Nucleotide excision repair (NER) was measured in human cell extracts incubated with either supercoiled or linearized damaged plasmid DNA as repair substrate. NER, as quantified by the extent of repair synthesis activity, was reduced by up to 80% in the case of linearized plasmid DNA compared with supercoiled DNA. An excess of undamaged linearized plasmid in the repair mixture did not interfere with DNA repair synthesis activity on a supercoiled damaged plasmid, indicating a cis-acting inhibiting effect.

In contrast, gaps on circular or linearized plasmids were filled in identically by the DNA polymerases operating in the extracts. When the extent of damage-dependent incision activity was measured, a ~70% reduction of repair incision activity by human cell extract was observed on linearized damaged plasmids. Recessed, protruding, or blunt ends were similarly inhibitory.

NER activity was partly restored when the extracts were preincubated with autoimmune human sera containing antibodies against the nuclear DNA end-binding heterodimer Ku. In addition, the inhibition of repair activity on linear damaged plasmids was released in extracts from rodent cells deficient in Ku activity but not in extracts from murine acid cells devoid of Ku-associated DNA-dependent kinase activity.

The genetic integrity of living organisms depend on their capacity to repair DNA damage produced by endogenous and exogenous agents. Among various repair mechanisms, nucleotide excision repair (NER) plays a major role by repairing a wide range of lesions in DNA, including UV photoproducts and base modifications by many carcinogenic or chemotherapeutic agents (1). The sequence of the NER process is conserved from bacteria to humans and consists of two broad steps: 1) lesion recognition, strand incision, and damaged oligonucleotide displacement, and 2) gap filling by DNA polymerization and ligation. Some 15–20 polypeptides might participate in the first sequence of the reaction in humans (2, 3). Significant insight into the NER mechanism has been obtained from the use of in vitro assays that reproduce the repair reaction by mixing protein extracts from mammalian cells with plasmid DNA bearing lesions like UV-C photoproducts or DNA adducts of cisplatin or acetylaminofluorene (4, 5). For example, it has been reported that the fragment excised by human cell-free extracts ranged in length from 27 to 29 nucleotides (6) and has 5'-phosphate and 3'-OH termini corresponding to enzymatic hydrolysis of mainly the 21st to 25th and the 3rd to 5th phosphodiester bonds, 5' and 3' to the lesion, respectively (7). These data were obtained by analyzing the released damaged oligonucleotide in a repair excision assay. Using a repair synthesis assay, a similar value was found when the length of the repair patch was estimated from the incorporation of radioactive label into plasmid DNA during the resynthesis step (8, 9). Repair synthesis requires the proliferating cell nuclear antigen (PCNA) that is probably loaded onto DNA by replication factor C, as shown recently in a reconstituted resynthesis reaction with partially purified components (10, 11). Although the DNA polymerase involved is thus PCNA-dependent, whether polymerase δ, polymerase ε, or both participate in the repair synthesis step is still unclear (12, 13).

It is commonly admitted that the incision/excision of the damaged DNA represents the limiting step of the repair reaction, since it is defective in most of the UV-sensitive mutant rodent cell lines; in addition, the cell lines of complementation groups A to G representative of the human cancer prone disease xeroderma pigmentosum (XP) exhibit a defect in NER that relies also on the recognition/incision step of the repair pathway (1).

Although the initial step in the repair process is limiting, there might be some situations where the gap-filling step could be impeded. In vitro studies concerning DNA polymerization with purified components of mammalian DNA polymerases δ and ε have shown that a circular structure of the double-stranded gapped template was critical for interaction of PCNA with DNA so that linear DNA could not serve as an efficient substrate (14). Since linear DNA has also been shown to be poorly repaired by NER both in vivo and in vitro with cell extracts (15, 16), it has thus been suggested that the impairment of NER on linear DNA might be due to the inability of the PCNA-dependent DNA polymerase concerned to perform the gap-filling step (16). However, the damage-dependent incision efficiency has not been determined in these repair experiments. On the other hand, we have shown recently that an impairment of both repair incision and DNA resynthesis activities could explain an NER defect, as observed in protein extracts of peripheral blood lymphocytes from healthy donors (17).

