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Recruitment of damaged DNA to the nuclear matrix in hamster cells following ultraviolet irradiation

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ABSTRACT

We examined the relationship between the nuclear matrix and DNA in the dihydrofolate reductase domain following irradiation of Chinese hamster cells with UV light. The fraction of matrix-bound DNA increased in transcribed and non-transcribed regions during a 3 h period after irradiation. However, no increase was observed with excision repair-deficient cells mutant for the ERCC1 gene. The major UV-induced lesion, the cyclobutane pyrimidine dimer, increased in frequency in the matrix-bound DNA 1 h after irradiation, in both transcribed and non-transcribed regions, but decreased subsequently. This phenomenon was also lacking in excision repair-deficient cells. These data demonstrate that recruitment of lesion-containing DNA to the nuclear matrix occurs following UV irradiation and suggest that this recruitment is dependent upon nucleotide excision repair. This is consistent with the concept of a 'repair factory' residing on the nuclear matrix at which excision repair occurs.

INTRODUCTION

DNA within the interphase nucleus is complexed with histones and other proteins in a highly compacted form known as chromatin. Also found within the nucleus is an insoluble three-dimensional network of proteinaceous, non-histone fibers called the nucleoskeleton. The nucleoskeleton is thought to play an architectural role in the nucleus by organizing higher order chromatin structure and is often referred to as the nuclear matrix or scaffold. It is now clear that many enzymatic functions affecting nucleic acids occur on the nucleoskeleton, including DNA replication, transcription and RNA splicing. Current models for the nucleus integrate the structural and functional properties of chromatin and the nucleoskeleton in a coordinated fashion (1–4).

A common approach for studying specific interactions between chromatin and the nucleoskeleton is to cleave the DNA with a nuclease, remove the bulk of DNA fragments from the insoluble skeleton and examine the properties of the DNA fraction that remains. One method involves extracting nuclei in a high salt solution, which removes the majority of histones and other soluble proteins from chromatin (5). The DNA may then be easily digested with a nuclease to generate a soluble fraction and a small, insoluble portion that sediments with this salt-extracted nucleoskeleton, termed the 'nuclear matrix' at this stage. An alternate approach is to extract nuclei in a hypotonic solution containing the detergent lithium diiodosalicylate (LIS) to remove histones and other proteins from the DNA (6). The DNA is then cleaved with a nuclease and a small fraction associated with the detergent-extracted nucleoskeleton, called a 'scaffold', is isolated. Both methods use non-physiological ionic conditions, so there is a possibility that DNA–nucleoskeleton interactions may be artifactually created or destroyed by the extraction procedure (7).

To reduce the potential for artifacts, a method has been developed to study nuclei under physiological salt conditions (8). Nuclei are prepared in physiological buffer with agarose beads, which prevents their aggregation and protects the chromatin from shearing. The DNA may then be cleaved with nuclease, albeit inefficiently, and the fraction not attached to the nucleoskeleton electroeluted in physiological buffer. Using this 'physiological salt' method, most of the conclusions drawn from studies of high salt nuclear matrices have been confirmed. DNA replicated during S phase is associated with the high salt nuclear matrix (9,10), the physiological salt nucleoskeleton (11) and replication 'factories' observed *in situ* by fluorescence and electron microscopy (12,13). Genes being transcribed by RNA polymerases I and II are located on the high salt nuclear matrix (14–19) and the physiological salt nucleoskeleton (20–22), as are nascent RNA transcripts and splicing intermediates (20,22–25), though the association of genes is not dependent upon the presence of the RNA. Active RNA polymerase II elongation complexes are also found on the high salt nuclear matrix (16,26) and physiological salt nucleoskeleton (20) and are observed *in situ* by fluorescence microscopy as discrete foci within the nucleus (27).

Cells exposed to chemical or physical agents that damage DNA are able to mitigate the toxic and mutagenic effects of lesions in the DNA by various mechanisms of lesion reversal, removal and tolerance (28). One ubiquitous mechanism, known as nucleotide excision repair (NER) (29,30), involves the recognition of a bulky lesion in DNA, incision of the strand of DNA containing the lesion both 3′ and 5′ of the lesion, removal of the lesion-containing oligonucleotide and DNA repair replication and ligation to close the resulting gap. This general mode of DNA repair is found in many organisms from bacteria and yeast to rodent and human cells (28). In mammals NER is tremendously complex and involves ~30 known polypeptides (31). Nucleotide

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excision repair can act to remove lesions from the bulk of a cell genome (global NER), but it can also act in a transcription-coupled fashion whereby lesions in a gene transcribed by RNA polymerase II are repaired at a faster rate or to a greater extent than are lesions in the overall genome. This sub-pathway, which has been observed for UV-induced lesions in bacteria (32), yeast (33) and mammalian cells (34,35), is due to enhanced repair of the transcribed DNA strand (36) and is dependent on active transcription (32,37–40).

Nucleotide excision repair may also occur in association with the nucleoskeleton (41,42). Mullenders *et al.* (43) found that in UV-irradiated human cells, DNA containing repair patches was enriched on the nuclear matrix. Furthermore, in cells deficient in the ability to perform global NER this effect was enhanced and in cells deficient in the ability to perform transcription-coupled NER the effect was lost. This suggested that repair patches synthesized in expressed genes are associated with the nuclear matrix as a consequence of the association of active genes with the matrix (43). In normal cells the phenomenon disappears at higher UV doses (42,44,45), where transcription-coupled repair may decrease relative to global DNA repair due to the inhibition of transcription (46). Repair patches in non-transcribed DNA must then either occur away from the matrix or occur on the matrix and be released soon after the repair synthesis step. There is evidence to suggest that repair patches, unlike newly replicated DNA resulting from S phase synthesis, are easily released from the nucleoskeleton under physiological conditions (47).

