Repair of UV Damage in Actively Transcribed Ribosomal Genes†

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ABSTRACT: Repair of UV-induced cyclobutane pyrimidine dimers (CPDs) was measured in the individual strands of transcriptionally active ribosomal genes in mouse Friend erythroleukemia cells. Ribosomal genes (rDNA) are multicopied, but only a fraction is transcriptionally active (or transcriptionally "poised"). Selective psoralen binding was used to separate the active fraction, which has an open chromatin structure, from inactive rDNA. *Eco*RI digestion was used to selectively release the active fraction from nuclei for DNA repair studies. UV dose response curves indicate there is no significant bias for CPD formation in either strand of both types of rDNA chromatin. More importantly, there was no evidence for transcription repair coupling in the individual strands of active and total rDNA. Indeed, over an 8 h period (one cell-cycle), repair of CPDs was almost nonexistent in either strand of active and total rDNA. Furthermore, the fraction of each chromatin structure remains constant during these repair times, suggesting that chromatin rearrangements observed during excision repair of nonnucleolar chromatin do not occur following UV damage of rDNA. However, CPDs are removed efficiently from the transcribed strand of the constitutively expressed c-*abl* gene (transcribed by Pol II), demonstrating that these cells are capable of transcription repair coupling.

Many studies have now shown that DNA repair of ultraviolet (UV)[1] light induced cyclobutane pyrimidine dimers (CPDs) in the genome is heterogeneous. Transcriptionally active chromatin is repaired at a faster rate than the genome overall [reviewed by Bohr (1991)]. This increased rate of repair is due primarily to efficient removal of damage from the transcribed strand of transcriptionally active RNA polymerase II (Pol II) genes and is thought to occur through transcription repair coupling (TRC) [reviewed by Hanawalt and Mellon (1993) and Friedberg *et al.* (1995)]. Preferential repair of the transcribed strand has been observed in *Escherichia coli* (Mellon *et al.*, 1989), yeast (Smerdon & Thoma, 1990), and mammalian cells (Mellon *et al.*, 1987), and appears to require elongation of the Pol II complex (Leadon & Lawrence, 1991; Christians & Hannawalt, 1992).

Determining if multicopied ribosomal genes (rDNA), which are transcribed by RNA polymerase I (Pol I), are repaired preferentially or strand-specifically is more complex because only a fraction of these genes is transcriptionally active (Conconi *et al.*, 1989). This fraction changes with cell type and, at least in the yeast *Saccharomyces cerivisiea*, with the cell cycle (Dammann *et al.*, 1993; J. Sogo, personal communication). Furthermore, it has been shown in a variety of different cell types that actively transcribing rDNA exists in a distinctly different chromatin structure than transcriptionally inactive rDNA, including *Tetrahymena* (Cech & Karrer, 1980), *Dictyostelium discoideum* (Ness *et al.*, 1983), mouse cells (Conconi *et al.*, 1989), *Xenopus* (Lucchini & Sogo, 1992), and the yeast *S. cerevisiae* (Dammann *et al.*, 1993). Most of these studies used differential binding of psoralen to distinguish between these two rDNA chromatin fractions.

Conconi *et al.* (1989) used psoralen binding and electrophoresis to observe a heterogeneity in chromatin structure within the rDNA copies in mouse Friend erythroleukemia cells. One chromatin fraction (about 60%) bound more psoralen and has a retarded migration on gels. When this fraction (from either *Dictyostelium discoideum* or yeast) is viewed by electron microscopy, it appears to be unfolded and presumably nucleosome-free (Sogo *et al.*, 1984; Dammann *et al.*,1993; Lucchini & Sogo, 1994). The other fraction bound less psoralen, and appears to contain canonical nucleosomes (Sogo *et al.*, 1984; Conconi *et al.*, 1989; Dammann *et al.*, 1993; Lucchini & Sogo, 1994). The more heavily cross-linked rDNA chromatin was shown to contain actively transcribing ribosomal genes, as this was the only fraction in which nascent rRNA (labeled during nuclear run-on) could be cross-linked to rDNA (Conconi *et al.*, 1989). These authors also showed that active/poised rDNA chromatin could be preferentially released by digesting nuclei with the restriction enzyme *Eco*RI. This enzyme has access to the active/poised fraction, but is blocked from digesting inactive rDNA in chromatin (presumably due to nucleosome shielding of enzyme recognition sites).

This differential access of *Eco*RI to rDNA chromatin provides a method to separate active/poised rDNA from inactive rDNA for repair studies. By combining this separation technique with the method developed by Bohr *et al.* (1985), we were able to measure damage induction and repair of UV-induced CPDs in each strand of the transcriptionally active subset of ribosomal genes.

MATERIALS AND METHODS

Cell Culture and Labeling. Mouse Friend erythroleukemia cells (FTG cell line; Concience & Meier, 1980) were a gift

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[1] Abbreviations: bp, base pair(s); CPD, *cis-syn* cyclobutane pyrimidine dimer; (6−4)PD, pyrimidine (6−4)pyrimidone photoproduct; TRC, transcription repair coupling; UV, ultraviolet; T4 endo V, T4 endonuclease V; Pol I, RNA polymerase I; Pol II, RNA polymerase II.

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from Dr. José Sogo, Eidgenössische Technische Hochschule, Zürich, Switzerland. These cells were cultured in suspension in T75 tissue culture flasks in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. Penicillin and streptomycin were added to the medium at concentrations of 100 units/mL and 100 μg/mL, respectively. Cells were grown at 37 °C in the presence of 10% CO_2. For repair experiments, cells were grown to a density of 2×10^5 cells/mL and then split 1:20 to a density of 10^4 cells/mL. Cells were prelabeled with 10 nCi/mL [^{3}H]dThd (New England Nuclear) about 16 h after splitting and harvested at a density of 5×10^5 cells/mL for mid-log phase cells.

254 nm UV Irradiation. For repair experiments, cells were pelleted by centrifugation (500*g*), washed with prewarmed 0.15 M NaCl, repelleted, and resuspended at 5×10^5 cells/mL, in 25 mL of 0.15 M NaCl. To prevent UV shielding, 25 mL of the cell suspension was poured into plastic Petri dishes (P150) to a depth of ~2 mm. Cells were irradiated with a low-pressure mercury lamp (Sylvania, Model G 30T8) providing predominantly 254 nm light. UV flux was determined using a Spectroline DM-254N UV meter (Spectronics Corp., Westbury, NY). After irradiation, cells were resuspended in prewarmed medium containing 10 μg/mL BrdUrd and 1 μg/mL FdUrd, and incubated for the specified repair times.

