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Repair and mutagenic potency of 8-oxoG:A and 8-oxoG:C base pairs in mammalian cells

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ABSTRACT

Replication of the oxidative lesion 8-oxo-7,8-dihydroguanine (GO) leads to the formation of both 8-oxo-7, 8-dihydroguanine:adenine (GO:A) and 8-oxo-7,8-dihydroguanine:cytosine (GO:C) pairs. The repair and mutagenic potency of these two kinds of base pairs were studied in simian COS7 and human MRC5V1 cells using the shuttle vector technology. Shuttle vectors carrying a unique GO residue opposite either a C or an A were constructed, then transfected into recipient mammalian cells. DNA repair resulting in G:C pairs and mutation frequency, were determined using resistance to digestion by the *Ngo*MI restriction enzyme for screening and DNA sequencing of suspect mutants. Results showed that the GO:C mismatch was well repaired since almost no mutations were detected in the plasmid progeny obtained 72 h after cell transfection. The GO:A pair was poorly repaired since only 32–34% of the plasmid progeny contained G:C whereas two thirds contained A:T at the original site. Repair kinetics measured with a non-replicating vector deleted by 13 bp at the SV40 replication origin, showed that GO:A was slowly repaired. Only 30% of the mispairs were corrected in 12 h. During this time 100% of the plasmids containing GO:A pairs were replicated as seen by the replication kinetics in a vector with an intact SV40 replication origin. These results show that, under our experimental conditions, replication is occurring before completion of DNA repair which explains the high mutagenic potency of the GO:A mispair.

INTRODUCTION

Considerable interest has arisen in recent years concerning the formation and consequences of oxidative DNA damage (1). This interest derives largely from the observation that the use of oxygen by aerobic metabolism is accompanied by the formation of reactive by-products. Free radical forms of oxygen that can damage cellular components constitute probably the most important source of spontaneous DNA damage: among these, 8-oxo-7,8-dihydroguanine (GO) has drawn the greatest interest. GO was first found in a hydroxy-radical-forming system (2), but has now been identified following ionizing radiation (3), and has been shown to be the product of an array of reagents that generate reactive oxygen species (1) and/or promote one electron oxidation of guanine. These include numerous chemical oxidants (3), photosensitizers (3,4), activated polynuclear leukocytes (3–5) and UV-A and UV-B radiations (6,7).

Oxidative DNA damage may be repaired in cells by a variety of repair enzymes. In both bacteria and mammalian cells, repair enzymes have been discovered which are active for removing GO (8–10). In particular, the *Escherichia coli* enzyme Formamidopyrimidine glycosylase (Fpg/MutM) is known to remove GO (4). A eukaryotic counterpart of this enzyme exists in yeast where the *ogg1* gene which encodes a DNA glycosylase that excises GO has been cloned, as well as in human where the homolog of the yeast gene has also been cloned (11–15). GO has miscoding potential *in vitro*: replicative DNA polymerases of both bacteria and mammalian cells, preferentially insert dAMP opposite GO in the template, whereas polymerases associated with repair processes, preferentially insert the correct nucleotide dCMP (16,17). The *in vitro* miscoding specificity is reflected *in vivo* by results from transfection with single-stranded DNA vectors containing a GO residue, which gives rise to GO to T transversions at a frequency of <1% after replication in *E.coli* (18). Little is known about the biological consequences of GO in human cells although recent studies in simian COS7 and CV-1 cells showed a mutation frequency of 4–5% using single-stranded shuttle vectors carrying a unique oxidized base (19,20).

The possible physical basis of GO mispairing has been discussed in terms of electronic properties, the conformation of the guanine being altered by its oxidation at the C-8 position (17). This alteration would facilitate GO:A and GO:C base pairings during DNA replication. These two base pairs have similar geometric form that could explain their preferential formation and most of the mutations found in *E.coli* and in eukaryotic cells. In *E.coli* a protein named MutY is able to correct errors arising from misincorporation of adenine opposite G or GO (21). This enzyme removes the adenine residue, leaving an abasic site (18–22), and cleaves the phosphodiester bond 3′ to the abasic site (23,24). The latter damaged DNA is finally repaired by DNA polymerase I with a marked preferential dC insertion (25).

