RNA Polymerase II Transcription Inhibits DNA Repair by Photolyase in the Transcribed Strand of Active Yeast Genes

Originally published in:
Nucleic Acids Research 25(19), https://doi.org/10.1093/nar/25.19.3795

Nucleic Acids Research, 1997, Vol. 25, No. 19 **3795–3800**

RNA polymerase II transcription inhibits DNA repair by photolyase in the transcribed strand of active yeast genes

Magdalena Livingstone-Zatchej, Andreas Meier, Bernhard Suter and Fritz Thoma*

Institut für Zellbiologie, ETH-Zürich, Hönggerberg, CH-8093 Zürich, Switzerland

Received July 9, 1997; Revised and Accepted August 11, 1997

ABSTRACT

Yeast uses nucleotide excision repair (NER) and photolyase (photoreactivation) to repair cyclobutane pyrimidine dimers (CPDs) generated by ultraviolet light. In active genes, NER preferentially repairs the transcribed strand (TS). In contrast, we recently showed that photolyase preferentially repairs the non-transcribed strands (NTS) of the URA3 and HIS3 genes in minichromosomes. To test whether photoreactivation depends on transcription, repair of CPDs was investigated in the transcriptionally regulated GAL10 gene in a yeast strain deficient in NER [AMY3 (*rad1*Δ)]. In the active gene (cells grown in galactose), photoreactivation was fast in the NTS and slow in the TS demonstrating preferential repair of the NTS. In the inactive gene (cells grown in glucose), both strands were repaired at similar rates. This suggests that RNA polymerases II blocked at CPDs inhibit accessibility of CPDs to photolyase. In a strain in which both pathways are operational [W303-1a (*RAD1*)], no strand bias was observed either in the active or inactive gene, demonstrating that photoreactivation of the NTS compensates preferential repair of the TS by NER. Moreover, repair of the NTS was more quickly in the active gene than in the repressed gene indicating that transcription dependent disruption of chromatin facilitates repair of an active gene.

INTRODUCTION

Cyclobutane pyrimidine dimers (CPDs) and 6-4 photoproducts (6-4PP) are the two major classes of stable DNA-lesions generated by ultraviolet light (UV). Unless repaired, these DNA-lesions may lead to blockage of transcription, mutations, cell death and cancer. CPDs can be repaired by two pathways, nucleotide excision repair (NER) and photoreactivation (reviewed in ref. 1).

NER is a ubiquitous multistep pathway in which numerous proteins are involved to execute damage recognition, excision of an oligonucleotide with the DNA lesion, and gap repair synthesis (reviewed in refs 2–4). In genes transcribed by RNA polymerase II, NER repairs the transcribed strand more quickly than the non-transcribed strand. This observation was originally made in human cells (5), and later extended to numerous other organisms including yeast (6–9). NER shares some proteins with the general transcription complex which links NER to transcription and partially explains why the transcribed strands of active genes are more quickly repaired than the non-transcribed strands or the genome overall. Preferential repair of the transcribed strand is frequently referred to as transcription coupled repair (TCR) although the coupling mechanism in eukaryotes remains to be elucidated (for references and discussion see 2,3).

As an alternative or additional pathway, many organisms including yeast *Saccharomyces cerevisiae* 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 (10,11). More recently, (6-4) photolyases have been identified in *Drosophila* (12,13), *Xenopus laevis* and rattlesnakes (14) suggesting that photolyases are widespread. Homologue genes were found in humans (12,15), but photoreactivation activity has not been reproducibly demonstrated in human cells (15–17). Although photoreactivation is a major repair pathway and the enzymes and the reaction mechanism of photolyases have been characterized in detail (reviewed in ref. 10), it was not examined so far how photolyase repairs transcriptionally active genes or how it recognizes DNA-lesions when DNA is packaged in chromatin.

