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Mutagenicity of oxidized DNA precursors in living cells: Roles of nucleotide pool sanitization and DNA repair enzymes, and translesion synthesis DNA polymerases

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Abstract

The base moieties of DNA precursors in the nucleotide pool are subjected to oxidative damage, and the formation of damaged DNA precursors is an important source of mutagenesis. 8-Hydroxy-2'-deoxyguanosine 5'-triphosphate, also known by the name of its keto-enol tautomer as 8-oxo-7,8-dihydro-2'-deoxyguanosine 5'-triphosphate, and 2-hydroxy-2'-deoxyadenosine 5'-triphosphate have been identified as the major products of *in vitro* oxidation reactions. The mutagenicities of these damaged precursors in living cells will be summarized in this review. In addition, the roles of the nucleotide pool sanitization and DNA repair enzymes, and the translesion synthesis DNA polymerases will be described.

Keywords: oxidized DNA precursor, 8-Hydroxy-dGTP, 2-Hydroxy-dATP, Translesion synthesis DNA polymerase, Nucleotide pool sanitization enzyme, DNA repair enzyme

1. Introduction

DNA lesions are formed by reactive oxygen species (ROS), and the mutational properties of many DNA lesions have been examined [1]. The formation of damaged DNA bases is recognized as being related to mutagenesis, carcinogenesis, neurodegeneration, and aging [2–4]. In addition to the base moieties of DNA, those of DNA precursors (2'-deoxyribonucleoside 5'-triphosphates) in the cellular nucleotide pool are also subjected to oxidative damage, and the formation of the damaged DNA precursors is another source of mutagenesis [5,6]. In *in vitro* oxidation reactions, guanine and adenine bases in dG(TP) and dA(TP), respectively, were more susceptible to oxidation than those in DNA [7,8], and 8-hydroxy-2'-deoxyguanosine 5'-triphosphate (8-OH-dGTP, 8-oxo-7,8-dihydro-2'-deoxyguanosine 5'-triphosphate) and 2-hydroxy-2'-deoxyadenosine 5'-triphosphate (2-OH-dATP) were identified as the major products of dGTP and dATP oxidation, respectively (Fig. 1). Moreover, intracellular DNA is bound with proteins, such as histones, and thus its reactivity would be lower than that of the “naked” DNA used in the *in vitro* oxidation reactions. 8-OH-dGTP was reportedly present at a concentration of 1–10% relative to unmodified dGTP, a remarkably high percentage, in the mitochondrial nucleotide pool [9].

The initial step of the mutation process by an oxidized DNA precursor is its incorporation into DNA. Since it competes with the normal nucleotides, which are present in much larger amounts in cells, its incorporation efficiency is very important.

The next step of the mutation process is insertion of a nucleobase opposite the damaged base in the DNA during the next round of replication. The template base paired with the damaged nucleotide (the first round of replication) and the nucleobase inserted opposite the damaged base (the next round of replication) are possibly different. In such a case, the oxidized precursor induces a mutation. For example, an A:T→C:G (A→C) mutation is induced when 8-OH-dGTP is incorporated opposite A and dCTP is inserted opposite 8-hydroxyguanine (8-OH-Gua, 8-oxo-7,8-dihydroguanine), as described below. In contrast, dUTP, formed from dCTP, is unlikely to be mutagenic, because dUTP is exclusively incorporated opposite A, and dATP is exclusively inserted opposite U. Thus, the kinds of DNA polymerases (pols) involved in the damaged nucleotide incorporation and the damaged base bypass are the determinant factors since mispair formation depends on natures of DNA pols.

Nucleotide pool sanitization and DNA repair function as defense systems against the mutagenesis caused by damaged nucleotides. Specific degradation of the oxidized DNA precursors by the nucleotide pool sanitization enzyme(s) could prevent their incorporation by DNA pols. Removal of the base damage derived from the damaged precursor by DNA repair machinery could restore the DNA to the status before the incorporation of the oxidized DNA precursor.

