Free Rad. Res., Vol. 29, pp. 487–497
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Excision Repair of 8-Hydroxyguanine in Mammalian Cells: The Mouse Ogg1 Protein as a Model

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Accepted by Prof. B. Halliwell

(Received 19 June 1998)

8-Hydroxyguanine (8-OH-Gua) is a major mutagenic lesion produced on DNA by the oxidative stress induced by either the endogen metabolism or the exposure to external agents. In bacteria and yeast this modified base can be removed by specific DNA glycosylases. Recently a human gene coding for an 8-OH-Gua DNA glycosylase/AP lyase has been identified by its homology to the yeast *OGG1*. This gene is located in human chromosome 3p25, a region commonly rearranged in various cancers, specially in lung tumor cells. We report here the cloning, by sequence homology to the yeast *OGG1*, of a mouse cDNA coding for a 8-OH-Gua DNA glycosylase with 84% and 38% identity to the human and yeast relevant proteins, respectively. The *Ogg1* gene is localized to the mouse chromosome 6E. The mouse *Ogg1* cDNA, when expressed in *Escherichia coli*, is capable of suppressing the spontaneous mutator phenotype of a DNA repair deficient *fpg mutY* strain. The mouse Ogg1 protein acts efficiently on duplexes in which the 8-OH-Gua is paired with a cytosine but is inactive on 8-OH-Gua : Ade pair, consistently with its proposed biological role in the avoidance of mutations. A comparison of the mouse enzyme with other eukaryotic Ogg1 enzymes is also presented. The isolation of this gene will allow the development of an animal model to study the effects of oxidative stress on carcinogenesis and degenerative diseases.

Keywords: Oxidative DNA damage, 8-hydroxyguanine, DNA repair, Ogg1 proteins

INTRODUCTION

It is now widely accepted that mutation events are at the origin of the cancer development process. It has been proposed that a mutator phenotype might be involved at some point in the multistage process of carcinogenesis.[1] This model has been actually confirmed by the finding that the hereditary nonpolyposis colorectal cancer is associated to defects in the gene coding for a homolog to the bacterial mismatch repair protein MutS.[2,3] Indeed, cells from these tumors have a hypermutator phenotype and the biochemical defect in the mismatch repair process was established.[4] These findings have been extended to other tumors with mutations in a second MutS homolog as well as in the homologs

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of MutL, another essential component in the biochemical mismatch repair machinery of *E. Coli*.[5,6] Impairments in the mechanisms of repair of DNA replication errors then give rise to an intrinsic genetic instability. This early step in carcinogenesis can lead to mutations in oncogenes or inactivation of tumor suppressor genes, altering the cell growth control.

Studies in bacterial systems have shown that replication errors are not the only origin for mutations. The DNA being a highly reactive chemical species, it is susceptible to damage by both exogenous and endogenous agents. A particularly important form of stress for all cells is the one produced by the presence of reactive oxygen species (ROS). These intermediates or by-products from many normal metabolic processes include hydrogen peroxide, hydroxyl radical and superoxide ion. They can also be generated by the action of exogenous agressors such as chemical mutagens and ionizing radiation. The interaction of the ROS with DNA can lead to several kinds of lesions like strand breaks, DNA-protein crosslinks or modified bases.[7] Some of these modifications can lead to the blockage of replication and therefore the lack of cell duplication. Other adducts however are tolerated by the replication machinery but have the potential to miscode or reduce the fidelity of the polymerases and therefore result in mutations. Thus, the oxidative damage in DNA has been implicated in a variety of pathologies, in particular cancer and ageing.[8]

An oxidatively damaged form of guanine, 8-hydroxyguanine (8-OH-Gua) is abundantly produced as a consequence of the normal or the induced oxidative stress.[8] The presence of this modified base in the DNA is highly mutagenic since, while it does not impede replication of the DNA, it preferentially pairs to adenine during replication[9] and therefore leads to the generation of G:C to T:A transversions.[10] The relevance of this kind of DNA damage has been unveiled by the study of two mutator genes in *E. coli*, *fpg* (*mutM*) and *mutY* (*micA*). Both genes code for DNA glycosylases that cooperate to prevent the mutagenic effects of 8-OH-Gua.[11,12] Inactivation of any of those two genes leads to a mutator phenotype characterized by the exclusive increase in G:C to T:A transversions.[13–15] The importance of this repair pathway has been shown by the extremely potent mutator phenotype of bacterial strains deficient in both activitites. *E. coli fpg* and *mutY* mutations act synergistically with respect to the frequency of G:C to T:A transversions.[10] This has led to the proposal of a sophisticated cellular system for the avoidance of the mutations induced by 8-OH-Gua[16,17] in bacteria.

