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Chromatin structure modulates DNA repair by photolyase *in vivo*

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Yeast and many other organisms use nucleotide excision repair (NER) and photolyase in the presence of light (photoreactivation) to repair cyclobutane pyrimidine dimers (CPDs), a major class of DNA lesions generated by UV light. To study the role of photoreactivation at the chromatin level *in vivo*, we used yeast strains which contained minichromosomes (YRpTRURAP, YRpCS1) with well-characterized chromatin structures. The strains were either proficient (*RAD1*) or deficient (*rad1*Δ) in NER. In contrast to NER, photolyase rapidly repairs CPDs in non-nucleosomal regions, including promoters of active genes (URA3, HIS3, DED1) and in linker DNA between nucleosomes. CPDs in nucleosomes are much more resistant to photoreactivation. These results demonstrate a direct role of chromatin in modulation of a DNA repair process and an important role of photolyase in repair of damaged promoters with presumptive effects on gene regulation. In addition, photoreactivation provides an *in vivo* test for chromatin structure and stability. In active genes (URA3, HIS3), photolyase repairs the non-transcribed strand faster than the transcribed strand and can match fast removal of lesions from the transcribed strand by NER (transcription-coupled repair). Thus, the combination of both repair pathways ensures efficient repair of active genes.

Keywords: chromatin/cyclobutane pyrimidine dimers/ DNA repair/nucleosome/photolyase

Introduction

In eukaryotic cells, DNA is folded around histone octamers into nucleosomes, connected by linker DNA and further condensed into higher-order chromatin structures. Since packaging affects the accessibility of DNA to proteins, all DNA processing reactions including transcription and DNA repair must be intimately coupled to, and might even be regulated by, structural and dynamic properties of chromatin. Indeed, nucleosomes positioned in promoter regions play a significant role in the regulation of transcription. Factors binding to promoter elements can compete with nucleosome formation during replication and establish 'preset' open promoters, or factors may lead to a disruption of nucleosomes ('remodelling') and generate a nuclease-sensitive region (NSR; Becker, 1994; Wallrath *et al.*, 1994). Furthermore, transcription elongation can lead to local dissociation and reassembly of histone octamers (e.g. genes transcribed by RNA polymerase II; Cavalli and Thoma, 1993; Cavalli *et al.*, 1996) or to a complete disruption or loss of nucleosome structures (e.g. rDNA genes transcribed by RNA polymerase I; Conconi *et al.*, 1989; Dammann *et al.*, 1993).

Cyclobutane pyrimidine dimers (CPDs) and 6-4 photoproducts (6-4 PD) are the two major classes of stable DNA lesions (pyrimidine dimers, PD) generated by UV light. Unless repaired, PDs may lead to blockage of transcription, mutations, cell death and cancer. Pyrimidine dimers are removed by two pathways: (i) nucleotide excision repair (NER); and (ii) photoreactivation. NER is a ubiquitous multistep pathway in which more than 30 proteins are involved to execute sequentially damage recognition, excision of an oligonucleotide with the pyrimidine dimer and gap repair synthesis (reviewed in Friedberg *et al.*, 1995). The major components have been identified and the basic reaction has been reconstituted on naked DNA substrates (Aboussekhra *et al.*, 1995). NER shares some proteins with the general transcription machinery which may link NER to transcription (transcription-coupled repair) and partially explains why the transcribed strand of an active gene is faster repaired than the non-transcribed strand or the genome overall (Friedberg, 1996; Sancar, 1996a). However, the relation between chromatin structure and NER is not resolved. We previously developed an assay to map CPDs along the DNA sequence and to compare it with the local chromatin structure determined by micrococcal nuclease (MNase) digestion and by indirect end-labelling (Smerdon and Thoma, 1990). This and a subsequent study (Bedoyan *et al.*, 1992) were done in a yeast strain containing a minichromosome (YRpTRURAP) with a defined chromatin structure as a model substrate (Thoma, 1986). These studies (Smerdon and Thoma, 1990; Bedoyan *et al.*, 1992) showed fast repair on the transcribed strand consistent with transcription-coupled repair, and slow repair in the non-transcribed strand. Repair was also efficient in a nuclease-sensitive promoter region of the URA3 gene, but slow in the nuclease-sensitive origin of replication (ARS1). Although those experiments indicated some modulation of NER by chromatin structure, they did not reveal clear differences between nuclease-sensitive regions and nucleosomes.

As an alternative or additional pathway to NER, a wide variety of organisms, including bacteria, fungi, plants, invertebrates and many vertebrates, can revert CPDs by CPD-photolyase in the presence of photoreactivating blue light (of wavelength 350–450 nm) restoring the bases to their native form (Yasui *et al.*, 1994; Sancar, 1996b). More recently, (6-4) photolyases have been identified in *Drosophila* (Todo *et al.*, 1993, 1996), *Xenopus laevis* and rattlesnakes (Kim *et al.*, 1996). A homologue gene was

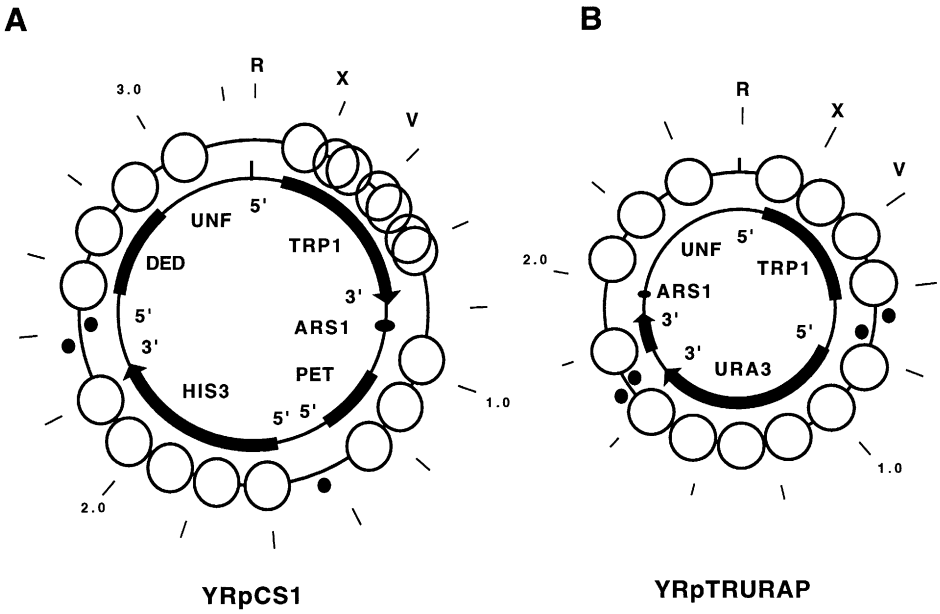


Fig. 1. Chromatin structures of the minichromosomes. (**A**) YRpCS1 of *S.cerevisiae* strain FTY117 and CSY1 contains the *pet56-HIS3-ded1* sequence with the HIS3 gene and the truncated DED1 and PET56 genes inserted in the UNF region of the TRP1ARS1 circle. UNF denotes the TRP1ARS1 region from the ARS1 consensus element (solid oval) to the *Eco*RI site. Nucleosome positions and nucleosome-free regions are shown as described (Losa *et al.*, 1990). The TRP1 gene in YRpCS1 shows overlapping nucleosome positions as in the TRP1ARS1 circle (Thoma *et al.*, 1984). (**B**) YRpTRURAP of strain JMY1 contains the URA3 gene inserted in the TRP1ARS1 circle. Nucleosome positions and nucleosome-free regions are shown as described (Thoma, 1986; Smerdon and Thoma, 1990). ● denote some polypyrimidine regions and polydT-tracts which are hot spots of CPD formation and which are fast-repaired by photolyase (outside is top strand; inside is bottom strand). Nucleosome positions (circles), the promoter regions (5′), the 3′ ends of the genes (3′), the ARS1 origin of replication (ARS1) are indicated. R (*Eco*RI), X (*Xba*I) and V (*Eco*RV) are restriction sites. Map units in basepairs (bp) are indicated in 0.2 kb steps.

