UC Davis
UC Davis Previously Published Works

Title
Antimutagenic specificities of two plant glycosylases, oxoguanine glycosylase and formamidopyrimidine glycosylase, assayed in vivo

Permalink
https://escholarship.org/uc/item/6s85n3t6

Journal
Biochemical and Biophysical Research Communications, 392(1)

Authors
Murphy, Terence M.
Guo, Ying-Yi

Publication Date
2010-01-11

eScholarship.org Powered by the California Digital Library
University of California

Biochemical and Biophysical Research Communications 392 (2010) 335–339

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Antimutagenic specificities of two plant glycosylases, oxoguanine glycosylase and formamidopyrimidine glycosylase, assayed in vivo

Terence M. Murphy *, Ying-Yi Guo

Department of Plant Biology, University of California, One Shields Avenue, Davis, CA 95616, United States

A R T I C L E I N F O

Article history:
Received 5 January 2010
Available online 11 January 2010

Keywords:
Arabidopsis thaliana
Base-excision repair
Base substitutions
Formamidopyrimidine-DNA glycosylase
Oxoguanine-DNA glycosylase

A B S T R A C T

The base-excision repair process protects genomes by removing and replacing altered bases in DNA. Two analogous glycosylases, oxoguanine glycosylase (OGG) and formamidopyrimidine glycosylase (FPG), can start the process by removing oxidized guanine, the most common modification that leads to misreading of DNA. Plants possess genes for both types of glycosylases. We have tested the hypothesis that the two enzymes in plants have diverged in their specificities by inserting the genes for each enzyme from *Arabidopsis thaliana* L. into *Escherichia coli* strains designed to indicate the frequencies of the six possible single-base changes. Both enzymes retain the ability to reduce the rate of GC → TA transversion mutations. Both enzymes also reduce the frequency of two other base-change mutations, GC → AT and AT → TA. We do not find a divergence in the repair capabilities of the two enzymes, as measured in *E. coli*, although surprisingly FPG appears to increase the rate of mutations in one particular strain.

© 2010 Elsevier Inc. All rights reserved.

Introduction

Modification of bases in DNA is a common occurrence in cells and one with potentially serious mutagenic consequences. One of the most common modifications is oxidation of guanine. 7,8-Dihydro-8-oxoguanine (8-oxo-G) base-pairs equally well with cytosine and adenine, leading to GC → TA transversions. Virtually all organisms have a glycosylase that recognizes and removes 8-oxo-G from DNA, leading to its replacement by unmodified G through the subsequent reactions of the base-excision repair pathway. In archaea, fungi, and animals, the effective glycosylase is oxoguanine glycosylase (OGG); in bacteria, it is formamidopyrimidine glycosylase (FPG). Plants, uniquely, have genes for both enzymes. Furthermore, plant cells have variants of the mRNA for FPG produced by alternative splicing [1,2].

The reason for the retention in plants of the genes for both OGG and FPG and the diversity of FPG variants is not known. It is reasonable to speculate that the presence of photosynthetic metabolism, with the resulting high concentration of diatomic oxygen and oxygen radicals, creates a particular need for protection of the genome against oxidative damage. However, knock-out mutants that lack genes for either OGG or FPG, or both, show no obvious phenotype over several generations [3], so the adaptive value of the genes must be subtle or important under unusual circumstances that have not yet been identified. It is also possible that the presence of two analogous genes has allowed one or both to alter the specificity of their protein products, allowing them to recognize additional base modifications. It is known that the glycosylases can have multiple specificities. Bacterial FPG was first shown to act on ring-opened purines [4].

To test the specificities of the plant enzymes, we have inserted cDNAs for OGG and FPG from *Arabidopsis thaliana* into *Escherichia coli* strains, devised by Cupples and Miller [5] to test for single-base substitution mutations, in which the endogenous FPG gene has been inactivated [6]. There are six Cupples and Miller [5] strains, each with a base-pair substitution in its *LacZ* gene that inactivates the product β-galactosidase. Reversion mutations at that base-pair can be easily detected by the recovery of β-galactosidase activity (Table 1). If reversions are initiated by damage to the DNA that can be repaired by plant OGG or FPG, we expect that strains containing the *Arabidopsis ogg* or *fpg* genes will show less frequent reversion. We report that for both OGG and the primary form of FPG a major activity is the prevention of GC → TA transversions, indicating that both genes retain their original specificities. There is also evidence for other anti- and pro-mutagenic activities of the two enzymes.