Preparation of Nuclei and DNA. Nuclei were prepared according to Marzluff and Huang (1984). DNA was purified from nuclei by digesting with proteinase K at a concentration of 200 μg/mL in 1% SDS at 46 °C for at least 6 h. Samples were extracted 2 times with a 1:1 mixture of Tris-buffered phenol and chloroform/isoamyl alcohol (24:1). DNA was precipitated in ethanol on ice by adding sodium acetate to a final concentration of 0.3 M and 2 volumes of 95% ethanol. The resulting pellets were washed in 70% ethanol to remove residual salt and resuspended in TE buffer (10 mM Tris, pH 8.0, and 1 mM EDTA).

Psoralen Photo-Cross-Linking. Psoralen cross-linking was performed according to Gale and Smerdon (1988b) and Conconi *et al.* (1989). Briefly, nuclei or DNA, at 4−6 A_{260} units, was suspended in 1.5 mL of storage buffer [5 mM $Mg(CH_3COO)_2$, 50 mM Tris, pH 8.0, 5 mM dithiothreitol, and 25% glycerol]. Psoralen was added to a final concentration of 10 μg/mL, and the samples were incubated on ice in the dark for 5 min. The sample was irradiated for 15 min on ice in polypropylene tubes 15 cm from a 450 W medium-pressure Hg lamp (Ace Glass Inc., Vineland, NJ). The light was filtered through pyrex to exclude wavelengths less than ~320 nm. This procedure was repeated 3 times to ensure extensive cross-linking (Gale & Smerdon, 1988b).

Electrophoresis of cross-linked fragments was performed on 25 cm long, 0.8% agarose gels in TBE buffer (Maniatis *et al.*, 1982) for 18 h at 100 V. Before transfer to membranes, cross-links were reversed by exposing gels to 254 nm UV for 12 min at a flux of 16 W/m^2 (Kochel & Sinden, 1987). This procedure was necessary in order to achieve maximal hybridization of probes to specific fragments on the membrane. DNA was transferred to Hybond N+ membranes (Amersham) using the alkaline transfer procedure (Ausubel *et al.*, 1988).

EcoRI Digestion of Nuclei. Nuclei were suspended in 10 mM $MgCl_2$, 50 mM NaCl, and 10 mM Tris (pH 8.0) and incubated for 1 h at 37 °C with 5 units of *Eco*RI/μg of DNA. Nuclei in storage buffer containing glycerol were washed 3 times in restriction enzyme buffer prior to addition of enzyme.

Isolation of Unreplicated DNA. Density gradient centrifugation was performed as described by Smith *et al.* (1981) and Bohr and Okumoto (1988). Samples were centrifuged at 55 000 rpm for at least 17 h in a Beckman Ti 70.1 rotor, and the gradients were fractionated into 0.2 mL fractions before pooling the parental density peak. In each repair experiment, unirradiated cells were incubated in medium containing 10 μM BrdUrd and 1 μM FdUrd for 8 h (the longest repair time). The DNA from these cells served not only as a control for nonspecific T4 endo V digestion but also as a control for the efficiency of BrdUrd density labeling of newly replicated DNA.

T4 Endonuclease V Digestion. The T4 endo V used in these experiments was a gift from Dr. R. Stephen Lloyd, University of Texas Medical Branch, Galveston, TX. A 1:200 dilution of the T4 endo V stock was added to 5−10 μg of DNA in T4 endo V digestion buffer (10 mM Tris-HCl, pH 8.0, 100 mM NaCl, 10 mM EDTA, and 10 mg/mL BSA). Also, 0.2 ng of linearized plasmid pHumA (see below) was added to correct for loading differences between gel lanes. The samples were digested for 1 h at 37 °C (Murad *et al.*, 1995).

Electrophoresis and Southern Blotting. Samples were electrophoresed in 0.6 or 0.8% alkaline agarose gels according to Ausubel *et al.* (1988). After electrophoresis, gels were neutralized and stained for photography by soaking in 1.5 M NaCl, 1 M Tris-HCl (pH 7.6), and 0.5 μg/mL ethidium bromide. Gels were then depurinated by soaking in 0.25 M HCl for 15 min. After acid treatment, gels were again soaked in neutralizing buffer described above. All gels were transferred to nylon membranes (Hybond N+, Amersham) using the alkaline transfer method according to Ausebel *et al.* (1988).

Preparation of Hybridization Probes. Plasmid pHumA was a gift from Drs. Brian McStay and Ronald Reeder, University of Washington, Seattle, WA. The plasmid contains a 7.2 kb *Eco*RI fragment encompassing the 3′ half of the transcribed region of human rDNA (Figure 1). Radioactively labeled double-strand DNA probes were synthesized by nick translation using the gel-purified 7.2 kb *Eco*RI fragment from pHumA. Nick translation reactions were done according to the manufacturer's directions (Promega, Madison, WI).

Plasmid pSPT28S was a gift from Drs. José Sogo (ETH, Zürich, Switzerland) and Antonio Conconi (WSU, Pullman, WA). It contains a 137 bp *Bam*HI/*Sst*I fragment from the 28S region of mouse rDNA inserted into a bidirectional promoter vector (Figure 1). Strand-specific probes were generated from plasmid pSPT28S, according to manufacturer's instructions included in the Riboprobe Gemini II Kit (Promega). Hybridization was performed in glass tubes in a hybridization chamber (Robbins Scientific) at 60 °C in the buffers of Church and Gilbert (1984) . Following hybridization, the membranes were washed 3 times in 0.1 × SSC (0.015 M NaCl, 1.5 mM sodium citrate) and 1% SDS at 60 °C. The membranes were exposed to preflashed X-ray film (Amersham) and intensifying screens (Dupont Cronex lightning plus) at −80 °C.

