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UvrA and UvrB enhance mutations induced by oxidized deoxyribonucleotides

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Abstract

Oxidatively damaged DNA precursors (deoxyribonucleotides) are formed by reactive oxygen species. After the damaged DNA precursors are incorporated into DNA, they might be removed by DNA repair enzymes. In this study, to examine whether a nucleotide excision repair enzyme, *Escherichia coli* UvrABC, could suppress the mutations induced by oxidized deoxyribonucleotides *in vivo*, oxidized DNA precursors, 8-hydroxy-2'-deoxyguanosine 5'-triphosphate and 2-hydroxy-2'-deoxyadenosine 5'-triphosphate, were introduced into *uvrA*, *uvrB*, and *uvrC* *E. coli* strains, and mutations in the chromosomal *rpoB* gene were analyzed. Unexpectedly, these oxidized DNA precursors induced mutations only slightly in the *uvrA* and *uvrB* strains. In contrast, effect of the *uvrC*-deficiency was not observed. Next, *mutT*, *mutT/uvrA*, and *mutT/uvrB* *E. coli* strains were treated with H₂O₂, and the *rpoB* mutant frequencies were calculated. The frequency of the H₂O₂-induced mutations was increased in all of the strains tested; however, the increase was three- to four-fold lower in the *mutT/uvrA* and *mutT/uvrB* strains than in the *mutT* strain. Thus, UvrA and UvrB are involved in the enhancement, but not in the suppression, of the mutations induced by these oxidized deoxyribonucleotides. These results suggest a novel role for UvrA and UvrB in the processing of oxidative damage.

Keywords: UvrA; UvrB; 8-hydroxy-dGTP; 2-hydroxy-dATP

1. Introduction

Replication errors by DNA polymerases and DNA lesions induced by mutagens cause mutations that are recognized as important steps in carcinogenesis [1-4]. The chemically modified DNA bases change the genetic information by base mismatch formation. DNA is damaged by many endogenous/environmental mutagens, such as reactive oxygen species (ROS), which are byproducts of respiration, ultraviolet and ionizing irradiation [3, 4]. The accumulation of DNA lesions seems to be involved in aging and neurodegeneration, in addition to mutagenesis and carcinogenesis.

The DNA lesions are removed by various DNA repair enzymes, as a defense system in prokaryotic and eukaryotic cells. DNA repair systems include base excision repair, nucleotide excision repair (NER), and mismatch repair [5-7]. In general, the DNA lesions formed by mutagens are removed by the base excision repair and NER systems, while the base mismatches that result from replication errors are corrected by the mismatch repair system.

Oxidative damage formed in DNA precursors is also considered to be important. Among them, oxidized dGTP and dATP, 8-hydroxy-2'-deoxyguanosine 5'-triphosphate (8-OH-dGTP) and 2-hydroxy-2'-deoxyadenosine 5'-triphosphate (2-OH-dATP), seem to be important lesions because of their mutagenicities [8]. Tajiri *et al.* reported that the accumulation of 8-hydroxyguanine (8-OH-Gua) by the incorporation of oxidized dGTP from the nucleotide pool contributed almost equally to the direct oxidation of G bases in DNA [9]. The damaged DNA precursors formed in the nucleotide pool would be hydrolyzed to the corresponding monophosphates by nucleotide pool sanitization enzymes before they are incorporated into DNA. *E. coli* MutT and its functional homologues in yeast *Saccharomyces cerevisiae* (YLR151c) and mammalian cells (MTH1)

hydrolyze 8-OH-dGTP [10-12]. Nucleotide pool sanitization activities have been identified for two *E. coli* proteins, Orf135 and Orf17 [13, 14]. The mutation frequency in *mutT*-deficient strains is 10- to 130-fold higher than that in wild type (wt) strains [9, 15, 16]. The disruption of the *YLR151c* gene in yeast causes a 14-fold increase in the spontaneous mutation frequency, as compared to wt [12]. Increased tumor formation in liver, lung, and stomach was found in MTH1 knock-out mice [17]. These findings verified that the nucleotide pool sanitization enzymes are involved in the elimination of oxidized deoxyribonucleotides *in vivo*, in addition to their enzymatic activities observed *in vitro*.

Alternatively, DNA repair enzymes may prevent the mutagenesis by the oxidized deoxyribonucleotides after they are incorporated into DNA. However, it is unclear whether oxidized deoxyribonucleotides are actually eliminated by DNA repair enzymes after their incorporation into DNA *in vivo*. Unlike the direct oxidation of bases in DNA, mutations induced by 8-OH-dGTP and 2-OH-dATP seem to be initiated by their incorporations opposite incorrect bases (A and G, respectively) [8]. In this study, we focused on an NER enzyme, the *E. coli* UvrABC nuclease. The NER enzyme is involved in the elimination of bulky DNA lesions, such as the cyclobutane thymine dimer and the (6-4) thymine [(6-4)TT] dimer [18-20]. In addition, it removes some forms of oxidative damage, such as an abasic (AP) site and thymine glycol, from DNA [21]. We examined whether the prokaryotic NER enzyme, UvrABC, is involved in the suppression of mutations induced by oxidized deoxyribonucleotides *in vivo*. Oxidized DNA precursors, 8-OH-dGTP and 2-OH-dATP, were introduced into *uvrA*, *uvrB*, and *uvrC* *E. coli* strains. In addition, *mutT*, *mutT/uvrA*, and *mutT/uvrB* *E. coli* strains were treated with hydrogen peroxide (H₂O₂). Unexpectedly, the deficiencies in UvrA and UvrB decreased, rather than increased, the

mutations by endogenous/exogenous, oxidized DNA precursors. Therefore, UvrA and UvrB may enhance the mutations induced by oxidized deoxyribonucleotides.

