Repair of oxidatively damaged guanine in *Saccharomyces cerevisiae* by an alternative pathway

Steven D. Bruner*, Huw M. Nash*, William S. Lane† and Gregory L. Verdine*

Background: Transversion mutations are caused by 8-oxoguanine (^{O}G), a DNA lesion produced by the spontaneous oxidation of guanine nucleotides, which mis-pairs with adenine during replication. Resistance to this mutagenic threat is mediated by the GO system, the components of which are functionally conserved in bacteria and mammals. To date, only one of three GO system components has been identified in the budding yeast *Saccharomyces cerevisiae*, namely the ^{O}G:C-specific glycosylase/lyase yOgg1. Furthermore, *S. cerevisiae* has been reported to contain a unique glycosylase/lyase activity, yOgg2, which excises ^{O}G residues opposite adenines. Paradoxically, according to the currently accepted model, yOgg2 activity should increase the mutagenicity of ^{O}G lesions. Here we report the isolation of yOgg2 and the elucidation of its role in oxidative mutagenesis.

Results: Borohydride-dependent cross-linking using an ^{O}G-containing oligonucleotide substrate led to the isolation of yOgg1 and a second protein, Ntg1, which had previously been shown to process oxidized pyrimidines in DNA. We demonstrate that Ntg1 has ^{O}G-specific glycosylase/lyase activity indistinguishable from that of yOgg2. Targeted disruption of the *NTG1* gene resulted in complete loss of yOgg2 activity and yeast lacking *NTG1* had an elevated rate of A:T to C:G transversions.

Conclusions: The Ntg1 and yOgg2 activities are encoded by a single gene. We propose that yOgg2 has evolved to process ^{O}G:A mis-pairs that have arisen through mis-incorporation of 8-oxo-dGTP during replication. Thus, the GO system in *S. cerevisiae* is fundamentally distinct from that in bacteria and mammals.

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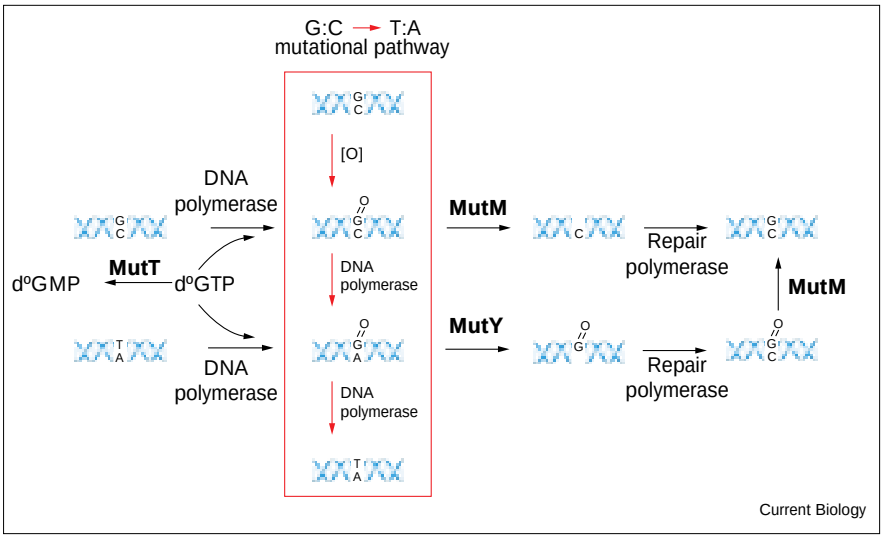
Background

Spontaneous oxidation of guanine nucleotides produces 8-oxoguanine (^{O}G), a lesion that mis-pairs with adenine during replication, thereby causing guanine : cytosine (G:C) to thymine : adenine (T:A) and A:T to C:G transversion mutations. Resistance to the mutagenic effects of ^{O}G is conferred by the GO system [1–3], the bacterial version of which comprises three protein components: MutM, a DNA glycosylase/lyase that recognizes ^{O}G:C and catalyzes excision of the ^{O}G; MutY, a DNA glycosylase that recognizes ^{O}G:A and catalyzes excision of the A; and MutT, a triphosphatase that sanitizes the nucleotide precursor pool of 8-oxo-dGTP (d^{O}GTP) by catalyzing its hydrolysis to d^{O}GMP and inorganic pyrophosphate. Human homologs of *MutT* and *MutY* have been cloned but, curiously, no mammalian protein having significant sequence similarity to MutM has been discovered to date. Instead, mammalian cells contain a protein, Ogg1, which functions as an 8-oxoguanine glycosylase/lyase but has no obvious structural similarity to MutM [4–9]. Thus, the components that make up the GO system are functionally, if not structurally, conserved in bacteria and mammals.

Circumstantial evidence suggests that the GO system in the budding yeast *Saccharomyces cerevisiae* may be configured differently from that in bacteria and mammals. Although a protein having structural and functional homology to mammalian Ogg1 has been cloned from *S. cerevisiae* (yOgg1) [10,11], no homologs of *MutT* or *MutY* are evident in its genomic sequence. Furthermore, *S. cerevisiae* appears to contain a second 8-oxoguanine DNA glycosylase activity, yOgg2, which is distinct from yOgg1 in its substrate specificity [10,12]. Namely, whereas yOgg1 selectively excises ^{O}G paired to C or (to a lesser extent) to T, yOgg2 has relatively little selectivity for the base paired to ^{O}G, and actually shows a slight preference for ^{O}G:A and ^{O}G:G mis-pairs. The potent activity of yOgg2 on ^{O}G:A mis-pairs presents a paradox, as such an activity would be expected to increase rather than decrease the mutagenicity of guanine oxidation (Figure 1). Indeed, this very reason has been widely invoked to explain the high discrimination of MutM and Ogg1 proteins against ^{O}G:A [4–6,8,9,13,14]. Interestingly, no protein having yOgg2-like activity has been observed in bacteria or in mammalian cells. Because *S. cerevisiae* is commonly used as a

Figure 1



Classical GO system (adapted from [1,2]). The pathway of ^{O}G-induced mutation of G:C to T:A is organized vertically, and is denoted by a red box; the ^{O}G repair/resistance pathway is organized horizontally. The three enzymatic components of the GO system are shown in bold; MutM is found in bacteria, but is replaced in mammalian cells by the functional homologue Ogg1.

model genetic system for the study of mutagenesis arising from ^{O}G-generators such as reactive oxygen species and ionizing radiation, it is important to elucidate the mechanisms that exist in yeast to counter this guanine oxidation. Progress on this front has been frustrated by the fact that only one component of the yeast GO system, yOgg1, has thus far been cloned.

Here we report the isolation, cloning and functional characterization of yOgg2. Tryptic peptides derived from yOgg2 were identical in sequence to segments of the recently discovered DNA glycosylase/lyase Ntg1 (also known as Scr1) [15,16]. This protein was previously assigned as the yeast ortholog of *Escherichia coli* endonuclease III, on the basis of sequence similarity and its ability to act on thymine glycol lesions in DNA, which result from pyrimidine-oxidising agents [15,16]. We confirm that recombinant Ntg1/yOgg2 does indeed nick DNA at thymine glycol lesions, but also show that it efficiently excises ^{O}G opposite A; endonuclease III does not exhibit this latter activity. Targeted disruption of the *NTG1* gene resulted in loss of ^{O}G:A-specific glycosylase/lyase activity, consistent with this gene encoding the yOgg2 protein. Yeast disrupted in the gene encoding the Ntg1/yOgg2 activity had an approximately 10-fold increase in the rate of A:T to C:G transversion mutations. As previously reported, disruption of *yOGG1* results in a G:C to T:A mutator phenotype; further disruption of *NTG1/yOGG2* in yeast disrupted for *yOgg1* diminished the rate of G:C to T:A transversions by 2.5-fold. These data suggest that the yOgg2 protein acts to protect yeast from ^{O}G residues that arise by replicative incorporation of d^{O}GTP rather than by oxidation of G residues in the DNA. The existence of such an alternative activity in yeast, together with the lack of MutY and MutT activities in this organism, indicates that the GO system in *S. cerevisiae* is configured in a way that is distinctly different from the corresponding system in bacteria and mammals.

Results

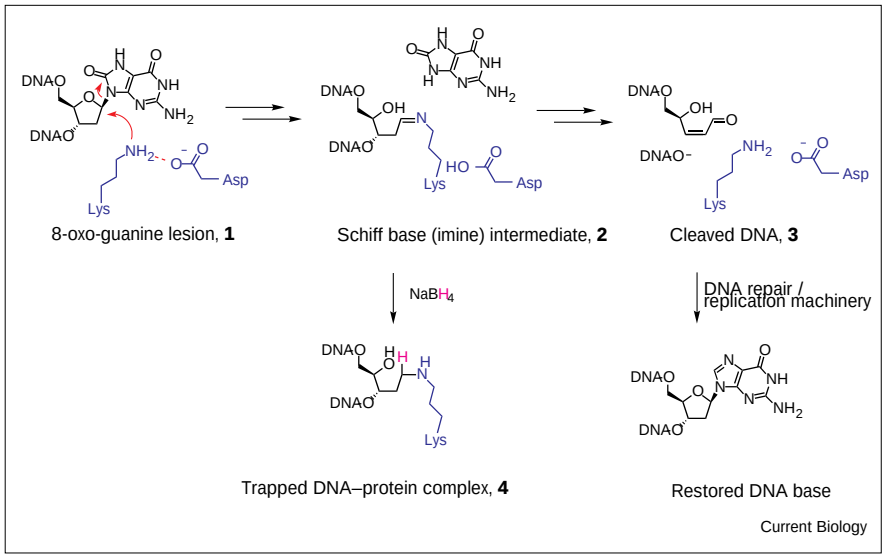
Isolation of yOgg2 using the borohydride trapping assay

We have used the borohydride trapping assay [17] to isolate yOgg2 and to detect and characterize DNA glycosylase/lyase activity throughout this study. This assay relies on the fact that all known DNA glycosylase/lyases, irrespective of their structure, use an enzyme-derived amine nucleophile to expel the aberrant base. This results in a Schiff-base intermediate which can be intercepted by borohydride, resulting in irreversible cross-linking (trapping) of the enzyme to DNA (Figure 2). Borohydride trapping assays using a 49 base pair ^{O}G:G-containing oligonucleotide substrate were used to detect yOgg2 activity in whole yeast cell extracts and to guide partial purification by batch diethylaminoethyl–cellulose (DEAE–cellulose) and BioRex70 column chromatography. Our strategy for isolation of yOgg2 relied on the fact that covalent attachment of the DNA substrate to the enzyme increases its net negative charge, thereby retarding its mobility on an anion-exchange column.