Plasmid pBS-Ty4 was a gift from Dr. Jean Wang (University of California, San Diego), and contains a 3.9 kb

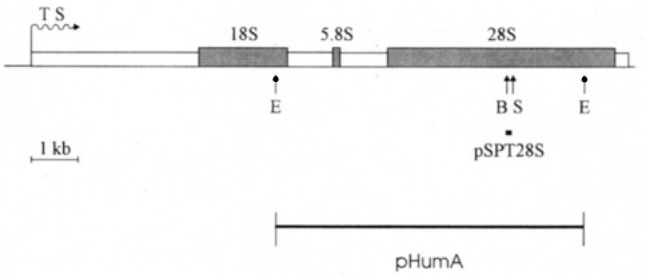

FIGURE 1: Map of the transcribed region of mouse ribosomal genes. Filled boxes indicate the positions of mature 18S, 5.8S, and 28S rRNAs. TS and arrow indicate transcriptional start and the direction of transcription. The *Eco*RI (E) arrows indicate the rDNA fragment used in this study, while the *Bam*HI (B) and *Sst*I (S) arrows show the position of the pSPT28S probe used in this study (short solid bar). The long solid bar indicates the map and position of pHumA.

cDNA clone of the mouse c-*abl* type IV transcript (Wang & Baltimore, 1983). Strand-specific RNA probes were generated from this plasmid, and membrane hybridizations were carried out as described previously (Murad *et al.*, 1995).

Quantitation of Autoradiograms. Quantitation of autoradiograms was performed either by scanning, using a laser densitometer (LKB Model 2222), or by video densitometry, using a Bioimage Systems (Visage 60) imager with the "Whole Band Analysis" software. The integrated intensity of T4 endo V treated bands was divided by the integrated intensity of T4 endo V untreated bands to determine the fraction of fragments free of CPDs (P_0). In each case, the intensities were normalized to the intensities of the respective loading controls. The average number of CPDs for each sample was determined using the Poisson expression [$-\ln(P_0)$] (Bohr & Okomoto, 1988).

RESULTS

The chromatin structure of the 6.5 kb *Eco*RI fragment (Figure 1) of rDNA in mouse Friend erythroleukemia cells was extensively characterized by Conconi *et al.* (1989). Therefore, we chose to examine CPD induction and repair in this fragment and in these cells. Also, this fragment lies completely within the actively transcribed 45S transcription unit of rDNA (Figure 1). It was shown by Conconi *et al.* (1989) that *Eco*RI digestion of nuclei preferentially releases the actively transcribing chromatin fraction of rDNA. Consequently, *Eco*RI digestion of nuclei from UV-irradiated cells and T4 endonuclease V (T4 endo V) treatment of the purified DNA followed by electrophoresis in alkaline agarose gels, blotting, and hybridization to strand-specific riboprobes permit the analysis of strand-specific damage induction and repair of transcriptionally active/poised rDNA. For comparison, DNA samples can be redigested with *Eco*RI, releasing all rDNA fragments, and total rDNA damage and repair can be assayed.

Psoralen Cross-Linking of Isolated Nuclei. To demonstrate the two different rDNA chromatin structures in mouse Friend cells, nuclei were processively cross-linked with psoralen. Nuclei, prepared from exponentially growing mouse Friend cells, were photoreacted with psoralen for different times (0, 0.5, 2, 6, and 30 min). Purified DNA was digested with *Eco*RI, separated on an agarose gel, transferred to a nylon membrane, and probed with nick-translated pHumA (Figure 1). Southern analysis of the digests reveals the processive cross-linking of both chromatin

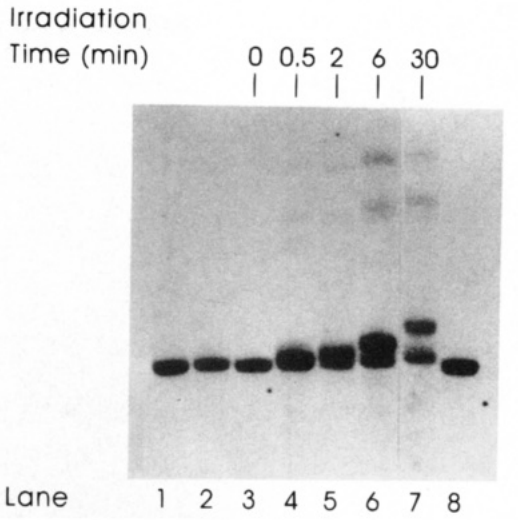

FIGURE 2: Psoralen cross-linking of nuclei during UVA irradiation. Nuclei, prepared from actively growing mouse Friend erythroleukemia cells, were incubated with psoralen in the dark for 5 min on ice. The sample was irradiated with UVA, and aliquots were removed at 0, 0.5, 2, 6, and 30 min (lanes 3−7). DNA was prepared, digested with *Eco*RI, and separated on a 0.8% agarose gel. After Southern transfer, the membrane was hybridized to nick-translated ^{32}P-labeled pHumA (see Figure 1). Lanes 1 and 8 are untreated, *Eco*RI-digested naked DNA, and lane 2 is DNA from nuclei, mock-treated with psoralen by adding 37 μL of ethanol only, and irradiated with UVA for 30 min.

fractions (Figure 2, lanes 3−7). As the length of UVA irradiation time increases, both active and inactive rDNAs accumulate cross-links, retarding their migration on the gel [e.g., see Carlson *et al.* (1982)]. As a control, nuclei were mock-treated with psoralen by adding ethanol alone (psoralen is dissolved in ethanol) and UV-irradiated for 30 min. The mock-photoreacted nuclei (lane 2) yield no evidence of altered migration of the *Eco*RI band when compared to untreated DNA (lanes 1 and 8).

Separation of rDNA Chromatin Fractions by EcoRI Digestion. To demonstrate the release of active/poised rDNA by *Eco*RI, we used the psoralen technique (Conconi *et al.*, 1989). Previously, we showed that (near) maximal psoralen cross-linking is achieved with a few succesive irradiations of chromatin (or nuclei) by a 450 W light source (Gale & Smerdon, 1988b). As shown in Figure 3, when nuclei are cross-linked in this manner and the DNA digested with *Eco*RI, the two bands of rDNA (designated F and S) are clearly resolved on native agarose gels (Figure 3, lane 2), in agreement with Conconi *et al.* (1989). The slower migrating band of the doublet (band S) is the active/poised fraction of rDNA, and the faster migrating band (band F) is the inactive fraction (Conconi *et al.*, 1989). The faint upper bands in lane 2 represent incompete digestion of some rDNA sequences, presumably due to cross-links at *Eco*RI restriction sites. As shown by Conconi *et al.* (1989), the density of psoralen cross-links in active/poised rDNA chromatin is similar to the density of psoralen cross-links obtained with isolated genomic DNA (Figure 3, lane 3), reflecting the open structure of transcriptionally active rDNA chromatin.

