Perturbations of enzymic uracil excision due to purine damage in DNA*

(carcinogens/DNA repair)

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ABSTRACT Phage PBS-2 DNA, which contains uracil in place of thymine, was selectively damaged and then used as substrate for purified *Bacillus subtilis* uracil-DNA glycosylase. This enzyme releases uracil from DNA in a limited processive manner. Irradiation by ultraviolet light (>305 nm) in the presence of isopropanol and a free radical photoinitiator introduced covalently bound 8-(2-hydroxy-2-propyl)purines into DNA. Methylation by dimethylsulfate yielded 7-methylguanine. Apurinic sites were produced by gentle heating of methylated DNA. Rates of enzymic release of uracil from DNA varied among these three substrates. The $V_{\max}$ was markedly decreased for DNA containing 8-(2-hydroxy-2-propyl)purines and apurinic sites but was unaffected by the presence of larger quantities of 7-methylguanine. This suggests that certain types of damaged DNA moieties may decrease the capacity for uracil excision. Therefore, interference with enzymic excision of this potentially mutagenic base may constitute a common mechanism of action of the reaction products of several unrelated DNA damaging agents.

Uracil-DNA glycosylase [dUra(DNA) glycosylase] specifically removes uracil from DNA. Such uracil may result either from incorporation in place of thymine during DNA synthesis or deamination of DNA cytosine (1), which may occur at physiological conditions (2). Left unrepaired, deaminated cytosines would result in transition mutations (3). *Escherichia coli* mutants deficient in dUra(DNA) glycosylase (*ung*⁻) are mutators, and *in vivo* deamination of cytosines is the source of these mutations (4, 5). This implies that dUra(DNA) glycosylase activity is necessary to preserve the integrity of DNA in the face of continuous potentially mutagenic damage. The finding that this enzyme is apparently ubiquitous supports the suggestions that this is its prime function (1, 4, 6).

The presence of UV photoproducts results in alteration of dUra(DNA) glycosylase activity toward phage PBS-2 DNA, which contains uracil in place of thymine. The rate of enzymic release of uracil from such UV-irradiated DNA decreases as the number of photodimers increases (7). This indicates that the presence of pyrimidine dimers in DNA may affect excision of uracil.

We investigated whether this alteration occurs with other types of DNA modifications. Three different types of purine damage were introduced into PBS-2 DNA, which was then used as substrate for dUra(DNA) glycosylase. Photoalkylation produced the modified purines 8-(2-hydroxy-2-propyl)guanine (HPG) and 8-(2-hydroxy-2-propyl)adenine (HPA) in DNA. 7-Methylguanine (mGua) was introduced by chemical alkylation of DNA by dimethylsulfate (Me_2SO_4), and apurinic sites were produced by gentle heating of such alkylated DNA. The rate of enzymic release of uracil was markedly decreased when the

DNA contained either apurinic sites or photoalkylated purines, but it was unaffected by the introduction of mGua. Thus, the presence of specific DNA modifications may decrease the capacity for uracil excision. This suggests the possibility that interference with the enzymic excision of this potentially mutagenic base might constitute a common mechanism of action of several DNA-damaging agents.

MATERIALS AND METHODS

DNA Preparations. Phage PBS-2 was grown in *B. subtilis*, and the DNA was purified and stored at 100 $\mu\text{g}/\text{ml}$ according to Lindahl *et al.* (8). PBS-2 DNA was labeled in uracil by addition of [³H]uridine (New England Nuclear; final concentration, 20 $\mu\text{Ci}/\text{ml}$; 1 Ci = 3.7×10^{10} becquerels) or [2-¹⁴C]uridine (New England Nuclear; final concentration, 1.0 $\mu\text{Ci}/\text{ml}$) 5 min after infection. PBS-2 DNA was also prepared with the purines radiolabeled by addition of [U-¹⁴C]adenine (Amersham; final concentration, 1.0 $\mu\text{Ci}/\text{ml}$) or [U-¹⁴C]guanosine (Amersham; final concentration, 0.3 $\mu\text{Ci}/\text{ml}$). Specific activities were: DNA labeled with [³H]uridine, 250,000 dpm/ μg ; DNA labeled with [¹⁴C]uridine, 49,000 dpm/ μg ; DNA labeled with [¹⁴C]adenine, 15,000 dpm/ μg with 98% of the label in adenine and 2% in guanine; DNA labeled with [¹⁴C]guanosine, 3,000 dpm/ μg with 50% of the label in sugar, 47% in guanine, and 3% in adenine.

