A processive versus a distributive mechanism of action correlates with differences in ability of normal and xeroderma pigmentosum group A endonucleases to incise damaged nucleosomal DNA

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A DNA endonuclease, isolated from the nuclei of normal human and xeroderma pigmentosum complementation group A (XPA) cells, which recognizes predominately pyrimidine dimers, was examined for the mechanism by which it locates sites of damage on UVC-irradiated DNA. In reaction mixtures with low ionic strengths (i.e. lacking KCl), the normal and XPA endonuclease locate sites of UV damage on both naked and reconstituted nucleosomal DNA by different mechanisms. On both of these substrates, the normal endonuclease acts by a processive mechanism, meaning that it binds non-specifically to DNA and scans the DNA for sites of damage, whereas the XPA endonuclease acts by a distributive one, meaning that it randomly locates sites of damage on DNA. However, while both the normal and XPA endonuclease can incise UVC irradiated naked DNA, they differ in ability to incise damaged nucleosomal DNA. The normal endonuclease showed increased activity on UVC treated nucleosomal DNA compared with naked DNA, whereas the XPA endonuclease showed decreased activity on the damaged nucleosomal substrate. Since a processive mechanism of action is sensitive to the ionic strength of the micro-environment, the KCl concentration of the reaction was increased. At 70 mM KCl, the normal endonuclease switched to a distributive mechanism of action and its ability to incise damaged nucleosomal DNA also decreased. These studies show that there is a correlation between the ability of these endonucleases to act by a processive mechanism and their ability to incise damaged nucleosomal DNA; the normal endonuclease, which acts processively, can incise damaged nucleosomal DNA, whereas the XPA endonuclease, which acts distributively, is defective in ability to incise this substrate.

Introduction

Unraveling the mechanisms by which DNA repair proteins locate and interact with their target sites is of critical importance in understanding the regulation of DNA repair processes in mammalian cells. A protein can locate target sites in DNA in two distinct ways: (i) a distributive mechanism, in which it has no affinity for non-target DNA and locates target sites by a random, three-dimensional diffusion process; or (ii) a processive mechanism, in which it has a non-specific binding capacity for non-target DNA and locates its recognition sites by a one-dimensional scanning or sliding process along the DNA molecule (reviewed in Refs 1–4). A processive mechanism has been observed for a number of proteins, including: Escherichia coli lac repressor (5,6), EcoR1 endonuclease (7,8), BamH1 methylase (9), bacteriophage λcro protein (10), the DNA helicase activity of E.coli recBCD enzyme (11), E.coli DNA polymerase (12–14) and prokaryotic and eukaryotic DNA polymerases (15–19). In addition, several enzymes involved in DNA repair [e.g. T4 endonuclease V (20,21), Micrococcus luteus UV endonuclease (22), the UvrABC nuclease (23) and E.coli and rat liver mitochondrial uracil–DNA glycosylases (24,25)] have been shown to act processively. There is also evidence that this latter mechanism is biologically significant: endonuclease V enhances UV resistance in repair-deficient E.coli, but this property is markedly reduced in mutants of endonuclease V in which the target search mechanism is distributive (26). Whether mammalian endonucleases involved in DNA repair act by a processive or a distributive mechanism has not been investigated, however, nor has whether chromatin structure plays any role in the mechanism by which these repair proteins locate sites of damage.