Here, we explore the precise nature of the inhibition of NER on a linear damaged plasmid substrate, and we show that it is due to negative interactions of repair proteins with DNA end-binding factors present in mammalian cell protein extracts.

**MATERIALS AND METHODS**

**Cell Lines**

Epstein-Barr virus-immortalized human lymphoblastoid cell line GM2253D (XP group D) was obtained from the NIGMS (National Institutes of Health) Genetic Mutant Cell repository (Coriell Institute, 31077 Toulouse, France).
Excision Repair and Double Strand Breaks

In Vitro Repair Reactions

Repair Synthesis Assay—Except as otherwise indicated, standard 50-μl reaction mixtures contained 200 ng each of damaged plasmid and untreated pHM plasmid, both linearized or supercoiled as indicated, cell extract protein (typically 200 μg) and 74 kBq of [\(\alpha\)-\(^{32}\)P]dCTP (110 TBq/mmol, Amersham Corp.) in reaction buffer containing 45 mM Hepes-KOH (pH 7.8), 7.4 mM MgCl\(_2\), 0.9 mM dithiothreitol, 0.4 mM EDTA, 60 mM potassium glutamate, 2 mM ATP, 20 μM each dGTP, dATP, and dTTP, 4 μM dCTP, 40 mM phosphocreatine, 2.5 μg of creatine phosphokinase (Type I, Sigma), 3.4% glycerol, and 18 μM of bovine serum albumin as described (5). Plasmid DNA was purified from reaction mixtures as described (5), linearized with EcoRI or HindIII as indicated, and electrophoresed overnight on a 1% agarose gel containing 0.5 μg/ml ethidium bromide. When necessary, plasmid DNA was recovered according to the same purification protocol, except that the linearization step was omitted.

Inversion Assay—Standard 50-μl reaction mixtures contained 200 ng each of damaged plasmid and untreated pHM plasmid, both linearized or supercoiled as indicated, cell extract protein (typically 200 μg), and the reaction buffer as above except that deoxyribonucleotides were omitted and 4.5 μM aminohydrolin was included as described (26). The reaction was carried out at 30 °C for 2 h. The reaction was stopped by the addition of EDTA to 25 mM, and the mixture was treated with 200 μg/ml proteinase K (37 °C, 1 h). The mixture was incubated in the presence of 0.5% SDS. Plasmid DNA was purified by phenol-chloroform extraction under gentle mixing conditions and ethanol-precipitated. DNA was then incubated for 10 min at 20 °C in 10 μl of reaction mixture containing 90 mM Hepes-KOH (pH 6.6), 10 mM MgCl\(_2\), 74 kBq of [\(\alpha\)-\(^{32}\)P]dCTP (110 TBq/mmol, Amersham), 2 mM dithiothreitol, 20 μM each dGTP, dATP, and dTTP, 2 μM dCTP, and 1 unit of E. coli DNA polymerase I large fragment (Life Technologies, Inc.). The reaction was stopped by adding EDTA to 50 mM and unlabeled dCTP to 1 mM. The mixture was treated with 50 μg/ml of bovine pancreatic ribonuclease A (37 °C, 10 min). DNA was purified by phenol-chloroform extraction, ethanol-precipitated, and then linearized with EcoRI or HindIII and electrophoresed overnight on a 1% agarose gel containing 0.5 μg/ml ethidium bromide.

Quantification of Repair—Data were quantified by densitography, scintillation counting of excised DNA bands, and densitometry of the photographic negative of the gel to normalize for plasmid DNA recovery in each reaction sample (Scanning Laser Densitometer, Biocom, France).