We have recently analysed photoreactivation in the URA3 and HIS3 genes of minichromosomes in yeast. Photoreactivation was found to be tightly modulated by chromatin structure. To our surprise, we noticed that photoreactivation was slower on the transcribed strands than on the non-transcribed strands, in contrast to NER (18). *Escherichia coli* RNA polymerase and mammalian RNA polymerase II are blocked at CPDs in the transcribed strand (19–21) and shield the CPD from recognition by photolyase *in vitro* (20). We therefore proposed that stalled RNA polymerase II might prevent accessibility of CPDs to photolyase *in vivo*. Here, we tested this hypothesis using the inducible GAL10 gene in yeast.

* To whom correspondence should be addressed. Tel: +41 1 633 3323; Fax: +41 1 633 1069; Email: thoma@cell.biol.ethz.ch

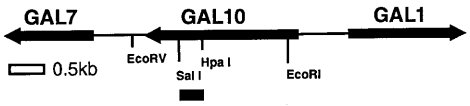

Figure 1. Map of the *GAL10* locus. Indicated are the GAL7, GAL1, GAL10 genes (arrows), relevant restriction sites (*Sal*I, *Hpa*I, *Eco*RI, *Eco*RV), the DNA segment used to generate strand specific probes (black bar), and a size marker (0.5 kb, open box). The map is derived from ref. 23.

MATERIALS AND METHODS

Yeast strains

W303-1a (*Mat***a**, *ade2-1, ura3-1, his3-11,15, trp1-1, leu2-3,112, can1-100*) was kindly provided by Dr R. Sternglanz. AMY3 (*Mat***a**, *ade2-1, ura3-1, his3-11,15, trp1-1, leu2-3,112, can1-100 rad1*Δ::*URA3*) was generated by deletion of part of the *RAD1* gene in W303-1a using a gene blaster construct (pR1.6, kindly provided by Dr L. Prakash). AMY3 exhibits a strong UV sensitivity typical for *rad1* strains (not shown).

Cultures and UV irradiation of yeast cells

Cells were grown in full media containing glucose (YPD) or galactose (YPG) (22) to a density of about 1×10^7 to 3×10^7 cells/ml, harvested, resuspended in minimal medium without amino acids to 3.5×10^7 cells/ml. Aliquots (250 ml) were transferred to plastic trays (22 cm × 31 cm) and irradiated at room temperature with 150 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 and the trays were placed on a metal cooling plate connected to a water bath. The temperature of the cell suspension during photoreactivation was ~23–26°C. Photoreactivation of 250–500 ml samples was done by using Sylvania Type F15 T8/BLB bulbs (peak emission at 375 nm) at 1.4 mW/cm^2 for 15–120 min. Samples (250 ml) were collected and chilled on ice. Cells were harvested by centrifugation and washed in 10 mM Tris–HCl, 1 mM EDTA (pH 8.0). Cells were converted to spheroplasts using Zymolyase and DNA was extracted following the QIAGEN Genomic Yeast DNA Isolation Protocol (QIAGEN Genomic DNA Handbook, September 1995).

Mapping of CPDs by indirect end labelling

DNA was cut with *Sal*I and *Eco*RI (Fig. 1) and repurified. Aliquots were incubated 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 or mock treated with the same buffer. The DNA was electrophoresed on 1.5% alkaline agarose gels, blotted to ZetaGT-membranes (BioRad), and hybridized to RNA-probes as described (6,18). Strand specific RNA probes were generated by *in vitro* transcription using a transcription kit (Stratagene) and appropriate DNA fragments (*Sal*I–*Hpa*I, Fig. 1) subcloned in a bluescript vector (Stratagene).

Quantification

The signals on the membranes were quantified using a PhosphorImager (Molecular Dynamics). In each lane, the signal in the intact restriction fragment was measured and divided by the signal of the whole lane (Figs 2 and 3) to give a signal normalized with respect to the overall DNA content in that lane [IRF(T4+); IRF(T4–)] (18). CPD content was calculated using the Poisson expression (5): –ln [IRF(T4+)/IRF(T4–)]. Initial damage (0 min repair) was set to 0% repair.

