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The fidelity of the human leading and lagging strand DNA replication apparatus with 8-oxodeoxyguanosine triphosphate

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ABSTRACT

A product of oxidative metabolism, 8-oxodeoxyguanosine triphosphate (8-O-dGTP), readily pairs with adenine during DNA replication, ultimately causing A·T→C·G transversions. This study utilized 8-O-dGTP as a probe to examine the fidelity of the leading and lagging strand replication apparatus in extracts of HeLa cells. Simian virus (SV) 40 T antigen-dependent DNA replication reactions were performed with two M13mp2 vectors with the SV40 origin located on opposite sides of the *lacZ*α sequence used to score replication errors. The presence of 8-O-dGTP at equimolar concentration with each of the 4 normal dNTPs resulted in a >46-fold increase in error rate for A·T→C·G transversions over that observed in the absence of 8-O-dGTP. A similar average error rate was observed on the (+) and (−) strands in both vectors, suggesting that the fidelity of replication by leading and lagging strand replication proteins is similar for the dA·8-O-dGMP mispair. Replication fidelity in the presence of 8-O-dGTP was reduced on both strands when an inhibitor of exonucleolytic proofreading (dGMP) was added to the reaction. These data suggest that the majority of dA·8-O-dGMP mispairs are proofread by both leading and lagging strand replication proteins.

INTRODUCTION

The normal metabolism of oxygen through a series of one electron reductions generates oxygen free radicals and other oxygen species within cells. These are capable of reacting with a variety of intracellular molecules including proteins, DNA and nucleoside triphosphates. One nucleoside triphosphate generated by oxidative metabolism is 8-oxodeoxyguanosine triphosphate (8-O-dGTP). Because this analog can pair with either cytosine or adenine, 8-O-dGTP produces A·T→C·G transversions if incorporated opposite adenine during DNA replication (1). In fact, previous *in vitro* studies have shown that 8-O-dGTP can be incorporated opposite adenine by exonuclease-deficient Klenow fragment (2) and the catalytic subunit of *E.coli* DNA polymerase III (3). Furthermore, studies in our laboratory have shown that the DNA synthesis fidelity of several DNA polymerases is reduced when 8-O-dGTP is included in the reaction. The Klenow DNA polymerase, T4 DNA polymerase, *Thermus thermophilus* DNA polymerase and mitochondrial DNA polymerase γ all misincorporate 8-O-dGTP opposite template adenine at a high rate (4). Using the SV40 model of human DNA replication *in vitro*, we also previously showed that 8-O-dGTP is misincorporated during double-stranded DNA replication (4). Because 8-O-dGTP may be produced *in vivo* under conditions of normal oxidative metabolism and as a result of oxidative DNA damage by agents such as ionizing radiation, misincorporation of 8-O-dGTP may contribute to spontaneous and induced mutation.

The studies presented here address several issues regarding the ability of the human replication complex to misincorporate 8-O-dGTP. In eukaryotes, much of what is known concerning DNA replication has been learned by studying the SV40 origin-dependent replication system *in vitro*. All the factors required for bidirectional replication of double-stranded DNA are provided by a host cell extract except for the SV40 T antigen (5,6 and, for review, see 7,8). This includes at least two DNA polymerases and a large number of accessory proteins. DNA polymerase α and its associated primase are required for initiating replication at the origin and to start each Okazaki fragment. DNA polymerase δ, assisted by RFC and PCNA, catalyzes most of the synthesis of the leading and lagging strands.

T antigen-dependent replication of DNA containing the SV40 origin in extracts of human cells is highly accurate (9). In fact, it is more accurate than is synthesis by purified DNA polymerase δ in the presence of PCNA or the four-subunit DNA polymerase α–primase complex (10). Furthermore, DNA polymerase δ is more accurate than DNA polymerase α, partly reflecting the fact that DNA polymerase δ has an associated 3′→5′ exonuclease activity, whereas highly purified human DNA polymerase α lacks such activity (for review, see reference 11). Because exonuclease-deficient DNA polymerases vary substantially in their ability to

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misincorporate 8-O-dGTP, and given the evidence that the proofreading exonuclease activity of the replicative T4 DNA polymerase can correct dA·8-O-dGMP mispairs (4), the mutagenic potential of 8-O-dGTP may vary during DNA replication in human cells, depending on which DNA polymerase is catalyzing polymerization.