In the first step of a two-step sequence, extracts containing yOgg2 were passed over Q_{ff} Sepharose (a strong anion-exchange chromatography resin). Fractions highly enriched in yOgg2 were subjected to borohydride trapping with the DNA substrate, and the reaction mixture was again passed over Q_{ff} Sepharose. The covalent yOgg2–DNA complex was found to elute at a position in the chromatogram which had been stripped of proteins by

Figure 2

Mechanism of ^{O}G repair initiated by eukaryotic 8-oxoguanine DNA glycosylase/lyases (Ogg proteins). The Schiff base intermediate formed during processing of a ^{O}G substrate by the enzyme can be intercepted by borohydride, thereby resulting in irreversible cross-linking (trapping) of the enzyme to DNA. The covalent enzyme–substrate intermediate that is formed initially (not shown) spontaneously rearranges to a ring-opened species, **2**, having a Schiff base (imine) linkage. In the normal catalytic cycle, intermediate **2** undergoes a series of further reactions, ultimately resulting in cleavage of the DNA backbone and release of the free enzyme. When this reaction is carried out in the presence of sodium borohydride, however, intermediate **2** is intercepted and reduced to yield **4**, a dead-end product that contains the protein irreversibly cross-linked to its substrate. Key catalytic residues of the enzyme are shown in purple. The hydride moiety donated by borohydride is shown in pink.

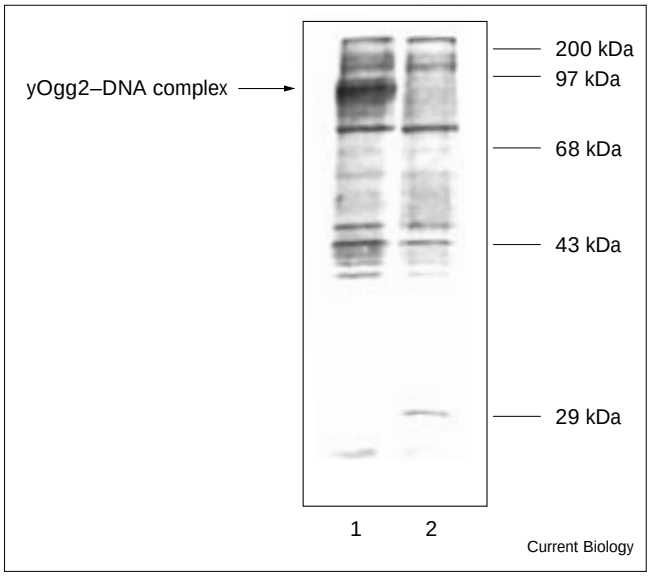


the first Q_{ff} Sepharose step, thereby providing the trapped complex at a high purity. Analysis of these fractions by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) revealed a broad major band with an apparent mobility in the range of about 80 to 90 kDa (Figure 3, lane 1). To determine whether this band contained DNA, we subjected an aliquot of the same material to digestion by *Serratia* endonuclease, a powerful non-specific DNA-cleaving enzyme, and found that the band was selectively removed (Figure 3, lane 2). The remainder of the trapped protein–DNA complex was loaded onto a single lane of an SDS-containing polyacrylamide gel, and the Coomassie-stained 80–90 kDa band was excised from the gel. Tryptic peptides obtained by in-gel digestion were then isolated and sequenced by capillary liquid chromatography/ion trap mass spectrometry. Of the peptide sequences thus obtained, the majority (12 out of 16) were identical to sections of the yOgg1 sequence, and the minority (4 out of 16) were identical to segments of the Ntg1 protein (encoded by the yeast open reading frame YAB5_YEAST, accession number 31378) [15,16]. These data could be explained by our isolation procedure having yielded a mixture of yOgg1 and yOgg2 trapped complexes: even though yOgg2 had a greater specificity than yOgg1 for the ^{O}G:G oligonucleotide used in the affinity tagging step, more yOgg1 appeared to be present in the trapped complex than Ntg1/yOgg2; this may have resulted from the relative instability of Ntg1/yOgg2 compared to yOgg1 (S.D.B., unpublished observations), and from the high concentrations of substrate DNA and extended reaction times used in preparative trapping experiments. The sequence information suggesting that yOgg2 is identical to Ntg1 was unexpected, as Ntg1 had been reported not to possess ^{O}G glycosylase activity [15].

Biochemical characterization of recombinant yOgg2

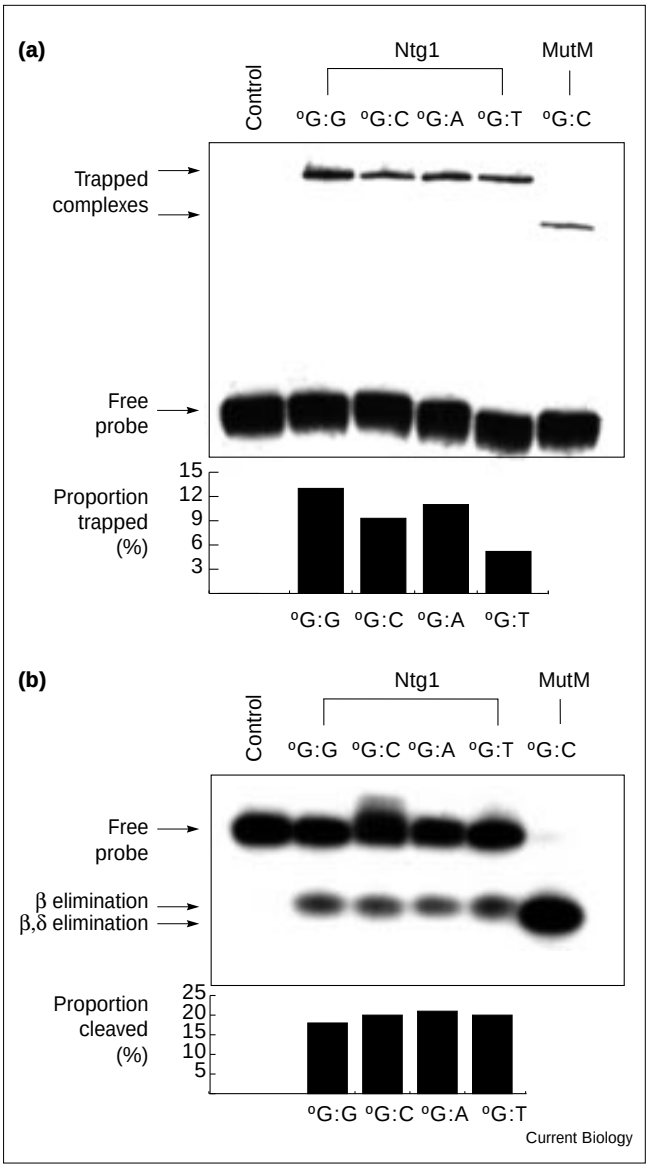
To verify that the Ntg1 protein possesses the enzymatic activity characteristic of yOgg2, we overproduced the protein in *E. coli* with a six histidine (His_6) amino-terminal tag and purified it by adsorption to and refolding on a Ni^{2+}–NTA matrix. In DNA trapping assays with a panel of ^{O}G:N substrates (in which N = A, C, G, or T), the His_6 fusion protein expressed by *E. coli* was found to trap all

Figure 3



SDS–PAGE of late-eluting Q_{ff} Sepharose fractions containing the borohydride-trapped yOgg2–DNA complex, before (lane 1) and after (lane 2) treatment with *Serratia* endonuclease. Note that the major ~80–90 kDa band in lane 1 is no longer evident in lane 2.

Figure 4



Borohydride trapping (a) and DNA cleavage (b) assays using the indicated ^{O}G:N-containing DNA substrates (probes) with recombinant His_6-tagged Ntg1 or *E. coli* MutM. The ^{O}G-containing strand was radiolabeled in these experiments and the complementary strand was unlabeled. Control denotes probe not exposed to a glycosylase. The proportion of added substrate trapped or cleaved is also represented as histograms for experiments using Ntg1. (b) Note that the Ntg1 and MutM DNA cleavage activities can be distinguished as they yield different elimination products.