RESULTS

To address the question whether preferential photoreactivation of the non-transcribed strand (NTS) depends on transcription, photoreactivation was studied in the inducible GAL10 gene (Fig. 1) in the NER deficient strain AMY3 (*rad1*Δ) (Fig. 2). GAL10 is either heavily transcribed or repressed when yeast cells are grown in galactose or glucose, respectively (23,24). Cells were UV irradiated in suspension with 150 J/m^2. Photoreactivation was done by exposing the cell suspension to photoreactivating light for 15–120 min at temperatures between 23 and 26°C. To control the contribution of NER to repair, aliquots of the irradiated cell suspension were incubated in the dark. To measure CPDs, DNA was extracted, mock treated or treated with T4-endonuclease V (T4-endoV) which cuts at CPDs (25). The cutting sites were displayed in the transcribed region (excluding promoter and 3′-ends) by indirect end labelling (6) from the *Sal*I restriction site towards the *Eco*RI site (Fig. 1) using strand specific probes. In contrast to the most frequently used procedure developed by Mellon, Spivak and Hanawalt (5), the indirect end labelling procedure displays CPDs along the DNA-sequence and allows investigation of site specific repair if necessary (6,18). Non-irradiated DNA (UV–) and mock treated DNA (T4–) give rise to an intact restriction fragment (Figs 2 and 3, top bands). In contrast, T4-endoV treatment of damaged DNA (UV+, T4+) generates a smear with several diffuse bands and top bands of reduced intensities (compare +T4 lanes and –T4 lanes, Fig. 2). The diffuse bands generated by T4-endoV cutting represent the CPD distribution in pyrimidine rich regions from the 3′ end of the gene (bottom of the lanes) towards the 5′ end of the gene (*Eco*RI site, top band). Since the lesions are distributed over a large region, the smear and CPD bands are relatively weak, but can be accurately quantified using PhosphorImager (18). The initial damage generated in galactose and glucose was ~0.3 CPD/kb (compare lanes 5 and 6, Fig. 2). With increasing repair time, the CPD bands disappeared and the intensities of the intact *Sal*I–*Eco*RI fragments increased. CPDs were quantified and their removal was displayed as a function of the repair time (Fig. 4).

When the repair of the GAL10 gene was analysed in AMY3 (*rad1*Δ) grown in galactose, photoreactivation was fast on the NTS with >70% of CPDs removed in 15 min (Fig. 2B; Fig. 4A, white circles). In the TS, photoreactivation was slow with <40% of CPDs removed in 15 min (Fig. 2A; Fig. 4A, black circles). More than 90% of CPDs are removed in 2 h from the NTS, but only ~75% from the TS. This result substantiates our previous observation on the URA3 and HIS3 genes in a minichromosome (18) and demonstrates that preferential repair of the NTS by photolyase does not depend on whether a gene is located in the chromosome or in a plasmid. The same observation was made for NER (7). Repair in the absence of photoreactivating light was negligible (–PR, 120 min) as expected for a *rad1*Δ mutant. Although photoreactivation in the transcribed strand is slow when compared with the non-transcribed strand, it must be emphasised,