Due to the ubiquitous presence of ROS, and therefore 8-OH-Gua, the existence of repair mechanisms similar to the bacterial one described above has been postulated in eukaryotes. In *Saccharomyces cerevisiae*, the *OGG1* gene encodes an 8-OH-Gua DNA Glycosylase activity that reduces the mutator phenotype of the *fpg mutY* double mutant of *E. Coli*.[18] The inactivation of this gene in yeast creates a mutator phenotype that is also specific for the generation of the G:C → T:A transversions characteristic of the presence of unrepaired 8-OH-Gua residues in DNA.[19] The yeast Ogg1 protein has 376 amino acids and, even though has an enzymatic activity similar to that of Fpg, it shows no sequence homology to the bacterial protein.[18,20] The Ogg1 protein is a DNA glycosylase/AP lyase that catalyzes both the release of 8-OH-Gua and the cleavage of DNA at the resulting AP site via a β-elimination reaction.[18,20,21] From these results it is clear that eukaryotes cells have a specific system to eliminate 8-OH-Gua from DNA through a base excision repair mechanism, and therefore avoid the mutagenic impact of ROS. The presence of such system in mammals has been confirmed by the recent cloning of *hOGG1*, a human homolog of the yeast enzyme.[22–28] This gene is located in human chromosome 3p25, a region frequently lost in various cancers, specially in lung tumors where loss of heterozygosity of markers or deletions in

this area are observed in a majority of cases.[29–32] Here we discuss the cloning of the murine homolog for the yeast and human genes and its properties.

MATERIALS AND METHODS

Strains and microbiological methods *E. coli* strains used are derived from CC104[33] (*ara*, Δ(*gpt-lac*), *rpsL*/F′ (*lacI*, *lacZ*, *proA*$^+$*B*$^+$)): PR180 (CC104 *mutY*::Kan^r; PR221 (CC104 *fpg*::Kan^r) and PR195 (CC104 *fpg*::Kan^r *mutY*::Kan^r). Strains were grown on either LB or minimal A media.[34] The homogeneous Ogg1 protein of *S. cerevisiae* was prepared as previously described.[18]

cDNA clone and sequencing A homolog of the *S. cerevisiae OGG1* gene was identified in the data base of the IMAGE consortium sequencing project.[35] The clone was obtained from the UK HGMP Resource Centre (Cambridge, UK) and sequenced on both strands using Sequenase version 2.0 (Amersham). To isolate the 5′ region of the cDNA the rapid amplification of cDNA ends (RACE) protocol was used on an anchor ligated cDNA (mouse testis Marathon Ready, Clontech).

Expression of the mouse cDNA in E. coli, determination of mutation frequencies, preparation of bacterial extracts and purification of the mouse Ogg1 protein Plasmid pPR68 was obtained by PCR amplification of the mouse *OGG1* open reading frame (ORF) sequence and subcloning into pKK223-3 (Pharmacia). PR195 cells harboring either this plasmid or the vector pKK223-3 were grown in LB broth containing 100 μg/ml ampicillin and 1 mM isopropyl-β-D-thiogalactoside (IPTG). To determine the spontaneous frequencies of rifampicin resistant mutants and of lactose revertants, appropriate dilutions of at least 15 overnight cultures, originally inoculated with 10^3 cells, were plated on either LB agar, LB agar with rifampicin (100 μg/ml), or minimal lactose (0.2%) agar. Colonies were counted the following day in the case of the LB plates and 48 h after plating for the minimal lactose plates.