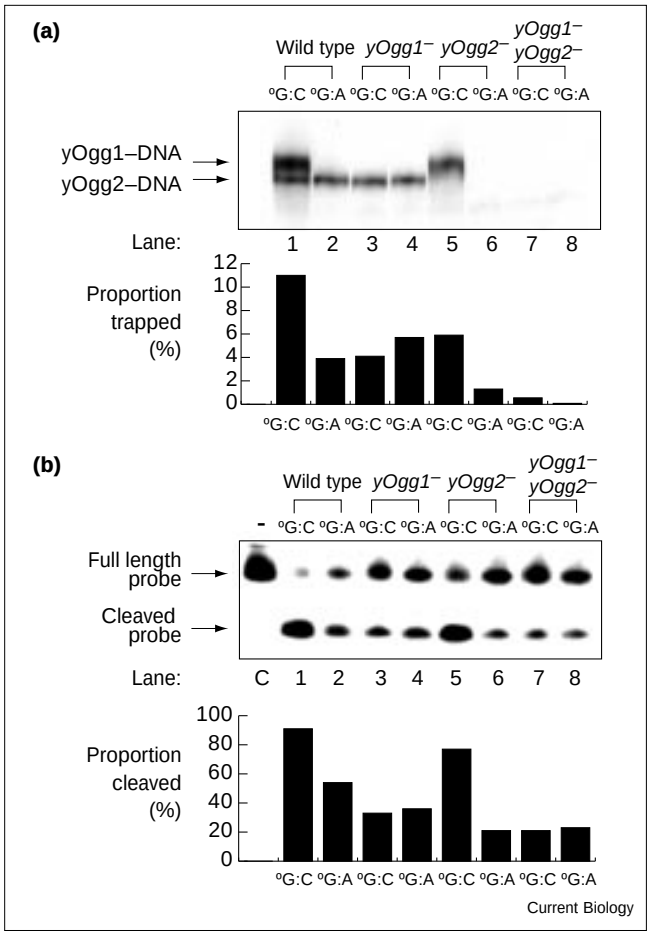
four substrates efficiently, but showed a slight preference for ^{O}G:G and ^{O}G:A over ^{O}G:T and ^{O}G:C (Figure 4a). The recombinant protein also cleaved all four substrates with roughly equal efficiency (Figure 4b). Both the cleavage and trapping specificities of the recombinant protein for ^{O}G:N substrates were essentially identical to those we have determined previously for yOgg2 in whole cell extracts [10]. Importantly, the yOgg2 activity found for the recombinant protein was unambiguously distinguished from the endogenous *E. coli* MutM protein by differences in the products of DNA cleavage; yOgg2 performs a β-elimination reaction and MutM performs a β,δ-elimination reaction. Moreover, the size of the trapped complex resulting from borohydride trapping of MutM was clearly distinguishable from that observed with the recombinant yeast protein. These data indicate that recombinant His_6-tagged Ntg1 protein bears the biochemical hallmarks of yOgg2 activity.

Targeted disruption of the gene encoding Ntg1/yOgg2 in *S. cerevisiae*

If Ntg1 and yOgg2 are encoded by the same gene, targeted disruption of *NTG1* should result in loss of yOgg2 activity. As we have previously shown [10], the covalent complexes formed in trapping assays between DNA and yOgg1 or yOgg2 have different mobilities in acrylamide gels, with the yOgg2 complex having slightly lower apparent molecular weight and yielding a sharper band than the yOgg1 complex (Figure 5a, lane 1). Consistent with this assignment, the upper band (yOgg1) trapped ^{O}G:C but not ^{O}G:A, whereas the lower band (yOgg2) trapped ^{O}G:C and ^{O}G:A with roughly equal efficiency (Figure 5a, lanes 1,2). Targeted disruption of *yOGG1* results in selective loss of the upper band, while the lower band remains unaffected (Figure 5a, lanes 3, 4) [10]. On the other hand, targeted disruption of the *NTG1* gene results in complete loss of yOgg2 trapping activity, while leaving yOgg1 activity undisturbed (Figure 5a, lanes 5,6). These data establish conclusively that yOgg2 activity is dependent upon the presence of the *NTG1* gene and we conclude that the Ntg1 and Ogg2 activities are encoded by the same gene (hereafter referred to as the *NTG1/yOGG2* gene). Targeted disruption of both the genes encoding yOgg1 and Ntg1/yOgg2 resulted in a virtually complete loss of ^{O}G-specific trapping activity (Figure 5a, lanes 7,8), indicating that these two proteins are the only glycosylase/lyases that play a major role in repair of ^{O}G lesions in yeast.

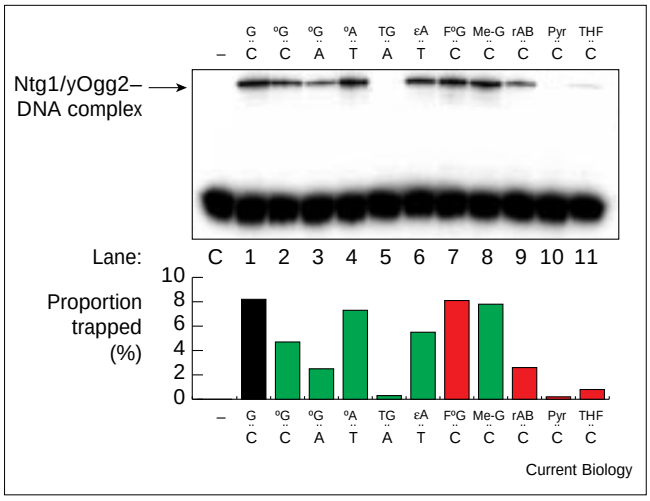
We also characterized *yOgg1*$^{-}$ and *Ntg1/yOgg2*$^{-}$ yeast cells for the ability to catalyze cleavage of ^{O}G residues paired to C and A. As we have shown previously, loss of yOgg1 activity results in a substantial decrease in ^{O}G:C-specific DNA cleavage, but only a slight change in the ^{O}G:A-specific processing (Figure 5b, compare lane 3 with lane 1 and lane 4 with lane 2) [10]. Conversely, loss of yOgg2 activity diminished ^{O}G:C-specific cleavage activity only slightly (Figure 5b, compare lane 5 with 1), but reduced ^{O}G:A cleavage activity substantially (Figure 5b, compare lane 6 with 8). Interestingly, yeast cells lacking both yOgg1 and Ntg1/yOgg2 still appeared to retain some ^{O}G-specific processing activity, which did not distinguish between ^{O}G:C and ^{O}G:A (Figure 5b, lanes 7,8). As no ^{O}G cleavage band is seen in the starting DNA control (Figure 5b, lane C), the

Figure 5



Borohydride trapping (a) and DNA cleavage (b) assays were performed as for Figure 4 using extracts from wild-type *S. cerevisiae* or yeast strains containing a disrupted *yOGG1* gene (*yOgg1*–), a disrupted *NTG1/yOGG2 gene* (*yOgg2*–) or both disrupted genes (*yOgg1*– *yOgg2*–). The control (C) in (b) contained the ^{O}G:A substrate but no yeast extract.

Figure 6



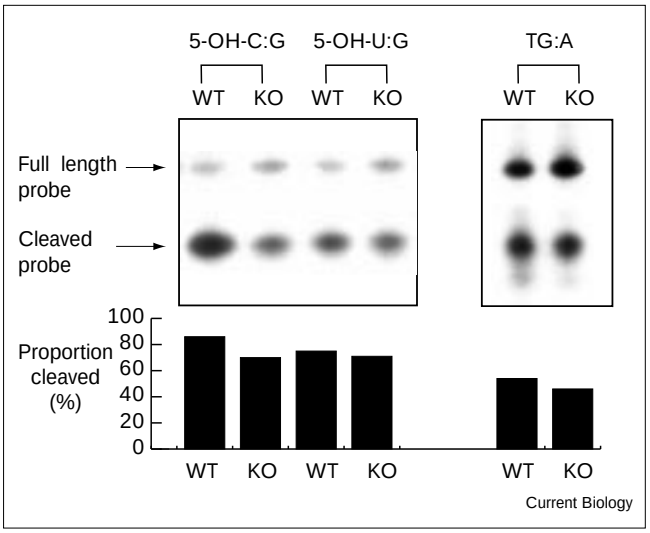
Competition trapping assays. A 100-fold molar excess of each unlabeled competitor oligonucleotide containing the potential yOgg2-interacting lesion indicated was added to trapping assays containing a radiolabeled ^{O}G:A-containing duplex substrate (only ^{O}G strand labeled). ^{O}A, 8-oxoadenine; TG, thymine glycol; εA, N1,N6-ethenoadenine; F^{O}G, 8-oxo-2′-flouro-2′-deoxyguanosine; Me-G, N7-methylguanine; rAB, reduced abasic site; Pyr, pyrrolidine transition-state analog; THF, tetrahydrofuran abasic site analog. Histograms are colored as follows: black, unmodified control; green, potential substrates; red, potential inhibitors. The control (C) included the radiolabeled substrate but no yeast extract and no competing oligonucleotide.

DNA-cleaving activity is clearly attributable to the cell extracts. These results suggest that *S. cerevisiae* may contain a monofunctional glycosylase capable of processing ^{O}G, especially under conditions in which yOgg1 and Ntg1/yOgg2 are lost.