We sought to investigate the relationship between damaged DNA, in both transcribed and non-transcribed regions, and the high salt nuclear matrix in both repair-proficient and NER-deficient hamster cells. We chose to examine DNA–matrix associations, which may reflect the earlier NER stages of recognition and incision. Using cells with an amplified dihydrofolate reductase (*DHFR*) domain we examined the association of restriction fragments in transcribed and non-transcribed regions of this domain with the nuclear matrix after UV irradiation. The fraction of DNA associated with the nuclear matrix increased, to a varying degree, in several regions of the domain after irradiation and the frequency of the predominant UV-induced lesion, the cyclobutane pyrimidine dimer (CPD), increased in matrix-bound DNA 1 h after irradiation, but then decreased at later times. These phenomena were not seen in cells mutant for ERCC1 (excision repair cross-complementing group 1), which is absolutely required for NER (48,49) and is thought to participate in the incision of DNA 5′ of the lesion (50,51). This suggests that the results we observed derive from a direct involvement of excision repair, rather than a generalized stress response. These data lend support to a model for global NER in which damage in the genomic DNA is recognized and brought into association with the nuclear matrix. Repair then occurs at a matrix-bound 'repair factory' consisting of an incision complex (including ERCC1), the transcription initiation factor TFIIH and enzymes for DNA synthesis and ligation. After repair synthesis is completed the DNA then loses its functional association with the matrix-bound repair factory.

MATERIALS AND METHODS

Cell culture and irradiation

B11 and UVL-10-PT cells are both Chinese hamster ovary (CHO) fibroblasts containing an ~50-fold chromosomal amplification of the *DHFR* gene region. B11 cells are repair proficient, whereas UVL-10-PT cells were derived from UVL-10 cells, which belong to excision repair cross-complementing group 1 (ERCC1) (48), previously called ERCC2. The UVL-10-PT cells were generously provided by M. S.Tang (M. D. Anderson Cancer Center, University of Texas). The cells were grown in minimal essential medium (Gibco) supplemented with 10% dialyzed fetal bovine serum, glutamine, non-essential amino acids and 0.5 µM methotrexate in a 37°C humidified atmosphere containing 5% CO_2. Two days before experimentation cells were split 1:6 into medium containing 0.1 mM thymidine and 1 µCi/ml [^{3}H]thymidine added to label the DNA. Ten hours before irradiation the medium was replaced with fresh medium of the same composition. At the time of irradiation the cells had not yet grown to confluence.

For irradiation, the medium was removed from the cells and reserved, then the cells were rinsed with phosphate-buffered saline (PBS) at 37°C and irradiated with a dose of 10 J/m^2 UV from a germicidal lamp (Westinghouse IL782–30) at an incident rate of 0.39 J/m^2/s at 254 nm, as determined by an International Light IL254 photometer. After irradiation the medium was replaced and cells were incubated for 0.5, 1, 2 or 3 h at 37°C. Cells incubated for 0 h were washed with ice-cold PBS immediately after irradiation and harvested. Irradiations were staggered for the various time points so that all cells were harvested at the same time.

Nuclear matrix isolation

The cells were washed with ice-cold PBS and harvested in PBS by scraping with a rubber policeman. Cells were pelleted and nuclei were prepared by vortexing in an ice-cold solution of 10 mM NaCl, 10 mM Tris–HCl, pH 7.4, 3 mM $MgCl_2$, 0.5% NP-40 and 0.2 mM phenylmethylsulfonyl fluoride (PMSF), as described (39). PMSF was added to all buffers at 0.2 mM in the following steps of the procedure, until DNA purification.

Nuclear matrix was prepared according to the protocol of Dijkwel and Hamlin (52). The nuclei were washed in cold 50 mM KCl, 5 mM Tris–HCl, pH 7.4, and 10 mM $MgCl_2$ and then extracted in 2 M NaCl. The pellet was then washed three times in cold 50 mM KCl, 20 mM Tris–HCl (pH 7.4), 5 mM $MgCl_2$ for *Kpn*I digestion. The pellet was digested in 5 ml buffer with 50 U/ml *Kpn*I for 1 h at 37°C and matrix-bound DNA was separated from unbound DNA by centrifugation. Digestion with *Kpn*I was then repeated with 70 U/ml for 1 h and again with 100 U/ml for 1 h. The matrix-bound DNA and pooled supernatant DNA were then purified.

Quantitative analysis

Purified DNA was quantified by fluorometry (53) and ^{3}H was measured by liquid scintillation counting. To probe for the presence of different DNA fragments, 1 µg each (by ^{3}H radioactivity) of matrix-associated DNA and supernatant DNA were re-cut with *Kpn*I, loaded on neutral agarose gels and electrophoresed. The DNA was transferred to Hybond N$^+$ membrane (Amersham) and probed for the DNA fragments indicated in Figure 1 using nick-translated ^{32}P-labeled probes. The radioactive membranes were exposed to Kodak X-omat AR film without intensifying screens, for times such that the band intensities remained within the linear range of the film. Autoradiographic bands on the films were quantified by densitometry using a Hewlett Packard ScanJet IIp flat bed scanner and NIH

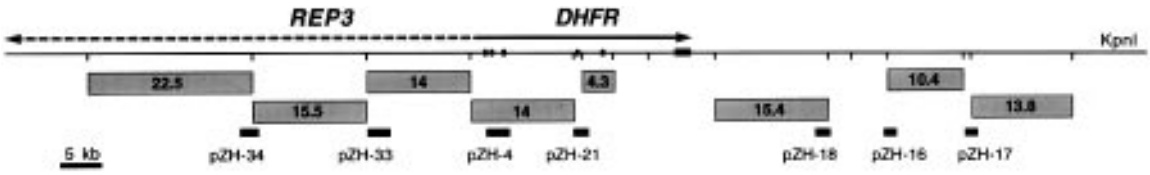

Figure 1. *Kpn*I map of the *DHFR* region of B11 and UVL-10-PT cells. The solid arrow depicts the *DHFR* transcription unit and the dashed arrow depicts the *REP3* transcription unit, which continues beyond the region shown. (Below) Sites of *Kpn*I incision are indicated as vertical ticks on the horizontal line and the exons of *DHFR* are shown as solid bars. The gray rectangles beneath represent the *Kpn*I restriction fragments studied, with the size in kilobases shown inside, and the DNA probes used for their detection are presented along the bottom.

Image software. The scanner was calibrated with NIH Image to provide a linear response using a Kodak standard gray scale. The 'association factor' of DNA with the nuclear matrix was calculated as the autoradiographic signal intensity of the matrix-bound DNA divided by the signal in total DNA. The signal in total DNA was derived as the weighted average of the matrix-bound and supernatant DNA signal intensities.