2. Materials and methods

2.1. Materials

The *E. coli* strains used in this study are listed in Table 1. The AB1157, AB1885, and AB1886 strains were obtained from the National Institute of Genetics, Stocks Research Center (Mishima, Japan) [22, 23]. The *mutT::Cm^r* locus of the TN1011 *E. coli* strain (Nunoshiba, unpublished) was transduced into AB1886, AB1885, and AB1157 by P1 transduction to yield KAM0001 (AB1886 but *mutT::Cm^r*), KAM0002 (AB1885 but *mutT::Cm^r*), and KAM0003 (AB1157 but *mutT::Cm^r*), respectively. KAM0004 (AB1157 but *uvrC::Tn10*) was constructed by P1 transduction of the *uvrC::Tn10* locus of the *E. coli* CAG12156 strain (*F⁻*, *uvrC279::Tn10*) (the National Institute of Genetics, Stocks Research Center). The pSF11 plasmid, containing the *uvrA* and *amp^r* genes and the pBR322 *ori*, was used as the *uvrA* expression vector [24]. The UvrA, UvrB, and UvrC proteins from *Thermus thermophilus* HB8 were overexpressed in *E. coli* and purified as reported previously ([25, 26], UvrC; unpublished data). 8-OH-dGTP and 2-OH-dATP were prepared as described previously [27, 28]. These purified deoxyribonucleotides each eluted as a single peak in both reverse-phase and anion-exchange high performance liquid chromatography (HPLC), and their purities were estimated to be more than 99% (data not shown). Oligodeoxyribonucleotides with 8-OH-Gua and 2-hydroxyadenine (2-OH-Ade) were synthesized by the phosphoramidite method,

and were purified using both reverse-phase and anion-exchange HPLC, as described previously [29]. Other oligodeoxyribonucleotides were purchased from Sigma Genosys Japan (Ishikari, Japan) in purified forms.

2.2. Introduction of oxidized deoxyribonucleotides

A single colony taken from an LB agar plate was inoculated into 7 mL of LB medium. The *E. coli* culture was incubated at 37°C until the turbidity at 570 nm reached 0.6. The culture was placed on ice for 10 min, and was divided into 500- μ L aliquots in microtubes. The microtubes were centrifuged at 13,000 \times g for 2 min at 4°C. Competent cells were prepared by a treatment with 0.1 M calcium chloride. To 50 μ L of the *E. coli* suspension, a deoxyribonucleotide solution (12.5 nmol) or H₂O was added, and then the mixture was placed on ice for 3 h. Prewarmed SOC medium (450 μ L) was then added, and cells were incubated at 37°C for 1 h. A portion of the suspension was transferred to an LB agar plate (titer plate), which was incubated at 37°C for 12 h. Another portion of the suspension was transferred to an LB agar plate containing rifampicin (100 μ g/mL) (selection plate), which was incubated at 37°C for 20 h. The *rpoB* mutant frequency was calculated according to the numbers of colonies on the titer and selection plates. *E. coli* cells harboring the pBR322 and pSF11 plasmids were grown in LB medium and on plates with ampicillin (50 μ g/mL). The statistical significance of the values was examined by Mann-Whitney U-test.

2.3. Mutant frequencies upon H₂O₂ treatment

A single colony taken from an LB agar plate was inoculated into 7 mL of LB medium. The *E. coli* culture was incubated at 37°C until the turbidity at 570 nm reached 0.2, and then 1 mL of the

culture was transferred to a microtube. After H₂O₂ (final concentration of 2 mM) or H₂O was added, the culture was incubated at 37°C for 30 min. The culture was then centrifuged at 13,000 X g for 2 min at room temperature. The pellet thus obtained was resuspended in 1 mL of prewarmed LB medium, cultured at 37°C for 2 h, and then placed on ice. The *rpoB* mutant frequency was calculated as described above.