found in humans (Todo *et al.*, 1996), suggesting that photolyases are widespread. CPD-photolyases recognize CPDs with a selectivity similar to that of sequence-specific DNA-binding proteins (Sancar *et al.*, 1987), which suggests that they might compete with histones for DNA accessibility in a similar way as do transcription factors. The *Escherichia coli* enzyme and the yeast enzyme recognize the same substrates, but the yeast enzyme shows a reduced number of phosphate contacts which could be advantageous for binding DNA in nucleosomes (Baer and Sancar, 1989). Injection of enzymes from *Anacystis* and *Saccharomyces* into human cells showed that both enzymes could act to some extent in chromatin and that the eukaryotic enzyme was more efficient in the removal of CPDs (Zwetsloot *et al.*, 1985). Although the enzymes and the reaction mechanism of photolyases have been characterized in detail (reviewed in Sancar, 1996b), a direct examination has not been made as to: (i) how CPDs are recognized by photolyase in chromatin; (ii) whether chromatin might affect photoreactivation; or (iii) how photolyase repairs transcriptionally active genes.

In contrast to the complex NER pathway, in which damage recognition and repair is carried out by different proteins, photoreactivation depends on a single enzyme and the reaction can be strictly controlled by presence or absence of photoreactivating light. Hence, monitoring CPD repair by photoreactivation allows direct conclusions to be made about the accessibility of CPDs to photolyase in chromatin *in vivo*. Here, we use yeast strains containing minichromosomes with well-characterized chromatin structures as model substrates to study the effect of different chromatin structures on the repair of CPDs by photolyase. We show a strong modulation of photoreactivation by chromatin structure, an active role of photolyase in repair of open gene promoters, and preferential repair of the non-transcribed strands. The results further document that photolyase is a useful tool with which to monitor chromatin structure in a living cell.

Results

Saccharomyces cerevisiae strains FTY117 and JMY1 are deficient in NER (*rad1*Δ) and contain the minichromosomes YRpCS1 and YRpTRURAP respectively (Figure 1). The chromatin structures of both minichromosomes have been determined previously using micrococcal nuclease (MNase) (Thoma, 1986; Losa *et al.*, 1990; Tanaka *et al.*, 1996). The minichromosomes contain several NSRs separated by positioned nucleosomes. NSRs are considered to be nucleosome-free or to contain disrupted nucleosomes. The NSRs include promoter regions of the DED1 gene joined to the 3′ end of the HIS3 gene, the divergent promoters of the PET56 and HIS3 gene, and the promoter and the 3′ end of the URA3 gene. Many NSRs contain poly dT-tracts and polypyrimidine regions (● in Figure 1). Poly dT-tracts are ubiquitous in yeast and serve as promoter elements to stimulate transcription (Struhl, 1985; Iyer and Struhl, 1995). Both minichromosomes contain an origin of replication (ARS1), which is structured as a NSR flanked by a nucleosome.

Chromatin structure of irradiated cells

Cells were irradiated in suspension with UV light (predominantly 254 nm) at a dose of 100 J/m^2 to generate approximately one CPD per DNA strand. For chromatin analysis, YRpCS1 minichromosomes were partially

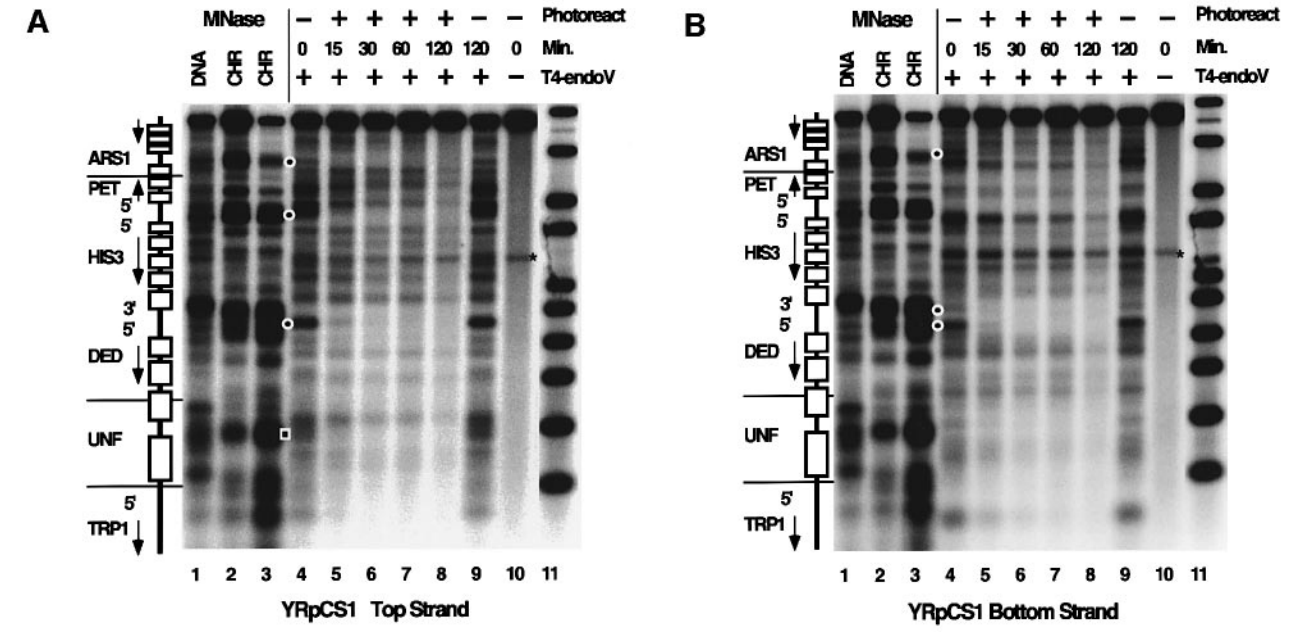
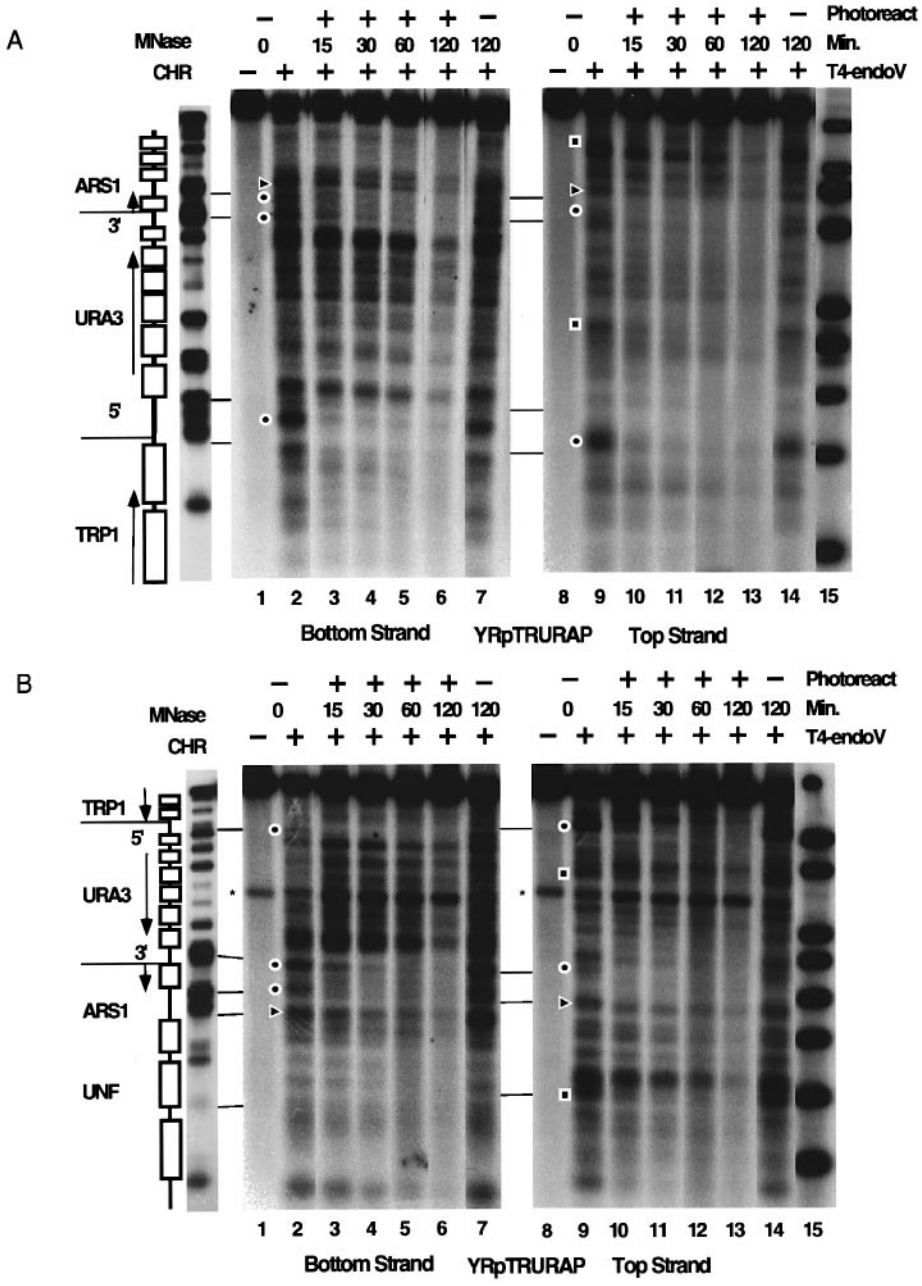


