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DNA Repair Excision Nuclease Attacks Undamaged DNA

A POTENTIAL SOURCE OF SPONTANEOUS MUTATIONS*

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Nucleotide excision repair is a general repair system that eliminates many dissimilar lesions from DNA. In an effort to understand substrate determinants of this repair system, we tested DNAs with minor backbone modifications using the ultrasensitive excision assay. We found that a phosphorothioate and a methylphosphonate were excised with low efficiency. Surprisingly, we also found that fragments of 23–28 nucleotides and of 12–13 nucleotides characteristic of human and *Escherichia coli* excision repair, respectively, were removed from undamaged DNA at a significant rate. Considering the relative abundance of undamaged DNA in comparison to damaged DNA in the course of the life of an organism, we conclude that, in general, excision from and resynthesis of undamaged DNA may exceed the excision and resynthesis caused by DNA damage. As resynthesis is invariably associated with mutations, we propose that gratuitous repair may be an important source of spontaneous mutations.

Nucleotide excision repair is a general repair system that removes damaged bases from DNA by dual incisions of the damaged strand at some distance from the lesion, releasing the damaged base in the form of 12–13-mers in prokaryotes and 24–32-mers in eukaryotes (1, 2). It is the major repair system for bulky base adducts, but it also acts on nonbulky lesions such as oxidized or methylated bases and, as such, functions as a backup system for DNA glycosylases, which have restricted substrate ranges (3, 4).

The wide substrate spectrum of the excision nuclease raises two interrelated questions: what is the substrate range of the enzyme system and how does the enzyme recognize substrate? Both of these questions have been addressed in numerous studies, and at present we have a basic understanding of damage recognition in both prokaryotes and eukaryotes (1, 2, 5, 6). With regard to substrate range, its limits remain to be defined. The excision nuclease, which originally was thought to be specific for bulky lesions, was later found to excise nonbulky adducts such as methylated bases but, apparently, failed to excise nucleotides with backbone modifications such as the C4′ pivaloyl adduct (5, 6). With the availability of more efficient *in vitro* systems (4, 7, 8) we decided to re-examine the question of recognition of backbone modifications. We found that both phosphorothioate and methylphosphonate backbone modifications were recognized as substrates by the human excision nuclease. This, in turn, led us to take a closer look at the effect of the enzyme system on undamaged DNA. We find that both the human and the *Escherichia coli* excision nucleases excise oligomers of 23–28 and 12–13 nucleotides, respectively, from undamaged DNA. This gratuitous excision and the inevitable repair synthesis that must follow could be potential sources of spontaneous mutations. Our data suggest that even in nondividing cells in which there is no DNA replication, there can be significant DNA turnover due to gratuitous excision and resynthesis and that this gratuitous "repair" may cause mutations in such cells, even when they are protected from all extrinsic and intrinsic DNA damaging agents.

MATERIALS AND METHODS

Substrates—Linear DNA substrates (136 or 140 bp[1] in length) were prepared with centrally located lesions as described previously (3). Unmodified oligonucleotides were obtained from Operon Technologies (Alameda, CA). The methylphosphonate-containing 12-mer, the phosphorothioate-containing 12-mer, and the 8-hydroxyguanine-containing (8-oxoG) 11-mer were purchased from Midland Scientific Reagent Company (Midland, TX). The sequence of the centrally located 12-mers was 5′-GAAGCTACGAGC with the phosphorothioate or methylphosphonate modifications between C5 and T6. The oligomer (5′-GTA[TT]ATG) containing the (6-4) photoproduct was prepared and high performance liquid chromatography-purified as described previously (9) and used to assemble a 136-bp substrate.

Repair Factors—Cell-free extracts (CFE, 10–20 mg/ml) were prepared as described previously (4) and stored at −80 °C in storage buffer (25 mM HEPES-KOH (pH 7.9), 100 mM KCl, 12 mM $MgCl_2$, 0.5 mM EDTA, 2 mM dithiothreitol, and 12.5% (v/v) glycerol). The Chinese hamster ovary cell lines were obtained from the American Type Culture Collection (Manassas, VA): CRL 1859 (AA8, wild type), CRL 1860 (UV41, XP-F mutant), and CRL 1867 (UV135, XP-G mutant). XPF·ERCC1 was purified using a previously described chromatographic scheme after expression in an insect cell system (8). The UvrABC proteins were purified as described elsewhere (10).

