Doctoral Thesis

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Repair of UV Induced DNA-Lesions by Photolyase in Yeast *S. cerevisiae* at the Chromatin Level

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For the degree of Doctor of Natural Sciences

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1. Summary

DNA lesions which are induced by the ultraviolet (UV) component of solar radiation interfere with biological processes like transcription and replication and, if not repaired, can lead to cell death, mutations and cancer. Cyclobutane pyrimidine dimers (CPDs) and, to a lesser extent, 6-4 pyrimidine pyrimidone photoproducts [(6-4)PPs], are the main classes of stable UV lesions. In many organisms, UV lesions are either removed by nucleotide excision repair (NER) or by photoreactivation. NER involves many different enzymes, exerting DNA damage recognition, excision of an oligonucleotide containing the lesion, DNA synthesis and ligation. NER is faster in the transcribed strand of active genes than in the non-transcribed strand and in inactive genes (transcription coupled repair). Photoreactivation is a process where a single enzyme, DNA photolyase, recognises a UV lesion and exerts repair using energy of blue light. Photolyases specific for CPDs are found in numerous microorganisms including yeast S. cerevisiae, in plants and also in animals including vertebrates. More recently, photolyases specific for (6-4)PPs were identified in several species. In eukaryotic cells, DNA is folded into nucleosomes and higher order chromatin structures. Regulatory regions are associated with regulatory proteins and active genes are occupied by transcribing polymerases. Modulation by chromatin structures was shown for the NER mechanism. The aim was to study how CPD-photolyase can recognise DNA lesions in chromatin and genes.

In the first part of the thesis (published by Suter, B., Livingstone, Z.M. and Thoma, F. (1997). EMBO J. 16. 2150-60), we analysed photoreactivation in the yeast minichromosomes YRpTRURAP and YRpCS1 by the indirect endlabeling technique, which allowed direct comparison with the chromatin structure. We found a distinct modulation of DNA-repair by photolyase in chromatin. Fast repair was found in nucleosome free regions and in linker DNA, whereas nucleosomal DNA was repaired more slowly. Fast repair of CPDs by photolyase in open promoters supports an important biological role in the restoration of these regulatory elements. In the transcribed URA3 and HIS3 genes, photoreactivation was fast in the non-transcribed strand and slow in the transcribed strand, demonstrating preferential repair of the non-transcribed strand. Analysis of photoreactivation in the induced and repressed chromosomal GAL10 gene demonstrated that this strand-specificity is dependent on transcription (Livingstone-Zatchej, M., Meier, A., Suter, B. and Thoma, F. (1997). Nucleic Acids Res 25. 3795-3800). Inhibition of photoreactivation in the transcribed strand can be explained by RNA-polymerases which are stalled at CPD lesions, thereby preventing access for photolyase. Fast repair in the non-transcribed strand by photolyase complements fast repair of the transcribed strand by NER, suggesting that the two repair pathways exert complementary roles in repair of active genes.
1. Summary

The minichromosomes YRpTRURAP and YRpCS1 contain the ARS1 origin of replication, which is nucleosome free but associated with protein complexes that regulate the initiation of DNA replication in a cell cycle dependent manner. In the second part of my thesis, I analysed the role of photolyase in repair of this regulatory region and its possible interference with DNA binding complexes. DNA damage formation and repair in ARS1 were analysed at nucleotide resolution using the primer extension technique and compared with the data for NER (R. Wellinger, ETH-thesis no. 11931). CPD repair by photolyase in the ARS1 region was much faster than repair by NER, suggesting a predominant role of photoreactivation for the removal of CPDs in ARS1. Furthermore, photoreactivation was heterogeneous within the nucleosome free region indicating that photoreactivation is affected by bound proteins. NER shows rather homogeneous repair at different sites. The different modulation of both mechanisms in ARS1 suggests that the sequences associated with factors are differently accessible to the single enzyme photolyase and to the complex NER mechanism. In YRpCS1 where the ARS1 region is weakly transcribed from the adjacent PET56 promoter, modulation of photoreactivation is altered compared with YRpTRURAP, where ARS1 is not transcribed. We suggest that ARS1 binding proteins may be transiently displaced by transcribing polymerases or by an altered chromatin structure.