Preparation of Damaged DNA Substrates. PBS-2 DNA was photoalkylated according to Livneh *et al.* (9), except that the samples were not flushed with nitrogen before irradiation. Actinometry (10) showed the incident dose to be 6.6×10^{-5} einstein $\text{cm}^{-2} \text{min}^{-1}$. Photoalkylated DNA was extensively dialyzed into 10 mM Tris-HCl/1 mM EDTA, pH 8.0. DNA was methylated with 10 mM Me_2SO_4 for 1 hr (11) and purified by passage through a Sephadex G-50 column in 10 mM Tris-HCl/1 mM EDTA, pH 8.0 (12). In some experiments, methylated PBS-2 DNA was partially depurinated by heat (13) followed by dialysis into the above buffer.

Analysis of DNA Damage. The degree of purine modification in DNA was assessed by quantitating labeled purines liberated by acid hydrolysis (14) utilizing paper chromatography (14) and HPLC. All HPLC for modified purines used a Waters chromatographic system and a Bio-Rad Aminex A-9 column (250 $\times$ 4 mm) maintained at 60°C. The column was eluted with ammonium formate pH 3.0 buffer at 0.8 ml/min; sample volume was 500 μl . In chromatography runs for the quantitation of

Abbreviations: HPA, 8-(2-hydroxy-2-propyl)adenine; HPG, 8-(2-hydroxy-2-propyl)guanine; dUra(DNA) glycosylase, uracil-DNA glycosylase; Me_2SO_4 , dimethylsulfate; mGua, 7-methylguanine.

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mGua yields in Me_2SO_4 -treated DNA, the elution buffer was 0.5 M and the guanine, mGua, and adenine peaks were detected at 23, 32, and 38 min after sample application, respectively. In the analysis for HPG, the buffer was 0.8 M and HPG, guanine and adenine peaks were detected at 14, 21, and 36 min, respectively. In the analysis for HPA, the elution buffer was 0.4 M and guanine, HPA, and adenine peaks were detected at 47, 53, and 85 min, respectively. The assessment of radioactivity and quantitation of concentrations in column elution fractions were as described (15). HPA marker was purchased from CDS Laboratories (Durango, CO). HPG marker was made by photoalkylation of guanine (16). Identities of both were confirmed by paper chromatography (9, 14). mGua was obtained from Vega Biochemicals (Tucson, AZ). The number of modified adducts per molecule were calculated from the known size and composition of PBS-2 DNA (17).

Photoalkylated DNA was assayed for uracil-containing dimers as described (7). Other pyrimidine damage was investigated by enzymic digestion of DNA to deoxynucleosides (18) and precipitation of the enzymes with 5% trichloroacetic acid. The supernatant was neutralized and analyzed by TLC (19) and HPLC, performed on a Waters C_{18} μ Bondapak column (250 $\times$ 4 mm) at room temperature; sample volume was 200 μ l. A 60-min 0–10% methanol gradient in 10 mM K_2HPO_4 (pH 7.4) was applied by using curve 10 on a Waters model 660 solvent programmer; the elution rate was 0.3 ml/min. The deoxyuridine peak appeared at 25 min into the gradient.

Enzymology. dUra(DNA) glycosylase was purified from *B. subtilis* according to Cone *et al.* (20). The reaction conditions and assay were those of Friedberg *et al.* (21). Enzyme assays, reaction mixtures, identification of the released product, and analysis of the Lineweaver–Burk plots were performed as described (7). Variations in activities against control substrates reflected different purifications and times of storage. Comparisons were made within data sets of control and damaged DNAs.

The possible processivity of dUra(DNA) glycosylase was investigated by DNA challenge experiments (22–25). Purified glycosylase (5 μ l) was incubated with 0.15 μ g of [^3H]uridine-labeled PBS-2 DNA in 40 μ l of 10 mM Tris·HCl, pH 8.0/1 mM EDTA plus 10 μ l of 100 mM KH_2PO_4 , pH 7.0/1 mM EDTA. After 30 sec in ice to allow binding of enzyme to substrate, 0.15 μ g of [^{14}C]uridine-labeled PBS-2 DNA was added and the reaction mixture was incubated at 37°C. At various intervals, enzymic activity was assayed by measuring release of labeled uracil into the acid-soluble fraction (26). In another experiment, the enzyme was incubated with [^3H]uridine-labeled PBS-2 DNA at 37°C for 4 min. An equal quantity of [^{14}C]uridine-labeled substrate was then added, and the released labeled uracil was assayed as above.