We then examined the preferential release of active rDNA chromatin by *Eco*RI from *un-cross-linked* nuclei (Conconi *et al.*, 1989). Nuclei not exposed to psoralen were digested with *Eco*RI and then photoreacted with psoralen to separate the active and inactive fractions (Figure 3, lane 5). An aliquot of this DNA was redigested with *Eco*RI to release

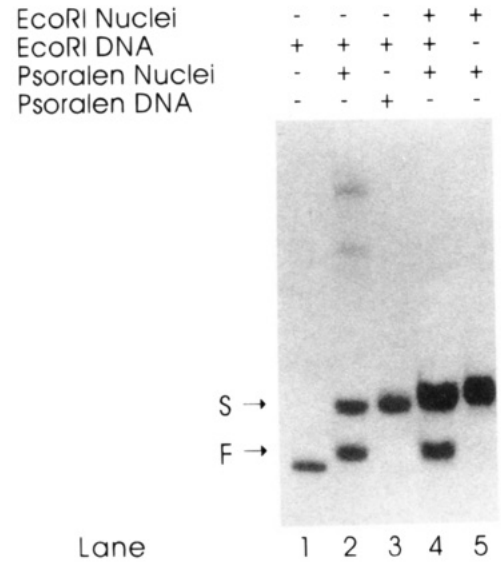

FIGURE 3: *Eco*RI has differential access to rDNA chromatin. DNA samples were prepared from nuclei with the following treatments: (lane 1) untreated nuclei, and *Eco*RI-digested DNA; (lane 2) psoralen-cross-linked nuclei, and DNA digested with *Eco*RI; (lane 3) DNA digested with *Eco*RI and then psoralen-cross-linked; (lane 5) *Eco*RI-digested nuclei and cross-linked with psoralen, prior to DNA isolation and loading on the gel. An aliquot of the DNA in lane 5 was redigested with *Eco*RI before being loaded on the gel (lane 4). Samples were separated on a 0.8% agarose gel, and transferred and hybridized to nick-translated ^{32}P-labeled pHumA (see Figure 1).

all of the rDNA fragments (Figure 3, lane 4). It is clear from these data that *Eco*RI digestion of nuclei yields only the active/poised fraction of rDNA (lane 5), and the inactive fraction is released upon redigestion of the isolated DNA (lane 4).

CPD Yield in Ribosomal Genes. Considering that the 6.5 kb *Eco*RI fragment is 68% G+C [e.g., see Hassouna *et al.* (1984)], we determined if (near) physiologic doses of UV light would yield sufficient DNA damage to allow accurate measurement of repair in this fragment. Therefore, we measured CPD induction in the individual strands of each chromatin fraction of rDNA following different UV doses. Actively growing cells were harvested and irradiated in suspension with 254 nm UV light at doses ranging from 0 to 50 J/m^2. Cells were immediately lysed; nuclei were prepared and digested with *Eco*RI, as described earlier. The DNA was isolated, and half of each sample was redigested with *Eco*RI (to assay the dose response of total rDNA). After treatment with (or without) T4 endo V, samples were separated on alkaline agarose gels and hybridized to strand-specific riboprobes (Figure 1). These riboprobes were initially tested by Northern analysis and found to be strand-specific for 28S rRNA (data not shown).

Induction of CPDs in the transcribed and nontranscribed strands of active/poised rDNA at different UV doses is shown in Figure 4A. A least-squares fit to the data yields slopes of 3.4×10^{-2} and 2.5×10^{-2} CPDs $(J/m)^{-2}$ per fragment for the transcribed and nontranscribed strands, respectively. The same analysis (Figure 4B) gives slopes of 3.0×10^{-2} and 2.6×10^{-2} CPDs $(J/m)^{-2}$ per fragment in the transcribed and nontranscribed strands, respectively, for total rDNA. These values predict a slight bias for induction of CPDs in the transcribed strand, in agreement with predictions from computer analysis (Murad, 1991) searching for possible sites

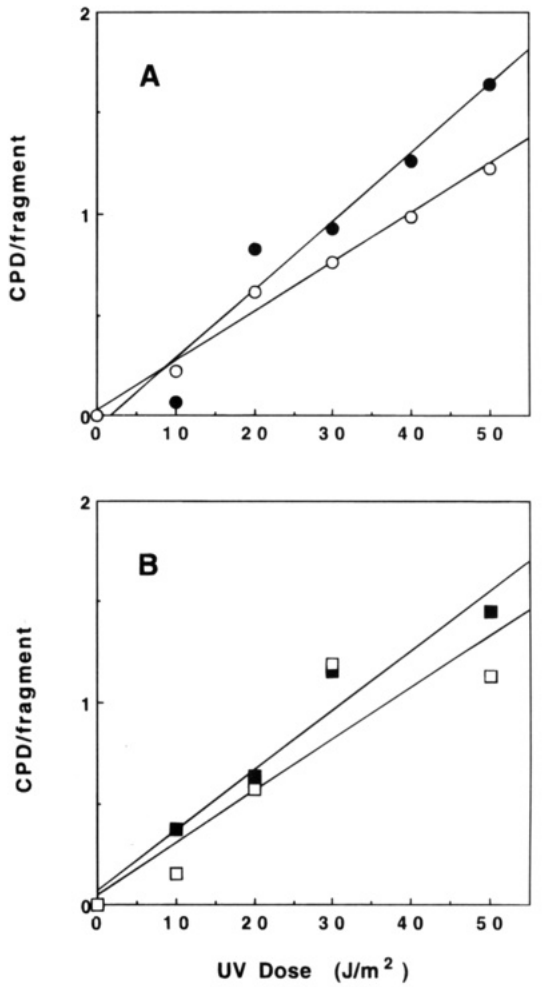

FIGURE 4: UV dose response of active/poised and total rDNA in mouse cells. Exponentially growing cells were exposed to 254 nm UV light at doses ranging from 0 to 50 J/m^2, and nuclei were immediately prepared and digested with *Eco*RI. DNA was then prepared, treated with or without T4 endo V, and separated on an alkaline agarose gel. Samples were transferred to a nylon membrane and hybridized to strand-specific riboprobes (from pSPT28S). Autoradiograms were scanned and the CPD/fragment determined (see Materials and Methods). Panel A: Lines represent least-squares fits to the data for the transcribed strand (●) and nontranscribed strand (○) of the active/poised fraction of rDNA. Panel B: Some of the DNA used in panel A was redigested with *Eco*RI, and samples were treated as described above. Lines are least-squares fits to the data for the transcribed strand (■), and nontranscribed strand (□) of total rDNA.

of CPD formation (data not shown). In addition, the data indicate there is no bias for CPD induction in either chromatin fraction. From these values, it was predicted that a UV dose of 20 J/m^2 will induce about 0.7 CPD/fragment, a sufficient level for accurate repair analysis (Bohr & Okumoto, 1988; Murad *et al.*, 1995).

