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# Inactivation of *OGG1* increases the incidence of G · C→T · A transversions in *Saccharomyces cerevisiae*: evidence for endogenous oxidative damage to DNA in eukaryotic cells

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**Abstract** The *OGG1* gene of *Saccharomyces cerevisiae* encodes a DNA glycosylase that excises 7,8-dihydro-8-oxoguanine (8-OxoG) and 2,6-diamino-4-hydroxy-5-*N*-methylformamidopyrimidine. To investigate the biological role of the *OGG1* gene, mutants were constructed by partial deletion of the coding sequence and insertion of marker genes, yielding *ogg1*::*TRP1* and *ogg1*::*URA3* mutant strains. The disruption of the *OGG1* gene does not compromise the viability of haploid cells, therefore it is not an essential gene. The capacity to repair 8-OxoG has been measured in cell-free extracts of wild-type and *ogg1* strains using a 34mer DNA fragment containing a single 8-OxoG residue paired with a cytosine (8-OxoG/C) as a substrate. Cell-free extracts of the wild-type strain efficiently cleave the 8-OxoG-containing strand of the 8-OxoG/C duplex. In contrast, cell-free extracts of the Ogg1-deficient strain have no detectable activity that can cleave the 8-OxoG/C duplex. The biological properties of the *ogg1* mutant have also been investigated. The results show that the *ogg1* disruptant is not hypersensitive to DNA-damaging agents such as ultraviolet light at 254 nm, hydrogen peroxide or methyl methanesulfonate. However, the *ogg1* mutant exhibits a mutator phenotype. When compared to those of a wild-type strain, the frequencies of mutation to canavanine resistance ($Can^R$) and reversion to $Lys^+$ are sevenfold and tenfold higher for the *ogg1* mutant strain, respectively. Moreover, using a specific tester system, we show that the Ogg1-deficient strain displays a 50-fold increase in spontaneously occurring G · C→T · A transversions compared to the wild-type strain. The five other base substitution events are not affected by the disruption of the *OGG1* gene. These results strongly suggest that endogeneous reactive oxygen species cause DNA damage and that the excision of 8-OxoG catalyzed by the Ogg1 protein contributes to the maintenance of genetic stability in *S. cerevisiae*.

**Key words** *Saccharomyces cerevisiae* · DNA repair · G · C→T · A transversions · 8-Oxoguanine · *OGG1* gene

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## Introduction

Reactive oxygen species (ROS) formed in cells either as by-products of aerobic metabolism or as a consequence of exposure to environmental mutagens have been suggested to play an important role in biological processes such as mutagenesis, carcinogenesis, and aging (Breimer 1990; Ames et al. 1993; Feig et al. 1994). Many ROS are highly reactive and cause various types of DNA damage, such as single- and double-strand breaks, base loss and base oxidation (Dizdaroglu 1991; Boiteux et al. 1992; Dizdaroglu et al. 1993). Several lines of evidence suggest that an oxidatively damaged form of guanine, 7,8-dihydro-8-oxoguanine (8-OxoG), is mutagenic in vivo and in vitro (Grollman and Moriya 1993). Oxidatively damaged DNA bases are mostly repaired by the base excision repair pathway (Boiteux 1993; Demple and Harrison 1994). The first step in this ubiquitous repair pathway is the recognition and removal of the altered base by a DNA glycosylase, leaving an abasic site (AP site). Subsequently, the AP site is incised and the repair is completed by phosphodiesterase, DNA polymerase, and DNA ligase (Lindahl 1995).

In *Escherichia coli*, two DNA glycosylases excise oxidized bases in DNA: the Nth protein which releases oxidized pyrimidines and the Fpg protein which releases oxidized purines (Boiteux 1993; Demple and Harrison 1994). The elucidation of the biological role of the Fpg protein in *E. coli* has been facilitated by the cloning of the *fpg* gene and the isolation of Fpg-deficient strains (Boiteux et al. 1987; Boiteux and Huisman 1989). The Fpg protein of *E. coli* is a DNA glycosylase which excises 8-oxopurines and 2,6-diamino-4-hydroxy-5-*N*-methylformamidopyrimidine (Fapy) (Chetsanga and Lindahl 1979; Boiteux et al. 1990, 1992; Tchou et al. 1991). In addition, the Fpg protein is endowed with an enzymatic activity that catalyzes cleavage at AP sites via $\beta$- and $\delta$-elimination reactions (O'Connor and Laval 1989; Bhagwat and Gerlt 1996). Inactivation of the *fpg* (*mut*M) gene of *E. coli* causes a moderate G · C→T · A mutator phenotype (Michaels et al. 1992; Duwat et al. 1995). Furthermore, the *fpg* (*mut*M) *mut*Y double mutant of *E. coli* is a strong mutator which specifically accumulates G · C→T · A transversions (Michaels et al. 1992). The MutY protein is a DNA glycosylase that excises adenine residues placed opposite 8-OxoG or guanine (Michaels and Miller 1992; Tsai-Wu et al. 1992). These results imply that Fpg and MutY proteins act synergistically to prevent mutations generated by endogenous ROS (Michaels and Miller 1992). The current hypothesis proposes that ROS attack DNA, yielding 8-OxoG, which causes mutations if not repaired by the Fpg/MutY repair system (Michaels and Miller 1992; Boiteux and Laval 1996).