Materials and methods

E. coli strains. Strains of *E. coli* with mutations in the *lacZ* gene for β-galactosidase [5] and a derivative with a knock-out mutation of the chromosomal gene for FPG produced with P1vir [6] were obtained from H. Schellhorn (McMaster Univ., Hamilton, Ontario, Canada). Growth on kanamycin confirmed the presence of the *fpg::kan* insertion.

* Corresponding author. Fax: +1 530 752 5410.
E-mail address: tmmurphy@ucdavis.edu (T.M. Murphy).

0006-291X/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2010.01.020

Table 1
Strains of *E. coli* used to test single-base mutations and base-excision repair.

Original *LacZ* mutants [5]	*LacZ* strains with fpg^- [6]	Amino acid at position 461	Base-pair mutation required to revert to activity
CSH100	HS1190	Glu	None
CSH101	HS1191	Term	AT → CG
CSH102	HS1192	Gly	GC → AT
CSH103	HS1193	Gln	GC → CG
CSH104	HS1194	Ala	GC → TA
CSH105	HS1195	Val	AT → TA
CSH106	HS1196	Lys	AT → GC

Plasmids. cDNAs were produced from mRNA templates of FPG variants in a flower mRNA library of *Arabidopsis thaliana* [7] as described [2]. Sequences of *fpg-1* and *fpg-2* cDNAs are registered as NCBI ID: AF099970 and AF099971, respectively. pTRC99a was obtained from Amersham Pharmacia Biotech, Inc. PCR copies of the cDNAs were inserted in the plasmid's multiple cloning site to give pTRC99a::*fpg-1*, and pTRC99a::*fpg-2* Like the expression plasmids described by [2], the PCR inserts were prepared with a N-terminal primer that used *E. coli*-preferred codons for the first six amino acids; unlike the expression plasmids, these inserts did not have poly-histidine C-terminal ends.

Using the same mRNA library, a cDNA coding for OGG was prepared and inserted in pTRC99a to give pTRC99a::*ogg*. The cDNA sequence is NCBI ID: AJ277400 [8].

For both FPG and OGG, the sequences of the inserts in the pTRC99a plasmids were confirmed directly by sequencing, conducted by the UC Davis College of Biological Sciences DNA Sequencing Center.

Transformation. E. coli strains were transfected with pTRC99a or with pTRC99a containing cDNAs for FPG-1, FPG-2, or OGG by electroporation Single colonies were selected on Luria–Bertani broth [9] containing kanamycin and ampicillin.

Mutation quantification. From overnight cultures, 1-ml cultures of each strain to be tested were diluted 1:100 and grown in Luria–Bertani broth at 37 °C for six to eight hours to stationary phase. Of these cultures, 100 μl were plated on 1.5% agar-solidified medium containing M9 salts (Gibco, BRL), 1 mM $MgSO_4$, 0.05 mM $CaSO_4$, and 0.2% lactose in 10-cm diameter plates. Plates were incubated at 37 °C. The appearance of colonies was recorded daily.

In each individual experiment, *LacZ* strains containing pTRC99a with an *ogg* or *fpg* insert were compared to a control with unmodified pTRC99a. The number of cells used for inoculation, estimated by dilution and plating of one full set (six *lacZ* mutations, four plasmids), was analyzed by two-factor analysis of variance. There was no significant effect of plasmid type on the stationary-phase concentrations of cells ($F_{3,15} = 0.46$); however, the *lacZ* strains did show significant differences ($F_{5,15} = 4.3$, $P < 0.05$; means ± SD ranging from 2.6 (±0.4) × 10^8 to 4.1 (±0.6) × 10^8). In previous experiments, the number of cells on lactose plates was found not to increase significantly over the period of the experiment [10]. Accordingly, the mutation rates for cells plated on lactose were calculated using the inoculation values specific for each *lacZ* strain.