In the third part of the thesis, I analysed photoreactivation in a yeast promoter (URA3) at nucleotide resolution. CPD-repair by photolyase shows fast repair rates at all sites in URA3 promoter region including sites for transcription factor binding and T-tracts [poly(dA-dT) sequences]. However, photoreactivation in the nucleosome positions flanking the URA3 promoter showed a distinct modulation. Repair of lesions in the internal nucleosomal region was slower compared with lesions close to the edge of the nucleosome. In contrast to NER (Wellinger, R.E. and Thoma, F. (1997). EMBO J 16. 5046-5056), photoreactivation is heterogeneous in the transcribed strand of the URA3 gene. We suppose that photoreactivation in the transcribed strand is affected by both transcription and chromatin structure.

Besides photoreactivation, I investigated the induction of UV damage in poly(dA-dT) sequences. Poly(dA-dT) sequences are upstream elements in constitutive yeast promoters and may exert a role in transcription by their structural properties. In vitro, T-tracts adopt an unusual structure different from normal B-DNA, which is reflected in the pattern of UV damage formation in DNA. To test whether T-tract structure exists in cellular chromatin, we compared UV damage formation in vitro and in vivo in poly(dA-dT) sequences of constitutive promoters (URA3 and DED1). Using the primer extension technique, UV damage formation at each dipyrimidine site within poly(dA-dT) sequences could be determined. UV damage patterns generated in the poly(dA-dT) tracts of the URA3 and DED1 promoters in vivo and in naked DNA were almost identical. However, when the DNA was irradiated in the presence of 50% DMSO, which disrupts T-tract structure, the pattern of damage formation was altered. We conclude that poly(dA-dT) sequences adopt T-tract structures in living cells. Assuming that the T-tract structure has
specific functions, it is conceivable that these functions will be affected if the structure is altered by the induction of DNA damage. Efficient photoreactivation will restore the structure of T-tracts and also their specific function. However, we found that not all T-tracts adopt the typical T-tract structure. The UV damage pattern in one T-tract of the *DED1* poly(dA-dT) sequence cannot be referred to a typical T-tract structure.
1. Zusammenfassung


Die beiden Minichromosomen YRpTRURAP und YRpCS1 enthalten als Replikationsursprung die ARS1 Sequenz. Diese ist, zwar frei von Nukleosomen, aber mit Proteinkomplexen assoziiert, welche, abhängig vom Zellzyklus, die DNA Replikation in die Wege leiten. Im zweiten Teil meiner Arbeit untersuchte ich die Rolle der Photolyase in der Reparatur dieser regulatorischen Region und ihre mögliche Behinderung durch an DNA bindende Komplexe. Dazu wurde die Bildung und die Reparatur der Schäden in ARS1 auf Nukleotidauflösung unter Verwendung der 'Primer Extension' Technik untersucht und mit den entsprechenden Daten für den NER verglichen (R. Wellinger, ETH-Diss Nr. 11931). CPDs in der ARS1 Region wurden viel schneller durch die Photolyase als durch NER repariert, was darauf hindeutet, dass Photoreaktivierung in der Reparatur von CPDs in der ARS1 Region eine vorherrschende Stellung einnimmt. Ferner war die Photoreaktivierung innerhalb der nukleosomenfreien Region heterogen, was auf eine Auswirkung von gebundenen Proteinen hindeutet. NER wies dagegen eine eher homogene Reparatur an verschiedenen Stellen auf. Die unterschiedliche Modulation beider Mechanismen in ARS1 legt nahe, dass die mit Faktoren assoziierte Sequenzen sich in ihrer Zugänglichkeit für die Photolyase und den komplexen NER-Mechanismus unterscheiden. In YRpCS1, wo die ARS1 Region vom anliegenden PET56 Promotor aus schwach transkribiert wird, war die Photoreaktivierung anders moduliert, als in YRpTRURAP, wo ARS1 nicht transkribiert wird. Wir vermuten, dass Proteine, welche an ARS1 binden, durch transkribierende Polymerasen vorübergehend entfernt werden könnten.