Repair of UV-Induced CPDs in the Individual Strands of rDNA. Actively growing mouse Friend cells were irradiated at 20 J/m^2 and allowed to repair for varying times. This cell line is fast growing with a cell division time of about 8 h (Conconi *et al.*, 1989), and repair times were chosen within one cell cycle. Cells were harvested at the specified repair times, and nuclei were prepared and digested with *Eco*RI to release the active/poised fraction of rDNA. Following DNA preparation, the samples were applied to CsCl density gradients to separate newly replicated DNA from parental DNA. The parental DNA was collected and either redigested

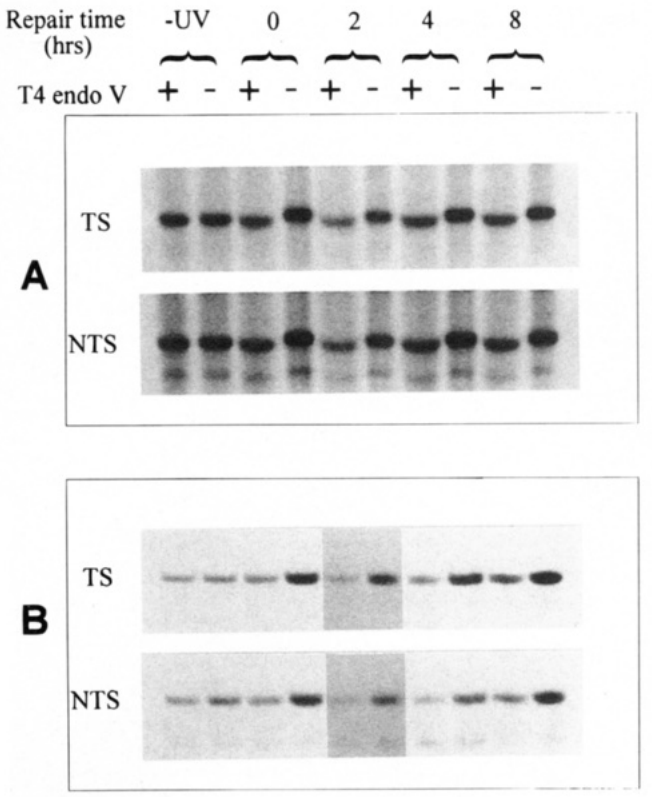

FIGURE 5: Repair of UV-induced CPDs in active/poised and total rDNA in mouse cells. Exponentially growing cells were labeled with 10 μM BrdUrd and 1 μM FdUrd, and irradiated with 20 J/m^2 UV light. Cells were harvested after repair times of 0, 2, 4, and 8 h. Nuclei were prepared and digested with *Eco*RI. Parental DNA was isolated by CsCl density gradient centrifugation and digested with or without T4 endo V. Samples were loaded on a 0.8% alkaline agarose gel, transferred, and hybridized to strand-specific riboprobes (from pSPT28S). Following autoradiography, the membranes were stripped and reprobed with the opposite strand. Panel A shows autoradiograms for both the transcribed (TS) and nontranscribed (NTS) strands of the active/poised fraction of rDNA. Panel B shows autoradiograms for each strand of total rDNA prepared by redigesting parental DNA (panel A) with *Eco*RI.

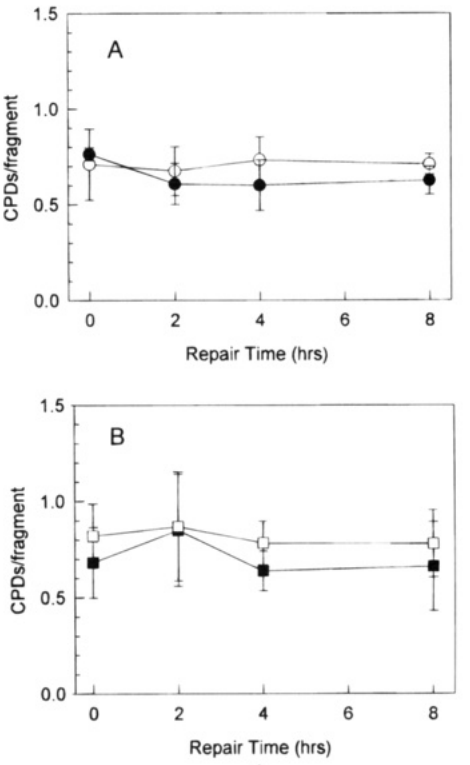

FIGURE 6: Repair of UV-induced CPDs in each strand of active/poised and total rDNA in mouse cells. Panels A and B show data from the analysis of autoradiograms (Materials and Methods), such as those shown in Figure 5. Panel A shows data obtained for the transcribed strand (●) and the nontranscribed strand (○) of active/poised rDNA. Data points represent the mean (±1 SD) of three independent experiments. Panel B shows data obtained for the transcribed strand (■) and the nontranscribed strand (□) of total rDNA. Data points represent the mean (±1 SD) of four independent experiments.

with *Eco*RI (to measure repair in total rDNA) or not redigested (to measure repair in active/poised rDNA), prior to digestion with T4 endo V. Representative autoradiograms for repair of active/poised and total rDNA are shown in Figure 5A and Figure 5B, respectively. [The faint band migrating ahead of the 6.5 kb band in the nontranscribed strand (NTS) was always detected, and assumed due to nonspecific hybridization.] These (and other) autoradiograms were analyzed by video densitometry to determine the fraction of CPDs repaired during the 8 h incubation time (Figure 6). As can be seen, there is little (or no) repair of CPDs in either strand of active/poised rDNA or total rDNA for the repair times investigated.

Repair of UV-Induced CPDs in the Individual Strands of a Pol II Gene. To determine if these cells were indeed capable of TRC in a Pol II gene, we analyzed the removal of CPDs from a 7.2 kb *Kpn*I fragment located at the 3′ end of the transcribing c-*abl* gene (Wang & Baltimore, 1983). Once again, exponentially growing Friend cells were irradiated with 20 J/m^2 UV light and allowed to repair for various times. As shown in Figure 7, both strands of c-*abl* showed significant CPD removal. By 8 h, 75% of the CPDs were removed from the transcribed strand, and 50% were removed from the nontranscribed strand. These data indicate not only that efficient repair of CPDs occurs in an active Pol II gene but also that TRC occurs in these cells.