The study of repair genes involved in preventing mutagenesis has facilitated the understanding of cancer susceptibility in man. An example of this is the demonstration that inherited susceptibility to heriditary nonpolyposis colon cancer is due to deficiencies in the human homologues of the bacterial mutator genes *mut*S or *mut*L (Kolodner 1995). Therefore, the identification of eukaryotic homologues of mutator genes such as *fpg* and *mut*Y may also provide valuable information concerning the biological impact of oxidative stress on the etiology of degenerative diseases in higher organisms. In *Saccharomyces cerevisiae*, the *OGG1* gene has been cloned by complementation of the mutator phenotype of the *fpg mut*Y mutant of *E. coli*. The *OGG1* gene encodes a protein of 376 amino acids with a molecular weight of 43 kDa. The Ogg1 protein has a DNA glycosylase activity that releases both 8-OxoG and Fapy from DNA (Auffret van der Kemp et al. 1996). Despite the absence of sequence homology, these results suggest that the Ogg1 protein is a functional eukaryotic homologue of the bacterial Fpg protein (Auffret van der Kemp et al. 1996). To assess the biological role of the *OGG1* gene of *S. cerevisiae*, we have constructed *ogg1*-disrupted strains and analyzed their phenotypes. The results show that cell-free extracts of *ogg1*-disrupted strains have no detectable activity that can cleave the 8-OxoG/C duplex. Furthermore, *ogg1*-disrupted strains exhibit a specific G · C→T · A mutator phenotype, thus providing evidence for the occurrence of endogenous oxidative DNA damage in eukaryotes.

## Materials and methods

### Strains, plasmids, enzymes, and microbiological methods

*S. cerevisiae* strains used in this study are listed in Table 1. Yeast strains were grown in standard media at 30° C with agitation. YPD medium (1% yeast extract, 0.5% bacto-peptone, 2% glucose), YPG medium (1% yeast extract, 0.5% bactopeptone, 3% glycerol), and YNBD minimal medium (0.7% yeast nitrogen base without amino acids, 2% glucose) were prepared as described (Cherest and Surdin-Kerjan 1992). Depending on the auxotrophic requirements of yeast strains, adenine (40 μg/ml), histidine (100 μg/ml), leucine (100 μg/ml), lysine (40 μg/ml), uracil or tryptophan (20 μg/ml) were added to minimal medium. Plasmid pYSB10 carrying the *OGG1* gene has been previously described (Auffret van der Kemp et al. 1996). The homogeneous Fpg protein of *E. coli* was purified as previously described (Boiteux et al. 1990). Transformation of *S. cerevisiae* was performed using the acetate lithium treatment (Gietz et al. 1992).

### Recombinant DNA methods

Plasmid purifications were performed as described by Ish-Horowicz and Burke (1981). Southern blot analyses were performed with genomic DNA prepared as described by Hoffman and Winston (1987). Probes were made radioactive by the random priming method (Hodgson and Fisk 1987). PCR amplifications were performed using the *Pfu* DNA polymerase. To disrupt the *OGG1* gene with the *TRP1* gene, the *Eco*RI-*Kpn*I fragment of plasmid pYSB10 was first cloned into pUC18 yielding the plasmid pOGG1-10. Plasmid pOGG1-10 was then digested with *Xho*I, treated with Klenow fragment to blunt the ends, dephosphorylated, and ligated with a blunt-ended *Bgl*II fragment of pFL39 containing the *TRP1*

**Table 1** *Saccharomyces cerevisiae* strains used in this study

| Strain | Genotype | Source |
|---|---|---|
| W303-1A | *MATa*, *ade2*, *his3*, *leu2*, *trp1*, *ura3*, *can1* | R. Rothstein |
| FF18733 | *MATa*, *his7*, *leu2*, *lys1*, *ura3*, *strp1* | F. Fabre |
| CD132 | *MATa*, *ade2*, *his3*, *leu2*, *trp1*, *ura3*, *can1*, *ogg1*::*TRP1* | This study |
| CD138 | *MATa*, *his7*, *leu2*, *lys1*, *ura3*, *ogg1*::*TRP1* | This study |
| YMH1 | *MATa*, *cyh2*, *cyc7*, *leu2*, *ura3* | M. Hampsey |
| YMH (2–7)[a] | *MATa*, *cyh2*, ***cyc1-22***[a], *cyc7*, *leu2*, *ura3* | M. Hampsey |
| YOG1 | *MATa*, *cyh2*, *cyc7*, *leu2*, *ogg1*::*URA3* | This study |
| YOG (2–7)[a] | *MATa*, *cyh2*, ***cyc1-22***[a], *cyc7*, *leu2*, *ogg1*::*URA3* | This study |