Thus, we performed this study to determine whether the biochemical asymmetry of the replication fork is reflected in the accuracy of replication of the leading and lagging strands. We take advantage of the unique miscoding potential of 8-O-dGTP and the observation that A·T→C·G transversions are rarely produced by the human replication apparatus with normal dNTPs (10), thus providing a low background on which to score 8-O-dGTP-induced mutations. The studies presented here demonstrate that the overall average fidelity of SV40 DNA replication in HeLa cell extracts in the presence of 8-O-dGTP is similar for the same sequence when replicated as either the leading or lagging strand. In addition, we provide evidence that the dA·8-O-dGMP mispair is proofread by both the leading and lagging strand replication apparatus.

MATERIALS AND METHODS

Materials

Bacterial strains and reagents. Escherichia coli strains CSH50, NR9099 and NR9162 have been described (12). The double-stranded M13mp2 DNA substrates containing the SV40 origin of replication located on either side of the wild-type *lacZα* sequence (ori right and ori left) and M13mp2A89 ori left have also been described previously (12). The 8-O-dGTP was prepared as described (4). SV40 T antigen was purchased from Molecular Biology Resources (Milwaukee, WI).

Methods

SV40 DNA replication reactions and product analysis. SV40 replication reactions and product analysis were carried out as described (12). Reactions (25 μl) were performed using 40 ng RFI M13mp2SV DNA, 1 μg SV40 T antigen and 75 μg HeLa cytoplasmic extract in the presence of 100 μM each dATP, dGTP, dTTP, [α-^{32}P]-dCTP (4000cpm/pmol) and 8-O-dGTP. In addition, reactions contained 4 mM ATP, 200 μM each CTP, GTP and UTP, 30 mM Hepes, pH 7.8, 7 mM $MgCl_2$, 40 mM creatine phosphate, 2.5 μg creatine phosphokinase and 15 mM sodium phosphate, pH 7.5. After incubation at 37°C for either 3 or 4 hours, reactions were terminated by adding EDTA to a final concentration of 15 mM. The extent of replication was determined by measuring TCA precipitable counts bound to a GF-C filter (12). Replication products were analyzed by diagnostic digestion with restriction endonucleases followed by separation by electrophoresis through a 1.1% agarose gel (12). For mutagenesis assays, aliquots of the reactions were treated with *Dpn I* to remove fully-methylated, unreplicated molecules. The DNA was then introduced into *E.coli* strain NR9162 by electroporation as described (12).

Mutagenesis assays. Infected cells were plated on minimal plates containing X-gal and IPTG (12). For the forward mutation assay, plates were scored for lighter blue or colorless plaque phenotypes in comparison to the wild-type dark blue phenotype. Mutant plaques were replated for verification of the phenotypes. ssDNA was isolated and sequenced as described previously (12). For the ori left vector, there are 25 detectable sites for A·T→C·G transversions on the (+) strand and 28 detectable sites on the (−) strand (ref. 12 and this study). The reversion assay scores single base substitutions within a TGA codon at nucleotide