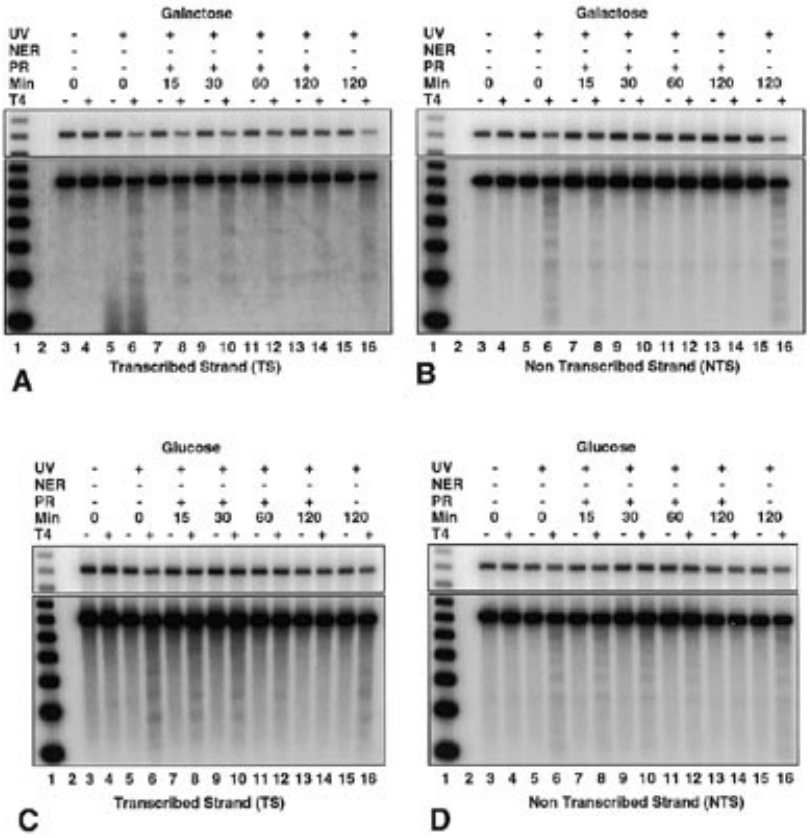

Figure 2. CPD repair in the GAL10 gene of AMY3 (*rad1*Δ). AMY3, which is deficient in NER (NER–), was grown in galactose (**A** and **B**) or glucose (**C** and **D**) where the GAL10 gene is transcribed or repressed, respectively. The cells were irradiated with ultraviolet light (150 J/m^2, UV+), exposed to photoreactivating light (PR+) for 15–120 min or kept in the dark (PR–). DNA was isolated, cut with T4-endoV (T4+) or mock treated (T4–), cut with *Eco*RI and *Sal*I, fractionated by alkaline agarose gel electrophoresis. The GAL10 specific regions were identified by blotting on membranes and hybridisation with strand specific probes (Fig. 1) for the transcribed strand (TS) and non-transcribed strand (NTS). Top panels are weak exposures of the top bands. Marker represents multiples of 256 bp (hybridized separately).

that it is as rapid as the 'fast' repair by transcription coupled NER (Fig. 3; Fig. 4C, black squares; see below).

When the experiment was repeated in glucose medium which represses transcription of the GAL10 gene by RNA polymerase II, photoreactivation was very similar on both strands (Fig. 2C and D; white and black circles in Fig. 4B). Hence, changing from a transcribed gene to a repressed gene results in a loss of strand specific repair by photolyase. These experiments show that the strand bias in photoreactivation depends on transcription by RNA polymerase II. Surprisingly, photoreactivation in the TS of the transcribed gene (Fig. 4A, black circles) was not only slower than in the NTS (white circles, Fig. 4A), but also slower than photoreactivation of both strands in the inactive gene (Fig. 4B, black and white circles). Thus, slow repair of the TS in galactose is consistent with an inhibition of photolyase accessibility by RNA polymerase II stalled at CPDs.

To address the role of photolyase and its contribution to repair in the presence of NER, photoreactivation experiments were performed with the W303-1a strain which is competent in NER (Fig. 3). In galactose and in the absence of photoreactivating light, the TS was more quickly repaired than the NTS (+NER –PR, Fig. 3A and B; Fig. 4C, black and white squares). The NTS and TS were repaired to ~20% and 80%, respectively, in 2 h. This preferential repair of the TS is characteristic for NER (frequently referred to as 'transcription coupled repair'). Most strikingly, however, when both pathways, photoreactivation and NER, were operational, both strands were repaired very rapidly and at similar rates (+PR +NER, Fig. 3A and B; Fig. 4C, white and black circles). This leads to repair of 70–80% of lesions in 15 min and ~90% in 1h. Repair of the TS by NER and photolyase (Fig. 4C, black circles) is dramatically enhanced compared with repair by NER alone (Fig. 4C, black squares) and it is also more quickly than photoreactivation alone (Fig. 4A, black circles). This indicates a relief of inhibition of photoreactivation by a functional NER and, in this sense, suggests a promoting role of NER for photorepair.