For experiments in which cells were treated with UV radiation, 100 μl of undiluted cultures were plated as described above on M9 medium containing lactose as carbon source. Each culture was also diluted appropriately and plated on M9 medium containing glucose. Pairs of plates (lactose and glucose) were irradiated together for 30 s at 1.86 W m^{-2} with unfiltered radiation from a Hg-vapor lamp.

Sequences of revertant lacZ genes. DNA from revertant colonies was extracted by heat treatment (95 °C for 5 min in 10 μl of 10 mM Tris, 1 mM ethylenediaminetetraacetic acid buffer, pH 8; 20 μl of water was added; and the suspension was centrifuged) and used as template in a polymerase chain reaction. With the primers, 5′CAAATAATATCGGTTGCGGAGGTG3′ and 5′AATATTGAA ACCCACGGCATGGTG3′, amplification produced 250-bp fragments. These were isolated by gel electrophoresis, and their sequences were determined by the UC Davis College of Biological Sciences DNA Sequencing Center.

Results

Fig. 1 compares the results for the fpg^- strains, HS1194 and HS1194(pTRC99a), with those for CSH104 (fpg^+). Colonies started to appear two days after inoculation and continued to appear until at least day 6. As reported by Palmer et al. [6] and apparent in a comparison of results for HS1194 with CSH104, the inactivation of the endogenous *fpg* gene increased the rate of appearance of colonies in HS1194. Each colony could be attributed to a G → T transversion that led to mutant (revertant) *lacZ* and active β-galactosidase.

No other *lacZ* mutant showed a marked difference in mutation rate between fpg^+ and fpg^- backgrounds (that is, rates were comparable between HS101 and CSH1191, HS102 and CSH1192, etc.). The number of reversion mutants was much higher in HS1194 than in any other fpg^- strain. The inclusion of the pTRC99a plasmid did not affect the reversion rate (Fig. 1). We, like Palmer et al. [6], found very few reversion mutations in HS1193 and HS1196 (Table 2).

Table 2 describes the effects of OGG, FPG-1, and FPG-2 on the appearance of revertant colonies after five days on minimal medium containing lactose. Fig. 2A illustrates the results for the case in which OGG and FPG activity is expected. There were significantly fewer revertants in HS1194(pTRC99a::*ogg*) and HS1194(pTRC99a:*fpg-1*) than in HS1194(pTRC99a): the presence of either OGG or FPG-1 reduced the number of revertants by approximately 75%. We infer that the plant OGG and FPG were each effective in initiating the base-excision repair process by removing oxidized G bases, thus blocking 8-oxo-G:A base pairing and consequent G → T transversions. In contrast, there was no evidence that FPG-2 had any significant effect in lowering the G → T reversion rate.

Although the numbers of mutants were not large, there was a consistent indication that the presence of OGG and FPG-1 reduced the number of revertants in HS1195 (AT → TA) and that FPG-1 (but not OGG) reduced the number of revertants in HS1192 (GC → AT) (Table 2). For HS1195, the numbers of colonies were sufficient to suggest a strong effect in lowering mutation rate (Fig. 2B). For HS1192, the number of revertant colonies was small and the effect of repair thus difficult to quantify.

To test the suggestion that OGG and FPG-1 blocked reversion in HS1192 and HS1195, we treated plated cells with UV radiation. The

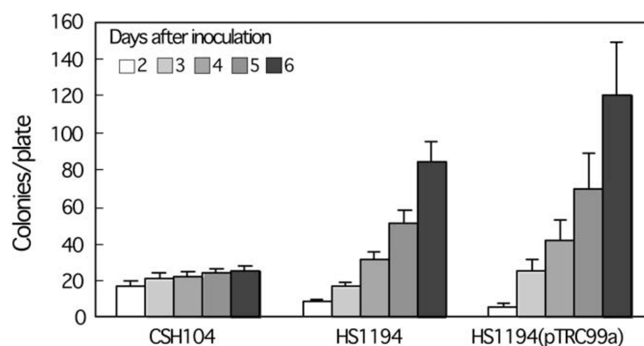

Fig. 1. Time course of appearance of $LacZ^+$ colonies. CSH104 is fpg^+; HS1194 is CSH104 fpg^- ($fpg::kan^r$); pTRC is HS1194 containing plasmid pTRC99a. All three strains revert to $LacZ^+$ by a G → T transversion.