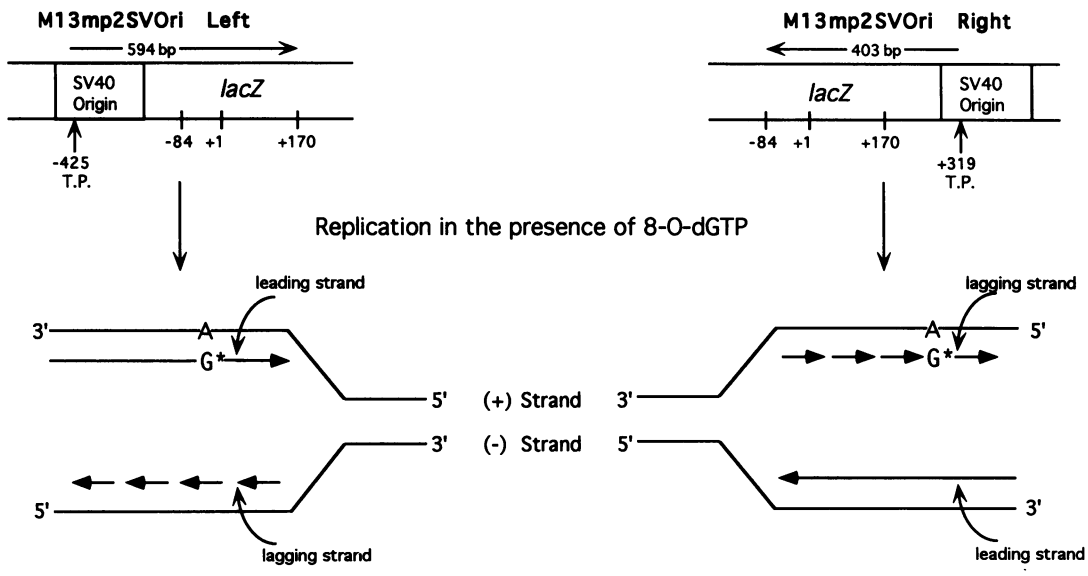

Figure 1. Strategy for discriminating between leading and lagging strand replication errors in the presence of 8-O-dGTP. See text in Results for a detailed description. The use of the two vectors M13mp2SV Ori left and Ori right for replication allows for a comparison of rates for the same error in the same sequence context when replicated by either the leading or lagging strand proteins. In the example shown, 8-O-dGMP is incorporated opposite a template adenine on the (−) strand. Thus, with the ori left vector this would result in a leading strand error, whereas this would be a lagging strand error for the ori right vector. Similarly, an error on the (+) strand would be a lagging strand error for ori left and a leading strand error for ori right. G* = 8-O-dGMP; T.P. = transition point. The transition point, as defined by Hay and DePamphilis (13), is the point within the SV40 origin (between nucleotides 5210 and 5211 in the SV40 genome) where there is a switch from discontinuous to continuous DNA synthesis.

positions 87, 88, and 89 in the *lacZ* gene in M13mp2 (where number 1 is the first transcribed base of the gene). There are 16 possible mispairs that can be detected as blue plaque revertants of the colorless plaque phenotype of the TGA codon (12). Two of these mispairs, at position 87 on the (−) strand and at position 89 on the (+) strand, result in A·T→C·G transversions.

RESULTS

Experimental approach for analysis of strand-specific errors

This study employs two vectors to analyze strand-specific errors generated during DNA replication. The M13mp2 vectors, designated ori left and ori right (12), both contain the SV40 origin but on opposite sides of the *lacZα* mutational target (Figure 1). The shortest distance the replication forks must proceed from the origin in order to fully replicate the *lacZα* gene target is 594 bp for the ori left vector and 403 bp for ori right. Given the 7.4 kb size of the vector, this is much shorter than the distance the other replication fork must traverse in order to fully replicate the gene target. Studies have shown the rate of replication fork movement to be similar in both directions (14, 15). Thus, one can infer which strand was replicated as the leading or lagging strand in each vector. Furthermore, the miscoding specificity of 8-O-dGTP (opposite template adenines) defines the strand into which the 8-O-dGTP is incorporated. Thus, rates can be obtained for the same error in the same sequence context when replicated by either leading or lagging strand replication proteins.

Replication of HeLa extracts in the presence of 8-O-dGTP

Parallel replication reactions were performed with the wild-type (i.e., blue) ori left and ori right substrates and a HeLa cell extract, either in the absence or presence of 8-O-dGTP at a concentration equal to that of each of the four normal dNTPs. The efficiency of replication was found to be similar to or only slightly diminished (≥10%) when 8-O-dGTP was included. Replication was T antigen-dependent and the products formed in reactions containing 8-O-dGTP were indistinguishable from those obtained from reactions performed in the absence of 8-O-dGTP (data not shown, but see reference 12).

Replicated DNAs were introduced into *E.coli* cells and the infected cells were plated to score the colors of the resulting M13 plaques, including light blue and colorless plaques resulting from replication errors . By electroporation of different mutant *E.coli* strains with DNA replicated in the presence of 8-O-dGTP, we had previously demonstrated the ability to quantitatively score misinsertion of 8-O-dGMP opposite template adenines (4). We had also previously shown that replication of the ori left DNA substrate in HeLa cell extracts with 8-O-dGTP was mutagenic (4). In the present study, this experiment was performed twice in parallel with replication of the ori right vector. A 3- to 4-fold increase in mutant frequency was observed for replication reactions containing 8-O-dGTP as compared to those that do not (Table 1). Similar mutant frequencies were obtained with the ori right and ori left substrates. No increase in mutant frequency was obtained with DNA from reactions containing 8-O-dGTP but lacking T antigen (data not shown), indicating that the base analog was not mutagenic in the absence of replication.