The analysis of CPD frequency was performed using the T4 endonuclease V method essentially as described (34,54). Since the time course following UV irradiation was short (3 h), however, no attempt was made to remove replicated DNA. Briefly, *Kpn*I re-cut matrix and supernatant DNA were divided into equal volumes and one half was treated with T4 endonuclease V to incise the DNA specifically at sites of CPDs. The treated and untreated DNA was denatured and electrophoresed in alkaline agarose gels, transferred to membranes and then probed with one of the four probes pZH-33, pZH-4, pZH-18 and pZH-17 (see Fig. 1), which detect *Kpn*I fragments of 14, 14, 15.4 and 13.8 kb in size respectively. The membranes were analyzed as above, except that intensifying screens were used. The dimer frequency in the DNA in the samples analyzed was calculated from the relative autoradiographic densities of the full-length DNA fragments in the untreated samples compared with the T4 endonuclease V-treated samples, using the Poisson expression as described (54). The results of two separate T4 endonuclease V assays were averaged to obtain the CPD frequencies for the DNA fragments in each experiment.

RESULTS

B11 and UVL-10-PT CHO fibroblasts were grown and irradiated with UV light as described in Materials and Methods. Cells were harvested and nuclei were prepared and extracted using a standard high salt nuclear matrix procedure (52). During the time between harvesting and high salt extraction (<2 h), when cells and nuclei were manipulated at 4°C, we found that there was no overall removal of either pyrimidine(6–4)pyrimidone photoproducts or CPDs (the two major UV-induced lesions), with an assay (55–57) using monoclonal antibodies (58) against each of the two kinds of photoproducts (data not shown). The matrices were washed and incubated with a restriction endonuclease to remove DNA not closely associated with the nuclear matrix. We used the enzyme *Kpn*I, which generates large restriction fragments, and our preparations contained 2–10% of the total DNA in the matrix fraction. Equal amounts of DNA from matrix and supernatant fractions were electrophoresed on agarose gels, transferred to nylon membranes and probed to measure the relative amounts of specific DNA fragments. A *Kpn*I restriction map of the *DHFR* region in these cells, including the probes used, is shown in Figure 1.

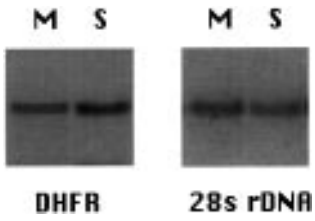

Figure 2. Autoradiograph of a Southern blot demonstrating enrichment of ribosomal DNA at the nuclear matrix in our preparations. Equal amounts of total matrix-bound (M) and supernatant (S) DNA from unirradiated B11 cells were loaded in each lane and probed with pZH-4 to detect the 14 kb *Kpn*I fragment in *DHFR* or probed with a 28s rDNA probe to detect a 7.7 kb *Kpn*I fragment containing the 28s rDNA gene. The preparation contained 7.6% total DNA in the matrix-bound fraction.

In studies with unirradiated B11 cells and a second CHO cell line (CHOC400) (D. R. Koehler and P. C. Hanawalt, manuscript in preparation) we found no conclusive evidence for strong high salt matrix attachment sites (association factor > 1) within the *REP3* gene or sequences downstream of *DHFR* or within the *DHFR* gene, as reported in conflicting studies (52,59). We obtained similar results using either *Kpn*I or *Bam*HI endonucleases and found that the association factor of the resulting DNA fragments was positively correlated with fragment size (most strongly in the transcribed region). A strong matrix attachment site residing downstream of *DHFR* was found in an *Eco*RI fragment previously described (52), yet we did not find this attachment site in *Kpn*I or *Bam*HI fragments overlapping the same region. We confirmed these results in B11 cells with the physiological salt method of Jackson *et al.* (8) using *Kpn*I, *Bam*HI and *Eco*RI restriction enzymes. In all cases ribosomal DNA was enriched on the high salt nuclear matrix (Fig. 2) and physiological nucleoskeleton, as reported by others using these procedures (19,21). Results with the excision repair-deficient UVL-10 PT cells were virtually identical. This is also evident in the association factors for DNA fragments observed immediately following UV irradiation (t = 0; Fig. 3B and C), in which the UVL-10-PT cells have a similar pattern of association as the B11 cells: no strong attachment sites, though slightly greater association factors for the fragments detected by probes pZH-34 and pZH-17. In preliminary experiments we found no significant difference in the association of *Kpn*I or *Bam*HI fragments with the nuclear matrix in unirradiated cells compared with cells harvested immediately after irradiation with 10 J/m^2 (t = 0 in the experiments below).

With B11 cells irradiated with 10 J/m^2 UV, the fraction of DNA fragments on the nuclear matrix (the 'association factor')

