Analysis of nucleotide excision repair by detection of single-stranded DNA transients

Carlos P. Rubbi and Jo Milner

YCR P53 Research Group, Department of Biology, University of York, York YO10 5DD, UK

1To whom correspondence should be addressed
Email: cpr2@york.ac.uk

Nucleotide excision repair (NER) removes bulky DNA lesions and is thus crucial for the protection against environmental carcinogens and UV light exposure. Deficiencies in NER cause increased mutation rates and chromosomal aberrations. Current methods for studying NER are mostly based on either quantification of lesion removal or detection of repair DNA synthesis. Both have their limitations: lesion removal is inaccurate at very short times post-lesion, where the fraction of removal is low. Repair synthesis is difficult to apply to normally cycling cells due to the need to discriminate repair from replicative DNA synthesis. To overcome these problems we developed a method for analysis of NER based on detection of transient single-stranded (ss) DNA stretches generated at the nucleotide excision step. Cells are metabolically labelled with BrdU, exposed to UV-irradiation and the ssDNA transients generated during excision repair are detected using an anti-BrdU antibody. The method allows single-cell microscopic analysis of the distribution of DNA repair sites as well as kinetic analysis of the DNA repair response. Studies using various DNA repair-deficient cell lines indicate that the detection method integrates a number of pre-synthesis nucleotide excision repair stages. Thus, assembled repair sites can be detected even when they may not lead to complete resolution of the DNA lesion. Using this approach, we show that repair helicase-deficient cells differ from endonuclease-deficient cells.

Introduction

DNA repair is a fundamental cellular process responsible for the maintenance of genomic integrity. Nucleotide excision repair (NER) is one of the best-understood DNA repair systems which eliminates a wide range of DNA lesions (1, for a recent review see ref. 2). The major contribution of NER is the repair of cis-syn cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidon photoproducts (6-4 PPs), produced by the UV-C (200–280 nm) and UV-B (280–320 nm) components of sunlight (1). NER comprises two partially overlapping pathways: global genomic NER (GGR), which eliminates DNA damage at any place within the chromatin, and transcription-coupled NER (TCR) which ensures fast and efficient repair of DNA damage at transcribed regions of the genome (1). Deficiencies in either pathway lead to genetic heterogeneity, hyper-photosensitivity and predisposition to skin cancer as seen in the autosomal recessive disorders Xeroderma pigmentosum (XP), Cockayne syndrome (CS) and trichothiodystrophy (TTD) (1).

The first step in the NER process is lesion detection, for which both the XPA protein and the XPC–HR23B complex have been proposed (2). This is followed by DNA melting and opening of the repair ‘bubble’ by the XPB and XPD helicases of TFIH, excision of the damaged DNA stretch (through the XPF and XPG endonucleases cutting 5’ and 3’ of the lesion, respectively) and re-synthesis of the removed stretch (DNA polymerases α and δ) (reviewed in refs 3,4).

NER is usually detected by measuring repair DNA synthesis or lesion removal (1). The former is typically measured by unscheduled DNA synthesis or BrdU density labelling, whereas for the latter loss of sensitivity to T4 endonuclease or anti-UV-lesion antibodies are used. DNA synthesis-based methods usually discriminate replicative synthesis from repair synthesis either by cell-cycle arrest or by replication inhibition; such approaches are, therefore, not applicable to normally cycling cells. Lesion removal methods refer their response to the maximum removal at completion and are thus relatively insensitive to the low proportions of lesion removal occurring at short times after lesion induction. Furthermore, certain bulky DNA lesions, such as those induced by adriamycin or actinomycin D, cause inhibition of DNA synthesis and, therefore, the repair process is compromised. Nonetheless, such techniques have generated important insights into the process of DNA repair in mammalian cells and, to date, UV photo-products are the best-studied DNA lesions repaired by the NER mechanism. These lesions provide a particularly useful experimental system for time-course analysis of NER, as they can be introduced by very short pulses of UV light and are efficiently removed.

Another approach increasingly used to study NER machinery is single-cell light microscopy analysis of the distribution of NER components in relation to active sites of NER (5–7). This approach is based on detection of sites of repair synthesis by the incorporation of labelled deoxynucleotides and, therefore, requires the discrimination of sites of replicative synthesis from those of repair.