For analysis of the enzymatic activities of the murine Ogg1 protein, cultures of PR221 cells harboring pPR68 were grown at 37°C in 20 ml of LB-broth medium containing 100 μg/ml of ampicillin until $OD600_{nm} = 1.0$ and supplemented with 1 mM IPTG. Cultures were then incubated at 37°C for 2.5 h under vigorous agitation. The cells were harvested by centrifugation, washed and stored at −80°C. Cell free extracts were prepared as previously described[11] and protein concentration was measured according to Bradford.[36] Partially purified mouse Ogg1 protein was prepared from *E. coli* cells PR221 harboring pPR68 (3 g, w/w). The purification procedure includes a QMA anion exchange column to separate nucleic acids from proteins. The active fractions were loaded onto an AcA44 (IBF-LKB) gel filtration column. Finally, active fractions were loaded onto a cationic MonoS HR5/5 column (Pharmacia). The Ogg1 activity eluted at 350 mM NaCl. Active fractions were pooled, dialyzed and stored at −20°C. The final fraction has a protein concentration of 250 μg/ml.

Determination of the 8-OH-Gua DNA glycosylase/AP lyase activity The 34-mer oligonucleotides containing a single 8-OH-Gua or a Gua residue at position 16 were synthesized as previously described.[37] To protect oligonucleotides from degradation in cell free extracts, the nucleotide at the 3′-end was inverted yielding a 5′-$(N)_n$-3′-P-3′-N-5′* sequence with two 5′-ends.[37] Complementary sequences with a cytosine, thymine, guanine or adenine opposite 8-OH-Gua were also synthesized. For the 8-OH-Gua DNA glycosylase activity, 1 μg of protein from the active Ogg1 fraction was incubated for 30 min at 37°C with 15 pmol of 34-mer oligonucleotides containing the various 8-OH-Gua base pairs. The products of the reactions were separated by HPLC and the free 8-OH-Gua was quantified by electrochemical detection.[18] For the AP lyase activity, the 34-mer oligonucleotides were labelled at both ends using [γ^{32}P]-ATP and T4

polynucleotide kinase. The assay mixtures (25 µl-final volume) contained 25 mM Tris-HCl pH 7.6, 2 mM Na_2EDTA, 50 mM KCl, 50 fmol of ^{32}P-labelled DNA duplex and bacterial cell free extracts or Ogg1 protein. The reactions were performed at 37°C for 30 min and the products were separated by 20% denaturing PAGE containing 7 M urea.

Fluorescence in situ hybridization (FISH) analysis Mouse chromosomes were prepared from normal fibroblast cultures after BrdU incorporation during the last 7 h before harvesting. The 1.4 kb cDNA clone of *Ogg1* was used as probe for FISH. The probe was labelled by nick-translation with biotin-11-dUTP (Sigma, France). A standard hybridization was performed as described.[38] The cDNA probe was used at a concentration of 20 ng/µl in 15 µl of hybridization buffer. Immunochemical detection of hybridization was performed using mouse anti-biotin antibodies (Dako, Denmark), biotin anti-mouse antibodies (Dako, Denmark) and streptavidin Rhodol green (Molecular Probes, Netherlands). Direct banding of BrdU substituted chromosomes was obtained by incubation in an alkaline solution of p-phenylenediamine (PPD11)[39] and stained with propidium iodide. Metaphases were observed under a fluorescent microscope (FXA, Nikon, Japan) and photographed on Ektakrome ASA 400 film (Kodak, USA).

RESULTS

Molecular Cloning of a Mouse cDNA with Homology to the Yeast OGG1

A mouse expressed tag sequence was identified in our database search for murine homologs of the yeast gene *OGG1* coding for an 8-OH-Gua DNA Glycosylase. The corresponding plasmid, from a mouse embryo cDNA library, carried an insert of 1532 bp. A 5′ RACE analysis identified 26 extra base pairs upstream of the end of the original clone. The compiled sequence (1558 bp) contains an open reading frame (ORF) that starts 225 bp from the 5′ end of the cDNA and codes for 345 amino acids (EMBL database accession no. Y 13479). A second putative initiation codon is found 30 bp from the first one. The downstream ATG has a sequence context somehow closer to the consensus sequence for initiation of translation in vertebrates mRNA.[40] A consensus polyadenylation site is present 21 base pairs from the start of the poly A. A putative nuclear localization sequence is observed in the carboxyl terminal region (residues 335–342) (Figure 1).