[a] Strains YMH2–YMH7 and YOG2–YOG7 differ from each other only by a single base substitution within codon Cys 22 of the *CYC1* gene (Hampsey 1991)

gene (Bonneau et al. 1991). The resulting plasmid was then digested with *Eco*RI and *Kpn*I and the product of the digestion was used to transform yeast wild-type strains. A similar procedure was used to disrupt the *OGG1* gene by *URA3*, except that we used a blunt-ended *Bgl*II fragment of pFL44 carrying the *URA3* gene (Bonneau et al. 1991). Correct disruption events were verified by Southern blot analysis and by PCR amplification of the locus using the oligonucleotides 5′-CACCGTTTTCTCGCGG-3′ and 5′-CTTTC-TCCACAAGGCAT-3′. Restriction endonucleases, DNA polymerases, DNA ligase, and polynucleotide kinase were from commercial sources and used as recommended by the manufacturers.

### Preparation of yeast cell-free extracts

The yeast strains were grown at 30° C in 200 ml of YPD medium until $OD_{600}$ = 1.0. Cells were centrifuged, washed with sterile water, centrifuged again, and pellets were stored at −80° C. For lysis, pellets were supplemented with 2 ml of acid-washed glass beads and 50 µl of phenylmethylsulfonyl fluoride (100 mM stock solution). The mixture was vortexed vigorously 4 times for 1 min each at 4° C. Then, 2 ml of buffer (25 mM TRIS-HCl pH 7.6, 5 mM disodium EDTA, 250 mM NaCl) was added and the mixture was gently vortexed. The lysate was centrifuged at 10 000 rpm for 15 min at 2° C in a JA20 (Beckman) rotor. The resulting supernatant was centrifuged again at 40 000 rpm for 45 min at 2° C in a 50 Ti (Beckman) rotor. The supernatant was the yeast cell-free extract. Protein concentration was determined using the Bradford (1976) method.

### Assay for the repair of 8-OxoG

The 34mer oligonucleotides containing a single 8-oxoG or a G residue at position 16 were synthesized as previously described (Castaing et al. 1993). The sequences used in this study are the following:
Oligo1: 5′-GGCTTCATCGTTATT(*8-OxoG*)ATGACCTGGTG-GATACCG-5′*
Oligo2: 5′-GGCTTCATCGTTATT(*G*)ATGACCTGGTGGATA-CCG-5′*

Complementary sequences with a C, T, G or A opposite 8-OxoG were also synthesized. 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. The 34mer oligonucleotides were labelled at both ends using $\gamma$-[$^{32}$P]ATP and T4 polynucleotide kinase. The $^{32}$P-labelled strand was hybridized with a complementary sequence by heating the mixture at 90° C for 10 min followed by slow cooling to room temperature. The assay mixtures (25 µl final volume) contained 25 mM TRIS-HCl pH 7.6, 2 mM disodium EDTA, 50 mM KCl, 50 fmol $^{32}$P-labelled DNA duplex, and limiting amounts of yeast cell-free extracts or Fpg protein. The reactions were performed at 37° C for 30 min and the products were separated in 20% denaturing polyacrylamide gels containing 7 M urea. The autoradiographs of the gels were scanned and quantitated as previously described (Castaing et al. 1993).

### Survival curves

Yeast strains were grown in YPD medium at 30° C with agitation to a cell density of $10^7$ cells/ml. The cells were harvested by centrifugation, resuspended in the same volume of phosphate-buffered saline (PBS) and exposed to the various treatments. For ultraviolet light irradiation, the cells were exposed for increasing lengths of time to a germicidal lamp with a maximum output at 254 nm at a dose rate of 3 J/m$^2$ per s. For methyl methanesulfonate (MMS) exposure, the cells were treated with increasing concentration of MMS for 20 min at 30° C with agitation. For hydrogen peroxide ($H_2O_2$) treatment, the cells were exposed to increasing concentration of $H_2O_2$ for 20 min at 30° C with agitation. After MMS and $H_2O_2$ treatment, cells were collected by centrifugation and resuspended in fresh PBS. For all three treatments, untreated and treated cell suspensions were diluted and 0.1 ml of the dilutions were spread on YPD plates and colonies were scored after 3 days at 30° C.

### Spontaneous mutation frequencies

Yeast strains were grown in 2 ml of YPD or YNBD medium at 30° C for 2 days to a cell density of approximately $10^8$ cells/ml. Cultures were obtained either by inoculation with a single colony or at a starting density of $10^3$ cells/ml. Cell density was measured by plating dilutions on YPD or YNBD agar plates and counting the colonies after 3 days at 30° C. The quantification of canavanine-resistant mutants (Can$^R$) was determined after plating 0.1 ml of undiluted culture on YNBD plates containing 60 µg/ml of canavanine sulfate (Sigma). For Lys$^+$ revertants, the cultures were concentrated tenfold before plating 0.1 ml on YNBD plates without lysine. The plates were incubated for 3–4 days at 30° C before counting the colonies.