To overcome these various problems we have sought to label sites of active NER by detection of transient stretches of single-stranded (ss) DNA generated during nucleotide excision. We reasoned that if the intact strand of DNA was labelled with bromodeoxyuridine (BrdU), the transient ssDNA stretches produced by the NER response to UV irradiation could be specifically detected with anti-BrdU antibodies, since these antibodies will not react with BrdU-substituted DNA in the double-stranded form. Visualization of ssDNA transients in vivo is possible, as shown by Raderschall et al. for sites of repair of double-strand DNA breaks (8), where ssDNA stretches ~1 kb long are generated. However, in the approach of Raderschall et al.,
both DNA strands were substituted with BrdU in order to maximize detectability: in our case substitution of thymidine by BrdU must occur in only one strand of DNA, since the BrdU base does not produce cyclobutane pyrimidine dimers (9), and thus a thymidine-containing strand must be present.

In the present work, we show that transient DNA gaps are detectable in nuclei following UV irradiation and demonstrate that the detection method is specific for nuclei undergoing NER. The method is applicable to normally cycling cells and allows analysis of DNA repair including, for example, colocalization of NER proteins with repair sites. Because nuclei can be distinguished as undergoing NER as soon as detectable levels of ssDNA are achieved, our method allows kinetic analysis of NER from times as short as 20 min post-lesion. Using NER-deficient cell lines we show that the detected fluorescent signal comprises several NER intermediates, ranging from the DNA melting step to the repair synthesis.

We conclude that detection of transient ssDNA is a useful approach to the molecular and kinetic analysis of NER at both the single-cell and the cell-population level.

Materials and methods

Cell culture and BrdU labelling
NIH 3T3 and Hep G2 (human hepatoma cells) were cultured in DMEM + 10% FCS. Normal diploid fibroblasts (NDF), XPA, XPF, XPC, XPG and CS-A fibroblasts were obtained from Coriell Repositories (Camden, NJ, USA) (Cat. GM00038B, GM01630, GM00671, GM01854, GM03021B and GM01856B, respectively) and cultured in MEM alpha + 15 or 20% FCS, as recommended. 1–3×10⁴ cells were loaded onto 13 mm diameter coverslips in 24 well plates and after attachment 30 μM BrdU (Sigma Chemical Co., Dorset, UK) was added for 6 (NIH 3T3), 8 (HepG2 cells) or 20 h (human fibroblasts) and released overnight in BrdU-free medium. For cell-cycle arrest, cells were subsequently incubated in medium with 0.1% FCS for 48 h. For irradiation, cells were rinsed in PBS, exposed to a UV-C germicide tube at a fluency of 2 W/m² for the appropriate times and immediately returned to culture medium.
Staining for immunofluorescence

Cells were rinsed twice in PBS and fixed. For wide-field fluorescence, cells were fixed with cold methanol for 20 min, dipped in cold acetone and transferred to PBS-T (PBS + 0.2% Tween 20). For confocal microscopy, cells were fixed with 4% paraformaldehyde for 15 min and washed with PBS-T. In all experiments a parallel sample was included for quantitation of cells that incorporated BrdU; this was done by treating coverslips with 1 M HCl for 10 min before blocking, and processing in the same conditions as the rest.

Fixed cells were blocked in PBS-T-S (PBS-T + 10% normal serum of the same species of the secondary antibody) for 20 min. All antibodies were diluted in PBS-T-S. Antibodies and dilutions used were 1:100 for ICR1 (rat anti-BrdU; Harlan Sera-Lab, Letchester, UK), 1:50 for both XPA and XPG antibodies (mouse monoclonals; NeoMarkers, Fremont, CA) and 1:100 for Ku p80 (mouse monoclonal; NeoMarkers). Rat-adsorbed biotinylated donkey anti-mouse IgG, mouse-adsorbed Cy3-donkey anti-rat IgG (Jackson Immuno-Research, West Grove, PA), FITC-rabbit anti-mouse IgG (Dako, Cambridge, UK) and FITC-goat anti-rat IgG (Sigma) were used diluted 1:100. Biotin was detected using streptavidin conjugated with dichlorotriazinyl-a-mino-iodo-fluorescein (dFITC) (Jackson, West Grove, PA). Coverslips were incubated on 20 µl drops of antibody on paraffin, for 1 h at reverse transcripase (RT) in a humid chamber, with four 2 min washings in PBS-T. Before mounting, nuclei were stained with 1 µg/ml Hoechst 33258. Samples were mounted using a Mowiol 4-88-based medium with 100 mg/ml DABCO. Wide-field observation was performed using a Carl Zeiss Axioplan 1 microscope equipped with Ar ion and He/Ne lasers and a X63 1.4NA PlanApochromatic objective. Three-dimensional images were collected at a Z interval of 0.3 µm. To check image registration, test objects were made by labelling an antigen (ssDNA) with two fluorochromes simultaneously. Line profiles were obtained using built-in functions of the Carl Zeiss software. Pearson’s correlation coefficients were calculated using our own software as described previously (10).