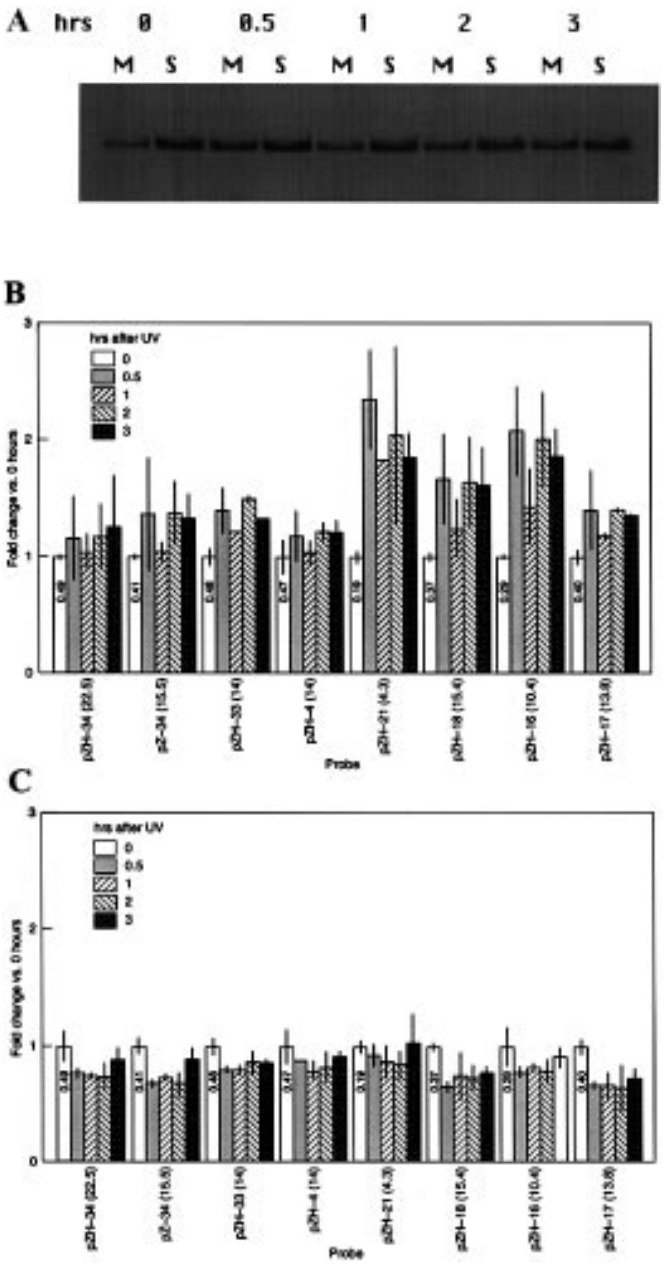

Figure 3. (**A**) Sample autoradiograph of a Southern blot showing the matrix-bound (M) and supernatant (S) DNA in the *DHFR* region of B11 cells following UV irradiation. Equal amounts of total DNA were loaded in each lane and probed with pZH-18 to reveal the 15.4 kb fragment. The time course is shown in hours. (**B**) Plot of the change in matrix association factor for DNA fragments in the *DHFR* region of B11 cells following UV irradiation. The probes used, with the *Kpn*I DNA fragment size (in kb) shown in parentheses, correspond from left to right with the map presented in Figure 1. The change in the matrix association factor after irradiation (see Materials and Methods) is normalized to the value at time zero for all probes used. The raw matrix association factors for each DNA fragment are shown within the zero time bars. The error bars represent the SEM for two independent biological experiments. In the first experiment, the percent total DNA in the matrix-bound faction at 0, 0.5, 1, 2 and 3 h was 2.4, 7.5, 1.9, 8.5 and 3.9 respectively; in the second experiment 3.3, 4.0, 4.1, 6.3 and 4.6 respectively. (**C**) As (B) but with UVL-10-PT cells. The error bars represent the SEM for two independent biological experiments. In the first experiment, the percent total DNA in the matrix-bound faction at 0, 0.5, 1, 2 and 3 h was 7.5, 4.2, 4.1, 3.7 and 5.1 respectively; in the second experiment 8.8, 5.1, 6.7, 2.2 and 9.4 respectively.

increased in many of the regions tested over the course of 3 h (Fig. 3A and B). The increase was smaller in the *REP3* and 5′ *DHFR* regions, but we do not know if this is significant since, in preliminary experiments using *Kpn*I and *Bam*HI restriction enzymes (not shown), the increase in DNA fragments on the matrix 1 h after irradiation was as great in the *REP3* region as in the region downstream of *DHFR* and similar to the 1.5- to 2-fold increase seen in the downstream region in the experiments presented in Figure 3A and B. The greatest increase was observed in the smallest *Kpn*I fragment (4.3 kb), but we do not believe fragment size is related to the magnitude of the increase, since similarly sized *Bam*HI fragments demonstrated the same increase (1.5- to 2-fold) in preliminary experiments as the larger DNA fragments. To determine whether the increase in matrix association factor following irradiation was related to NER, we performed the same experiments with UVL-10-PT cells. UVL-10-PT cells are deficient in all NER due to a mutation in the ERCC1 gene and are currently the only NER mutant cell line available with an amplified *DHFR* domain. We found no increase, but rather a slight decrease (<35%), in the association factor for DNA in the *DHFR* domain following UV irradiation (Fig. 3C).

We might expect the total DNA bound to the nuclear matrix from repair-proficient cells to increase after irradiation, if the phenomenon of enhanced matrix-bound DNA we observed in the *DHFR* region occurs throughout the genome. However, using several restriction enzymes or DNase I, we found that the variation in total DNA in the matrix fraction in separate samples varied too widely (from 2–10% in the experiments shown in Fig. 3) to reveal a 2-fold increase in total nuclear DNA on the matrix. We also found that this variation bears no apparent relation to the fraction of *DHFR* DNA bound to the nuclear matrix calculated by Southern blot (see Fig. 3 legend). Presumably, the variation in total DNA in the matrix fraction is due to the completeness of the endonuclease reaction in different samples.

To detect the presence of UV-induced lesions in the matrix-bound and supernatant DNA, we analyzed DNA from the same experiments shown in Figure 3 with a standard assay that uses T4 endonuclease V to incise DNA specifically at sites of CPDs (see Materials and Methods). We studied four restriction fragments in the *DHFR* region, 13.8–15.4 kb in size, detected by the probes pZH-33, pZH-4, pZH-18 and pZH-17 (Fig. 1). The UV dose used (10 J/m^2) induced about one CPD each in these DNA fragments. With B11 cells the frequency of CPDs in the matrix-bound fraction of DNA increased by 50% within 1 h after irradiation and then decreased subsequently (Fig. 4A and B). The lack of change in CPD frequency in the supernatant DNA at 1 h is expected, since the matrix-bound DNA represents only 2–10% of the total DNA and a corresponding change in the supernatant DNA would not be distinguishable due to the standard error we encountered in these studies. With repair-deficient UVL-10-PT cells there does not appear to be any remarkable change in the CPD frequency in matrix-bound and supernatant DNA following irradiation (Fig. 4C), except for a drop at 0.5 h in the DNA fragment detected by pZH-4. Again, these changes and lack thereof bear no apparent relation to the percent of total genomic DNA isolated in the matrix-bound fractions in the individual experiments (see Fig. 3 legend).