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Recently the human homolog gene to *mutY* gene (*hMYH*) has been cloned and sequenced (26). The human gene has been mapped to the short arm of chromosome 1.

GO:C is formed when the oxidative lesion is either generated in a double-stranded DNA after an oxidative stress induced in cells (for example ionizing radiation) or by incorporation of the oxidized dGTP precursor during DNA replication. GO:A is formed during DNA replication by insertion of A opposite GO or by incorporation of 8-oxodGTP opposite A (27). For both GO:C and GO:A mispairs, activities of MutY and Fpg repair enzymes, or their eukaryotic homologs, give rise to G:C base pair formation. The objective of the present study was to investigate the repair and the mutation frequency of GO:A and GO:C mispairs in simian and human cells. In order to perform this study, cells were transfected with a double-stranded replicative DNA shuttle vector carrying a unique GO:C or GO:A mismatch in the human *Ha-ras* context, and DNA of the plasmid progeny was analyzed. For the first time, plasmid replication and repair kinetics enabled us to evaluate more fully the outcome of GO:A mispair in mammalian cells.

MATERIALS AND METHODS

Experimental protocol

Double-stranded DNA shuttle vectors carrying a unique GO opposite either a C or an A were constructed, then transfected into either simian or human cells for replication and/or repair. Three days later, plasmid DNA was extracted from the mammalian cells and then shuttled into bacteria in order to individualize the progeny molecules. They were then screened for mutations induced in eukaryotic cells. Repair kinetics of GO:A were analyzed following a similar experimental protocol using a plasmid deleted in the SV40 replication origin. Progeny molecules were analyzed from 2 to 72 h after transfection.

Replication kinetics of GO:A carrying plasmid DNA were performed by a Southern blotting technique to quantify replicated DNA from 2 to 48 h after transfection.

Plasmids and oligonucleotides

The pS189 plasmid was a generous gift from Dr Seidman. It possesses, as shown in Figure 1, the replication origins of SV40 and of πAN7 for replication in eukaryotic and prokaryotic cells, respectively. The M13K07 helper phage allows single-stranded DNA plasmid production as described in the procedure of Analects (Pharmacia). pS189 deleted at the SV40 replication origin was constructed by digesting plasmid DNA with *Pvu*II whose restriction sites are shown in Figure 1. The *Pvu*II fragment was replaced by the identical fragment deleted by 13 bp at the SV40 replication origin, coming from the pLAS-wt plasmid, a generous gift from L. Daya-Grosjean (28).

Synthetic oligonucleotides used to construct the mono-modified plasmids were from Genset (Paris, France) and purified in a denaturing 20% polyacrylamide gel. The modified 19mer oligonucleotide carrying a unique GO contains a small fragment of the human *Ha-ras* gene from codon 10 to codon 14 with the lesion located on the second guanine of codon 12 as shown in Figure 1. This modified oligonucleotide was produced and purified as previously described (29,30).

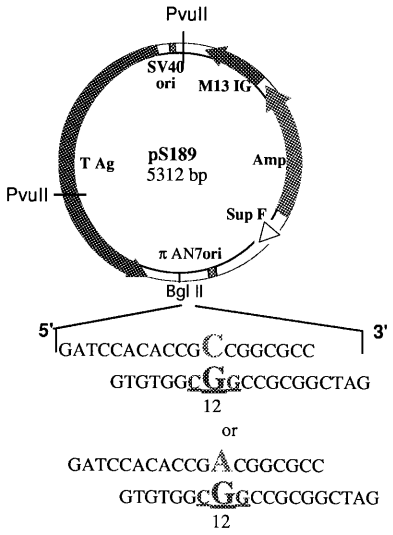

Figure 1. Genetic map of the pS189 plasmid containing a unique GO. The unique GO lesion opposite either a cytosine or an adenine, is located on codon 12 of the human *Ha-ras* sequence corresponding to codons 10–14 of this gene, inserted at the single *Bgl*II restriction site. Eukaryotic (SV40 ori) and prokaryotic (πAN7 ori) replication origins are shown. It also contains MI3 origin allowing single-stranded DNA production, T antigen, supF and ampicillin resistance genes. The *Pvu*II restriction sites used to delete the SV40 ori as described in Materials and Methods are indicated.