### Base substitution analysis

Yeast strains (YMH1–7) and isogenic *ogg1*::*URA3* derivatives [YOG1–7 (see Table 1)] were grown in 25 ml of YPD medium for 2 days at 30° C. Cultures were concentrated 100-fold and 0.1 ml was plated onto YPG (complete medium containing 3% glycerol) plates and colonies were counted after 10 days at 30° C.

## Results

### Expression of the *OGG1* gene of *S. cerevisiae*

The *OGG1* gene of *S. cerevisiae* expressed in *E. coli* encodes a DNA glycosylase that releases 8-OxoG and Fapy from damaged DNA and cleaves DNA at AP sites via a $\beta$-elimination reaction (Auffret van der Kemp et al. 1996). A northern blot analysis of total RNA extracted from a yeast wild-type strain reveals that a radioactive *ogg1* probe hybridizes with a single transcript of about 2.0 kb, demonstrating that the *OGG1* gene is transcribed in vivo (data not shown). Furthermore, comparison of the signal intensities obtained with *ogg1*- and actin-specific probes suggests that the *OGG1* gene is expressed at a very low level under normal yeast growth conditions. To detect the Ogg1 protein in yeast cell-free extracts, we have used a biochemical assay which relies on the cleavage of a 34mer DNA duplex containing a single 8-OxoG (Castaing et al. 1993; De Oliveira et al. 1994). The 8-OxoG-containing strand was labelled with $^{32}$P at both ends and annealed with a complementary sequence that has one or other of the four DNA bases opposite 8-OxoG, yielding 8-OxoG/N duplexes. Figure 1 shows that cell-free extracts of *S. cerevisiae* possess an enzymatic activity that cleaves the 8-OxoG/C duplex at the position of the 8-OxoG residue. The positions of the bands corresponding to the products generated by cleavage at the 8-OxoG residue by the homogeneous Fpg protein of *E. coli* are indicated (Fig. 1). In contrast, 8-OxoG/T, 8-OxoG/G, 8OxoG/A duplexes and single-stranded 8-OxoG containing DNA are not cleaved at a detectable rate under these assay conditions (Fig. 1).

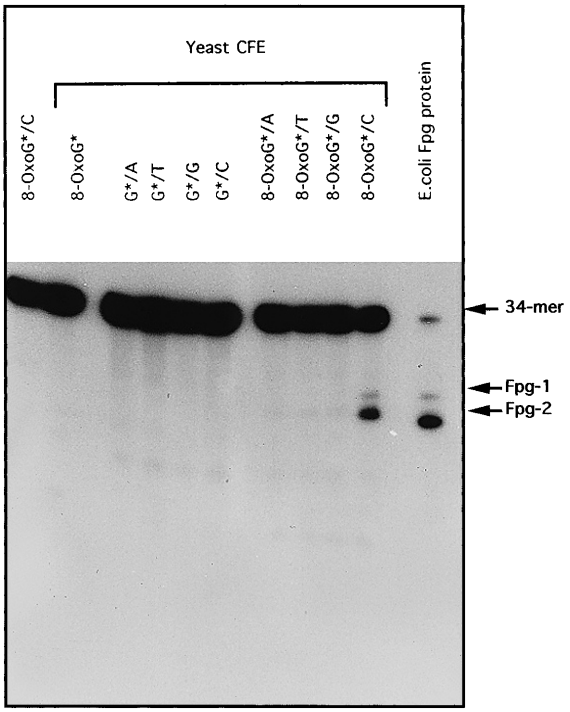


**Fig. 1** Cleavage by cell-free extracts of *Saccharomyces cerevisiae* of DNA duplexes containing a single 7,8-dihydro-8-oxoguanine (8-OxoG) mismatched with one of the four DNA bases. The 8-OxoG- or $G_{16}$-containing strands were labelled with $^{32}P$ (*) and annealed with one of the four complementary sequences carrying A,T,C or G opposite to the 8-OxoG or $G_{16}$. These substrates were incubated with yeast cell-free extracts (CFE) (25 μg protein) and the products of the reaction were analyzed by PAGE in the presence of 7 M urea. Since both ends of the 8-OxoG-containing strand were $^{32}P$-labelled, two labelled fragments are generated after cleavage at 8-OxoG. Control lane shows the cleavage at the 8-OxoG residue by 1 ng of purified Fpg protein of *Escherichia coli*. *Fpg-1* and *Fpg-2* indicate the two products generated by cleavage at 8-OxoG by the Fpg protein (Auffret van der Kemp et al. 1996)

Control experiments show that the four duplexes with a guanine ($G_{16}$) mismatched with one of the four DNA bases, G/N duplexes, are not incised (Fig. 1). Therefore, the substrate specificity of the 8-OxoG repair activity detected in yeast cell-free extracts is very similar to that of the purified Ogg1 protein (Auffret van der Kemp et al. 1996). Furthermore, cell-free extracts of a yeast strain overexpressing the *OGG1* gene from the multicopy plasmid pYSB10, exhibit a tenfold increase in 8-OxoG/C cleavage activity (data not shown). These data suggest that the enzymatic activity that cleaves the 8-OxoG/C duplex in yeast cell-free extracts indeed corresponds to the Ogg1 protein activity encoded by the *OGG1* gene.