The predicted protein coded by the longest ORF has molecular weight of 38,857 Da and a calculated isoelectric point of 9.07. Sequence comparison with the yeast Ogg1 (Figure 1) reveals a 38% identity (48% similitude) at the amino acid level. Among the regions most conserved is the stretch between residues 240 and 270 that carries essential residues for the yeast enzymatic activity, in particular Lys 241.[21] This conserved region also includes a helix–hairpin–helix motif, characteristic of the DNA binding site from the *E. coli* endonuclease III.[41] Comparison to the human homolog (Figure 1) shows that the mouse enzyme is 84% identical to the human Ogg1. This cDNA clone was used as a probe on a Northern blot carrying polyA$^+$ RNA from mouse tissues to detect a single band corresponding to a 1.5 kb transcript in all the tissues tested (data not shown).

Genetic Complementation of a Bacterial Mutator Phenotype by the Murine Ogg1

To express the Ogg1 protein in *E. Coli* the *Ogg1* ORF was cloned into the expression vector pKK223-3. The resulting plasmid (pPR68) was introduced by transformation into the bacterial strain PR195 (*fpg mutY*). This strain exhibits a very high mutator phenotype due to its incapacity to eliminate the mutagenic 8-OH-Gua from its DNA.[10] In particular it allows to detect