Table 2
Frequency of spontaneous reversions in the presence of plant OGG and FPG variants, compared to control strains without a basic endonuclease.

Plasmid	Strain/base change to revert					
	HS1191 T → G A → C	HS1192 G → A C → T	HS1193 C → G G → C	HS1194 C → A G → T	HS1195 T → A A → T	HS1196 A → G T → C
OGG	6.7 ± 4.5(4)[a]	7.4 ± 4.9(2)	0 ± 0(2)	133 ± 21(4)	0 ± 0(2)	0 ± 0(2)
Control	2.9 ± 0.9(4)	10 ± 3(3)	0 ± 0(2)	412 ± 81(4)	10 ± 4(3)	0 ± 0(2)
FPG-1	105 ± 54(4)	0 ± 0(3)	1 ± 1(2)	157 ± 87(4)	1.5 ± 0.8(4)	0 ± 0(2)
FPG-2	9 ± 6(4)	11 ± 4(3)	4 ± 1(2)	664 ± 27(4)	10 ± 2(4)	0 ± 0(2)
Control	7.6 ± 5.4(4)	7.2 ± 5.5(3)	0 ± 0(2)	707 ± 18(4)	13 ± 4(4)	0 ± 0(2)

[a] Each tabulated value is the average number of revertant colonies on a lactose plate ± SE (number of independent experiments) observed five days after plating, divided by an estimate of the maximum number of cells on the plate, times 10^9.