Excision Assay—In vitro removal of oligonucleotides was measured with the excision assay, which measures the release of radiolabeled fragments from substrate DNA (11). For experiments with the mammalian excision nuclease, the reaction mixtures contained 3 fmol of radiolabeled substrate DNA, 12.6 fmol of pBR322, and 50 μg of CFE in 25 μl of reaction buffer (17 mM HEPES-KOH (pH 7.9), 12 mM Tris-HCl (pH 7.5), 35 mM KCl, 44 mM NaCl, 5.8 mM $MgCl_2$, 0.3 mM EDTA, 0.34 mM dithiothreitol, 2–4 mM ATP (except where indicated otherwise), and 2.5% glycerol with bovine serum albumin at 200 μg/ml) and were incubated at 30 °C for 60 min. For complementation assays, 25 μg of each repair-deficient CFE was premixed on ice and used in the reaction, or 20 ng of XPF·ERCC1 was added to 50 μg of XP-F-deficient CFE. For experiments with the *E. coli* excision nuclease, the reactions contained 3 fmol of radiolabeled substrate DNA, 5 nM UvrA, 20 nM UvrB, and 50 nM UvrC in 25 μl of reaction buffer (50 mM Tris (pH 7.5), 50 mM KCl, 10

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[1] The abbreviations used are: bp, base pair(s); CFE, cell-free extract; XP, xeroderma pigmentosum; ERCC, excision repair cross-complementing; 8-oxoG, 8-hydroxyguanine; nt, nucleotide(s).

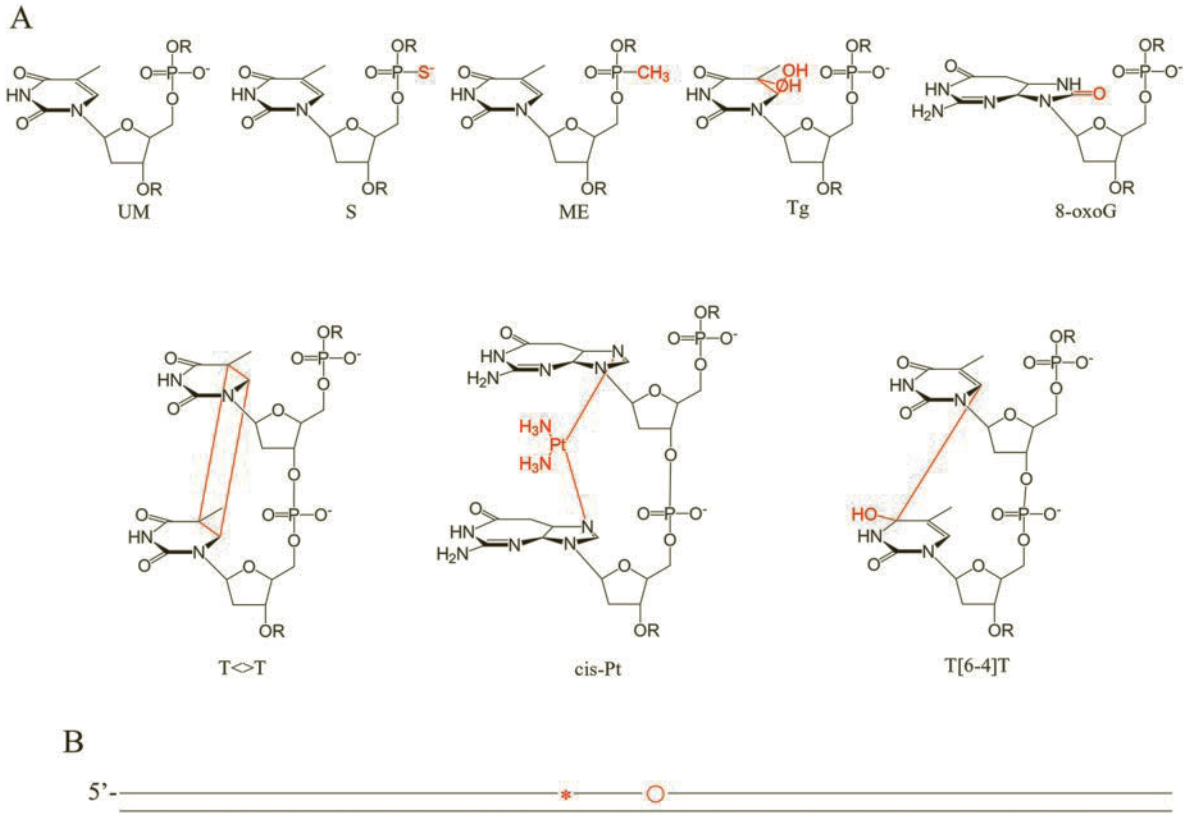

FIG. 1. **Structures of DNA modifications incorporated into excision repair substrates.** *A*, the phosphorothioate (*S*)- and methylphosphonate (*ME*)-adducted thymidines introduce minor alterations into the sugar-phosphate backbone and are shown next to undamaged (unmodified, *UM*) thymidine. Thymine glycol (*Tg*) and 8-oxoguanine (*8-oxoG*) are damaged bases generated by reactive oxygen species; these lesions cause minor helical distortions. The *lower panel* illustrates three bulky, helix-distorting lesions introduced either by ultraviolet radiation, such as cyclobutane thymine dimer (*T<>T*) and (6-4) photoproduct (*T[6-4]T*), or by the chemotherapeutic agent cisplatin, 1,2-d(GpG) diadduct (*cis-Pt*). Only unmodified, phosphorothioate, methylphosphonate, and T[6-4]T substrates were used in the current study; the other substrates have been tested previously (1). *B*, schematic illustration of the substrates. The duplex of 136/140 bp without lesion or with a lesion at the position indicated by a *circle* and a radiolabel at the position indicated by an *asterisk* was prepared as described previously (3).