Rearrangement of rDNA Chromatin following UV Irradiation. We considered the possibility that damaged, inactive rDNA chromatin could unfold prior to repair, as occurs in bulk chromatin (Smerdon, 1989), and become accessible to *Eco*RI digestion. If this were the case, the inactive fraction of rDNA could mimic the active/poised rDNA in our repair studies. Therefore, we measured *Eco*RI accessibility to rDNA chromatin during repair using psoralen as a probe for chromatin structure. After irradiating cells (20 J/m^2) and incubating them for varying repair times, they were harvested, and the nuclei were prepared and digested with *Eco*RI and photoreacted with psoralen. Equal amounts of the isolated DNA were either redigested or not with *Eco*RI and separated on neutral agarose gels. The autoradiogram in Figure 8A (lanes 2, 4, 6, 8, and 10) shows there is little difference in the access of *Eco*RI to active rDNA during repair. Redigestion with *Eco*RI following DNA preparation (lanes 1, 3, 5, 7, and 9) releases the inactive fraction of rDNA (see Figure 3). Figure 8B shows the fraction of active/poised rDNA determined for each repair time, as well as for undamaged cells (open circle). These results suggest that the fraction of active/poised rDNA remains constant over the repair times investigated.

DISCUSSION

We have examined the induction and removal of CPDs from the individual strands of both active/poised and total

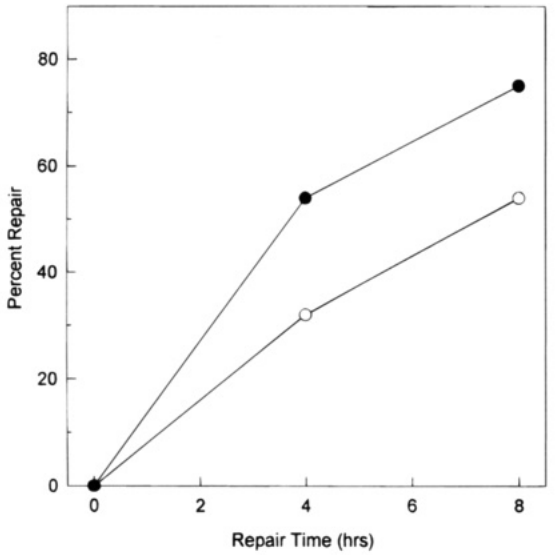

FIGURE 7: Strand-specific repair of the c-*abl* gene in mouse cells. Exponentially growing cells were UV-irradiated at 20 J/m^2, and harvested at repair times of 0, 4, and 8 h. DNA was prepared and digested with *Kpn*I. Parental DNA was purified on CsCl gradients, and digested with or without T4 endo V. Samples were electrophoresed on a 0.8% alkaline agarose gel, transferred, and hybridized to strand-specific riboprobes from plasmid pBS-Ty4. Autoradiograms were scanned, and the CPD per fragment was determined (see Materials and Methods) for the transcribed strand (●) and nontranscribed strand (○).

rDNA in mouse Friend erythroleukemia cells. As stated earlier, determining if transcription repair coupling is associated with Pol I transcribed genes is complicated because only a fraction of the rDNA copies are transcriptionally active. This fraction varies from <20% in tomato leaves and mouse kidney cells to almost 65% in mouse Friend cells (Conconi *et al.*, 1992; Wiesendanger, 1994; J. Sogo, personal communication). Indeed, we have recently observed that confluent human diploid fibroblasts also have an active/poised rDNA fraction of <30% (L. Fritz and M. Smerdon, unpublished results). Thus, the active/poised fraction of rDNA may be significantly lower than the inactive fraction of rDNA, and must be separated from total rDNA to assay repair in the transcribed fraction alone.

Christians and Hanawalt (1993) reported a lack of strand-specific repair in total rDNA in human and CHO cells. They observed no repair of CPDs in either strand of rDNA within 24 h in CHO cells, which agrees with our observations. In addition, repair of CPDs in rDNA was detected in human cells; however, it was less efficient than that reported for the genome overall. It should be noted that the rDNA fragments analyzed by these authors contained a large portion (over half) of intergenic spacer region, whose chromatin structure and transcriptional activity have not yet been characterized. Indeed, as stated by these authors, "probably only about 25% of the transcribed strand of the restriction fragments examined was actually transcribed" (Christians & Hanawalt, 1993). Also, transcriptionally active rDNA sequences were not assayed independently nor was the fraction of active/poised rDNA determined for either CHO or human cells. Instead, the effect of rDNA transcription on repair was measured by comparing repair rates of total rDNA in actively growing cells to those in stationary cells.

To further characterize the effect of rDNA transcription on repair, Christians and Hanawalt (1994) examined the

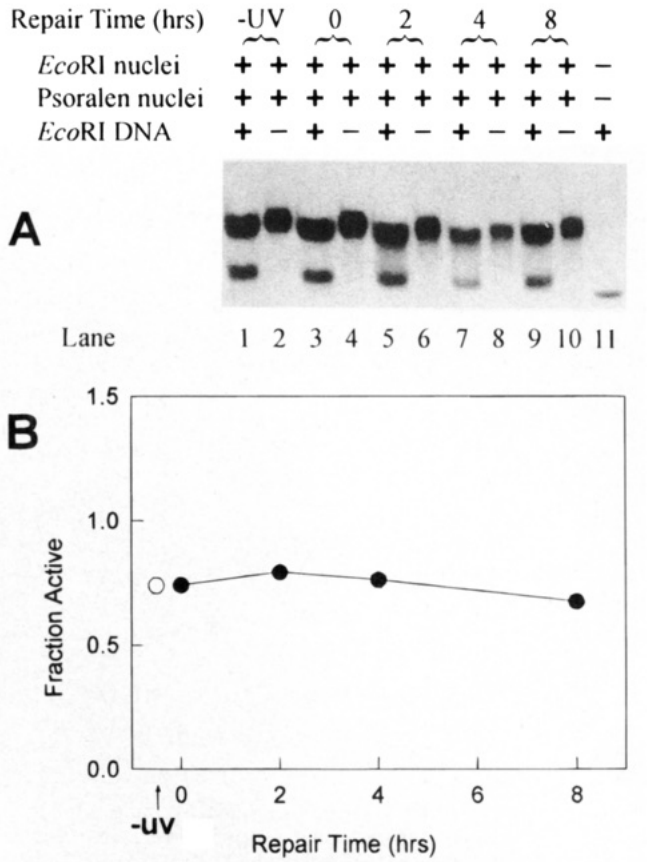

FIGURE 8: Accessibility of *Eco*RI and psoralen to rDNA during UV repair. Exponentially growing cells were irradiated with 20 J/m^2 UV light, and harvested at repair times of 0, 2, 4, and 8 h. Isolated nuclei were first digested with *Eco*RI and then photoreacted with psoralen. Following DNA preparation, equal amounts of DNA were either redigested (lanes 1, 3, 5, 7, 9) or not (lanes 2, 4, 6, 8, 10) with *Eco*RI and separated on a 0.8% agarose gel. The autoradiogram in panel A shows the migration of rDNA liberated by *Eco*RI in each case. Panel B shows the fraction of active/poised rDNA determined for UV-irradiated (●) and nonirradiated (○) samples. Values represent the mean of two independent experiments.