Fig. 2. Chromatin structure and CPD repair by photolyase in minichromosome YRpCS1. (**A**) Top strand. (**B**) Bottom strand. The bottom strand is the transcribed strand of the TRP1 and HIS3 genes. FTY117 cells were UV-irradiated with 100 J/m^2. Chromatin structure was analysed by micrococcal nuclease digestion (MNase) of DNA (lane 1) and chromatin (CHR, lanes 2 and 3) extracted from irradiated cells. Photoreactivation (+ Photoreact) was for 15 to 120 min (lanes 5–8). CPD distribution and repair was analysed by T4-endoV cleavage (+ T4-endoV, lanes 4–9). Lane 10 is irradiated DNA (same as lane 4) without T4-endoV cleavage. An aliquot of cells was kept in the dark for 120 min (lane 9). Cleavage sites for MNase and T4-endoV are shown by indirect end-labelling from the *Xba*I site (Figure 1). A schematic interpretation of chromatin structure is shown (left side). Chromatin regions of 140 to 200 bp that are protected against MNase cleavage represent positioned nucleosomes (rectangles), cutting sites between nucleosomes represent linker DNA, long regions with multiple cutting sites represent NSRs (ARS1; 5′PET-5′HIS3; 3′HIS3-5′DED; 5′TRP1). 5′ and 3′ ends of genes, direction of transcription (arrows) are indicated. ● and ■ indicate fast repair in NSRs and linker DNA, respectively. * denote cross-hybridization with genomic DNA. Size markers (in bp, lane 11) are 261, 460, 690, 895, 1122, 1291, 1796, 2093, 2719 and 3347. (**C**) CPD repair in the top and bottom strand. The initial damage (0 min) was 1.2 ± 0.2 CPDs in the top and bottom strand. The average and standard deviation of four gels are shown. +UV, –UV, indicates damaged and non-damaged samples; +366, –366, photoreactivated and non-photoreactivated samples.

purified from FTY117 cells, digested with MNase, and the cutting sites were displayed by indirect end-labelling and compared with those obtained in naked DNA (Figure 2). The pattern revealed positioned nucleosomes separated by linker DNA and nuclease-sensitive regions. The pattern was indistinguishable from that obtained from non-irradiated cells (Losa *et al.*, 1990; Tanaka *et al.*, 1994). Hence, irradiation produced no detectable effect on the chromatin structure of YRpCS1 (Figure 2). In contrast to standard procedures which map MNase cuts by non-denaturing gel electrophoresis (Thoma *et al.*, 1984), Figure 2 shows a Southern blot of an alkaline gel hybridized with an RNA probe specific for the top strand and re-hybridized with a probe specific for the bottom strand. Both strands show an indistinguishable cutting pattern (compare Figure 2A and B), demonstrating that MNase preferentially generates double-strand cuts in linker DNA between nucleosomes and that single-strand nicking on the nucleosome surface is not detected.

CPD repair by photolyase

Photoreactivation was done by exposure of the cell suspension to photoreactivating light for 15 to 120 min. A control sample was kept in the dark for 120 min. DNA was extracted, mock-treated or treated with T4-endonuclease V (T4-endoV) which cuts at CPDs (Gordon and Haseltine, 1980). The cutting sites were displayed by indirect end-labelling using alkaline gel electrophoresis (Smerdon and Thoma, 1990; Figures 2–4). Unirradiated DNA (not shown) and mock-treated DNA showed a background smear due to nicking of DNA during preparation (–T4-endoV lanes; Figures 2–4). In contrast, T4-endoV-treated DNA revealed numerous bands of different intensities (+ T4-endoV lanes; Figures 2–4). These bands can be