Cell lines and growth conditions

Monkey COS7 and human MRC5V1 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 15% (v/v) fetal calf serum, fungizone and antibiotics. MRC5V1 fibroblasts transformed by the SV40 virus were from C. Arlett's laboratory (Brighton, UK).

Construction of the closed circular double-stranded plasmid DNA containing a unique GO:C or GO:A pair

This was carried out as already described by Pletsa *et al.* (31). A single-stranded modified pS189 DNA carrying an adenine (A) or cytosine (C) was constructed by inserting a 19mer oligonucleotide complementary to the synthetic 19mer oligonucleotide harboring the GO into the original pS189 DNA. A gapped plasmid was then constructed by hybridizing single-stranded pS189 (A) or (C) in 1× SSC with a double-stranded pS189 DNA linearized at the *Bgl*II site. The mixture was heated at 98°C for 10 min, cooled slowly to 65°C, allowed to renature for 1 h and then ethanol precipitated. Samples (20 µg) of the gapped duplex plasmid DNA formed, were hybridized overnight with 5 µg of the 5′ ^{32}P-phosphorylated GO carrying oligonucleotide (GATC GGC GCC G**GO**C GGT GTG) in T4 ligation buffer containing 1 mM ATP, then ligated for 20 min with 400 U T4 DNA ligase (Biolabs) at 12°C. Finally, covalently closed molecules were purified by isopycnic centrifugation on an ethidium bromide/cesium chloride gradient. Fractions collected from the gradient were analyzed on a 1% agarose gel and those corresponding to the closed circular double-stranded DNA vector were pooled, dialyzed against TE (1 mM Tris pH 7.8, 1 mM EDTA) and then ethanol precipitated.

For repair studies we used the plasmid deleted at its eukaryotic replication origin and the mono-modified plasmid DNA was constructed according to an identical protocol.

Transfection of cells

Transfection into different cell lines was mediated using the cationic liposome Dotap's procedure (Boehringer). Cells were then incubated for 3 days and collected. Extrachromosomal plasmid DNA was recovered by a small-scale alkaline lysis method (32). Digestion with *Bgl*II restriction enzyme was performed in order to eliminate any progeny molecules arising from replication of the double-stranded DNA that may have escaped the initial *Bgl*II digestion during the construction process and may not have carried the modified oligonucleotide. Finally, digestion with *Dpn*I restriction enzyme eliminates any double-stranded unreplicated rescued molecule. This step was not used with the non-replicating vector.

Bacteria and transformation

Escherichia coli XL1 blue bacteria were used for single-stranded DNA production. DH5α recA bacteria used as plasmid host for mutagenesis assay, were transformed by electroporation. For each transformation, one tenth of the DNA recovered from mammalian cells was added to 40 µl of bacterial suspension. The mixture was transferred into a cold BioRad gene pulser cuvette (0.2 cm) and electroporations were performed with a Sedd Cell Ject apparatus (BioRad) under 40 µF, 192 Ohm and 2500 V. Colonies were selected by ampicillin resistance carried by the plasmid.

DNA repair kinetics require the use of *E.coli* PR195 *mutY*$^{-}$/*fpg*$^{-}$ strain (Δ*lac-pro F′ pro lacI lacZ, mutY::kan, fpg::kan, Tn10*), a generous gift from S. Boiteux (CEA, Fontenay-aux-Roses, France) to amplify the ori SV40 deleted plasmid progeny and to avoid any repair of GO mispairs in the bacteria.

Screening procedure and mutation frequency determination

DNA from individual bacterial colonies was mostly prepared using the Jetsar genomed miniplasmid purification system (Bioprobe). Mutations induced by the lesion were screened using *Ngo*MI digestion which cleaves only when the G:C base pair is present at the site of the GO. Three to five independent transformations were carried out for each experiment. *Ngo*MI resistant colonies were sequenced by the chain elongation terminating method using Sequenase 2.0 kit from Amersham and mutation frequency was determined.