## Disruption of the *OGG1* gene of *S. cerevisiae*

To demonstrate that the *OGG1*gene codes for the repair activity detected in yeast cell-free extracts (Fig. 1), we disrupted the chromosomal copy of the *OGG1*gene and measured cleavage of an 8-OxoG/C duplex in extracts of the resulting strain. The *Eco*RI-*Kpn*I fragment of pYSB10 containing the entire *OGG1*gene was subcloned in pUC18 yielding the plasmid pOGG1-10. The [*Xho*I fragment] of the *OGG1* gene in pOGG1-10, specifying codons 130–176, was deleted and replaced by the selectable marker, the *TRP1*gene (Fig. 2). This construct was used to transform haploid yeast wild-type strains. Stable $Trp^+$ transformants were selected. Southern blot analysis of the genomic DNA isolated from one of these transformants was performed to control the disruption of the *OGG1*gene. Digestion of genomic DNA with *Eco*RI, *Kpn*I, and *Eco*RV, generates two fragments of 1.9 kbp and 1.3 kbp in the mutant strain, as expected given the position of the *Eco*RV site within the *TRP1* sequence (Fig. 2B). These data demonstrate that the *OGG1* gene has been disrupted by the expected simple rearrangement event described in Fig. 2A. Similar constructions with the *URA3* gene as selectable marker were obtained and verified. The haploid *ogg1* disruptant grows normally, implying that *OGG1* is not an essential gene.

As shown in Fig. 3, cell-free extracts from the *ogg1*::*TRP1* mutant strain contain no detectable activity that catalyzes the cleavage of the 8-OxoG/C duplex. In contrast, under the same assay conditions, the same amount of protein from the wild-type strain catalyzes

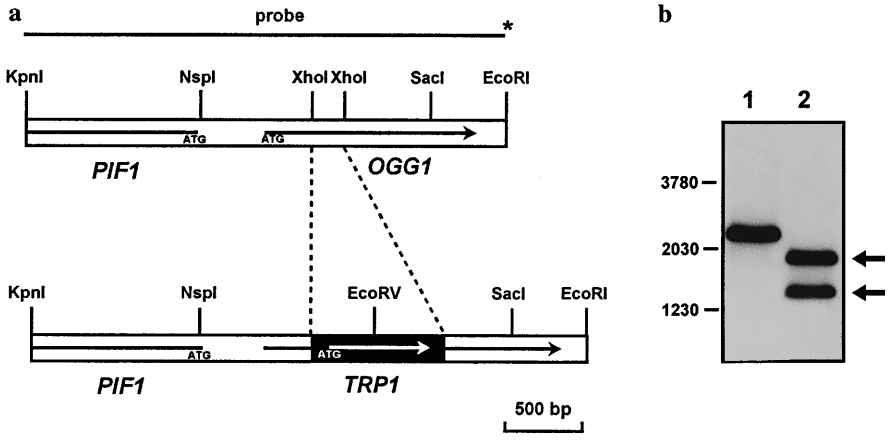


**Fig. 2a, b** Disruption of the *OGG1* gene of *S. cerevisiae*. **a** Physical map of the genomic *OGG1* and *ogg1*::*TRP1* regions. **b** Southern blot analysis of the haploid *OGG1* and *ogg1* disrupted strains. *Eco*RI *Eco*RV *Kpn*I triple digests of genomic DNA extracted from *OGG1* and *ogg1* disrupted strains were probed with the *Kpn*I-*Eco*RI fragment. *Lane 1*, wild-type strain (W303-A). *Lane 2*, *ogg1*::*TRP1*-disrupted strain (CD132)

