Tight correlation between inhibition of DNA repair *in vitro* and transcription factor IIIA binding in a 5S ribosomal RNA gene

Antonio Conconi, Xiaoqi Liu,
Lilia Koriazova¹, Eric J.Ackerman¹ and
Michael J.Smerdon²

Department of Biochemistry and Biophysics, Washington State University, Pullman, WA 99164-4660 and ¹Molecular Biosciences Department, Pacific Northwest National Laboratory, P7-56, Box 999, Richland, WA 99352, USA

²Corresponding author
e-mail: smerdon@mail.wsu.edu

UV-induced photoproducts (cyclobutane pyrimidine dimers, CPDs) in DNA are removed by nucleotide excision repair (NER), and the presence of transcription factors on DNA can restrict the accessibility of NER enzymes. We have investigated the modulation of NER in a gene promoter using the *Xenopus* transcription factor IIIA (TFIIIA)–5S rDNA complex and *Xenopus* oocyte nuclear extracts. TFIIIA alters CPD formation primarily in the transcribed strand of the 50 bp internal control region (ICR) of 5S rDNA. During NER *in vitro*, CPD removal is reduced at most sites in both strands of the ICR when TFIIIA is bound. Efficient repair occurs just outside the TFIIIA-binding site (within 10 bp), and in the absence of 5S rRNA transcription. Interestingly, three CPD sites within the ICR [+56, +75 (transcribed strand) and +73 (non-transcribed strand)] are repaired rapidly when TFIIIA is bound, while CPDs within ~5 bases of these sites are repaired much more slowly. CPDs at these three sites may partially displace TFIIIA, thereby enabling rapid repair. However, TFIIIA is not completely displaced during NER, at least at sites outside the ICR, even though the NER complex could be sterically hindered by TFIIIA. Such inefficient repair of transcription factor binding sites could increase mutation frequency in regulatory regions of genes.

Keywords: DNA repair/5S rDNA/TFIIIA/transcription/UV damage

Introduction

The major DNA lesions induced by ultraviolet (UV) light (at 250–320 nm) are *cis-syn* cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6–4) pyrimidone photodimers [(6–4)PDs] (Cadet *et al.*, 1992; Friedberg *et al.*, 1995). These two DNA photoproducts, like other adducts that perturb DNA secondary structure, are efficiently processed in cells by the nucleotide excision repair (NER) pathway (reviewed in Sancar, 1996). At physiological UV doses, (6–4)PDs form between 10 and 30% of the total pyrimidine dimers in mammalian cells (depending on DNA sequence) and are repaired much faster than CPDs

(Mitchell and Nairn, 1989). Both UV photoproducts cause mutations in actively growing cells (Brash, 1988).

As first discovered in Chinese hamster ovary cells (Bohr *et al.*, 1985), and subsequently in several other organisms (Mellon and Hanawalt, 1989; Smerdon and Thoma, 1990), NER occurs preferentially in many transcriptionally active genes (reviewed in Friedberg, 1996). In addition, the transcribed strand (TS) of many transcriptionally active genes is repaired more rapidly than the non-transcribed strand (NTS; Hanawalt and Mellon, 1993). In mammalian cells, only RNA polymerase II (pol II)-transcribed genes show both preferential repair and strand-specific repair, suggesting that NER is coupled to RNA pol II transcription. In fact, CPD removal from RNA polymerase I (pol I)-transcribed rDNA is very slow and without strand-specificity in rodent and human cells (Christians and Hanawalt, 1993; Fritz and Smerdon, 1995; Fritz *et al.*, 1996). In addition, it was recently reported that there is a lack of preferential and strand-specific DNA repair in RNA polymerase III (pol III)-transcribed genes of human fibroblasts (Dammann and Pfeifer, 1997). Conversely, in growing yeast cells, NER efficiently removes CPDs from rDNA sequences (Verhage *et al.*, 1996) and yeast cells were shown to preferentially repair the NTS of a pol III transcribed gene (Aboussekhra and Thoma, 1998).

Chromatin structure has been implicated in several nuclear DNA processing events (reviewed in Wolffe, 1995) including DNA repair (reviewed in Smerdon and Conconi, 1999). For instance, modulation of DNA repair was found within nucleosomes of human cells (Jensen and Smerdon, 1990). Other protein–DNA complexes, like stalled RNA polymerases at sites of base damage, were also shown to inhibit repair of CPDs (Selby and Sancar, 1990). Furthermore, transcription factors bound to DNA regulatory elements can alter CPD formation both *in vivo* (Selleck and Majors, 1987; Tornaletti and Pfeifer, 1995) and *in vitro* (Wang and Becker, 1988; Liu *et al.*, 1997); and CPDs formed in promoter DNA elements can inhibit transcription factor binding (Tommasi *et al.*, 1996; Liu *et al.*, 1997). Finally, CPD removal was found to be slow in the promoters of the *PGK1* and *Jun* genes of human cells (Gao *et al.*, 1994; Tu *et al.*, 1996).

To evaluate the modulation of NER by a tightly bound transcription factor, we followed repair of CPDs in the *Xenopus borealis* 5S rRNA gene complexed with transcription factor IIIA (TFIIIA). The 5S rRNA gene is 120 bp long and the minimal promoter element required for accurate initiation by RNA pol III is a 50 bp internal control region (ICR) (Wolffe, 1994; see Figure 1). The ICR is the binding site for TFIIIA, a 38 kDa protein composed of nine tandemly repeated zinc fingers and an additional C-terminal domain. Three elements were identified in the ICR: the A-box at the 5' end; the C-box at the 3' end; and the intermediate element (IE) between

Fig. 1. Schematic diagram of the *X. borealis* 5S rRNA gene fragment used in this study. The locations of *Hind*III (H) and *Eco*RI (E) restriction sites are shown. The arrow indicates the 5S rDNA, the direction of transcription and the transcription start site (+1). The large open box represents the ICR of the 5S rDNA, and the smaller hatched boxes represent the positions of the A-box, IE and C-box, from left to right, respectively. The different fragment lengths are in base pairs (bp).

these boxes (Figure 1, shaded boxes). The three N-terminal zinc fingers of TFIIIA bind to the C-box (+80 to +91), the three central zinc fingers bind to the IE (+67 to +72), and the three C-terminal zinc fingers bind to the A-box (+50 to +64). Upon binding, TFIIIA makes strong contacts, mainly with the NTS (Hayes and Tullius, 1992). After formation of the TFIIIA–5S rDNA unit, TFIIIC and TFIIIB sequentially bind and this multiprotein complex directs initiation of transcription. Consequently, this complex bound to the 5S rDNA is in the direct path of the transcribing RNA pol III. Despite this steric obstruction, it was shown that the 5S rRNA transcription complex remains bound to the 5S rDNA during transcription (Bogenhagen *et al.*, 1982; Wolffe *et al.*, 1986), although this complex is disrupted during DNA replication (Wolffe and Brown, 1986).