In vitro experiments using purified DNA pols, nucleotide pool sanitization enzymes, and DNA repair proteins have provided quite useful data on the possibilities of

their stimulatory and suppressive roles in the mutation process. Experiments with synthetic 2'-deoxyribonucleoside 5'-triphosphates in living cells could provide direct evidence for their roles in the process. In this review, the author will summarize the mutagenicity of oxidized DNA precursors in living cells, and the roles of the nucleotide pool sanitization and DNA repair enzymes, and the translesion synthesis (TLS) DNA pols.

2. Mutations induced by oxidized DNA precursors in living cells

2.1. Induced mutations in bacterial cells

The mutational properties of 8-OH-dGTP and 2-OH-dATP in *Escherichia coli* cells were examined by the direct introduction of these damaged DNA precursors into bacterial cells treated with CaCl_2 and the isolation of *lacI/lacO^c* and *rpoB* mutants [10,11]. The introduction of oxidized dGTP and dATP increased the occurrence of substitution mutations, in contrast to the treatments with unmodified dGTP and dATP. An A:T→C:G transversion was the substitution mutation found most frequently in the 8-OH-dGTP-induced mutants [10,11]. The most frequent substitution mutation was a G:C→T:A transversion in the case of 2-OH-dATP. The A:T→C:G and G:C→T:A transversions observed for 8-OH-dGTP and 2-OH-dATP, respectively, would be initiated by their misincorporations opposite A and G (Fig. 2). These interpretations are supported

by the results of *in vitro* incorporation experiments using *E. coli* DNA pol III, the replicative DNA pol [12–14].

In addition, A:T→C:G and G:C→T:A transversion mutations are spontaneous mutations found in an *E. coli* strain lacking superoxide dismutases and a repressor for iron-uptake systems, and Nunoshiba *et al.* concluded that these mutations would be caused by 8-OH-dGTP and 2-OH-dATP [15]. Thus, the 8-OH-dGTP and 2-OH-dATP formed in the nucleotide pool are suggested to be important sources of the mutations induced by ROS in bacterial cells.

The mutational properties of other damaged DNA precursors produced by ROS and reactive nitrogen species have been reported [16,17]. In addition, Fapy-dGTP, *N*⁶-(2-deoxy- α,β -D-ribofuranosyl)-2,6-diamino-5-formamido-4-hydroxypyrimidine 5'-triphosphate), another oxidation product of dGTP, is nonmutagenic in *E. coli* (Kamiya *et al.*, unpublished results).

2.2. *Induced mutations in mammalian cells*

The mutagenicity of 8-OH-dGTP in mammalian cells was examined by the introduction of 8-OH-dGTP plus shuttle plasmid DNA containing the *supF* gene into simian COS-7 and human 293T cells [18,19]. The oxidized form of dGTP induced A:T→C:G substitution mutations in mammalian cells, as in *E. coli* cells. In contrast, we did not detect significant mutation induction by the introduction of 2-OH-dATP (Satou *et*

al., unpublished results). Thus, the mutagenic potential of the oxidized form of dATP is limited in mammalian cells. In agreement with these results, the mutagenicity of 2-OH-dATP was substantially lower than that of 8-OH-dGTP in *in vitro* DNA replication with a HeLa cell extract [20,21].

3. Roles of Y-family DNA polymerases in mutation induction

The initial step of the mutation process by an oxidized DNA precursor is its incorporation into DNA, and DNA pols play important roles in induced mutation formation. The TLS DNA pols, including *E. coli* DNA pol IV (DinB) and human DNA pols η (XPV) and ι , reportedly incorporate 8-OH-dGTP opposite A with quite high frequencies *in vitro* [22–24]. The incorporation of 2-OH-dATP opposite T and G also occurs with similar efficiencies by the TLS DNA pols [22–24]. These results suggest that TLS pols are involved in the misincorporation of 8-OH-dGTP and 2-OH-dATP in cells. To examine this possibility, these oxidized nucleotides were introduced into *E. coli* strains deficient in the TLS DNA pols, DNA pols IV and V (UmuD'₂C). Moreover, 8-OH-dGTP was introduced into human 293T cells, in which the TLS DNA pols were knocked-down by siRNAs.