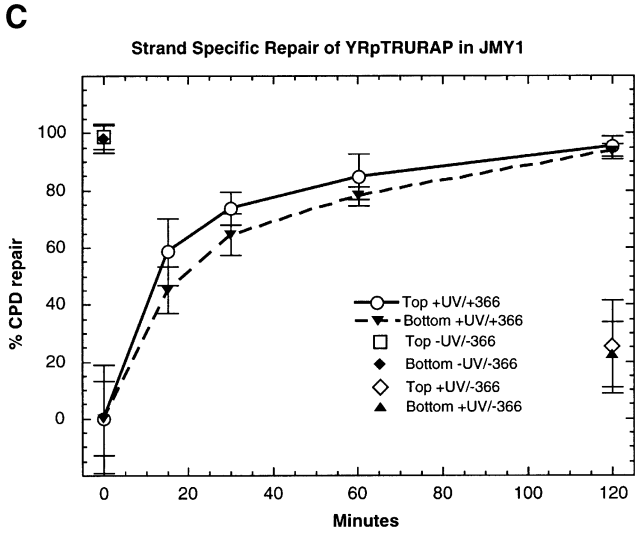


Fig. 3. Chromatin structure and CPD repair by photolyase in minichromosome YRpTRURAP. UV irradiation of JMY1 cells, photoreactivation, and analysis of CPD distribution and repair was as described in Figure 2. Mapping was from the *Xba*I site: (**A**) clockwise using strand-specific probes generated from the *Xba*I–*Eco*RV fragment of TRP1; (**B**) counter-clockwise using strand-specific probes generated from the *Eco*RI–*Xba*I fragment of TRP1. Chromatin structure was determined by MNase digestion of non-irradiated cells (FTY23 containing YRpTRURAP) and cutting sites were mapped from the *Xba*I site using non-denaturing agarose gel electrophoresis. A schematic interpretation is shown as described in Figure 2. ● and ■ indicate fast repair in NSRs and linker DNA, respectively. ▲ indicate slow repair in ARS1. * denote cross-hybridization with genomic DNA. Size markers (in bp) are 199, 429, 634, 861, 1030, 1535, 1832, 2017, 2432 [(A), lane 15], and 261, 460, 690, 895, 1122, 1291, 1796, 2093 [(B), lane 11]. (**C**) CPD repair in the top and bottom strand. The initial damage (0 min) was 1.0 ± 0.2 CPDs in the top strand and 1.2 ± 0.2 CPDs in the bottom strand. The average and standard deviation of eight gels are shown.

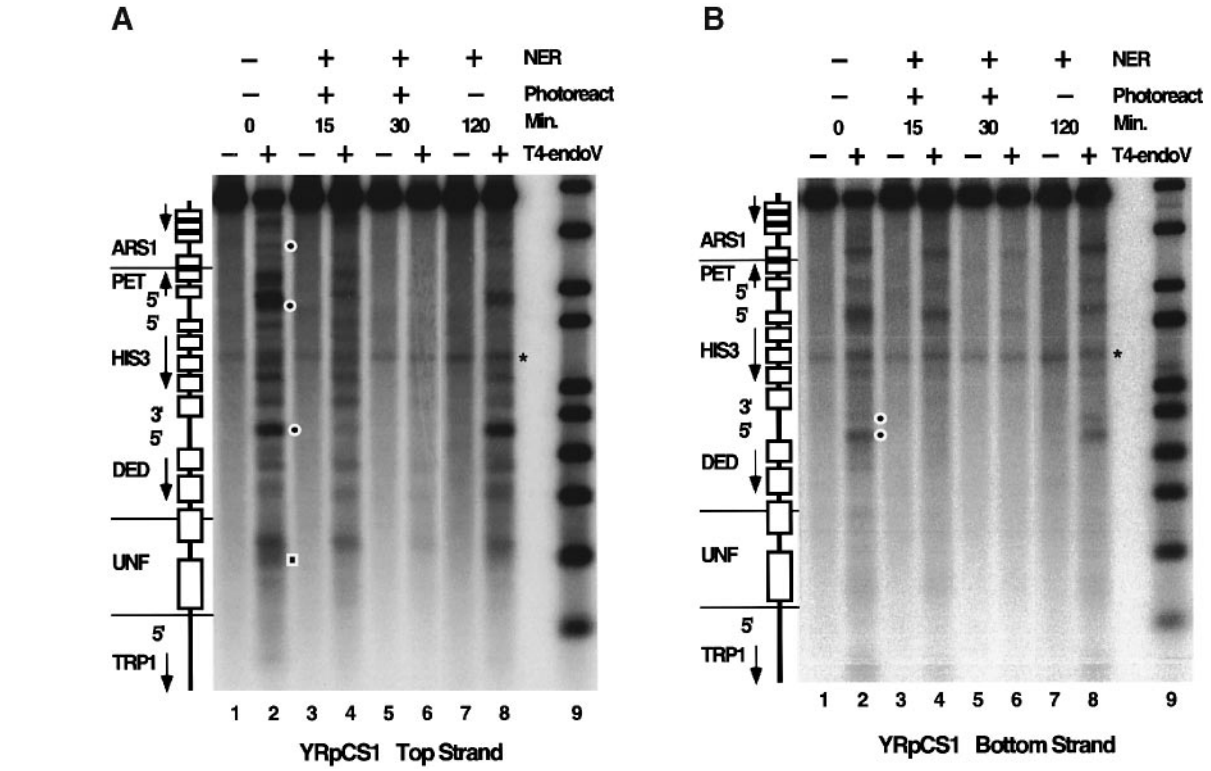


Fig. 4. Photoreactivation and NER in YRpCS1. UV irradiation of CSY1 cells, photoreactivation, and analysis of CPD distribution and repair was as described in Figure 2. Mapping was from the *Xba*I site for the top strand (**A**) and bottom strand (**B**). (**C**) CPD repair in the top and bottom strand. The initial damage (0 min) was 0.8 ± 0.2 CPDs in the top and bottom strand. The average and standard deviation of four gels are shown.

assigned to dipyrimidines and polypyrimidine tracts in the DNA sequence. Many strong bands correspond to T-tracts in the promoter regions of the DED1-, HIS3-, PET56- and URA3- genes, demonstrating that these tracts are hot spots of CPD formation. For example, the strong bands in the promoter region of the DED1 gene (● in 5′ DED, Figure 2) represent CPDs in 5′-CTTTCCTTTTTTCTT-TTTGCTTTTTCTTTTTTTTTCTCTT-3′ (top strand, Figure 2A) and in 3′-CTCTTTTTTTTATATTTTCTCTAC-CTCCTTGCCCTTTTTC-5′ (bottom strand, Figure 2B). Similarly, the strong bands in the 5′ regions of the PET56 and HIS3 genes represent CPDs in 5′-TCCTTT-CCCGCAATTTTCTTTTTCTATTACTCTTGGCCTCCT-CTAGTACACTCTATATTTTTTTATGCCTCGGTAAT-GATTTTCATTTTTTTTTTTCCACCTAGCGGATGACT-CTTTTTTTTTCTT-3′ (top strand, Figure 2A). In the promoter region of the URA3 gene, the strong bands (●, Figure 3A) reflect CPDs in 5′-CTTTTCAATTCATCATT-TTTTTTTTATTCTTTTTTTTGATTTCGGTTTCCTTG-AAATTTTTTTG-3′ (top strand) and 3′-CTTTAAAA-AAACTAAGCCATTAGAGGCTTGTCTTCCTTCTTG-CTTCCTTCCTCGTGTCTGAATCTA-5′ (bottom strand). T-tracts are ubiquitous promoter elements of yeast genes (Struhl, 1985; Iyer and Struhl, 1995). Hence, UV light efficiently damages these promoter elements and thereby could affect gene expression.

Upon irradiation with photoreactivating light, >90% of CPDs were removed from both strands within 120 min. During incubation in the dark for 120 min (dark control), no repair was observed in YRpCS1 (Figure 2C). Hence, in the presence of photoreactivating light the CPDs were repaired by photolyase. (In YRpTRURAP, the dark control

sample showed ~20% less CPDs; Figure 3C. This could be due to a lower initial damage, since that particular sample was irradiated in a separate vessel, or alternatively due to incomplete protection against photoreactivating light.)