We have initiated studies on the mechanism(s) whereby protein–DNA interactions modulate NER in chromatin. We have chosen the combination of the well characterized *Xenopus* TFIIIA–5S rRNA gene complex (Wolffe, 1994) as repair ‘substrate’, and the vigorous *in vitro* repair activity of *Xenopus* oocyte nuclear extracts (Oda *et al.*, 1996) to follow NER at specific sites of a protein–DNA complex. The *Xenopus* nuclear extracts carry out NER synthesis and completely remove CPDs in the dark (Oda *et al.*, 1996). Thus, NER (and not photolyase) accounts for removal of CPDs in these extracts. In addition, the structure of a complex between six zinc fingers of TFIIIA and 31 bp of the ICR was recently solved (at 3.1 Å resolution; Nolte *et al.*, 1998). This allows for direct comparison of specific structural features of the TFIIIA–5S rDNA complex with CPD removal by NER. Moreover, the 5S rDNA sequence forms a well positioned nucleosome *in vitro* (Wolffe, 1995) and can be used to study modulations of DNA repair by histones. Here we show that, apart from the specific sites TTC(+56) and TC(+75) in the TS, and TT(+73) in the NTS, CPD removal in the 5S rRNA gene is dramatically reduced at many sites in the 50 bp ICR sequence when TFIIIA is bound. This reduction occurs in both strands of the ICR while efficient NER occurs in each strand just outside the TFIIIA binding region. Our data also indicate that TFIIIA is not displaced by the NER machinery (presumably a large, multi-protein complex) during removal of CPDs at sites outside the ICR sequence, even though some DNA repair patches must extend into this region. Moreover, all these processes occur in the absence of transcription of the 5S rDNA.

Results

Stability of the TFIIIA–5S rDNA complex during NER *in vitro*

A cloned 214 bp fragment was used in these studies and contained all of the *X. borealis* 5S rRNA gene (120 bp), 60 bp of flanking DNA regions and 34 bp of plasmid DNA (Liu *et al.*, 1997; Figure 1). Since the conditions used for the formation of a stable TFIIIA–5S rDNA complex (Del Rio and Setzer, 1991; Liu *et al.*, 1997) differ from those used for NER by the *Xenopus* oocyte nuclear extracts *in vitro* (Saxena *et al.*, 1990; Oda *et al.*, 1996), it was necessary to examine the stability of the complex during the repair reaction. This was assayed by incubating 5S rDNA with TFIIIA under conditions optimized for both the stability of the TFIIIA–5S rDNA complex and the repair of CPDs by the extract *in vitro*. In these experiments, the 214 bp 5S rDNA fragment was irradiated with 500 J/m² of UV light (primarily 254 nm) to produce an average of ~1.3 CPDs/214 bp fragment, with ~0.9 CPD in the TS and ~0.4 CPD in the NTS (Liu *et al.*, 1997; see Figure 5). The TFIIIA complex was then formed with the UV-irradiated 5S rDNA. As shown previously (Liu *et al.*, 1997), this level of DNA damage does not inhibit TFIIIA binding to the 5S rRNA gene.

The stability of the TFIIIA–5S rDNA complex during NER *in vitro* was analyzed by DNA gel shift and DNase I footprinting. For these experiments, the TS of the 214 bp DNA fragment was ³²P end-labeled. After TFIIIA complex formation with UV irradiated (or unirradiated) DNA fragments, *Xenopus* oocyte extracts were added to some of the samples for different times and the presence of the TFIIIA–5S rDNA complex was monitored. The results of the gel shift analysis show that the TFIIIA–5S rDNA complex was present throughout the DNA repair reaction (Figure 2A, upper panel, compare lane 5 with lanes 7, 9, 11 and 13). These gels were quantified by phosphorimager analysis and the data confirmed the stability of the complex during the 4 h *in vitro* repair reaction (e.g. 93.4 ± 3.2% of the DNA was complexed with TFIIIA throughout the reaction shown in Figure 2).

The DNase I footprinting assays examined whether TFIIIA maintained correct binding to the 5S rDNA (at the ICR) during the DNA repair reaction. The results shown in Figure 2A, lower panel, suggest that the vast majority of the 5S rDNA molecules were correctly complexed with TFIIIA before and during the 4 h repair incubation at room temperature. Similar experiments were performed for the NTS (Figure 2B) and these data support the results found for the TS.

To investigate whether rapid displacement and reassociation of TFIIIA occurs during the repair reaction, repair assays were carried out in the presence of unlabeled competitor 5S rDNA (to trap unbound protein) and reduced amounts of TFIIIA. The amount of TFIIIA–5S rDNA complex formed, over the unreacted free components, is strictly dependent on the ratio of the starting concentrations of TFIIIA and 5S rDNA. In the experiments described above, TFIIIA was present in ~10 times molar excess of 5S rDNA (see Materials and methods). Under these conditions, almost all of the 5S rDNA was complexed with TFIIIA (Figure 2, upper panels) and a considerable amount of unbound TFIIIA existed in the reaction. At a

Fig. 2. TFIIIA binding to 5S rDNA during DNA repair *in vitro*. (A) The transcribed strand of a 214 bp DNA fragment containing the 5S rRNA gene was 32 P end-labeled before UV irradiation at 500 J/m². The TFIIIA–5S rDNA complex was formed by incubating UV damaged DNA with TFIIIA at room temperature for 30 min. *Xenopus* oocyte nuclear extracts were added at different times after complex formation and the presence of complex was monitored by both gel mobility shift (upper panel) and DNase I footprinting (lower panel). Lanes 1 to 5: the TFIIIA–5S rDNA complex was formed onto undamaged DNA (lane 2) or onto UV irradiated DNA (lanes 3 and 5) and incubated at 22°C for 4 h in the absence of oocyte extracts. In parallel, free DNA (lanes 1 and 4) was treated under the same conditions. Lanes 6 to 13: UV irradiated DNA with (lanes 7, 9, 11 and 13) or without (lanes 6, 8, 10 and 12) TFIIIA were incubated for 1, 2, 3 and 4 h in the presence of *Xenopus* oocyte nuclear extracts. Open bar at the right represents the TFIIIA-binding site (or ICR) within the 5S rRNA gene. (B) Similar experiments as in (A) with the NTS of the 214 bp DNA fragment 32 P end-labeled. Lanes 14 and 15 correspond to lanes 12 and 13 in (A), and the open bar at right represents the TFIIIA-binding site (or ICR) within the 5S rRNA gene.

ratio of 3.5 TFIIIA molecules for each 5S rDNA fragment, ~70% of the labeled 5S rDNA formed complexes (Figure 3, lane 1). Interestingly, when oocyte extracts were added to the reactions, even more complex was formed with both irradiated and non-irradiated 5S rDNA (Figure 3; compare lane 1 with lanes 2 and 4). This is probably due to residual TFIIIA present in the extracts and/or additional factors present in the extracts that stabilize the TFIIIA–5S rRNA complex (Wolffe, 1988; Hayes *et al.*, 1989). When a 10-fold excess of unlabeled competitor 5S rDNA fragment is added to the reaction, the ratio of bound:free labeled rDNA should decrease if TFIIIA is totally displaced during CPD repair. Indeed, in the absence of repair (–UV) and in the presence of high amounts of competitor 5S rDNA, we observed ~30% less TFIIIA bound to the labeled complex after the 2 h incubation (Figure 3, lane 5). However, no additional displacement was observed by this assay during repair (+UV) *in vitro* (Figure 3, lane 3). Since the ‘off-rate’ of TFIIIA binding is slow, as indicated by Figure 3, lane 5 (also see Romaniuk, 1990), these

