in *Saccharomyces cerevisae* expressing human DNA TOP1. In this case, the majority of the plasmid DNA can undergo rapid relaxation. The large increase in the plasmid linking number suggests major chromatin structural reorganization in response to TOP I-mediated DNA damage.

INTRODUCTION

Chromatin represents a structural barrier to the repair of DNA damage (1–3). However, very little is known about the state of chromatin during DNA repair. It is reasonable to assume that, upon DNA damage, chromatin must undergo changes to allow repair enzymes to access damaged DNA sites. Indeed, many studies have suggested that chromatin loses its nucleosomal structure immediately following repair, as based on nuclease digestion studies on repair-labeled DNA (4–6). Reassembly of nucleosomes in repaired genomic regions can be demonstrated to occur gradually (6). However, nothing is known about the process that leads to nucleosome disassembly prior to or during repair.

Unlike most other DNA damaging agents, camptothecin (CPT) induces a highly specific type of DNA damage and is therefore particularly useful for studying DNA damage responses. CPT induces topoisomerase I (TOP1)-mediated DNA damage by specifically blocking the religation step in the enzyme-catalyzed breakage/religation cycles on DNA (7,8). Many cellular effects of CPT, such as induction of sister-chromatid exchanges and chromosome aberrations, G_2 cell cycle arrest, elevation of c-*fos* and c-*jun* mRNAs and p53, and activation of NF-κB, are characteristic of DNA damage responses (9–13). However, different from other DNA damaging agents, CPT delivers protein-linked single-strand breaks primarily to actively transcribed regions (8,14–17). Consequently, CPT is particularly useful for probing repair of DNA damage within the transcribed regions. Indeed, Cockayne cells (both CSA and CSB), which are defective in transcription-coupled repair, have been shown to be hypersensitive to CPT (18).

In this study, the state of chromatin was monitored by measuring the linking number of an SV40-based episomal DNA vector in COS cells treated with CPT. Within minutes, a large increase in linking number of the episomal DNA was observed, suggesting major chromatin reorganization as a rapid response to TOP1-mediated DNA damage. Studies in *Saccharomyces cerevisae* have also demonstrated similar plasmid linking number change in response to CPT. These results suggest major chromatin structural change in response to TOP1-mediated DNA damage. Preliminary genetic studies have ruled out the involvement of RAD6 in this process.

MATERIALS AND METHODS

DNA, cells, yeast strains and other reagents

pSV2cat was propagated in *Escherichia coli* HB101. Closed circular DNA was prepared by the alkali lysis method followed by Quiagen column purification. 20(S)-CPT [unless otherwise indicated, 20(S)-CPT was used throughout this study] and 20(R)-CPT were kind gifts from Dr Monroe Wall (Research Triangle Park, NC). VM-26 (teniposide), 3-aminobenzamide and cycloheximide were purchased from Aldrich Chemical Co. DMSO, aphidicolin and Proteinase K were purchased from Boehringer Mannheim. Fetal bovine serum and tissue culture media were purchased from Gibco-BRL. COS-1 and COS-7 cells were obtained from ATCC. The human β-actin gene probe was purified from pHFbA-1 as a 2.1 kb *Xho*I fragment containing the human β-actin cDNA (19). The human topoisomerase I (hTOP1) cDNA was purified as an *Eco*RI fragment from pETT1B (20). The human β-globin cDNA was purified as a 1.7 kb *Bam*HI fragment from pLY4 (21). All DNA fragments were ^{32}P-labeled by the random-primer DNA labeling method (22).

YCpGAL1-hTOP1 is a single-copy plasmid containing hTOP1 cDNA under the control of the GAL1 promoter (23). The yeast 2μ-derived plasmid Yep96 carries a synthetic yeast Ub gene under the control of the copper-inducible CUP1 promoter (24). Plasmid HA-UbK63R, in which Lys63 of ubiquitin

*To whom correspondence should be addressed. Tel: +1 732 235 4592; Fax: +1 732 235 4073; Email: lliu@umdnj.edu

is replaced with arginine, is identical to Yep96 except that it bears a haemagglutinin (HA) tag on the N-terminus of Ub (25). *Saccharomyces cerevisiae* strain JN362a (*MATa, ade1, ura3-52, leu2, is7, trp1, tyr1, ise2*) and its isogenic Δrad6 derivative, JN362aΔ*rad6* (*MATa, ade1, ura3-52, leu2, is7, trp1, tyr1, ise2, rad6::LEU2*) are constructed by Dr J. Nitiss.

Cell culture and transfection

SV40 T-antigen expressing Simian monkey kidney cells, COS-1 and COS-7 (26), were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum at 37°C in a 5% CO_2 incubator. Subconfluent cells were transfected with pSV2cat DNA by the DEAE-dextran method (27). Cells were washed twice with pre-warmed phosphate-buffered saline (PBS) and then transfected with 250 ng of pSV2cat DNA mixed with DEAE-dextran. After frequent agitation for 3–5 h, cells were washed twice with PBS and incubated with pre-warmed DMEM-10 at 37°C for continued growth. CPT treatment or UV irradiation was performed within 48–72 h post-transfection.

Cell harvest and isolation of episomal DNAs

Following CPT treatment, transfected COS-cells in 35 mm dishes were washed once with ice-cold PBS. DNAs were isolated by the Hirt extraction procedure (28). The DNA pellet was dissolved in 65 μl of 20 mM Tris, pH 8.0 and 50 mM EDTA. One-third of each DNA sample was analyzed by electrophoresis in 1% agarose gel and 0.5× Tris–phosphate electrophoresis (TPE) buffer containing the indicated concentration of chloroquine. Gels were stained with ethidium bromide and then prepared for Southern transfer onto Zeta GT membrane filters. pUC19 DNA was used to prepare the ^{32}P-labeled probe by the random-primer labeling method (22).