Fast repair in nuclease-sensitive regions and linker DNA; slow repair in nucleosomes

Inspection of the results at individual sites or clusters of CPDs very strikingly reveals two classes of repair: fast repair, when CPDs are removed within 15 to 30 min (● in Figures 2 and 3); and slow repair, when CPDs remain detectable for up to 60–120 min. A comparison of CPD repair with the chromatin analysis shows that fast repair correlates strictly with the accessibility of DNA to MNase (bands in chromatin lanes), and slow repair corresponds to inaccessibility to MNase (no bands in chromatin lanes). This is best observed in Figure 2, where chromatin analysis and CPD repair are displayed on the same gel. Hence, chromatin structure regulates the accessibility to CPDs as it regulates the accessibility to MNase.

The locations of fast-repaired sites (●) correspond to NSRs in chromatin. This includes repair of CPDs in T-tracts of the promoter region of DED1 (5′ end) and 3′ end of HIS3 (Figure 2, both strands), the common promoter region of HIS3 and PET56 (5′ ends; Figure 2, top strand). Similarly, the promoter and 3′ end of the URA3 gene are rapidly repaired (Figure 3, both strands). This result strongly suggests a direct role of photolyase in repair of 'open' chromatin regions, in particular of active gene promoters.

Sites that are slowly repaired strictly co-localize with regions which are resistant to MNase cleavage and represent positioned nucleosomes (open rectangles in schematic drawings, Figures 2 and 3). This is best observed in the five nucleosomes of the HIS3 gene, in the PET region (Figure 2) as well as in the URA3 gene (Figure 3, bottom strand). In the UNF region of the minichromosomes, one site on the top strand was fast repaired (■ in Figures 2A and 3B), while a site nearby was slowly repaired. The fast-repair sites correspond to linker region between two positioned nucleosomes, while the slow-repair sites are located within a nucleosome. Similarly, a CPD site that is fast-repaired mapped in the linker between the second and third nucleosome of URA3 (■ in Figure 3A, top strand). Hence, nucleosomes apparently restrict the accessibility of CPDs to photolyase, but they do not represent a complete block.

Repair in the ARS1 region was more heterogeneous showing fast (●, Figures 2 and 3) and slowly repaired sites (▲). The slow site on the top strand includes the B1 and B2 elements of ARS1 and is located in the NSR. The site on the bottom strand includes the ARS1 consensus sequence (A element) located at the edge of a nucleosome (Thoma *et al.*, 1984; Thoma, 1986; Losa *et al.*, 1990). It is possible that photoreactivation in these sites is modulated by the protein complex at the origin of replication associated with these elements (Diffley and Cocker, 1992).

Repair of nuclease-sensitive regions: a role of photolyase

In wild-type yeast, both repair pathways, NER and photoreactivation, are active under daylight conditions. The results described above in NER-deficient strains suggest a role of photolyase in repair of open NSRs, including promoters of active genes. To address the role and contribution of photolyase in presence of NER, a photoreactivation experiment was performed with the CSY1 strain (Figure 4). CSY1 is wild-type for NER and photolyase and contains the minichromosome YRpCS1 (Figure 1A; Losa *et al.*, 1990).

Initially, repair in the CSY1 strain was much faster than in the NER-deficient strains FTY117 and JMY1 (Figure 4C). About 70–80% of CPDs were repaired after only 15 min from both the top and bottom strands and few CPDs remained after 30 min (Figure 4A and B, lanes 2, 4 and 6; also Figure 4C). For comparison, photolyase alone achieved 70–80% repair only after ~1 h (Figures 2C and 3C). Dark repair alone removed only ~62% and 73% of CPDs from the top and bottom strands, respectively, within 120 min (Figure 4A and B, lanes 8; also Figure 4C).

Inspection of site-specific repair reveals that CPDs in the nuclease-sensitive promoter regions of the DED1 and HIS3/PET56 genes (●, Figure 4) are repaired within 15 min under photoreactivating conditions (lane 4) which is as fast as in the absence of NER (Figures 2 and 3). In contrast to photoreactivation, a large fraction of the CPDs persists in those NSRs during dark repair (NER) for 120 min (Figure 4A and B, lanes 8), although under those conditions already more than half of all the CPDs were removed from each strand. Hence, NER itself does not preferentially repair CPDs in NSRs, which is consistent with our previous observations in YRpTRURAP (Smerdon and Thoma, 1990). It is possible that some factors (transcription factors?) inhibit NER but not photoreactivation. In summary, these results clearly demonstrate that photolyase and not NER plays an important role in rapid repair of 'open' chromatin structures. Although photolyase and NER might compete for the same substrates, there is no obvious inhibition of photoreactivation by NER in the nuclease-sensitive regions.

Photoreactivation in transcribed genes

The bottom strand is the transcribed strand of the TRP1, HIS3 and DED1 sequences in YRpCS1, while the PET56 promoter induces transcripts from the top strand (Tanaka *et al.*, 1994). The bottom strand is the transcribed strand for the major transcripts of URA3 and TRP1 sequences in YRpTRURAP, but some transcripts were also detected from the top strand outside of the URA3 region (Bedoyan *et al.*, 1992).

Several observations indicate that photoreactivation repairs the non-transcribed strand faster than the transcribed strand. First, photoreactivation in the absence of NER appeared to show a small enhancement of repair of the top strand of YRpCS1 (Figure 2C) or YRpTRURAP (Figure 3C). Second, dark repair in CSY1 removed ~62% from the top strand (Top +UV/–366) and 73% of CPDs from the bottom strand (Bottom +UV/–366) of YRpCS1 (Figure 4C), which is consistent with preferential repair of the transcribed strand by NER (transcription-coupled repair). However, photoreactivation in presence of NER shows almost identical repair curves for both strands (Figure 4C), which indicates that fast repair of the non-transcribed strand by photolyase can match the fast repair