Fig. 3. Effect of competitor 5S rDNA on stability of the TFIIIA–5S rDNA complex during DNA repair *in vitro*. Approximately 10 ng (or 7 nM) of end-labeled 214 bp 5S rDNA fragments was irradiated (lanes 1 to 3, +UV) or unirradiated (lanes 4 and 5, –UV) with 500 J/m² of UV light and incubated with 25 nM TFIIIA. The protein–DNA complexes were then incubated at 22°C for 2 h with (lanes 2 to 5, +Extract) or without (lane 1, –Extract) extracts, and with (lanes 3 and 5, +Competitor) or without (lanes 1, 2 and 4, –Competitor) competitor DNA [~100 ng (or 70 nM) unlabeled 214 bp 5S rDNA fragment]. The presence of TFIIIA–5S rDNA complex was monitored by gel mobility shift (upper panel), and results of quantitation (mean $\pm$ 1 SD of three experiments) for these gels, is shown in the lower panel.

results suggest that during repair of most CPD sites in the 214 bp fragment, TFIIIA is not released to bind the competitor DNA. However, it is important to note that partial displacement of TFIIIA molecules (e.g. displacement of one or a few zinc fingers) present at a site undergoing CPD removal would not be detected by this analysis.

Transcription of 5S rDNA during DNA repair *in vitro*

During the course of these studies, we noted that different batches of oocyte extracts contained varying amounts of an unidentified DNA exonuclease activity. To reduce the exonuclease activity, several types of exogenous DNA were tested in the repair reactions (data not shown), and the best source was restriction enzyme digested calf thymus DNA (see Materials and methods). This carrier DNA effectively inhibited the exonuclease activity (without markedly destabilizing the TFIIIA–5S rDNA complex) and was used in all of the repair reactions reported here. Some of the other carrier DNA tested (e.g. short DNA fragments and oligonucleotides) were also effective inhibitors of the exonuclease activity but severely destabilized the TFIIIA–5S rDNA complex (data not shown). Therefore, we examined transcription of the 5S rDNA with and without this carrier DNA present.

It was previously shown that 5S rDNA sequences are transcribed *in vitro* in the presence of *Xenopus* oocyte extracts and exogenously added ribosomal nucleotide triphosphates (rNTPs) (Birkenmeier *et al.*, 1978). However, no rNTPs were added to our repair assays. Thus, we investigated whether the 5S rDNA is transcribed under the conditions used for DNA repair *in vitro*. Undamaged 5S rDNA was incubated in the presence of oocyte extracts,

Fig. 4. The 5S rRNA gene is not transcribed during *in vitro* DNA repair. About 10 ng of unlabeled (non UV-irradiated) 214 bp 5S rDNA fragment was incubated with *Xenopus* oocyte nuclear extracts in the presence of [32 P]UTP. The reactions were incubated for 2 h at 22°C either in the absence (lane 1, -CT DNA) or presence (lanes 2 and 3, +CT DNA) of carrier (CT) DNA, with (lanes 1 and 3, +NTPs) or without (lane 2, -NTPs) addition of exogenous NTPs. To left of lane 1, horizontal lines denote migration of specific size markers (in bases), arrow denotes 5S rRNA transcript, and asterisks denote several non-specific transcripts observed only with 5S rDNA fragment alone (lane 1).

as described for the DNA repair reactions, except that [32 P]UTP was added to the samples. The RNA was extracted and analyzed on denaturing polyacrylamide gels (Figure 4). Transcription of the 5S rRNA gene was observed only when ATP, CTP, GTP and [32 P]UTP were added to the repair reaction and the carrier DNA was omitted (Figure 4, lane 1). In the presence of all four rNTPs and carrier DNA, only a smear of unspecific transcripts is observed (Figure 4, lane 3). Therefore, the large amount of carrier DNA added to inhibit exonuclease activity is responsible for non-specific transcription in these experiments, in agreement with previous studies (Birkenmeier *et al.*, 1978). Importantly for the present study, very little (or no) labeled 5S rRNA was observed when [32 P]UTP was the only rNTP added to the oocyte extracts (Figure 4, lane 2). Since these latter conditions are those used in our DNA repair reactions, there is little (if any) transcription of the 5S rRNA gene during repair incubations. Furthermore, the non-specific transcription of the carrier DNA (Figure 4, lane 3) does not allow us, at the present time, to study the *in vitro* DNA repair of transcribing 5S rRNA genes.

Effect of TFIIIA binding on repair of CPDs in the 5S rDNA fragment

Modulation of NER by TFIIIA binding was analyzed with the *in vitro* assay developed by Saxena *et al.* (1990). The 5S rDNA was 32 P end-labeled specifically in each strand and irradiated with 500 J/m² of UV light (as described above). Aliquots of UV-irradiated 5S rDNA were incubated with TFIIIA to form the complex or treated under the same conditions in the absence of TFIIIA. DNA repair was initiated by adding *Xenopus* oocyte nuclear extracts to these samples, and reactions were incubated at room temperature for different times (1, 2, 3 and 4 h). Controls

Fig. 5. Overall repair of CPDs in the 214 bp 5S rDNA fragment. The TS or the NTS of the 214 bp DNA fragment was 32 P end-labeled and the fragment was UV-irradiated at 500 J/m². The TFIIIA-5S rDNA complex was formed as described in Figure 2. *Xenopus* oocyte nuclear extracts were added at different times to the TFIIIA-5S rDNA complex and to the free 5S rDNA, and incubated in parallel to mock-treated samples. *In vitro* DNA repair was carried out at 22°C for 1, 2, 3 and 4 h. The purified DNA was treated with T4 endo V, separated on a 8% sequencing gel and quantitative analysis were done as described in the Material and methods. After UV irradiation at 500 J/m², ~0.9 and ~0.4 CPD was introduced into the TS (circles) and NTS (triangles), respectively. Removal of CPDs from the naked fragment (open symbols) and TFIIIA-5S rDNA complex (filled symbols) was determined for different repair times (1, 2, 3 and 4 h). Data are averages of two independent experiments.

for both the 5S rDNA and the TFIIIA-5S rDNA complex were incubated for 4 h under the same conditions, except that no extract was added. Aliquots from these reactions were analysed to confirm the presence of the TFIIIA complex and the absence of high exonuclease activities (Figure 2). The rest of the samples were stripped of protein and digested with T4 endonuclease V (T4 endo V), an enzyme that makes single-strand cuts at CPD sites (Dodson and Lloyd, 1989). The samples were then run on DNA-sequencing gels.

