Transcription Affects the Rate but Not the Extent of Repair of Cyclobutane Pyrimidine Dimers in the Human Adenosine Deaminase Gene*

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Ultraviolet light (UV) induces two major DNA adducts, the cyclobutane pyrimidine dimer and the pyrimidine (6–4) pyrimidone photoprodut. Both types of damage are removed by DNA excision repair. Especially in the case of cyclobutane pyrimidine dimers, ample evidence has been presented that repair of this lesion occurs nonrandomly in the genome.

Preferential repair of dimers in transcriptionally active genes has now been well documented in a wide array of organisms ranging from Escherichia coli (Mellon and Hanawalt, 1989) and the lower eukaryote Saccharomyces cerevisiae (Terleth et al., 1989, 1990) to rodent (Bohr et al., 1985; Madhani et al., 1986) and human cells (Mellon et al., 1986; Venema et al., 1990a). An exception is found in Drosophila embryonal cell lines which exhibit efficient repair of both active and inactive loci (De Cock et al., 1991). Efficient removal of DNA damage from transcribed sequences is presumed to enhance cellular survival by enabling the cell to express essential genes before removal of the bulk of the damage. This concept is supported by the finding that Cockayne’s syndrome cells are UV-sensitive due to a deficiency in preferential repair of active genes (Venema et al., 1990b).

The biological advantage of preferential repair is evident, but the mechanism by which it occurs is still largely unknown. Initially, the presumed explanation for the accelerated repair of active genes was the open chromatin configuration of such genes which renders them more accessible to DNA repair enzymes. This model was challenged by the observation of strand-specific repair, i.e. within active genes the transcribed strand was repaired much faster and more efficiently than the nontranscribed strand (Mellon et al., 1987). The most dramatic effect was seen in Chinese hamster (Mellon et al., 1987) and xeroderma pigmentosum complementation group C cells (Venema et al., 1991). Both cell types exhibited efficient repair of the transcribed strand, whereas virtually no dimers were removed from the nontranscribed strand. These results appeared to favor a model in which the transcription process itself is the primary target for repair enzymes. This was supported by the finding of strand-specific repair in E. coli (Mellon and Hanawalt, 1989) which does not possess a “eukaryote-like” chromatin structure. In normal human cells, the difference in repair rates of both strands was only 2–3-fold. The nontranscribed strand was repaired at a rate and to an extent similar to that of the genome overall, suggesting that preferential repair of active genes was due to selective repair of the transcribed strand. These cells therefore also performed efficient repair of nontranscribed sequences.

To further identify the factors which are involved in preferential repair of active genes, we investigated removal of cyclobutane pyrimidine dimers in a cell line derived from a patient suffering from severe combined immunodeficiency (SCID). The disease was caused by a deletion in the adeno-
sine deaminase (ADA) gene in both alleles. The deletion covered the promoter and the first exon of the gene. It was previously shown that this mutation resulted in a true null allele having no detectable transcription (Berkvens et al., 1987). This enabled us to analyze removal of dimers in two ways: first, the rate and extent of repair of the nontranscribed ADA gene were compared with those of the wild type ADA gene. Secondly, we compared dimer removal in the same cell line between the nontranscribed ADA gene and two repressed loci, 754 and coagulation factor IX.

**EXPERIMENTAL PROCEDURES**

**Cell Lines and Culture Conditions**—The cell line used in this study was derived from a female SCID patient. Fibroblasts were cultured in Ham's F10 medium (without hypoxanthine and thymidine) supplemented with 15% fetal calf serum and antibiotics in a humidified 2.5% CO₂ atmosphere. Routinely, cells were grown to confluence and then split 1:3. To prelabel the cells 0.1 μCi/ml [³H]thymidine (100 mCi/mmol) was added to the medium, and the cells were cultured for 3 days. The medium was then replaced by fresh label-free medium and cell growth was continued to confluence. Prior to UV irradiation, prelabeled cells were seeded in 94-mm petri dishes and cultured for 10 days with regular medium changes to reach stationary phase.

**Analysis of Cyclobutane Pyrimidine Dimer Removal**—Removal of cyclobutane pyrimidine dimers from specific DNA sequences was analyzed as described previously (Venema et al., 1990a) except for one modification, which is presented below. Cells were irradiated with 10 J/m² UV (254 nm) and incubated for up to 24 h. After incubation, cells were lysed and high molecular weight DNA was purified by phenol and chloroform extractions followed by ethanol precipitation.