The substrate specificity of Ntg1/yOgg2

Ntg1 was originally characterized on the basis of its ability to nick OsO_4-treated DNA, a property like that of *E. coli* endonuclease III [15]. Ntg1 has also been shown to process the defined endonuclease III substrate dihydrouracil [16]. Unlike endonuclease III, however, Ntg1 catalyzes efficient base excision on N7-methylated formamidopyrimidine (ring-opened N7-methylguanine) lesions, an activity usually associated with 8-oxoguanine DNA glycosylases, including yOgg1 [11,18]. Surprisingly, however, Ntg1 has been reported not to process any of the four ^{O}G:N lesions [15], a finding that is inconsistent with those reported here. To investigate further the substrate specificity of Ntg1/yOgg2, we examined the ability of various unlabeled lesion-containing oligonucleotides to compete against borohydride trapping of recombinant Ntg1/yOgg2 with a radiolabeled ^{O}G:A substrate (Figure 6). Among the potential substrates examined (Figure 6, green), thymine glycol was clearly the most effective competitor (Figure 6, lane 5), suggesting this is the most efficient substrate for Ntg1/yOgg2. ^{O}G:A also competed effectively against trapping by the enzyme (Figure 6, lane 3), however, and so did ^{O}G:C, though to a lesser extent (Figure 6, lane 2). The alternative oxidized purine 8-oxoadenine failed to compete for trapping (Figure 6, lane 4), as did the alkylated lesions N1,N^{6}-ethenoadenine and 7-methylguanine (Figure 6, lanes 6,8, respectively). A series of potential inhibitors that mimic intermediates in the glycolase/lyase catalysis pathway were also tested for their ability to compete against substrate trapping by Ntg1/yOgg2 (Figure 6, red); of these, the pyrrolidine and tetrahydrofuran abasic site analogs competed most strongly. The substrate analog 8-oxo-2′-flouro-2′-deoxyguanosine paired to C failed to inhibit Ntg1/yOgg2 (Figure 6), just as this oligonucleotide failed to inhibit any Ogg1 ortholog [4,10] or MutM (T. Kawate and G.L.V., unpublished observations).

Figure 7



DNA cleavage assays of whole-cell extracts from wild-type (WT) *S. cerevisiae* and 'knockout' cells bearing a targeted deletion of *NTG1/yOGG2* (KO). The radiolabeled DNA substrates contained a single 5-hydroxycytosine opposite G (5-OH-C:G), 5-hydroxyuracil opposite G (5-OH-U:G), or thymine glycol opposite A (TG:A).

Additional endonuclease-III-like activity in *S. cerevisiae*

To assess the impact of deleting *NTG1/yOGG2* on the competence of yeast to excise oxidatively damaged pyrimidines, we carried out DNA-cleavage assays on whole-cell extracts of wild-type and *Ntg1/yOgg2*$^-$ yeast (Figure 7). The DNA substrates for these assays were duplex oligonucleotides bearing a single oxidatively damaged pyrimidine: 5-hydroxycytosine, 5-hydroxyuracil, or thymine glycol. Although the capacity of yeast to repair all three lesions was diminished by deletion of *NTG1/yOGG2*, the 'knockout' cells nonetheless maintained a robust DNA-nicking activity in each case. Thus, it appears that yeast contain additional repair activities other than Ntg1/yOgg2 capable of handling oxidized pyrimidines efficiently.

The role of Ntg1/yOgg2 in the prevention of oxidative mutagenesis *in vivo*

The current model for replication and repair of oxidative DNA damage suggests that any repair protein that excises the ^{O}G residue in an ^{O}G:A base pair, such as Ntg1/yOgg2, should increase the rate of G:C to T:A transversion mutations (Figure 1). Were this the case, then *Ntg1/yOgg2*$^-$ yeast should acquire G:C to T:A transversions less frequently than wild-type yeast. To determine whether this is indeed the case, we used the canavanine reversion assay, which scores for resistance to the arginine analog canavanine, acquired through mutational disruption of the arginine permease gene [19]. For purposes of comparison, we also examined the mutation rate of *yOgg1*$^-$ yeast and yeast disrupted for both *yOGG1* and *NTG1/yOGG2*. Remarkably, loss of Ntg1/yOgg2 did not decrease the frequency of resistance to canavanine, but rather increased it by two-fold (Table 1). As previously reported, canavanine reversion is increased 12-fold by loss of yOgg1 activity. Yeast having disruption of both yOgg1 and Ntg1/yOgg2 functions showed no greater rate of canavanine reversion than cells having lost yOgg1 alone.

Table 1

Canavanine reversion assay.

Yeast strain	Number of colonies per 10^8 cells
Wild type	50
Ntg1/yOgg2$^-$	100
yOgg1$^-$	600
yOgg1$^-$ *Ntg1/yOgg2*$^-$	600

To characterize the nature of the mutations that result from loss of Ntg1/yOgg2 function, we used the iso-cytochrome c reversion assay, which scores for the ability of cells to grow on glycerol due to mutagenic restoration of a codon encoding a key Cys residue in iso-cytochrome c [20]. Loss of yOgg2 function resulted in a significant increase in the rate of A:T to C:G transversion mutations (Table 2). Paradoxically, the rate of G:C to A:T transitions actually decreased in *Ntg1/yOgg2*$^-$ yeast. Consistent with previous observations, *yOgg1*$^-$ yeast exhibited a substantial (~25-fold) increase in the rate of G:C to T:A transversions [21], but for unknown reasons also show a nearly 10-fold elevation in the rate of G:C to A:T transitions. Disruption of both *NTG1/yOGG2* and *yOGG1* significantly decreased the mutagenic effects of disrupting

Table 2

Iso-cytochrome c reversion assay.

	Yeast strain			
Mutation	WT	*yOgg2*$^-$	*yOgg1*$^-$	*yOgg1*$^-$ *yOgg2*$^-$
A:T→C:G	< 1	**10**	< 1	< 1
G:C→T:A	2	< 2	**50**	**20**
A:T→G:C	1	1	1	1
G:C→A:T	**14**	< 1	**130**	**50**
G:C→C:G	< 1	< 1	< 1	**4**
A:T→T:A	< 1	< 1	< 1	< 1

The number of revertant colonies per 10^8 cells is shown for wild-type (WT) and single and double gene knockout yeast strains. Each of the six tester strains used in these assays contains a different mutation in codon 22 (wild-type Cys) of the iso-cytochrome c gene, which must undergo a specific reversion mutation in order to restore the function of this essential protein. Thus each of the six possible mutational pathways can be scored by survival of the corresponding tester strain [20]. Numbers in bold indicate significant increases in the mutation rate.

yOGG1 alone, both with respect to G:C to T:A transversions and G:C to A:T transitions (Table 2). The double-knockout cells also had a slightly increased rate of G:C to C:G transversions, which was not seen in either single-knockout strain.

Discussion

S. cerevisiae 8-oxoguanine DNA glycosylase activities

The first ^{O}G repair activity reported in *S. cerevisiae* had the paradoxical property of being specific for ^{O}G residues opposite G residues [12]. It was not determined whether the activity used a glycolytic or endonucleolytic mechanism; indeed, an earlier report had indicated the existence of ^{O}G-specific human repair enzymes that used either mechanism [22]. The existence of 8-oxoguanine DNA glycosylase/lyase activity was ultimately established by the isolation, cloning and biochemical characterization of yOgg1 [10,11]. ^{O}G:G was found to be a very poor substrate for yOgg1, however, suggesting the existence of additional ^{O}G repair activities in *S. cerevisiae* that have distinct specificities from yOgg1. Definitive evidence in favor of this hypothesis was first gained when we used borohydride trapping assays on cell extracts from *yOgg1*$^{-}$ yeast, which clearly revealed the existence of a second ^{O}G-specific glycosylase/lyase, which we designated yOgg2 [10]. Unlike yOgg1, yOgg2 had only a modest specificity for the base opposite ^{O}G, and actually preferred ^{O}G:A and ^{O}G:G over ^{O}G:C and ^{O}G:T [10]. Here we have reported the molecular characterization of yOgg2.

Isolation of yOgg2 by affinity tagging

We initially identified yOgg2 by its irreversible cross-linking to ^{O}G-containing substrates in the presence of sodium borohydride — an assay that capitalizes upon the covalent catalysis mechanism employed by glycolase/lyases [10]. This borohydride trapping procedure has not only been used to characterize DNA glycosylase/lyases, but has also proven an invaluable guide to the isolation of eukaryotic glycosylase/lyases by providing definitive evidence for which band on an SDS-containing polyacrylamide gel corresponds to the repair enzyme of interest [10,23]. To our knowledge, however, this paper reports the first instance in which borohydride trapping *per se* has been used to purify a glycosylase/lyase. We have taken advantage of the fact that attachment of a polyanionic oligonucleotide to yOgg2 increases its retention on an anion-exchange chromatography column, relative to that of the free enzyme. In the first step of a two-step procedure, we enriched yeast extracts for yOgg2 and at the same time cleansed them of all late-eluting material by passage through Q_{ff} Sepharose. Trapping of yOgg2 to DNA then shifted the mobility of the protein to a portion of the chromatogram that had been cleansed in the first step, thus affording substantial purification of the protein as a covalent complex with its DNA substrate. This affinity tagging strategy is likely to be useful in purification of other DNA-binding proteins, including processing enzymes such as methyltransferases and glycosylase/lyases.