RESULTS

Comparison of DNA Repair Synthesis in Vitro on Linearized versus Supercoiled Plasmid DNA—In order to compare the repair efficiency in vitro on a damaged linear or supercoiled plasmid DNA substrate, a repair synthesis reaction with HeLa cell-free extracts was performed with each form of a UV-irradiated plasmid DNA mixed with the corresponding linear (L) or supercoiled (SC) undamaged control plasmid (Fig. 1A). When NER operated on supercoiled DNA, conversion of superhelical plasmid molecules to relaxed covalently closed circular DNA (CC) occurred in the presence of cell extracts as well as preferential nicking (OC) of the UV-treated plasmids, as judged from the stained agarose gel (Fig. 1B, lane 1); linear plasmids were not degraded during the 2-h incubation time, and part of the DNA was converted to higher molecular weight species that we have identified as homodimers or mixed plasmid dimers (M, Fig. 1B, lane 2). An identical multimerizing activity has already been described with HeLa nuclear extracts (27); the similarity with the activity we observed was confirmed because in both cases the joining reaction was accompanied by changes in sequence at the junction, since these dimers could no longer be digested by the restriction enzyme used initially to linearize the plasmids (Fig. 1C, lane 2) nor other enzymes cutting within the same multiconing site (data not shown).

When repair synthesis was assessed by the yield of specific DNA bands, densitometry of the photographic negative of the gel to normalize for plasmid DNA recovery in each reaction sample (Scanning Laser Densitometer, Biocom, France).
mobility nor DNA synthesis as judged by the identical radiolabeling in the linear undamaged plasmid as compared with the supercoiled homolog DNA.

In order to quantify the repair synthesis inhibition on linear DNA, we next performed various repair experiments with variable repair protein concentrations and incubation times (Fig. 2, A and B). For 100–300 µg of extract protein, the inhibition extends from 65 to 80% (Fig. 2A). While the radiolabel incorporation in the supercoiled damaged plasmid was linear up to 60 min of incubation time, it had already reached a plateau at 30 min for the linear substrate, leading to a 80% inhibition of repair synthesis at 120 min (Fig. 2B). Under the same repair conditions, an identical repair inhibition was found with linear DNA substrates treated with cisplatin or 8-methoxypsoralen (data not shown).

The distribution of repair synthesis patches along the DNA molecule was addressed using restriction nuclease digestion for both supercoiled and linear UV-irradiated plasmid substrates (Fig. 3A). In both cases, the analysis of five fragments (ranging from 166 to 1111 bp) showed that the radiolabel incorporation increased linearly with the DNA fragment size (Fig. 3B), corresponding broadly to an even distribution of repair patches along the plasmid DNA molecule.

**DNA Repair Synthesis In Vitro on Linearized Plasmid DNA**—The proliferating cell nuclear antigen (PCNA) is involved in the DNA synthesis step of the NER process in vitro as a cofactor of DNA polymerase δε (9, 28). It has been described with purified components that the loading of PCNA on linear DNA was impeded but that an excess of PCNA protein could partly overcome this defect (14). We have then added an excess of PCNA purified from calf thymus (gift of Dr. Ulrich Hübsher, Zürich, Switzerland) up to 900 ng with no effect on the repair synthesis extent on either linear or supercoiled DNA (data not shown).

In order to determine if the NER process on linear damaged DNA was blocked at the synthesis stage, we first compared the synthesis efficiency catalyzed by cell-free extracts in the presence of a small gap on linear or circular plasmid DNA (Fig. 4A). Contrary to the repair synthesis inhibition reported above, the extracts yielded equivalent radiolabel incorporation on both forms of gapped DNA. In order to mimic more closely the repair synthesis reaction, we next performed the following experiment; supercoiled plasmids damaged by various agents (UV light, cisplatin, or 8-methoxypsoralen) were incised in the presence of HeLa whole cell extracts, and the incised intermediates were purified (see "Incision Assay"). Half of the incised plasmids was linearized, while the other half was mock-treated under the same linearization conditions, and then each sample was radiolabeled in the presence of incision-deficient but DNA synthesis-proficient XPD cell extracts, as described elsewhere (17). The DNA synthesis that occurred under these conditions has been shown to reflect the repair synthesis step of the NER process (17). No significant difference was observed with the three damaging agents in the DNA synthesis by XP extracts on linear or circular preincised plasmid substrates (Fig. 4B).