mM $MgCl_2$, and 2 mM ATP with bovine serum albumin at 100 μg/ml) and were incubated at 30 °C for 60 min. Following the reaction, the DNA was extracted with phenol:chloroform, and the deproteinized DNA was precipitated with ethanol, resuspended in formamide/dye mixture, and resolved in 10% polyacrylamide gels containing 7 M urea (sequencing gels) to separate excision products from substrate DNA. DNA was visualized by autoradiography or by scanning on a model 860 Storm PhosphorImager (Molecular Dynamics), and the intensity of signal was analyzed with ImageQuant software (version 5.0, Molecular Dynamics). The extent of excision for each reaction was determined from the percentage of signal migrating as ~23–28-mers (12–13-mers for *E. coli* excinuclease) relative to the signal for full-length DNA (signal in the ~110–140-mer range, which contains 80–90% of the total radioactivity in the lane). Because of the significant DNA degradation observed with CFE-based reactions, we adjusted for nonspecific nuclease activity by determining the percentage of signal in an equal sized area that migrated in the ~30–38-mer range and subtracting this background value from the percentage excision calculated for fragments migrating in the ~23–28-mer range.

Assay for Cryptic Oxidative DNA Damage—Oligonucleotides, either as purchased from the supplier (Operon Technologies) or after being subjected to mock kinase and ligase reactions followed by purification via denaturing polyacrylamide gel electrophoresis and annealing, were hydrolyzed with formic acid; the hydrolysates were lyophilized, trimethylsilylated, and analyzed for 8-oxoG by isotope dilution mass spectrometry as described previously (12). The analysis was kindly performed by Dr. Miral Dizdaroglu (National Institute of Standards and Technology, Gaithersburg, MD).

RESULTS

Excision of DNA Backbone Modifications—To define the substrate range of human excision nuclease, a variety of lesions have been tested. Fig. 1 shows some of the substrates that we have used in this and our previous studies. Prior work has shown that all base lesions and even mismatches tested were excised by the human excision nuclease, albeit with greatly different efficiencies (3, 4). However, attempts to detect excision from DNA with backbone modifications failed, suggesting that these lesions might not be substrates for the human excision nuclease (5, 6). Recently, we have improved the efficiency and sensitivity of the excision assay (13, 14), and we wished to test DNA with backbone modifications in our assay system. Fig. 2 shows that DNA containing either a phosphorothioate or a methylphosphonate in the backbone are recognized and excised by the human excision nuclease, suggesting that not only base modifications but also backbone modifications, which cause modest helical distortions, can be substrates for the excision nuclease.

Repair of Undamaged DNA by Human Excision Nuclease—The difference between the backbones of unmodified DNA with phosphodiester and phosphorothioate linkages is minor (15). Hence, the excision of an oligomer carrying the phosphorothioate bond was unexpected and led us to consider the possibility that the excision nuclease system, which is capable of recognizing such subtle perturbations in the duplex structure, may act in a similar manner on unmodified DNA with a low but finite probability. When we tested the unmodified DNA in our assay system, we found that fragments 23–28 nucleotides in length were removed from this substrate as well (Fig. 3). That these oligomers are generated by the excision nuclease system and not by nonspecific degradation of DNA by contaminating nucleases is supported by three lines of evidence. First, the excision nuclease is the only known mammalian nuclease that