Specificity and distribution of 8-O-dGTP-induced mutations

For determination of the base substitution specificity, strand specificity and distribution of 8-O-dGTP-induced mutations, we performed DNA sequence analysis of collections of independent mutants, analyzing the *lacZα*-complementation target sequence from position −84 through +170. Ninety mutants were sequenced for the ori left vector and 121 mutants for the ori right vector. Of these, sequence changes were found in 46 ori left and 84 ori right mutants. No changes were observed in the remaining mutants, which may contain replication errors downstream of the sequence we analyzed (e.g., see 17,19). These mutants were not further characterized because of the extensive sequencing effort required.

Among the 130 sequenced mutants in which changes were found, A·T→C·G transversions were the primary mutations recovered (37 with the ori left substrate and 68 with the ori right substrate, Table 2). This transversion is consistent with the expected misincorporation of 8-O-dGMP opposite template adenines. Knowledge of the number of template adenines at which A·T→C·G transversions are detectable (4,12) permits a quantitative calculation of the error rate per detectable adenine-site replicated. Previous SV40 replication studies in HeLa cell extracts with normal dNTPs have shown that A·T→C·G transversions are rarely generated by the human replication complex (error rate $\leq 1.1\times10^{-6}$, ref. 10). With the ori left substrate, this rate is increased >46-fold when 8-O-dGTP is present (to 51×10^{-6}, Table 2). A similar A·T→C·G transversion error rate was observed with both substrates. Rates for most of the remaining base substitutions and for one base deletion errors were not higher than those previously reported for replication of ori left in the presence of 100 μM dNTPs (10). The only exception is a small increase in the C·G→A·T

Table 1. Summary of mutant frequencies for replication with 8-O-dGTP

Conditions	Plaques	Mutants	Mutant Frequencies ($\times10^{-4}$)
Ori Left			
= dNTPs	–	–	7.7+4 (10)[a]
+ 8-O-dGTP	11,988	28	23[b]
	12,748	71	56[b]
	17,474	35	20
Ori Right			
= dNTPs	–	–	13+5 (4)[a]
+ 8-O-dGTP	28,758	131	46
	30,484	94	31

[a]Data is an average of experiments described here and previously (4, 9, 10, 16, 17, 18, 19). Mutant frequencies shown are expressed as average values + S.E.M.. The number of experimental determinations is in parentheses.
[b]Data taken from reference 4.

transversion error rate observed with the ori right vector. This could represent misincorporation of dAMP opposite template 8-O-dG in a second round of DNA replication (note that in these experiments, replication products were not treated with restriction endonuclease *MboI* to destroy products resulting from two or more rounds of replication.)

The distribution of A·T→C·G transversions are depicted in Figure 2. These were scored at 31 of 53 detectable sites with

Table 2. 8-O-dGTP-induced base substitution and frameshift error rates

	Ori Left			Ori Right		
	Sites	Mutants	Error Rate ($\times 10^{-6}$)[a]	Sites	Mutants	Error Rate ($\times 10^{-6}$)[a]
Substitutions						
A·T→C·G	53	37	51	59	68	72
A·T→G·C	46	3	5	46	0	≤1.4
A·T→T·A	40	0	≤1.8	40	1	2
C·G→A·T	42	2	3	42	9	13
C·G→T·A	48	1	2	54	3	3
G·C→C·G	28	0	≤2.6	28	0	≤2.2
Frameshifts	250	2	0.6	250	4	1

The error rate for A·T→C·G transversions with 100 μM normal dNTPs is $\leq 1.1 \times 10^{-6}$ (10).
[a]Error rates are defined as errors made per detectable nucleotide incorporated. Error rates were calculated by dividing the number of mutants per class by the total number of mutants sequenced and multiplying by the overall average mutant frequency. This value was divided by 0.5 (the probability of expressing an error), and then divided by the number of detectable sites for each class (for ori left, see reference 12 and this paper). Because the detectable sites for the ori right vector are the same as for the ori left vector as well as additional sites reported in reference 17 and this paper, the ori right vector is more sensitive for detection of *lacZα*-complementation mutants (18,20 and 21).