DISCUSSION

We have shown that changes occur in the association of DNA with the nuclear matrix in the *DHFR* domain of CHO fibroblasts

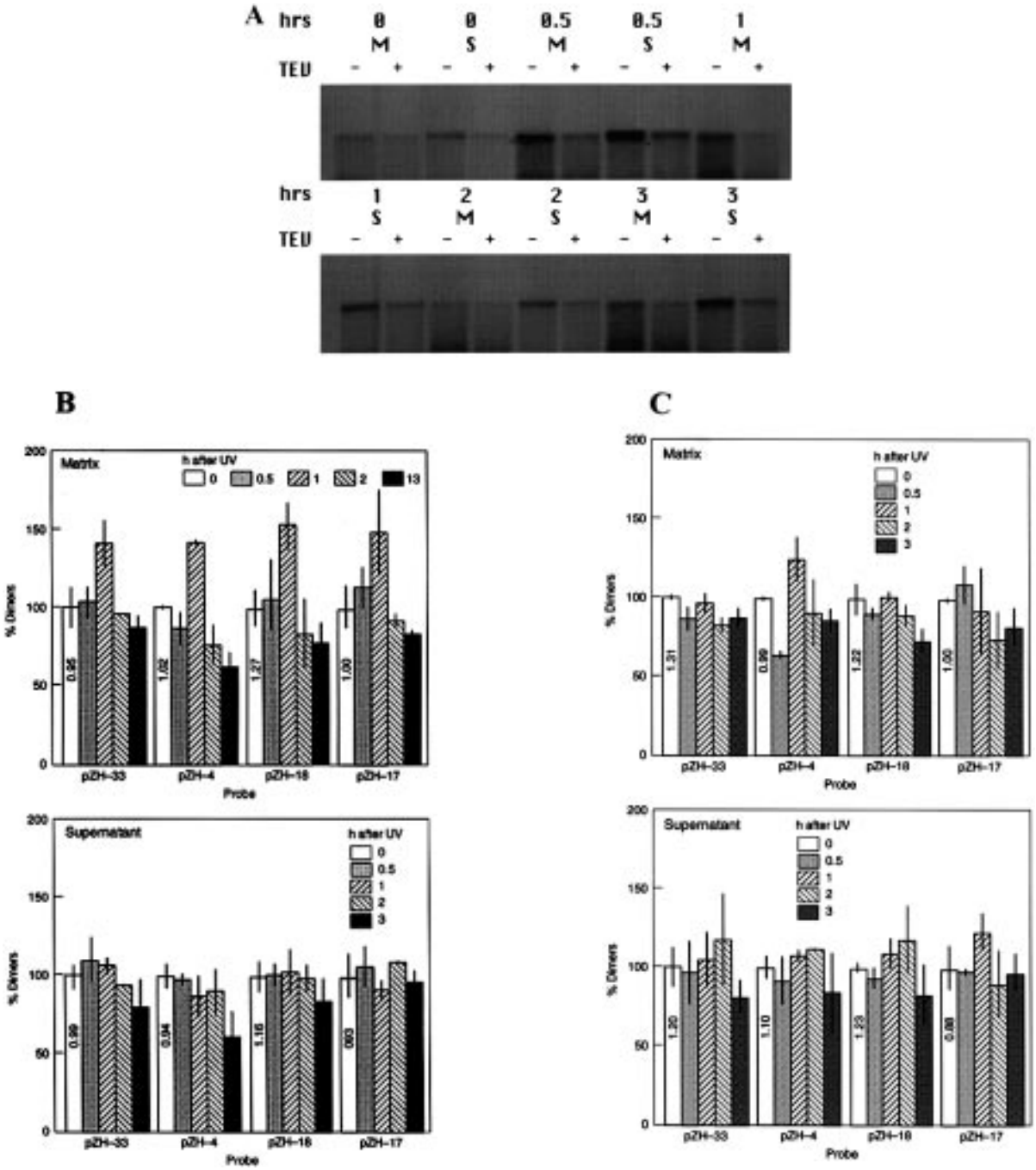

Figure 4. (**A**) Sample autoradiographs of Southern blots demonstrating detection of CPDs in matrix-bound (M) and supernatant (S) DNA following UV irradiation. Each sample was divided into two and digested with T4 endonuclease V (+) or mock digested (–). The blots shown were probed with pZH-33 to reveal the 14 kb fragment. The time course is shown in hours. (**B**) Plot of the change in the frequency of CPDs in the matrix-bound and supernatant DNA of B11 cells following UV irradiation. The four probes used detect *Kpn*I DNA fragments as indicated in Figure 1. The CPD frequency is normalized to 100% for the value at time zero for each probe used. The raw values for initial CPD frequency, per DNA fragment, are shown within the zero time bars. (Top) CPD frequency in matrix-bound DNA. (Bottom) CPD frequency in supernatant DNA. The error bars represent the SEM for two independent biological experiments. For each experiment, the CPD frequency was calculated as the average from two separate determinations using the T4 endonuclease V assay. (**C**) As (B) but with UVL-10-PT cells.

after UV irradiation. First, the matrix association factor for DNA in the *DHFR* region was increased, up to 2.5-fold in some areas, over a period of 3 h following UV, in both transcribed and non-transcribed regions of the domain (Fig. 3A and B). Second, the frequency of CPDs increased 50% in the matrix-bound DNA 1 h after irradiation and then declined, in both the transcribed and non-transcribed regions (Fig. 4A and B). Neither of these effects were evident in a CHO fibroblast deficient in excision repair (Figs 3C and 4C), suggesting that the phenomena observed are not a generalized response to cellular stress, but are specifically due to the action of NER. For a comparison of the significance of the magnitude of the changes we observed, consider the finding that the fraction of nuclear matrix-bound vitellogenin II DNA is increased only 3-fold in chick liver after a primary or secondary

stimulation of vitellogenin transcription with estradiol (15). This estradiol treatment resulted in a 120-fold (primary) or 1400-fold (secondary) increase in vitellogenin mRNA levels (15). We do not know if the small (<35%) drop in the matrix association factor for some DNA fragments in the repair-deficient UVL-10-PT cells after irradiation is significant (Fig. 3C). There was no change in matrix associations of DNA from repair-proficient cells immediately after irradiation (data not shown), so it seems unlikely that alterations in chromatin structure due to the presence of photodimers leads to the loss of matrix association.

Since the observed increase in matrix-bound DNA and CPD frequency occurs in both transcribed and non-transcribed regions of DNA, we consider these effects related to global genomic DNA repair, but not necessarily transcription-coupled repair (36). We sought to investigate whether this phenomenon occurs in the genome as a whole using a monoclonal antibody-based method that we and others have successfully applied to detect CPDs and pyrimidine(6–4) pyrimidone photoproducts in the DNA of bacteria (57,60), yeast (56), maize (55) and mammalian cells (61; D. R. Koehler, unpublished data). Our matrix-associated and supernatant DNA prepared by high salt fractionation, however, was refractory to repeated attempts at analysis using this technique.