The DNA was digested with either BclI, EcoRI, or KpnI (Pharmacia LKB Biotechnology Inc.) and purified by phenol and chloroform extraction, followed by concentration with n-butanol and ethanol precipitation (since the cells used in this study were in the confluent G₀ state, it is not necessary to density label the DNA with bromodeoxyuridine in order to separate replicated DNA by CsCl density centrifugation). Equal amounts of DNA were either treated or mock-treated with the dimer-specific enzyme T4 endonuclease V (Nakabeppu et al., 1982) and electrophoresed in parallel on an alkaline agarose gel. The DNA was transferred to Hybond-N° membranes (Amersham) by Southern blotting and hybridized with ³²P-labeled gene-specific probes. After autoradiography, films were scanned using a Bio-Rad video densitometer. T4 endonuclease V causes nicking of fragments which contain cyclobutane pyrimidine dimers. The number of dimers present in a fragment at each time point was calculated from the amount of fragments not nicked by T4 endonuclease V, using the Poisson expression.

**DNA Probes**—The Factor IX probe was a genomic 440-bp HindIII fragment covering exons 2 and 3 (Anson et al., 1984). The 754 probe was a genomic 2.0-kb HindIII fragment (Hofker et al., 1986). Both probes were prepared by random primer extension (Feinberg and Vogelstein, 1983).

**Preparation of Strand-specific Probes**—Three ADA cDNA PstI fragments (Berkvens et al., 1987) and a 690-bp HindIII-EcoRI genomic fragment from intron V of the DHFR gene (Will and Dornick, 1986) were subcloned in SsEV/U19 vectors (Biermat et al., 1989). The orientation of all DNA fragments was confirmed by sequence analysis. The SSEV vector contains a polylinker which is able to form a stem-loop structure in the single-stranded form. This stem-loop structure contains an EcoRI site and thereby allows for the formation of dimers present in a fragment at each time point was calculated from the amount of fragments not nicked by T4 endonuclease V, using the Poisson expression.

**RESULTS**

**Transcriptional Status of the ADA Gene**—The cell line used in this study was derived from a female SCID patient and was homozygous for a deletion of 3.2 kb in the 5' end of the ADA gene which removed the promoter and the first exon (Berkvens et al., 1987). A partial restriction map is shown in Fig. 1 which also shows the location and extent of the deletion. The deletion runs from position -1480 to +1770 relative to the transcriptional start site. This deletion results in the absence of any detectable ADA-specific RNA detected in blood cells from the patient. When repair was measured with probes recognizing the template (32) or nontemplate strand (31) of the 20.0-kb ADA BclI fragment, respectively, DNA was isolated from cells at various times after irradiation with 10 J/m² UV and treated (+) or not (-) with the dimer-specific enzyme T4 endonuclease V. The presence of cyclobutane pyrimidine dimers results in a decrease in the amount of full length fragments. Repair is detected as a reappearance of the full size band.

**Dimer Removal in the ADA Gene**—We wanted to establish the effect of the absence of transcription on the efficiency and strand specificity of dimer removal from the ADA gene. This was measured using T4 endonuclease V to specifically detect cyclobutane pyrimidine dimers. Digestion of DNA from UV-irradiated cells with this enzyme results in a single-stranded nick at the site of a dimer. This degradation is visualized on a denaturing agarose gel as a decrease in the amount of full-length DNA. Removal of dimers will result in less degradation of the T4 endonuclease V-treated DNA. Repair in specific DNA fragments can be analyzed by Southern blotting and hybridization with a gene-specific probe.

The results of such an analysis for the ADA BclI fragment are shown in Fig. 2. When repair was measured with probes recognizing the template and nontemplate strand, we observed efficient repair of dimers in both strands. Within 24 h after UV treatment, the band in the lane containing T4 endonuclease V treated DNA has almost returned to the density of the band in the nontreated lane. The results were quantified by densitometry scanning of the autoradiograms (Fig. 3). Both the template and the nontemplate strand are...
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Fig. 3. Repair in the ADA BclI fragment. The results of autoradiograms such as the one shown in Fig. 2 were quantified by densitometry and the dimer frequency was calculated from the relative band densities in the lanes containing treated and nontreated DNA. The percentage of repair was then calculated from the dimer frequency at each time point. ○, template strand; □, nontemplate strand. The values are the average of three independent experiments. The standard error is indicated.