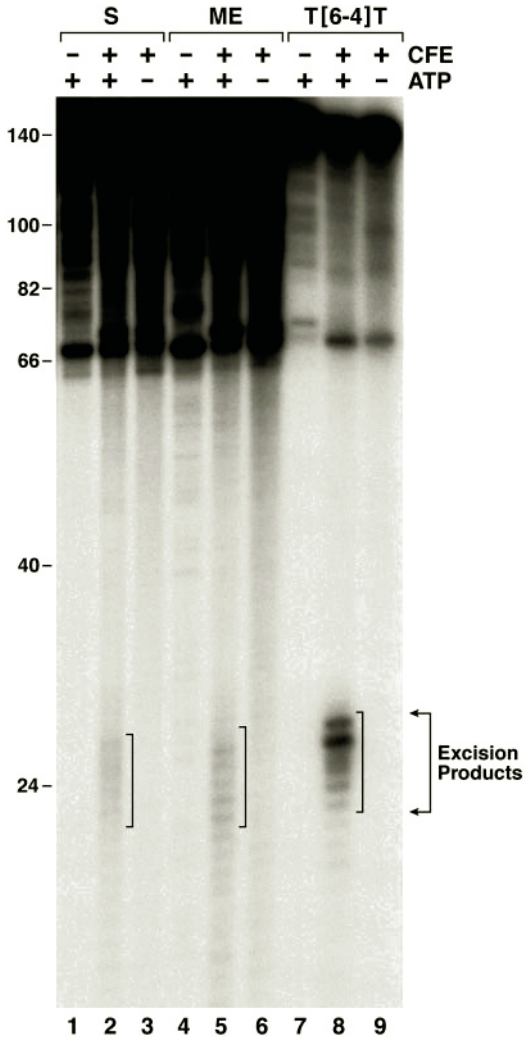

FIG. 2. **Excision of oligonucleotides containing DNA backbone modifications by mammalian excision nuclease.** Substrate DNAs were incubated for 60 min in 25-μl reaction mixtures containing 50 μg of AA8 CFE plus 3 fmol of DNA substrate and then resolved in a 10% polyacrylamide sequencing gel; *brackets* indicate the location of excision products. Excision products were only observed in complete reactions (*i.e.* those containing both CFE and ATP, *lanes 2*, *5*, and *8*). The observed percentages of excision (n = 3–4 experiments) were 0.31 ± 0.1 for phosphorothioate (*S*), 0.43 ± 0.21 for methylphosphonate (*ME*), and 12.8 ± 3.5 for T[6-4]T. Only 1/10 of the reactions were loaded onto the lanes containing the T[6-4]T substrate.

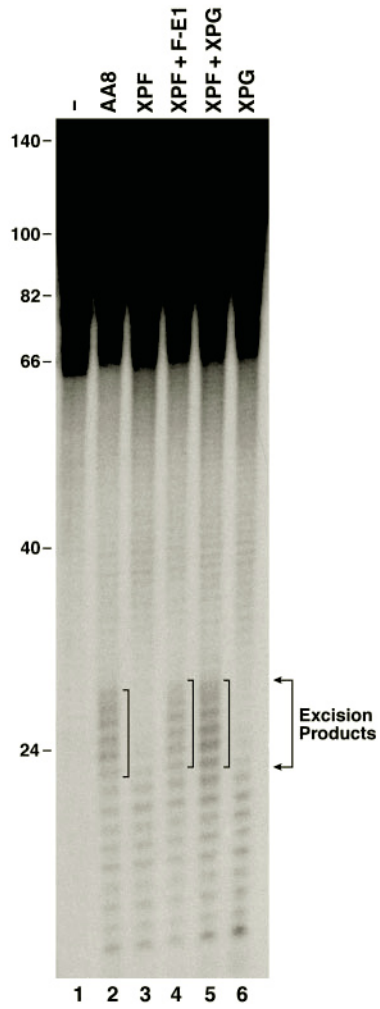

FIG. 3. **Excision of undamaged DNA by the mammalian excision nuclease.** Substrate DNA prepared with unmodified oligonucleotides was incubated for 60 min in 25-μl reaction mixtures either lacking or containing cell-free extracts prepared from normal cells (*AA8*), repair-deficient cell lines (*XPF* or *XPG*), or XPF extract supplemented with purified protein or with extract prepared from an XPG cell line. The figure shows an autoradiograph obtained after resolution of DNA samples in a 10% polyacrylamide sequencing gel; *brackets* indicate the location of excision products. Excision products were not observed in the absence of cell extracts (*lane 1*) or when substrate was incubated with extracts prepared from repair-deficient cell lines lacking excision nuclease subunits (*lanes 3* and *6*); but the defect in XPF extracts was restored to wild type levels (*lane 2*) by the addition of recombinant XPF·ERCC1 (*F-E1*) heterodimer (*lane 4*) or by coincubation with the XPG cell extract (*lane 5*). The observed percentages of excision were 0.09, 0.07, and 0.11, respectively, for AA8, XPF extract complemented with recombinant protein and XPF extract mixed with XPG cell extract; the apparent presence of excision products in *lanes 1*, *3*, and *6* were not above background levels. In separate experiments (data not shown, n = 3) conducted under the same conditions, the percentages of excision were 0.07 ± 0.02 for undamaged DNA and 10.4 ± 2.3 for T[6-4]T photoproduct when substrates were incubated with AA8 CFE.

cuts out oligomers in the range of 23–28 nucleotides from a duplex. Second, the excision of 23–28-mers is ATP-dependent as is the excision nuclease in removing damaged bases. Finally, extracts from cells lacking the XPG or XPF subunits of the excinuclease fail to release 23–28-mers from undamaged DNA. Moreover, the excision activity can be restored by supplementing extract from the mutant cell line with the missing subunit. In conclusion, the data in Fig. 3, considered in its entirety, show that the human excision nuclease is capable of excising oligomers of 27 nt nominal length from undamaged DNA. Similar levels of excision were observed when the centrally located oligomer with ^{32}P label in the 140–143-bp duplex was an undamaged 15-mer 5′-TCCTCCTCGCCTCCT or 20-mer, 5′-GCTCGAGCTAAATTCGTCAG (data not shown). Thus, it appears that excision of undamaged DNA occurs in at least three different sequence contexts.