RESULTS

Characterization of Damaged DNAs. Photoalkylation of PBS-2 DNA yielded linear increases, with time, of HPA (Table 1) and HPG. Analysis by both paper chromatography and HPLC showed increases in HPA yield with time of photoalkylation.

Table 1. Analysis of HPA in photoalkylated PBS-2 DNA

Irradiation, min	HPA, % of total adenine	
	By paper chromatography	By HPLC
10	0.09	0.05
20	0.36	0.27
30	0.59	0.39
40	0.84	0.49

However, the yield as determined by paper chromatography was consistently greater than that obtained on HPLC. In all cases, the discrepancy in HPA radioactivity was accounted for by two uncharacterized peaks. Neither xanthine nor hypoxanthine was formed. The yield of HPG was higher than that of HPA. Five minutes of photoalkylation modified 0.7% of guanine to HPG; 10 and 20 min of photoalkylation yielded 1.6% and 3.9% guanine modification, respectively. There were no significant differences in recovery between the two methods of analysis, and no other type of modified guanine was detected. The relative yields of photoalkylated purines here differ from those reported for *E. coli* and PM2 phage DNAs, in which HPG and HPA are formed in equal quantities (9, 14).

Uracil-containing photodimers were also formed during photoalkylation. After 5 min of irradiation, 0.2% of the radioactivity of the DNA labeled in uracil was present as dimers. This increased to 0.5%, 1.0%, and 1.5% with 10, 20, and 40 min of photoalkylation, respectively. The low level of uracil dimers produced by 10 min of photoalkylation has no detectable effect on dUra(DNA) glycosylase activity (7). All nondimer material in [^{14}C]uridine-labeled DNA was identified as deoxyuridine by enzymic hydrolysis followed by HPLC and TLC. No photoalkylated derivatives of cytosine have been reported (27). Therefore, the major products of photoalkylation of PBS-2 DNA were photoalkylated purines. As with native thymine-containing DNAs, no photoalkylated pyrimidines were detected (9, 14). This is consistent with the suppression of photoalkylation of pyrimidines by equimolar purines, reported for both uracil and thymine bases (28) and for PM2 DNA (9). Yields of HPG, HPA, and uracil dimers were reproducible under the conditions used.

DNA alkylation by Me_2SO_4 yields mGua as the major product (11, 29). Analysis by HPLC showed modification of 3.8% of guanine to mGua, with about 50% of these residues released by heat. Both the yield of mGua produced by Me_2SO_4 alkylation and of apurinic sites that resulted from heating such alkylated DNA were the same for PBS-2 DNA as for calf thymus or *B. subtilis* DNA (11, 13). Therefore, quantitative substitution of uracil for thymine in DNA does not greatly alter purine reactivity or the stability of methylated purines.

Enzymic Release of Uracil from Damaged DNAs. The activities of dUra(DNA) glycosylase from *B. subtilis* against PBS-2 DNAs photoalkylated for 5 and 10 min and control DNAs are shown in Fig. 1. Assays with photoalkylated substrates were performed with concentrations of DNA uracil identical to those of the control preparations. Damaged DNAs contained 0.7% and 1.6% HPGs and 0.2% and 0.5% photodimers, respectively, with a small number of HPAs. The enzyme V_{max} was decreased with photoalkylated substrates compared with control DNAs. There was a 17% decrease in V_{max} against PBS-2 DNA containing 600 modified purines per molecule and a 69% reduction against substrate containing 1,540 modified purines (Fig. 1). Therefore, the amount of inhibition of enzyme activity is dependent upon the extent of DNA damage. There were no purines released from DNA as determined by paper chromatography and TLC (7, 9, 14, 26).