We have isolated from the nuclei of normal human lymphoblastoid cells several chromatin-associated DNA endonuclease complexes that selectively recognize different types of DNA damage (27–31). We have shown that these complexes contain endonucleases, which produce dual incisions at sites of adducts on damaged DNA (31), as well as damage-recognition proteins (32). These endonuclease complexes have in all likelihood a relationship with the proteins known to be involved in the incision step of nucleotide excision repair (reviewed in Ref. 33). One of the complexes we have isolated recognizes cyclobutane pyrimidine dimers produced by short wavelength ultraviolet (UV*C*) light (29,30) and monoadducts produced by psoralen plus long wavelength (UVA) light (28,31,34). This endonuclease complex displays enhanced activity on both types of damaged DNA when the DNA is assembled into nucleosomes compared to its activity on damaged naked (i.e. non-nucleosomal) DNA (30,34–36). We have also isolated this same complex from cells of patients with the DNA repair deficient, cancer-prone, genetic disease, xeroderma pigmentosum, complementation group A (XPA) (28,34,36). In XPA patients, a direct link has been found between the development of skin cancer and a deficiency in the ability to repair lesions in DNA produced by UV light (reviewed in Refs 37,38). This repair defect lies in the initial, damage recognition and incision step in the repair process (37,38). We have shown that the endonuclease complex isolated from XPA cells has levels of endonucleolytic activity similar to those of the corresponding normal complex on naked DNA containing monoadducts induced by psoralen plus UVA light (28,34). However, in contrast to the normal endonuclease complex, the XPA complex shows decreased activity on psoralen damaged DNA when it has been assembled into nucleosomes (34–36).

*Abbreviations: XPA, xeroderma pigmentosum, complementation group A; UVC, short wavelength ultraviolet light; UVA, long wavelength ultraviolet light.

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In the present paper, we report that these endonuclease complexes from normal human and XPA cells act by different mechanisms on UVC damaged DNA. The normal endonuclease locates sites processively regardless of whether the substrate is damaged naked or damaged nucleosomal DNA. The XPA endonuclease locates sites distributively on both types of damaged DNA and is also defective in its ability to interact with UVC damaged nucleosomal DNA. We further demonstrate that at increasing salt concentrations the normal endonuclease converts from a processive to a distributive mechanism of action and loses the ability to incise damaged nucleosomal DNA. These results show then that there is a correlation between the ability of these endonucleases to act by a processive mechanism and their ability to incise damaged nucleosomal DNA.

Materials and methods

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Analysis of mode of recognition of sites of damage by the endonucleases

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Incision of damaged DNA

Fig. 1. Action of chromatin-associated DNA endonuclease complexes from XPA and normal human cells on UVC light irradiated DNA. Endonuclease activity was assessed on DNA irradiated with 45 J/m² UVC light. These values have had subtracted from them the enzyme activity on undamaged DNA (0.05 ± 0.01 breaks). Vertical lines represent ± SEM of six experiments (two to three extractions from each of two different normal and two different XPA cell lines).

Results

Endonuclease activity on UVC irradiated non-nucleosomal DNA

As we have previously shown for normal human cells (30), XPA cells have a DNA endonuclease complex, pl 7.6, which is active on UVC irradiated DNA and has a level of activity similar to that of the normal complex (Figure 1). Both normal and XPA endonuclease complexes were over three times as active on UVC irradiated naked DNA as they were on undamaged naked DNA. Increasing the dosage of UVC irradiation resulted in a similar increase in the number of incisions produced by the normal and XPA endonucleases on DNA (data not shown). Pretreatment of UVC irradiated plasmid DNA with E. coli photolyase resulted in an ~90% decrease in the activity of the normal and XPA endonucleases (data not shown) consistent with our previous finding (30) that cyclobutane pyrimidine dimers are the predominant type of damage recognized by these endonucleases. Two different normal and two different XPA cell lines were examined and data were obtained from two to three different extractions from each cell line; in all cases the results were the same.

Endonuclease activity on UVC irradiated nucleosomal DNA

The influence of nucleosome structure on the activity of the normal and XPA endonucleases was examined using the reconstituted system that we have previously characterized; it consisted of a plasmid DNA containing the entire SV40 genome and either core or total histones (27,39,45). The number of pyrimidine dimers present in core and total nucleosomal DNA, before reaction with each endonuclease complex, was 4.7 and 4.6, respectively, representing a 10 and 12% reduction compared with damaged naked DNA.