Repair of Undamaged DNA by E. coli Excinuclease—The subunits of human and *E. coli* excinucleases do not share any homology, yet the reaction mechanisms of the two systems are remarkably similar (1): ATP-independent damage recognition followed by ATP-dependent unwinding of DNA and formation of a stable preincision complex and finally dual incisions at phosphodiester bonds several base pairs removed from the damage site. Hence, the finding that the human excision nuclease performs standard dual incisions on backbone modified and undamaged DNA led us to re-examine the effect of *E. coli* excinuclease on these substrates as well. The results, presented in Fig. 4, show that it does excise a characteristic 12-nucleotide oligomer from DNA with phosphorothioate or methylphosphonate modifications. This excision is also observed with undamaged DNA, albeit at lower efficiency. As with the

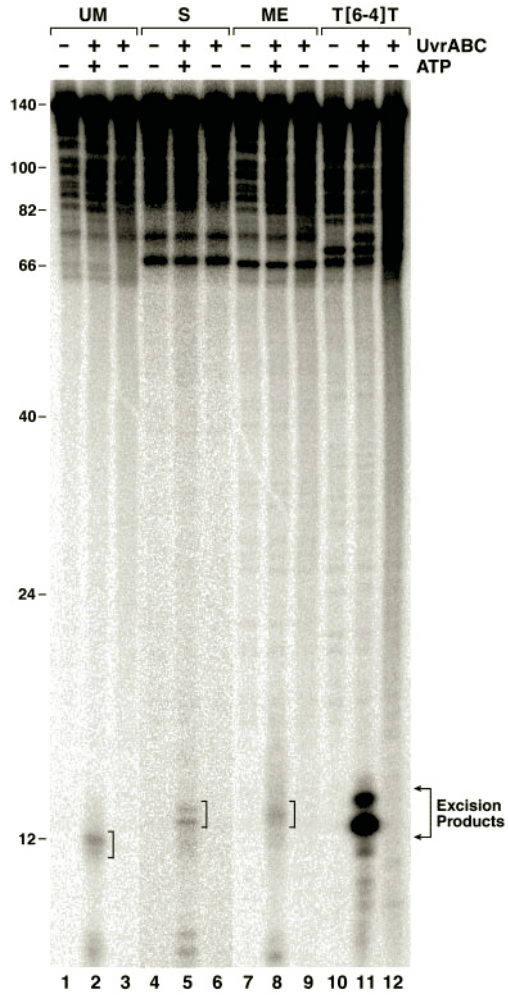

FIG. 4. **Excision of oligonucleotides containing undamaged DNA and backbone modifications by the *E. coli* excision nuclease.** Undamaged (*UM*) and phosphorothioate (*S*)- and methylphosphonate (*ME*)-adducted thymidines were incorporated into DNA substrates, and the (6-4) photoproduct (*T[6-4]T*) was used as a reference lesion. Substrate DNA (3 fmol) was incubated for 60 min in 25-μl reaction mixtures containing 5 nM UvrA, 20 nM UvrB, and 50 nM UvrC in reaction buffer with and without ATP as indicated. The figure shows an autoradiograph obtained after resolution of DNA samples in a 10% polyacrylamide sequencing gel; *brackets* indicate the location of excision products. Note that the major excision products from undamaged and T[6-4]T substrates are 12 nt in length. The photoproduct causes one nucleotide slower migration than expected. The phosphorothioate and methylphosphonate modifications may also cause slightly anomalous migration of the oligomer. Excision products were only observed in complete reactions (*i.e.* those containing both UvrABC and ATP, *lanes 2*, *5*, *8*, *11*), and the observed percentages of excision were 0.68 ± 0.4 (n = 7) for undamaged, 0.94 ± 0.6 (n = 6) for phosphorothioate, 1.23 ± 0.5 (n = 2) for methylphosphonate, and 50.6 ± 12.5 (n = 5) for T[6-4]T substrates.

human excision nuclease, excision was also observed in a 140-bp duplex when the centrally located oligomer was a 20-mer, 5′-GCTCGAGCTAAATTCGTCAG (data not shown). Thus, it appears that removal of oligomers of defined lengths from damaged or undamaged DNA is a general property of excision nucleases.