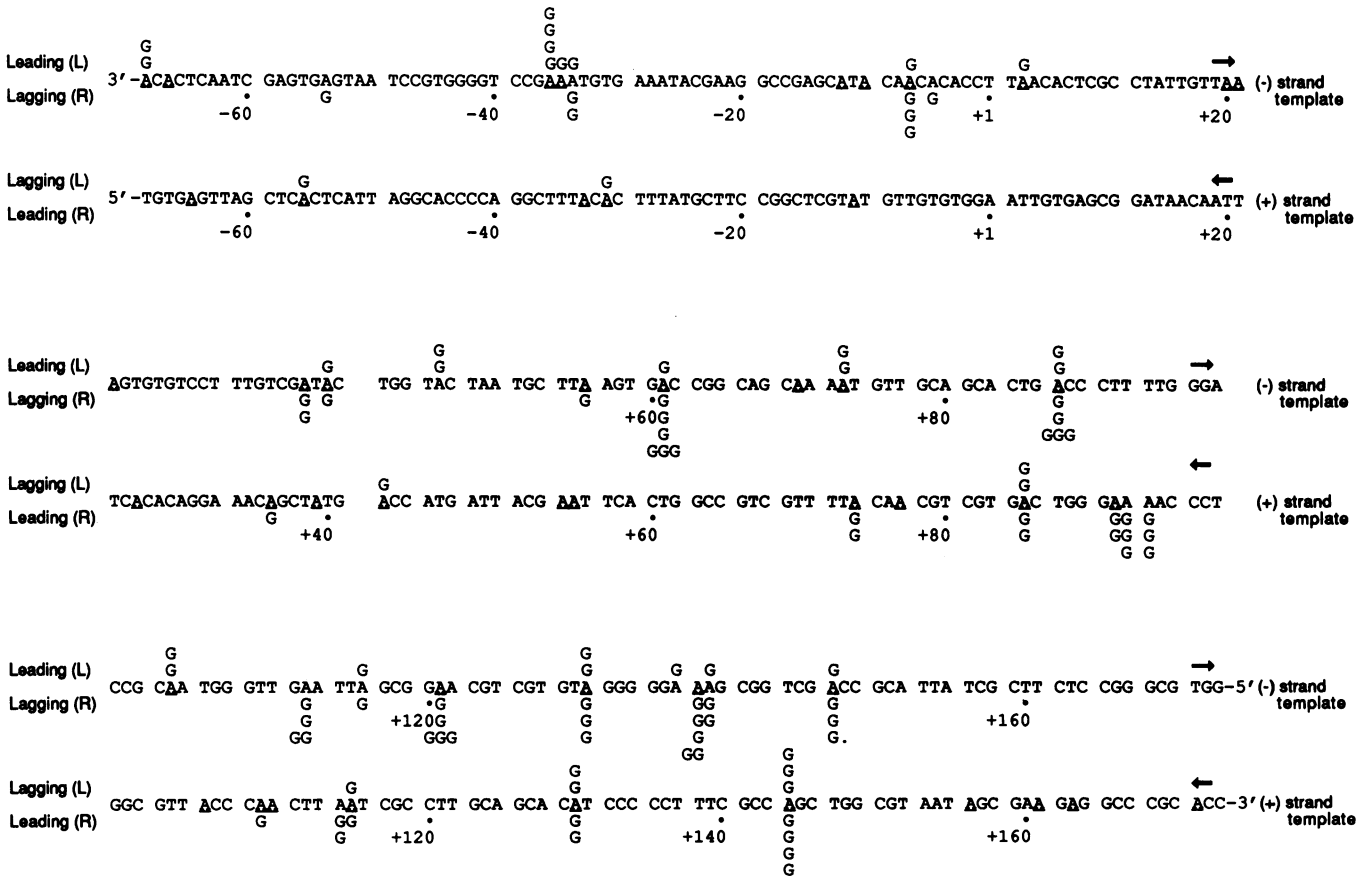

Figure 2. Spectra of mutations generated during replication in the presence of 8-O-dGTP. The spectra of mutations generated with the ori right (R) and ori left (L) vectors are shown above and below the three lines of primary DNA sequence. Leading and lagging strand assignments are based on the strategy outlined in the Results and Figure 1. Mutations are shown as the dA·8-O-dGMP mispair thought to be responsible for the A·T→C·G transversions observed with 8-O-dGTP. Sites at which the dA·8-O-dGMP mispairs would result in phenotypically-detectable A·T→C·G transversions with the ori left vector are underlined.