UV irradiation of COS cells

COS-7 cells transfected with pSV2cat DNA were irradiated with UV light at 48–72 h post-transfection. The media were removed from dishes thoroughly by suction and cells were irradiated in the UV Stratalinker (Model 2400) with 254 nm wavelength germicidal light at 30, 100 or 300 J/m^2 as indicated. Irradiated cells were replenished with pre-warmed DMEM-10 medium and incubated in a CO_2 incubator at 37°C for various time periods.

Digestion of chromatin in isolated nuclei with *Staphylococcus aureus* nuclease

Cells were either treated with 10 μM CPT at 37°C for 10 min, or UV-irradiated at 100 J/m^2 and then incubated at 37°C for 15 min. Nuclei of treated cells were then isolated by the following procedure. Briefly, cells were lysed by a lysis solution containing 10 mM NaCl, 10 mM sodium phosphate, pH 7.5, 3 mM $MgCl_2$, 0.5 mM dithiothreitol (DTT), 0.5 mM PMSF, 5 mM N-ethylmaleimide (NEM) and 0.6% NP-40. Nuclei were scraped off the dishes with rubber policemen and washed twice with the digestion buffer (10 mM Tris, pH 8.0, 25 mM $CaCl_2$ and 50 mM NaCl). *Staphylococcus aureus* nuclease was added to nuclei. Nuclease digestion was carried out at 37°C for 10 min. The reactions were terminated by adding an SDS-lysis buffer (1% SDS, 50 mM EDTA, 10 mM HEPES, pH 7.5 and 1 mg/ml Proteinase K) followed by incubation at 65°C for 1 h. DNAs were isolated by extraction with phenol/chloroform followed by precipitation with ethanol. DNA analysis by electrophoresis and Southern blotting were performed as described above.

Yeast growth, CPT treatment and DNA isolation

Yeast strains were transformed with various plasmids according to Gietz *et al.* (29). Strains were grown at 30°C in minimal medium (YNB) containing 0.67% yeast nitrogen base without amino acids (Difco) supplemented with appropriate nutrients (SD medium). Strains were inoculated into 2% glucose SD medium and grown to saturation at 30°C. Cells were then transferred into 2% raffinose SD medium (1:100 dilution). When cells reached 0.5 at O.D.600, 2% galactose was added to induce hTOP1. To induce HA-UbK63R overexpression, 100 μM $CuSO_4$ was added together with galactose. Eight hours post-induction, cells were treated with CPT [100 μM in 1% DMSO and 25 mM 4-(2-hydroxyethyl)-1-peperazineethane-sulfonic acid (pH 7.2)] for 30 min.

For DNA isolation, 25 ml of yeast cell culture was mixed with 2 ml of ice-cold 0.5 M EDTA (pH 8.0) and 25 ml of an ice-cold termination solution (95% ethanol, 3% toluene and 20 mM Tris, pH 8.0). The mixture was then centrifuged at 3000 r.p.m. at 4°C for 5 min. Cell pellets were suspended with a spheroplasting solution (1 M sorbitol, 25 mM EDTA, pH 8.0, 50 mM β-mercaptoethanol and 1 mg/ml zymolyase). After 30 min incubation at 37°C, the spheroplast was pelleted and plasmid DNAs were recovered from cell pellets as described (30).

RESULTS

CPT induces rapid and reversible relaxation of episomal DNAs in COS cells

In order to study the effect of TOP1-mediated DNA damage on the chromatin structure, the linking number of episomal DNAs in COS cells treated with CPT was monitored. As shown in Figure 1, within 3 min, CPT induced a large change in the linking number of the episomal pSV2cat DNA, resulting in the faster migration of a new species labeled R in 1% agarose gel containing 5 μM chloroquine. This new species R was identified to represent partially relaxed topoisomers by comparing its electrophoretic pattern in the absence and presence of varying concentrations of chloroquine (data not shown) as well as by two-dimensional gel electrophoresis (Fig. 3). Based on the separation between the supercoiled DNA (S) and relaxed DNA (R), the linking number increase was estimated to be greater than 10. This large change in DNA linking number is characteristic of major chromatin structural reorganization and possibly indicates partial or complete disassembly of multiple nucleosomes (31). This relaxation phenomenon appeared to be restricted to a 5–15% population of the total intracellular pSV2cat DNA in COS cells. As shown in Figure 1, after 10 min of CPT treatment, ~15% of pSV2cat DNA was relaxed and no further increase in the proportion of this relaxed population was observed. Similarly, a dose response curve has revealed a similar saturation phenomenon with the plateau being 15% of relaxed population (data not shown). This relaxation phenomenon was shown to be due to the formation of TOP1-mediated DNA damage since the inactive enantiomer of CPT, 20(R)-CPT, failed to induce relaxation even at higher concentrations (data not shown). Most interestingly, plasmid relaxation appeared to require continued presence of CPT. Upon removal of CPT