2.4. Preparation of DNA substrates containing oxidative damage

ODN-1 (5'-dGGCACCAATAACTGCCTT-3'), ODN-2 (5'-dGGTGGCCTGXCGCATTCCCCAA-3'), and ODN-3 (5'-dTACGACTCATTATAGGGAGA-3'), where X represents either 8-OH-Gua or 2-OH-Ade, were used for the construction of 60 mer oligodeoxyribonucleotides containing the oxidized bases. Scaffold-1 (5'-dCAGGCCACCAAGGCAGTTA-3') and Scaffold-2 (5'-dAGTCGTATTGGGG-3') were used as scaffold oligodeoxyribonucleotides. The 5' ends of ODN-2 and ODN-3 were phosphorylated with T4 polynucleotide kinase (Toyobo, Osaka, Japan). After annealing the mixture of these oligodeoxyribonucleotides, T4 DNA Ligase (Takara, Kusatsu, Japan) was added. The ligated products were dissolved in 95% formamide and purified on a denaturing 9% polyacrylamide gel. The 60 mer oligodeoxyribonucleotides recovered from the gel were passed through an NAP5 column (GE Healthcare, Piscataway, New Jersey, USA) to remove the urea from the gel. The resulting oligodeoxyribonucleotides were annealed to the complementary strand, ODN-4 (5'-dTCTCCCTATAATGAGTCGTATTGGGGAATGCGYCAGGCCACCAAGGCAGTTATTGTGCC-3'), to yield 60-bp duplex DNAs with 8-OH-Gua and 2-OH-Ade (Fig. 1 A). The 5' end of ODN-1 or ODN-4 was labeled with Cy5. The 59-bp duplex DNA with the (6-4)TT dimer was

prepared by a similar procedure (Fig. 1 B).

2.5. *UvrABC* incision assay

The substrates containing 8-OH-Gua and 2-OH-Ade (20 nM) were incubated in 50 μ L of buffer (50 mM Tris-HCl (pH 7.5), 100 μ M FeSO₄, 10 mM MgCl₂, 50 mM KCl, 1 mM ATP, 5 mM DTT) at 60°C for 5 min. The UvrA (120 nM), UvrB (720 nM), and UvrC (300 nM) proteins were then added, and the mixtures were further incubated at 60°C for 2 h [30]. Reactions were terminated by the addition of EDTA (final concentration of 20 mM). The sample was dissolved in 95% formamide, heated at 100°C for 10 min, and fractionated by 12% denaturing PAGE. The incision efficiency was analyzed with a fluorescent image analyzer (FLA-2000 Fluorescent Image Analyzer, Fuji Photo Film, Tokyo, Japan).

3. Results

3.1. *Reduced mutation frequencies in uvrA and uvrB strains upon the direct introduction of oxidized deoxyribonucleotides*

It was previously demonstrated that the direct incorporation of 8-OH-dGTP and 2-OH-dATP, added to an *E. coli* suspension, induced chromosomal gene mutations [8]. In addition, these oxidized DNA precursors were introduced into *E. coli* strains deficient in nucleotide pool sanitization enzymes and specialized DNA polymerases, to examine their *in vivo* functions [16, 31, 32]. In this study, we examined whether an NER enzyme, the *E. coli* UvrABC nuclease, suppresses

mutations induced by oxidized deoxyribonucleotides *in vivo*. Oxidized deoxyribonucleotides, 8-OH-dGTP and 2-OH-dATP, were introduced into wt, *uvrA*, and *uvrB* *E. coli* strains, and the mutant frequencies for the chromosomal *rpoB* gene were calculated. Growth rates for these strains, and numbers of colonies on titer plates were very similar (data not shown).

In the wt strain, the mutant frequencies were increased to 14×10^{-8} and 16×10^{-8} upon the treatments with 12.5 nmol of 8-OH-dGTP and 2-OH-dATP, respectively, while the mutant frequency was 5.0×10^{-8} with the H₂O treatment (Table 2, Expt. 1). Thus, 8-OH-dGTP and 2-OH-dATP induced mutations in the wt strain, as reported previously [8]. Remarkably, the deficiency in UvrA or UrvB resulted in the reduction in the mutations caused by these oxidized deoxyribonucleotides. In the *uvrA* strain, the mutant frequencies upon the treatments with H₂O, 8-OH-dGTP and 2-OH-dATP were five-, seven-, and eight-fold lower than those in the wt strain. Similar results were obtained with the *uvrB* strain (Table 2, Expt. 1). The introduction of 8-OH-dGTP and 2-OH-dATP induced mutations only slightly in the *uvrA* and *uvrB* strains.

We then examined whether the introduction of the pSF11 plasmid, carrying the *uvrA* gene, into the *uvrA* *E. coli* strain could restore the “sensitivity” to the exogenous deoxyribonucleotides. The pBR322 plasmid was transfected into the *uvrA* strain and was used as a control. Both strains were treated with the oxidized deoxyribonucleotides, and the *rpoB* mutant frequencies were examined. The mutant frequencies in the *uvrA* cells carrying the pBR322 plasmid upon the treatments with the damaged DNA precursors were similar to those in the *uvrA* cells without the plasmid (Table 2, Expt. 2). On the other hand, the mutant frequencies in the *uvrA* cells carrying the pSF11 plasmid were 12×10^{-8} upon the treatments with 12.5 nmol of 8-OH-dGTP and 2-OH-dATP. Thus, the introduction of 8-OH-dGTP and 2-OH-dATP significantly induced mutations in the *uvrA* cells

carrying the pSF11 plasmid, as in the wt strain, but not in the *uvrA* cells carrying the pBR322 plasmid. These results suggest that UvrA and UvrB enhanced the mutagenesis induced by the exogenous, damaged DNA precursors.