We expected that the lack of a TLS DNA pol would result in fewer mutations induced by 8-OH-dGTP and 2-OH-dATP, if the DNA pol was involved in their misincorporation. In agreement with this speculation, the mutation induced by

2-OH-dATP occurred less frequently in the *dinB E. coli* strain than in the wild-type (wt) strain, suggesting the involvement of DNA pol IV in the mutagenesis by 2-OH-dATP [25]. Meanwhile, the DinB-deficiency had no effect on the mutagenesis by 8-OH-dGTP. Unexpectedly, in the case of DNA pol V, both 8-OH-dGTP and 2-OH-dATP induced mutations more efficiently in the *umuDC* strain than in the wt strain [25]. When additional pol V was expressed from a plasmid in the wt strain, fewer mutations caused by 8-OH-dGTP and 2-OH-dATP were detected. These results suggested that pol V suppressed the mutagenesis induced by these oxidized 2'-deoxyribonucleotides. Interestingly, the DNA pol activity was not required for the suppressive effects of DNA pol V. These results suggested that *E. coli* DNA pol IV is involved in the mutagenesis by 2-OH-dATP, and that the *umuDC* gene products play suppressive role(s) in the mutagenesis by both 8-OH-dGTP and 2-OH-dATP. DNA pol IV seems to facilitate the mutagenicity of 2-OH-dATP through the misincorporation of 2-OH-dATP [25]. However, the possibility that DNA pol IV is involved in the incorporation of dTTP opposite 2-hydroxyadenine (2-OH-Ade) and/or the extension from the 3'-terminal 2-OH-Ade residue during the second round of replication remains to be determined.

To examine the effects of the knock-downs of specialized DNA pols in human cells, we treated 293T cells with siRNAs against DNA pols η , ι , and ζ , and REV1, and introduced 8-OH-dGTP into the knocked-down cells [19]. The knock-downs of DNA pols η and ζ , and REV1 decreased the frequency of A:T→C:G mutations caused by

8-OH-dGTP. In contrast, the 8-OH-dGTP–induced mutations were not affected by the knock-down of DNA pol ι . When DNA pols η plus ζ and REV1 plus DNA pol ζ were knocked-down (double knock-down experiments), the A:T→C:G mutations induced by 8-OH-dGTP were further reduced. In contrast, the induced mutation frequency was not decreased by the double knock-down of DNA pol η plus REV1. These results suggested that DNA pols η and ζ , and REV1, but not DNA pol ι , are involved in the misincorporation of 8-OH-dGTP opposite A, and that REV1-DNA pol η and DNA pol ζ work in different steps.

Alternatively, these DNA pols might contribute to the insertion of dCTP opposite 8-OH-Gua after 8-OH-dGTP is incorporated into DNA (Fig. 2). To examine this possibility, we introduced plasmid DNA containing an 8-OH-Gua:A pair, an intermediate in the mutagenic process of 8-OH-dGTP, into cells with knocked-down TLS DNA pols. The knock-down of DNA pol η only slightly decreased the frequency of mutations induced by 8-OH-Gua:A [26]. Moreover, minor, if any, effects were observed for the knock-downs of DNA pol ζ and REV1. Thus, the human DNA pols η and ζ , and REV1 were suggested to be involved in 8-OH-dGTP incorporation into the nascent strand.

4. Prevention of induced mutagenesis by nucleotide pool sanitization enzymes

The specific degradation of damaged DNA precursors (2'-deoxyribonucleoside 5'-triphosphates) is the first defense system against their induced mutagenesis. In 1992,

Maki and Sekiguchi found that the *E. coli* MutT (NudA) protein specifically catalyzed the hydrolysis of 8-OH-dGTP to the corresponding 5'-monophosphate [12]. *E. coli* and mammalian cells possess multiple MutT-type enzymes that contain the “MutT signature/phosphohydrolase module/Nudix box” [27–29], and their hydrolytic activities for oxidized DNA precursors have been observed *in vitro*. The *E. coli* MutT and Orf17 (NudB) proteins degrade the 2'-deoxyribonucleoside 5'-diphosphates, as well as the 5'-triphosphates, to the 5'-monophosphates [30,31]. The human NUDT5 protein catalyzes the hydrolysis of damaged 2'-deoxyribonucleoside 5'-diphosphates to the monophosphate derivatives, while the hydrolysis of the 5'-triphosphates is only weakly catalyzed by the NUDT5 protein [32,33].