Confocal microscopy and image analysis

For confocal microscopy we used a LSM 410 system (Carl Zeiss, Herts, UK) equipped with Ar ion and He/Ne lasers and a X63 1.4NA PlanApochromatic objective. Three-dimensional images were collected at a Z interval of 0.3 µm. To check image registration, test objects were made by labelling an antigen (ssDNA) with two fluorochromes simultaneously. Line profiles were obtained using built-in functions of the Carl Zeiss software. Pearson’s correlation coefficients were calculated using our own software as described previously (10).

Results

ssDNA is detectable after UV-irradiation in BrdU-substituted cells

The rationale for labelling ssDNA transients is depicted in Figure 1A. Cells were cultured in a saturating concentration of BrdU (30 µM) in order to maximize the incorporation of hapten. The incorporation time was shorter than a complete cell cycle in order to avoid double-strand BrdU substitution in both DNA strands which would limit the formation of pyrimidine dimers (see Introduction). The cells were then released into BrdU-free medium for approximately half a cell cycle to ensure that only one DNA strand was labelled and that labelling was not biased towards a particular phase of the cell cycle. Single-strand labelling and even distribution of BrdU label throughout the cell cycle were confirmed by flow cytometry (Figure 1F and G).

Confocal microscopy sections of UV-irradiated nuclei stained for DNA repair confirmed a punctuated pattern where individual NER sites formed resolution-limited spots (see below and Discussion) (Figure 1B). The nuclear staining, absent in non-irradiated controls (Figure 1C), was observed at UV-C fluencies of 5 J/m² or higher, as shown in Figure 1D and C for 10 and 40 J/m², respectively. For irradiations of 20 J/m² or lower, some cytoplasmic staining was observed, which, although clearly distinguishable from the nuclear staining, prompted us to work with 40 J/m² for easier counting. Staining was specific for UV-C irradiation (<280 nm), as no staining was detected when irradiation was performed in identical conditions, but with a filter to cut off wavelengths shorter than 290 nm (data not shown).

NER proteins co-localize with sites of transient ssDNA

To confirm that the detected ssDNA sites corresponded to NER activity we sought to demonstrate the presence of associated NER proteins. To this end we simultaneously labelled ssDNA sites and either the XPA or the XPG repair proteins. These two proteins were chosen because the monoclonal antibodies available produced detectable signals in the dual-labelling system used, where cross-species absorption of the secondary antibodies reduces the sensitivity. Nonetheless, both XPA and XPG signals were very low and for these proteins streptavidin-biotin amplification was used. As the signals of both the repair proteins and the BrdU-labelled DNA in the dual-label system were significantly lower than in single labelling systems, we believe that not all XPA- or XPG-containing sites (and possibly not all BrdU sites) were labelled. The co-localization of both XPA and XPG proteins with ssDNA sites was strong, as shown in Figure 2A and B, respectively. The yellow dots in the images correspond to the overlap of green (XPA or XPG) and red (ssDNA) dots. The line plots in Figure 2A’ and B’ show the extensive coincidence of green and red peaks. More importantly, the positive central peak in the corresponding Pearson’s correlation plots (rₚ, Figure 2A” and B”’) indicates that the co-localization is statistically significant (see Materials and methods and ref. 10). The widths of the peaks also agree with those expected for the correlation of resolution-limited spots (not shown).

As a negative control we applied an identical analysis to the Ku protein. Ku is involved in the repair of double-strand DNA breaks (11) and immunostaining with a monoclonal antibody against the p80 subunit of Ku showed no co-localization with BrdU-labelled sites (Figure 2C). These results demonstrate that the ssDNA labelling method specifically targets the NER machinery.

Kinetic analysis of ssDNA transients in NER-proficient and deficient cells

Next we evaluated whether the rate of onset of ssDNA-positive nuclei would be indicative of NER proficiency. To this end we compared human skin NDF with skin fibroblasts derived from Xeroderma pigmentosum complementation groups A and C (XPA and XPC) and Cockayne syndrome A and B (CS-A and CS-B). XPA cells are deficient in both NER pathways, whereas XPC and CS-A cells are deficient in GGR and TCR, respectively (3).