In formulating a hypothesis to explain these changes in nuclear matrix–DNA association we must first consider the differential processing of the two major kinds of UV-induced lesions in the cell. Hamster cells remove CPDs from the transcribed strands of active genes, but are generally deficient in removal of these lesions from the overall genome (34,36,62,63). In contrast, the second most frequent UV-induced lesion, the pyrimidine(6-4)pyrimidone photoproduct, is removed very rapidly from the overall genome, reaching completion in ~3 h (61; D. R. Koehler, unpublished data) and this removal may be enhanced in active genes (64). Therefore, regardless of whether CPDs or pyrimidine(6-4)pyrimidone photoproducts or both lesions are responsible for the NER-dependent increase in matrix-bound DNA observed in Figure 3A and B, we must be aware that CPDs are not subject to complete repair in non-transcribed regions of the genome. Additionally, the increase in CPD frequency we observed in matrix-bound DNA 1 h after UV (Fig. 4A and B) may involve NER, but probably does not result in successful repair of these lesions, especially in the non-transcribed regions. The difference in time course between the increase in matrix-bound DNA, which remains elevated for at least 3 h, and the increase in CPD frequency only at 1 h is mysterious, but could be related to differential processing of the two major UV lesions in CHO cells. In human cells, pyrimidine(6–4)pyrimidine photoproducts are rapidly repaired and CPDs are also removed efficiently (though more slowly) from the total genome and both lesions are removed more rapidly from genes in a transcription-coupled manner (35,36,46,65).

Knowing the characteristics of DNA repair in human and hamster cells, our results can be integrated with those of Mullenders *et al.* (43) to form a more complete model of the role of the nuclear matrix in NER. In repair-proficient human cells, after a UV dose of 5 J/m^2, Mullenders *et al.* (43) found that DNA repair patches were preferentially located close to the nuclear matrix for up to 2 h after irradiation. Additionally, patches created shortly after irradiation could not be chased from the matrix over the course of 1 h. In xeroderma pigmentosum group C (XP-C) cells, which are deficient in all NER that is not transcription-coupled (46,66,67), repair patches were even more frequently found near the nuclear matrix. In Cockayne's syndrome (CS) cells, which lack transcription-coupled repair but have no demonstrated defect in global NER (68,69), there was a slight depletion of repair patches near the nuclear matrix. At a higher UV dose of 30 J/m^2 the enrichment of repair patches at the matrix is lost in normal cells, but still apparent in XP-C cells (42,45). This may be due to an overall inhibition of transcription and thus transcription-coupled repair, relative to global DNA repair, at the higher dose (46).

The data of Mullenders *et al.* (43) likely indicate that repair patches created in genes, both by the rapid transcription-coupled mechanism and by the total genomic repair system, remain associated with the nuclear matrix by virtue of their location in actively transcribed sequences (43). Repair patches created in transcriptionally silent DNA then either occur distantly from the matrix or also occur on the matrix and are released after completion. Our data support the latter hypothesis. Our observation that the matrix association factor for DNA in the *DHFR* domain increased after UV in an NER-dependent fashion and in transcriptionally active and silent regions is consistent with damaged DNA being recruited to the matrix for repair. The high salt treatment used to prepare nuclear matrix may precipitate functional DNA associations with NER proteins on the matrix during the excision repair event, but before repair synthesis has been completed. After repair synthesis, the lesion-free DNA would then be released from the matrix. In fact, under physiological conditions repair patches are easily removed from the nucleoskeleton, in marked contrast to replicative S phase DNA synthesis (47). The increase in CPD frequency observed on the matrix 1 h after UV suggests that the 'defect' in hamster cells that prevents significant global CPD repair occurs in a step subsequent to recruiting the DNA to the matrix, such as later recognition and/or incision stages of NER.

It is of interest to speculate on the role of ERCC1 in the association of UV-damaged DNA with the nuclear matrix. The initial recognition of DNA damage probably involves the XPA protein, which is defective in persons with xeroderma pigmentosum group A (70,71). ERCC1 has been shown to interact with XPA protein both *in vitro* and *in vivo* (72,73). ERCC1 may also enhance the DNA damage binding ability of XPA *in vitro* (74) and ERCC1 interaction with XPA is required for NER in cell extracts (75). Additionally, there is evidence *in vitro* for a complex involving XPA, ERCC1 and ERCC4 (XPF) (76–78). ERCC1 is thought to participate with XPF protein in the incision of the DNA 5′ of the damaged region (50,51). The incision 3′ of the damage is likely made by the XPG protein (51,79). The XPB and XPD proteins, which are tightly associated components of the transcription initiation factor TFIIH, are essential for all NER (80). Excision repair may also depend on an interaction between XPA and TFIIH (81). Thus excision repair *in vivo* may involve the formation of a large complex in which lesions are recognized by a factor such as XPA, possibly enhanced by ERCC1, and brought into association with an excision/synthesis complex which involves XPA, ERCC1, XPF, XPG, TFIIH (containing XPB and XPD), RPA, PCNA, RFC, DNA polymerase(s), DNA ligase(s) and an activity called IF7 (31). In the absence of a blocked RNA polymerase II elongation complex (i.e. in the bulk of the genome), XPC protein would also be required for repair; it is necessary for NER *in vitro* (31) and has been demonstrated to associate with TFIIH (80). Repair of an RNA polymerase-

blocking lesion within a gene may require the additional involvement of CS factors CSA and CSB (37). Some critical components of NER may reside permanently on the nucleoskeleton, such as TFIIH, which is also required for the initiation of transcription by RNA polymerase II.

Our data lend support to the concept of a 'repair factory' (82,83) localized on the nucleoskeleton, comprised of many or most of the enzymes involved in the excision and repair synthesis stages of NER. Excision repair, like transcription and replicative DNA synthesis, occurs very inefficiently *in vitro* and may require proper spatial orientation of the ~30 polypeptides (31) involved. Human cell-free systems for studying NER, in which UV-damaged plasmids are incubated with cell extracts, typically result in the removal of <5% of the lesions. *In situ* DNA repair occurs at many discrete foci in the nucleus that are unrelated to DNA and UV lesion density, co-localize with some but not all transcription foci and contain PCNA (82). It would be informative to localize proteins involved in repair and transcription, such as TFIIH, in relation to the nucleoskeleton under physiological conditions and to examine the distribution of these proteins after high salt extraction to prepare nuclear matrix.