To address the contribution of the nucleotide pool sanitization enzymes to the prevention of mutations induced by 8-OH-dGTP and 2-OH-dATP, *E. coli* cells deficient in MutT and Orf135 (NudG) were treated with these damaged nucleotides. Exogenous 8-OH-dGTP induced mutations more efficiently in an *mutT* strain than in the wt strain [11], providing the first direct evidence that the MutT protein suppresses the mutations by 8-OH-dGTP *in vivo*. Meanwhile, the frequencies of the mutations caused by 2-OH-dATP were similar in the *mutT* and wt strains, in agreement with the fact that MutT does not act on 2-OH-dATP *in vitro* [34]. The Orf135 protein catalyzes the hydrolysis of 2-OH-dATP, and that of 8-OH-dGTP somewhat less efficiently, [35]. In line with these *in vitro* results, exogenous 2-OH-dATP caused mutations more efficiently in an *orf135* strain than in the

wt strain [36]. Conversely, the 8-OH-dGTP–induced mutations were similar in both strains. The importance of the phosphohydrolase activity, particularly the 2-OH-dATPase activity, was shown by the expression of various Orf135 mutants in the *orf135* strain [37,38]. Thus, the MutT and Orf135 proteins primarily degrade 8-OH-dGTP and 2-OH-dATP, respectively, in *E. coli* cells.

The Orf17 protein, another *E. coli* MutT-type enzyme, catalyzes the hydrolysis of 8-OH-dATP, and those of 8-OH-dGTP and 2-OH-dATP less efficiently [31]. Plasmid DNA containing the *orf17* gene in the antisense orientation was introduced into *E. coli* cells [39], due to the severe growth inhibition caused by the destruction of the *orf17* gene. When 8-OH-dGTP and 2-OH-dATP were introduced, their mutagenicities were slightly, but insignificantly, higher in the knock-down cells than in the control cells (Hori et al., unpublished data).

The mammalian MTH1 (NUDT1) and MTH2 (NUDT15) proteins catalyze the hydrolysis of 8-OH-dGTP *in vitro* [40–42]. The human NUDT5 protein hydrolyzes 8-hydroxy-2'-deoxyguanosine 5'-diphosphate (8-OH-dGDP, 8-oxo-7,8-dihydro-2'-deoxyguanosine 5'-diphosphate) to the 5'-monophosphate derivative, while it hydrolyzes 8-OH-dGTP only at very low levels [32,33]. To examine the roles of the three MutT-type proteins in human cells, we treated 293T cells with siRNAs against MTH1, MTH2, and NUDT5, and introduced 8-OH-dGTP into the knocked-down cells [43]. The knock-downs of all of the MutT-type proteins enhanced the A:T→C:G

substitution mutations. These results suggested that the three MutT-type enzymes act as a defense against the mutagenesis induced by oxidized dGTP and exhibit mutually complementary roles in its elimination from the nucleotide pool. Although the biological significance of the unique activity of NUDT5 is still open to debate, our results indicated its contribution to nucleotide pool sanitization. 8-OH-dGTP and 8-OH-dGDP are interconvertible in cells [44], and the removal of either nucleotide may contribute to mutation prevention to similar degrees. Tsuzuki *et al.* reported that greater numbers of tumors were spontaneously formed in the lungs, livers, and stomachs of MTH1-deficient mice, as compared with wt mice [45]. It would be interesting to examine the phenotypes of animals deficient in MTH2 and NUDT5.