NDFs showed a bimodal onset of ssDNA transients (Figure 3, filled circles). This bimodal profile was present in all NER proficient cell lines studied (data not shown). Time courses were never followed beyond ~6 h in order to avoid the possible entry of cells into apoptosis, which might produce false-positive signals due to DNA breakdown. The drop in percentages of positive nuclei observed at 1.5–2 h post-irradiation indicates that ssDNA transients are resolved. Thus, the first peak spans 1–1.5 h after irradiation, which is coincidental with the reported times for both TCR and global removal of 6-4 photoproducts (6-4PPs) (12,13).

Comparison with NDFs, XPC, CS-A and CS-B cells showed significantly reduced percentages of positive nuclei as expected for TCR- and GGR-deficient cells (Figure 3). This observation further confirms the specificity of the method for the detection of sites of active NER. In addition, it is interesting
Fig. 2. Sites of ssDNA co-localize with NER proteins. Dual staining for ssDNA and XPA (A), XPG (B) and Ku p80 (C). In each figure, the image represents a single confocal section of a nucleus stained with FITC (green) for the protein and with Cy3 (red) for ssDNA. Line plots (A', B' and C') indicate the intensity profiles of the red and green images at the indicated horizontal lines. Plots (A'', B'' and C'') correspond to the Pearson's correlation coefficients at different shifts of the green image over the red. Central peaks indicate high correlation when the images are aligned (see Materials and methods). XPA and XPG were detected using biotinylated secondary antibody. Ku was detected with FITC-labelled secondary antibody. Bar: 5 µm.
to note that both TCR-deficient cell lines (CS-A and CS-B) show an almost identical profile where the bimodality is lost. This suggests that the first phase observed in normal and XPC cells is mostly contributed to by TCR. Nevertheless, both CS-A and CS-B cells show a significant number of positive cells at short times (30 min) post-irradiation, which are most probably generated by repair of 6-4PPs (12).

A fraction of nuclei of XPA cells always stained positive for ssDNA transients both in non-irradiated controls and at all time points post-UV (Figure 3, open circles). The relatively high percentage of non-irradiated XPA cells that stain positive suggests that these cells normally display a background level of DNA damage. This is in agreement with the observations of Hurt et al. (14) that XPA cells show naturally existing gaps in their DNA. This also agrees with recent reports that suggest that XPA is not an absolute requirement for the initiation of DNA repair helicase activity. As XPC cells do not have a full complement of helicases and thus cannot proceed to the excision step, this indicates that NER-associated ssDNA transients become detectable as soon as the lesion-containing double-stranded DNA melts, i.e. without requiring excision of the lesion-containing DNA stretch (see Figure 5).

When the staining intensities of XPG cells were compared with those of NDFs or XPB cells a striking difference was found. At 30 min post-UV, NDFs show comparatively low staining (Figure 4B) which is even lower in XPB cells (Figure 4C), whereas a small number of XPG cells show unusually high labelling intensity (Figure 4D). At 4 h post-UV, these differences are more marked, with NDFs showing normal staining, XPB cells maintaining a very low intensity (Figure 4F) and XPG cells showing an extremely high intensity staining (Figure 4G).

As only a fraction of ssDNA-positive XPG cells showed high intensity staining, we reasoned that unresolved 5′ single-strand breaks introduced by the functional XPF might be stalling DNA replication in S phase cells. This would not occur in XPB cells, where DNA continuity is preserved. To test this hypothesis, we arrested NDFs and XPG cells by serum starvation immediately after BrdU incorporation and repeated the time-course experiments following UV irradiation. NDFs showed similar ssDNA intensities 4 h after irradiation in arrested cells as in normally cycling cells (Figure 4H). In contrast, serum starvation had a marked effect on the XPG cells in that high intensity nuclei were absent in arrested XPG cells (Figure 4I). This indicates that the high intensity nuclei observed in normally cycling XPG cells (Figure 4G) correspond to ssDNA lesions generated when S phase nuclei attempt to replicate through unresolved, unilaterally excised NER complexes.