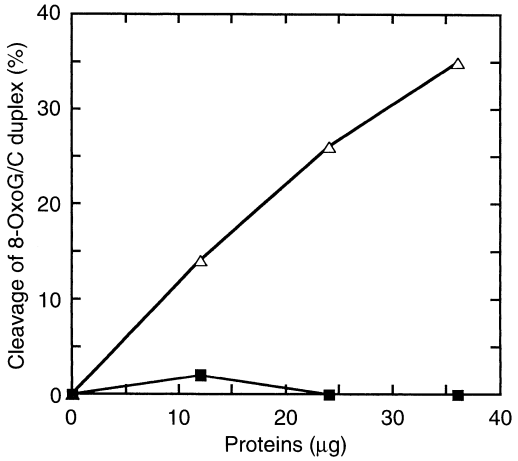


**Fig. 3** Cleavage of the 8-OxoG/C duplex by cell-free extracts of wild-type and Ogg1-deficient strains. Enzymatic activity was measured in cell-free extracts of the FF18733 wild-type (Δ) and CD138 *ogg1*::*TRP1*strains (■). The reactions were carried out at 37° C for 30 min using $^{32}$P-labelled 8-OxoG/C duplex as a substrate (see legend to Fig. 1). Cleavage of the 8-OxoG/C duplex was quantitated by scanning the autoradiographs of the gels

the cleavage of 35% of the 8-OxoG/C duplex present in the reaction mixture (Fig. 3). The introduction of plasmid pYSB10 into the *ogg1*-disrupted strain produced about tenfold more activity than that measured in a wild-type strain (data not shown). These results demonstrate that most, if not all, of the enzymatic activity capable of cleaving the 8-OxoG/C duplex in yeast cell-free extracts corresponds to the Ogg1 protein activity.

## Biological function of the *OGG1* gene of *S. cerevisiae*

The construction of *ogg1*-disrupted strains was an important step toward the understanding of the physiological function of the *OGG1* gene of *S. cerevisiae*. The sensitivity of the *ogg1* strain to three different classes of DNA-damaging agents was investigated. Figure 4 shows that the *ogg1* mutant is not unusually sensitive to the methylating agent, MMS (Fig. 4a), to the oxidizing agent, $H_2O_2$ (Fig. 4b) or to ultraviolet light at 254 nm (Fig. 4c). Thus, the *OGG1* gene appears not to be essential for counteracting the lethal action of the DNA lesions induced by these various treatments.

However, the *OGG1* gene could have a role in the maintenance of genetic stability, as is the case for the bacterial *fpg* gene (Michaels and Miller 1992; Castaing et al. 1993). To test whether the *OGG1* gene product may protect yeast cells from the mutagenic action of endogenous reactive chemicals, we have compared the spontaneous mutation frequencies of an *ogg1*-disrupted strain with an isogenic wild-type strain. Spontaneous mutagenesis was determined as the frequency of appearance of canavanine-resistant mutants or Lys$^+$ revertants in cultures of a *S. cerevisiae* (*lys1-1* Can$^S$) strain grown under normal conditions. Can$^R$ mutants result from any mutation that inactivates the arginine

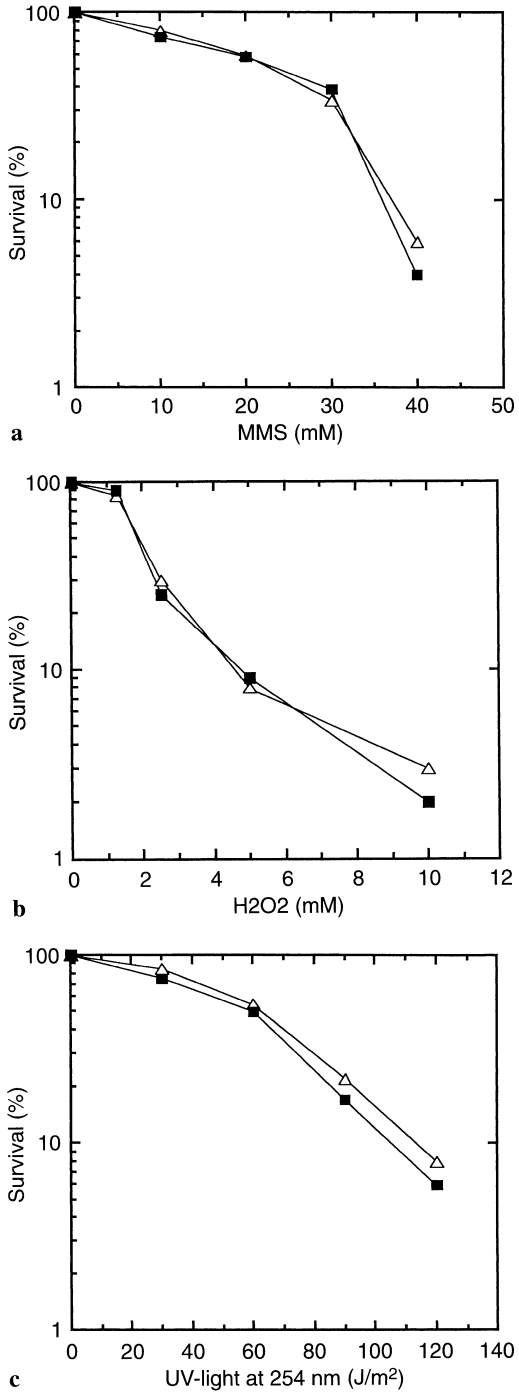


**Fig. 4a–c** Sensitivity of strains FF18733 (*OGG1*, Δ) and CD138 (*ogg1*::*TRP1*, ■) to DNA-damaging agents. **a** Methyl methanesulfonate (*MMS*). **b** Hydrogen peroxide ($H_2O_2$). **c** Ultraviolet (*uv*) light at 254 nm

permease gene. Lys$^+$ revertants of the *lys1-1* ochre allele are due either to intragenic events or to extragenic informational suppressors (Cassier-Chauvat and Fabre 1991; Kunz et al. 1991). The results show that frequencies for both Can$^R$ mutants and Lys$^+$ revertants are significantly higher in the *ogg1* mutant strain than