In contrast to photoalkylated DNA, the rate of enzymic uracil release was practically unaffected by the presence of 3.8% of guanine as mGua. Activities against control and methylated DNAs are compared in Fig. 2. The V_{max} for the methylated DNA was practically identical to that for the control. No purines were released. Glycosylase activity against partially depurinated DNA is shown in Fig. 3. The DNA contained 1.9% of its guanine as mGua and an equal percentage as apurinic sites. This resulted in a 60% reduction of the V_{max} . The enzyme released only free uracil as demonstrated by TLC (19, 26).

FIG. 1. Double-reciprocal plots of *B. subtilis* dUra(DNA) glycosylase activity with DNA photoalkylated for 5 min (A) or 10 min (B). $\circ$, Photoalkylated substrate; $\bullet$, control (native) PBS-2 DNA substrate. The reaction time was 10 min. (A) Apparent K_m s for dUMP in DNA: $3.7 \pm 0.07 \times 10^{-7}$ M for photoalkylated substrate; $4.0 \pm 0.07 \times 10^{-7}$ M for control DNA. V_{max} was 1.05 ± 0.04 nmol of uracil released per min per ml of enzyme protein for the photoalkylated DNA and 1.26 ± 0.04 for the control DNA. (B) Apparent K_m s for dUMP in DNA: $2.5 \pm 1.1 \times 10^{-7}$ M for photoalkylated substrate; $4.5 \pm 1.0 \times 10^{-7}$ M for control DNA. V_{max} was 0.23 ± 0.03 nmol of uracil released per min per ml of enzyme protein for the photoalkylated DNA and 0.90 ± 0.01 for the control DNA.

Enzyme Processivity. The basis for the observed alterations of dUra(DNA) glycosylase activity against damaged PBS-2 DNAs was investigated. The enzyme might be distributive, dissociating from DNA after release of each uracil, or proces-

FIG. 2. Double-reciprocal plot of *B. subtilis* dUra(DNA) glycosylase activity with alkylated ($\circ$) and control (native) ($\bullet$) PBS-2 DNA substrates. The reaction time was 10 min. Apparent K_m s for dUMP in DNA: $1.6 \pm 1.0 \times 10^{-7}$ M for the methylated substrate; $3.7 \pm 1.0 \times 10^{-7}$ M for the control DNA. V_{max} was 1.29 ± 0.07 nmol of uracil released per min per ml of enzyme protein for the methylated DNA and 1.37 ± 0.06 for the control DNA.

FIG. 3. Double-reciprocal plot of *B. subtilis* dUra(DNA) glycosylase activity with depurinated ($\circ$) and control (native) ($\bullet$) PBS-2 DNA substrates. The reaction time was 10 min. Apparent K_m s for dUMP in DNA: $1.3 \pm 1.2 \times 10^{-7}$ M for the partially depurinated substrate and $1.3 \pm 0.5 \times 10^{-7}$ M for the control DNA. V_{max} was 0.16 ± 0.03 nmol of uracil released per min per ml of enzyme protein for the partially depurinated DNA and 0.40 ± 0.02 for the control DNA.

sive, moving along the DNA chain and continuously releasing uracil. dUra(DNA) glycosylase was allowed to bind to [3 H]uridine-labeled DNA; an equal amount of [14 C]uridine-labeled DNA was added and the reaction mixture was heated to 37°C . A distributive enzyme would be expected to release both uracils at the same rate. If the enzyme were processive, release of the [3 H]uracil should precede the release of [14 C]uracil by a constant increment, reflecting the length of DNA nucleotide processed. Fig. 4 shows that the latter is the case. Release of [3 H]uracil preceded that of [14 C]uracil by a constant increment of about 5% of the radioactivity present at all reaction times. Reversing the sequence by first binding the enzyme to [14 C]-labeled DNA followed by addition of [3 H]-labeled DNA reversed the order of release.