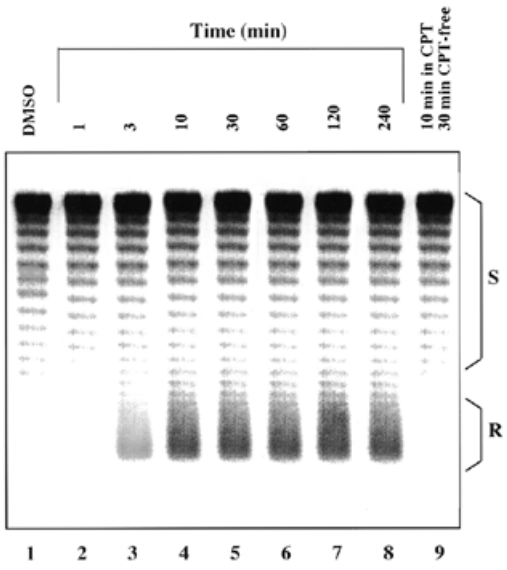

Figure 1. Reversible change in the linking number of episomal DNAs in COS cells treated with CPT. COS-1 cells were cultured in 100 mm Petri dishes to subconfluency and then transfected with pSV2cat DNA as described in Materials and Methods. Sixty hours post-transfection, cells were treated with 25 μM CPT. Cells were harvested at 1, 3, 10, 30, 120 and 240 min following CPT treatment. DNA samples were subjected to electrophoresis in 0.5× TPE buffer containing 5 μM chloroquine. Southern analysis was performed using ^{32}P-labeled pSV2cat DNA as the probe. Lane 1, control, no CPT treatment; lanes 2–8, DNA samples from cells treated with CPT for 1, 3, 10, 30, 120 and 240 min, respectively; lane 9, transfected cells were treated with CPT for 10 min followed by incubation in CPT-free medium for another 30 min. The relaxed (R) and supercoiled (S) DNA populations were marked.

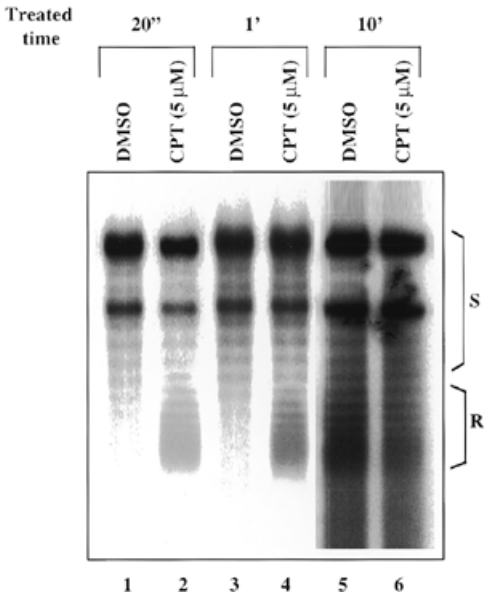

Figure 2. CPT induces DNA relaxation in isolated nuclei. Nuclei were prepared from COS cells transfected with pSV2cat as described in Materials and Methods. Isolated nuclei were treated with CPT (5 μM) at 37°C for varying time period and then subjected to electrophoresis (with 5 μM chloroquine) and Southern analysis. Lanes 1, 3 and 5, DMSO controls (no CPT) for 20 s, 1 and 10 min incubation, respectively; lanes 2, 4 and 6, nuclei treated with 5 μM CPT for 20 s, 1 and 10 min, respectively.

from the culture medium, the relaxed population disappeared within 10 min at 37°C. The relaxed DNA population is either degraded or more likely converted back to the supercoiled population.

A number of metabolic inhibitors including 3-aminobenzamide (3-AB) [an inhibitor of poly(ADP-ribose) polymerase], aphidicolin (an inhibitor of DNA polymerase α and δ), dichloro-D-ribofuranosylbenzimidazole (DRB) (an RNA polymerase II inhibitor) and cycloheximide (a protein synthesis inhibitor), have been used during co-treatment with CPT and none affected CPT-induced relaxation.

To further study this phenomenon, nuclei were prepared from pSV2cat transfected COS cells. Addition of CPT (5 μM) to isolated nuclei similarly resulted in relaxation of pSV2cat DNA (Fig. 2, lanes 2 and 4). Relaxation was rapid. Within 20 s of CPT addition, a portion of pSV2cat DNA was relaxed, suggesting that relaxation is a direct response to TOP1-mediated DNA damage. Unexpectedly, upon prolonged (10 min) incubation of nuclei, pSV2cat DNA was also relaxed in the absence of CPT (Fig. 2, lane 5).

UV irradiation induces relaxation of episomal DNAs

We have tested the effect of topoisomerase II (TOP2)-mediated DNA damage on the linking number of episomal DNAs. Surprisingly, VM-26 (100 μM), a specific TOP2 poison, had no detectable effect on the linking number of episomal DNA in COS cells (data not shown). However, irradiation of transfected COS-7 cells with the 254 nm UV light induced the relaxed population (Fig. 3, see topoisomer population labeled

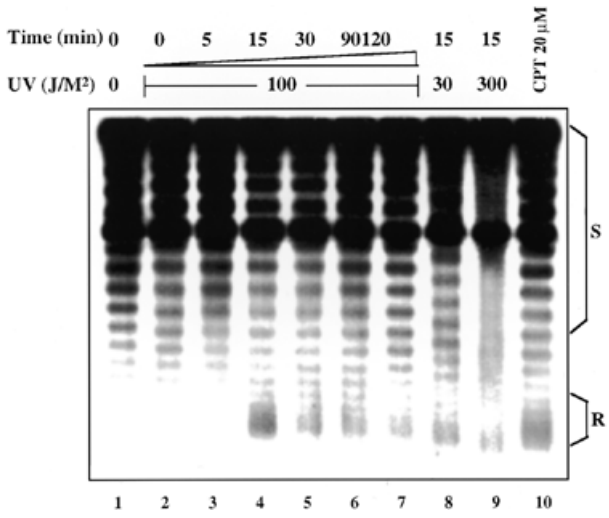

Figure 3. UV irradiation induces an increase in the linking number of episomal DNA in COS cells. pSV2cat DNA transfected COS-7 cells were irradiated at various UV doses and subsequently incubated at 37°C for various time periods as indicated on top of each lane. Lanes 2–7, COS-7 cells irradiated with 100 J/m^2 and then incubated at 37°C for 0, 5, 15, 30, 90 and 120 min, respectively; lane 8, COS-7 cells were irradiated with a UV dose of 30 J/m^2 and then incubated at 37°C for 15 min; lane 9, COS-7 cells were irradiated with a UV dose of 300 J/m^2 and then incubated at 37°C for 15 min; lane 10, COS-7 cells were treated with 20 μM of CPT for 15 min.