**Table 2** The *ogg1*::*TRP1* mutant strain of *S. cerevisiae* is a mutator. Values reported for frequencies of canavanine-resistant (*Can*$^R$) mutants and Lys$^+$ revertants are the average of at least ten independent cultures. Plasmid pYSB10 carries the *OGG1* gene of *S. cerevisiae*. (*ND* Not determined)

| Strain | *OGG1* genotype | Can$^R$/10$^7$ | Lys$^+$/10$^7$ |
|---|---|---|---|
| FF18733 | Wild type | 21 +/− 4 | 2 +/− 1 |
| CD138 | *ogg1*::*TRP1* | 148 +/− 29 | 23 +/− 5 |
| Harboring plasmid PYSB10 (*OGG1*) | | | |
| FF18733 | Wild type | 30 +/− 9 | ND |
| CD138 | *ogg1*::*TRP1* | 25 +/− 11 | 3 +/− 2 |

in the wild-type strain (Table 2). The frequencies of Can$^R$ and Lys$^+$ events are 7- and 11-fold higher in *ogg1* than in the wild-type strain, respectively (Table 2). Finally, the mutator phenotype of the *ogg1* mutant is completely suppressed by the expression the *OGG1* gene cloned on plasmid pYSB10 (Table 2). These results show that the *OGG1* gene is a new mutator locus in *S. cerevisiae* that prevents mutations resulting from endogenous DNA damage.

### Specificity of the mutator phenotype of the *ogg1* mutant

The results show that inactivation of the *OGG1* gene of *S. cerevisiae* confers a mutator phenotype. To analyze the specificity of this mutagenesis, we used the reporter system designed by Hampsey (1991), which allows the detection of all possible base pair substitutions. This system relies on a set of isogenic *S. cerevisiae* strains (YMH2–7) that differ by only one single base substitution within the cysteine 22 codon of the *CYC1* gene coding for iso-1-cytochrome *c*. Since the tester strains are also deleted for the *CYC7* gene encoding iso-2-cytochrome *c*, they are not able to grow on a non-fermentable carbon source. The frequency of Cyc$^+$ revertants was measured by plating on agar medium containing glycerol as a unique carbon source. A set of six isogenic *ogg1*::*URA3* strains, (YOG2–7) was constructed (Table 1). The frequency of Cyc$^+$ reversions was measured for the 12 strains that correspond to each of the six possible base substitutions in a wild-type or an *ogg1* genotype (Table 3). Analysis of base pair substitutions in this system shows that, with the exception of G · C → A · T transitions, all reversion events occur at very low frequencies in the wild-type strains (Table 3; Hampsey 1991). Comparison between wild-type and *ogg1* strains reveals a 50-fold excess in G · C→T · A transversion events in the *ogg1*-disrupted strain as compared with the parental strain (Table 3). In contrast, all other base substitutions are observed at similar frequencies in wild-type and *ogg1* strains. These results demonstrate that the Ogg1-deficient strain is a specific G · C→T · A mutator. Together, the substrate specificity of the Ogg1 protein and the mutator specificity of *ogg1*-disrupted strains indicate that transversion mutations result from the formation of 8-OxoG residues in yeast chromosomal DNA.

**Table 3** Specificity of base substitution caused by the disruption of the *OGG1* gene of *S. cerevisiae*. A total of $5 \times 10^8$ cells, determined by plating dilutions on YPD (complete medium containing 2% glucose), was plated on YPG (complete medium containing 3% glycerol) plate and colonies were scored after 10 days at 30° C. Reversion frequencies were normalized for $10^9$ cells. Each value represents the average of three independent experiments

| Base Pair Substitution | Mutation frequency ($\times 10^{-9}$) YMH2–7 (*OGG1*) | YOG2–7 [*ogg1*::*URA3*] | YOG$_n$/YMH$_n$ |
|---|---|---|---|
| G·C→A·T | 69 | 53 | 0.8 |
| A·T→T·A | 3 | < 1 | < 0.3 |
| G·C→T·A | 2 | 105 | 52.5 |
| G·C→C·G | 2 | 3 | 1.5 |
| A·T→C·G | < 1 | < 1 | (1) |
| A·T→G·C | 2 | < 1 | < 0.5 |