Taken together, the above results suggested that the inhibition of the NER process on linear DNA relied rather on the incision/excision step.

**Comparison of DNA Repair Incision Activity In Vitro on**
Linearized versus Supercoiled Plasmid DNA—In order to analyze the first stage of the NER reaction independently from the polymerization and ligation steps, we had previously set up an assay in which DNA repair synthesis was blocked in a mixture of cell-free extracts with damaged and undamaged plasmid DNA; the purified incised intermediates were then quantitatively radiolabeled by the large fragment of *E. coli* DNA polymerase I (Klenow polymerase) (26, 29). We applied this method to the quantification of repair incision activity by HeLa extracts on linear DNA as compared with supercoiled plasmid (Fig. 5). For the three DNA-damaging agents tested (8-methoxypsoralen and cisplatin in Fig. 5A and UV-C light in Fig. 5B) the radiolabeling was obviously decreased for the damaged plasmids incised in a linear form (Fig. 5, A and B). As a control experiment, we tested that radiolabeling by Klenow polymerase per se was insensitive to whether the plasmid DNA substrate was in linear or circular form (data not shown). The data clearly indicated an inhibition of the HeLa extract incision activity on linear substrates with a residual activity below 40% under these repair conditions (Fig. 5C). Moreover, recessed, protruding, or blunt ends were similarly inhibitory, although the unspecific radiolabel incorporation in both damaged and undamaged plasmids by Klenow polymerase was lower in the presence of recessed free ends.

FIG. 3. Distribution of DNA repair synthesis patches. A, 300 ng each of UV-irradiated pBS plasmid either supercoiled or linearized with *Hind*III were incubated under standard repair synthesis conditions for 2 h at 30 °C with 150 μg of HeLa protein extract. Plasmids were then purified and restricted first with a mixture of *Sma*I and *Hin*III, which cut within the polycloning site upstream and downstream the *Hind*III site, respectively, allowing the loss of the unspecific radiolabel at the *Hind*III ends. Then both plasmids were linearized with *Dde*I. The five fragments produced were separated on a 10% polyacrylamide gel. The bands were excised from the dried gel, and the radioactivity was counted. A, autoradiography of the dried gel. Lane 1, supercoiled UV-irradiated pBS plasmid; lane 2, UV-irradiated pBS linearized with *Hind*III. Lane 2 was exposed longer in order to compensate for the lower incorporation. B, quantification of data from A.

FIG. 4. DNA synthesis with human cell extracts on linearized plasmid DNA. A, DNA synthesis with HeLa cell extract on gapped linear or circular plasmid. 200 ng each of gapped pBS and control pHM plasmids were incubated under standard repair synthesis conditions for 2 h at 30 °C with HeLa protein extracts as indicated. Before incubation, both gapped and control plasmids were either in a circular form or linearized with EcoRV. The figure shows femtomoles of dCMP incorporated into plasmid DNA. For each sample, incorporation was normalized for the amount of DNA recovered. B, DNA synthesis with repair-deficient XP-D cell extract on purified preincised damaged plasmids. Damaged pBS plasmid (200 ng for UV and cisplatin (CDDP) treatments or 350 ng for 8-methoxypsoralen (8-MOP) treatment) and 200 ng of untreated pHM control plasmid were incubated for 2 h at 30 °C with 200 μg of HeLa protein extract. Incubation was carried out in the absence of added dNTP and with aphidicolin under standard repair incision conditions. Each sample was incubated in duplicate. After purification under the standard procedure, one sample of plasmid- incised intermediates was linearized with EcoRV, and the other was mock-treated. After phenol-chloroform extraction and ethanol precipitation, each DNA sample, either in a circular (C) or linear (L) form, was incubated with 150 μg of XP-D protein extract under standard repair synthesis conditions with [γ-32P]dCTP for 2 h at 30 °C. Plasmids were then purified, linearized with EcoRV, and electrophoresed. Top, photograph of the ethidium bromide-stained agarose gel; bottom, autoradiograph of the dried gel.