R). Like CPT, UV-induced relaxation of a small fraction of pSV2cat DNA in COS cells (Fig. 3, compare lanes 4 and 10). Interestingly, relaxation of pSV2cat DNA only occurred after 15 min of incubation at 37°C following UV irradiation. With-

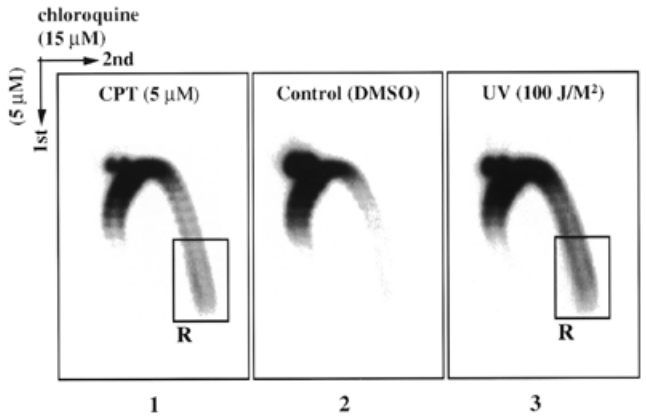

Figure 4. Two-dimensional gel electrophoresis of pSV2cat DNA isolated from CPT-treated COS cells. pSV2cat DNA was isolated from COS cells treated with 5 μM CPT for 10 min and then analyzed by two-dimensional gel electrophoresis in the presence of chloroquine (5 and 15 μM for the first and second dimensions, respectively). Panel 1, DNA isolated from cells treated with CPT; panel 2, DNA isolated from cells treated with 1% DMSO (control, no CPT); lane 3, DNA isolated from cells irradiated with 100 J/m^2 UV (254 nm) followed by 15 min post-irradiation incubation.

out post-irradiation incubation, no relaxation of pSV2cat DNA was observed (Fig. 3, lane 2). It was also observed that the relaxed population, albeit at reduced levels, persisted even after 120 min of post-irradiation incubation at 37°C (Fig. 3, lane 7). Relaxation of pSV2cat DNA in UV irradiated cells was confirmed by two-dimensional gel electrophoresis (Fig. 4).

Probing chromatin structural changes with *Staphylococcal* nuclease

The observed large linking number change of episomal DNAs treated with CPT suggests possible chromatin structural alteration. In order to probe the chromatin structural change, nuclei were isolated and the chromatin structure probed with *S.aureus* nuclease. As shown in Figure 4, the overall rate of digestion of chromatin in isolated SW620 nuclei, as revealed by staining with ethidium bromide, was not altered by CPT treatment (Fig. 5). Similarly, when gels were probed with ^{32}P-labeled human β-actin cDNA, hTOP1 cDNA or human β-globin cDNA, no major difference in the rates of digestion with or without CPT treatment was observed (Fig. 5). This experiment has been repeated with similar results in a number of cell lines including COS-7, HT29 and Cockayne cells (both CSA and CSB). The slightly faster rates of digestion in non-Cockayne cells (i.e. COS-7, HT29 and SW620) treated with CPT were reproducibly observed using β-actin and TOP1 cDNA probes but not the β-globin cDNA probe. The significance of this observation is unclear. UV irradiation (100 J/m^2) had essentially the same effect as CPT in all cell lines tested (data not shown).

CPT induces plasmid relaxation in yeast

We have tested whether this plasmid relaxation phenomenon can be reproduced in yeast. Using the CPT permeable strain JN362a and under the condition that hTOP1 was overexpressed (see +hTOP1 lanes in Fig. 6A), we were able to demonstrate that CPT (100 μM) indeed induced plasmid relaxation (see + CPT lanes in Fig. 6A). In this case, over 70% of the

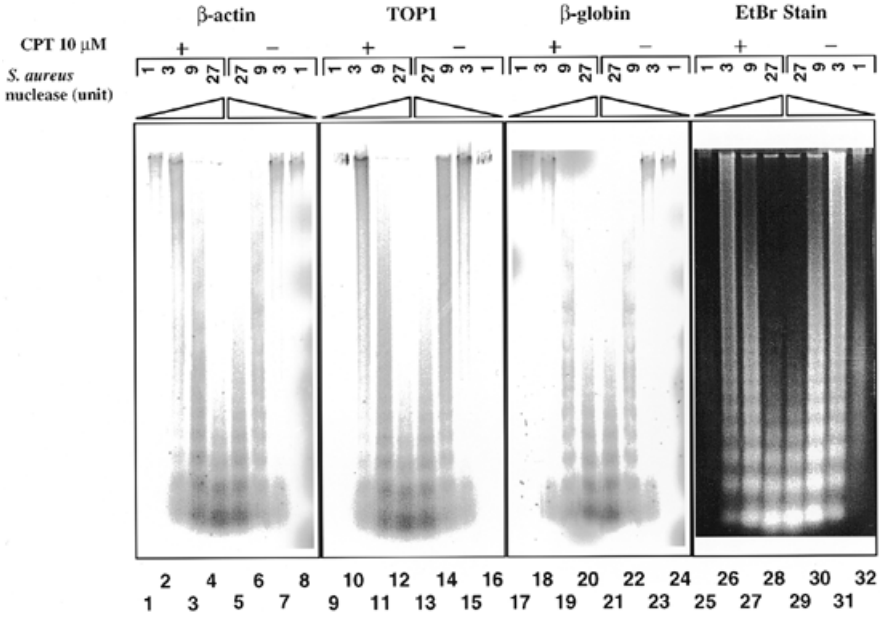

Figure 5. Probing CPT-induced chromatin structural change by *Staphylococcal* nuclease digestion. SW620 cells were treated with 10 μM CPT for 15 min. Nuclei were then isolated as described in Materials and Methods. Isolated nuclei were treated with CPT (20 μM) for 15 min and then digested with *S.aureus* nuclease as described in Materials and Methods. Digested DNA was analyzed by electrophoresis in 1% agarose gel and Southern blotting with various ^{32}P-labeled DNA probes. Lanes 1–8, probed with β-actin cDNA; lanes 9–16, probed with hTOP1 cDNA; lanes 17–24, probed with β-globin cDNA; lanes 25–32, total DNA as revealed by ethidium staining.