Taken together, these data indicate that the transient ssDNA method detects a number of NER intermediates spanning the DNA melting step through to lesion removal and DNA repair synthesis. Thus, intermediate NER complexes can be detected even when the final outcome may not be the resolution of the UV lesion. The NER steps detected by ssDNA transients are represented in schematic form in Figure 5.

Detection of DNA lesions other than photoproducts

NER of lesions different from UV photoproducts, particularly adducts, can be difficult to evaluate as (i) repair DNA synthesis

### Detection of NER sites

Transient ssDNA comprises several stages of the NER ‘bubble’

The clearly visible staining for ssDNA transients (see for example Figure 1B and D) was surprisingly intense for the proposed model depicted in Figure 1A. With the observation that XPA cells also showed some staining (see above), we hypothesized that the method might be detecting NER stages earlier than the nucleotide excision step and probably as early as the opening of the double strand. To analyse this we repeated the time-course analyses with XPB and XPD cells (deficient in the NER helicases) and XPF and XPG cells (deficient in the NER endonucleases; 1,3).

Figure 4A shows the time-dependent responses of XPB, XPG and XPF cells in comparison with NDFs. Similar results to XPB were obtained for XPD cells (not shown). All mutant cell lines initially showed significantly reduced percentages of ssDNA-positive nuclei, albeit higher than non-irradiated controls. At longer times larger percentages of ssDNA-positive cells were observed, consistent with the accumulation of initiated but unresolved NER complexes, and in agreement with deficiencies in repair helicase or endonuclease activity. As XPB cells do not have a full complement of helicases and thus cannot proceed to the excision step, this indicates that NER-associated ssDNA transients become detectable as soon as the lesion-containing double-stranded DNA melts, i.e. without requiring excision of the lesion-containing DNA stretch (see Figure 5).

As only a fraction of ssDNA-positive XPG cells showed high intensity staining, we reasoned that unresolved 5′ single-strand breaks introduced by the functional XPF might be stalling DNA replication in S phase cells. This would not occur in XPB cells, where DNA continuity is preserved. To test this hypothesis, we arrested NDFs and XPG cells by serum starvation immediately after BrdU incorporation and repeated the time-course experiments following UV irradiation. NDFs showed similar ssDNA intensities 4 h after irradiation in arrested cells as in normally cycling cells (Figure 4H). In contrast, serum starvation had a marked effect on the XPG cells in that high intensity nuclei were absent in arrested XPG cells (Figure 4I). This indicates that the high intensity nuclei observed in normally cycling XPG cells (Figure 4G) correspond to ssDNA lesions generated when S phase nuclei attempt to replicate through unresolved, unilaterally excised NER complexes.

Taken together, these data indicate that the transient ssDNA method detects a number of NER intermediates spanning the DNA melting step through to lesion removal and DNA repair synthesis. Thus, intermediate NER complexes can be detected even when the final outcome may not be the resolution of the UV lesion. The NER steps detected by ssDNA transients are represented in schematic form in Figure 5.

Detection of DNA lesions other than photoproducts

NER of lesions different from UV photoproducts, particularly adducts, can be difficult to evaluate as (i) repair DNA synthesis
may be impaired or (ii) DNA adducts have to be discriminated from their free forms, thus making single-cell analysis difficult. To evaluate the applicability of the ssDNA method to non-UV DNA lesions, we performed time-course analyses of the onset of ssDNA-positive nuclei after treatment with the DNA binding drugs adriamycin and actinomycin D. Figure 6 shows that both treatments produced a clearly detectable response but with markedly different kinetics. In the case of adriamycin (Figure 6, open circles) the response appears to plateau without noticeable reduction to ssDNA-stained nuclei, most probably due to the continuous presence of the drug during the assay. The response of actinomycin D was not detectable until 2 h and appeared noticeable only at longer times (Figure 6, filled circles).

Discussion
The data presented here show that detection of ssDNA transients generated during the NER process is possible and provides a novel and useful approach to study NER. The specificity of the method for NER is demonstrated by (i) the co-localization of ssDNA sites with both the XPA and XPG NER repair proteins, together with the lack of co-localization with the Ku protein and (ii) the reduction in the percentage of ssDNA-positive nuclei in UV-treated cells deficient for NER.

The technique has three features that make it a useful complement or alternative to existing methods for analysis of DNA repair. First, it is applicable to normally cycling cells and does not require cell-cycle arrest or the use of DNA synthesis inhibitors. Secondly, it allows for single-cell analysis, and in particular for high-resolution microscopy for the localization of NER components. Thirdly, it is particularly useful for studying kinetics of NER. The technique monitors the appearance of positively stained nuclei instead of a change (e.g. loss of lesions) relative to a maximum, a technique that is inherently error-prone at initial, low values. For this reason, it is especially useful for kinetic analyses at short time post-DNA damage.