Overall repair of the 5S rDNA fragment was measured from the fraction of full-length (214 base) fragment resistant to T4 endo V cleavage. As shown in Figure 5, the overall rate of removal of CPDs from each strand of the 5S rDNA fragment is essentially unchanged with or without TFIIIA bound. Since only ~23% of the 214 bp rDNA fragment is complexed with TFIIIA (at the ICR), and <1 CPD/strand (on average) is induced at this UV dose (Figure 5), this result could be explained if modulation of NER is limited to repair of CPDs only in the ICR. Therefore, NER must occur efficiently at most CPD sites in the 214 bp rDNA fragment when TFIIIA is bound and present in 10-fold molar excess of the rDNA fragment (see Material and methods).

Modulation of NER at individual CPD sites by TFIIIA binding is shown in Figure 6. From these data it is clear that CPDs in the TS disappear with increasing repair time (Figure 6A, compare lanes 4 and 5 with lanes 6 to 13). Moreover, CPDs present outside the ICR are repaired at similar rates in both the naked rDNA and the TFIIIA-5S rDNA complex (Figure 6A, compare lanes 6, 8, 10 and 12 with lanes 7, 9, 11 and 13, above and below the shaded bar). On the contrary, there is a striking difference between these samples in repair of CPDs inside the ICR (Figure 6A,

Fig. 6. DNA repair of the transcribed strand (**A**) and non-transcribed strand (**B**) of UV-damaged 5S rDNA *in vitro*. UV irradiation, formation of the TFIIIA-5S rDNA complex and *in vitro* DNA repair were carried out as described in Figure 2. The free 5S rDNA (-TFIIIA) and the TFIIIA-5S rDNA complex (+TFIIIA) were incubated at room temperature without (lanes 1 to 5, -Extracts) or with (lanes 6 to 13, +Extracts) *Xenopus* oocyte extracts. DNA repair was followed for 1, 2, 3, and 4 h (lanes 6 to 13, +Extract) and compared with mock-treated samples (lanes 4 and 5, -Extract). The DNA samples were treated with T4 endo V and separated on denaturing gels as described in Figure 5. Lanes 1 to 13 are as in Figure 2. Shaded bar at right in each panel denotes the TFIIIA-binding site (ICR). Sites from -26 to +99 for the TS (**A**) and +96 to +3 for the NTS (**B**) denote the position (in bases) from the transcription start site (+1; see Figure 1).

sites within the shaded bar region). These results demonstrate that binding of TFIIIA to the 5S rDNA dramatically retards NER, but only within the internal 50 bp ICR promoter element. A similar repair experiment for the labeled NTS is shown in Figure 6B. As found for the TS, CPDs are removed faster outside of the ICR when TFIIIA is bound.

The time course of removal of individual CPDs was measured at 32 different sites inside and outside the ICR region, using peak deconvolution for accurate area analysis (Materials and methods). The removal curves for 20 different CPD sites in the TS and 12 different CPD sites in the NTS are presented in Figure 7A and B, respectively. Note that the ordinates (%CPDs) differ from that in Figure 5, where the absolute number of CPDs/fragment is plotted. Those panels designated with more than two pyrimidines [e.g. TCTCCT(-54)] show the average values for band clusters that were not well resolved on the gels.

From the time courses for removal of CPDs in the TS (Figure 7A), it is clear that there is a tight correlation between TFIIIA binding and inhibition of DNA repair in the ICR. All the CPD sites located outside of the ICR in the complex [e.g. from TCTCCT(-54) to CTTTC(+25), and from CC(+98) to TT(+101)] are repaired at similar rates to naked 5S rDNA (Figure 7A, closed and open circles, respectively). On the other hand, most of the CPD sites inside the ICR are repaired much slower when TFIIIA is bound [Figure 7A, panels CCTTC(+51) to TCT(+90)]. Indeed, CPDs at CCC(+66) and CC(+71) show little

(or no) repair during the 4 h incubation when TFIIIA is bound. Interestingly, there are two CPD sites in the TS of the ICR [TTC(+56) and TC(+75)] that are rapidly repaired when TFIIIA is bound (also see Figure 6A, asterisks). These two sites are in the A-box (+50 to +64) and between the IE (+67 to +72) and C-box (+80 to +91) for TTC(+56) and TC(+75), respectively (Figure 7A).

The repair kinetics of CPD sites in the NTS of the 5S rRNA gene are shown in Figure 7B. Again, repair of most CPDs outside the ICR is not modulated by the presence of TFIIIA, while repair at one site [TCTC(+43 to +46)] at the edge of the A-box is partially inhibited by bound TFIIIA. Four of the five CPD sites located inside the ICR of the NTS are repaired more slowly when TFIIIA is bound (Figure 7B, compare open and closed circles). However, as with site TC(+75) of the TS, the CPD at TT(+73) is rapidly repaired in the ICR (Figure 6B, asterisk and Figure 7B). This site is also located in the region between the C-box and IE, close to TC(+75) in the TS (Figure 7A).

Repair heterogeneity in the TFIIIA-5S rDNA complex

A summary of the CPD removal rates in the 5S rRNA gene is shown in Figure 8. The percent of CPDs remaining after 2 h repair is plotted for each CPD site in the TS (Figure 8, lower panel) and NTS (upper panel), with and without TFIIIA bound. When the %CPDs remaining was larger for the TFIIIA-5S rDNA complex than for the free

Fig. 7. Time courses of repair of 20 different CPD sites in the transcribed strand (**A**) and 12 different CPD sites in the non-transcribed strand (**B**) of the 5S rDNA. The level of CPDs at each site was set to 100% at t_0 . Data represent the mean of three (**A**) or two (**B**) *in vitro* DNA repair experiments. In each panel, the time course of removal of CPDs from free 5S rDNA fragments (○) and TFIID-5S rDNA complexes (●) is shown. The DNA sequence and position of CPD sites (in bases from transcription start) are indicated at the top of each panel. The shaded bars above some panels denote CPD sites within the A-box, IE and C-box, that are part of the ICR (see Figure 1). The shaded bar over site TCTC(+46) in (**B**) denotes the position of this sequence at the edge of the A-box.

Fig. 8. CPDs remaining in each strand of 5S rDNA after 2 h repair *in vitro*. Bars represent the percent of CPDs at specific sites remaining after 2 h repair incubation *in vitro*. Data represent averages of three (for TS) or two (for NTS) independent experiments for CPD sites in the TS (lower panel) and NTS (upper panel), where error bars in the lower panel denote maximum SD (for free DNA or complex) of the mean. For both strands, if the %CPDs remaining in the TFIIIA-5S rDNA complex was greater than that in the naked 5S rDNA (i.e. slower repair in complex), the difference is in red. If the %CPDs remaining in the TFIIIA-5S rDNA complex was less than that in the naked 5S rDNA (i.e. faster repair in complex), the difference is in blue. If the values were zero %CPDs remaining after 2 h for both samples, an arrow was included for 'zero bars' in the NTS.