3.2. Reduced mutation frequency in *uvrA*- and *uvrB*-deficient *mutT* strains

The above-mentioned results revealed the effects of *uvrA*- and *uvrB*-deficiencies on the mutations induced by exogenous, oxidized deoxyribonucleotides. We then focused on the mutations induced by endogenous 8-OH-dGTP, accumulated because of a *mutT*-deficiency. MutT, a nucleotide pool sanitization enzyme, catalyzes the hydrolysis of 8-OH-dGTP and the corresponding diphosphate, 8-hydroxy-2'-deoxyguanosine 5'-diphosphate, and the mutation frequency in *mutT*-deficient strains is 10- to 130-fold higher than that in wt strains [9, 10, 15, 16, 33]. Since the strong mutator phenotype of cells defective in the *mutT* gene was completely suppressed under anaerobic conditions [34], MutT seems to play a crucial role in the removal of 8-OH-dGTP from the nucleotide pool. We examined the effects of the UvrA- and UvrB-deficiencies on the mutations caused by endogenous 8-OH-dGTP spontaneously accumulated in *mutT* cells. Moreover, the oxidation of dGTP would increase under the oxidative stress produced by the exposure to H₂O₂ in *mutT* cells. G is highly susceptible to oxidative stress, as it has the lowest redox potential of all four bases [35]. Indeed, the mutation frequency in the *mutT* cells was one order of magnitude higher than that in the wt cells, when these bacterial cells were treated with H₂O₂ (Hori *et al.*, unpublished results), indicating the enhanced accumulation of 8-OH-dGTP in the *mutT* cells exposed to H₂O₂. We examined the effects of the UvrA- and UvrB-deficiencies on mutations caused by endogenous 8-OH-dGTP in *mutT* cells treated with H₂O₂.

E. coli mutT, *mutT/uvrA*, and *mutT/uvrB* strains were treated with 2 mM H₂O₂, and the *rpoB* mutant frequencies were examined. The mutation frequencies were increased in all strains tested with 2 mM H₂O₂. The mutant frequency upon the H₂O₂ treatment was 41×10^{-7} in the *mutT* strain (Table 3, Expt. 1). The deficiency in the *uvrA* or *uvrB* gene resulted in the reduction in the mutations caused by oxidized dGTP endogenously accumulated in the *mutT* cells. The increase in the mutant frequency was three- and four-fold lower in the *mutT/uvrA* and *mutT/uvrB* strains than in the *mutT* strain. As with the exogenous 8-OH-dGTP treatment, the presence of UvrA and UvrB enhanced the mutations caused by the endogenous 8-OH-dGTP.

The pSF11 plasmid carrying the *uvrA* gene was transfected into the *mutT/uvrA E. coli* cells, and the transformed cells were treated with H₂O₂. The *mutT/uvrA* cells with the pBR322 plasmid were used as a control. Each *E. coli* strain was treated with 2 mM H₂O₂, and the *rpoB* mutant frequencies were observed. The mutant frequencies obtained upon the treatment were similar between the *uvrA* cells carrying the pBR322 plasmid and the *uvrA* cells (Table 3, Expt. 2). On the other hand, the mutant frequency observed upon the H₂O₂ treatment was increased to 37×10^{-7} in the *mutT/uvrA/pSF11* cells. The mutant frequency upon the treatment in the *mutT/uvrA/pBR322* cells was three-fold lower than that in the *mutT/uvrA/pSF11* cells. These results suggest that UvrA and UvrB were involved in the enhancement of the mutations induced by the endogenous, oxidized deoxyribonucleotide.

3.3. Incision activity of UvrABC toward a DNA substrate containing oxidative damage

The UvrABC nuclease incises the damaged DNA strand and prevents the toxicity and mutations caused by the damage. The fact that the deficiencies in UvrA and UvrB increased the mutations by

oxidized DNA precursors prompted us to hypothesize that UvrABC incises the undamaged DNA strand, instead of the strands containing 8-OH-Gua and 2-OH-Ade, formed by the incorporation of 8-OH-dGTP and 2-OH-dATP, respectively. If the incorrect incision occurs, then an A base opposite 8-OH-Gua is removed and dCTP is incorporated opposite 8-OH-Gua by DNA polymerase I, resulting in the enhancement of the A:T→C:G transversion [36, 37]. Likewise, the G:C→T:A mutation is enhanced by the putative incision when DNA polymerase I inserts dTTP, instead of the original G [38].

We examined this possibility by using the purified UvrABC nuclease from *Thermus thermophilus* HB8, a thermophilic bacterium, *in vitro*. The amino acid sequences of *T. thermophilus* UvrABC share 70% homology to those of *E. coli* UvrABC, suggesting that the mechanism of NER in *T. thermophilus* is similar to that in *E. coli* [25, 26, 39]. UvrC catalyzes both incisions, the first incision at the 5th phosphodiester bond on the 3' side of the lesion X (3'-incision) and the second incision at the 8th phosphodiester bond on the 5' side of the lesion X (5'-incision, Fig. 1 A). The 5' ends of damaged and undamaged strands of the 60-bp DNA duplexes containing 8-OH-Gua and 2-OH-Ade were labeled with Cy5 (Fig. 1 A). After the addition of *T. thermophilus* UvrABC, the reaction mixture was incubated at 60°C for 2 h.