The fluorescent signal detected is surprisingly strong when considering that the ssDNA stretches produced during NER

![Fig. 4. Differential NER response by XPB and XPG cells. (A) Time-course analysis of the ssDNA response by NDFs (filled circles), XPB (triangles up), XPG (open circles) and XPF (triangles down) cells. Conditions are the same as in Figure 3. (B, C and D) Thirty minute post-irradiation staining for ssDNA of NDF, XPB and XPG cells, respectively. (E, F and G) As before, 4 h post-irradiation. (H and I) NDF and XPG cells, respectively, arrested by serum starvation and stained 4 h after UV irradiation. Primed letters correspond to Hoechst-stained nuclei.](image-url)
Detection of NER sites

are estimated to be ~28 nucleotides long (1,3). We reason that this fluorescence intensity is achieved because individual repair sites are clustered in ‘repairsomes’ (17–19), similar to the other nuclear ‘factories’, replicosomes and trans-

scriptosomes. The sizes of transcription and replication factories observed by electron microscopy are in the range of 70 and 100–300 nm, respectively (20,21). Considering that this size range is also likely to be valid for repairsomes, and that confocal imaging followed by deconvolution has a resolution of 200 nm or better, we conclude that we are resolving individual repair factories. As shown in Figures 3 and 4, the method appears to detect the initiation of an NER response, even when the cells are not fully competent for NER. This implies that the supramolecular organization of repair sites into ‘repairsomes’, as discussed above, is already present at the earliest stages of NER.

The fact that ssDNA is produced in repair-deficient cell lines indicates that for full evaluation of repair-proficiency the method should be used in combination with another approach that can account for efficient lesion removal and re-synthesis. Nevertheless, it allows for easy monitoring of the onset of NER in UV-induced and non-UV-induced DNA lesions and can demonstrate differential NER kinetics for these agents. For example, as shown in Figure 6, actinomycin D lesions show slower kinetics, attributable to GGR. This is consistent with preferential binding to ribosomal DNA by this drug, where bulky DNA lesions are repaired by GGR (22).

Because the method responds to pre-resolution NER complexes, it has revealed a significant difference in the consequences of deficiencies in repair helicases, as opposed to endonucleases (Results and Figure 4). Thus, ssDNA stretches accumulate heavily in endonuclease-deficient XPG cells, giving high intensity staining in the individual cells (Figure 4D) (also XPF cells; data not shown). We interpret this to reflect DNA replication clashes with unresolved NER complexes. Helicase-deficient XPB cells (and also XPD cells; data not shown) also initiate NER following UV irradiation, as shown by the time-dependent appearance of ssDNA, although it is known that these cells are highly inefficient at removing UV-generated DNA lesions (Figure 4A). However, as opposed to endonuclease-deficient XPG cells, helicase-deficient cells do not appear to perturb DNA replication. This is shown by the low levels of ssDNA staining displayed by these cells at all times post-irradiation (Figure 4). Volker et al. (15) recently showed that the repairsome is assembled even when some of its components are non-functional, with the possible exception of deficiency in the recognition complex XPC–hHR23B, which appears to be crucial for the assembly of global repairsomes (15,16). This suggests that the phenomenon of increased intensity of ssDNA sites observed in cycling cells (Figure 4) is not due to an incomplete or aberrant formation of the repairsome, but to the status of the damaged DNA when a replication complex is encountered.

It thus appears that replication can proceed in the presence of UV photoproducts (and even in the presence of assembled repair complexes) as long as the continuity of the DNA strands is maintained. This observation implies that the mutagenicity of helicase-deficiency syndromes may be different from that of endonuclease-deficiency. In repair helicase-deficiencies, translesion synthesis appears to be possible and hence mutations will be dependent on the fidelity of this mechanism. In repair endonuclease-deficiencies, replication appears to stall significantly at sites with assembled NER complexes, which may lead to chromosomal aberrations.

Acknowledgements

We are grateful to James Ford, Jørgen Larsen and Mats Ljungman for their helpful comments, to Andrei Okorokov for careful reading of the manuscript.
References


Received May 2, 2001; revised August 8, 2001; accepted August 24, 2001