5. Roles of DNA repair proteins

As described above, the nucleotide pool sanitization enzymes function as the first defense against the mutagenesis by damaged DNA precursors. Alternatively, DNA repair enzymes may prevent the mutagenesis by the oxidized 2'-deoxyribonucleotides after their incorporation into DNA.

We examined this possibility by introducing 8-OH-dGTP into *E. coli* cells deficient in the *mutY*, *nth*, and *nei* genes encoding DNA glycosylases, and analyzing the mutations in the chromosomal *rpoB* gene (we did not examine the corresponding *mutM* strain, due to its significant growth delay as compared to the wt strain) [46]. Exogenous

8-OH-dGTP increased the *rpoB* mutant frequency more efficiently in the *nth* strain than in the wt strain. In agreement with the results of the 8-OH-dGTP introduction experiments, the spontaneous mutant frequency in an *mutT/nth* strain was 2-fold higher than that in the corresponding *mutT* strain. Our data suggested that endonuclease III (Nth) functions as the second defense against 8-OH-dGTP, and are consistent with the previous report that endonuclease III excises 8-OH-Gua in DNA containing an 8-OH-Gua:A pair *in vitro* [47]. In contrast, the mutations induced by exogenous 2-OH-dATP were similar in all of the strains tested. Thus, the *E. coli* MutY protein and endonucleases III and VIII do not seem to act as the defense against the mutagenesis by 2-OH-dATP.

The *E. coli* UvrA and UvrB proteins, which are involved in nucleotide excision repair, enhanced the mutations induced by 8-OH-dGTP and 2-OH-dATP by an unknown mechanism(s) [48].

To examine the roles of DNA glycosylases in the mutagenesis by 8-OH-dGTP in human cells, we introduced 8-OH-dGTP into 293T cells, in which the target DNA glycosylases, OGG1, MUTYH, NTH1, or NEIL1, were knocked-down by siRNAs [49]. The mutations induced by 8-OH-dGTP were reduced by the knock-down of MUTYH, but the knock-downs of the other glycosylases had no effect on the mutation frequencies. This result suggests that the 8-OH-Gua:A pair is efficiently converted to the G:C pair by the excision of the A opposite 8-OH-Gua by MUTYH. This speculation is supported by the finding that an 8-OH-Gua:A pair in plasmid DNA induced the A:T→C:G mutation

two-fold less frequently in the MUTYH-knocked-down cells than in the control cells. Thus, human OGG1, NTH1, and NEIL1 do not act as a defense against the mutagenesis by 8-OH-dGTP. Conversely, MUTYH enhances mutations by 8-OH-dGTP in the nucleotide pool.

6. Conclusions

Damaged DNA precursors formed in the nucleotide pool by the action of ROS contribute to mutation induction in living cells. Some TLS DNA pols are involved in the incorporation of the oxidized nucleotides (Table 1). The nucleotide pool sanitization enzymes function as the first defense against the damaged DNA precursors, and most of the DNA repair enzymes do not prevent the induced mutations. A thorough understanding of the roles of these proteins is necessary to clarify the effects of oxidized DNA precursors.

Conflict of interest statement

The author declares that there are no conflicts of interest.

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Figure Legends

Fig. 1. Structures of 8-OH-dGTP and 2-OH-dATP. The equilibrium between the keto (left) and hydroxy (right) forms shifts to the keto form for 8-OH-dGTP. The proportion of the two forms of 2-OH-dATP is affected by the environment [50,51].

Fig. 2. Proposed models for mutations induced by (A) 8-OH-dGTP and (B) 2-OH-dATP. G*, 8-OH-Gua; A*, 2-OH-Ade.