A similar result was obtained by letting enzymic excision of uracil to proceed for 4 min on the [3 H]-labeled substrate before adding the [14 C]-labeled DNA. If the enzyme were distributive, release should be quickly initiated on the second substrate, with a reduction of the rate of uracil excision from the first and the

FIG. 4. Release of uracil from native PBS-2 DNA by *B. subtilis* dUra(DNA) glycosylase. After incubation of the enzyme for 0.5 min with DNA labeled with [3 H]uridine ($\circ$), an equal amount of DNA labeled with [14 C]uridine (Δ) was added and the reaction mixture was incubated at 37°C for the times indicated. In a separate experiment the enzyme was allowed to react for 4 min with the [3 H]-labeled DNA ($\bullet$) before addition of an equal quantity of [14 C]-labeled DNA (Δ). A control experiment ($\times$) shows the reaction with [3 H]-labeled DNA in the absence of any additional [14 C]-labeled substrate. Each point represents the average of four assays done in substrate excess.

sum should equal that when the enzyme acted on only one substrate. Should the enzyme be processive, excision from the second substrate would be delayed, with continuous release of uracil from the first substrate. Fig. 4 shows that appreciable release of uracil from the second substrate did not begin until 6 min after its addition. After that, rates of uracil release were equivalent. Reversing the sequence, by first allowing the enzyme to react with ^{14}C -labeled DNA for 4 min before addition of the ^3H -labeled DNA resulted in an identical delay of uracil excision from the second substrate.

These experiments suggest that *B. subtilis* d(Ura)DNA glycosylase is processive. However, because not all uracil is released from one substrate DNA before initiation of excision from the second substrate, processivity is limited. About 5% of substrate uracil is released before the enzyme dissociates and binds to another substrate DNA molecule. The inhibitions observed here might be due to blockage of processive movement, perhaps as a consequence of structural distortion of DNA or of a high affinity of the glycosylase for these forms of DNA damage. This would result in tight binding of the enzyme to forms of DNA damage other than those involving uracil (7).

DISCUSSION

These results demonstrate that purines can be damaged reproducibly in uracil-containing DNA. These damaged DNAs were used as substrates for purified d(Ura)DNA glycosylase. Simultaneous comparisons were made between the damaged and control DNA substrates. The effects of the presence of the three different types of DNA damage on the rate of enzymic uracil excision are compared in Table 2. The inhibition index is the ratio of $V_{\max}$ of the d(Ura)DNA glycosylase with the undamaged control DNA to its $V_{\max}$ with the damaged substrate; therefore, the index increases as the latter $V_{\max}$ is decreased.

The major product of Me_2SO_4 alkylation of DNA is mGua; other products were not analyzed here, but it is obvious that the introduction of 3,200 mGua molecules per molecule does not affect enzymic uracil excision. Conversion of half of these mGua molecules to apurinic sites caused a 60% reduction in $V_{\max}$. No apurinic/aprimidinic endonuclease activity was detectable under these conditions. However, purification of the pyrimidine dimer-DNA glycosylase from T4-infected *E. coli* to homogeneity yielded a single polypeptide containing both glycosylase and apurinic/aprimidinic site endonuclease activities (30–32). Such endonuclease activity by d(Ura)DNA glycosylase, with binding to apurinic sites, would account for the observed reduction of the $V_{\max}$ with a partially depurinated substrate. Purification to homogeneity is necessary to determine if this hypothesis is correct. Although this d(Ura)DNA glycosylase has been extensively purified, a completely homogenous prepara-

tion has not been obtained (20, 33).

It is unlikely that local denaturation of DNA at damaged sites is responsible for the decrease of enzymic uracil release. Purified glycosylases excise uracil at a higher rate from single-stranded DNA or polynucleotide than from native DNA (8, 20). Therefore, single-stranded regions due to damaged or missing purines would increase, rather than decrease, the rate of uracil release from DNA. This suggests other mechanisms for these observed effects.

Alkylation of purines at the C-8 position markedly affected enzymic uracil excision. The effects of bound acetylaminofluorene moieties at the C-8 position on DNA guanine on other enzyme activities have been studied. Such adducts act as blocks to the Klenow fragment of *E. coli* DNA polymerase I (34). A number of DNA polymerases terminate immediately at or preceding such lesions in DNA segments (35). The presence of these adducts irreversibly inhibits rat brain cytosine-5-methyltransferase by blocking movement of this processive enzyme along the DNA (36). The inhibition was similar in extent to the decrease of d(Ura)DNA glycosylase activity observed here against DNA containing about the same number of C-8 guanine adducts. It therefore is possible that the blockage of DNA processive enzymes might be a common effect of C-8 modified guanines. Further investigations, with other types of C-8 modified purines, are necessary to establish if this is the case.