5S rDNA (slower repair), the difference is plotted as a red bar (e.g. most CPD sites in the TS from +45 to +95; Figure 8). If the %CPDs remaining was smaller for the complex (faster repair), the difference is plotted as a blue bar (e.g. CPD sites in the TS between -35 and -15; Figure 8). At each site, the solid portion of each bar denotes the lowest value of %CPDs remaining in either sample. In addition, sites with zero CPDs remaining after 2 h in both samples are denoted with either error bars (TS) or arrow heads (NTS) (Figure 8).

Three different observations can be made from these data. First, the repair of free 5S rDNA (not complexed with TFIIIA) *in vitro* is heterogeneous, where some CPD sites are repaired more rapidly than others (Figure 8, solid bars). For example, >50% of the CPDs remain after 2 h repair at CCC(+66) in the TS and at CCC(+30) in the NTS, while other CPDs (in both strands) show 100% repair after this time [e.g. CTTTC(+25) in the TS (error bars) and CTTTT(+122) in the NTS (arrow heads); Figure 8]. Secondly, repair of CPDs in the TFIIIA-5S rDNA complex is inhibited almost exclusively within the ICR (Figure 8, red bars). As mentioned earlier, repair at one site in the NTS [TCTC(+43 to +46)], just at the edge of the ICR, is also impeded by the TFIIIA complex. Moreover, three CPDs are rapidly repaired [TTC(+56) and TC(+75) in the TS, TT(+73) in the NTS] in the TFIIIA complex. Thirdly, one site in the TS and outside

the ICR [CCTTTTC(-15)] may actually be repaired more rapidly in the complex.

Discussion

Regulation of the 5S rRNA gene and interactions of TFIIIA with the ICR sequence have been well characterized (Hayes and Tullius, 1992; Wolffe, 1994). Recently, the crystal structure of the six N-terminal zinc fingers of TFIIIA bound to 31 bp of the 5S rRNA gene promoter was solved to 3.1 Å resolution (Nolte *et al.*, 1998). Furthermore, this sequence forms a precisely positioned nucleosome *in vitro* (Wolffe, 1995). Since *Xenopus* oocyte nuclear extracts have a vigorous NER activity *in vitro* (Oda *et al.*, 1996) and the process of nucleosome loading following NER synthesis has been shown to be present in *Xenopus* cell extracts (Gaillard *et al.*, 1996), the *Xenopus* 5S rDNA complex provides an ideal model substrate to study NER of chromatin in a homologous *in vitro* system.

In the present report, we first analyzed the stability of the TFIIIA-5S rDNA complex during *in vitro* DNA repair. It has been shown previously that many rounds of RNA synthesis can occur *in vitro* without dislodging the TFIIIA protein (Bogenhagen *et al.*, 1982; Wolffe *et al.*, 1986). On the other hand, disruption of the TFIIIA complex occurs during *in vitro* DNA replication (Wolffe and Brown, 1986). Here, we suggest that removal of most CPDs in the 214 bp DNA fragment does not displace TFIIIA (Figure 3). However, it is important to consider the level of sensitivity of these experiments. Assuming a Poisson distribution of UV damaged fragments, ~73% of all molecules contained at least one CPD (for an average of 1.3 CPDs/214 bp fragment) and the remaining fragments were undamaged. Since the majority of CPDs in the 5S rDNA fragment were almost completely removed after 4 h incubation with the oocytes extracts (Figure 5), complete displacement of TFIIIA during repair of CPDs should be measurable by the competition experiment presented in Figure 3. No displacement of TFIIIA was observed, suggesting that the majority of TFIIIA-5S rDNA complexes are not dissociated during NER. However, only ~26% of the 5S rDNA molecules contained a CPD in the ICR following the UV dose used (~1.3 CPDs/fragment). Since 68% of the CPDs were removed from this region during the repair reaction, a maximum change of 18% in bound 5S rDNA is expected if TFIIIA is displaced only during repair of this region. Although no decrease was observed in the bound fraction (Figure 3), the error in our measurements (1 SD of $\pm 9\%$) was too close to this maximum change. Therefore, it is possible that among the damaged 5S rDNA fragments there are a few CPD sites where TFIIIA is completely displaced during NER. The existence of such a minor population of displaced TFIIIA would not be detected in our assay.

Displacement of TFIIIA would solve the paradox of how a large repair complex can generate a repair patch where TFIIIA is bound to DNA. However, we suggest that TFIIIA may only be partially displaced during DNA repair, as previously shown for transcription (Bogenhagen *et al.*, 1982; Wolffe *et al.*, 1986). For this scenario, one or more of the multiple contact sites (zinc fingers) between TFIIIA and DNA could be disrupted without dislodging the entire complex. However, experiments measuring real-

time kinetics of TFIIIA binding must be performed before this issue can be completely resolved.

In addition to CPDs, (6–4)PDs were also shown to form in the 5S rDNA sequence (Liu *et al.*, 1997). Even though these photoproducts form a much larger distortion in the DNA helix than CPDs (Kim *et al.*, 1995) and are more rapidly removed by NER (Mitchell and Nairn, 1989), at the UV dose used for these experiments these lesions did not displace TFIIIA (Liu *et al.*, 1997). Since the *Xenopus* oocyte nuclear extracts repair (6–4)PDs (Oda *et al.*, 1996) and since we did not detect displacement of TFIIIA during repair *in vitro*, we presume that removal of (6–4)PD damage also does not require complete displacement of the protein.

It was recently shown that strand specific repair of the pol III-transcribed tRNA^{Ser} and tRNA^{Val} genes does not occur in human fibroblasts (Dammann and Pfeifer, 1997). In the present study, we also did not observe any major difference in repair rates between the two strands of the 5S rRNA gene (Figure 5). However, in the previous report the tRNA genes were actively transcribing (Dammann and Pfeifer, 1997), while no transcription of the 5S rDNA was observed during our repair reactions (Figure 4), although NER was efficient in both rDNA strands (Figure 5). Upon induction of transcription, by adding the four rNTPs to the *in vitro* repair essays, non-specific RNA was synthesized from the large amount of carrier DNA in the oocyte extracts (Figure 4). This effect did not allow us to follow repair of the transcribing 5S rRNA gene.

We have analyzed CPD removal at specific sites in the TFIIIA–5S rDNA complex. The results show that NER of the 5S rRNA gene is very slow in the ICR when TFIIIA is bound. Furthermore, despite the fact that TFIIIA makes contacts mainly with the NTS of the ICR (Hayes and Tullius, 1992; Nolte *et al.*, 1998), the slow repair occurred in both strands. This probably reflects steric hindrance of repair proteins by TFIIIA from gaining access to CPD sites in the complex. In fact, the crystal structure of the six N-terminal zinc fingers of TFIIIA complexed with 31 bp of the ICR (containing the IE and the C-box sequences) (Nolte *et al.*, 1998) shows that fingers 1, 2 and 3 bind tightly within the major groove of the DNA in the C-box. Furthermore, it was previously shown biochemically that the three C-terminal zinc fingers (not in the crystal; Nolte *et al.*, 1998) bind the major groove of the DNA in the A-box very similar to the N-terminal zinc fingers (Hayes and Tullius, 1992). Because of these strong interactions with the two distal elements of the ICR (A- and C-boxes), TFIIIA may present a formidable obstacle to the NER machinery in gaining access to CPDs in these regions. CPDs at all but one site [TTC(+56) in the TS] in either strand of the A- and C-boxes were repaired more slowly in the TFIIIA–5S rDNA complex than in free DNA.