Topological analysis of reconstituted nucleosomes indicated that the number of nucleosomes per DNA molecule increased linearly as the ratio of histone/DNA was increased up to a histone/DNA ratio of 1, at which point ~23 ± 1 nucleosomes (either core or total) formed per DNA molecule (Figure 2A and B). This number remained constant at higher histone/DNA ratios. Examination of endonuclease activity on these UVC irradiated substrates showed that as the number of nucleosomes increased, the normal endonuclease activity increased and that of the XPA endonuclease decreased (Figure 2C and D). When there were 23 core or total nucleosomes present, activity of the normal endonuclease activity was increased 2.3-fold and 1.4-fold, respectively, compared with its activity on damaged naked DNA (Figures 2C and 2D). In marked contrast, on these same substrates the XPA endonuclease activity was increased slightly on damaged core and by ~50% on damaged total nucleosomal DNA (Figures 2C, 2D and 3A). No differences were found in the activities of either the XPA or the normal endonucleases on undamaged core or total nucleosomal DNA compared with undamaged naked DNA (data not shown).

Mechanism of location of target sites on damaged naked DNA

Two methods were utilized for examining the mechanism by which the normal and XPA DNA endonucleases locate sites of damage on naked DNA. These were measurement of: (i) the rate of conversion of form I DNA to form II and form III DNA; and (ii) endonuclease activity in the presence of a UVC irradiated competitor DNA. The basis of the first method is
Fig. 3. Activity of the DNA endonucleases from normal and XPA cells on reconstituted nucleosomal DNA at 0 mM and 70 mM KCl concentrations. The normal (A) and the XPA (B) endonuclease complex were incubated for 180 min with UVC-irradiated naked DNA (0.1 µg) or DNA (0.1 µg) reconstituted at a 1:1 ratio with core or total histones. The reaction solution had a KCl concentration of 0 mM (■) or 70 mM (□). Samples were electrophoresed on 1.0% agarose gels and the number of breaks per DNA molecule determined. Vertical lines represent ± SEM for five experiments (1–2 extractions from each of two different normal and two different XPA cell lines).

Fig. 4. Analysis of the incision activity of the normal and XPA endonucleases on UVC-irradiated naked DNA at 0 mM and 70 mM KCl concentrations. The normal (A and C) and the XPA (B and D) endonuclease complex (0.5 µg) were incubated for the indicated times with plasmid DNA (pWT830/pBR322) (0.1 µg) irradiated with UVC light (250 mW/cm² for 98 s). The reaction solutions contained either 0 mM KCl (A and B) or 70 mM KCl (C and D). The three topological forms of DNA were separated on a 1% agarose gel. The percentage of form I (■), form II (○), and form III (●) DNA in each sample was determined. Vertical lines represent ± SEM for 3–4 experiments (1–2 extractions from each of two different normal and two different XPA cell lines).

that if an endonuclease acts by a processive mechanism of action, it will bind non-specifically to DNA and scan the molecule, incising at each site of damage. Form I DNA will decrease with the concomitant formation of nicked, circular form II DNA and then linear form III DNA, which should increase linearly over time as incisions occur in close proximity on opposite DNA strands (21). If the endonuclease acts by a distributive mechanism of action, a three-dimensional search will occur in which random incisions are made at sites of damage. Form I DNA will decrease with time, but accumulation of form III will lag until enough random breaks are produced to linearize the molecule (21).

As shown in Figure 4A, incubation of the normal endonuclease with UVC irradiated plasmid DNA in reaction mixtures with low ionic strengths (i.e. lacking KCl), resulted in a loss in form I DNA and a linear increase in form III DNA over the period of time examined, which was indicative of a processive mechanism of action. The XPA endonuclease also produced a decrease in form I DNA on this substrate; however, there was little or no accumulation of form III DNA during this time period (Figure 4B). These results indicate that the XPA endonuclease acted in a distributive manner.