Under the conditions described in the Materials and Methods section, the incision activity on the DNA substrate containing the (6-4)TT dimer was 70% (Figs. 1 B and 2 V). 24mer (5'-incision) and 37mer (3'-incision) products were observed due to uncoupled 5'- and 3'-incisions. On the other hand, neither the damaged nor undamaged strand of the DNA substrates containing 8-OH-Gua and 2-OH-Ade was incised (Fig. 2 I-IV). Thus, the hypothesis that UvrABC incises the undamaged strand of DNA duplexes containing 8-OH-Gua and 2-OH-Ade is unlikely.

3.4. Effects of *uvrC*-deficiency on mutations induced by oxidized deoxyribonucleotides

We examined whether UvrC could promote the mutations induced by oxidized deoxyribonucleotides, like UvrA and UvrB, since we did not observe the expected incision of the undamaged strands. 8-OH-dGTP and 2-OH-dATP were introduced into *uvrC E. coli* cells, and the mutant frequencies were examined. In the *uvrC* strain, the mutant frequencies upon the treatments with 8-OH-dGTP and 2-OH-dATP were increased (Table 2, Expt. 3). The effect of the UvrC-deficiency was not observed, in contrast to the UvrA- and UvrB-deficiencies. These results suggest that UvrA and UvrB play the major role in the mutation enhancement.

4. Discussion

Nucleotide pool sanitization enzymes are expected to be involved in the elimination of oxidized deoxyribonucleotides, and their antimutator functions have been shown *in vivo* [16, 31, 40]. It is not apparent, however, whether DNA repair enzymes could eliminate oxidized deoxyribonucleotides after their incorporation into DNA. In this study, we examined the possibility that the NER enzyme, UvrABC, would suppress the mutations induced by oxidized deoxyribonucleotides *in vivo*.

When we introduced oxidized DNA precursors, 8-OH-dGTP and 2-OH-dATP, into the wt, *uvrA*, and *uvrB E. coli* strains, the oxidized DNA precursors induced mutations in the wt cells, and the induction was only slight in the *uvrA* and *uvrB* cells (Table 2, Expt. 1). The low mutation frequencies upon the 8-OH-dGTP and 2-OH-dATP treatments in the *uvrA* cells were increased to

the levels in the wt cells when the *uvrA* gene on the pSF11 plasmid was introduced (Table 2, Expt. 2). Therefore, UvrA and UvrB were involved in the increase in the mutations induced by 8-OH-dGTP and 2-OH-dATP. In addition, we analyzed the nucleotide sequence of the *rpoB* gene in rifampicin-resistant colonies derived from each strain (wt, *uvrA*, *uvrB*, *uvrA/pBR322*, and *uvrA/pSF11*). Although A:T→C:G and G:C→T:A transversion mutations were induced by 8-OH-dGTP and 2-OH-dATP in all of the *E. coli* strains, the frequencies of these transversions were lower in the *uvrA*, *uvrB*, and *uvrA/pBR322* strains (data not shown). This result supports the unexpected finding that the lack of UvrA or UvrB reduced the mutations induced by the damaged DNA precursors. The UvrABC nuclease is a DNA repair enzyme, and it suppresses the mutations and elevates the survival rates under UV-irradiation conditions [23, 41]. However, the results obtained in this study suggest that UvrA and UvrB are involved in the enhancement, but not in the suppression, of the mutations induced by 8-OH-dGTP and 2-OH-dATP.

To confirm this interpretation, the mutation frequencies induced by 8-OH-dGTP accumulated endogenously, not by exogenous 8-OH-dGTP, were measured. In addition to the spontaneous mutation frequency, the mutation frequency upon H₂O₂ treatment was one order of magnitude higher in the *mutT* strain than in the wt strain (Hori *et al.*, unpublished results), indicating that the concentration of intracellular 8-OH-dGTP (the MutT substrate) increased with the H₂O₂ treatment. Thus, the *mutT*, *mutT/uvrA*, and *mutT/uvrB* *E. coli* strains were treated with H₂O₂, and the mutation frequencies were examined. The mutation frequencies were lower in the *mutT/uvrA* and *mutT/uvrB* strains than in the *mutT* strain (Table 3, Expt. 1). The mutation frequency upon the H₂O₂ treatment was enhanced when the *uvrA* gene on the pSF11 plasmid was introduced into the *mutT/uvrA* cells (Table 3, Expt. 2). These results were similar to those obtained when the cells were treated with

exogenous 8-OH-dGTP, as described above. In all cases of the introduction of exogenous 8-OH-dGTP and 2-OH-dATP, and the H₂O₂ treatment of the *mutT* strains, the mutation frequencies were decreased in the absence of functional *uvrA* and *uvrB* genes. These results suggest that UvrA and UvrB enhance the mutations induced by these oxidized deoxyribonucleotides.

We examined whether this result was dependent on the target gene. The frequencies of *gyrA* mutations, as detected by nalidixic acid resistance, were examined after the H₂O₂ treatment of the *mutT*, *mutT/uvrA*, and *mutT/uvrB* strains. The *gyrA* mutant frequencies in the *mutT/uvrA* and *mutT/uvrB* cells were lower than that in the *mutT* cells, as in the case of the *rpoB* gene (data not shown). Thus, the reduced mutation frequencies in the *uvrA*- and *uvrB*-deficient cells were independent of the target genes.