removal of CPDs from rDNA in *Xeroderma pigmentosum* complementation group C (XPC) cells and Cockayne's syndrome complementation groups A and B (CSA and CSB) cells. These cells were chosen because XPC cells, although repair-deficient in the genome overall (Kantor *et al.*, 1990), were shown to exhibit normal levels of TRC in Pol II transcribed sequences (Venema *et al.*, 1991; Evans *et al.*, 1993). Conversely, CS cells display normal levels of excision repair in the genome overall (Mayne *et al.*, 1982), but lack TRC (Venema *et al.*, 1990). Christians and Hanawalt (1994) observed lower than normal repair of CPDs in rDNA in CSA and CSB cells, and no repair of CPDs in XPC cells. Once again, the same rDNA fragment (containing >50% intergenic spacer region) was analyzed in these cells, and the fraction of transcriptionally active rDNA sequences was not determined.

Vos and Wauthier (1991) examined rDNA repair of psoralen cross-links in a human−mouse cell hybrid. In these cells, the mouse rDNA was shown to be transcriptionally active, whereas the human rDNA was silent. However, the fraction of mouse rDNA that was transcriptionally active was not measured. They reported inefficient repair of psoralen cross-links and monoadducts in both the mouse and human rDNA sequences. However, they observed that the (pre-

sumed active) mouse rDNA genes were 5 times more susceptible to psoralen modification than the inactive human rDNA.

Finally, Stevnsner *et al.* (1993) measured repair of lesions induced by UV light, alkylating agents, and cisplatin in total rDNA in CHO cells. They reported inefficient repair of CPDs, and monoadducts and diadducts induced by nitrogen mustard. However, they observed efficient repair of methyl methanesulfonate adducts and cisplatin interstrand cross-links. Repair in the individual strands of rDNA was not measured in this study. Also, as in the other reports, the active/poised fraction of rDNA was not isolated for separate determination of damage induction or repair.

It has been established that rodent cells repair the bulk of their DNA inefficiently (e.g., Bohr *et al.*, 1985). Thus, it was postulated that cell survival after UV irradiation is dependent on efficient repair of transcriptionally active genes (Bohr *et al.*, 1985; Madhani *et al.*, 1986). Moreover, it has been shown that efficient repair is confined to the transcribed strand of active genes [reviewed by Hanawalt and Mellon (1993) and Friedberg *et al.* (1995)]. Therefore, it is surprising to observe inefficient repair of either strand of the active/poised fraction of rDNA. It has been recently reported that the proteins complementing XPD and XPB not only are involved in transcription repair coupling but also are subunits of the transcription factor TFIIH (Schaeffer *et al.*, 1993; Drapkin *et al.*, 1994). Since TRC was not observed in rDNA (Figure 6), yet was observed in the Pol II transcribed c-*abl* gene (Figure 7), an explanation for this difference is that these factors are not found in the nucleolus. To date, there have been no reports of repair proteins associated with Pol I transcription factors. Furthermore, the nucleolus may act as a barrier to the large, multisubunit "repairosome" required for nucleotide excision repair (Friedberg *et al.*, 1995). Such a barrier does not exist for all repair proteins, however, since we have recently found that bleomycin-induced strand breaks are repaired very efficiently in rDNA (L. Fritz, C. Suquet, and M. Smerdon, manuscript in preparation).

It has been demonstrated that TRC requires a translocating polymerase [reviewed in Friedberg *et al.* (1995)]. Even though log phase cells were used in all repair experiments, it is possible that rDNA transcription is down-regulated in response to UV damage and elongation does not occur in UV-damaged ribosomal genes. However, rDNA sequences are highly conserved [see Gonzalez *et al.* (1990) and references cited therein). One must assume, therefore, that some repair mechanism(s) is (are) operational for this set of genes. It has been reported that biased gene conversion (Hillis *et al.*, 1991) and interchromosomal recombination (Worton *et al.*, 1988) are involved in the concerted evolution of rDNA. Perhaps these are the mechanisms responsible for maintaining the integrity of rDNA sequences.

Finally, since chromatin rearrangements can occur during excision repair [reviewed by Smerdon (1989)], we were concerned that damaged, inactive rDNA could unfold and become accessible to *Eco*RI digestion. Using *Eco*RI digestion of nuclei combined with psoralen as a probe for chromatin structure, we measured the ratio of active/poised rDNA to inactive rDNA as a function of repair time. This analysis indicates that the fraction of active/poised rDNA to total rDNA remains constant over the repair times investigated. These results indicate that even the first stages of nucleotide excision repair (i.e., chromatin rearrangement) do not occur in rDNA.

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REFERENCES

Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., & Struhl, K. (1988) in *Current protocols in molecular biology*, John Wiley and Sons, New York.

Bohr, B. A. (1991) *Carcinogenesis 12*, 1983−1992.

Bohr, V. A., & Okumoto, D. S. (1988) in *DNA repair: A laboratory manual of research procedures* (Friedberg, E. C., & Hanawalt, P. C., Eds.) pp 347−366, Marcel Dekker, Inc., New York.

Bohr, V. A., Smith, C. A., Okumoto, D. S., & Hanawalt, P. C. (1985) *Cell 40*, 359−369.

Carlson, J. O., Pfenninger, O., Sinden, R. R., Lehman, J. M., & Pettijohn, D. E. (1982) *Nucleic Acids Res. 10*, 2043−2063.

Cech, T. R., & Karrer, K. M. (1980) *J. Mol. Biol. 136*, 395−416.

Christians, F. C., & Hanawalt, P. C. (1992) *Mutat. Res. 274*, 93−101.

Christians, F. C., & Hanawalt, P. C. (1993) *Biochemistry 32*, 10512−10518.

Christians, F. C., & Hanawalt, P. C. (1994) *Mutat. Res. 323*, 179−187.

Church, G. M., & Gilbert, W. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 1991−1995.