Southern blotting

Replication studies were performed using a standard protocol for Southern blotting (33). The molecular probe used was a pS189 specific ^{32}P-labelled 20mer oligonucleotide. Extent of replication was quantified using a Storm 860 Imager (Molecular Dynamics).

RESULTS

Analysis of the replicative progeny from GO:A or GO:C carrying plasmid DNA

Previous studies in simian cells have shown that replication of single-stranded DNA containing a unique GO residue was associated with the production of G:C and T:A pairs (19,20). This observation implies that replicative intermediates contain either GO:C or GO:A base pairs. We have therefore studied the outcome of these two types of mispairs located on codon 12 of human *Ha-ras* (normally GGC), in simian and human cells. In order to characterize our constructs (see Materials and Methods) an aliquot fraction of the GO:C and GO:A molecules was analyzed by electrophoresis in a 1% agarose gel (Fig. 2A). Lanes 3 and 4 corresponding to the GO:C or GO:A molecules before purification by isopycnic centrifugation on cesium chloride gradient, show mainly the circular covalently-closed molecules with their dimer form accompanied by a band corresponding to gapped duplex DNA. Single-stranded in excess has been eliminated using an anion exchange resin column (Qiagen). Digestion of the GO:C construct with the Fpg protein (lane 5) or digestion of the GO:A construct with a bacteria extract containing the MutY activity (lane 6), results in the formation of relaxed circular plasmid DNA showing that all covalently closed circular molecules carry the GO. Purification of the GO:C construct by centrifugation on a cesium chloride gradient is shown in Figure 2B. Gradient fractions were analyzed on a 1% agarose gel and those containing the closed circular molecules were pooled and used to transfect mammalian cells. Seventy-two hours after transfection into cells of the GO:C and GO:A purified covalently closed circular constructs (lanes 7 and 8, respectively), neither the rescued plasmid DNA from GO:C nor the GO:A plasmid, is

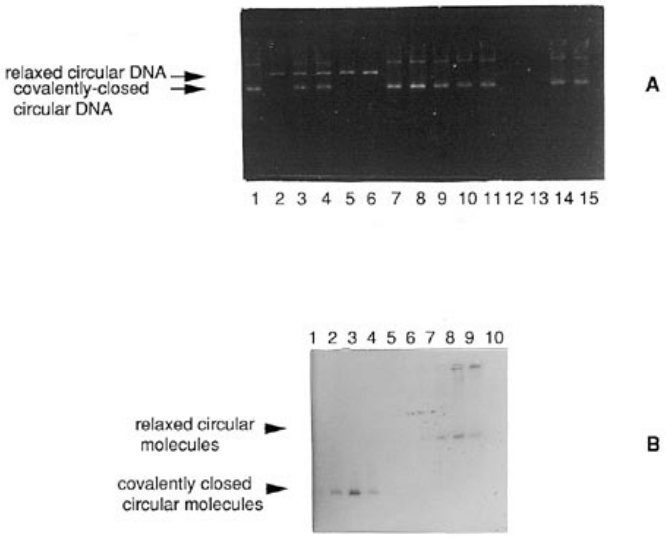

Figure 2. (**A**) Characterization of the GO:C construct (lane 3) and GO:A construct (lane 4). Digestion by Fpg of GO:C and digestion by a MutY activity of GO:A are shown in lanes 5 and 6, respectively. DNA rescued from human cells of GO:C (lane 7) is digested by Fpg (lane 9), *Mbo*I (lane 12) or *Dpn*I (lane 14). DNA rescued from human cells of GO:A (lane 8) is digested by Fpg (lane 10), a MutY activity (lane 11), *Mbo*I (lane 13) or *Dpn*I (lane 15). Lanes 1 and 2 correspond, respectively, to the covalently closed circular and the relaxed circular forms of the pS189 vector DNA. (**B**) Autoradiograph of a 1% agarose gel showing aliquots of some fractions from the isopycnic centrifugation on cesium chloride gradient of a GO:C construct [shown in lane 3 of (A)]. The 19mer carrying GO oligonucleotide was ^{32}P 5′-labelled. Fractions were collected from the bottom of the tube and loaded on the agarose gel in 1 mM Tris, 20 mM Na acetate, 2 mM EDTA pH 7.8 adjusted with CH_3COOH. Fractions 2–5 were used to transfect mammalian cells.