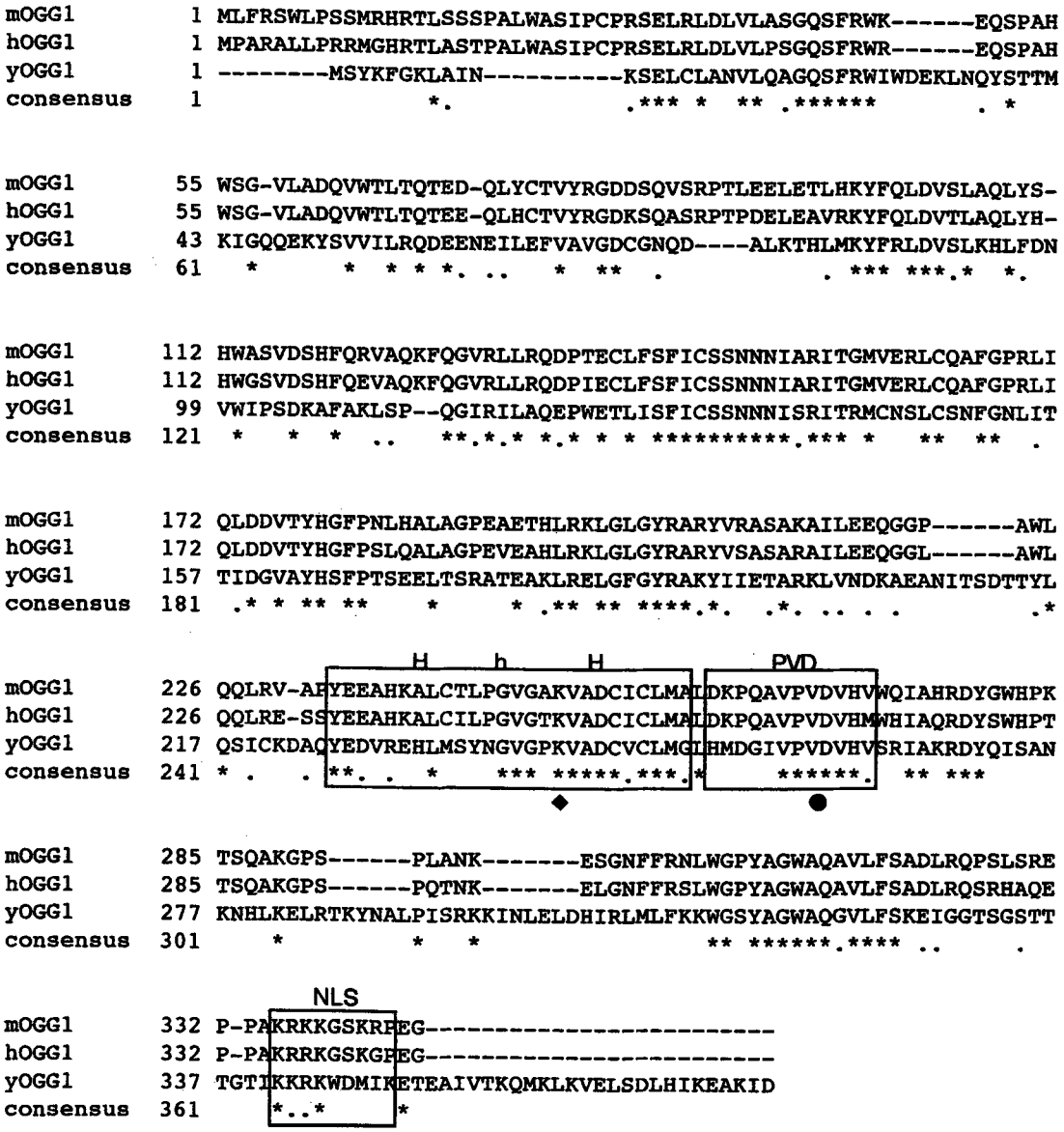

FIGURE 1 Sequence alignment of the mouse (Y13479), human (Y11731) and yeast (U44855) Ogg1 proteins. Asterisks indicate amino acids that are identical for the three proteins. The regions corresponding to the helix–hairpin–helix motif (H-h-H), the proline/valine domain (PVD) and the putative nuclear localization signal (NLS) are boxed. Amino acids involved in the catalytic mechanism of the yeast Ogg1 protein are indicated, Lys241 (◆) and Asp260 (●), respectively.

specifically the G:C to T:A transversions through the reversion of the *lacZ* mutation. We have then assessed the mutation frequencies for two markers: mutation to rifampicin resistance and reversion to Lac^+ in PR195 carrying either the vector pKK223-3 or the plasmid coding for *Ogg1* (pPR68). Table I demonstrates that the expression of the murine gene is able to reduce the mutation frequencies for both markers in the *mutY fpg* mutator strain. This complementation of the mutator phenotype of a *mutY fpg* strain suggests that the murine *Ogg1* gene is

TABLE I Spontaneous mutagenesis of the mutant strain *E. coli fpg mutY* expressing the mouse *Ogg1* gene

Bacterial strain	Plasmid	$Rif^R/10^8$	$Lac^+/10^8$
PR180 (*mutY*)	none	23+/−2	6.4+/−1.1
PR195 (*fpg mutY*)	vector	131+/−17	1160+/−185
PR195 (*fpg mutY*)	*Ogg1*	51+/−10	39+/−9

Numbers represent the spontaneous mutation frequencies as determined by the method of the median[48] on 20 independent cultures of each strain.

involved in the repair of mutagenic lesions. More specifically, because it prevents the generation of G:C to T:A transversions and its homology to the yeast *OGG1*, it is likely to be involved in the removal of the 8-OH-Gua residues from the DNA.

Enzymatic Activities of the Murine Ogg1 Protein

The mouse Ogg1 protein was partially purified from *E. coli* strain PR221 harboring the plasmid pPR68. The excision of 2,6-diamino-4-hydroxy-5-N-methylformamidopyrimidine (Fapy) was used as an activity assay in the course of the purification procedure.[11] To test whether the 8-OH-Gua in DNA is a substrate for the glycosylase activity of Ogg1, the protein fraction was incubated with double stranded oligonucleotides (34-mers) carrying a single 8-OH-Gua residue in one strand mispaired with any of the four DNA bases in the complementary strand. The products of the reactions were analyzed using HPLC with electrochemical detection. The mouse Ogg1 protein releases a product that coelutes with the 8-OH-Gua marker molecule (data not shown). These results show that the expressed Ogg1 has a DNA glycosylase activity capable of releasing 8-OH-Gua as a free base. Figure 2A shows that 8-OH-Gua is readily released when paired to Cyt or Thy. No significant release of the modified base was detected when opposite Gua or Ade. This preference of Ogg1 for 8-OH-Gua:Cyt pairs is characteristic of this family of