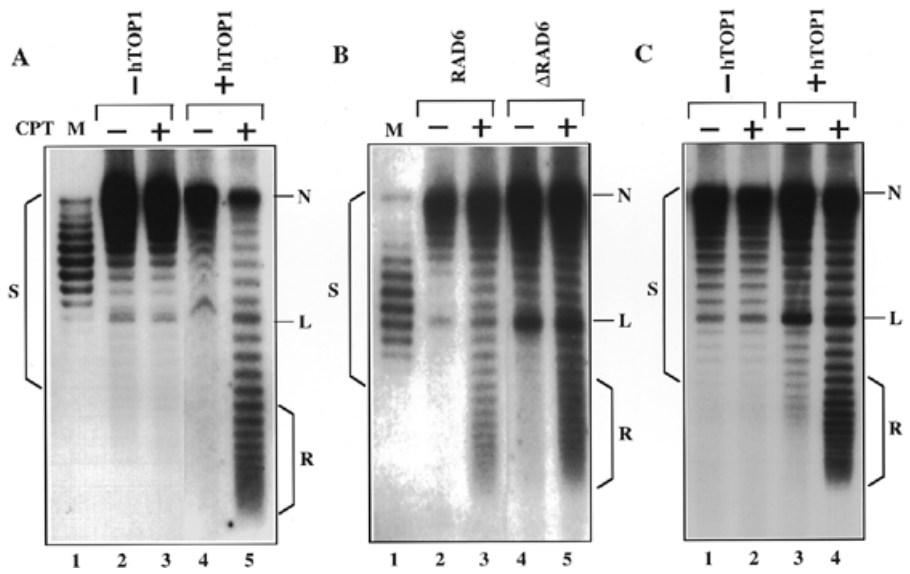

Figure 6. CPT induces plasmid relaxation in yeast. All the strains used here were transformed with plasmid YCpGAL1-hTOP1 in which hTOP1 cDNA is under the control of the GAL1 promoter. Cells were grown in 2% glucose SD medium and then transferred (1:100 dilution) into the same SD medium containing 2% raffinose. When O.D.600 reached 0.5, 2% glucose or 2% galactose (for hTOP1 induction) was added into the log phase cultures. After 8 h of glucose repression (–hTOP1) or galactose induction (+hTOP1), cells were treated with 100 μM CPT (+CPT) for 30 min at 30°C. Yeast plasmid DNA was then extracted and analyzed by electrophoresis in 1% agarose gel (0.5× TPE buffer containing 8 μM chloroquine). Southern blotting was carried out using the ^{32}P-labeled Yep96 DNA. (**A**) The endogenous 2μ plasmid was directly measured in JN362a transformed with YCp-hTOP1 using the ^{32}P-labeled Yep96 DNA probe (the larger YCp-hTOP1 plasmid is not shown in the gel); (**B**) JN362a (labeled RAD6) and its isogenic Δrad6 derivative (labeled ΔRAD6) were transformed with YCpGA1-hTOP1. The endogenous 2μ plasmid detected by the ^{32}P-labeled Yep96 DNA probe was shown. (**C**) Yeast CPT-permeable strain JN362a (RAD6) was transformed with both YCpGAL1-hTOP1 and a yeast 2μ-derived plasmid HA-UbK63R carrying the yeast Ub mutant (UbK63R). $CuSO_4$ (100 μM) was added to all culture media at the time of galactose (or glucose) addition. The plasmid HA-UbK63R DNA has the same size as the endogenous 2μ DNA (6318 versus 6206 bp). Consequently, both plasmid DNAs were detected by the ^{32}P-labeled Yep96 DNA probe as shown.

population was shown to be converted into the relaxed form (see species marked R in Fig. 6). The requirement for overexpression of hTOP1 is probably due to the fact that yeast TOP1 is much less sensitive to CPT than hTOP1. We have also tested the potential role of RAD6 and ubiquitin in CPT-induced plasmid relaxation. RAD6, an E2 enzyme involved in ubiquitin–protein conjugation, has been shown to be involved in DNA repair. As shown in Figure 6B, plasmid relaxation was observed in both RAD6 and Δrad6 strains. In addition, overexpression of a dominant negative mutant Ub (HA-UbK63R) had no effect on plasmid relaxation, suggesting that at least Lys63 of Ub is not required for this process.

In order to examine the chromatin structure, yeast JN362a transformed with hTOP1 expression plasmid was treated with CPT and then permeabilized for digestion with *S.aureus* nuclease. As shown in Figure 7, the *S.aureus* nuclease digestion pattern remained the same with or without CPT treatment.