modified by treatment with Fpg (Fig. 2A, lanes 9 and 10). The rescued plasmid from the GO:A construct is also not modified by the bacteria extract containing the MutY activity (Fig. 2A, lane 11), showing the lesion was no longer present. Digestion with *Mbo*I or *Dpn*I restriction enzymes (Fig. 2A, lanes 12 and 14 for GO:C and lanes 13 and 15 for GO:A) indicates that all rescued molecules have been replicated in mammalian cells.

The rescued molecules from cells were then digested by *Dpn*I in order to eliminate any unreplicated molecules, then shuttled into bacteria as described in Materials and Methods. Moreover, to ensure that mutations were not due to translesion synthesis of remaining oxidative lesions in bacteria, *Dpn*I digested DNA extracts from mammalian cells transfected with GO:C construct were digested by 100 ng of Fpg or not digested, then used to transform recA bacteria. No differences in the number of bacterial clones, in mutation frequency or in the specificity of the induced mutations, were detected. Moreover, direct transformation of bacteria with our vector containing either a unique GO:C or GO:A mispair led to a mutation frequency of <1 and 3%, respectively. We can therefore conclude that the vast majority of the observed mutations, if not all, have been produced in mammalian cells.

Figure 3 shows the molecular analysis of the plasmid progeny DNA obtained after transfection of both simian COS7 and human MRC5V1 cells with a plasmid DNA containing a unique GO inserted opposite either a cytosine or an adenine. Transfection with a GO:C construct leads to progeny molecules containing almost exclusively a G:C base pair at the site of the lesion. For example, <1% of progeny molecules produced in human cells, among >100 clones analyzed, contained a T:A base pair at the site of the lesion. GO:A containing vectors resulted in plasmid progeny with approximately one third of G:C and two thirds of T:A base pairs at the site of the lesion. No significant difference was noticed between human MRC5V1 and simian COS7 cell lines (Fig. 3).

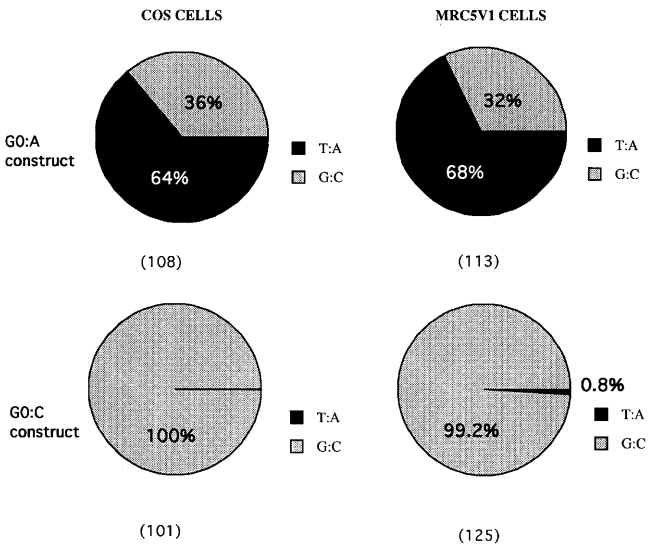

Figure 3. Distribution of G:C and T:A at either GO:A or GO:C sites after replication in COS7 cells and MRC5V1 human cells. The oxidized base is located on the second base of codon 12 of the human *Ha-ras* sequence. The number of sequences analyzed is given in brackets.