Combined NER and photoreactivation of the NTS (Fig. 4C, white circles) is dramatically enhanced compared with NER alone (Fig. 4C, white squares), but it reaches similar levels to those obtained by photoreactivation alone (Fig. 4A, white circles). Hence, an intact NER pathway appears to have no obvious effect on photoreactivation of the NTS. In conclusion, the similar repair kinetics of the NTS and TS observed in RAD1 cells

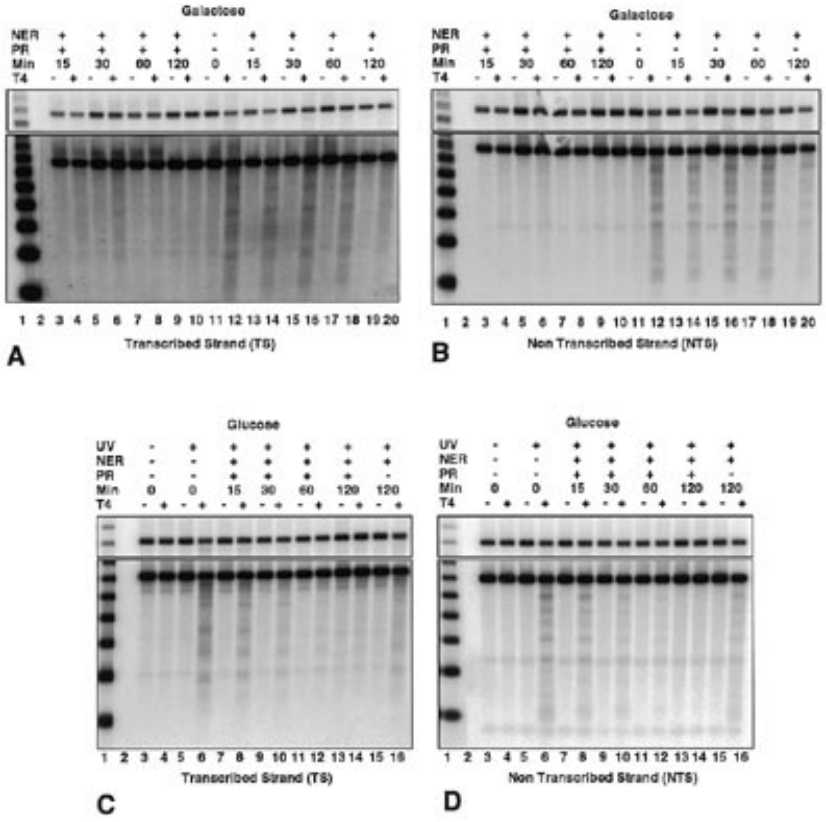

Figure 3. CPD repair in the GAL10 gene of W303-1a (*RAD1*). W303-1a, which is proficient in NER (NER+) and photoreactivation, was grown in galactose (**A** and **B**) or glucose (**C** and **D**), irradiated with 150 J/m^2, and exposed to photoreactivating light (PR+) or kept in the dark (PR–). CPDs were analysed on the transcribed strand (TS) and non-transcribed strand (NTS) as described in Figure 2. Marker represents multiples of 256 bp (hybridized separately).

demonstrate that photoreactivation on the NTS can complement preferential repair of CPDs by NER on the TS.

When the experiment with the W303-1a strain was repeated in glucose medium, again, both strands were repaired at similar rates (Fig. 3C and D; Fig. 4D, black and white circles). Hence, irrespective of transcription, both strands are repaired at similar rates and more quickly than by NER alone.