DISCUSSION

The large linking number increase of episomal DNAs observed in COS cells treated with CPT is intriguing. The most likely explanation is that episomal DNAs have undergone rapid chromatin reorganization. The increase of greater than 10 linking number in the episomal DNA suggests that at least 10 nucleosomes have been disassembled or significantly altered so that wrapping of DNA around the nucleosome cores is prevented (31). An alternative explanation is that TOP1 is actively involved in maintaining the negatively supercoiled state of a population of nucleosome-free episomal DNA (32,33). In this case, TOP1 must selectively remove positive supercoils which is normally generated due to RNA transcription (34,35). Inhibition of TOP1 would result in more positively supercoiled DNA (i.e. an increase in the linking number). We consider this alternative explanation unlikely for at least the following four reasons. First, purified TOP1 does not exhibit preferential activity on positively supercoiled DNA (36). Second, CPT is an excellent TOP1 poison but a rather poor activity inhibitor of TOP1 (8). Third, positively supercoiled episomal DNAs have never been detected even at the highest CPT concentrations used in our studies. Fourth, active transcription does not seem to be required for the observed linking number change since the transcription inhibitor DRB had no effect on this process. In addition, CPT was shown to induce the same change in linking number in the absence of active transcription in isolated nuclei (Fig. 2) (note that no rNTPs were added to isolated nuclei during the reaction).

It is interesting that only a small fraction of the episomal DNA in COS cells is relaxed by CPT or UV treatment. Previous studies have shown that only a small fraction of SV40 DNA in infected monkey cells is transcriptionally active and are torsionally stressed upon isolation (32,33). The amount of this

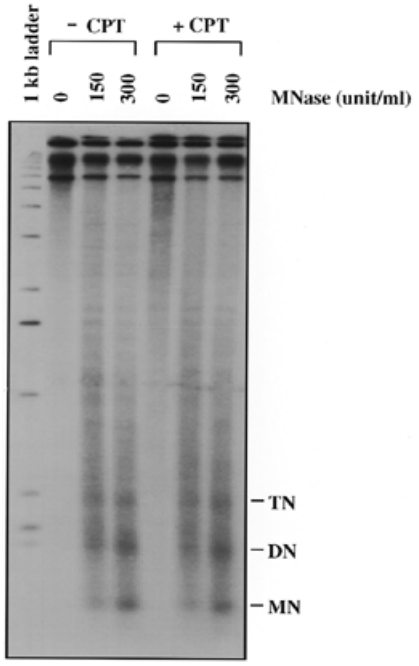

Figure 7. *Staphylococcus aureus* nuclease digestion of chromatin in permeabilized yeast cells. Yeast JN362a transformed with YCpGAL1-hTOP1 was cultured in 2% glucose SD medium. Expression of hTOP1 by galactose induction and CPT (100 μM) treatment were performed as described in the Figure 6 legend. Yeast cells were then permeabilized using NP-40 as described except that CPT and *S.aureus* nuclease were included in the permeabilization solution (47). *Staphylococcus aureus* nuclease digestion was performed at 30°C for 5 min. Digestion was terminated by the addition of SDS, RNase A and proteinase K as described (47). Digested DNA was then isolated by phenol/chloroform extraction followed by ethanol precipitation. DNA samples were analyzed by electrophoresis in 2% agarose gel and Southern blotting with ^{32}P-labeled Yep96 DNA. The positions of mononucleosomes (MN), dinucleosomes (DN) and trinucleosomes (TN) are marked.

fraction varies depending on the post-infection time (37). Addition of purified TOP1 has been shown to result in relaxation of this small population of transcriptionally active SV40 minichromosomes in isolated nuclei (32,33). It seems possible that the small fraction (e.g. 5–15%) of the relaxed episomal DNA we have observed in transfected COS cells represents the transcriptionally active fraction. Either CPT or UV treatment is able to effect relaxation of this population.

The effect of UV irradiation on the linking number change of the episomal DNA is quite similar to that of CPT. Only a fraction of the episomal DNA is converted to the relaxed state in COS cells. Interestingly, relaxation of episomal DNA (as measured by the linking number increase) peaked at 15 min post-irradiation in COS cells irradiated with 100 J/m^2. At this UV dose, multiple UV lesions are introduced into the episomal DNA template. This is most evident by inspecting the smeared distribution of topoisomers in cells irradiated with a UV dose of 300 J/m^2 (Fig. 3, lane 9), which indicates that all episomal DNA molecules have been heavily damaged. However, even at 300 J/m^2, only a fraction of the episomal DNA is relaxed (Fig. 3, lane 9). This result clearly indicates that the majority of the UV-damaged episomal DNA molecules retain their intact chromatin structure. Only a fraction (<10%) of the episomal DNA molecules are relaxed.

To test whether the chromatin structure in response to TOP1-mediated DNA damage, we have performed nuclease digestion studies. The results from *Staphylococcal* nuclease digestion studies in isolated nuclei indicate that bulk chromatin is not altered in COS cells treated with either CPT or UV. Likewise, both active (e.g. hTOP1, β-actin) and inactive (β-globin) genes are arranged in regular nucleosomal arrays. These results appeared to contradict the plasmid relaxation results. One possible explanation is that nucleosomes within actively transcribed regions may undergo some form of disassembly (to explain plasmid relaxation as revealed by the linking number measurement) without complete dissociation of histones from the DNA templates (to explain the nucleosomal arrays as revealed by nuclease digestion). This type of chromatin structural alteration has been demonstrated for ATP-dependent chromatin remodeling complexes such as SWI/SNF (38–42).

Chromatin reorganization observed in our studies could result from histone modifications. Amongst a myriad of histone modifications, histone ubiquitination appears attractive. First, ubiquitinated histones H2A and H2B are known to be associated with active chromatin (43). Second, CPT is known to induce rapid multi-ubiquitination of TOP1 (44). UV irradiation is also known to induce ubiquitination of the largest subunit of RNA polymerase II (45). Third, RAD6, an E2 enzyme known to be involved in DNA repair, can catalyze ubiquitin conjugation to histone H2A *in vitro* (46). Consequently, we have tested the role of RAD6 in plasmid relaxation in yeast. Our results have clearly shown that RAD6 is not responsible for plasmid relaxation in response to CPT.