DNA replication kinetics of the GO:A carrying vector DNA

A replication kinetics study of the GO:A carrying plasmid DNA after transfection in human MRC5V1 or simian COS7 cells was undertaken. Plasmid DNA was rescued from cells at different times, then digested with the *Dpn*I restriction enzyme which cuts methylated DNA, unreplicated in mammalian cells. Southern blotting from rescued DNA at times 2, 4, 6, 12, 24 and 48 h was carried out and plasmid DNA was visualized with an appropriate ^{32}P-labelled probe. Taking into account that each replication event produces two *Dpn*I resistant molecules from one input plasmid, the ratio of *Dpn*I resistant DNA to total DNA represents the fraction of the transfected DNA that was replicated in eukaryotic cells.

Results presented in Figure 4A show that replication of the transfected plasmid DNA is a fast event. Six hours after transfection *Dpn*I resistant DNA already appears with the percentage of replicated DNA increasing progressively with time to reach 100% of the input plasmid 12 h after transfection.

DNA repair kinetics of the GO:A carrying vector DNA

pS189 plasmid deleted at its eukaryotic replication origin as described in Materials and Methods, was used to construct a GO:A mismatch containing vector DNA. At different times after transfection, plasmid DNA was rescued from the cells and used to transform *mutY*$^{-}$/*fpg*$^{-}$ double mutant bacteria unable to eliminate the oxidative lesion. We wanted to know the global GO:A repair resulting in the G:C base pair. DNA repair was determined by the frequency of vectors resistant to *Ngo*MI digestion recovered in the progeny, normalized to the frequency of vectors resistant to *Ngo*MI digestion observed in bacteria directly transformed with the original GO:A vector. *Ngo*MI digestion cleaves only when the G:C base pair is present at the site of the GO. Efficient elimination of any contaminant DNA input before extracting plasmid DNA from cells was performed by treatment of cell cultures with DNase I (not shown). Repair kinetics presented in Figure 4B show that repair of GO:A mispair to G:C is relatively slow. No significant repair is seen 6 h after transfection. Only 35% of the mismatches are correctly repaired 12 h after transfection. It takes as long as 72 h to observe complete error-free repair, and to detect only G:C base pairs.

The fact that 100% of the GO:A molecules were repaired 72 h after transfection implies that all rescued molecules used to transform bacteria do not contain any more GO.

DISCUSSION

Results presented in Figure 3 show that transfection of a GO:A mispair carrying plasmid in simian COS7 or human MRC5V1 cells, gives rise to a progeny composed of one third of G:C and two thirds of T:A base pairs at the site of the original GO:A base pair. What mechanisms could explain their formation ?

To generate a G:C progeny, the original GO:A plasmid has to be fully repaired. According to the mechanism which has been described in *E.coli* (18,34), the adenine has to be removed by the mammalian homolog of MutY (hMYH protein in human cells). This should be followed by the insertion of a cytosine residue opposite the GO during DNA repair, or during the subsequent DNA replication leading to a GO:C. Finally, substitution of the GO with a guanine residue requiring the intervention of the mammalian homolog of Fpg (hOGG1 protein in human cells)