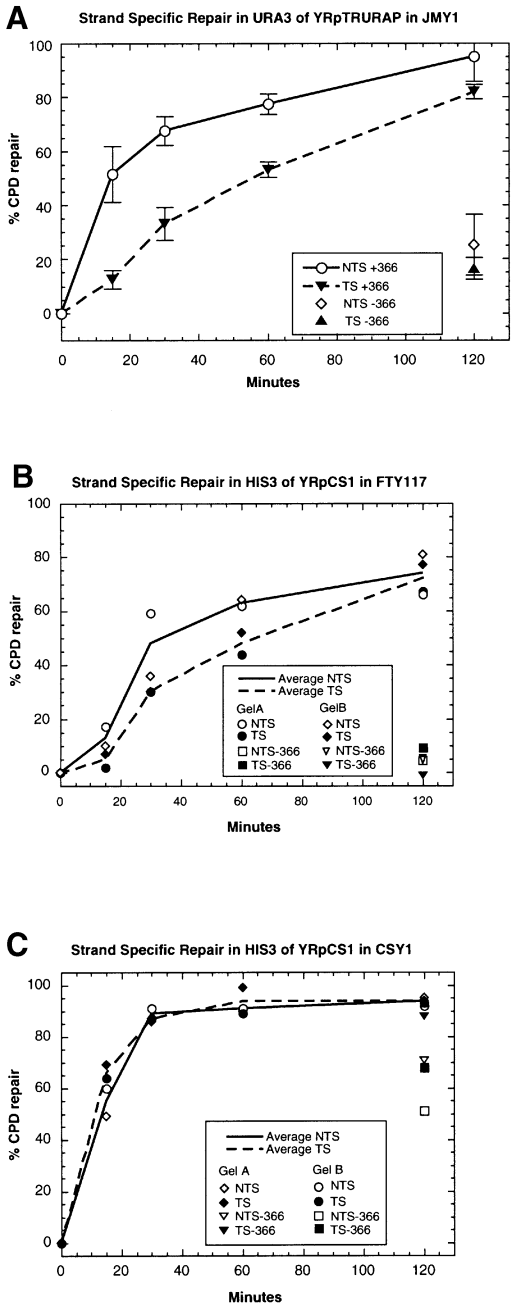


Fig. 5. Strand-specific photoreactivation of the transcribed regions of the HIS3 and URA3 genes. (**A**) URA3 of YRpTRURAP in JMY1. (**B**) HIS3 of YRpCS1 in FTY117. (**C**) HIS3 of YRpCS1 in CSY1. The transcribed regions correspond to the nucleosomal regions (see Figures 2 and 3), but exclude the nuclease-sensitive and fast-repaired promoters and 3′ ends. +UV, –UV, indicates damaged and non-damaged samples; +366, –366, photoreactivated and non-photoreactivated samples. NTS, non-transcribed strand (top strand); TS, transcribed strand (bottom strand). The average and standard deviation of four gels are shown in (A), and of two gels in (B) and (C).

of the transcribed strand by NER. Third, in the absence of NER, the top strand (non-transcribed) of the URA3 gene in YRpTRURAP appears to be faster repaired than the bottom strand (Figure 3A and B). The effect on the HIS3 gene in YRpCS1 is not obvious from visual inspection of the gels (Figure 2).

We therefore quantified CPD removal over the transcribed regions of the URA3 and HIS3 genes (Figure 5). This includes the nucleosomal region (Figures 2 and 3), but excludes the nuclease-sensitive promoters and 3′ ends. In both genes of the NER-deficient strains FTY117 and JMY1, the non-transcribed strands were faster repaired by photolyase than the transcribed strands (Figure 5A and B). The effect was more pronounced in the URA3 gene. In CSY1, when NER and photolyase are active, both strands of the HIS3 gene showed similar repair curves (Figure 5C). The dark repair control showed the expected preferential repair of the transcribed strand by NER. Hence, fast repair of the non-transcribed strand by photolyase can match fast repair of the transcribed strand by NER. In other words, fast repair of the non-transcribed strand by photolyase is directly opposite to the preferential repair of the transcribed strand by NER.

Discussion

CPD repair by photolyase is modulated by chromatin structure

The strict correlation between photoreactivation and MNase accessibility provides substantial insight into a DNA repair process as well as into structural and dynamic properties of chromatin. We conclude that CPD repair by photolyase in the living cell is tightly modulated by chromatin structure, which apparently restricts the accessibility of DNA lesions to photolyase (illustrated schematically in Figure 6). Only CPDs that are located in linker DNA or in NSRs are rapidly repaired, while CPDs in nucleosomes are slowly repaired. In contrast to these photoreactivation results, previous results on NER in the same substrate (YRpTRURAP) (Smerdon and Thoma, 1990; Bedoyan *et al.*, 1992), and in particular the results shown in Figure 4, do not reveal a preference of NER for CPD repair in nuclease-sensitive regions. Hence, the photoreactivation results are to our knowledge the first data that show a clear modulation of a DNA repair process by the local chromatin structure.

Photoreactivation in nucleosomes

Nucleosomes have an inhibitory effect on photoreactivation. The fact, however, that most CPDs in nucleosomes were repaired within 120 min, can be explained by structural and dynamic properties of nucleosomes. Changes in nucleosome positions, e.g. by sliding of histone octamers along the DNA sequence (Figure 6B) or transient unfolding or disruption (Figure 6C) could allow the inaccessible CPD lesions to become accessible to photolyase. Consistent with such a rearrangement of nucleosomes, it was found that nucleosome positions can be altered *in vitro* (‘nucleosome mobility’; for references see Meersseman *et al.*, 1992) and *in vivo* in yeast (Thoma, 1986) and that nucleosomes in various yeast sequences [TRP1, URA3 (Thoma *et al.*, 1984; Thoma, 1986; Thoma and Zatchej, 1988), 5S rDNA (Buttinelli *et al.*, 1993)] can occupy multiple positions. High-resolution mappings of the URA3 gene in the genome and in YRpTRURAP showed that the positions may vary by a few base pairs (illustrated in Figure 6A and B; Tanaka *et al.*, 1996). It is presumed that those positions exist in an equilibrium. A shift of a nucleosome by five base pairs, or half a helical turn of DNA, rotates the inner surface of nucleosomal DNA outside and, hence, could affect the accessibility of DNA