In contrast to the N- and C-terminal zinc finger cassettes, the three middle zinc fingers (4–6) of TFIIIA make few contacts with the ICR (Hayes and Tullius, 1992; Nolte *et al.*, 1998). Fingers 4, 5 and 6 run along one side of the DNA helix and form a more open and extended structure (Nolte *et al.*, 1998). Of these, only finger 5 makes contact with bases in the major groove of the IE (Nolte *et al.*, 1998). Namely, the phosphate of T(+69) makes electrostatic contacts with His155 and Lys144, the G(+70) makes

hydrogen-bond contacts with Arg154 and G(+71) makes hydrogen-bond contacts with Arg151 (Nolte *et al.*, 1998). In correlation with this structure, the two CPDs formed at bases 67–71 [CC(+71) in the TS and CCT(+69) in the NTS] are slowly repaired when TFIIIA is bound to the ICR.

Considering the strong interactions between TFIIIA and the ICR (Hayes and Tullius, 1992; Nolte *et al.*, 1998), there was surprisingly little inhibition of repair of CPDs immediately outside the ICR. In fact, the time course for repair at most of these sites is almost identical for the complex and free 5S rDNA (Figure 7). Thus, repair proteins must not encounter resistance in accessing CPD sites right next to the TFIIIA binding region (ICR), even when they are within one turn of the DNA helix from the ICR [e.g. Figure 7A, compare TCT(+90) and CC(+98) in the TS]. This is intriguing since repair patches at these sites (24–32 bp) should extend into the ICR region (i.e. DNA covered by TFIIIA). Taken together, these results suggest that TFIIIA binding inhibits formation of the pre-incision complex at CPD sites, where both lesion recognition and subsequent incision are influenced by the DNA chromatin environment. Once this complex is formed, additional NER proteins must be able to partially dissociate TFIIIA from the ICR to carry out NER.

Two CPD sites in the TS [TTC(+56) and TC(+75)] and one CPD site in the NTS [TT(+73)] of the ICR were rapidly repaired in the presence of TFIIIA (Figure 6, asterisk and Figure 7). One explanation for these results is that the formation of CPDs in these sites may reduce (or inhibit) binding of zinc fingers 8 and 5 (Hayes and Tullius, 1992; Nolte *et al.*, 1998). Such an effect could open up a portion of the TFIIIA complex and allow an easier access to CPD sites in this region by the pre-incision complex. Our previous findings support this possibility (Liu *et al.*, 1997). When the TFIIIA–5S rDNA complex is formed before UV irradiation, CPD formation at TTC(+56) and TC(+75) of the TS is strongly inhibited relative to the yield for naked rDNA (Liu *et al.*, 1997). This suggests that the binding of fingers 8 and 5 is incompatible with the presence of CPDs in their binding sites. Moreover, in the co-crystal structure of the complex (Nolte *et al.*, 1998), the pyrimidine base of T(+74) makes hydrogen-bond contacts with Leu148 and the phosphate of T(+74) makes electrostatic contacts with Ser150 of finger 5. Therefore, a CPD at TC(+75) could abolish these contacts and prevent finger 5 from binding.

DNA bending in the complex may also allow faster repair at TTC(+56) and TC(+75) in the TS, and TT(+73) in the NTS. Indeed, in the co-crystal structure there are three significant bends in the helical axis of the ICR fragment. One of these (16.7°) occurs at base pair (+70), where finger 5 makes hydrogen-bond contact via Arg154 (Nolte *et al.*, 1998). This is the exact site [CC(+71)] at which we observe enhancement of CPD formation upon TFIIIA binding (Liu *et al.*, 1997), while CPD formation at all other sites in the ICR was either inhibited or essentially unaffected (Liu *et al.*, 1997). Furthermore, CPDs that form close to this site [TC(+75) and TT(+73)] may not allow the 16.7° bend at C(+71) required for optimal binding of finger 5. This could create a locally open structure and explain the fast repair of these two CPDs when TFIIIA is present [Figure 8, panels TC(+75)

and TT(+73)]. Interestingly, these two photo-dimers [TC(+75) and TT(+73)] are less than half of a turn of the DNA helix downstream of the bending site [CC(+71)]. On the other hand, CPD formation at two sites present just upstream of the bending site [CCT(+69) and CCC(+66)] are not modulated by TFIIIA binding (Liu *et al.*, 1997) and are repaired more slowly in the TFIIIA complex. It is possible that the additional bending induced at each of these four closely positioned CPD sites may or may not be compatible with TFIIIA binding. Indeed, this compatibility may depend on their juxtaposition to the bend at base pair (+70). Thus, the DNA bending induced at CPDs TC(+75) and TT(+73) could destabilize finger 5 and allow fast NER in a very localized portion of the 5S rDNA, while CPDs within half a turn of the DNA helix [CCT(+69) and CCC(+66)] could have much less effect on finger 5 binding and are repaired more slowly.

In summary, 13 out of 16 CPDs present in the promoter of the 5S rRNA gene are very slowly removed when transcription factor TFIIIA is bound. Two of the three rapidly repaired sites in the complex [TC(+75) in the TS and TT(+73) in the NTS] are within 4 bp of a marked bend in the helix axis seen in a co-crystal structure of a fragment of TFIIIA complexed to a fragment of the promoter. The third CPD site [TTC(+56) in the TS] is only approximately half a helical turn from CPD sites that are repaired much more slowly [CCTTC(+51) and TCCC(+61) in the TS]. Furthermore, immediately outside the TFIIIA binding site (ICR), most CPDs are removed from the TFIIIA–5S rDNA complex with the same efficiency as free 5S rDNA. As the occurrence of UV-induced mutations depends on both the DNA damage frequency and rate of DNA repair, these studies are of particular importance for understanding the initiation of mutations in regulatory regions of genes.

Materials and methods

Preparation and UV irradiation of the 5S rDNA fragment

Plasmid pKS-5S (Mann *et al.*, 1997) was linearized with either *EcoRI* or *HindIII* (Life Technologies) to label the TS and the NTS, respectively. After dephosphorylation by alkaline phosphatase (Boehringer Mannheim), the linearized plasmid DNA was end-labeled with T4 polynucleotide kinase (Life Technologies) and digested with a second restriction enzyme (*HindIII* for TS-labeling and *EcoRI* for NTS-labeling), producing a single end-labeled 214 bp DNA fragment containing the *X. borealis* 5S rDNA sequence (Liu *et al.*, 1997; see Figure 1). After separation on a 2% preparative agarose gel, the end-labeled 214 bp DNA fragment was recovered and resuspended in TE buffer [10 mM Tris pH 7.5 and 1 mM EDTA], at a concentration of ~2 µg/ml. Aliquots of 5 µl were UV-irradiated (predominantly 254 nm) under four low-pressure Hg lamps (Sylvania, Model G30T8) at a flux of ~15 W/m², measured with a Spectroline DM-254N UV meter (Spectronics Corp.).