The basis of the second method for determination of mode of location of sites of damage is that, if an endonuclease is acting in a processive manner, it will scan a DNA molecule, incising at multiple sites of damage before dissociating and associating with another DNA molecule. The presence of a competitor DNA, added after the endonuclease has associated with its substrate DNA, will therefore have little effect on its activity on the substrate DNA. If the endonuclease acts in a distributive manner, however, it will randomly search and incise both the substrate and competitor DNAs at sites of damage. Therefore, its activity on the substrate will rapidly decrease when a competitor is added since it will be incising the competitor as well. Since the competitor and non-competitor DNAs used in the present study differed in size, they could be readily separated by gel electrophoresis and the three topological forms of each quantitated.

The normal or the XPA endonuclease complex was incubated with the UVC irradiated plasmid DNA for 40 min before addition of the competitor DNA in order to give sufficient time for each complex to associate with the DNA. As shown in Figure 5A, the activity of the normal endonuclease on the UVC irradiated plasmid DNA was the same in the presence and absence of UVC irradiated competitor DNA, which was indicative of a processive mechanism of action. In contrast, the number of incisions produced by the XPA endonuclease on UVC irradiated plasmid DNA decreased after the competitor DNA was added (Figure 5B). After 140 min incubation with the competitor DNA, the activity of the XPA endonuclease on the UVC irradiated plasmid was ~60% of its activity on this substrate when no competitor was present. In addition, the XPA endonuclease cleaved much more of the UVC irradiated competitor DNA than did the normal endonuclease (Figure 5A and B). Even though the method of analysis employed here, which measures only initial cleavages, underestimates the activity of processively acting enzymes, these data indicate that a much smaller proportion of competitor DNA molecules were cleaved (regardless of the number of times each was
Incision of damaged DNA

Fig. 5. Analysis of normal and XPA endonuclease incision of UVC-irradiated naked substrate DNA in the presence of a competitor DNA. The normal (A and C) or the XPA (B and D) endonuclease complex (0.5 µg) were incubated with UVC-irradiated plasmid DNA (pWT830/pBR322) (0.1 µg) for 40 min. A UVC-irradiated competitor DNA (pGM-3zf) (0.1 µg) was then added to the reaction and incubation continued for the indicated times. The reaction solutions contained either 0 mM KCl (A and B) or 70 mM KCl (C and D). Samples were electrophoresed on 1.1% agarose gels to resolve the topological forms of DNA. The number of breaks per DNA molecule that each endonuclease produced on the substrate and competitor DNAs was calculated. Endonuclease activity on: ■, substrate DNA; ○, substrate DNA in the presence of competitor; △, competitor DNA. Vertical lines (A and B) represent ± SEM for four experiments (1–2 extractions from two different normal and two different XPA cell lines). Values (C and D) represent the average of three experiments with the deviation from the mean <5%.

6A and B shows that the number of incisions the normal endonuclease produced, over a period of time, on either UVC-irradiated core or total nucleosomal DNA, was the same as when no competitor was added. The number of incisions produced by the XPA endonuclease on the UVC-damaged nucleosomal substrates was reduced, however, after addition of the competitor DNA (Figure 6C and D). After 140 min the number of incisions created on UVC-irradiated core or total nucleosomal DNA when the competitor was present was only ~60% and 50%, respectively, of the number produced on either type of damaged nucleosomal DNA in the absence of the competitor. These data also show that the number of UVC-irradiated competitor DNA molecules cleaved by the XPA endonuclease was ~2.5 times greater than those cleaved by the normal endonuclease (Figure 6C and D versus 6A and B). These results indicate that the XPA endonuclease is locating sites of damage on nucleosomal DNA in a distributive manner and the normal endonuclease complex in a processive manner, just as they do on damaged non-nucleosomal DNA. All of these studies using competitor DNA were repeated at least four times using at least two different normal and XPA endonuclease extractions.

When the mechanism of location of target sites by the normal and XPA endonucleases was examined utilizing the assay in which formation of form III DNA was measured, the results were the same as those shown in Figure 4A and B for UVC-damaged naked DNA (data not shown). These results
are again indicative that nucleosome structure does not affect the mechanism by which the normal and XPA endonucleases locate sites of damage on DNA.