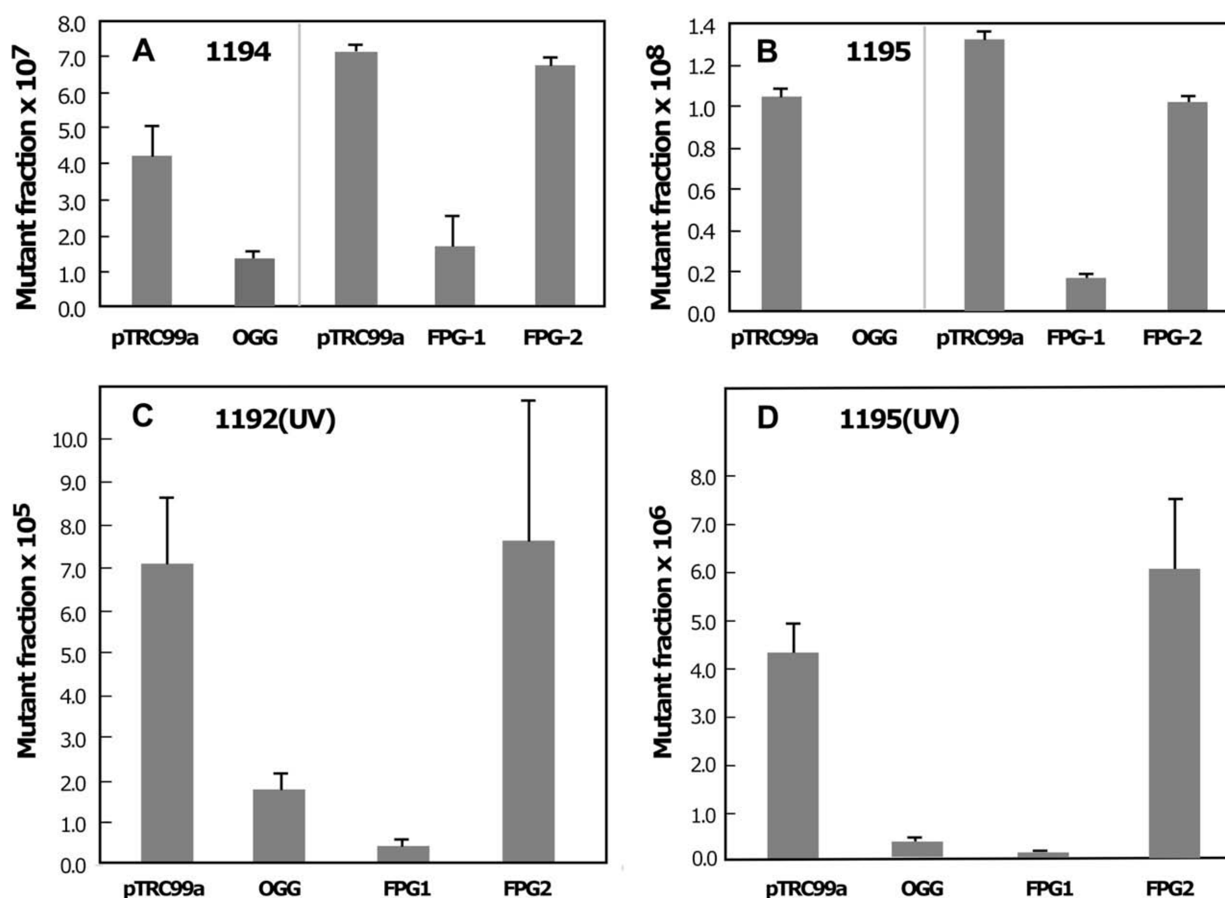

Fig. 2. Rates of appearance of revertant *LacZ*⁺ colonies in *E. coli* strains containing pTRC99a (control—expressing no glycosylase), pTRC99a::*ogg*, pTRC99a::*fpg-1*, or pTRC99a::*fpg2*. A, B: cells not irradiated; C, D: cells treated with far-UV radiation.

treatment reduced the viability of the cells by 95% with no substantial difference in survival among HS1191 strains containing the different plasmids (data not shown). The UV treatment increased the rates of reversion of HS1192(pTRC99a) and HS1195(pTRC99a) by factors of 10,000 and 325, respectively (compare Tables 2 and 3). With this increase, it became clear that both OGG and FPG-1 were effective in reducing reversion in both strains. To check whether the reversion of irradiated cells was equivalent to reversion of unirradiated cells, we extracted DNA from five revertant colonies each of HS1192(pTRC99a) and HS1195(pTRC99a) and determined their *lacZ* base sequences around the critical base (Fig. 3). In all cases, there was evidence for mutation to a triplet that would transcribe and translate to an active glu in the LacZ protein. In both HS1192 and HS1195, some colonies showed mixed bases at the critical position, indicating either that colonies contained both revertant and non-revertant cells or that cells contained both revertant and non-revertant plasmids, a finding not observed in previous studies involving spontaneous reversions [10]. In one HS1192 revertant, two adjacent bases were mutated.

Cells of HS1191(pTRC99a:*fpg-1*) consistently showed a higher number of revertants than did HS1191(pTRC99a) or HS1191 (pTRC99a:*fpg-2*) (Tables 2 and 3). The mutations were stable, in that they maintained their *lacZ*⁺ phenotype on repeated transfer to new media. However, sequence analysis of PCR fragments from the *lacZ* genes of five revertants showed the expected AT → CG mutations at position 461 in only three of the five (data not shown). The mutational changes leading to the other reversions are not known.

Table 3
Frequency of UV-radiation-induced reversions in the presence of plant OGG and FPG variants, compared to control strains without a basic endonuclease.