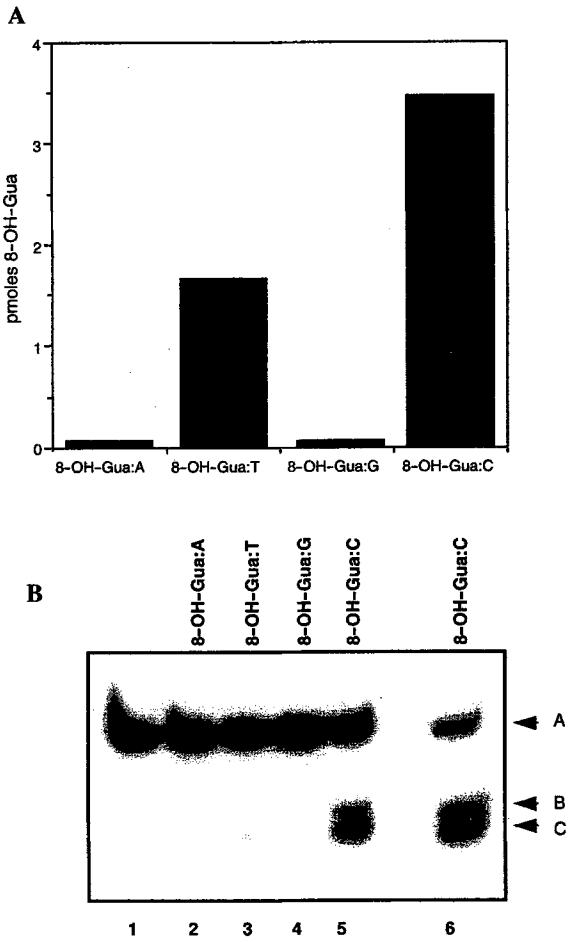

FIGURE 2 (A) Substrate specificity of the glycosylase activity of the mouse Ogg1 protein. one µg of protein from the active Ogg1 fraction was incubated for 30 min at 37°C with 15 pmol of 34-mer oligonucleotides containing the various 8-OH-Gua base pairs. The products of the reactions were separated by HPLC and the free 8-OH-Gua was quantified by electrochemical detection. (B) Substrate specificity of the glycosylase/AP lyase activity of the mouse Ogg1 protein. The partially purified mouse Ogg1 (lanes 2–5) (20 ng of the active fraction) or 5 ng of the yeast Ogg1 (lane 6) were incubated with oligonucleotides carrying the indicated 8-OH-Gua base pairs and ^{32}P labelled on the strand carrying the modified base. After 30 min at 37°C, the products of the reactions were separated by gel electrophoresis. Band A: 34-mer substrate; bands B and C: products of the cleavage at the lesion site. Lane 1 corresponds to the 8-OH-Gua:Cyt oligonucleotide incubated without a protein fraction.

enzymes[18,20,22–24,26,27] and consistent with its postulated physiological role to avoid transversions induced by the presence of 8-OH-Gua in DNA.[16,17]

The mouse Ogg1 protein was then tested for its capacity to cleave 34-mer double-stranded substrates containing a single 8-OH-Gua mispaired with a Cytosine. The substrate is labelled at both ends of the strand with the modified guanine.[37] Figure 2B shows that this protein fraction can incise the DNA strand carrying the oxidized base paired with a cytosine to yield two labelled products. The products of the reaction are consistent with a mechanism involving the glycosylase step followed by a β-elimination reaction.[21] Therefore the 8-OH-Gua DNA glycosylase activity of Ogg1 is associated to a nicking activity. The subsequent addition to the reaction mixture of endonuclease IV from *E. Coli* does not increment the amount of cleavage products (data not shown), suggesting that the lyase activity from Ogg1 is not limiting with respect to the glycosylase activity. The substrate specificity of the combined glycosylase/lyase activities was explored by analyzing the cleavage of 34-mer DNA duplexes containing 8-OH-Gua opposite to each of the four normal DNA bases. A marked preference for the substrate carrying the 8-OH-Gua:Cyt base pair is observed, while poor cleavage is detected on the 8-OH-Gua:Thy carrying duplexes (Figure 2B). As it was shown for the glycosylase activity, the DNAs carrying either 8-OH-Gua:Gua or 8-OH-Gua:Ade base pairs are not substrates for the incision activity. No cleavage at all was detected on duplexes carrying a normal Gua paired to each of the four canonic DNA bases (data not shown).

Chromosomal Localization of the Mouse Ogg1 Gene

Fluorescence *in situ* hybridization on mouse metaphase chromosomes, using the *Ogg1* probe, revealed recurrent spots on chromosome 6, bands 6E or 6F1. Sixty percent of the 50 metaphases analyzed exhibited signal on one or both chromatids at this position, the mapping on 6E being more probable than that on 6F (Figure 3).