It was reported that undamaged DNA may be incised by the human and *E. coli* NER system [42]. We hypothesized that the complementary strand, not the strand containing oxidized base damage, is incised by UvrABC, thus causing the increase in the *in vivo* mutation frequencies in the NER-proficient strains. However, purified UvrABC from *T. thermophilus* HB8 incised neither the damaged nor undamaged strand, when DNAs containing 8-OH-Gua and 2-OH-Ade were incubated with UvrABC (Fig. 2). This result excludes the possibility that UvrABC fixes the mutations induced by oxidatively damaged deoxyribonucleotides by the cleavage of the undamaged, complementary strand.

In addition, this result is in agreement with the finding that the UvrC-deficiency did not affect mutant frequencies upon the treatment with 8-OH-dGTP and 2-OH-dATP, in contrast to the UvrA- and UvrB-deficiencies (Table 2, Expt. 3). Thus, it seems that UvrA and UvrB mainly contribute to the mutation enhancement. These results prompted us to examine the hypothesis that UvrA and

UvrB bind to DNA containing 8-OH-Gua and 2-OH-Ade, and inhibit their removal by other DNA repair systems. However, UvrA and UvrB have similar affinities for oligodeoxyribonucleotides containing 8-OH-Gua and 2-OH-Ade and for unmodified DNA *in vitro* (data not shown). Previously, the absence of both recognition and incision of 8-OH-Gua-modified DNA duplexes by purified UvrA, UvrB, and UvrC was reported [43].

Mfd, which is also known as TRCF (transcription repair coupling factor), recognizes RNA polymerase stalled at a DNA lesion, removes the transcription complex, and recruits the NER complex to the site [44, 45]. Mfd shares both the sequence and structural similarity with UvrB [46]. These regions in Mfd may interact with UvrA and UvrB. UvrA was found to be involved in illegitimate recombination by interacting with RecQ helicase [47]. In addition, the double *polA/uvrA* and *polA/uvrB* *E. coli* mutants are not viable [48 49], suggesting a functional interaction among UvrA, UvrB, and DNA polymerase I. Thus, the decrease in the mutant frequencies in the *uvrA* and *uvrB* strains may be related to the loss of the interactions with other proteins involved in DNA repair.

The control mutant frequencies in the *uvrA* and *uvrB* strains were lower than that in the wt strain (Table 2, Expt. 1) although the opposite tendency was previously reported [50]. In addition, the introduction of the pSF11 plasmid carrying the *uvrA* gene into the *uvrA* strain increased the control mutant frequency (Table 2, Expt. 2). Molecular mechanism(s) of the low mutant frequencies in the *uvrA* and *uvrB* strains observed in this study are currently unknown. G:C→A:T transition was the most frequently observed mutation in the wt, *uvrA*, *uvrB*, and *uvrA/pSF11* strains (data not shown).

In this study, we observed that the deficiencies in UvrA and UvrB decreased the mutagenesis

induced by oxidized DNA precursors. Similar data were reported for mutagen-induced mutations. When a plasmid containing $^1\text{O}_2$ -induced damage was transfected into wt and *uvrA* cells, the mutant frequency was two-fold lower in the *uvrA* cells than in the wt cells [51]. Therefore, UvrA and UvrB may be involved in the enhancement of mutations for some DNA lesions, although the mechanism of the enhancement remains to be solved.

In conclusion, UvrA and UvrB are involved in the promotion of the mutations induced by oxidized deoxyribonucleotides. These proteins might play various roles in DNA maintenance beyond NER, and the promotion of mutations may be induced by these functions. The elucidation of this mechanism would clarify the functions of the proteins involved in DNA repair. In addition, the examination of other repair pathways possibly involved in the prevention of the mutagenesis induced by 8-OH-dGTP and 2-OH-dATP, mutagenic deoxyribonucleotides [52], is important. Experiments along this line are in progress.

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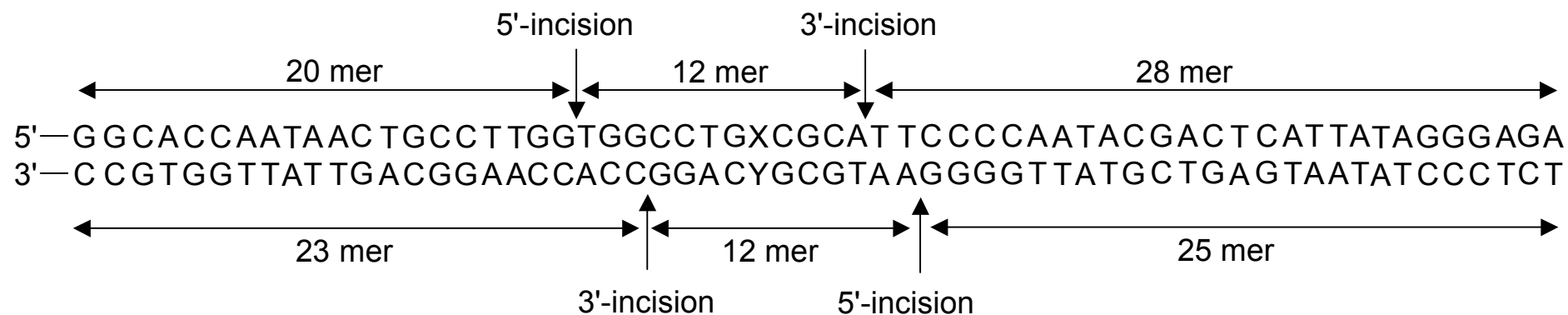
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Figure Legends