Damage in "Undamaged DNA"—Although we have interpreted the excision from our DNA oligomers prepared without targeted lesions as arising from undamaged DNA, it is virtually impossible to have a DNA preparation free of damage (16–18). This is because both the nucleobases and the phosphodiester bonds are rather reactive and prone to modification by both extrinsic and intrinsic agents. Thus, a given DNA preparation always contains a certain amount of lesions, the level of which would be dependent on a variety of factors, including the source of DNA and the method of purification. In particular, it is practically impossible to prepare DNA without oxidative base damage. Hence, it could be argued that the excision we observe from our nominally undamaged DNA may be due to low levels of oxidative damage introduced during the handling of DNA.

TABLE I
Level of 8-oxoG in undamaged synthetic DNA

Oligonucleotides	8-OxoG/G
Unprocessed[a]	5.4×10^{-4}
Processed[b]	5.2×10^{-4}

[a] Oligonucleotides were used as received from the supplier.
[b] Oligonucleotides were used after mock kinase and ligase reactions and purification through a sequencing gel followed by electroelution and ethanol precipitation.

To address this concern we measured the level of 8-oxoG in our synthetic oligonucleotides that had been subjected to essentially the same treatment as our radiolabeled substrate. 8-OxoG is the most common oxidative stress lesion (16, 18) and, among the major oxidative base lesions, it is the most efficient substrate for human excision nuclease (4). Thus, we reasoned that if excision from undamaged DNA arises from cryptic lesions, most of it would have been caused by 8-oxoG. The rate of excision from undamaged DNA is 5–10% the rate of removal of a single 8-oxoG in the same duplex (Ref. 4 and data not shown). Hence, if excision from the undamaged DNA were to arise from cryptic 8-oxoGs in our substrate, it would be expected that the 9 guanines, which are close enough to the radiolabel to give rise to radiolabeled 23–28-mer products, would be in the form of 8-oxoG in 5–10% of the undamaged DNA. This would mean an 8-oxoG/G ratio in the undamaged DNA substrate of (0.05 to 0.10)/9 = 5.5×10^{-3} to 1.1×10^{-2}. As shown in Table I, 8-oxoG is present at a level of 5.2×10^{-4} to 5.4×10^{-4} in our DNA. Hence cryptic 8-oxoG contributes in the range of 5–10% to the excision signal from our undamaged DNA. Thymine glycol, urea, and other oxidative lesions, which are less frequent than 8-oxoG (16, 18), and are excised less efficiently by the human excision nuclease (4), are expected to contribute to the signal from undamaged DNA even less. It should be noted, however, that our quantitation of 8-oxoG was performed with nonradiolabeled DNA. An argument could therefore be made that with radiolabeled DNA the 8-oxoG level would be greater due to DNA damage caused by radioactive decay, and thus there would be higher contributions to the excision signal from damage. However, we think this is unlikely to be the source of gratuitous excision for the following reason. The DNA molecules in which the decay occurs are no longer detectable in the excision assay, and the likelihood that low level β-decay would damage other DNA molecules, especially in the presence of EDTA in the storage buffer, is infinitesimally small. Thus, it can be reasonably concluded that most of the excision signal we detect with undamaged DNA is produced by the attack of the excision nuclease on undamaged DNA as a consequence of the intrinsic property of the action mechanism of the excision nuclease system.

DISCUSSION

Our findings raise two questions: why and how is the undamaged DNA attacked by the excision nuclease, and what is the biological role of gratuitous DNA repair? These questions are addressed below.

Attack of Excision Nuclease on Undamaged DNA—The precise mechanism of damage recognition by human excision nuclease is not known. Hence, at present, it is not possible to answer the questions of why and how the enzyme attacks