the ori left vector and at 29 of 59 detectable sites with the ori right vector. They are distributed throughout the mutational target, but not completely randomly. For example, zero or one mutants were observed at many detectable template adenine positions (underlined adenines in Figure 2), while four, five or six independent mutants were observed at several other locations.

To determine whether there is an overall strand-specific difference for the observed A·T→C·G transversions, a comparison was made of the error rates per detectable adenine-site replicated for the (+) and (−) strands, when replicated as either the leading or lagging strand (Table 3). The differences are less than 2-fold, indicating that the overall average fidelity of misincorporation of 8-O-dGMP is similar for the leading and lagging strand replication proteins. However, some template adenines yielded more A·T→C·G transversions when replicated as the lagging strand as compared to when replicated as the leading strand (e.g., see positions 61, 112, 121, 138) or the reverse (position −36). These reflect 4- to 5-fold differences in error rate at these sites.

Exonucleolytic proofreading of the dA·8-O-dGMP mispair

To determine if the dA·8-O-dGMP mispair is exonucleolytically proofread during replication, replication reactions were performed using a *lacZα* opal codon reversion substrate (9). This substrate was used because previous studies with undamaged dNTPs indicated that base substitutions at this sequence are proofread (20) and because both possible A·T→C·G transversions are detectable. T antigen-dependent replication of the M13mp2A89 substrate in cell extracts in the presence of 8-O-dGTP resulted in a 20-fold increase in the reversion frequency relative to that obtained with the four normal dNTPs alone (Table 4, experiment 1, compare 310×10^{-6} to 15×10^{-6}). A repeat of this experiment (Table 4, experiment 2) resulted in a similar reversion frequency. Sequence analysis of 20 revertants (Table 5) revealed that all were A·T→C·G transversions, consistent with the expected misincorporation of 8-O-dGMP opposite template adenine at position 87 on the (−) strand (7 mutants) or position 89 on the (+) strand (13 mutants).

The addition of monophosphate has been shown to reduce the fidelity of the human replication complex in cell extracts (20). We have interpreted this result as indicating diminished exonucleolytic proofreading capacity during replication, because, as the end product of proofreading, deoxyribonucleoside monophosphates are known to inhibit the 3′→5′ exonuclease activities of purified DNA polymerases (for review, see reference 22). As a control in the present study, we performed replication reactions in the presence of excess dCTP to force formation of specific dA·dCMP mispairs at the opal codon (see reference 20). Under these conditions, addition of dGMP resulted in a 10-fold increase in reversion frequency (Table 4, last experiment), confirming the earlier observations and suggesting that proofreading of normal mispairs occurs at the opal codon. When dGMP was added to reactions containing 8-O-dGTP, the resulting reversion frequency was increased 3-fold in comparison to

Table 3. Leading and lagging strand error rates for dA·8-O-dGMP mispairs

	Detectable sites	Occurrences	Error rate ($\times10^{-6}$)
Ori Left[a]			
+Strand (Lagging)	25	11	32
−Strand (Leading)	28	26	68
Ori Right[b]			
+Strand (Leading)	28	23	52
−Strand (Lagging)	31	45	91

[a]For sites at which A·T→C·G transversions can be detected in the ori left vector, see reference 12 and Figure 1.
[b]See Table 2 for a description of detectable sites for the ori right vector.