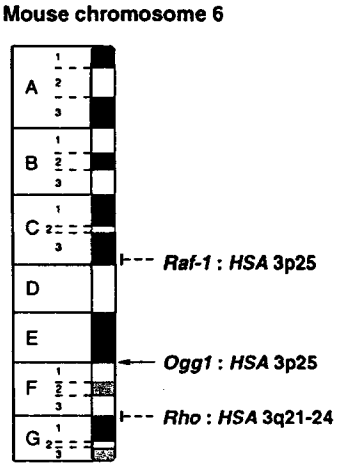

FIGURE 3 Chromosomal localization of the *Ogg1* gene as determined by FISH. Schematic representation of mouse chromosome 6. The localizations of the genes *Raf1*, *Rho*, and *Ogg1* are shown. The localization of the human homolog on chromosome 3 is indicated following the name of the gene.

DISCUSSION

ROS formed in cells either as by-products of aerobic metabolism or as a consequence of exposure to environmental mutagens can attack DNA or its precursors yielding oxidatively damaged bases and strand-breaks. Several lines of evidence suggest that an oxidatively damaged guanine, 8-OH-Gua, is critical in terms of mutagenesis.[7,8] Its capacity to pair preferentially with adenine when present in the DNA specifically generates G:C to T:A transversions during the normal replication of the genetic material. In *E. coli* the system that prevents mutations of this kind to occur is composed of three distinct enzymatic activities coded by the genes *mutT*, *fpg* and *mutY*. MutT is an 8-OH-dGTPase whose function is to sanitize the nucleotide pool. Fpg and MutY are DNA glycosylases that act through the base excision repair pathway to eliminate the 8-OH-Gua and the mispaired adenine respectively. In yeast only the functional homolog of *fpg* (*OGG1*) has been identified and surprisingly it shows no sequence homology to the bacterial gene. No homologs

have yet been detected for the other two components of the 8-OH-Gua repair pathway even though the sequencing of the entire genome has been completed. In mammalian cells the homologs to MutY and MutT have been cloned.[42,43] Recently the human *OGG1* gene has been identified and shown to be highly homologous to the yeast gene. Here we report the cloning of a mouse gene (*Ogg1*) homologous to the yeast *OGG1*.

The mouse *Ogg1* is located on chromosome 6E while its human homolog, *OGG1* has been mapped to band 3p25.[22] The location of a number of genes located on mouse chromosome 6 (MMU6) is also known in the human karyotype. Briefly, genes from human chromosome 7 (HSA7) are proximally located in MMU6. One gene from HSA2 is located in the median part (region C) of MMU6. Finally two genes, *RAF1* and *RHO*, located in bands HSA3p25 and HSA3q21–24, are found in bands MMU6 C3 and MMU6 F3 respectively. *Ogg1*, whose human homolog has been mapped to HSA3p25, is located on band MMU6E or F1, i.e. between *Raf1* and *Rho*. This confirms that chromosomes MMU6 and HSA3 share homologies. However, *Ogg1* is fairly distant from *Raf1* in MMU6 whereas they are very close to each other in band HSA3p25. Furthermore, *Ogg1* is closer to *Rho* than to *Raf1*. This complex situation is probably a consequence of chromosomal rearrangements which occurred during Primates and Rodentia evolution, a number of which can be reconstructed. An ancestral mammalian chromosome was a large acrocentric, formed by equivalents of HSA3 and HSA21 chromosomes.[44] As a consequence of this, the genes lying in the same region could have been separated and located on different arms. During Muridae evolution, *Ogg1*, *Rho* and *Raf1* probably remained synthenic, but were translocated with sequences from chromosomes equivalent to HSA7, HSA2, HSAl2 and HSA10. Data from linkage studies show that sequences from HSA12 and HSA10 are located between *Raf1* and *Rho*. They now seem to be located between *Raf1* and *Ogg1*, *Ogg1* and *Rho* remaining fairly close together, perhaps as in the ancestral mammalian chromosome.