Fig. 1 Substrates containing (A) 8-OH-Gua and 2-OH-Ade, and (B) (6-4)TT dimer. The phosphodiester bonds possibly incised by UvrABC are indicated by vertical arrows. (A) The damaged base (X), its pairing base (Y), and the position of the Cy5 label are shown in the table under the sequence. (B) TT represents the (6-4)TT dimer. The damaged strand was labeled with Cy5.

Fig. 2 Incision activities of UvrABC for DNA duplexes containing 8-OH-Gua (I and II) and 2-OH-Ade (III and IV). The DNA substrates (20 nM) were 5'-terminally labeled with Cy5 on the non-damaged complementary strand (I and III) or the damaged strand (II and IV), and were incubated with UvrABC (120 nM UvrA, 720 nM UvrB, 300 nM UvrC) at 60°C for 2 h. The DNA containing a (6-4)TT dimer was used as the positive control for UvrABC (V). Products were analyzed by denaturing 12% PAGE.

(A)



Substrate	X	Y	Strand labeled with Cy5
I	8-OH-Gua	A	non-damaged complementary strand
II	8-OH-Gua	A	damaged strand
III	2-OH-Ade	G	non-damaged complementary strand
IV	2-OH-Ade	G	damaged strand

(B)

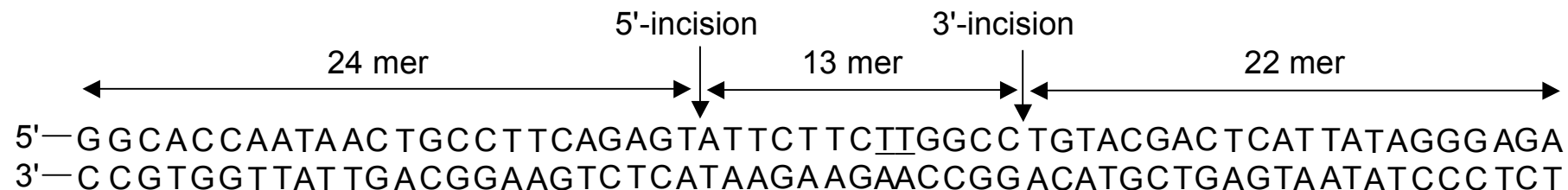


Fig. 1

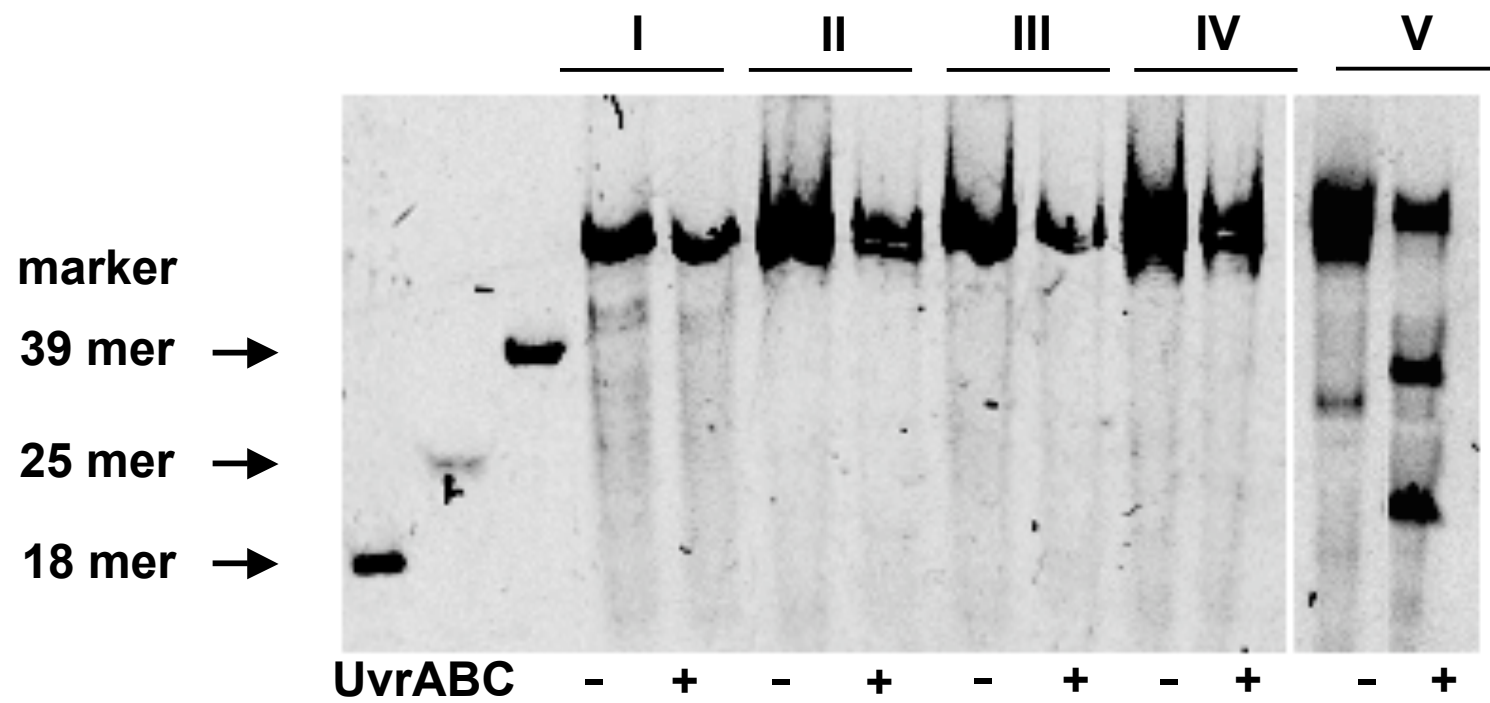


Fig. 2

Table 1 *E. coli* strains used in this study.

Strains	Relevant genotype	Origin or source
AB1157	<i>thr-1, leuB6, thi-11, lacY1, galk2, ara-14, xyl-5, mtl-1, proA2, his-4, argE3, rpsL31, tsx-33, supE44, flaND</i>	[22]
AB1885	As AB1157 but <i>uvrB5</i>	[23]
AB1886	As AB1157 but <i>uvrA6</i>	[23]
KAM0001	As AB1886 but <i>mutT::Cm^r</i>	This study
KAM0002	As AB1885 but <i>mutT::Cm^r</i>	This study
KAM0003	As AB1157 but <i>mutT::Cm^r</i>	This study
KAM0004	As AB1157 but <i>uvrC279</i>	This study

Table 2 Mutant frequencies in various *E. coli* strains upon treatment with oxidized deoxyribonucleotides^a.

	Mutant frequency (X 10 ⁻⁸)		
	None	8-OH-dGTP	2-OH-dATP
Expt. 1			
AB1157 (wt)	5.0 (3.8-11)	14 ^c (6.6-20)	16 ^c (13-25)
AB1886 (<i>uvrA</i>)	1.0 ^d (0.6-1.9)	2.0 ^{b, d} (1.4-3.4)	2.1 ^{c, d} (1.0-2.7)
AB1885 (<i>uvrB</i>)	1.3 ^d (0.9-2.3)	2.3 ^d (1.4-4.5)	1.5 ^d (0.9-3.6)