FIG. 5B, lane *KpnI*. In addition, the location of the linearization cut does not affect the repair inhibition, since repair was similarly inhibited on UV-damaged pBS plasmid linearized at the *Sca*I site located 1.1 kilobase pairs upstream from the polycloning site (data not shown).

Involvement of Ku Protein in NER Inhibition on Linear DNA in Vitro—Total linear damaged and control plasmid DNA in the repair mixture contributed 0.36 pmol of free ends. This number of DNA termini was not sufficient to account for the repair inhibition, since 2-fold this number of DNA ends in the mixture as linearized undamaged plasmid did not inhibit the repair reaction on supercoiled damaged DNA (Fig. 6). Therefore, the repair inhibition was most probably a cis-acting phenomenon related to the presence of the DNA lesions on the linear repair substrate.

In order to explain this poor repair on linear DNA, we hypothesized a negative cis-acting effect between DNA end binding proteins and repair proteins on the same DNA molecule. Since Ku nuclear heterodimer represents the major DNA end
inhibition on linear DNA (Fig. 7). As compared with control sera (a kind gift from Dr. J. Hardin, Columbia Medical College, Augusta, GA), we explored the potential role of Ku in the repair reaction. Ku was initially discovered as an autoantigen in patients with autoimmune diseases (31). Human sera from these individuals contain large amounts of autoantibodies to both subunits of Ku, and they have been shown to inhibit Ku DNA binding activity in vitro (32). Using anti-Ku human antisera (a kind gift from Dr. J. Hardin, Columbia Medical College, Augusta, GA), we explored the potential role of Ku in the repair reaction on linear DNA (Fig. 7). As compared with control reactions without serum or with a human control antiserum (anti-ribonucleoprotein), two anti-Ku sera from two unrelated individuals partly reconstituted the damage-specific repair signal in the linear substrate (Fig. 7, lanes L, in the presence of sera anti-Ku 1 and 2); repair synthesis increased on the linear damaged plasmid, while unspecific incorporation decreased on the linear undamaged plasmid, leading to a 2.5-fold increase in repair activity on the linear UV-damaged plasmid (the damage-dependent incorporation in linear UV-damaged pBS plasmid yielded 50 and 129 fmol of dCMP incorporated in the presence of control and anti-Ku sera, respectively). The stimulating effect of the anti-Ku sera was restricted to repair on the linear DNA, since we observed no significant change in the repair activity on the supercoiled plasmid (Fig. 7, lanes SC). However, even in the presence of anti-Ku antibodies, the repair efficiency on linear DNA remained below the repair activity on supercoiled plasmid (129 and 268 fmol of dCMP incorporated, respectively), observed no significant change in the repair activity on the linear undamaged plasmid (129 fmol of dCMP incorporated, respectively), possibly due to incomplete inhibition of the abundant nuclear Ku activity.

In order to overcome the limitation of the use of antibodies, we then performed similar experiments with extracts from Ku-deficient mutant cells. Mutant CHO cell lines belonging to

![Figure 5](image_url)