Table 4. Effect of dGMP on 8-O-dGTP-induced base substitution reversion frequencies

Conditions	dGMP	Plaques scored[a] Revertants	Total ($\times10^{-3}$)	Reversion frequency ($\times10^{-6}$)
Experiment 1				
= dNTPs	−	7	460	15
= dNTPs, 8-O-dGTP	−	85	260	310
= dNTPs, 8-O-dGTP	2 mM	187	180	1100
Experiment 2				
= dNTPs, 8-O-dGTP	−	104	410	260
= dNTPs, 8-O-dGTP	2 mM	239	390	610
Experiment 3				
200 mM dCTP	−	4	450	9
200 mM dCTP	2 mM	20	210	97

Control reactions performed in the absence of T antigen (i.e. no replication) resulted in a reversion frequency of 8×10^{-6}. Similarly, the addition of 8-O-dGTP to reactions lacking T antigen resulted in a reversion frequency of 4×10^{-6}.
[a]Prior to introduction into NR9162 cells by electroporation, reaction products were digested with the restriction enzymes *DpnI* (to inactivate unreplicated fully methylated molecules) and *MboI* (to remove unmethylated molecules resulting from more than one round of replication). Thus, the plaques scored result from products which have been through only one round of replication.

reactions lacking 8-O-dGTP. The result was reproducible in two separate experiments (Table 4, experiments 1 and 2). These data suggest that about 75% of the dA·8-O-dGMP mispairs are proofread by the human replication complex. Sequence analysis of 20 revertants from reactions containing both 8-O-dGTP and dGMP revealed (Table 5) a six-fold dGMP-dependent increase in error rate for A·T→C·G transversions at position 87 (a lagging strand error with this vector). The same comparison for position 89 (a leading strand error) yielded a two-fold increase.

DISCUSSION

The substantial increase in the rate of A·T→C·G transversions resulting from the presence of 8-O-dGTP in replication reactions (Table 2 and reference 4) suggests that the replication apparatus in human cell extracts readily misincorporates this base analog opposite template adenines. The present study to examine this mutagenic potential in greater detail was prompted by the following facts. First, replication of double-stranded DNA is an asymmetric process wherein the leading strand is replicated continuously while the lagging strand is replicated as a series of Okazaki fragments initiated by RNA primers that are later replaced with DNA. Second, replication requires more than one DNA polymerase (e.g., see 5–8). At least one of these, DNA polymerase α, lacks a tightly associated proofreading exonuclease (23) activity and is not highly accurate for errors occurring with normal dNTPs (10). In contrast, another essential replicative DNA polymerase (δ) does have associated proofreading exonuclease activity (23) and is more accurate than DNA polymerase α (10). Third, previous studies of the mutagenic potential of 8-O-dGTP demonstrated that several different DNA polymerases readily misincorporate the analog opposite a template adenine to yield the characteristic A·T→C·G at a rate much higher than that obtained for misincorporation of normal dGTP opposite dA (10). However, these polymerases differed greatly in their ability to misincorporate 8-O-dGTP, and data obtained with one of them (the T4 DNA polymerase) suggested that its associated exonuclease activity proofreads the dA·8-O-dGMP mispair. These observations suggest that the mutagenic potential of 8-O-dGTP might vary during replication, either by template position or even at the same template adenine depending on whether it is replicated as the leading or lagging strand. Given that A·T→C·G transversions are rarely generated during replication (10), here we have used the unique base substitution specificity of 8-O-dGTP as a molecular biomarker to examine the site- and strand-specificity of replication errors.

The results in Figure 2 reveal that the 8-O-dGTP misincorporations occur at many different template positions in the target sequence during both leading and lagging strand replication. Although errors are widely distributed over the target, four, five or even six independent mutants containing A·T→C·G transversions were recovered at some sites (e.g., adenines in Fig. 2 at nucleotide positions −36, 61, 87, 112, 121, 138 and 144) while no mutants were recovered at 21 other template adenines where this transversion error is known to yield a mutant plaque phenotype. Thus, as with purified DNA polymerases (4), misincorporation of 8-O-dGTP by the multiprotein replication machinery is not completely random. This implies that the mutagenic potential of this damaged precursor may not be constant in the genome *in vivo*.