Plasmid	Strain/base change to revert					
	HS1191 T → G A → C	HS1192 G → A C → T	HS1193 C → G G → C	HS1194 C → A G → T	HS1195 T → A A → T	HS1196 A → G T → C
OGG	8 ± 5(5) [a]	170 ± 38(9)	2.3 ± 2.0(6)	160 ± 79(6)	2.4 ± 1.1(9)	0.3 ± 0.1(6)
FPG-1	120 ± 45(6)	33 ± 18(9)	0 ± 0(6)	800 ± 380(6)	1.0 ± 0.8(8)	35 ± 26(6)
FPG-2	9 ± 4(6)	760 ± 350(9)	8.4 ± 2.8(6)	310 ± 100(6)	60 ± 16(9)	5.8 ± 5.5(6)
Control	2.1 ± 0.7(6)	700 ± 160(9)	5.6 ± 3.9(6)	380 ± 230(5)	42 ± 6(8)	1.5 ± 1.2(6)

[a] Each tabulated value is the average of the rate of reversion (number of revertant colonies on a lactose plate observed five days after plating, divided by the number of plated cells calculated from the colonies on a glucose plate) ± SE (number of independent experiments) times 10^7. Pairs of lactose and glucose plates were irradiated together.

1192(pTRC) CCC/GGG (gly) → CTC/GAG (glu)

71 … 130

0 TCGTGATTAGCGCCGTGGCCACTC**C**CATTCCCCAGCGACCAGATGATCACACTCGGGTGA
1 TCGTGATTAGCGCCGTGGCCACTC**T**CATTCCCCAGCGACCAGATGATCACACTCGGGTGA
2 TCGTGATTAGCGCCGTGGCCACTC**Y**CATTCCCCAGCGACCAGATGATCACACTCGGGTGA
3 TCGTGATTAGCGCCGTGGCCACTC**Y**CATTCCCCAGCGACCAGATGATCACACTCGGGTGA
4 TCGTGATTAGCGCCGTGGCCACT**TT**CATTCCCCAGCGACCAGATGATCACACTCGGGTGA
5 TCGTGATTAGCGCCGTGGCCACTC**Y**CATTCCCCAGCGACCAGATGATCACACTCGGGTGA

Y=C/T

1195(pTRC) CAC/GTG (val) → CTC/GAG (glu)

71 … 130

0 TCGTGATTAGCGCCGTGGCCACTC**A**CATTCCCCAGCGACCAGATGATCACACTCGGGTGA
1 TCGTGATTAGCGCCGTGGCCACTC**T**CATTCCCCAGCGACCAGATGATCACACTCGGGTGA
2 TCGTGATTAGCGCCGTGGCCACTC**T**CATTCCCCAGCGACCAGATGATCACACTCGGGTGA
3 TCGTGATTAGCGCCGTGGCCACTC**K**CATTCCCCAGCGACCAGATGATCACACTCGGGTGA
4 TCGTGATTAGCGCCGTGGCCACTC**T**CATTCCCCAGCGACCAGATGATCACACTCGGGTGA
5 TCGTGATTAGCGCCGTGGCCACTC**T**CATTCCCCAGCGACCAGATGATCACACTCGGGTGA

K=G/T

Fig. 3. Base sequences of *lacZ* genes from position 71 to position 130 for original and revertant strains of HS1192(pTRC99a) and HS1195(pTRC99a). In each case, the original sequence is labeled '0'; revertant mutants, selected for growth on lactose medium, are labeled '1' to '5'.

Discussion

The results of these experiments confirm that both OGG and FPG-1 enzymes coded by their corresponding *Arabidopsis* genes are capable of reducing the frequency of G → T transversions. These results were predicted by earlier reports of their ability in vitro to cleave DNA containing 8-oxo-G (OGG: [8,11]; FPG: [2,12]. 8-Oxo-G base-pairs equally well with A and C. To the degree that the A is not removed from an 8-oxo-G:A base-pair by the *MutY* gene product, adenine-DNA glycosylase, the next round of replication will yield a stable T:A base-pair (and an active β-galactosidase if this base-pair is in the appropriate position in the *lacZ* gene). OGG and FPG-1 reduce the frequency of G → T transversions by removing the 8-oxo-G and thus preventing the formation of the 8-oxo-G:A base-pair (Fig. 4).