Our working model for this interesting phenomenon is shown in Figure 8. In this model, we propose that SV40 minichromosomes exist in two populations, the active (susceptible fraction) and the inactive (inert fraction) chromatin. DNA damage in active but not inactive chromatin induces major chromatin reorganization. The nature of this reorganization is unclear. However, based on both linking number measurement and nuclease probing, the reorganized chromatin may involve extensive disassembly of multiple nucleosomes without histone dissociation. The reorganized chromatin is detected as relaxed DNA due to passive activity of a topoisomerase. Apparently, the majority of plasmid DNA molecules are susceptible to relaxation in response to DNA damage in yeast. Consequently, yeast may offer an excellent system for both genetic and biochemical analysis of chromatin dynamics in response to DNA damage. Clearly, further studies are necessary to reveal the nature of this rapid chromatin reorganization reaction as an early response to TOP1-mediated DNA damage.

ACKNOWLEDGEMENTS

We are grateful to Dr Kawai Lau for his many helpful discussions and assistance during the course of this work, and Drs John Nitiss and R. Haguensauer-Tsapis for providing us with the yeast strains. This work was supported by NIH grants GM27731 (to L.F.L.), CA39662 (to L.F.L.) and CA72878 (to H.Z.).

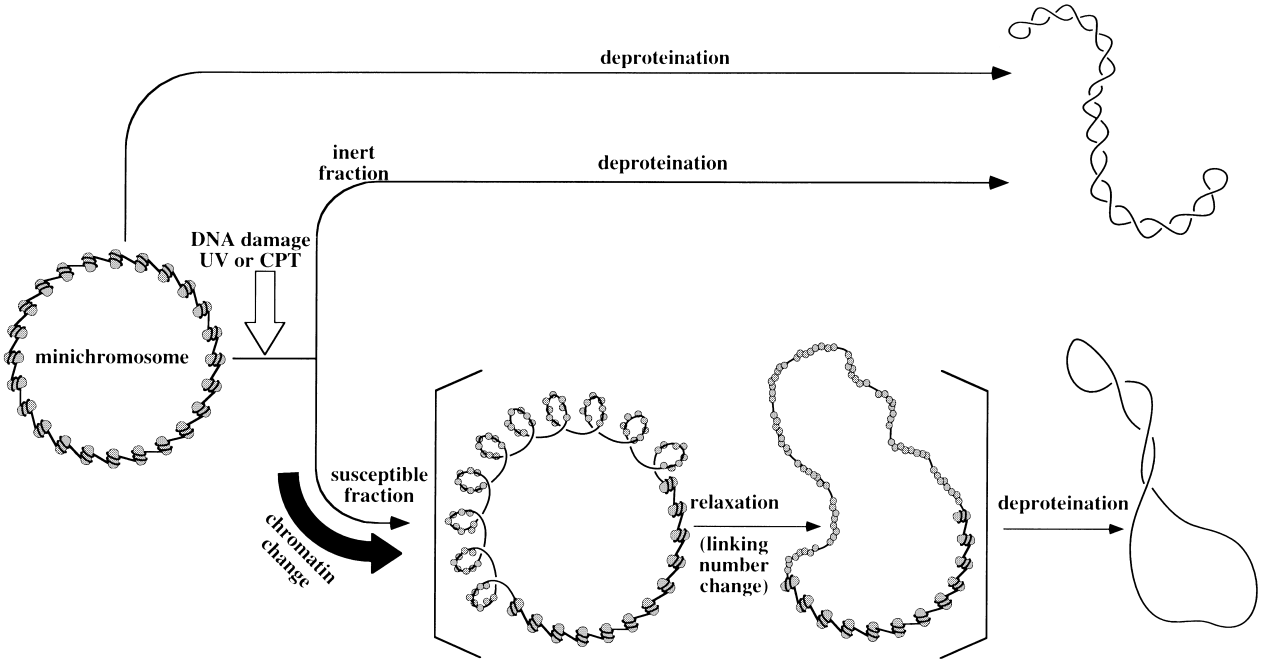

Figure 8. A working model for chromatin reorganization induced by DNA damage. In COS cells, two populations of the SV40 minichromosomes exist, the inert and the susceptible fractions. The susceptible fraction, which probably represents transcriptionally active population of the SV40 minichromosomes, can undergo rapid and extensive chromatin structural alteration in response to DNA damage by either UV or CPT treatment. Chromatin structural alteration most likely involves disassembly of a group of nucleosomes without the loss of histones. This chromatin structural alteration is detected by the linking number change of the circular episome due to passive activity of a topoisomerase.