Ntg1, the yeast ortholog of endonuclease III, possesses 8-oxoguanine DNA glycosylase/lyase activity

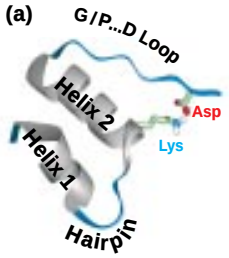
The protein–DNA complex that was isolated using the borohydride trapping procedure was found to contain two yeast polypeptides, one of which was yOgg1, and the other of which was the Ntg1 protein, previously identified as a *S. cerevisiae* ortholog of *E. coli* endonuclease III. Ntg1 has previously been shown to nick DNA-containing lesions induced by oxidation and ionizing radiation [15], and to catalyze base excision on DNA substrates containing defined dihydrouracil residues [16] and N-formamidopyrimidine (FaPy) lesions [15]. Ntg1 was reported not to act on synthetic oligodeoxynucleotides containing ^{O}G opposite any of the four complementary bases [15]. The lack of ^{O}G glycosylase activity in Ntg1 was perplexing, because all other known base-excision DNA repair proteins that act on FaPy lesions also repair the structurally similar ^{O}G adduct [6,11,13].

Having isolated Ntg1 on the basis of its borohydride trapping to an ^{O}G-containing substrate, we thus reinvestigated the issue of whether this polypeptide contains an intrinsic G^{O} glycosylase/lyase activity. In our hands, recombinant Ntg1 was found to undergo efficient borohydride trapping to all four ^{O}G:N substrates, and to catalyze efficient base-excision and strand cleavage on these as well. Furthermore, the trapping of Ntg1 to DNA was susceptible to competition by unlabeled substrates containing either thymine glycol, a prototypical Ntg1 substrates, or ^{O}G:A. Finally, targeted disruption of the *NTG1* gene in *S. cerevisiae* results in complete loss of ^{O}G:A-specific trapping activity, and removes one of the two ^{O}G:C-specific trapping activities, both of which were attributed to yOgg2. Thus, three independent lines of data establish that Ntg1 repairs ^{O}G residues in DNA, and that it is responsible for the yOgg2 activity of yeast cells. We therefore suggest that Ntg1 be designated henceforth as Ntg1/yOgg2.

Structural relatives of yOgg1 and Ntg1/yOgg2

The yeast 8-oxoguanine DNA glycosylase/lyases yOgg1 and Ntg1/yOgg2 belong to a structural superfamily of base-excision DNA repair proteins [10], the hallmark of which is an active site HhH–GPD motif comprising a helix–hairpin–helix (HhH) sub-motif [24,25] followed by a Gly/Pro-rich loop and an absolutely conserved aspartate residue (the G/P...D loop) [10,26,27]. The archetypal members of the HhH–GPD superfamily, endonuclease III and AlkA (*E. coli* 3-methyladenine DNA glycosylase), represent the two classes of mechanistically related yet distinct enzymes, glycosylase/lyases and monofunctional glycosylases, respectively. These two classes can readily be distinguished by sequence inspection of the HhH–GPD motif: glycosylase/lyases contain an invariant

Figure 8



(b)

	Helix 1	Hairpin	Helix 2	G/P...D Loop
Scer Ogg2	INELLG	LPGVG	PKMAYLTLQKA	WGKI-EGICVDVHV
Scer Ogg1	REHLMS	YNGVG	PKVADCVCLMG	LHMD-GIVPVDVHV
Hsap Ogg1	HKALCI	LPGVG	TKVADCICLMA	LDKP-QAVPVDVHM
Ecol EndoIII	RAALEA	LPGVG	RKTANVVLNTA	FGWP--TIAVDTHI
Ecol MutY	FEEVAA	LPGVG	RSTAGAILSLS	LGKH--FPILDGNV
Ecol AlkA	MKTLQT	FPGIG	RWTANYFALRG	WQAKDVFLPDDYLI

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The active site HhH–GPD motif of a superfamily of base-excision DNA repair proteins including yOgg2 and several Ogg1 orthologs. (a) Backbone ribbon trace of endonuclease III (endo III), with the catalytic Lys and Asp residues shown in licorice representation. (b) Sequence alignments of *S. cerevisiae* (*Scer*), human (*Hsap*) and *E. coli* (*Eco1*) HhH–GPD proteins with the hairpin and G/P...D loop shown in blue and the catalytic Lys (or other residue at this position) and Asp residues shown in green and red, respectively.

Lys residue at the second position of helix 2 of the HhH motif. Consistent with its known indispensability for catalysis [4,24,28], this Lys residue has recently been shown to serve as the catalytic nucleophile/Schiff-base donor in human Ogg1, a role that is presumably common to all glycosylase/lyases of the HhH–GPD superfamily [29]. The observation that Ntg1/yOgg2 undergoes efficient borohydride trapping is sufficient to establish that the protein is a glycosylase/lyase that uses an amine nucleophile for covalent catalysis, and indeed the protein contains the signature Lys residue at position 243 (Figure 8).

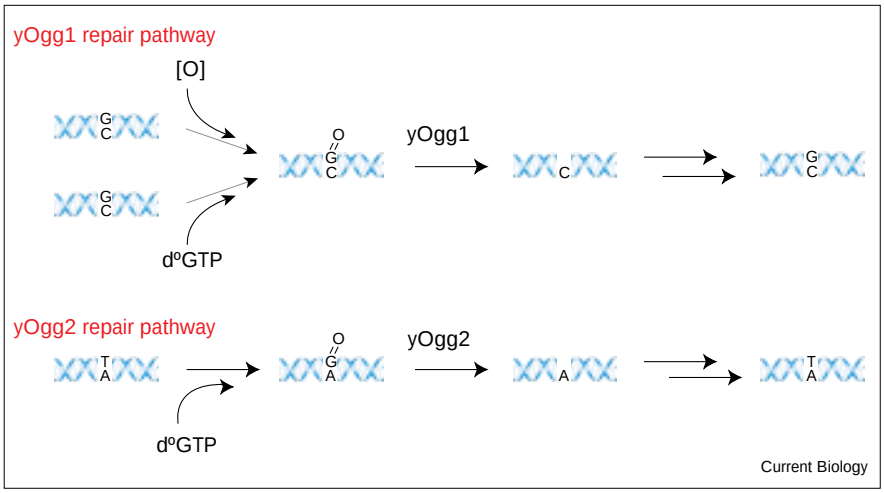
Several close relatives of Ntg1/yOgg2 have been identified through biochemical experiments and sequence comparisons. One of these is encoded by the yeast open reading frame *YOL043c* (also known as *SCR2*; accession number 66728) and has 36% identity to Ntg1/yOgg2. Whereas Ntg1/yOgg2 lacks the [Fe_4S_4] cluster found in endonuclease III, the *YOL043c*-encoded protein evidently contains such a cluster. Human, bovine and *Schizosaccharomyces pombe* glycosylase/lyases having endonuclease-III-like activity have recently been cloned, all of which contain sequence motifs and spectroscopic features characteristic of an [Fe_4S_4] cluster [23,30–32]. Because the *YOL043c*-encoded protein contains the active site Lys residue of glycosylase/lyases but does not trap any ^{O}G:N substrate in *yOgg1*$^{-}$ *Ntg1/yOgg2*$^{-}$ yeast (Figure 5 and data not shown), we can rule out the possibility that this protein possesses ^{O}G glycosylase/lyase activity. These observations suggest that one form of endonuclease III, the form that containing the [Fe_4S_4] cluster, is conserved throughout evolution. A second form, which does not contain an [Fe_4S_4] cluster, has been found only in yeast and is represented by only one known protein, Ntg1/yOgg2. We believe that Ntg1/yOgg2 serves a unique functional role in yeast as a key component of an alternative system for resistance to guanine oxidation.

An alternative GO system in *S. cerevisiae*

The GO system has evolved to protect organisms against the mutagenic effects of guanine oxidation caused by the reactive oxygen species to which they are constantly exposed. As classically elucidated in *E. coli* (Figure 1), the GO system consists of three enzymes that act coordinately: MutM, a glycosylase/lyase whose primary function is to cleave ^{O}G residues opposite C, before they are mis-replicated; MutY, a monofunctional glycosylase that cleaves A residues opposite ^{O}G, following mis-replication; and MutT, a triphosphatase that sanitizes the nucleotide precursor pool of d^{O}GTP. The existence of MutT ensures that the vast majority of ^{O}G residues in the bacterial genome are produced by DNA oxidation rather than by replication using d^{O}GTP as a DNA building block. Functional, if not structural, homologs of all three GO system components have been identified in mammalian cells, suggesting that the basic configuration of the system is conserved throughout evolution.

This being the case, it is unusual that *S. cerevisiae* appears to contain a functional homolog of MutM (yOgg1), but no known counterpart of MutY or MutT. No open reading frames bearing the sequence hallmarks of bacterial and mammalian MutT and MutY are evident in the yeast genome and, more importantly, no biochemical activities corresponding to those of MutY or MutT have been reportedly observed in *S. cerevisiae*. Furthermore, *S. cerevisiae* contains an enzymatic activity unique to this organism — the ability to excise ^{O}G residues opposite A, a process catalyzed by Ntg1/yOgg2. These considerations suggest that the GO system in *S. cerevisiae* may possess an alternative configuration, which requires only the function of yOgg1 and Ntg1/yOgg2 (Figure 9). Because *S. cerevisiae* contains no MutT activity, it must be equipped to handle ^{O}G residues that are incorporated into the genome via replication, in addition to those that result from DNA oxidation. Assuming that yOgg1 activity is efficient enough to repair ^{O}G:C lesions that arise through DNA oxidation before they are mis-replicated, the majority of ^{O}G:A pairs will have been generated by mis-incorporation of d^{O}GTP opposite A during replication (A:T→A:^{O}G in Figure 9). If the d^{O}GTP incorporation route does indeed give rise to significant numbers of A:^{O}G lesions in *S. cerevisiae* then, firstly, MutY

Figure 9



The alternative GO system of *S. cerevisiae*. The lack of MutT in *S. cerevisiae* permits ^{O}G residues to become incorporated into the yeast genome by the use of d^{O}GTP as a DNA building block. Most ^{O}G:A mis-pairs thus arise in *S. cerevisiae* by d^{O}GTP incorporation opposite A; in bacteria and humans, ^{O}G:A mis-pairs arise through dATP incorporation opposite ^{O}G. To accommodate this difference in the predominant route of ^{O}G incorporation, yeast process ^{O}G:A mis-pairs by excising the ^{O}G residue (Ntg1/yOgg2 activity), whereas bacterial and mammalian cells excise the A (MutY activity; refer to Figure 1).

activity should be subject to contraselection during evolution, because it would accelerate rather than prevent mutations; and, secondly, a base-excision repair activity specific for ^{O}G:A would be expected to decrease rather than increase the rate of spontaneous A:T to C:G transversions. The yOgg2 knockout data presented here provide direct support for the latter assertion; furthermore, the available evidence suggests that *S. cerevisiae* contains no MutY function (S.D.B. and G.L.V, unpublished observations).