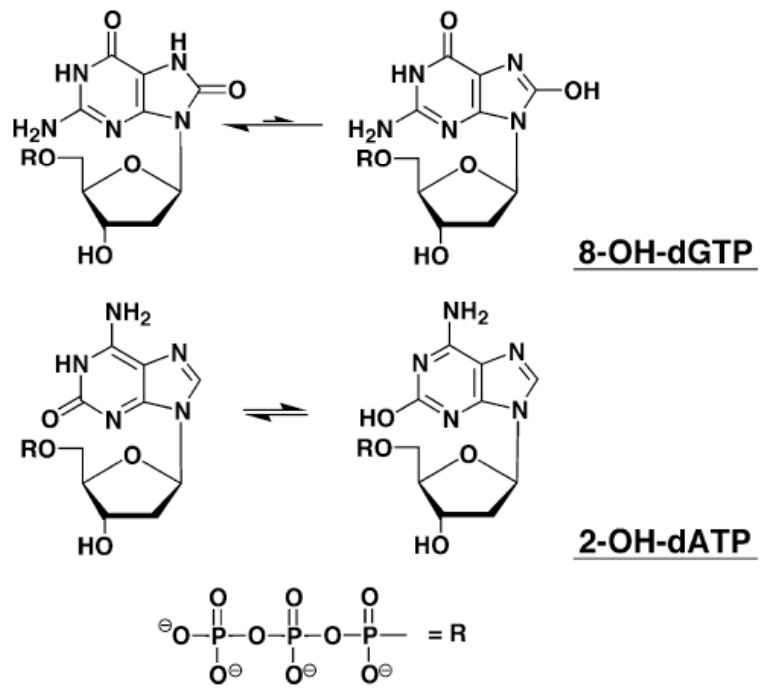
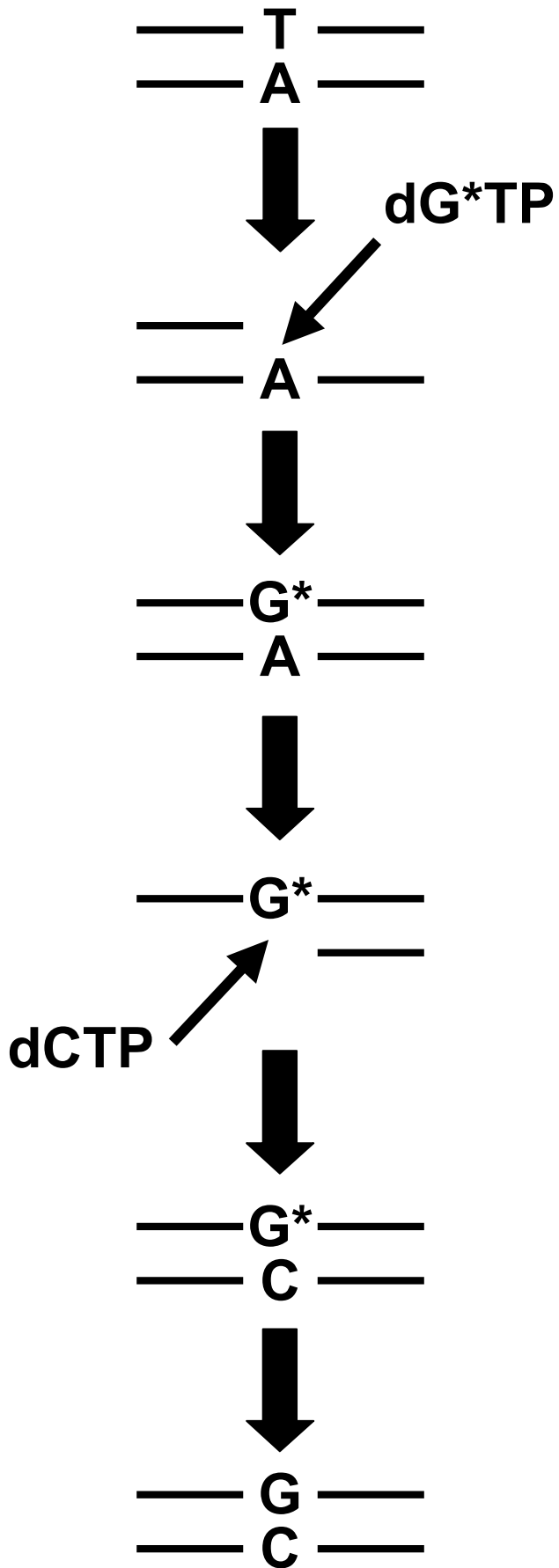