FPG-2 had no consistent activity in reducing G → T transversions. Gao and Murphy [2] earlier reported that FPG-2 had a limited amount of activity in vitro on depurinated, UV-treated, and methylene-blue-treated DNA (but not on 8-oxo-G-containing oligonucleotides). FPG-2 contains the N-terminal domain and the H2TH motif in the C-terminal domain, but lacks the FPG-1 C-terminal sequence that corresponds to the bacterial zinc-finger motif [13]; this motif contributes to stabilization of the enzyme–DNA complex [14]. Other products of the *Arabidopsis fpg* gene splicing variants found from cloning and RT-PCR screens were not tested, but most of these are predicted to lack essential active site amino acids or have other major disruptions in their C-terminal amino acid sequences [1], so it seems unlikely that they would be active.

The present experiments were performed in order to determine whether evolution of the *Arabidopsis Fpg* or *Ogg* genes produced enzymes with specificities other than, or in addition to, the removal of 8-oxo-G. In unirradiated cells we found a strong effect of FPG-1 in reducing GC → AT transitions in HS1192 and AT → TA transversions in HS1195 and of OGG in reducing AT → TA transversions in HS1195 (Table 2). In irradiated cells, the results were similar, except that the effect of OGG on GC → AT transitions in HS1192 became evident. FPG from *E. coli* is known to recognize and remove Fapy-A and other altered bases in vitro [15], but Palmer et al. [6] did not find substantial differences in the reversion rates of far-UV-irradiated HS1192 or HS1195 strains possessing or lacking the *E. coli fpg* gene. Thus these results imply that *Arabidopsis* FPG-1 and OGG recognize and remove a more extensive set of altered bases than does *E. coli* FPG. However, it is not yet clear what these altered bases are or whether the processes leading to reversion to *lacZ*$^+$ are as direct for HS1192 and HS1195 as are those for HS1194 (Fig. 4).

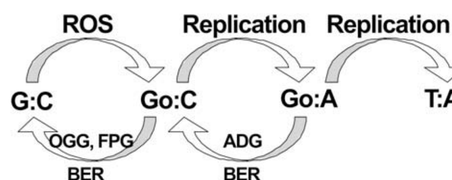

Fig. 4. Processes leading to a G:C → T:A transversion in HS1194 through the formation of 8-oxo-G (Go). ADG, adenine-DNA glycosylase; BER, base-excision repair; ROS, reactive oxygen species.

Surprisingly, FPG-1 appeared to *increase* the frequency of reversion of the *lacZ* gene in HS1191 (Tables 2 and 3). Some of these reversions were apparently due to second-site mutations, because base-sequence analysis indicated no difference at position 461. In natural systems, the enzymes of base-excision repair act in complexes (e.g., [16]); it may be that the presence of the plant FPG-1 affects the operation of the repair DNA polymerase. We note that if FPG-1 stimulated AT → CG transversion, it would have the effect of both *limiting* HS1194 reversion and *promoting* HS1191 reversion.

Because only plants among eukaryotes have the *fpg* gene, it is probable that they obtained the gene in the evolutionary events that led to the incorporation of plastids into primordial plant cells. This raises the question of how the various glycosylases are targeted to the locations of the three genomes, nucleus, mitochondrion, and plastid, in the plant cell. The mammalian *ogg* gene produces several initiation and splicing variants of mRNA [17], some products of which are targeted to the mitochondria and some to the nucleus. In contrast, the *Arabidopsis ogg* gene produces only one type of mRNA by splicing [8]. To our knowledge, there is as yet no evidence for the targeting of plant OGG or FPG proteins to various organelles, but *Arabidopsis* OGG is predicted through sequence analysis to be localized in mitochondria or plastids [18] and *Arabidopsis* FPG-1 has a predicted nuclear localization signal [12]. These predictions are opposite to what might be expected from evolutionary considerations. Direct measurements are essential to clarify this point.

Acknowledgment

The authors are grateful to Joline Shu for work on preliminary experiments that led to this study.

References

[1] T.M. Murphy, M.-J. Gao, Multiple forms of formamidopyrimidine-DNA glycosylase produced by alternative splicing in *Arabidopsis thaliana*, J. Photochem. Photobiol. B. Biology 61 (2001) 87–93.

[2] M.-J. Gao, T.M. Murphy, Alternative forms of formamidopyrimidine-DNA glycosylase from *Arabidopsis thaliana*, Photochem. Photobiol. 73 (2001) 128–134.