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REFERENCES

1 Razin,S.V. and Gromova,I.I. (1995) *Bioessays*, **17**, 443–450.
2 Cook,P.R. (1994) *Bioessays*, **16**, 425–430.
3 Berezney,R. (1991) *J. Cell Biochem.*, **47**, 109–123.
4 Jackson,D.A. (1995) *Bioessays*, **17**, 587–591.
5 Berezney,R. and Coffey,D.S. (1974) *Biochem. Biophys. Res. Commun.*, **60**, 1410–1417.
6 Mirkovitch,J., Mirault,M.-E. and Laemmli,U.K. (1984) *Cell*, **39**, 223–232.
7 Jackson,D.A., Dickinson,P. and Cook,P.R. (1990) *EMBO J.*, **9**, 567–571.
8 Jackson,D.A. and Cook,P.R. (1985) *EMBO J.*, **4**, 913–918.
9 Berezney,R. and Coffey,D.S. (1975) *Science*, **189**, 291–293.
10 Vaughn,J.P., Dijkwel,P.A., Mullenders,L.H. and Hamlin,J.L. (1990) *Nucleic Acids Res.*, **18**, 1965–1969.
11 Jackson,D.A. and Cook,P.R. (1986) *EMBO J.*, **5**, 1403–1410.
12 Hassan,A.B. and Cook,P.R. (1993) *J. Cell Sci.*, **105**, 541–550.
13 Hozak,P., Hassan,A.B., Jackson,D.A. and Cook,P.R. (1993) *Cell*, **73**, 361–373.
14 Robinson,S.I., Nelkin,B.D. and Vogelstein,B. (1982) *Cell*, **28**, 99–106.
15 Jost,J.-P. and Seldran,M. (1984) *EMBO J.*, **3**, 2005–2008.
16 Razin,S.V., Yarovaya,O.V. and Georgiev,G.P. (1985) *Nucleic Acids Res.*, **13**, 7427–7444.
17 Farache,G., Razin,S.V., Rzeszowska-Wolny,J., Moreau,J., Targa,F.R. and Scherrer,K. (1990) *Mol. Cell. Biol.*, **10**, 5349–5358.
18 Ciejek,E.M., Tsai,M.J. and O'Malley,B.W. (1983) *Nature*, **306**, 607–609.
19 Keppel,F. (1986) *J. Mol. Biol.*, **187**, 15–21.
20 Jackson,D.A. and Cook,P.R. (1985) *EMBO J.*, **4**, 919–925.
21 Dickinson,P., Cook,P.R. and Jackson,D.A. (1990) *EMBO J.*, **9**, 2207–2214.
22 Thorburn,A. and Knowland,J. (1993) *Biochem. Biophys. Res. Commun.*, **191**, 308–313.
23 Zeitlin,S., Parent,A., Silverstein,S. and Efstratiadis,A. (1987) *Mol. Cell. Biol.*, **7**, 111–120.
24 Ciejek,E.M., Nordstrom,J.L., Tsai,M.J. and O'Malley,B.W. (1982) *Biochemistry*, **21**, 4945–4953.
25 Ross,D.A., Yen,R.W. and Chae,C.B. (1982) *Biochemistry*, **21**, 764–771.
26 Razin,S.V. and Yarovaya,O.V. (1985) *Exp. Cell Res.*, **158**, 273–275.
27 Jackson,D.A., Hassan,A.B., Errington,R.J. and Cook,P.R. (1993) *EMBO J.*, **12**, 1059–1065.
28 Friedberg,E.C., Walker,G.C. and Siede,W. (eds) (1995) *DNA Repair and Mutagenesis*. ASM Press, Washington, DC.
29 Hoeijmakers,J.H. (1993) *Trends Genet.*, **9**, 173–177.
30 Hoeijmakers,J.H. (1993) *Trends Genet.*, **9**, 211–217.
31 Aboussekhra,A., Biggerstaff,M., Shivji,M.K., Vilpo,J.A., Moncollin,V., Podust,V.N., Protic,M., Hubscher,U., Egly,J.M. and Wood,R.D. (1995) *Cell*, **80**, 859–868.
32 Mellon,I. and Hanawalt,P.C. (1989) *Nature*, **342**, 95–98.
33 Smerdon,M.J. and Thoma,F. (1990) *Cell*, **61**, 675–684.
34 Bohr,V.A., Smith,C.A., Okumoto,D.S. and Hanawalt,P.C. (1985) *Cell*, **40**, 359–369.
35 Mellon,I., Bohr,V.A., Smith,C.A. and Hanawalt,P.C. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 8878–8882.
36 Mellon,I., Spivak,G. and Hanawalt,P.C. (1987) *Cell*, **51**, 241–249.
37 Hanawalt,P.C. (1994) *Science*, **266**, 1957–1958.
38 Leadon,S.A. and Lawrence,D.A. (1992) *J. Biol. Chem.*, **267**, 23175–23182.
39 Christians,F.C. and Hanawalt,P.C. (1992) *Mutat. Res.*, **274**, 93–101.
40 Sweder,K.S. and Hanawalt,P.C. (1992) *Proc. Natl. Acad. Sci. USA*, **89**, 10696–10700.
41 McCready,S.J. and Cook,P.R. (1984) *J. Cell Sci.*, **70**, 189–196.
42 Harless,J. and Hewitt,R.R. (1987) *Mutat. Res.*, **183**, 177–184.
43 Mullenders,L.H., van Kesteren van Leeuwen,A.C., van Zeeland,A.A. and Natarajan,A.T. (1988) *Nucleic Acids Res.*, **16**, 10607–10622.
44 Mullenders,L.H., van Kesteren,A.C., Bussmann,C.J., van Zeeland,A.A. and Natarajan,A.T. (1984) *Mutat. Res.*, **141**, 75–82.
45 Mullenders,L.H., van Kesteren,A.C., Bussmann,C.J., van Zeeland,A.A. and Natarajan,A.T. (1986) *Carcinogenesis*, **7**, 995–1002.
46 van Hoffen,A., Venema,J., Meschini,R., van Zeeland,A.A. and Mullenders,L.H. (1995) *EMBO J.*, **14**, 360–367.
47 Jackson,D.A., Balajee,A.S., Mullenders,L. and Cook,P.R. (1994) *J. Cell Sci.*, **107**, 1745–1752.
48 Clarkson,J.M., Mitchell,D.L. and Adair,G.M. (1983) *Mutat. Res.*, **112**, 287–299.
49 Biggerstaff,M. and Wood,R.D. (1992) *J. Biol. Chem.*, **267**, 6879–6885.
50 Bardwell,A.J., Bardwell,L., Tomkinson,A.E. and Friedberg,E.C. (1994) *Science*, **265**, 2082–2085.
51 Matsunaga,T., Mu,D., Park,C.H., Reardon,J.T. and Sancar,A. (1995) *J. Biol. Chem.*, **270**, 20862–20869.
52 Dijkwel,P.A. and Hamlin,J.L. (1988) *Mol. Cell. Biol.*, **8**, 5398–5409.
53 Cesarone,C.F., Bolognesi,C. and Santi,L. (1979) *Anal. Biochem.*, **100**, 188–197.
54 Bohr,V.A. and Okumoto,D.S. (1988) In Friedberg,E.C. and Hanawalt,P.C. (eds), *DNA Repair: A Laboratory Manual of Research Procedures*. Marcel Dekker, New York, NY, Vol. 3, pp. 347–366.
55 Stapleton,A.E., Mori,T. and Walbot,V. (1993) *Plant Mol. Biol. Rep.*, **11**, 230–236.
56 Sweder,K.S., Chun,R., Mori,T. and Hanawalt,P.C. (1996) *Nucleic Acids Res.*, **24**, 1540–1546.
57 Koehler,D.R., Courcelle,J. and Hanawalt,P.C. (1996) *J. Bacteriol.*, **178**, 1347–1350.
58 Mori,T., Nakane,M., Hattori,T., Matsunaga,T., Ihara,M. and Nikaido,O. (1991) *Photochem. Photobiol.*, **54**, 225–232.
59 Kas,E. and Chasin,L.A. (1987) *J. Mol. Biol.*, **198**, 677–692.
60 Mori,T., Shimoi,K., Sasaki,Y.F., Wakabayashi,K., Nagao,M. and Kinae,N. (1993) *Carcinogenesis*, **14**, 1475–1478.
61 Numata,M., Hata,H., Shiomi,T., Matsunaga,T., Mori,T., Nikaido,O., Yasui,A. and Oikawa,A. (1993) *Cancer Res.*, **53**, 495–499.
62 Zelle,B., Reynolds,R.J., Kottenhagen,M.J., Schuite,A. and Lohman,P.H. (1980) *Mutat. Res.*, **72**, 491–509.
63 van Zeeland,A.A., Smith,C.A. and Hanawalt,P.C. (1981) *Mutat. Res.*, **82**, 173–189.
64 Thomas,D.C., Okumoto,D.S., Sancar,A. and Bohr,V.A. (1989) *J. Biol. Chem.*, **264**, 18005–18010.
65 Mitchell,D.L., Haipek,C.A. and Clarkson,J.M. (1985) *Mutat. Res.*, **143**, 109–112.