Influence of KCl concentration on mechanism of location of sites of damage

Enzymes that act in a processive manner have been reported to switch to a distributive one when the KCl concentration is increased (20,21,26). When the KCl concentration was raised to 70 mM, the normal endonuclease produced an increased rate of loss of form I DNA and a delayed accumulation of form III DNA (Figure 4C) indicating that such a switch had occurred. This conversion to a distributive mechanism of action was also detected by addition of a UVC irradiated competitor DNA. The normal endonuclease produced fewer incisions on damaged naked DNA (Figure 5C), or core or total nucleosomal DNA (data not shown) in the presence of the competitor than in its absence. The mechanism of target search by the XPA endonuclease remained distributive at 70 mM KCl (i.e. there was no change in results obtained, using either of the two types of assay methods, compared with those obtained at 0 mM KCl) (Figures 4D and 5D).

Increasing the KCl concentrations has also been shown to differentially affect the activity of enzymes that act in a processive versus a distributive manner (21,26). This was found to be true for the normal and XPA endonucleases. At a KCl concentration of 70 mM, the number of incisions made by the normal endonuclease on UVC irradiated naked DNA was ~1.4-fold greater than when the KCl concentration was 0 mM (Figure 3A), whereas incisions made by the XPA endonuclease were only ~40% of those produced at 0 mM KCl (Figure 3B). At this KCl concentration, incisions produced by the normal endonuclease on core and total nucleosomal DNA decreased to ~90% and 45%, respectively, of those produced on damaged naked DNA (Figure 3A) while the XPA endonuclease produced no incisions on these substrates (Figure 3B).

Discussion

The present results show that normal and XPA cells have endonucleases that locate sites of damage in DNA by different mechanisms and support a model in which the normal endonuclease acts by a processive mechanism and the XPA endonuclease by a distributive one. This conclusion is based on the following three observations. First, interaction of the normal endonuclease with UVC irradiated DNA results in an increase in form III DNA, with a concomitant loss of form I DNA, which is indicative of a processive mechanism of action. The XPA endonuclease, on the other hand, produces little or no accumulation of form III DNA, while form I DNA decreases, indicative of a distributive mechanism of action. Second, when a UVC irradiated competitor DNA is added, the normal endonuclease remains associated with and continues to incise its damaged substrate, producing few incisions on the competitor. The XPA endonuclease, however, shows a decrease in incisions generated on its damaged substrate as it produces incisions on the competitor DNA. These findings are again indicative of processive and distributive mechanisms of action, respectively. Third, when the KCl concentration is raised to 70 mM, the normal endonuclease switches from a processive to a distributive mode of action. This sensitivity to KCl concentration is characteristic of proteins exhibiting a processive mechanism of action (8,11,19–21,25). We have also found that an endonuclease complex, pI 4.6, which recognizes psoralen plus UVA induced DNA interstrand cross-links (28,31,36), acts processively, whereas the XPA complex acts distributively (unpublished observations). Taken together, these results indicate that these mechanisms for location of target sites may be general ones used by normal and XPA endonucleases involved in repair of different types of DNA damage.

Both the normal and XPA endonucleases continued to use the same mechanism of action (i.e. processive and distributive, respectively) on damaged nucleosomal DNA as they did on damaged naked DNA. In addition, there is a correlation between a distributive mechanism of action and decreased ability to interact with damaged nucleosomal DNA. The evidence for this is of two types. First, the XPA endonuclease, which acts distributively, has markedly decreased ability to incise damaged nucleosomal DNA (almost three-fold on damaged core nucleosomal DNA) compared with the normal endonuclease. Second, when the normal endonuclease is induced to act in a distributive manner (i.e. at 70 mM KCl), it also shows reduced ability to interact with damaged nucleosomal DNA.