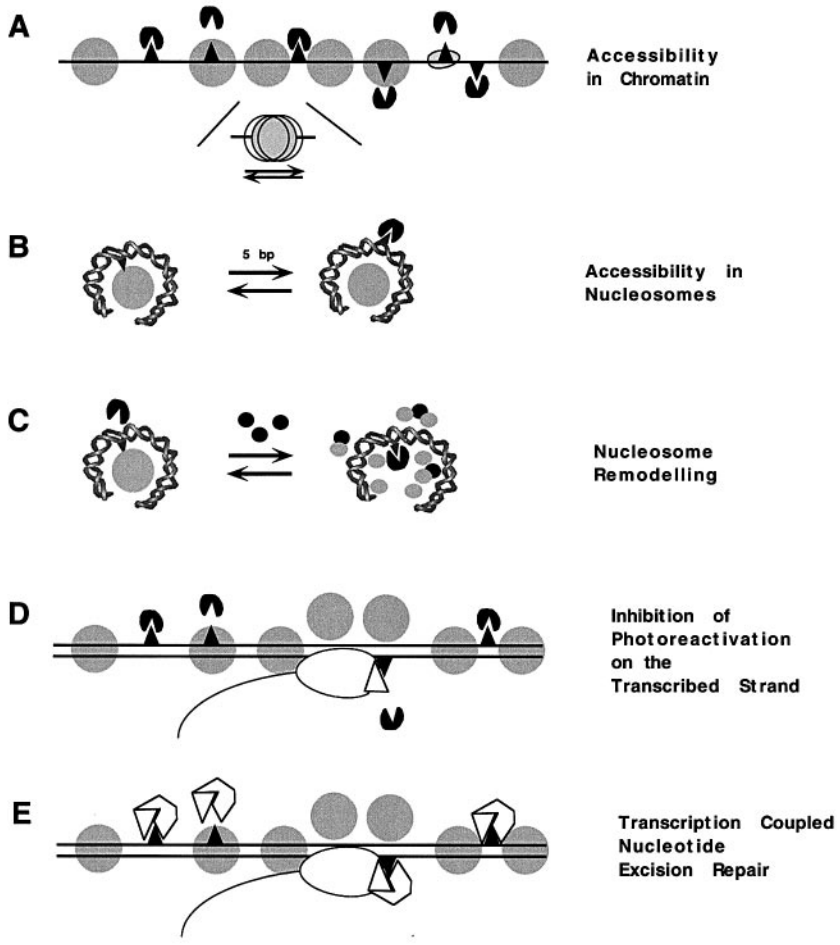


Fig. 6. CPD repair in chromatin by photolyase. (**A**) Photolyase preferentially recognizes CPDs in linker DNA and nuclease-sensitive regions, while DNA-binding proteins and nucleosomes limit the accessibility. Nucleosomes may occupy multiple positions (overlapping circles). Multiple positions are in a dynamic equilibrium (arrows). ▲ represent CPDs; grey circles represent nucleosomes; the Packman symbols represent photolyase. (**B**) Changing a nucleosome position by 5 bp turns the inner surface of DNA outside and alters the accessibility of DNA lesions. One turn of nucleosomal DNA is shown (adapted from Richmond *et al.*, 1984). The grey circle represents the histone octamer. (**C**) Remodelling factors (●) may lead to a partial or complete disruption of nucleosome structure and enhance DNA damage recognition. Grey circles represent histones. (**D**) RNA polymerase II blocked at a CPD on the transcribed strand may prevent access to photolyase, explaining slow repair of the transcribed strand compared with the non-transcribed strand. (**E**) RNA polymerase II blocked at a CPD on the transcribed strand promotes assembly of the NER machinery, explaining preferential repair of the transcribed strand. Sharing of proteins between the transcription machinery and the NER (stippled polygon) is indicated (white triangle).

lesions (Figure 6B; Thoma *et al.*, 1993). A nucleosome shift may also move a DNA lesion into linker DNA. Nucleosome cores isolated from UV-irradiated human cells showed a periodic formation or accommodation of CPDs at sites where the minor groove faces outside (Gale *et al.*, 1987). However, no change in that periodic pattern was observed during NER (Jensen and Smerdon, 1990) which demonstrated that CPDs were removed at nearly equal rates from the inner and outer surfaces of DNA in nucleosomes. Although we do not know how NER recognizes CPDs, these results are consistent with dynamic properties of nucleosomes that make lesions accessible. Interestingly, a recent experiment on CPD formation in a reconstituted nucleosome showed that distortions generated by CPDs are tolerated and that a disruption of histone–DNA interactions is required to alter the rotational setting on the nucleosomal surface (Schieferstein and Thoma, 1996). Hence, nucleosome positions *in vivo* are apparently more dynamic than that particular nucleosome studied *in vitro*.

Promoter activation by transcription factors may require a nucleosome remodelling activity which leads to a disruption of nucleosomes and facilitates factor binding (reviewed in Peterson and Tamkun, 1995). By analogy, we should consider that DNA repair in nucleosomes may require a similar activity to facilitate CPD recognition (Figure 6C). There is no evidence so far that photoreactivation depends on such a complex. However, the nucleosome disruption hypothesis is attractive for NER, where numerous proteins assemble to execute DNA incision, removal of the damaged fragment, and gap filling. The fact that new repair patches are nuclease-sensitive and not folded in canonical nucleosomes implies a nucleosome disruption or rearrangement process in the earlier steps of NER (Smerdon, 1989).

Could replication explain slow repair in nucleosomes? One round of DNA replication after CPD induction and without repair would double the amount of DNA and reduce the CPD content in DNA 2-fold. This would give the impression of 50% CPD removal. Since our dark

repair control in the NER-deficient strains (120 min without photoreactivating light) shows very low levels of CPD removal (e.g. 0% in YRpCS1, Figure 2C), we can assume that, if at all, only a minor fraction of minichromosomes could have been replicated. This contrasts with >95% repair by photoreactivation. Even if nucleosome disruption during replication would make all CPDs accessible to photolyase, this could not account for the high repair level observed under photoreactivating conditions. Hence, replication as an argument for slow repair in nucleosomes can be excluded.

Finally, *rad1*Δ strains are very sensitive to UV irradiation. Less than 1% of the FTY117 and JMY1 cells survived and formed colonies after irradiation with 100 J/m^2 and no remarkable change occurred as a consequence of photoreactivation. Survival of the wild-type CSY1 was close to 50%. Since it is not known how long the cells survive, DNA repair in nucleosomes could be explained by a loss of nucleosome structure due to histone degradation. However, chromatin analysis by MNase digestion after 120 min photoreactivation in FTY117 showed intact chromatin (data not shown), thus excluding chromatin degradation as a cause for nucleosome repair. We conclude that repair in nucleosomes is possible due to dynamic properties and not due to replication or chromatin degradation.

Photolyase: a molecular tool to study chromatin structure in vivo

Chromatin analysis by nuclease digestion requires a disruption of cells and sometimes a partial purification of chromatin. We are therefore always concerned that those procedures could affect chromatin composition as well as nucleosome arrangement and stability. Hence, we are very pleased to see the tight correlation of CPD repair by photolyase *in vivo* with MNase digestion in chromatin *in vitro*. This substantiates that the *in vitro* analysis of chromatin structure by MNase digestion indeed reflects a chromatin structure as it exists in living cells, at least at this level of resolution.

Figure 2 shows that chromatin analysis after irradiation was indistinguishable from that obtained from unirradiated cells (reported previously by Losa *et al.*, 1990). The fraction of minichromosomes with a CPD at a specific site is very low, but chromatin analysis by nuclease digestions reveals an averaged structure of the whole chromatin population. Hence, it is impossible to analyse by nuclease digestions whether a CPD at a particular site results in an altered chromatin structure. However, the tight correlation of MNase accessibility and photoreactivation strongly suggests that CPD induction does not grossly alter chromatin structure *in vivo*. This is also consistent with the observation that reconstituted nucleosomes irradiated *in vitro* can tolerate distortions imposed by DNA damage (Schieferstein and Thoma, 1996). In summary, this work shows that photolyase can be used as a molecular tool to address accessibility of DNA in chromatin of living cells.

Promoter repair: an important role for photolyase

What are the roles of photolyase? Most striking is the rapid repair of nuclease-sensitive promoter regions. Such repair occurs in the absence (Figures 2 and 3) or presence (Figure 4) of NER and clearly distinguishes photoreactivation from NER. T-tracts are ubiquitous promoter elements of constitutively expressed yeast genes (Struhl, 1985; Iyer and Struhl, 1995) and these promoters are located in NSRs (Losa *et al.*, 1990). Transcription factors bound to DNA may reduce or enhance CPD formation (Pfeifer *et al.*, 1992; Tornaletti and Pfeifer, 1995), but CPDs might also interfere with factor binding. Since T-tracts are hot spots of CPD formation (Figures 2 and 3; Brunk, 1973), UV-mediated damage may inactivate promoters. Hence, efficient photoreactivation in NSRs strongly supports a role of photolyase to repair CPD-lesions in active ('open') promoters, thereby regenerating gene regulation.

Similarly, photorepair of some sites in the nuclease-sensitive ARS1 region was fast, while repair of other sites was slow. Since the same regions are slowly repaired by NER (Smerdon and Thoma, 1990), photolyase might improve regeneration of an active ARS region.