DISCUSSION

Inhibition of photoreactivation by stalled RNA polymerases

Why is photoreactivation slow in the transcribed strand? The comparison between photoreactivation of cells grown in glucose and galactose demonstrates that preferential repair of the NTS and slow repair of the TS depend on transcription. *In vitro* experiments showed that *E.coli* polymerase (26) and human RNA polymerase II are blocked by CPDs on the transcribed strand (20,21). Blocked human RNA polymerase covers ~40 nt around the dimer in a nearly symmetrical way (21) and prevents access of *E.coli* photolyase to the DNA lesion (20) but has no effect on excision repair (21). Hence, slow photorepair of the TS of transcribed genes *in vivo* is most likely due to RNA polymerase II transcription complexes which are stalled at CPDs and inhibit access to photolyase. Repression of transcription (in the *rad1*Δ strain) enhances photoreactivation on the transcribed strand which is consistent with a relief of inhibition. The inhibition, however, is not complete, which allows photorepair of 50% of CPDs within 50–60 min. (AMY3, Fig. 4A). Let's make the following assumptions. (i) Each CPD in the TS blocks an RNA polymerase. (ii) There is sufficient photolyase in a cell to rapidly recognize the CPDs as soon as they are released from the stalled complex (by mechanisms described below). This assumption is justified, since CPDs are repaired extremely fast when located in 'open' chromatin (promoter regions) or linker DNA [repair in <15 min (18)]. (iii) Reloading of RNA polymerases on the damage is slower than photoreactivation. Considering these points and the photorepair curve of the transcribed strand (Fig. 4A, dark circles), the time at which half of the CPDs are repaired reflects the half-life of a stalled RNA polymerase II complex, which is in the range of 50–60 min. This result is probably an overestimation, since the half-life could be shorter, if reloading of polymerases on the CPD is fast. Photoreactivation in the TS of the active gene is even slower than repair in the inactive gene, where DNA is folded in an inactive chromatin structure. Hence, inhibition of photorepair by stalled polymerases seems to be stronger than inhibition of repair by nucleosomes. In this perspective, a half-life of stalled polymerases of 50–60 min appears to be surprisingly long. However, it is remarkably shorter

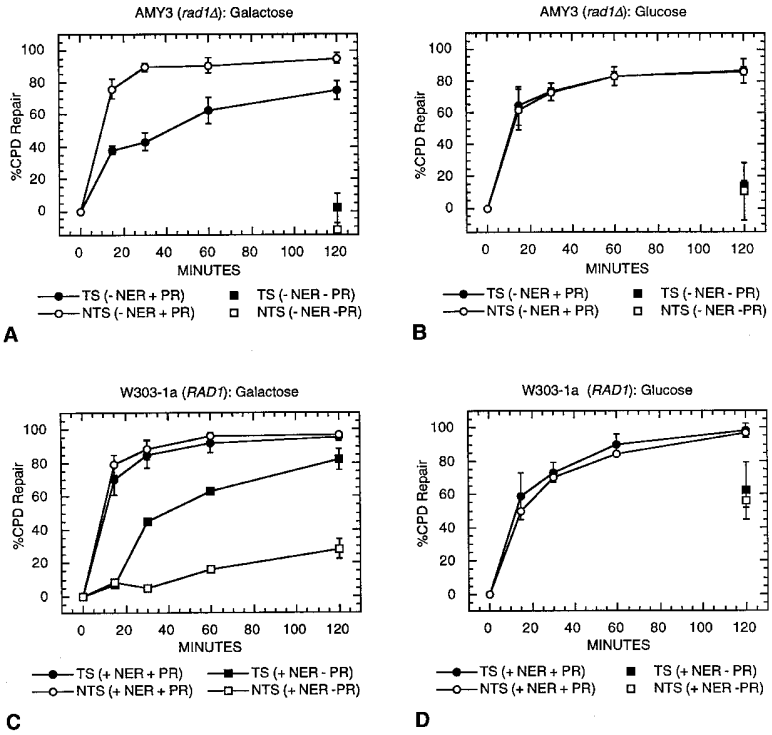

Figure 4. Summary of CPD repair in AMY3 and W303-1a. (**A**) AMY3 (*rad1*Δ) in galactose. (**B**) AMY3 (*rad1*Δ) in glucose. (**C**) W303-1A (*RAD1*) in galactose. (**D**) W303-1A (*RAD1*) in glucose. Data are given as an average with standard deviations from three to eight gels (C, two experiments; A, B and D, one experiment). Note, that the repair curves of TS and NTS in glucose are very similar (B and D) as well as the curves of NTS in A and C. The NER data of 15–60 min in (C) (black and open squares) are from one gel each (Fig. 3A and B). [One data set in (B) was obtained from analysis of the *Eco*RV–*Eco*RI fragment (Fig. 1), but the result was indistinguishable from the analyses of the shorter fragment (*Sal*I–*Eco*RI).]