Conconi, A., Widmer, R. M., Koller, T., & Sogo, J. M. (1989) *Cell 57*, 753−761.

Conconi, A., Sogo, J. M., & Ryan, C. A. (1992) *Proc. Natl. Acad. Sci. U.S.A. 89*, 5256−5260.

Conscience, J.-F., & Meier, W. (1980) *Exp. Cell Res. 125*, 111−119.

Dammann, R., Lucchini, R., Koller, T., & Sogo, J. M. (1993) *Nucleic Acids Res. 21*, 2321−2338.

Drapkin, R. J., Reardon, T., Ansarl, A., Huang, J.-C., Zawel, L., Ahn, K., Sancar, A., & Reinberg, D. (1994) *Nature 368*, 769−772.

Evans, M. K., Robbins, J. H., Ganges, M. B., Tarone, R. E., Nairn, R. S., & Bohr, B. A. (1993) *J. Biol. Chem. 268*, 4839−4847.

Friedberg, E. C. (1985) in *DNA repair*, W. H. Freeman and Company, New York.

Friedberg, E. C., Walker, G. C., & Siede, W. (1995) in *DNA Repair and Mutagenesis*, ASM Press, Washington, D.C.

Gale, J. M., & Smerdon, M. J. (1988a) *J. Mol. Biol. 204*, 949−958.

Gale, J. M., & Smerdon, M. J. (1988b) *Biochemistry 27*, 7197−7205.

Gale, J. M., Nissen, K. A., & Smerdon, M. J. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 6644−6648.

Gonzalez, I. L., Chambers, C., Gorski, J. L., Stambolian, D., Schmickel, R. D., & Sylvester, J. E. (1990) *J. Mol. Biol. 212*, 27−35.

Hanawalt, P., & Mellon, I. (1993) *Curr. Opin. Cell Biol. 3*, 67−69.

Hassouna, N., Michot, B., & Bachellerie, J.-P. (1984) *Nucleic Acids Res. 12*, 3563−3583.

Hillis, D. M., Moritz, C., Porter, C. A., & Baker, R. J. (1991) *Science 251*, 308−310.

Kantor, G. J., Barsalou, L. S., & Hanawalt, P. C. (1990) *Mutat. Res. 235*,171−180.

Kochel, T. J., & Sinden, R. R. (1987) *J. Mol. Biol. 205*, 91−102.

Leadon, S. A., & Lawrence, D. A. (1991) *Mutat. Res. 255*, 67−78.

Long, E. O., & Dawid, I. B. (1980) *Annu. Rev. Biochem. 49*, 727−764.
Lucchini, R., & Sogo, J. M. (1992) *Mol. Cell. Biol. 12*, 4288−4296.
Lucchini, R., & Sogo, J. M. (1994) *Mol. Cell. Biol. 14*, 318−326.
Madhani, H. D., Bohr, V. A., & Hanawalt, P. C. (1986) *Cell 45*, 417−423.
Maniatis, T., Fritsh, E. F., & Sambrook, J. (1982) in *Molecular cloning, a laboratory manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
Marzluff, W. F., & Huang, R. C. C. (1984) in *Transcription and Translation: A Practical Approach* (Hames, B. D., & Higgins, S. J., Eds.) pp 89−129, IRL Press, Oxford.
Mayne, L. V., Lehmann, A. R., & Waters, R. (1982) *Mutat. Res. 106*, 179−189.
Mellon, I., & Hanawalt, P. C. (1989) *Nature 342*, 95−98.
Mellon, I., Spivak, G., & Hanawalt, P. C. (1987) *Cell 51*, 241−249.
Murad, A. O. (1991) *Selective DNA damage and repair in a glucocorticoid inducible gene*, Ph.D. Thesis, Washington State University, Pullman, WA.
Murad, A. O., de Cock, J., Brown, D. W., & Smerdon, M. J. (1995) *J. Biol. Chem. 270*, 3949−3957.
Ness, P. J., Labhart, P., Banz, E., Koller, T., & Parish, R. W. (1983) *J. Mol. Biol. 166*, 361−381.
Schaeffer, L., Roy, R., Humbert, S., Moncollin, V., Vermeullin, W., Hoeijmakers, J. H. J., Chambon, P., & Egly, J.-M. (1993) *Science 260*, 58−63.
Smerdon, M. J. (1989) in *DNA repair mechanisms and their biological implications in mammalian cells* (Lambert, M. W., & Laval, J., Eds.) pp 271−294, Plenum Press, New York.
Smerdon, M. J., & Thoma, F. (1990) *Cell 61*, 675−684.
Smerdon, M. J., Lan, S. Y., Calza, R. E., & Reeves, R. (1982) *J. Biol. Chem. 257*, 13441−13447.
Smith, C. A., Cooper, P. K., & Hanawalt, P. C. (1981) in *DNA repair: A laboratory manual of research procedures* (Friedberg, E. C., & Hanwalt, P. C., Eds.) pp 289−305, Marcel Dekker, Inc., New York.
Sogo, J. M., Ness, P. J., Widmer, R. M., Parish, R. W., & Koller, T. (1984) *J. Mol. Biol. 178*, 897−928.
Stevnsner, T., May, A., Petersen, L. N., Larminat, F., Pirsel, M., & Bohr, V. A. (1993) *Carcinogenesis 14*, 1591−1596.
Venema, J., Mullenders, L. H. F., Natarajan, A. T., van Zeeland, A. A., & Mayne, L. V. (1990) *Proc. Natl. Acad. Sci. U.S.A. 87*, 4707−4711.
Venema, J., van Hoffen, A., Karcagi, V., Natarajan, A. T., van Zeeland, A. A., & Mullenders, L. H. F. (1991) *Mol. Cell. Biol. 11*, 4128−4234.
Vos, J.-M. H., & Wauthier, E. L. (1991) *Mol. Cell. Biol. 11*, 2245−2252.
Wang, J. Y. J., & Baltimore, D. (1983) *Mol. Cell. Biol. 3*, 773−779.
Wiesendanger, B. (1994) *The proportion of non-nucleosomal, transcribable and nucleosomal, non-transcribable ribosomal RNA gene copies is cell type-specific*, Ph.D. Thesis, No. 10801, Eidgenössische Technische Hochschule, Zürich, Switzerland.
Worton, R. G., Sutherland, J., Sylvester, J. E., Willard, H. F., Bodrug, S., Dube, I., Duff, C., Kean, V., Ray, P. N., & Schmickel, R. D. (1988) *Science 239*, 64−67.

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