Photoreactivation and transcription

In NER, the transcribed strand of the URA3 gene in YRpTRURAP was repaired faster than the non-transcribed strand (Smerdon and Thoma, 1990). This process is now known as transcription-coupled repair (Hanawalt *et al.*, 1994; Selby and Sancar, 1994; Figure 6E). In this work we noticed that, during photoreactivation in NER-deficient strains, the transcribed strands of the URA3 and HIS3 genes were more slowly repaired than the non-transcribed strands (Figure 5). Both *E.coli* RNA polymerase and mammalian RNA polymerase II are blocked at a CPD in the transcribed strand (Selby and Sancar, 1993; Donahue *et al.*, 1994). Sharing of proteins between the eukaryotic RNA polymerase II transcription machinery and NER may lead to a more rapid assembly of the repair complex at a stalled polymerase and thus explains the preferential repair of the transcribed strand (Figure 6E; for reviews see Hanawalt *et al.*, 1994; Ma *et al.*, 1995; Sancar, 1996a). In contrast, however, *in vitro* experiments have shown that RNA polymerase II blocked at a CPD on the transcribed strand shielded the CPD from recognition by photolyase (Donahue *et al.*, 1994). Hence, our photoreactivation results could indicate that stalled RNA polymerases prevent accessibility of CPDs to photolyase *in vivo* (Figure 6D). Experiments are currently directed to address this topic in more detail.

In conclusion, cells that are exposed to sunlight are simultaneously exposed to damage-inducing radiation and photoreactivating light. This study shows that chromatin structure modulates DNA repair by photolyase. Furthermore, it provides evidence for a role of photolyase in the efficient repair of open chromatin structures, and in particular of regions that are important for gene regulation. In contrast, NER is comparably slow in those regions. Furthermore, the fast repair of the non-transcribed strand by photolyase can match the fast removal of lesions from the transcribed strand by NER. Hence, the combination of both repair pathways ensures efficient repair of the genome and active genes. This rapid repair is particularly important for unicellular organisms (e.g. yeast) to enhance survival, but is also likely to be important in those cells of more complex organisms that are exposed to sunlight.

A number of organisms and tissues that are never exposed to sunlight express photolyase, suggesting a non-

photoreactivation function for photolyase (references in Ozer *et al.*, 1995). In the dark, photolyase stimulates removal of UV damage by NER in yeast (Sancar and Smith, 1989) and in *E.coli* (Yamamoto *et al.*, 1983). Furthermore, photolyase binds to other lesions (e.g. *cis*-diamminedichloroplatinum adducts) and either inhibits NER of those lesions in *S.cerevisiae* (Fox *et al.*, 1994) or enhances it in *E.coli* (Ozer *et al.*, 1995). Those observations suggest an interaction between NER and photolyase, most likely at the level of DNA damage recognition. Hence, knowing the characteristics of CPD recognition by photolyase in chromatin could provide further insight into the damage recognition process of NER and into the interaction between these two repair mechanisms.

Materials and methods

Yeast strains

JMY1 [MAT**a**, *his3-Δ1 trp1-289 rad1-Δ ura3-52* YRpTRURAP(*URA3 ARS1*)] was a gift of Drs M.Smerdon and J.Mueller; FTY117 [MAT**a** *his3-Δ1 trp1-289 rad1-Δ ura3-52* YRpCS1(*HIS3 TRP1 ARS1*)] was generated from JMY1 by selecting for plasmid loss and subsequent transformation with YRpCS1. CSY1 [MATα *his3-1 trp1 ura3-52 gal2 gal10* YRpCS1(*HIS3 TRP1 ARS1*)] was described by Losa *et al.* (1990).

Cultures and UV irradiation of yeast cells

Yeast cultures were grown at 30°C in minimal media (2% dextrose, 0.67% Yeast Nitrogen Base without amino acids supplemented with the appropriate amino acids or uracil (Sherman *et al.*, 1986) to a density of ~3×10^7 cells/ml, harvested, and resuspended in minimal medium to 4×10^7 cells/ml. 250 ml aliquots were transferred to plastic trays (22×31 cm) to provide a 4 mm thin cell suspension and irradiated at room temperature with 100 J/m^2 of UV light (predominantly 254 nm) generated by germicidal lamps (Sylvania, Type G15 T8). After irradiation, the medium was supplemented with the appropriate amino acids or uracil.

Chromatin analysis

After irradiation of yeast cells, minichromosomes were partially purified, digested with MNase and the cutting sites were mapped by indirect end-labelling as described (Thoma and Simpson, 1985; Thoma, 1986) except that alkaline gels and strand-specific probes were applied.

Photoreactivation

After damage induction, photoreactivation of 250–500 ml samples was done by using Sylvania Type F15 T8/BLB bulbs (peak emission at 375 nm) at 1.5 mW/cm^2 for 15 to 120 min. During photoreactivation the temperature increased from 23°C to 26°C. 250 ml samples were collected and chilled on ice. Cells were harvested by centrifugation, resuspended in Zymolyase Reaction Buffer (250 mM EDTA, pH 8.0, 1 M sorbitol, 20 mM β-mercaptoethanol, 1 mM phenylmethylsulphonyl fluoride), and converted to spheroplasts by using 3 mg Zymolyase 100T (Seikagaku Kogyo Co., Tokyo, Japan) at 30°C. All steps from irradiation to lysis of spheroplasts were carried out in rooms equipped with gold fluorescent light. Spheroplasts were collected by centrifugation, washed in 12 ml 1 M sorbitol, 10 mM EDTA, lysed in 7.5 ml 1% SDS, 50 mM Tris, pH 7.4, 20 mM EDTA and incubated with 750 µg proteinase K for 2 h at 65°C. After addition of 3 ml of 3 M potassium 5 M acetate and incubation on ice, insoluble material was pelleted, RNA was removed by RNase digestion, and DNA was extensively purified by several phenol extractions and by using Elutip-d (Schleicher and Schüll). For mapping of CPDs by indirect end-labelling, DNA was cut to completion with *Xba*I, re-purified on Elutips and cut with T4-endonuclease V in 20 mM Tris, pH 7.4, 10 mM EDTA, 0.1 M NaCl, 0.1 mg/ml bovine serum albumin. The DNA was electrophoresed on 1.5% alkaline agarose gels (20×25 cm, Gibco-BRL), blotted to ZetaGT-membranes (Bio-Rad), and hybridized to RNA-probes as described (Smerdon and Thoma, 1990). Strand-specific RNA probes were generated from the *Eco*RI–*Xba*I or *Xba*I–*Eco*RV fragments of TRP1 subcloned in a bluescript vectors (Stratagene). The membranes were exposed to X-ray films and PhosphorImager screens. The band intensities were quantified using a PhosphorImager (Molecular Dynamics). The cutting sites were mapped using the DIGIGEL program (DNASTAR).

Quantifications

Strand-specific repair of minichromosomes (CPDs/top strand and CPDs/bottom strand) was calculated using the Poisson expression $\{-\ln [IF_{(+T4\text{-}endoV)}/IF_{(-T4\text{-}endoV)}]\}$ (Mellon *et al.*, 1987), where IF is the intact restriction fragment of the linearized minichromosome DNA. Appropriate corrections were made for background and gel loading variations.

Quantification of the transcribed regions of URA3 and HIS3 genes was carried out using the indirect end-label approach; the signal in a lane represents total DNA, including undamaged linearized minichromosome DNA, DNA cut at CPDs, and randomly nicked DNA. The gene regions were determined by indirect end-labelling (see above) and compared with the chromatin data (see Figures 2 and 3).

Repair in a defined region (Figure 5) was determined as follows. First, the CPD content was measured at each repair time as the signal in that region and divided by the signal of the whole lane (+T4 endoV) to yield a value normalized with respect to the DNA content in that lane. Second, background signal was determined from the same region in the corresponding –T4 lane and divided by the signals of the whole lane. Third, the normalized background was subtracted from the normalized signal. The background corrected with the loading factor was subtracted. Fourth, to generate repair curves, the values were normalized with respect to the initial damage (0 min, 100%).

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