Fig. 1

A)



B)

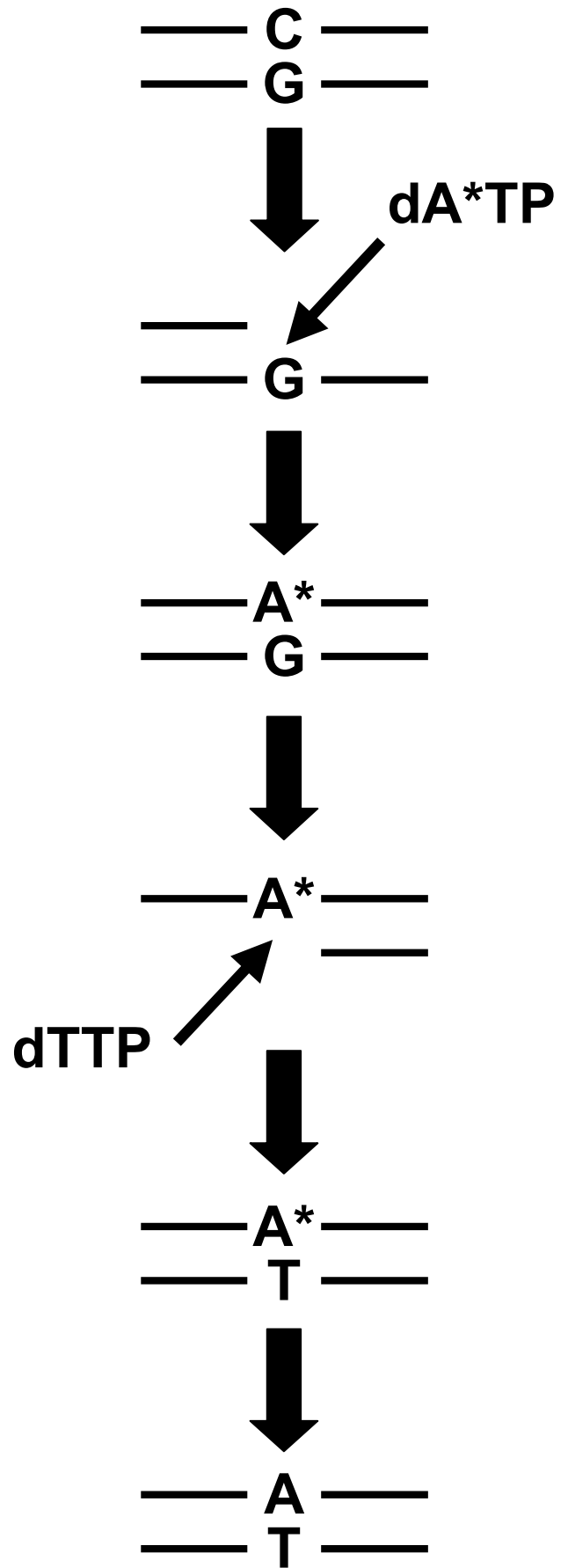


Fig. 2

Table 1. Cellular proteins affecting mutagenesis by oxidized DNA precursors

Function	Oxidized DNA precursor (Organism)		
	8-OH-dGTP (<i>E. coli</i>)	8-OH-dGTP (human cells)	2-OH-dATP (<i>E. coli</i>)
Degradation	MutT	MTH1, MTH2, NUDT5	Orf135
Incorporation into DNA	pol V (suppressive)?	pol η, pol ζ, REV1 (stimulatory)	pol IV (stimulatory)? pol V (suppressive)?
Removal of incorporated oxidized base	endonuclease III	?	?
Removal of adenine/guanine base opposite 8-OH-Gua/2-OH-Ade	?	MUTYH	?
Incorporation of dCTP/dTTP opposite 8-OH-Gua/2-OH-Ade	pol V (suppressive)?	pol η (stimulatory)	pol IV (stimulatory)? pol V (suppressive)?