One of the primary objectives of this study was to determine the relative fidelity of replication of the same DNA sequence when replicated by either the leading or lagging strand replication machinery. The data in Table 3 suggest that the overall average error rates for leading and lagging strand replication differ by less than two-fold. One scenario that is consistent with this fact is as follows. Recent studies of SV40 origin-dependent replication using purified components suggest that polymerase δ is involved in extending Okazaki fragments primed by polymerase α and is responsible for leading strand synthesis (5,6). DNA polymerase α, an inaccurate replicative polymerase (10) and primase, which is very inaccurate (24), are likely to be responsible for initiation of replication at the origin and for priming DNA on the retrograde arms of the replication fork. However, excision of RNA primers, with possible concomitant removal of some DNA as well (25) may eliminate any misincorporation by these more error-prone enzymes. Although the polymerase responsible for filling in the gaps produced by excision of RNA primers is not known, studies have shown that DNA polymerase δ is able to perform this task (5). Thus, DNA polymerase δ may perform the bulk of DNA synthesis on both strands, yielding similar average 8-O-dGTP misincorporation rates on the leading and lagging strands. Note, however, that this rationale for similar error rates does not explain earlier results obtained using a large excess of dTTP (19). In that study, the rate of misincorporation of dTTP opposite template guanine and cytosine bases on the (+) strand was much higher for the lagging than for the leading strand apparatus. Comparison of the data sets in the two studies thus suggests that the two halves of the replication machinery do not discriminate equally against all types of errors.

The results in Table 2 can also be compared to those obtained using a different form of damaged substrate, UV-irradiated DNA (18). In that study of the fidelity of replicative bypass of cyclobutane pyrimidine dimers in the template, no difference was observed in the fidelity of replication by the leading and lagging strand apparatus when the rate was averaged for all sites. However, the distribution of errors was found to be significantly different for the same sequence when replicated as the leading or the lagging strand. As noted above, misincorporation of 8-O-dGTP by the replication machinery is not completely random either, and we further note several sites wherein the misincorporation rate was four- to five-fold higher with one

Table 5. Effect of dGMP on A·T→C·G error rates at the two positions of the opal codon

dGMP	Position $T_{87}GA$ Number of mutants	Error rate[a] ($\times 10^{-6}$)	Position TGA_{89} Number of mutants	Error rate[a] ($\times 10^{-6}$)
–	7	220	13	410
2 mM	12	1300	7	750

[a]Error rates were determined as described in Table 2.

substrate as compared to the other (Figure 2, positions -36, 61, 112, 121 and 138). Although in this study the number of sequenced mutants obtained at any one site is too small to withstand a rigorous statistical test for a significant difference (18), the differences at these five sites are consistent with the possibility that, at some specific sites, the same error is generated at a different rate when a template base is copied by leading or lagging strand replication proteins. This concept is strongly supported by our earlier study with excess dTTP, where two clear and reproducible site-specific differences were observed in the fidelity of leading and lagging strand replication (19).

Studies with purified prokaryotic polymerases have addressed the issue of whether the dA·8-O-dGMP mispair may be exonucleolytically proofread. The 8-O-dG(template)·dAMP mispair does not appear to be proofread by the large fragment of *E.coli* DNA polymerase I (25). In addition, this enzyme does not efficiently edit the reciprocal dA(template)·8-O-dGMP mispair either (4). However, fidelity measurements suggest that the majority of these latter mispairs are proofread by the replicative T4 DNA polymerase (4). Because of the importance of proofreading to genome stability, we examined whether dA·8-O-dGMP mispairs were subject to proofreading by the human replication complex. The addition of dGMP, an inhibitor of exonucleolytic proofreading, resulted in an overall 3-fold increase in reversion frequency for a TGA codon. These data provide evidence that the dA·8-O-dGMP mispair is proofread by the human replication complex. Furthermore, the sequence analysis of revertants (Table 5) suggests that proofreading of this mispair occurs on both the leading and lagging strands. Similarly, previous studies have shown the human replication complex to edit normal mispairs efficiently on both strands (20). It will be of interest to determine whether proofreading of the dA·8-O-dGMP mispair occurs to a similar extent on both strands of the replication fork.

The amount of 8-O-dGTP present in human cells may be modulated in part by hydrolysis of this base analog by the human mutT homolog (27, 28). In addition, once incorporated into DNA, 8-O-dG is subject to removal by DNA glycosylases thereby preventing accumulation of this oxidized base in DNA (29). Therefore, the presence of several lines of defense against mutations resulting from this base analog whether it is present as the nucleoside triphosphate or in DNA, suggests that this damaged base is biologically important (for reviews see 30, 31). The increased error rate for A·T→C·G transversions when 8-O-dGTP is present during replication occurs despite the proofreading ability of the human replication apparatus, suggesting that this analog may be an important contributor to mutation *in vivo*.

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