The predicted amino acid sequence of Ogg1 reveals that this enzyme is a member of the family of DNA glycosylases/AP lyases sharing a common ancestor gene with the *E. coli* endonuclease III. Characteristic of this family is the HhH-PVD motif present between residues 245 and 280 from the mouse Ogg1. The net preference for DNA substrates carrying an 8-OH-Gua : Cyt base pair is consistent with the role of this enzyme in the elimination, before replication, of the mutagenic damage created by oxydation of the Gua, as it was shown for Fpg in the bacterial system. This parallel with the *E. Coli* system is confirmed by the complementation of the mutator phenotype of an *fpg mutY* double mutant strain when mOgg1 is expressed in it. The biochemicals studies reported here, consistently with those reported recently,[24] clearly show that Ogg1 is endowed with both an 8-OH-Gua DNA glycosylase activity that generates an apurinic site and a subsequent nicking activity on substrates carrying 8-OH-Gua : Cyt base pairs. The products of the nicking reactions on duplex DNA containing the oxidized form of Gua are consistent with the catalysis by Ogg1 of a β-elimination reaction as found for the yeast enzyme.[21] Complementary studies using the trapping assay have led to similar conclusions.[27] Moreover, as it is the case with Fpg and the yeast Ogg1, the mouse Ogg1 can also catalyze the excision of Fapy residues from DNA. The properties of the yeast, mouse and human Ogg1 proteins are summarized in Table II.

The analysis of the sequence changes in p53, a tumor supressor gene very commonly mutated in many cancers, showed that, unlike in most other tumors, in lung cancer there is a strong bias for the presence of G to T tranversions.[45] By analogy to the bacterial and yeast systems[15,19] this type of mutations would be expected in mammalian cells incapable of eliminating

TABLE II Comparison of the properties of the yeast and mammalian Ogg1 proteins

	Yeast Ogg1	Mouse Ogg1	Human Ogg1[a]
Gene localization	Chromosome XIII	6E	3p25
Protein (amino acids)	376	345	345
Molecular mass (kDa)	42.8	38.9	38.8
Isoelectric point	8.83	9.07	8.89
Putatif Motifs			
HhH	yes	yes	yes
NLS	yes	yes	yes
Enzymatic activities			
DNA glycosylase	yes	yes	yes
Me-FapyGua:Cyt	yes	yes	yes
FapyGua:Cyt	yes	nd	nd
8-OH-Gua:Cyt	yes	yes	yes
8-OH-Gua:Ade	no	no	no
AP *lyase*	yes: β-elimination	yes: β-elimination	yes: β-elimination
Mutant phenotype	mutator GC → TA	nd	nd
Complementation			
E. coli fpg mutY	yes	yes	yes
S. cerevisiae ogg1	yes	nd	yes

[a]Human nuclear Ogg1 protein. nd: not determined. The data reported in this table are collected from Refs. [18–28].

8-OH-Gua from their DNA and therefore likely to be deficient in the *OGG1*. Moreover, a role for oxidative lesions in the mutagenic effects of benzo (α) pyrene, a carcinogenic component of cigarette smoke, has been proposed by studies performed in bacterial strains deficient in MutY and Fpg functions.[46] These facts together with the mapping of the human *OGG1* to chromosome 3p25 are intriguing. A combination of cytogenetic and molecular studies have implicated loss of heterozygosity or deletions in chromosome 3p in all major types of lung cancers.[29,31] So the presence in this locus of a DNA repair gene, whose bacterial and yeast functional homologs are mutator genes that specifically prevent G:C to T:A transversions, make it a good candidate for a long sought cancer predisposition gene. Supporting this view, we have found mutations in the *OGG1* gene in 2 out of 25 human lung tumors studied.[47] These two mutations were base pair substitutions generating missense mutations in the Ogg1 protein. Our results indicate that only the mutant allele of *OGG1* was expressed in the tumoral tissue, suggesting a process of sequential inactivation of the two alleles of *OGG1*. Finally, the cloning of the mouse *Ogg1* gene will allow the construction of an *ogg1*$^-$/*ogg1*$^-$ animal model.

To conclude, these results indicate that the Ogg1 protein protects the genome from the deleterious action of oxidative DNA damage. In turn, inactivation of *OGG1* gene in mammalian cells will provide an important strategy to evaluate the biological impact of oxidative stress on several biological processes such as cancer, ageing and other degenerative diseases.

Acknowledgements

This research was supported by the Commissariat à l'Energie Atomique (CEA), the Centre National de la Recherche Scientifique (CNRS) (ACC-SV8 to S.B.) and the Comité de Radioprotection of Electricité de France (EDF).

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