In the normal endonuclease complex, processivity may be mediated by the endonuclease itself, as is done by T4 endonuclease V (20,21), M.luteus UV endonuclease (22) and EcoRI endonuclease (7,8), or by a separate accessory protein found to be true for the normal and XPA endonucleases. At a KCl concentration of 70 mM, the number of incisions made on damaged naked DNA (Figure 5C), or core or total nucleosomal DNA (data not shown) in the presence of the competitor than in its absence. The mechanism of target search by the XPA endonuclease remained distributive at 70 mM KCl (i.e. there was no change in results obtained, using either of the two types of assay methods, compared with those obtained at 0 mM KCl) (Figures 4D and 5D).

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The present results show that normal and XPA cells have endonucleases that locate sites of damage in DNA by different mechanisms and support a model in which the normal endonuclease acts by a processive mechanism and the XPA endonuclease by a distributive one. This conclusion is based on the following three observations. First, interaction of the normal endonuclease with UVC irradiated DNA results in an increase in form III DNA, with a concomitant loss of form I DNA, which is indicative of a processive mechanism of action. The XPA endonuclease, on the other hand, produces little or no accumulation of form III DNA, while form I DNA decreases, indicative of a distributive mechanism of action. Second, when a UVC irradiated competitor DNA is added, the normal endonuclease remains associated with and continues to incise its damaged substrate, producing few incisions on the competitor. The XPA endonuclease, however, shows a decrease in incisions generated on its damaged substrate as it produces incisions on the competitor DNA. These findings are again indicative of processive and distributive mechanisms of action, respectively. Third, when the KCl concentration is raised to 70 mM, the normal endonuclease switches from a processive to a distributive mode of action. This sensitivity to KCl concentration is characteristic of proteins exhibiting a processive mechanism of action (8,11,19–21,25). We have also found that an endonuclease complex, pI 4.6, which recognizes psoralen plus UVA induced DNA interstrand cross-links (28,31,36), acts processively, whereas the XPA complex acts distributively (unpublished observations). Taken together, these results indicate that these mechanisms for location of target sites may be general ones used by normal and XPA endonucleases involved in repair of different types of DNA damage.

Both the normal and XPA endonucleases continued to use the same mechanism of action (i.e. processive and distributive, respectively) on damaged nucleosomal DNA as they did on damaged naked DNA. In addition, there is a correlation between a distributive mechanism of action and decreased ability to interact with damaged nucleosomal DNA. The evidence for this is of two types. First, the XPA endonuclease, which acts distributively, has markedly decreased ability to incise damaged nucleosomal DNA (almost three-fold on damaged core nucleosomal DNA) compared with the normal endonuclease. Second, when the normal endonuclease is induced to act in a distributive manner (i.e. at 70 mM KCl), it also shows reduced ability to interact with damaged nucleosomal DNA.

In the normal endonuclease complex, processivity may be mediated by the endonuclease itself, as is done by T4 endonuclease V (20,21), M.luteus UV endonuclease (22) and EcoRI endonuclease (7,8), or by a separate accessory protein found to be true for the normal and XPA endonucleases. At a KCl concentration of 70 mM, the number of incisions made T4 DNA polymerase on damaged DNA (Figure 3A) while the defective in the XPA endonuclease complex (Lambert et al., XPA endonuclease produced no incisions on these substrates in preparation). Moreover, we have also shown that in cells derived from patients with Fanconi anemia, complementation group A, the endonuclease complex, pI 4.6, which has specificity for DNA interstrand cross-links, has a defective damage-recognition protein (32), but nonetheless both acts processively (Lambert et al., unpublished observations) and interacts as does its normal counterpart with damaged nucleosomal DNA (40). Since the XPA gene encodes a protein that preferentially binds to DNA containing UVC induced 6–4 photoproductase by a distributive one. This conclusion is based on the following three observations. First, interaction of the normal (49,50), it may be that processivity and nucleosomal interaction, both of which are defective in the XPA complexes, are not mediated by the XPA protein. It is thus possible that more than one gene may be affected in XPA, which is consistent with the model of ‘co-recessive inheritance’, proposed by us (51,52).