REFERENCES

1. Mathis,G. and Althaus,F.R. (1990) *Carcinogenesis*, **11**, 1237–1239.
2. Wang,Z.G., Wu,X.H. and Friedberg,E.C. (1991) *J. Biol. Chem.*, **266**, 22472–22478.
3. Gaillard,P.H., Martini,E.M., Kaufman,P.D., Stillman,B., Moustacchi,E. and Almouzni,G. (1996) *Cell*, **86**, 887–896.
4. Lieberman,M.W. (1982) *Basic Life Sci.*, **20**, 303–314.
5. Smerdon,M.J. (1983) *Biochemistry*, **22**, 3516–3525.
6. Sidik,K. and Smerdon,M.J. (1984) *Carcinogenesis*, **5**, 245–253.
7. Porter,S.E. and Champoux,J.J. (1989) *Nucleic Acids Res.*, **17**, 8521–8532.
8. Hsiang,Y.H., Hertzberg,R., Hecht,S. and Liu,L.F. (1985) *J. Biol. Chem.*, **260**, 14873–14878.
9. Lim,M., Liu,L.F., Jacobson-Kram,D. and Williams,J.R. (1986) *Cell Biol. Toxicol.*, **2**, 485–494.
10. Tsao,Y.P., D'Arpa,P. and Liu,L.F. (1992) *Cancer Res.*, **52**, 1823–1829.
11. Nelson,W.G. and Kastan,M.B. (1994) *Mol. Cell. Biol.*, **14**, 1815–1823.
12. Kharbanda,S., Rubin,E., Gunji,H., Hinz,H., Giovanella,B., Pantazis,P. and Kufe,D. (1991) *Cancer Res.*, **51**, 6636–6642.
13. Piret,B. and Piette,J. (1996) *Nucleic Acids Res.*, **24**, 4242–4248.
14. Zhang,H., Wang,J.C. and Liu,L.F. (1988) *Proc. Natl Acad. Sci. USA*, **85**, 1060–1064.
15. Fleischmann,G., Pflugfelder,G., Steiner,E.K., Javaherian,K., Howard,G.C., Wang,J.C. and Elgin,S.C. (1984) *Proc. Natl Acad. Sci. USA*, **81**, 6958–6962.
16. Gilmour,D.S., Pflugfelder,G., Wang,J.C. and Lis,J.T. (1986) *Cell*, **44**, 401–407.
17. Stewart,A.F., Herrera,R.E. and Nordheim,A. (1990) *Cell*, **60**, 141–149.
18. Squires,S., Ryan,A.J., Strutt,H.L. and Johnson,R.T. (1993) *Cancer Res.*, **53**, 2012–2019.
19. Gunning,P., Ponte,P., Blau,H. and Kedes,L. (1983) *Mol. Cell. Biol.*, **3**, 1985–1995.
20. D'Arpa,P., Machlin,P.S., Ratrie,H.D., Rothfield,N.F., Cleveland,D.W. and Earnshaw,W.C. (1988) *Proc. Natl Acad. Sci. USA*, **85**, 2543–2547.
21. Wilson,J.T., Wilson,L.B., deRiel,J.K., Villa-komaroff,L., Efstratiadis,A., Forget,B.G. and Weissman,S.M. (1978) *Nucleic Acids Res.*, **5**, 563–581.
22. Feinberg,A.P. and Vogelstein,B. (1983) *Anal. Biochem.*, **132**, 6–13.
23. Bjornsti,M.A., Benedetti,P., Viglianti,G.A. and Wang,J.C. (1989) *Cancer Res.*, **49**, 6318–6323.
24. Ecker,D.J., Khan,M.I., Marsh,J., Butt,T.R. and Crooke,S.T. (1987) *J. Biol. Chem.*, **262**, 3524–3527.
25. Galan,J. and Haguenauer-Tsapis,R. (1997) *EMBO J.*, **16**, 5847–5854.
26. Gluzman,Y. (1981) *Cell*, **23**, 175–182.
27. Pagano,J.S. and McCutchan,J.H. (1969) *Prog. Immunobiol. Stand.*, **3**, 152–158.
28. Hirt,B. (1967) *J. Mol. Biol.*, **26**, 365–369.
29. Gietz,R.D., Schiestl,R.H., Willems,A.R. and Woods,R.A. (1995) *Yeast*, **11**, 355–360.
30. Mann,W. and Jeffery,J. (1989) *Anal. Biochem.*, **178**, 82–87.
31. Germond,J.E., Rouviere-Yaniv,J., Yaniv,M. and Brutlag,D. (1979) *Proc. Natl Acad. Sci. USA*, **76**, 3779–3783.
32. Luchnik,A.N., Bakayev,V.V., Zbarsky,I.B. and Georgiev,G.P. (1982) *EMBO J.*, **1**, 1353–1358.
33. Luchnik,A.N., Bakayev,V.V., Yugai,A.A., Zbarsky,I.B. and Georgiev,G.P. (1985) *Nucleic Acids Res.*, **13**, 1135–1149.
34. Liu,L.F. and Wang,J.C. (1987) *Proc. Natl Acad. Sci. USA*, **84**, 7024–7027.
35. Wu,H.Y., Shyy,S.H., Wang,J.C. and Liu,L.F. (1988) *Cell*, **53**, 433–440.
36. Liu,L.F. and Miller,K.G. (1981) *Proc. Natl Acad. Sci. USA*, **78**, 3487–3491.
37. Jackson,D.A. and Cook,P.R. (1993) *J. Cell Sci.*, **105**, 1143–1150.
38. Peterson,C.L. and Tamkun,J.W. (1995) *Trends Biochem. Sci.*, **20**, 143–146.
39. Cairns,B.R., Lorch,Y., Li,Y., Zhang,M., Lacomis,L., Erdjument-Bromage,H., Tempst,P., Du,J., Laurent,B. and Kornberg,R.D. (1996) *Cell*, **87**, 1249–1260.
40. Cairns,B.R., Erdjument-Bromage,H., Tempst,P., Winston,F. and Kornberg,R.D. (1998) *Mol. Cell.*, **2**, 639–651.
41. Bazett-Jones,D.P., Cote,J., Landel,C.C., Peterson,C.L. and Workman,J.L. (1999) *Mol. Cell. Biol.*, **19**, 1470–1478.
42. Cote,J., Peterson,C.L. and Workman,J.L. (1998) *Proc. Natl Acad. Sci. USA*, **95**, 4947–4952.
43. Nickel,B.E., Allis,C.D. and Davie,J.R. (1989) *Biochemistry*, **28**, 958–963.
44. Desai,S.D., Liu,L.F., Vazquez-Abad,D. and D'Arpa,P. (1997) *J. Biol. Chem.*, **272**, 24159–24164.
45. Ratner,J.N., Balasubramanian,B., Corden,J., Warren,S.L. and Bregman,D.B. (1998) *J. Biol. Chem.*, **273**, 5184–5189.
46. Swerdlow,P.S., Schuster,T. and Finley,D. (1990) *Mol. Cell. Biol.*, **10**, 4905–4911.
47. Cheng,T.-H., Li,Y.-C. and Gartenberg,M.R. (1998) *Proc. Natl Acad. Sci. USA*, **95**, 5521–5526.