than the half-life of a human RNA polymerase II stalled at a CPD *in vitro* [~20 h (21)]. This indicates to us, that the *in vivo* situation is more dynamic. We do not yet know how photolyase finally gets access to the CPDs *in vivo*. One possibility is that RNA polymerase II is released from the template DNA. Alternatively, it is possible that RNA polymerase II moves backwards with the help of RNA hydrolyzing factors (TFIIS of humans or yeast, GreA and GreB of *E.coli*; reviewed in ref. 27) and retracts from the damaged site (28,29).

Repair of the NTS: chromatin effects?

We noticed that, in particular in the first 30 min, the NTS in the active gene was always more quickly repaired than either strand in the inactive gene. This effect was observed for photoreactivation alone [AMY3 (*rad1*Δ), Fig. 4A and B] as well as for the combined action of NER and photolyase [W303-1a (*RAD1*), Fig. 4C and D]. Hence, this effect is either independent of an operational NER pathway or the contribution of the NER pathway is too small to be detected under those conditions. The enhanced repair of the NTS in galactose can be explained by an altered chromatin structure in the transcribed GAL10 gene. Analysis of chromatin structures in the GAL1, GAL10 and in an artificial GAL-URARIB gene revealed positioned nucleosomes in the inactive genes and rearranged nucleosomes on the transcribed genes. The rearrangement was interpreted as a dissociation of nucleosomes in front of the polymerase and a rapid reassembly behind it (24,30). Whether this altered chromatin structure of the transcribed state persists after damage induction is unknown and cannot be experimentally tested, since the lesions are distributed all over the gene. However, the unexpected fast repair of the NTS argues in favour of an altered structure with rearranged nucleosomes. Additionally, we consider that transcription is not completely stopped, since the initial damage was only ~0.5 CPD/gene and transcription resumes with increasing repair time. *In vitro* experiments have shown that lesions in the non-transcribed strand can be passed by RNA polymerase (20,26). Therefore ongoing transcription would transiently disrupt the nucleosome structure and, hence, enhance the accessibility of CPDs to photolyase.

Complementary roles for NER and photolyase in gene repair

We have previously shown that photolyase repairs open promoters of active genes within a few minutes, more quickly than NER, hence demonstrating a role for photolyase in promoter repair (18). Efficient repair of DNA lesions is required to reduce the risk of mutagenesis, cell death and cancer (31). In the genes, which are actively transcribed by RNA polymerase II, NER preferentially repairs the transcribed strand (Fig. 4C), but leaves the NTS slowly repaired. Photolyase takes care of CPDs in the NTS, but repair in the TS is slower. It is the combination of both repair pathways which establishes efficient repair of both strands and, hence, reduces the risk of mutations in active genes (Fig. 4C). In the repressed GAL10 gene, photolyase provides efficient

repair even in the absence of NER and seems to be the major pathway to remove CPDs. In conclusion, the combination of both repair pathways ensures efficient repair of the genome and active genes and prevents the risk of sunlight induced mutagenesis.

ACKNOWLEDGEMENTS

We thank Dr A.Aboussekhra for discussions, Dr R.S.Lloyd for T4-endoV, Dr R.Sternglanz for a yeast strain, Dr L.Prakash for plasmid DNA, Dr C.Weissmann for access to the Phosphor-Imager, and Drs T.Koller and U.Suter for continuous support. This work was supported by grants from the Swiss National Science Foundation and by the Swiss Federal Institute of Technology Zürich (ETH) (to FT).