S. cerevisiae is widely used as a model genetic system for the analysis of DNA damage and repair and for diseases such as cancer and aging. Future studies concerning the role of oxidative lesions in these processes should take into account the fact that *S. cerevisiae* uses a fundamentally distinct system from that in bacteria and mammals to protect against guanine oxidation.

Materials and methods

Oligonucleotide duplex substrates/competitors

Oligonucleotides were synthesized by standard chemistry and purified by 20% denaturing PAGE. Radiolabeling reactions were performed with T4 polynucleotide kinase (New England Biolabs) and γ-[^{32}P]ATP. The radiolabeled strand of each duplex is the top strand as shown below. Duplexes were prepared by annealing with a 10-fold molar excess of the complementary strand, in 100 mM NaCl/1 × TE. The phosphoramidites of 8-oxo-dG [33,34], 8-oxo-dA [35], 1,N^{6}εdA [36], THF [37], rAB (H.M.N. and G.L.V, unpublished) and Pyr [38] were synthesized according to published procedures. Duplex DNA containing thymine glycol was synthesized as described [39]. The phosphoramidite of 8-oxo-2′-flouro-2′-deoxyguanine was synthesized by an unpublished procedure (T. Kawate and G.L.V., unpublished). Oligonucleotides containing 5-hydroxycytosine and 5-hydroxyuracil were a generous gift from J.M. Essigmann. Sequences of oligonucleotides are available as Supplementary material (published with this paper on the internet).

Purification of the yOgg2–DNA covalent complex

S. cerevisiae (ard1Δ/pep4Δ) was grown to an OD_{600} of 1.5–2.0 in YPD (yeast extract/peptone/dextrose) media at 30°C. All operations were conducted at 4°C unless otherwise stated. Cells were pelleted by centrifugation and washed once with double-distilled water (ddw). Yeast were lysed by pouring cell paste (100 ml wet volume) slowly into liquid nitrogen, the resulting solid was added to a Waring blender containing liquid nitrogen. The mixture was blended at high speed for 3 × 2 min and mixed with 2 volumes (200 ml) of lysis buffer (20 mM Tris, pH 7.4, 500 mM NaCl, 10% glycerol, 1 mM DTT, 1 mM EDTA, 1 mM PMSF, 100 μM benzamidine, 0.5 μg/ml leupeptin, 2 μg/ml aprotinin and 1 μg/ml pepstatin), and the solution was stirred until homogeneous. The crude lysate was clarified by centrifugation at 5000 *g*, for 30 min. DEAE cellulose (6 ml dry volume) was added to the suspension and was stirred in a plastic beaker for 30 min. The DEAE cellulose was removed by centrifugation at 5000 *g* for 30 min. The DEAE clarified supernatant was diluted with two volumes (600 ml) of buffer A (20 mM Tris, pH 7.4, 10% glycerol, 1 mM EDTA, 10 mM β-mercaptoethanol, 1 mM PMSF), then passed through a Q_{ff} Sepharose column (3 × 7 cm) at 3 ml/min. The Q_{ff} flow-through was diluted threefold with buffer A (~3000 ml final volume) and loaded onto a BioRex 70 column (5 × 10 cm) at 3 ml/min. The column was washed with 200 ml of buffer A and eluted with a linear gradient of 300 ml buffer A to buffer A plus 1 M NaCl. Fractions were assayed for the presence of yOgg2 by borohydride trapping using radiolabeled ^{o}G:G-containing duplex DNA.

Fractions containing yOgg2 (120 ml) were pooled and dialyzed overnight against buffer A plus 100 mM NaCl. The sample was passed through a Q_{ff} Sepharose column (1 × 2 cm) and collected in a 250 ml siliconized Erlenmeyer flask. Sodium cyanoborohydride was added to a final concentration of 50 mM along with 20 nmol 49-mer ^{o}G/G-containing duplex DNA. The trapping reaction was stirred at 23°C for 2 h, then was cooled to 4°C. The trapping reaction mixture was loaded onto a Q_{ff} Sepharose column (0.5 × 1 cm) at 2 ml/min. The column was washed with 200 ml buffer A plus 100 mM NaCl, then eluted with a 25 ml gradient of buffer A plus 100 mM NaCl to buffer A plus 2 M NaCl, with the fractions being collected in siliconized glass tubes. Fractions containing the DNA–protein complex (as determined by SDS-PAGE analysis of samples containing radiolabeled DNA) were pooled and concentrated by ultrafiltration using a Centricon-10 unit. The filter was washed with 1 × SDS–PAGE loading dye at 100°C. To identify which of the protein bands contains a covalently attached DNA molecule, two separate 10 μl aliquots of the filter wash were taken and to each was added 40 μl of 50 mM Tris, pH 8.0, 1 mM $MgCl_2$. To one of the samples was added 1 μl *Serratia* endonuclease (benzonase, EM Merck). The two samples were incubated for 2 h at 37°C and analyzed by SDS–PAGE with silver staining. The remainder of the filter wash (100 μl) was loaded onto one lane of a SDS–PAGE gel. After staining

with Coomassie R-250, the band corresponding to the DNA–protein complex was excised, and subjected to in-gel carboxyamidomethylation and digestion with trypsin.

Peptide sequencing by capillary HPLC electrospray ionization ion trap mass spectrometry

A single aliquot of the in-gel digest mixture was analyzed by capillary (180 μm × 15 cm column, LC Packings) reverse-phase chromatography coupled to the electrospray ionization (ESI) source of a Finnigan LCQ quadrupole ion trap mass spectrometer. Peptides were eluted from the column with a linear gradient of 5–40% acetonitrile in 0.1% acetic acid/0.02% trifluoroacetic acid at a flow rate of 2.25 μl/min. Ionization was assisted with a coaxial sheath liquid of 70% methanol/0.05% acetic acid. Spectra were acquired as successive sets of three scan modes: full scan MS over the m/z range 395–1118 amu, followed by two data dependent scans on the most abundant ion in that full scan. These data dependent scans allowed the automatic acquisition of a high resolution (zoom) scan to determine charge state and exact mass, and MS/MS spectra for peptide sequence information. Base peak relative ion abundance corresponded to a load of 60–170 fmol by comparison with a standard peptide mixture analyzed under identical conditions. Interpretation of the resulting MS/MS spectra of the peptides was facilitated by searching the yeast_nrpep protein database with the algorithm Sequest [40].

Cloning, overexpression, purification and refolding of Ntg1/yOgg2

The *NTG1/yOGG2* gene was PCR amplified from yeast genomic DNA (strain FY250) with Deep Vent DNA polymerase (New England Biolobs) and the following primers: 5′-TCCGCGAGCTCATGCAAAAGGATCAGTAAATACTCATCT-3′, 5′-TCTACTCGAGTTAGTCCTCTACTTTAACAGAAATATCATTTTCCAG-3′. The resulting expression cassette was cloned into the *Sac*1/*Xho*1 sites of the vector pET30a (Novagen). Positive clones were transformed into competent BL21(DE3) *E. coli* (Novagen). Ntg1/yOgg2 was purified from 0.5 l cultures, grown at 37°C, to an OD_{600} of 0.5 and induced with 0.5 mM IPTG. The growth of induced cells was continued for 5 h at 37°C. Cells were pelleted by centrifugation and the resulting pellet was solubilized in 5 ml of 6 M GuHCl buffer (6 M guanidinium HCl, 100 mM sodium phosphate pH 8.0, 10 mM Tris, pH 8.0, 20 mM imidazole) for 2 h. Cellular lysate was clarified by centrifugation at 10,000 *g* for 20 min, the supernatant was incubated with 1.0 ml Ni-NTA resin (Qiagen) for 45 min. The resin was washed with 25 ml of 6 M GuHCl buffer, then with 25 ml 8 M urea buffer (8M urea, 500 mM NaCl, 20% glycerol, 10 mM Tris, pH 7.4). The resin-bound protein was refolded by passage over the resin of a 200 ml linear gradient of 8 M to 1 M urea buffer at 1.5 ml/min and room temperature. The refolded protein was washed with 10 ml 1 M urea buffer and eluted stepwise with 1 M urea buffer plus 25 mM to 500 mM imidazole. Fractions were analyzed by SDS–PAGE, and shown to be greater than 95% homogeneous based on Coomassie R-250 staining. Fractions containing refolded Ntg1/yOgg2 were supplemented with 1 mM EDTA and 10 mM β-mercaptoethanol.