**Fig. 5.** Damage-dependent DNA incision with HeLa cell extracts on linearized versus supercoiled plasmid DNA. A, UV-damaged pBS (UV) and untreated pHM plasmids were linearized by various restriction enzymes as indicated. 200 ng each of pBS and pHM plasmids, both in either linear (L) or control circular (CC) form, were incubated for 2 h at 30 °C with 200 µg of HeLa protein extract under standard repair incision conditions. After purification, plasmids were radiolabeled in the presence of Klenow polymerase (1 unit, 10 min, 20 °C). Plasmids were then purified, linearized with EcoRV, and electrophoresed. Top, photograph of the ethidium bromide-stained agarose gel; bottom, autoradiograph of the dried gel. B, 200 ng each of pBS damaged with 8-methoxypsoralen (8-MOP) or cisplatin (CDDP) and untreated pHM control plasmids (both in either linear (L) or circular (CC) form) were incubated in an incision reaction as described in part A. C, quantification of the incision activity in linear as compared to supercoiled damaged plasmid DNA. The figure shows the ratio of femtromoles of dCMP incorporated into linear versus supercoiled damaged plasmids by Klenow polymerase. For each sample, incorporation was normalized for the amount of DNA recovered, and the amount of background incorporation in pHM control plasmid was subtracted from incorporation in pBS plasmid. Values are the mean of three experiments, with error bars representing S.D.
IR-sensitive group 5 lack Ku activity, while this activity is restored to normal level in resistant revertant lines (33, 34). We next compared repair activity on linear and circular plasmid DNA in the presence of nuclear extracts from CHO-K1, xrs6, and xrs6rev cell lines (parental, IR-sensitive, and revertant cell lines, respectively) (Fig. 8A). Repair activity by the extracts from the parental cell lines showed a 50% inhibition on linear plasmid damaged with various agents as compared with circular DNA. However, linear and circular damaged plasmids were nearly identically repaired by the Ku-deficient xrs6 extracts. In contrast, the Ku-proficient extracts from the revertant xrs6rev cell line showed the same 50% inhibition of repair activity on linear DNA as the extracts from the parental line.

Once bound to DNA, Ku has been shown to recruit a 450-kDa polypeptide to form the DNA-dependent protein kinase (DNA-PK), which can then phosphorylate various proteins in the vicinity (30). We thus decided to examine whether Ku and DNA-PK as the ultimate protein responsible for the repair inhibition that we observed on linear DNA. Since it was demonstrated recently that extracts from the mouse scid cell line lacked DNA-PK activity but were Ku-proficient (33–35), repair activity by nuclear extracts from parental BALB/c and scid mouse cell lines was assessed on linear DNA as compared with linear plasmid substrate (Fig. 8B). An identical 50–55% repair inhibition on linear DNA was found with each DNA-PK-proficient and DNA-PK-deficient extract.

**DISCUSSION**

Here, we show that the NER process catalyzed in vitro with mammalian cell protein extracts is strongly inhibited on linear plasmid substrate. Legerski et al. (15) have already reported that UV photoproducts in plasmid DNA microinjected in frog oocytes were repaired 50 times more rapidly in circular molecules than in linear DNA. It has also been observed that linearized DNA was a poor substrate when repair synthesis was carried out in vitro (16). However, our results extend these observations, since we demonstrate that this inhibition does not rely on an impaired DNA repair synthesis activity but results from a reduced NER incision activity on linear damaged DNA. Huang and Sancar (36) concluded that the efficiency of DNA adduct removal was the same from linear or closed circular DNA by analyzing the release of radiolabeled fragments carrying the lesions in the presence of HeLa cell-free extract. However, 1) their experiments were carried out with a small amount of protein extract (100 μg) due to the interfering degradation of the excised oligonucleotide (such protein concentration shows only a modest repair inhibition on linear DNA) (Fig. 2A); and 2) most of the given efficiencies for the release of the damaged oligonucleotide from linear DNA fragments ranging from 164 to 618 bp were about 50% or less when compared with closed circular DNA substrate (36).