Our finding that XPA endonucleases can incise UVC damaged naked but not nucleosomal DNA is in agreement with studies of Mortelmans et al. (53) and Kano and Fujiwara (54). In apparent disagreement with our findings, several studies, which have utilized an in vitro repair system involving whole cell extracts and reaction buffers containing 70 mM KCl, have concluded that XPA extracts are unable to carry out repair synthesis on UVC damaged naked DNA (55–57). Two factors may account for this disagreement. First, whole cell extracts, which have been shown to reconstitute naked DNA into structures which resemble nucleosomes (58), were used in these experiments. Though the percentage of nucleosomes formed in those systems may be low (57), our results show that the XPA endonuclease has decreased activity, especially
when compared to the normal activity, on UVC damaged DNA when only a few nucleosomes are present. Second, our results indicate that at 70 mM KCl the XPA endonuclease shows reduced activity on damaged naked DNA and no activity at all on damaged nucleosomal DNA, compared with the normal endonuclease that has activity on all these substrates. Thus the possible presence of nucleosomes as well as the presence of 70 mM KCl in the reactions could account for the decreased levels of repair synthesis by XPA cell extracts, compared with normal extracts observed by other investigators (55,56). In another study, in which 70 mM KCl was not added, normal cell extracts showed decreased ability to carry out repair synthesis on UVC irradiated SV40 chromosomes compared with naked DNA (59), which again disagrees with our findings. However, that system included at the start of the reaction an undamaged naked competitor DNA. We have found that addition of such a competitor at the start of our reactions also results in decreased activity of the normal endonuclease on damaged nucleosomal compared with damaged naked DNA (unpublished observations). Since the normal endonuclease acts processively, it initially binds non-specifically to DNA and this binding would be expected to occur on competitor DNA as well as on nucleosomal DNA, thus accounting for the reduced endonuclease activity observed on damaged nucleosomal DNA when an undamaged competitor is present. Although the physiological salt concentration of a mammalian cell is >70 mM (60), it is quite possible that in vivo the normal endonuclease acts by a processive mechanism. T4 endonuclease V has been shown to act processively in vivo under normal physiological conditions, although the E. coli intracellular ionic concentration is considerably greater than 40 mM, above which this enzyme acts distributively in vitro (21). The presence of other cellular proteins, monovalent anions other than chloride, as well as fluctuations in binding and/or compartmentalization of ions such as Na+ and K+ could account for the in vivo and in vitro differences observed (61,62). In addition, it should be noted that Cl− is not the major monovalent anion present in cells and at NaCl or KCl concentrations in which Na+ or K+ levels are similar to those found in the cell, the Cl− levels are well above the physiological range and are detrimental to protein–DNA interactions (61,62). Since processivity is dependent upon electrostatic interactions between a protein and DNA, local changes in the ionic environment could potentially regulate whether an enzyme acts processively or distributively and these changes in turn could ultimately affect the ability of the enzyme to interact with damaged nucleosomal DNA.

A processive scanning capability of a repair protein has been proposed to significantly increase the rate and efficiency of the repair process. The importance of this mechanism for DNA endonuclease involved in repair is demonstrated by a study of Dowd and Lloyd (26) in which mutants of T4 endonuclease V, containing site-specific amino acid substitutions, showed loss of ability to act processively. These mutant enzymes had catalytic activity similar to that of the wild-type enzyme, but lacked ability to restore survival of repair-deficient E. coli after UVC irradiation. The present study shows that lack of processivity of a human DNA endonuclease correlates with a defect in its ability to incise UVC damaged nucleosomal DNA. This in turn correlates with increased sensitivity of XPA patients to the deleterious effects of UV radiation (37,38). Thus a processive mechanism of action appears to be biologically important in both E. coli and man.

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