Targeted disruption of the yOGG1 *and* NTG1/yOGG2 *genes*

Yeast containing targeted disruptions were constructed using the general protocol of Baudin *et al*. [41]. Primer sequences are available as Supplementary material. The PCR-amplified cassette was transformed into FY250 yeast (MATα, *URA3-52*, *HISΔ200*, *LEU2Δ1*, *TRP1Δ63*) or YMH(2-7) using the lithium acetate/PEG technique [42]. Positive clones were selected for by plating on appropriate dropout plates, individual clones were analyzed by PCR amplification of the targeted gene followed by restriction mapping.

Preparation of whole cell yeast extracts, DNA trapping and cleavage assays

Whole cell yeast extracts were prepared as described [10], total protein concentrations were normalized using Bradford assay before use in trapping or cleavage assays. Cleavage reactions were performed in a final volume of 20 μl under the conditions 50 mM Tris, pH 7.4, 5 mM EDTA, 100 mM NaCl and 1 nM radiolabeled substrate DNA duplex. Reactions contained 3 μl recombinant protein or normalized cellular extract and were incubated for 30 min at 30°C. Reactions were stopped by adding 10 μl of 90% formamide loading dye and heating at 75°C for 5 min. Samples were loaded onto a 20% (19:1) 1 × TBE denaturing acrylamide gel. The gel was exposed to film and phosphorimaging plates for imaging and quantification. DNA trapping reactions using sodium borohydride were preformed as previously described [10].

Canavanine forward mutation assays

Cultures (10 ml) of wild-type (FY250), *yOgg1*⁻, *Ntg1/yOgg2*⁻ and *yOgg1*⁻ *Ntg1/yOgg2*⁻ yeast were grown in YPD media at 30°C with shaking. After the cells had reached stationary phase (~18 h), they were pelleted by centrifugation at 4°C and washed two times with sterile ddw. The cells were resuspended in 2 ml sterile ddw, and cell concentrations were normalized by measuring the absorbances at 600 nm. Tenfold serial dilutions were made in sterile ddw, and 100 μl of undiluted, 10^{-1} diluted, and 10^{-2} diluted cells were spread on 2% glucose complete minimal agar plates lacking L-arginine and containing 60 μg/ml L-canavanine (Sigma). The plates were incubated at 30°C for ~2.5 days and the colonies were then counted. The total number of cells used in each experiment was ascertained by spreading 100 μl of 10^{-6}-fold dilutions on YPD plates.

Iso-1-cytochrome c (CYC1) reversion assays

Cultures (10 ml) of wild-type (FY250), *yOgg1*⁻, *Ntg1/yOgg2*⁻ and *yOgg1*⁻ *Ntg1/yOgg2*⁻ were grown in YPD media at 30°C with shaking. After the cells had reached stationary phase (~18 h), they were pelleted by centrifugation at 4°C and washed two times with sterile ddw. The cells were resuspended in 2 ml sterile ddw, and the concentrations were normalized by measuring the absorbances at 600 nm. Tenfold serial dilutions were made in sterile ddw, and 100 μl of undiluted, 10^{-1} diluted, and 10^{-2} diluted cells were spread on 3% glycerol/0.1% glucose complete minimal (YPGD) agar plates [20]. The plates were incubated at 30°C for ~7 days and the colonies were then counted. The total number of cells used in each experiment was ascertained by spreading 100 μl of 10^{-6}-fold dilutions on YPD plates.

Supplementary material

Sequences of the oligonucleotide duplex substrate/competitors and of the primers used for targeted disruption of the *yOGG1* and *NTG1/yOGG2* genes are published with this paper on the internet.

Acknowledgements

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References

1. Michaels ML, Miller JH: The GO system protects organisms from the mutagenic effect of the spontaneous lesion 8-hydroxyguanine (7,8-dihydro-8-oxoguanine). *J Bacteriol* 1992, 174:6321-6325.
2. Grollman AP, Moriya M: Mutagenesis by 8-oxoguanine: an enemy within. *Trends Genet* 1993, 9:246-249.
3. Friedberg EC, Walker GC, Siede W: *DNA Repair and Mutagenesis*. Washington, D.C.: ASM Press; 1995.
4. Lu R, Nash HM, Verdine GL: A DNA repair enzyme that excises oxidatively damaged guanines from the mammalian genome is frequently lost in lung cancer. *Curr Biol* 1997, 7:397-407.
5. Radicella JP, Dherin C, Desmaze C, Fox MS, Boiteux S: Cloning and characterization of ***hOGG1***, a human homolog of the ***OGG1*** gene of ***Saccharomyces cerevisiae***. *Proc Natl Acad Sci USA* 1997, 94:8010-8015.
6. Rosenquist TA, Zharkov DO, Grollman AP: Cloning and characterization of a mammalian 8-oxoguanine DNA glycosylase. *Proc Natl Acad Sci USA* 1997, 94:7429-7434.