Formation of the TFIIIA–5S rDNA complex

Recombinant TFIIIA protein was prepared as described previously (Liu *et al.*, 1997). To form the TFIIIA–5S rDNA complex, 10 ng of UV-irradiated 5S rDNA fragment (~7 nM) were incubated with TFIIIA (~100 nM) at 22°C for 30 min in the presence of 20 mM Tris pH 7.5, 7 mM MgCl₂, 10 µM ZnCl₂, 70 mM KCl, 1 mM dithiothreitol (DTT), 0.1% Nonidet P-40 (NP-40), 100 µg/ml bovine serum albumin (BSA), 5% glycerol, 0.5 mM phenylmethylsulfonyl fluoride (PMSF) and carrier DNA (~2.5 µg calf thymus DNA, double digested with *EcoRI* and *BamHI* to render fragments of an average size of ~2 kb).

DNA repair and 5S rDNA transcription *in vitro*

The *in vitro* repair experiments were performed as described (Oda *et al.*, 1996), with some minor modifications. Briefly, 8 µl of the repair buffer

[3 mM DTT, 120 µM dNTPs, 80 mM KCl, 8 mM MgCl₂, 0.1 mM EDTA, 10% glycerol, 10 mM HEPES pH 7.4 and 1% polyvinylpyrrolidone (PVP)-360] were added to each of the 10 µl samples containing the TFIIIA–5S rDNA complex (see above). The repair reactions were started at 22°C by adding 2 µl of *Xenopus* oocyte nuclear extracts (Oda *et al.*, 1996). Transcription of the 5S rRNA gene was initiated in these mixtures by adding ATP, CTP, GTP (500 µM each) and [³²P]UTP (100 µM, ~5 µCi/mmol; New England Nuclear) to the repair reactions.

DNA mobility shift and DNase I footprinting assays

For the gel mobility shift assays, 2 µl aliquots from the 20 µl DNA repair reactions were directly applied to a nondenaturing 6% polyacrylamide gel containing 5% glycerol. After pre-electrophoresis for 10 min at room temperature, gel electrophoresis of the samples was run for 1 h at 120 V in 25 mM Tris and 0.2 M glycine (pH 8.0) as the running buffer. DNase I (~0.04 U; Life Technologies) was added to 5 µl aliquots of the DNA repair reactions and incubated at room temperature for 2 min or 30 s, for the samples that did or did not contain *Xenopus* oocyte extracts, respectively. The DNase I digestions were stopped with 2.5 mM EDTA and 0.25% SDS. After phenol extraction and ethanol precipitation, DNA was fractionated on 8% polyacrylamide–8 M urea sequencing gels. Gels were vacuum dried before exposure to Phosphor-Imager screens (Molecular Dynamics).

CPD mapping

The remaining 13 µl of the DNA repair reactions were extracted with phenol and then precipitated in ethanol. After resuspension in 5 µl of T4 endo V digestion buffer (20 mM Tris pH 7.4, 10 mM EDTA pH 8, 100 mM NaCl and 100 µg/ml BSA), a 1 µl aliquot of a 100-fold dilution of T4 endo V stock (~400 ng/ml) was added to each sample and incubated at 37°C for 1 h. The reactions were stopped by the addition of gel loading buffer (98% formamide, 10 mM EDTA, 0.2% Bromophenol Blue and 0.2% xylene cyanol), heat denatured and analyzed by sequencing gel electrophoresis.

Quantitative analysis

Peak deconvolution of individual bands on the gels was performed by PeakFit 4.0 software (SPSS Inc., Chicago, IL), and was used to analyze the band intensity at each CPD site. Loading differences were corrected by normalizing the intensity of each band to the sum of all bands in a lane. Thus, the value of each band was expressed as the percentage of the total intensity of a lane. The average CPDs per strand was obtained from the intensity of the intact fragment (i.e. P₀), assuming a Poisson distribution of UV damaged fragments, as described previously (Bohr and Okumoto, 1988).

Acknowledgements

We thank Drs R.S.Lloyd for T4 endo V and D.Setzer for *Escherichia coli* strain BL21. This study was supported by NIH grant ES02614 (to M.J.S.) from the National Institute of Environmental Health Sciences, and by PNNL Laboratory Directed Research and Development funds (to E.J.A.) from the Department of Energy.

References

- Aboussekhra, A. and Thoma, F. (1998) Nucleotide excision repair and photolyase preferentially repair the nontranscribed strand of RNA polymerase III-transcribed genes in *Saccharomyces cerevisiae*. *Genes Dev.*, **12**, 411–421.
- Birkenmeier, E.H., Brown, D.D. and Jordan, E. (1978) A nuclear extract of *Xenopus laevis* oocytes that accurately transcribes 5S RNA genes. *Cell*, **15**, 1077–1086.
- Bogenhagen, D.F., Wormington, W.M. and Brown, D.D. (1982) Stable transcription complexes of *Xenopus* 5S RNA genes: a means to maintain the differentiated state. *Cell*, **28**, 413–421.
- Bohr, V.A. and Okumoto, D.S. (1988) *DNA Repair: A Laboratory Manual of Research Procedures*. Marcel Dekker Inc., New York, NY, pp. 347–366.
- Bohr, V.A., Smith, C.A., Okumoto, D.S. and Hanawalt, P.C. (1985) DNA repair in an active gene: removal of pyrimidine dimers from the DHFR gene of CHO cells is much more efficient than in the genome overall. *Cell*, **40**, 359–369.
- Brash, D.E. (1988) UV mutagenic photoproducts in *Escherichia coli* and human cells: a molecular genetics perspective on human skin cancer. *Photochem. Photobiol.*, **48**, 59–66.