## Discussion

The *OGG1* gene of *S. cerevisiae* encodes a DNA glycosylase that excises 8-OxoG a major product of oxidative damage, from DNA, (Auffret van der Kemp et al. 1996). To investigate the biological role of the *OGG1* gene, we have constructed yeast mutants where the *OGG1* gene has been inactivated by deletion of an internal 130-bp fragment and insertion of a selectable gene. Cell-free extracts of the resulting *ogg1* mutant strain do not possess detectable activity catalyzing the cleavage of a 34mer DNA duplex containing a single 8-OxoG placed opposite a cytosine (8-OxoG/C). These results indicate that the Ogg1 protein is the only enzyme that excises 8-OxoG in *S. cerevisiae*. However, the contribution of other repair systems in vivo cannot be formally excluded. For example, the potential action of nucleotide excision repair (NER) cannot be detected in our assay, which uses a short DNA fragment as a substrate in the presence of disodium EDTA.

The Ogg1-deficient strains are not unusually sensitive to chemical methylation, ultraviolet radiation at 254 nm or oxidation by $H_2O_2$. These results imply that the Ogg1 protein does not play an essential role in the removal of DNA lesions that are associated with the lethal action of these treatments in vivo. Similarly, the *fpg* mutant of *E. coli* does not exhibit unusual sensitivity to the same DNA-damaging treatments (Boiteux and Huisman 1989). The lack of sensitivity of the *ogg1* mutant strain may be due to the fact that the Ogg1 protein only repairs non-lethal lesions such as 8-OxoG in DNA (Grollman and Moriya 1993).

We show that the Ogg1-deficient strain is a mutator. The mutator phenotype was revealed using three independent reporter systems, Can$^R$ mutations, Lys$^+$ and Cyc$^+$ reversions. Analysis of the spectrum of base sub-

stitutions with the $Cyc^+$ reversion system shows that the inactivation of the *OGG1* gene specifically enhances the incidence of G · C→T · A transversions by more than 50-fold. This result implies that $Lys^+$ revertants of the *lys1-1* ochre allele are external suppressor mutations, as previously observed for a *rad18* mutant which is also a G · C→T · A mutator (Cassier-Chauvat and Fabre 1991, Kunz et al. 1991). The fact that the *ogg1* strain is a mutator supports the notion that the Ogg1 protein is the major enzymatic activity that excises 8-OxoG from DNA in *S. cerevisiae*. The role of the NER system is not critical, since *ogg1* strains show a mutator phenotype in a NER proficient background. Furthermore, an *ogg1 rad14* double mutant strain does not exhibit a hypermutator phenotype when compared to the *ogg1* single mutant strain (our unpublished results). Taken together, these results demonstrate that the biological function of the Ogg1 protein is required to repair endogenous mutagenic DNA damage, probably 8-OxoG, thus preventing G · C→T · A transversions.

These results and those previously reported by Auffret van der Kemp et al. (1996), establish that the *OGG1* gene from *S. cerevisiae* is indeed the functional homologue of the bacterial *fpg* gene, although no significant sequence similarities could be detected between their encoded products. In fact, the *OGG1* gene appears to be related to the endonuclease III gene of *E. coli*, nth (Nash et al. 1996). In *E. coli*, the Fpg and MutY proteins form a DNA repair system that prevents mutagenesis induced by the presence of 8-OxoG in DNA: the Fpg protein catalyzes the excision of 8-OxoG from DNA and the MutY protein is a DNA glycosylase that removes adenine residues paired with 8-OxoG (Michaels and Miller 1992; Boiteux and Laval 1996). If a similar system exists in *S. cerevisiae*, it is likely that the *E. coli* MutY protein and the yeast MutY functional homologue are highly divergent or unrelated enzymes, as is the case for the Ogg1 and Fpg proteins, since no MutY sequence homologue could be identified within the *S. cerevisiae* genome, whose sequence is now entirely established. In human cells, 8-OxoG is released from DNA by a DNA glycosylase activity (Bessho et al. 1993) and a human homologue of the *mutY* gene of *E. coli* has been cloned (Slupska et al. 1996). In conclusion, these data indicate that enzymatic functions responsible for the repair of 8-OxoG lesions in DNA are highly conserved in bacteria, yeast, and man.

One might imagine that eukaryotic genomes would be less prone to endogenous reactive chemicals because of their cellular compartmentation. The nuclear membrane should protect genomic DNA from the cytoplasm where the majority of cellular reactions take place. However, the fact that yeast cells lacking the Ogg1 protein display a mutator phenotype demonstrates that the chromosomal DNA is a target for endogenous ROS in eukaryotes, supporting the hypothesis that associates oxidative stress with degenerative diseases in humans. Finally, by analogy with studies showing that mutator genes correlate with cancer predisposition (Kolodner 1995), a defect in the human homologue of the yeast *OGG1* gene could provoke susceptibility to spontaneous or oxidative stress-induced cancer in humans.

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