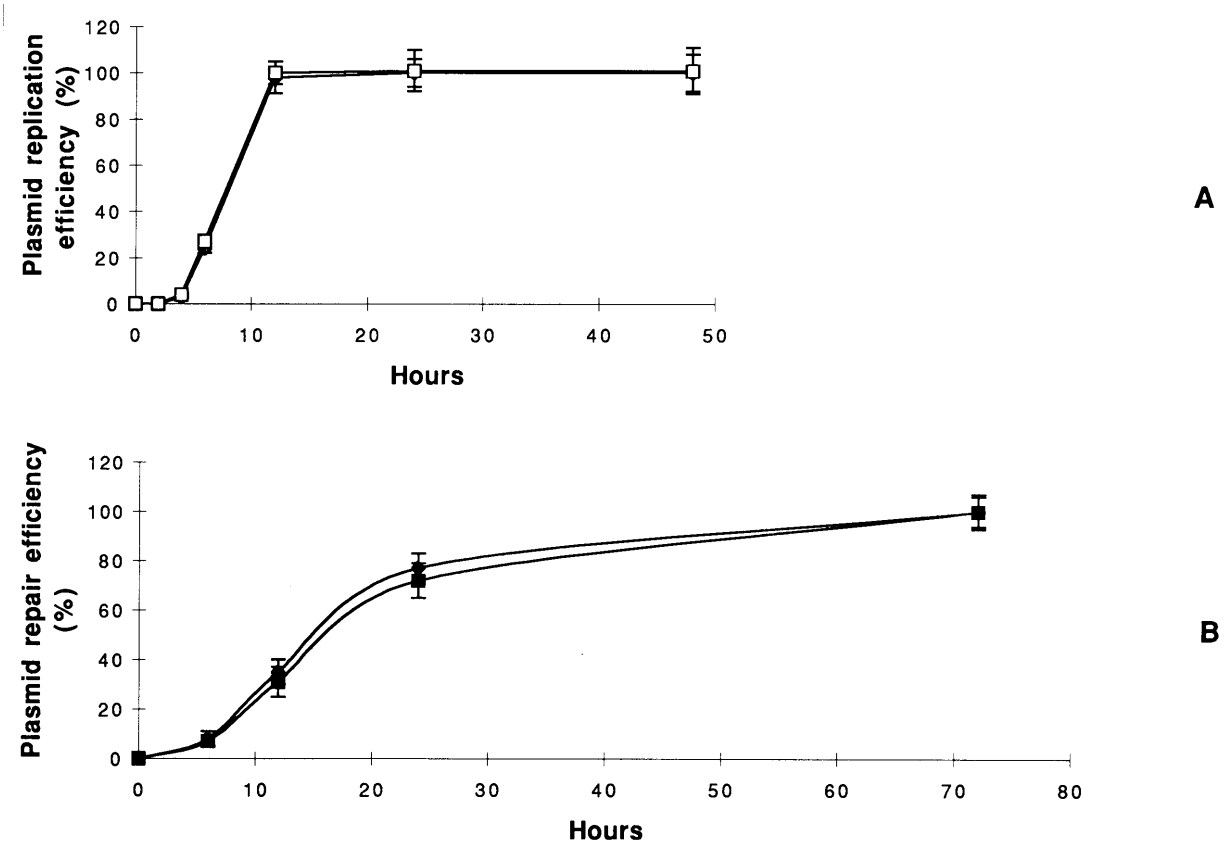

Figure 4. (**A**) Replication kinetics of the GO:A construct in COS7 (◇) cells and human MRC5V1 (□) cells from 2 to 48 h after transfection of the replicative plasmid DNA. DNA replication efficiency corresponds to the percentage of DNA extracted from cells at the various times and resistant to the *Dpn*I restriction enzyme. (**B**) Repair kinetics in COS7 (◆) and MRC5V1 (■) cells of the GO:A construct. Repair efficiency of the non-replicating plasmid was performed from 2 to 72 h after transfection. It was normalized to the repair observed after a direct transformation into *mutY*$^{-}$/*fpg*$^{-}$ bacteria as described in the text. The bars correspond to the standard deviation.

must occur. Our experiments, where GO:C constructs were transfected into mammalian cells, show that the latter step involving the repair of the GO:C pair (Fig. 3), is very efficient since a very low number of mutants were found in the plasmid progeny. It appears, therefore, that the eukaryotic Fpg protein homolog is active with great efficiency under our experimental conditions. Hence, the limiting step in repairing GO:A appears to be the MutY protein homolog, not the Fpg protein homolog, after metabolism of the GO:A base pair to GO:C. It should be noted that if GO:A pair arises from an incorporation of GO opposite an adenine during DNA replication the processing of the mismatch is mutagenic.