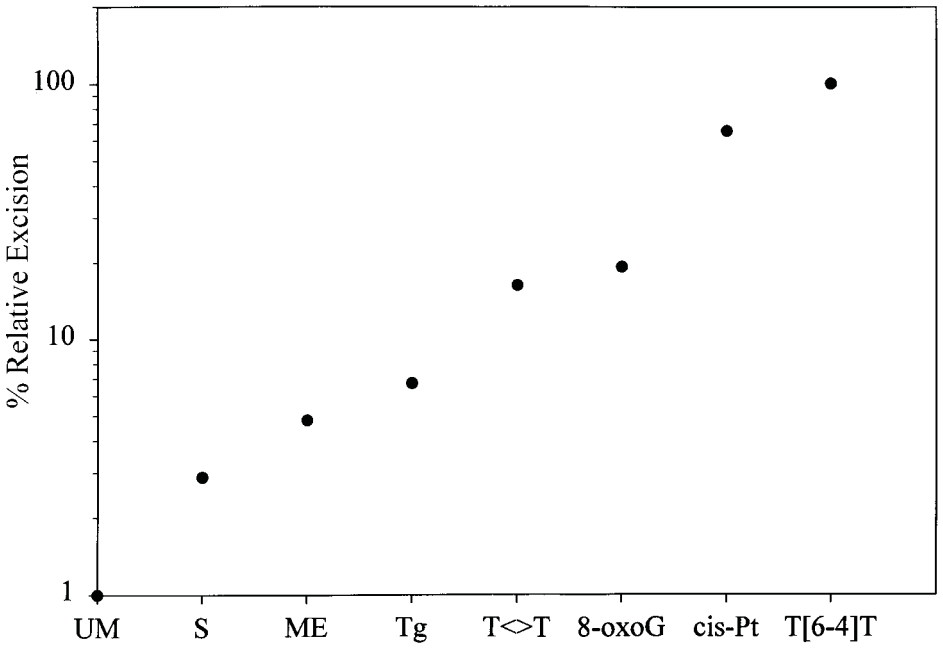

FIG. 5. **Relative levels of oligonucleotide excision by the mammalian system.** To express the relative efficiency of nucleotide excision for various DNA substrates, the average percent excision observed for the T[6-4]T photoproduct was defined as 100, and values for the excision of other lesions were normalized relative to this value. For comparison, all excision reactions were under substrate-limiting conditions, and the sources of excision nuclease were cell-free extracts prepared from mammalian cells. This figure incorporates data generated in the present study and in previously published work where (6-4) photoproduct or cyclobutane thymine dimer were used as references (4, 37); abbreviations are the same as in Fig. 1. Note that the spacing of the lesions along the *x* axis is for the sake of clarity and is not meant to imply a special relationship between the various structures and their efficiency as substrates.

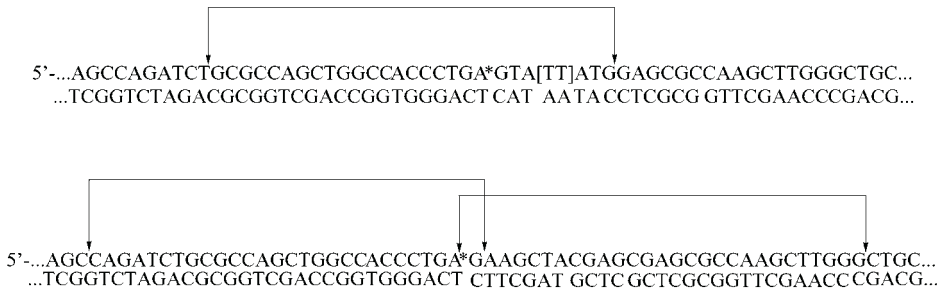

FIG. 6. **Dual incisions releasing labeled oligonucleotides from undamaged and damaged DNA.** The (6-4) photoproduct is released mainly by incisions at the 4th phosphodiester bond 3′ and the 24th phosphodiester bond 5′ to the lesion. With undamaged DNA any combination of incisions about 28 nt apart, bracketing the label, release the appropriate size fragments. The dual incisions representing the extreme locations for releasing the label are shown, and any combination of sites between these two extremes will release the radiolabel from undamaged DNA.

undamaged DNA in any detail. Based on the structure of a preincision DNA-enzyme complex, which contains a subset of the repair factors and partially unwound and kinked DNA (1, 2, 7, 13), it has been proposed that any DNA structure that is amenable to unwinding and kinking and otherwise assuming the conformation existing in the ultimate preincision complex might function as a substrate (1, 5, 6). Since even undamaged DNA can assume the conformation of the preincision complex with low but finite probability (1), it is not surprising that undamaged DNA is a substrate for excision nucleases. Indeed, there have been reports on incision of undamaged DNA by the *E. coli* excinuclease (19, 20). In one of those studies, however, uniformly radiolabeled plasmid DNA was used in a nicking assay that is incapable of detecting an excinuclease mode of action (19). The second study used linear DNA uniformly labeled with ^{32}P as a substrate in an excision assay, and 9-nt-long oligomers were released instead of the characteristic 12–13-nt-long oligomers (20). Later work revealed that the 9-mers are released by a potent 3′-exonuclease action of the UvrABC proteins at a nick or a double-strand break (21, 22), and hence the product was not released by an excinuclease type of action (dual incisions in one strand). In this study we present unambiguous evidence that both the human and the *E. coli* excision nucleases attack undamaged DNA in the typical excinuclease mode.