Fig. 4. Removal of cyclobutane pyrimidine dimers from the 18.5-kb ADA EcoRI fragment. Data were obtained as described in Fig. 3. ○, ADA template strand; □, non-ADA template strand.

Fig. 5. Dimer removal in the 20-kb DHFR KpnI fragment. ■, transcribed strand; □, nontranscribed strand.

Fig. 6. Removal of cyclobutane pyrimidine dimers from two X-chromosomal loci. Repair was measured in a 16.7-kb KpnI fragment of the coagulation factor IX gene (▼) and in a 14-kb EcoRI fragment of the 754 locus (▲).

Fig. 7. Time after UV (h)

DISCUSSION

The finding of preferential repair of transcribed genes and (later on) the selective repair of the transcribed strand has posed questions about the mechanism of this repair. Data...
obtained with *E. coli* and Chinese hamster cells seemed to indicate that the transcription machinery itself was somehow involved in DNA repair or perhaps in the targeting of repair enzymes. Human repair deficient XP-C cells only repair the transcribed strand of active genes, indicating that also in human cells repair of the transcribed strand is (at least partly) independent of repair of the bulk of the genome (Venema et al., 1991). In contrast, Cockayne’s syndrome (CS) cells are deficient in preferential repair of active genes. The transcribed and nontranscribed strand of active genes are repaired to the same extent, which is characteristic of that of X-chromosomal, inactive genes.² In CS cells, the repair level of active genes appears to drop to a level which is even lower than that observed in the nontranscribed strand of normal cells.

A possible way to elucidate the role of transcription in preferential DNA repair would be to analyze repair in the presence of transcription inhibitors. Indeed, recently Leadon et al. (1990) found an increase in repair in the transcribed strand of active genes, indicating that also in CS cells preferential repair of the transcribed strand in an active gene is important to compare the repair data found for the ADA gene with those observed in two inactive, tissue-specific genes. It was shown that repair in the ADA EcoRI fragment was measured in two restriction fragments together encompassing over 38 kb of DNA. It was shown that repair of the nontranscribed ADA gene is substantial, with about 80% of the dimers removed in 24 h. However, the initial rapid repair associated with the transcribed strand in the wild type ADA gene is absent. Instead, almost no repair is observed at early times after UV irradiation. When these results are compared to those obtained for the normal ADA gene (Venema et al., 1991), it can be concluded that the repair level of both strands in the absence of transcription has dropped to that seen for the nontranscribed strand in the normal ADA gene. The same slow repair kinetics are found at early times after UV irradiation, although in this study the repair levels after 8 h seem to be somewhat higher. In the EcoRI fragment, partly containing 3′ flanking sequences, there appears to be strand specificity toward the complementary strand, which is likely to be caused by a convergent transcription unit (Lattier et al., 1989).

As a control, dimer removal was also analyzed in the DHFR gene. It was found that preferential repair of the transcribed strand occurred in an internal KpnI fragment. At early times after treatment, repair in this strand is clearly faster than that of the nontranscribed strand. This result is in agreement with previous data obtained with another human cell line (Venema et al., 1991). The repair rate for the DHFR gene is slower than that of the normal ADA gene. This could be due to the fact that repair in the DHFR gene was measured in a single repair experiment which gave somewhat lower repair values. When repair in the ADA EcoRI fragment was measured in the same experiment, the repair values were comparable to those obtained for the DHFR gene. The final data for the ADA gene, however, is the average of three independent experiments. Based on these results, we conclude that the cell line used in this study is capable of performing preferential repair of the transcribed strand in an active gene. It was also important to compare the repair data found for the ADA gene with those observed in two inactive, tissue-specific genes. The results indicate that the extent of dimer removal in the

² J. Venema, A. van Hoffen, A. T. Natarajan, A. A. van Zeeland, and L. H. P. Mullenders, unpublished results.

³ R. J. Sakkers, personal communication.
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