REFERENCES

1 Friedberg, E. C., Walker, G. C. and Siede, W. (1995) *DNA Repair and Mutagenesis.* ASM Press, Washington, DC.
2 Friedberg, E. C. (1996) *Annu. Rev. Biochem.*, **65**, 15–42.
3 Sancar, A. (1996) *Annu. Rev. Biochem.*, **65**, 43–81.
4 Wood, R. D. (1996) *Annu. Rev. Biochem.*, **65**, 135–167.
5 Mellon, I., Spivak, G. and Hanawalt, P. C. (1987) *Cell*, **51**, 241–249.
6 Smerdon, M. J. and Thoma, F. (1990) *Cell*, **61**, 675–684.
7 Sweder, K. S. and Hanawalt, P. C. (1992) *Proc. Natl. Acad. Sci. USA*, **89**, 10696–10700.
8 Leadon, S. A. and Lawrence, D. A. (1992) *J. Biol. Chem.*, **267**, 23175–23182.
9 Verhage, R., Zeeman, A. M., de Groot, N., Gleig, F., Bang, D. D., van de Putte, P. and Brouwer, J. (1994) *Mol. Cell. Biol.*, **14**, 6135–6142.
10 Sancar, A. (1996) *Science*, **272**, 48–49.
11 Yasui, A., Eker, A. P. M., Yasuhira, S., Yajima, H., Kobayashi, T., Takao, M. and Oikawa, A. (1994) *EMBO J.*, **13**, 6143–6151.
12 Todo, T., Ryo, H., Yamamoto, K., Toh, H., Inui, T., Ayaki, H., Nomura, T. and Ikenaga, M. (1996) *Science*, **272**, 109–112.
13 Todo, T., Takemori, H., Ryo, H., Ihara, M., Matsunaga, T., Nikaido, O., Sato, K. and Nomura, T. (1993) *Nature*, **361**, 371–374.
14 Kim, S. T., Malhotra, K., Taylor, J. S. and Sancar, A. (1996) *Photochem. Photobiol.*, **63**, 292–295.
15 Hsu, D. S., Zhao, X. D., Zhao, S. Y., Kazantsev, A., Wang, R. P., Todo, T., Wei, Y. F. and Sancar, A. (1996) *Biochemistry*, **35**, 13871–13877.
16 Li, Y. F., Kim, S. T. and Sancar, A. (1993) *Proc. Natl. Acad. Sci. USA*, **90**, 4389–4393.
17 Sutherland, B. M. and Bennett, P. V. (1995) *Proc. Natl. Acad. Sci. USA*, **92**, 9732–9736.
18 Suter, B., Livingstone-Zatchej, M. and Thoma, F. (1997) *EMBO J.*, **16**, 2150–2160.
19 Selby, C. P. and Sancar, A. (1993) *J. Bacteriol.*, **175**, 7509–7514.
20 Donahue, B. A., Yin, S., Taylor, J. S., Reines, D. and Hanawalt, P. C. (1994) *Proc. Natl. Acad. Sci. USA*, **91**, 8502–8506.
21 Selby, C. P., Drapkin, R., Reinberg, D. and Sancar, A. (1997) *Nucleic Acids Res.*, **25**, 787–793.
22 Sherman, F., Fink, G. R. and Hicks, J. B. (1981) *Methods in Yeast Genetics.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
23 St. John, T. P. and Davis, R. W. (1981) *J. Mol. Biol.*, **152**, 285–315.
24 Cavalli, G. and Thoma, F. (1993) *EMBO J.*, **12**, 4603–4613.
25 Gordon, L. K. and Haseltine, W. A. (1980) *J. Biol. Chem.*, **255**, 12047–12050.
26 Selby, C. P. and Sancar, A. (1990) *J. Biol. Chem.*, **265**, 21330–21336.
27 Kassavetis, G. A. and Geiduschek, E. P. (1993) *Science*, **259**, 944–945.
28 Hanawalt, P. and Mellon, I. (1993) *Curr. Biol.*, **3**, 67–69.
29 Selby, C. P. and Sancar, A. (1994) *Microbiol Rev.*, **58**, 317–329.
30 Cavalli, G., Bachmann, D. and Thoma, F. (1996) *EMBO J.*, **15**, 590–597.
31 Tornaletti, S. and Pfeifer, G. P. (1994) *Science*, **263**, 1436–1438.