It is very likely that certain DNA sequences would be more susceptible to attack by excision nuclease than others. We have tested three different random sequences and found a similar level of excision by the excision nuclease. A more extensive survey, however, is likely to identify certain sequences and conformations with increased susceptibility to excision nuclease. Indeed, a recent study (23) has shown that the poly(purine:pyrimidine) tract present in the polycystic kidney disease gene (*PKD1*) when present in a supercoiled plasmid is efficiently processed by the *E. coli* excinuclease. An extreme case of the effect of DNA conformation on gratuitous repair is the form of gratuitous repair that has been proposed to occur as a side product of transcription-repair coupling (24, 25). It has been speculated that when RNA polymerase stalls at natural transcriptional pause sites the transcription-coupled repair machinery is activated in a manner similar to RNA polymerase stalling at a lesion and that such activation of the transcription-coupled repair system leads to gratuitous and potentially mutagenic repair. Currently there is no experimental evidence for gratuitous repair initiated by stalled RNA polymerase. However, there are several reports that show that transcribed DNA is mutated at higher frequency than nontranscribed DNA (26–29). Whether this increased mutation frequency is due to transcription-coupled gratuitous DNA repair or the increased susceptibility of single-stranded DNA in the transcription bubble to various DNA damaging agents is not known at present.

Biological Relevance of Gratuitous Repair—We suspect that gratuitous excision repair has no beneficial effect for the organism. Removal and replacement of undamaged DNA by nucleotide excision repair is the price the cell has to pay to have an

omnipotent DNA repair enzyme capable of handling a virtually infinite variety of lesions. This excision and resynthesis may not be totally innocuous, since it may introduce spontaneous mutations into undamaged DNA as is shown in the following calculation.

Fig. 5 compares the relative efficiency of human excision nuclease on a variety of lesions and on undamaged DNA. As is apparent, with the unique substrate and assay system we use, undamaged DNA is excised at a rate of about 1% that of the (6-4) photoproduct, which is the best natural substrate for the enzyme and is used as the "gold standard" for other substrates. However, in calculating the susceptibility of undamaged DNA to excision nuclease activity with the (6-4) photoproduct as a reference, a correction factor must be introduced for the relative abundance of the targets. Essentially all of the excision products from the (6-4) substrate arise from a single lesion, whereas the excision products from undamaged DNA arise from dual incisions over about a 50-nucleotide region in a variety of combinations that bracket the radiolabel (Fig. 6). Hence, in calculating the efficiency of the enzyme on an undamaged nucleotide, a correction factor of 50 is introduced, making the actual efficiency of an undamaged base relative to that of a (6-4) photoproduct equal to about $1/(50 \times 100) = 2 \times 10^{-4}$. This might seem insignificant, but if one considers that every nucleotide in the human genome complement is a potential target for attack by the excision nuclease, the level of excision of undamaged DNA becomes significant. The maximum rate of excision of (6-4) photoproducts under substrate saturating condition has been estimated to be 2.7×10^3/min/diploid human cell (30). Assuming that the relative rates we obtained *in vitro* are applicable to the *in vivo* environment, it is predicted that every minute $(2.7 \times 10^3) \times (2 \times 10^{-4}) = 5.4 \times 10^{-1}$ undamaged nucleotides would be subject to excinuclease action, and since each excision event removes about 25 nucleotides, it is calculated that $5.4 \times 10^{-1} \times 25 = 13.5$ nucleotides/min are removed by the human excision nuclease. This, in turn, means excision and replacement of about 2×10^4 nucleotides per day per human cell. This value is comparable with the nucleotide turnover that occurs under physiological conditions as a result of base excision repair processing of damaged bases (10^4 to 10^5 per cell per day) arising from depurination, deamination, oxidation, and methylation (31, 32). Thus, it is conceivable that gratuitous nucleotide excision repair contributes to DNA turnover as much as base excision repairs acting on spontaneous DNA lesions. Gratuitous repair is not necessarily restricted to the nucleotide excision repair system. It has been shown that certain DNA glycosylases also attack undamaged DNA causing gratuitous repair which, under special conditions, can be mutagenic (33, 34). Mismatch repair, like nucleotide excision repair, has a wide substrate range and many mechanistic similarities to nucleotide excision repair (35, 36) and conceivably may perform gratuitous repair. Since the mismatch repair patches, as a rule, are much larger than those of base or nucleotide excision repair, this system as well may contribute to spontaneous mutagenesis.

Conclusion—In this paper we have shown that DNA with minor backbone modifications and nominally unmodified (undamaged) DNA are attacked by the human and *E. coli* excision nucleases. The concern that the nominally undamaged DNA may in fact contain some cryptic damage can never be unequivocally eliminated. We feel, however, that the excision we observe from undamaged DNA does represent attack on truly undamaged DNA for the following reasons. First, using an analytical probe for the most common spontaneous lesion in DNA, 8-oxoG, we demonstrate that the level of this lesion in our synthetic substrate is well below the level required to account for the level of excision we observe for such undamaged substrate. Second, the fact that even such a minor modification as the replacement of an oxygen by a sulfur in the backbone increases the susceptibility of DNA to the excision nuclease leads to the reasonably logical conclusion that substrate and nonsubstrate DNA are not quantized for the excision nuclease. Instead it suggests that DNA structures ranging from gross distortions to no distortion represent the two extremes of the continuum of excision nuclease substrates.

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