7. Arai K, Morishita K, Shinmura K, Kohno T, Kim SR, Nohmi T, *et al*.: Cloning of a human homolog of the yeast ***OGG1*** gene that is involved in the repair of oxidative DNA damage. *Oncogene* 1997, 14:2857-2861.
8. Aburatani H, Hippo Y, Ishida T, Takashima R, Matsuba C, Kodama T, *et al*.: Cloning and characterization of mammalian 8-hydroxyguanine-specific DNA glycosylase/apurinic, apyrmidinic lyase, a functional MutM homologue. *Cancer Res* 1997, 57:2151-2156.
9. Roldan-Arjona T, Wei YF, Carter KC, Klungland A, Anselmo C, Wang RP, *et al*.: Molecular cloning and functional expression of a human cDNA encoding the antimutator enzyme 8-hydroxyguanine-DNA glycosylase. *Proc Natl Acad Sci USA* 1997, 94:8016-8020.
10. Nash HM, Bruner SD, Schärer OD, Kawate T, Addona TA, Sponner E, *et al*.: Cloning of a yeast 8-oxoguanine DNA glycosylase reveals the existence of a base-excision DNA-repair protein superfamily. *Curr Biol* 1996, 6:968-980.
11. van der Kemp PA, Thomas D, Barbey R, de Oliveira R, Boiteux S: Cloning and expression in ***Escherichia coli*** of the ***OGG1*** gene of ***Saccharomyces cerevisiae***, which codes for a DNA glycosylase that excises 7,8-dihydro,8-oxoguanine and 2,6-diamino-4-hydroxy-5-N-methylformamidopyramidine. *Proc Natl Acad Sci USA* 1996, 93:5197-5202.
12. de Oliveira R, van der Kamp PA, Thomas D, Geiger A, Nehls P, Boiteux S: Formamidine DNA glycosylase in the yeast ***Saccharomyces cerevisiae***. *Nucleic Acids Res* 1994, 22:3760-3764.
13. Tchou J, Kasai H, Shibutani S, Chung M-H, Laval J, Grollman AP, Nishimura S: 8-Oxoguanine (8-hydroxyguanine) DNA glycosylase and its substrate specificity. *Proc Natl Acad Sci USA* 1991, 88:4690-4694.
14. Tchou J, Bodepudi V, Shibutani S, Antoshechkin I, Miller J, Grollman AP, Johnson F: Substrate specificity of Fpg protein. *J Biol Chem* 1994, 269:15318-15324.
15. Eide L, Bjorås M, Pirovano M, Alseth I, Berdal KG, Seeberg E: Base excision of oxidative purine and pyrimidine DNA damage in ***Saccharomyces cerevisiae*** by a DNA glycosylase with sequence similarity to endonuclease III from ***Escherichia coli***. *Proc Natl Acad Sci USA* 1996, 93:10735-10740.
16. Augeri L, Lee Y-M, Barton AB, Doetsch PW: Purification, characterization, gene cloning and expression of ***Saccharomyces cerevisiae*** redoxyendonuclease, a homolog of ***Escherichia coli*** endonuclease III. *Biochemistry* 1997, 36:721-729.
17. Dodson ML, Schrock RD III, Lloyd RS: Evidence for an imino intermediate in the T4 endonuclease V reaction. *Biochemistry* 1993, 32:8284-8290.
18. Dizdaroglu M, Laval J, Boiteux S: Substrate specificity of the ***Escherichia coli*** endonuclease III: excision of thymine- and cytosine-derived lesions in DNA produced by radiation-generated free radicals. *Biochemistry* 1993, 32:12105-12111.
19. Ono BI, Ishino Y, Shinoda S: Nonsense mutations in the ***can1*** locus of ***Saccharomyces cerevisiae***. *J Bacteriol* 1983, 154:1476-1479.
20. Hampsey M: A tester system for detecting each of the six base-pair substitutions in ***Saccharomyces cerevisiae*** by selecting for an essential cysteine in iso-1-cytochrome c. *Genetics* 1991, 128:59-67.
21. Thomas D, Scot AD, Barbey R, Padula M, Boiteux S: Inactivation of ***OGG1*** increases the incidence of G·C to T·A transversions in ***Saccharomyces cerevisiae***: evidence for endogenous oxidative damage to DNA in eukaryotic cells. *Mol Gen Genet* 1997, 254:171-178.
22. Besho T, Tano K, Kasai H, Ohtsuka E, Nishimura S: Evidence for two DNA repair enzymes for 8-hydroxyguanine (7,8-dihydro-8-oxoguanine) in human cells. *J Biol Chem* 1993, 268:19416-19421.
23. Hilbert TP, Boorstein RJ, Kung HC, Bolton PH, Xing D, Cunningham RP, Teebor GW: Purification of a mammalian homolog of ***Escherichia coli*** endonuclease III: identification of a bovine pyrimidine hydrate-thymine glycol DNA-glycosylase/AP lyase by irreversible cross linking to a thymine glycol-containing oligodeoxynucleotide. *Biochemistry* 1996, 35:2505-2511.
24. Thayer MM, Ahern H, Xing DX, Cunningham RP, Tainer JA: Novel DNA binding motifs in the DNA repair enzyme endonuclease III crystal structure. *EMBO J* 1995, 14:4108-4120.
25. Doherty AJ, Serpell LC, Ponting CP: The helix-hairpin-helix DNA-binding motif: a structural basis for non-sequence-specific recognition of DNA. *Nucleic Acids Res* 1996, 24:2488-2497.
26. Labahn J, Scharer OD, Long A, Ezaz-Nikpay K, Verdine GL, Ellenberger TE: Structural basis for the excision repair of alkylation-damaged DNA. *Cell* 1996, 86:321-329.
27. Yamagata Y, Kato M, Odawara K, Tokuno Y, Nakashima Y, Matsushima N, *et al*.: Three-dimensional structure of a DNA repair enzyme, 3-methyladenine DNA glycosylase II, from ***Escherichia coli***. *Cell* 1996, 86:311-319.
28. Girard P-M, Guibourt N, Boiteux S: The Ogg1 protein of ***Saccharomyces cerevisiae***: a 7,8-dihydro-8-oxoguanine DNA glycosylase/AP lyase whose lysine 241 is a critical residue for catalytic activity. *Nucleic Acids Res* 1997, 25:3204-3211.
29. Nash HM, Lu R, Lane WS, Verdine GL: The critical active-site amine of the human 8-oxoguanine DNA glycosylase, hOgg1: direct identification, ablation and chemical reconstitution. *Chem Biol* 1997, 4:693-702.
30. Hilbert TP, Chaung W, Boorstein RJ, Cunningham RP, Teebor GW: Cloning and expression of the cDNA encoding the human homologue of the DNA repair enzyme, ***Escherichia coli*** endonuclease III. *J Biol Chem* 1997, 272:6733-6740.
31. Aspinwall R, Rothwell DG, Roldan-Arjona T, Anselmino C, Ward C, Cheadle JP, *et al*.: Cloning and characterization of a functional human homolog of ***Escherichia coli*** endonclease III. *Proc Natl Acad Sci USA* 1997, 94:109-114.
32. Roldán-Arjona T, Anselmo C, Lindahl T: Molecular cloning and functional analysis of a ***Schizosaccharomyces pombe*** homologue of ***Escherichia coli*** endonuclease III. *Nucleic Acids Res* 1996, 24:3307-3312.
33. Roelen HCPF, Saris CP, Brugghe HF, van den Elst H, Westra JG, van der Marel GA, van Boom JH: Solid-phase synthesis of DNA fragments containing the modified base 7-hydro-8-oxo-2'-deoxyguanosine. *Nucleic Acids Res* 1991, 19:4361-4369.
34. Bodepudi V, Iden CR, Johnson F: An improved method for the preparation of the phosphoramidites of modified 2'-deoxynucleotides: incorporation of 8-oxo-2'-deoxy-7H-guanosine into synthetic oligomers. *Nucleosides Nucleotides* 1991, 10:755-761.
35. Guy A, Duplaa A-M, Harel P, Téoule R: Synthesis and characterization of DNA fragments bearing an adenine radiation product: 7,8-dihydroadenin-8-one. *Helvetica Chimica Acta* 1988, 71:1566-1571.
36. Srivastava SC, Raza SK, Misra R: 1,N6-etheno deoxy and ribo adenosine and 3,N4-etheno deoxy and ribo cytidine phosphoramidites. Strongly fluorescent structures for selective introduction in defined sequence DNA and RNA molecules. *Nucleic Acids Res* 1994, 22:1296-1304.
37. Takeshita M, Chang C-N, Johnson F, Will S, Grollman AP: Oligodeoxynucleotides containing synthetic abasic sites. *J Biol Chem* 1987, 262:10171-10179.
38. Schärer OD, Verdine GL: Specific binding of the DNA repair enzyme AlkA to a pyrrolidine-based inhibitor. *J Am Chem Soc* 1995, 117:6623-6624.
39. Kao JY, Goljer I, Phan TA, Bolton PH: Characterization of the effects of a thymine glycol residue on the structure, dynamics, and stability of duplex DNA by NMR. *J Biol Chem* 1993, 268:17787-17793.
40. Eng JK, McCormick AL, Yates JR III: An approach to correlate tandem mass spectral data of peptides with amino acid sequences in a protein database. *J Am Soc Mass Spectrom* 1994, 5:976-989.
41. Baudin A, Ozier-Kalogeropoulos O, Denouel A, Lacroute F, Cullin C: A simple and efficient method for direct gene deletion in ***Saccharomyces cerevisiae***. *Nucleic Acids Res* 1993, 21:3329-3330.
42. Hill J, Donald IG, Griffiths DE: DMSO-enhanced whole cell transformation. *Nucleic Acids Res* 1991, 19:5791.

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Supplementary material

Repair of oxidatively damaged guanine in *Saccharomyces cerevisiae* by an alternative pathway

Steven D. Bruner, Huw M. Nash, William S. Lane and Gregory L. Verdine

Current Biology March 1998, 8:393–403

Materials and methods

Sequences of oligonucleotide duplex substrates/competitors

^{O}G:A	5′-GGATAGTGTCCA	^{0}G GTTACTCGAAGC-3′
	3′-CCTATCACAGGT	A CAATGAGCTTCG-5′
^{O}G:C	5′-GGATAGTGTCCA	^{0}G GTTACTCGAAGC-3′
	3′-CCTATCACAGGT	C CAATGAGCTTCG-5′
^{O}G:G	5′-GGATAGTGTCCA	^{0}G GTTACTCGAAGC-3′
	3′-CCTATCACAGGT	G CAATGAGCTTCG-5′
^{O}G:G	5′-GGATAGTGTCCA	^{0}G GTTACTCGAAGC-3′
	3′-CCTATCACAGGT	T CAATGAGCTTCG-5′
8-oxoA:T	5′-GGATAGTGTCCA	^{0}A GTTACTCGAAGC-3′
	3′-CCTATCACAGGT	T CAATGAGCTTCG-5′
1,N6εA:T	5′-GGATAGTGTCCA	εA GTTACTCGAAGC-3′
	3′-CCTATCACAGGT	T CAATGAGCTTCG-5′
G:C	5′-GTGAACCTGAGC	G TAGCTCAGTAAC-3′
	3′-CACTTGGACTCG	C ATCGAGTCATTG-5′
rAB:C	5′-GTGAACCTGAGC	rAB TAGCTCAGTAAC-3′
	3′-CACTTGGACTCG	C ATCGAGTCATTG-5′
THF:C	5′-GGATAGTGTCCA	THF GTTACTCGAAGC-3′
	3′-CCTATCACAGGT	C CAATGAGCTTCG-5′
pyr:C	5′-GGATAGTGTCCA	pyr GTTACTCGAAGC-3′
	3′-CCTATCACAGGT	C CAATGAGCTTCG-5′
βF–^{O}G:C	5′-GGATAGTGTCCA	F^{0}G GGTACTCGAAGC-3′
	3′-CCTATCACAGGT	C CCATGAGCTTCG-5′
Thymine	5′-GCAGCAGCACAG	^{0}T CAGCAAGGAACG-3′
glycol:A	3′-CGTCGTCGTGTC	A GTCGTTCCTTGC-5′
7-Me-G:C	5′-CGC MeG	AATT C GCG-3′
	3′-GCG C	TTAA MeG CGC-5′

5-HO-C:G

5′-AATTGCGATCTAGCTCGCCAG ^{0}C AGCGACCTTATCTGATGA-3′
3′-TTAACGCTAGATCGAGCGGTC G TCGCTGGAATAGACTACT-5′

5-HO-U:G

5′-AATTGCGATCTAGCTCGCCAG ^{0}U AGCGACCTTATCTGATGA-3’
3′-TTAACGCTAGATCGAGCGGTC G TCGCTGGAATAGACTACT-5’

49-mer ^{O}G:G

5′-GGCAAGTCTGATGGATAGTGTCCA ^{0}G GGTACTCGAAGCAGTTCGAACTGG-3′
3′-CCGTTCAGACTACCTATCACAGGT G CCATGAGCTTCGTCAAGCTTGACC-5’

Primers used for targeted disruption of the yOGG1 *and* NTG1/yOGG2 *genes*

The *HIS3* gene was amplified from a plasmid using the following set of primers, which contain regions of homology within the *NTG1/yOGG2* gene: 5′-GAGGTTGTCCCTCAACCCGTGGATATCGACTGGGTTA-AATCGCTGGAGCAGATTGTACTGAGAGTGCAC-3′, 5′-GTATTTC-ACAACCTTTGGATAACTCATGATATTTTGCTGGATTTCCTCCTTAC GCATCTGTGCGGTATTTC-3′. The *LEU2* gene was amplified from a plasmid using the following primers, which are homologous to yOGG1: 5′-ATGTCTTATAAATTCGGCAAACTTGCCATTAATAAAAGTGAGC-TACTCTTGGCCTCCTCTAG-3′, 5′-CTAATCTATTTTTGCTTCTTTGA-TGTGAAGATCAGACAATTCAACTCGTTCAGAATGACACG-3′.