- Cadet,J., Anselmino,C., Douki,T. and Voituriez,L. (1992) Photochemistry of nucleic acids in cells. *J. Photochem. Photobiol.*, **B15**, 277–298.
- Christians,F.C. and Hanawalt,P.C. (1993) Lack of transcription-coupled repair in mammalian ribosomal RNA genes. *Biochemistry*, **32**, 10512–10518.
- Dammann,R. and Pfeifer,G.P. (1997) Lack of gene- and strand-specific DNA repair in RNA polymerase III-transcribed human tRNA genes. *Mol. Cell. Biol.*, **17**, 219–229.
- Del Rio,S. and Setzer,D.R. (1991) High yield purification of active transcription factor IIIA expressed in *E.coli*. *Nucleic Acids Res.*, **19**, 6197–6203.
- Dodson,M.L. and Lloyd,R.S. (1989) Structure-function studies of the T4 endonuclease V repair enzyme. *Mutat. Res.*, **218**, 49–65.
- Friedberg,E.C. (1996) Relationships between DNA repair and transcription. *Annu. Rev. Biochem.*, **65**, 15–42.
- Friedberg,E.C., Walker,G.C. and Siede,W. (1995) *DNA Repair and Mutagenesis*. ASM Press, Washington, DC.
- Fritz,L.K. and Smerdon,M.J. (1995) Repair of UV damage in actively transcribed ribosomal genes. *Biochemistry*, **34**, 13117–13124.
- Fritz,L.K., Suquet,C. and Smerdon,M.J. (1996) Strand breaks are repaired efficiently in human ribosomal genes. *J. Biol. Chem.*, **271**, 12972–12976.
- Gaillard,P.H., Martini,E.M., Kaufman,P.D., Stillman,B., Moustacchi,E. and Almouzni,G. (1996) Chromatin assembly coupled to DNA repair: a new role for chromatin assembly factor I. *Cell*, **86**, 887–896.
- Gao,S., Drouin,R. and Holmquist,G.P. (1994) DNA repair rates mapped along the human *PGK-1* gene at nucleotide resolution. *Science*, **263**, 1438–1440.
- Hanawalt,P.C. and Mellon,I. (1993) Stranded in an active gene. *Curr. Biol.*, **3**, 67–69.
- Hayes,J.J. and Tullius,T.D. (1992) Structure of the TFIIIA–5S DNA complex. *J. Mol. Biol.*, **227**, 407–417.
- Hayes,J., Tullius,T.D. and Wolffe,A.P. (1989) A protein–protein interaction is essential for stable complex formation on a 5S RNA gene. *J. Biol. Chem.*, **264**, 6009–6012.
- Jensen,K.A. and Smerdon,M.J. (1990) DNA repair within nucleosome cores of UV-irradiated human cells. *Biochemistry*, **29**, 4773–4782.
- Kim,J.K., Patel,D. and Choi,B.S. (1995) Contrasting structural impacts induced by *cis-syn* cyclobutane dimer and (6–4) adduct in DNA duplex decamers: implication in mutagenesis and repair activity. *Photochem. Photobiol.*, **62**, 44–50.
- Liu,X., Conconi,A. and Smerdon,M.J. (1997) Strand-specific modulation of UV photoproducts in 5S rDNA by TFIIIA binding and their effect on TFIIIA complex formation. *Biochemistry*, **36**, 13710–13717.
- Mann,D.B., Springer,D.L. and Smerdon,M.J. (1997) DNA damage can alter the stability of nucleosomes: effects are dependent on damage type. *Proc. Natl Acad. Sci. USA*, **94**, 2215–2220.
- Mellon,I. and Hanawalt,P.C. (1989) Induction of the *Escherichia coli* lactose operon selectively increases repair of its transcribed DNA strand. *Nature*, **342**, 95–98.
- Mitchell,D.L. and Nairn,R.S. (1989) The biology of the (6–4) photoproduct. *Photochem. Photobiol.*, **49**, 805–819.
- Nolte,R.T., Conlin,R.M., Harrison,S.C. and Brown,R.S. (1998) Differing roles for zinc fingers in DNA recognition: structure of a six-finger transcription factor IIIA complex. *Proc. Natl Acad. Sci. USA*, **95**, 2938–2943.
- Oda,N., Saxena,J.K., Jenkins,T.M., Prasad,R., Wilson,S.H. and Ackerman,E.J. (1996) DNA polymerases α and β are required for DNA repair in an efficient nuclear extract from *Xenopus* oocytes. *J. Biol. Chem.*, **271**, 13816–13820.
- Romaniuk,P.J. (1990) Characterization of the equilibrium binding of *Xenopus* transcription factor IIIA to the 5S RNA gene. *J. Biol. Chem.*, **265**, 17593–17600.
- Sancar,A. (1996) DNA excision repair. *Annu. Rev. Biochem.*, **65**, 43–81.
- Saxena,J.K., Hays,J.B. and Ackerman,E.J. (1990) Excision repair of UV-damaged plasmid DNA in *Xenopus* oocytes is mediated by DNA polymerase α (and/or δ). *Nucleic Acids Res.*, **18**, 7425–7432.
- Selby,C.P. and Sancar,A. (1990) Transcription preferentially inhibits nucleotide excision repair of the template DNA strand *in vitro*. *J. Biol. Chem.*, **265**, 21330–21336.
- Selleck,S.B. and Majors,J. (1987) Photofootprinting *in vivo* detects transcription-dependent changes in yeast TATA boxes. *Nature*, **325**, 173–177.
- Smerdon,M.J. and Thoma,F. (1990) Site-specific DNA repair at the nucleosome level in a yeast minichromosome. *Cell*, **61**, 675–684.
- Smerdon,M.J. and Conconi,A. (1999) Modulation of DNA damage and DNA repair in chromatin. In Moldave,K. (ed.), *Progress in Nucleic Acids Research and Molecular Biology*. Vol. 62. Academic Press, San Diego, CA, pp. 227–255.
- Tommasi,S., Swiderski,P.M., Tu,Y., Kaplan,B.E. and Pfeifer,G.P. (1996) Inhibition of transcription factor binding by ultraviolet-induced pyrimidine dimers. *Biochemistry*, **35**, 15693–15703.
- Tornaletti,S. and Pfeifer,G.P. (1995) UV-light as a footprinting agent: modulation of UV-induced DNA damage by transcription factors bound at the promoters of three human genes. *J. Mol. Biol.*, **249**, 714–728.
- Tu,Y., Tornaletti,S. and Pfeifer,G.P. (1996) DNA repair domains within a human gene: selective repair of sequences near the transcription initiation site. *EMBO J.*, **15**, 675–683.
- Verhage,R.A., van de Putte,P. and Brouwer,J. (1996) Repair of rDNA in *Saccharomyces cerevisiae*: *RAD4*-independent strand-specific nucleotide excision repair of RNA polymerase I transcribed genes. *Nucleic Acids Res.*, **24**, 1020–1025.
- Wang,Z. and Becker,M.M. (1988) Selective visualization of gene structure with ultraviolet light. *Proc. Natl Acad. Sci. USA*, **85**, 654–658.
- Wolffe,A.P. (1988) Transcription factor TFIIC can regulate differential *Xenopus* 5S RNA gene transcription *in vitro*. *EMBO J.*, **7**, 1071–1079.
- Wolffe,A.P. (1994) The role of transcription factors, chromatin structure and DNA replication in 5S RNA gene regulation. *J. Cell Sci.*, **107**, 2055–2063.
- Wolffe,A.P. (1995) *Chromatin Structure and Function*. Academic Press, San Diego, CA.
- Wolffe,A.P. and Brown,D.D. (1986) DNA replication *in vitro* erases a *Xenopus* 5S RNA gene transcription complex. *Cell*, **47**, 217–227.
- Wolffe,A.P., Jordan,E. and Brown,D.D. (1986) A bacteriophage RNA polymerase transcribes through a *Xenopus* 5S RNA gene transcription complex without disrupting it. *Cell*, **44**, 381–389.

Received November 3, 1998; revised and accepted January 14, 1999