As far as the generation of T:A from GO:A is concerned, two mechanisms could be involved. First, T:A induction could be the consequence of the mammalian homolog of Fpg removing the oxidized base on GO:A, leaving an abasic site opposite the adenine. Repair of the latter lesion would lead to an insertion of a thymine opposite the adenine. However, the efficiency of the human homolog glycosylase/Ap lyase of the bacterial Fpg protein was shown to be very low under these conditions, the enzyme removing the GO only when it was paired with cytosine or thymine (15); such a phenomenom seems therefore unlikely. Second, T:A could be simply the replicative consequence of GO:A before its repair by MutY homolog as described in the preceding paragraph, the A carrying strand used as template giving rise to T:A pairing. This mechanism seems more probable. The majority of T:A pairs were found in the plasmid DNA progeny at the site of GO:A under our experimental conditions. This implies that most of the GO:A carrying molecules transfected into mammalian cells were replicated before DNA repair had occured. DNA replication kinetics described in Figure 4A shows that replication starts 6 h after transfection in eukaryotic cells and that 12 h later, ~100% of the molecules are replicated. The repair of the GO:A pair, carried out by DNA glycosylases, has so far not been shown to be dependent on DNA replication in mammalian cells. Moreover, in non-growing bacteria, Fpg or MutY proteins efficiently repair the GO (34). This is why we used a non-replicating vector to evaluate repair of the GO:A base pair. In Figure 4B, DNA repair kinetics show that in 12 h only 25–30% of transfected GO:A carrying plasmid DNA deleted by 13 bp in the SV40 origin, and consequently unable to replicate, are repaired. This difference could explain, as suggested above, the majority of T:A base pairs observed in the progeny at the original GO:A pair, replication of the mismatch occuring before its repair. In the absence of efficient repair, 50% of molecules should at least give rise to T:A base pairs.

Another factor may favour the predominance of T:A recovery in the progeny. Replication of a GO carrying strand is less

efficient than that of an intact DNA strand. It has been previously shown (20) that replication of a single-stranded GO carrying vector DNA in eukaryotic cells is only 70% efficient compared to an intact vector DNA. Moreover, the GO strand is replicated with a mutation frequency of ~4–5%, giving rise to G to T transversions, increasing consequently the formation of T:A base pairs. Combination of the lower efficiency of replication of the GO strand versus the A strand with the mutation frequency associated with the replication of the GO strand, may explain the final percentage of T:A of ~66% in the GO:A progeny.

As mentioned above, MutY and its human homolog hMYH, are the key enzymes for the repairing of the GO:A pair. MutY is involved in the repair of G:A as well as GO:A pairs (35) although the relative efficiency of the prokaryotic enzyme for GO:A and G:A is still under discussion. According to Lu *et al*. (36) MutY cleaves G:A 3-fold more efficiently than GO:A, whereas Bulychev *et al*. (37) showed that GO:A is the preferred natural substrate for MutY. Repair of G:A mismatches has also been studied in eukaryotic cells. Using a SV40 DNA probe harboring a single mispair, Brown and Jiricny (38) showed that G:A mismatches were poorly repaired in simian COS7 cells. Only 39% of mismatches were repaired before replication in eukaryotic cells. Arcangeli *et al*. (39) also obtained a low repair rate of G:A mispair in NIH3T3 cells. The G:A mismatch located at codon 12 of human *Ha-ras* is correctly repaired but with an efficiency of only 35% after replication in NIH3T3 and is therefore most likely to undergo a mutational event. The results we report here with the GO:A mispair in simian and human cells are therefore in agreement with previous studies using a G:A mismatch in simian and murine cells, assuming that it was the adenine which was incorrectly incorporated during DNA replication. In this case we show that mutations induced are the result of a competition between DNA repair and DNA replication.

Our study shows that the presence of a unique GO:C base pair in a double-stranded DNA leads to a low level of mutations (<1%) in simian and human cells, whereas a unique GO:A base pair leads to a high level of targeted T:A base pairs. Under our experimental conditions, the eukaryotic MutY homolog activity could be the limiting step for repairing the oxidative lesion in mammalian cells. The processing of the GO:A replicative intermediate explains the mutational potency of the oxidative lesion. In more general terms, our results suggest that accumulation of oxidative lesions, induced either spontaneously or after exposure to physical or chemical agents, leading to erroneous replication, could produce mutations implicated in phenomena such as ageing, progressive neurological deterioration or cancer induction.

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