Expt. 2			
AB1886/pBR322	0.97 (0.58-1.6)	1.2 (0.66-1.8)	0.85 (0.52-2.0)
AB1886/pSF11 (<i>uvrA</i> ⁺)	2.9 ^d (1.6-3.7)	12 ^{c, d} (7.7-16)	12 ^{c, d} (9.4-13)
Expt. 3			
AB1157 (wt)	6.9 (4.8-7.5)	22 ^b (19-35)	36 ^b (23-64)
KAM0004 (<i>uvrC</i>)	8.9 (7.4-13)	15 ^b (13-24)	17 ^b (12-27)

^a Experiments were repeated at least seven times in Expt. 1 and eleven times in Expt. 2. Experiments were repeated five times for AB1157 and eleven times for KAM0004 in Expt. 3. There was no difference in numbers of colonies on titer plates and growth rates for the strains used in each experiment. The data are expressed as median and numbers in parentheses represent 95% confidence limits for the median measured in individual experiments.

^b P<0.05, ^c P<0.01 (significant difference versus with H₂O (None) treatment, Mann-Whitney U-test)

^d P<0.01 (significant difference versus the same treatment group of wt in Expt. 1 and AB1886/pBR322 in Expt. 2, Mann-Whitney U-test)

Table 3 H₂O₂-induced mutant frequencies in various *E. coli* strains^a.

	Mutant frequency (X 10 ⁻⁷)	
	0 mM	2 mM
Expt. 1		
KAM0003 (<i>mutT</i>)	4.2 (3.6-7.3)	41 ^b (18-47)
KAM0001 (<i>mutT/uvrA</i>)	3.4 (3.1-5.4)	13 ^{b, c} (11-21)
KAM0002 (<i>mutT/uvrB</i>)	3.7 (2.6-4.1)	10 ^{b, c} (6.8-13)
Expt. 2		
KAM0001/pBR322	3.2 (2.6-4.1)	12 ^b (6.9-19)
KAM0001/pSF11 (<i>uvrA</i> ⁺)	17 ^{b, c} (14-20)	37 ^{b, c} (32-59)

^aExperiments were repeated at least eighteen times in Expt. 1 and sixteen times in Expt. 2.

There was no difference in numbers of colonies on titer plates and growth rates for the strains used in each experiment. The data are expressed as median and numbers in parentheses represent 95% confidence limits for the median measured in individual experiments.

^bP<0.01 (significant difference versus with 0 mM treatment, Mann-Whitney U-test)

^cP<0.01 (significant difference versus with the same treatment group of KAM0003 in Expt. 1 and KAM0001/pBR322 in Expt. 2, Mann-Whitney U-test)