These experiments demonstrate that enzymic excision of uracil from DNA can be reduced by a relatively much smaller quantity of C-8 purine adducts or apurinic sites. The amount of uracil present in the DNA of prokaryotes or eukaryotes, however transiently, cannot approach the quantitative substitution of uracil for thymine present in PBS-2 DNA, nor can the extensive amount of DNA damage assayed in these experiments be compatible with cell survival. However, in all cases the number of modified purines or apurinic sites was more than 2 orders of magnitude less than the number of substrate uracils. Therefore, relatively small numbers of damaged purines markedly affect enzymic uracil repair. In order to be potentially mutagenic, uracil must arise via deamination of DNA cytosine (1, 3). Such hydrolytic deamination preferentially occurs in a single-stranded DNA; the rate of deamination in native DNA is 0.3–0.5% that of denatured DNA (1).

Damaged moieties in single-stranded DNA may block processivity of the glycosylase into the nucleotide tract containing uracil, thereby obstructing repair. Some types of lesions tend to be localized in single-stranded regions of the genome. Denatured DNA is more susceptible to binding of acetylaminofluorene moieties at the C-8 position of guanine than is native DNA, and therefore such specific regions of the genome may suffer preferential damage *in vivo* by that carcinogen (37). Similarly, the rate of purine losses in single-stranded DNA is 4 times that in double-stranded DNA (38). It therefore is possible that both uracil and other DNA lesions are localized along the genome. Such clustering would make d(Ura)DNA glycosylase inhibition by other damaged moieties a possible significant source of transition mutations. The biological significance of *in vivo* deamination of uracil to cytosine has been demonstrated in *E. coli* mutants lacking d(Ura)DNA glycosylase (4). The extent of such deamination in human DNA has not been determined because no similar human mutant cell lines are available (39, 40). Therefore, the efficient repair of uracil renders impossible the detection of deamination of cytosine.

The effects of one form of DNA damage on repair of a second, unrelated, type of lesion have been assessed by using measurements of repair in cultured cells. In one study of repair of the DNA damages caused by UV irradiation and the carcinogen *N*-acetoxy-2-acetylaminofluorene in xeroderma pigmentosum

Table 2. Inhibition of d(Ura)DNA glycosylase activity by DNA damage

Damaged adduct		
No. per PBS-2 DNA molecule	Type	Inhibition index
600	8-(2-Hydroxy-2-propyl)purines	1.2
1,540	8-(2-Hydroxy-2-propyl)purines	3.2
3,200	mGua	1.1
1,600	Apurinic sites	2.5
1,600	mGua	
9,000	Uracil dimers (7)	
		1.9

Calculations were based on a molecular weight of 2×10^8 and a base composition of 28% guanine + cytosine for PBS-2 DNA.

cells, inhibitory effects were found to be exerted by the products of one form of damage on the cellular repair enzymes for the other type (41). These results indicate a similar effect by photoalkylated purines and apurinic sites on the excision of uracil. The enzyme preparation used here has been extensively studied, and no activity against any DNA purines has been reported (20, 21). Likewise, the purified dUra(DNA) glycosylase used in these experiments had no activity against any alkylated DNA purines. One form of DNA damage may interfere with repair of a totally unrelated form of damaged DNA moiety without their necessarily sharing a common repair enzyme or pathway. Therefore, the amount of DNA repair synthesis elicited by two separate agents that damage DNA may not necessarily indicate the number of repair pathways involved. This constitutes another of the many factors by which two separate genotoxic agents may interact simultaneously in living cells and affect DNA repair, as pointed out by others (41–44).

Because deamination of DNA cytosine to uracil can occur either spontaneously or due to environmental agents, dUra(DNA) glycosylase is necessary for the prevention of the accumulation of mutagenic damage (1, 4, 5). A number of DNA modifications may interfere with uracil excision. In addition to pyrimidine dimers (7), both photoalkylated purines and apurinic sites alter such glycosylase activity. The dUra(DNA) glycosylases purified from *E. coli* and from HeLa cells remove 5-fluorouracil from DNA, but at 1/20th and 1/30th the rate of uracil removal, respectively (45, 46, 47). This suggests the possible interference of 5-fluorouracil moieties in DNA with uracil excision. It therefore is possible that a wide variety of persistent damaged DNA moieties may perturb the enzymic excision of uracil from DNA. Should such uracil have arisen from deamination of DNA cytosine, such transition mutations may arise as a consequence of totally unrelated forms of genotoxic damages.

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