[3] T.M. Murphy, What is base excision repair good for?: knockout mutants for FPG and OGG glycosylase genes in *Arabidopsis*, Physiol. Plant. 123 (2005) 227–232.

[4] C.J. Chetsanga, T. Lindahl, Release of 7-methylguanine residues whose imidazole rings have been opened from damaged DNA by a DNA glycosylase from *Escherichia coli*, Nucleic Acids Res. 6 (1979) 3673–3684.

[5] C.G. Cupples, J.H. Miller, A set of *lacZ* mutations in *Escherichia coli* that allow rapid detection of each of the six base substitutions, Proc. Natl. Acad. Sci. USA 86 (1989) 5345–5349.

[6] C.M. Palmer, D.M. Serafini, H.E. Schellhorn, Near ultraviolet radiation (UVA and UVB) causes a formamidopyrimidine glycosylase-dependent increase in G to T transversions, Photochem. Photobiol. 65 (1997) 543–549.

[7] D. Weigel, J. Alvarez, D.R. Smyth, M.F. Yanofsky, E.M. Meyerowitz, LEAFY controls floral meristem identity in *Arabidopsis*, Cell 69 (1992) 843–859.

[8] A.L. Dany, A. Tissier, A functional OGG1 homologue from *Arabidopsis thaliana*, Mol. Genet. Genomics 265 (2001) 293–301.

[9] J. Sambrook, E.F. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual, second ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989.

[10] J. Shu, H.E. Schellhorn, T.M. Murphy, Stationary phase-induction of G → T mutations in *Escherichia coli*, Mutation Res. 596 (2006) 106–112.

[11] T. Morales-Ruiz, M. Birincioglu, P. Jaruga, H. Rodriguez, T. Roldan-Arjona, M. Dizdaroglu, *Arabidopsis thaliana* Ogg1 protein excises 8-hydroxyguanine and 2.6-diamino-4-hydroxy-5-formamidopyrimidine from oxidatively damaged DNA containing multiple lesions, Biochemistry 42 (2003) 3089–3095.

[12] T. Ohtsubo, O. Matsuda, K. Iba, I. Terashima, M. Sekiguchi, Y. Nakabeppu, Molecular cloning of *AtMMH*, an *Arabidopsis thaliana* ortholog of the *Escherichia coli mutM* gene, and analysis of functional domains of its product, Mol. Gen. Genet. 259 (1998) 577–590.

[13] M. Sugahara, T. Mikawa, T. Kumasaka, M. Yamamoto, R. Kato, K. Fukuyama, Y. Inoue, S. Kuramitsu, Crystal structure of a repair enzyme of oxidatively damaged DNA, MutM (Fpg), from an extreme thermophile, *Thermus thermophilus* HB8, EMBO J. 19 (2000) 3857–3869.

[14] A. Banerjee, W.L. Santos, G.L. Verdine, Structure of a DNA glycosylase searching for lesions, Science 311 (2006) 1153–1157.

[15] M. Dizdaroglu, Substrate specificities and excision kinetics of DNA glycosylases involved in base-excision repair of oxidative DNA damage, Mutation Res. 531 (2003) 109–126.

[16] E. Parlanti, G. Locatelli, G. Maga, E. Dogliotti, Human base excision repair complex is physically associated to DNA replication and cell cycle regulatory proteins, Nucleic Acids Res. 35 (2007) 1569–1577.

[17] K. Nishioka, T. Ohtsubo, H. Oda, T. Fujiwara, D. Kang, K. Sugimachi, Y. Nakabeppu, Expression and differential intracellular localization of two major forms of human 8-oxoguanine DNA glycosylase encoded by alternatively spliced OGG1 mRNAs, Mol. Biol. Cell 10 (1999) 1637–1652.

[18] J.L. Heazlewood, J. Tonti-Filippini, R.E. Verboom, A.H. Millar, Combining experimental and predicted datasets for determination of the subcellular location of proteins in *Arabidopsis*, Plant Physiol. 139 (2005) 598–609.