We have shown that the impairment of repair incision on linear DNA could reasonably be attributed to some negative effect of the DNA end-binding heterodimer Ku on the repair protein complex, since this inhibition was released when anti-Ku inhibiting antibodies were added to Ku-proficient extracts or when repair was carried out with extracts from Ku-deficient cells. The Ku 1:1 dimer of 70- and 86-kDa polypeptides binds without sequence preference to double-stranded DNA ends with 5’- or 3’-protruding ends or blunt ends (37); accordingly, we found a similar repair incision inhibition on damaged plasmids bearing either type of ends. More generally, the following biochemical properties have been reported for Ku heterodimer: 1) recognition of transitions from double to single-stranded DNA (38), 2) translocation along the DNA in an ATP-independent manner allowing several Ku dimers to bind to a single DNA molecule and form a multimeric complex (39), and 3) 5’–3’ ATP-dependent DNA unwinding activity (40).

In addition, Ku is a component of the DNA-dependent protein kinase with a 450-kDa protein partner corresponding to the catalytic subunit (DNA-PKcs) (32); the large kinase subunit is recruited to DNA by Ku binding and then acquires the capacity to phosphorylate on serine/threonine of various proteins. The DNA-PK protein substrates have been identified essentially in vitro, and most of them have DNA binding properties (30); they include several transcription factors, SV40 T-antigen, p53, Ku dimer, and replication protein A, which has also been identified as a phosphorylation substrate in vivo (41). Recently, the analysis of the molecular defects in CHO mutant cell lines sensitive to IR and defective in V(D)J recombination have established that Ku and DNA-PKcs are implicated in the mechanism of double strand break repair (42).

The Ku end-binding activity but not the protein kinase property of the DNA-PK complex accounted for the NER inhibition on linear DNA, since this inhibition was only released with extracts from Ku p86 (xrs6) but not DNA-PKcs (scid) mutant cell lines. This result illustrates the specific properties of the Ku dimer as opposed to the whole DNA-PK complex. Accordingly, although each defect in end-binding or kinase activities has been shown to induce a similar broad phenotype (IR sensitivity and V(D)J recombination defect), detailed analysis of the corresponding mutant cell lines revealed some differences, e.g. more severe anomalies of double strand break junctions in V(D)J recombination or special sensitivity to DNA topoisomerase II inhibitors (42). Taken together, these results and ours indicate that some of the activities of the DNA-PK complex rely specifically on the DNA binding activity of the Ku component.

The fact that the NER inhibition on damaged DNA by free ends was a cis effect might indicate that the basis for this inhibition relied on negative interactions at the sites of damage between Ku subunits and DNA repair proteins. Such a situation is reminiscent of the inhibiting effect of DNA-proteins of the high mobility group family on the repair by NER of the major 1,2-intrastrand class of cisplatin/DNA adducts due to their affinity for this type of lesion (43). However, while a yeast-defective mutant for a major high mobility group protein exhibited an increase resistance to cisplatin (44), no report has been made.
for the Ku mutants of an increased resistance to agents whose lesions are repaired by the NER pathway. Accordingly, we have not found any affinity of Ku protein for closed circular DNA damaged with UV-C light or cisplatin used as competitors for Ku binding to a linear probe in gel shift experiments (data not shown). The presence of free ends on damaged DNA and the translocating property of Ku probably allow a local high concentration of Ku protein at the site of damage. Which of the lesion recognition, incision, and/or oligonucleotide displacement steps are impeded remains to determined.

Here, we have reported that the repair by NER of lesions at a distance shorter than ~1.5 kilobase pair from a DNA end is inhibited in vitro. Whether a double strand break causes NER inhibition under in vitro conditions where the distance between lesions should be much larger remains to be established. Site-specific DNA cleavage has been monitored in yeast and used to study DNA synthesis errors associated with double strand break repair (45). Such tools might be useful to study the NER efficiency in the vicinity of a double strand break. According to our results, we can suggest that lesions processed via the formation of double strand breaks might negatively interfere with the repair of adjacent lesions. Since the occurrence of double strand breaks has been reported in the processing of interstrand cross-links by psoralen compounds (46, 47), this phenomenon might also contribute to the cytotoxicity of such DNA lesions.

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