66 Kantor,G.J., Barsalou,L.S. and Hanawalt,P.C. (1990) *Mutat. Res.*, **235**, 171–180.
67 Venema,J., van Hoffen,A., Natarajan,A.T., van Zeeland,A.A. and Mullenders,L.H. (1990) *Nucleic Acids Res.*, **18**, 443–438.
68 Venema,J., Mullenders,L.H., Natarajan,A.T., van Zeeland,A.A. and Mayne,L.V. (1990) *Proc. Natl. Acad. Sci. USA*, **87**, 4707–4711.
69 van Hoffen,A., Natarajan,A.T., Mayne,L.V., van Zeeland,A.A., Mullenders,L.H. and Venema,J. (1993) *Nucleic Acids Res.*, **21**, 5890–5895.
70 Jones,C.J. and Wood,R.D. (1993) *Biochemistry*, **32**, 12096–12104.
71 Robins,P., Jones,C.J., Biggerstaff,M., Lindahl,T. and Wood,R.D. (1991) *EMBO J.*, **10**, 3913–3921.
72 Li,L., Elledge,S.J., Peterson,C.A., Bales,E.S. and Legerski,R.J. (1994) *Proc. Natl. Acad. Sci. USA*, **91**, 5012–5016.
73 Park,C.H. and Sancar,A. (1994) *Proc. Natl. Acad. Sci. USA*, **91**, 5017–5021.
74 Nagai,A., Saijo,M., Kuraoka,I., Matsuda,T., Kodo,N., Nakatsu,Y., Mimaki,T., Mino,M., Biggerstaff,M., Wood,R.D., Sijbers,A., Hoeijmakers,J.H.J. and Tanaka,K. (1995) *Biochem. Biophys. Res. Commun.*, **211**, 960–966.
75 Li,L., Peterson,C.A., Lu,X. and Legerski,R.J. (1995) *Mol. Cell. Biol.*, **15**, 1993–1998.
76 Biggerstaff,M., Szymkowski,D.E. and Wood,R.D. (1993) *EMBO J.*, **12**, 3685–3692.
77 van Vuuren,A.J., Appeldoorn,E., Odijk,H., Yasui,A., Jaspers,N.G., Bootsma,D. and Hoeijmakers,J.H. (1993) *EMBO J.*, **12**, 3693–3701.
78 van Vuuren,A.J., Appeldoorn,E., Odijk,H., Humbert,S., Moncollin,V., Eker,A.P., Jaspers,N.G., Egly,J.M. and Hoeijmakers,J.H. (1995) *Mutat. Res.*, **337**, 25–39.
79 O'Donovan,A., Davies,A.A., Moggs,J.G., West,S.C. and Wood,R.D. (1994) *Nature*, **371**, 432–435.
80 Drapkin,R., Reardon,J.T., Ansari,A., Huang,J.C., Zawel,L., Ahn,K., Sancar,A. and Reinberg,D. (1994) *Nature*, **368**, 769–772.
81 Park,C.H., Mu,D., Reardon,J.T. and Sancar,A. (1995) *J. Biol. Chem.*, **270**, 4896–4902.
82 Jackson,D.A., Hassan,A.B., Errington,R.J. and Cook,P.R. (1994) *J. Cell Sci.*, **107**, 1753–1760.
83 Svejstrup,J.Q., Wang,Z., Feaver,W.J., Wu,X., Bushnell,D.A., Donahue,T.F., Friedberg,E.C. and Kornberg,R.D. (1995) *Cell*, **80**, 21–28.