Oxidative Damage and Mutagenesis in *Saccharomyces cerevisiae*: Genetic Studies of Pathways Affecting Replication Fidelity of 8-Oxoguanine

Arthur H. Shockley,* David W. Doo,* Gina P. Rodriguez,* and Gray F. Crouse*,†,1
*Department of Biology and †Winship Cancer Institute, Emory University, Atlanta, Georgia 30322

ABSTRACT Oxidative damage to DNA constitutes a major threat to the faithful replication of DNA in all organisms and it is therefore important to understand the various mechanisms that are responsible for repair of such damage and the consequences of unrepaired damage. In these experiments, we make use of a reporter system in *Saccharomyces cerevisiae* that can measure the specific increase of each type of base pair mutation by measuring reversion to a Trp+ phenotype. We demonstrate that increased oxidative damage due to the absence of the superoxide dismutase gene, *SOD1*, increases all types of base pair mutations and that mismatch repair (MMR) reduces some, but not all, types of mutations. By analyzing various strains that can revert only via a specific CG → AT transversion in backgrounds deficient in Ogg1 (encoding an 8-oxoG glycosylase), we can study mutagenesis due to a known 8-oxoG base. We show as expected that MMR helps prevent mutagenesis due to this damaged base and that Pol η is important for its accurate replication. In addition we find that its accurate replication is facilitated by template switching, as loss of either *RAD5* or *MMS2* leads to a significant decrease in accurate replication. We observe that these *ogg1* strains accumulate revertants during prolonged incubation on plates, in a process most likely due to retromutagenesis.

OXIDATIVE damage to DNA has long been recognized as an important source of DNA damage and subsequent mutagenesis (Bjelland and Seeberg 2003; Imlay 2003; Evans *et al.* 2004; Nakabeppu *et al.* 2006; D'Errico *et al.* 2008; Imlay 2008; Kryston *et al.* 2011; Stone *et al.* 2011). Oxidative damage has been proposed as an important contributor to cancer (Nakabeppu *et al.* 2006; Paz-Elizur *et al.* 2008; Maynard *et al.* 2009; Tudek *et al.* 2010; Kryston *et al.* 2011; Melis *et al.* 2011; Stone *et al.* 2011), neurological diseases (Fishel *et al.* 2007; Lovell and Markesbery 2007; Trushina and Mcmurray 2007; D'Errico *et al.* 2008; Ventura *et al.* 2010; Jeppesen *et al.* 2011; Liu and Wilson 2012), and, somewhat more controversially, aging (Burhans and Weinberger 2007; Maynard *et al.* 2009; Gredilla *et al.* 2010; Tudek *et al.* 2010; Speakman and Selman 2011). Oxidative damage to the DNA bases is a primary concern for mutagenesis due to the propensity of damaged bases to miscode, and a large number of different oxidation products of DNA bases have been analyzed (Wallace 2002; Evans *et al.* 2004; Neeley and Essigmann 2006; Stone *et al.* 2011). Of all of the various oxidatively damaged bases, 8-oxoG is perhaps the most significant because of its abundance and frequent mispairings to yield GC → TA transversions (Evans *et al.* 2004; Neeley and Essigmann 2006; Beard *et al.* 2010; van Loon *et al.* 2010; Zahn *et al.* 2011). The central role of 8-oxoG in mutagenesis is reflected in the fact that there are specific glycosylases that excise 8-oxoG from DNA.

Much oxidative damage is due to reactive oxygen species (ROS) generated within the cell itself, particularly from the mitochondrion (Karthikeyan and Resnick 2005; Kim *et al.* 2006; Linford *et al.* 2006; Lambert and Brand 2009). Most organisms have several enzymes devoted to detoxifying ROS; one important enzyme is superoxide dismutase (SOD), the only enzyme that can detoxify superoxide (Gralla and Valentine 1991; Longo *et al.* 1996; Serra *et al.* 2003; Van Raamsdonk and Hekimi 2012). Base excision

doi: 10.1534/genetics.113.153874
Manuscript received May 31, 2013; accepted for publication July 11, 2013
Available freely online through the author-supported open access option.
Supporting information is available online at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.113.153874/-/DC1.
[1]Corresponding author: Department of Biology, 1510 Clifton Rd., Emory University, Atlanta, GA 30322. E-mail: gcrouse@emory.edu

repair (BER) is the first line of defense against oxidatively damaged bases in the genome (Dizdaroglu 2005; D'Errico *et al.* 2008; Maynard *et al.* 2009; van Loon *et al.* 2010). Ogg1 is a BER glycosylase that recognizes and removes an 8-oxoG paired with C (Nash *et al.* 1996; van der Kemp *et al.* 1996; Huang and Kolodner 2005; Klungland and Bjelland 2007; Tsuzuki *et al.* 2007; Paz-Elizur *et al.* 2008). Another important repair mechanism for oxidative DNA damage is MMR. We demonstrated the general effect of MMR on oxidative damage in yeast (Earley and Crouse 1998) and subsequently the specific effect of MMR and 8-oxoG in yeast was demonstrated (Ni *et al.* 1999). Experiments in many organisms have reinforced the finding that MMR plays an important and conserved role in prevention of mutations due to oxidative damage (Mure and Rossman 2001; Boiteux *et al.* 2002; Colussi *et al.* 2002; Gu *et al.* 2002; Mazurek *et al.* 2002; Shin and Turker 2002; Slupphaug *et al.* 2003; Wyrzykowski and Volkert 2003; Russo *et al.* 2004; Huang and Kolodner 2005; Zlatanou *et al.* 2011). However, those experiments were not designed to measure the effect of oxidative damage on specific base pair mutations and the effect of MMR on formation of those mutations. Oxidatively damaged bases, such as 8-oxoG, have generally not been thought to affect replication. However, we have recently found (Rodriguez *et al.* 2013) that 8-oxoG can induce template switching in which the replicating 3′ end invades the sister strand, either by strand invasion or a fork regression, thereby bypassing the damaged base (Li and Heyer 2008). Polyubiquitination of PCNA by a complex of Ubc13–Mms2–Rad5 appears to be necessary for template switching (Chang and Cimprich 2009).

Increased ROS can also have effects through damage to RNA. Although most of the interest in 8-oxoG has understandably been in its direct involvement in DNA mutation, it was also found *in vitro* that RNA polymerases could frequently misincorporate an A opposite 8-oxoG during the process of transcription (Chen and Bogenhagen 1993; Viswanathan and Doetsch 1998; Doetsch 2002). Later, it was shown that such transcriptional mutagenesis could occur *in vivo* in *Escherichea coli* (Brégeon *et al.* 2003) as well as in mammalian cells (Saxowsky *et al.* 2008). It has been proposed that mutagenic transcription of damaged DNA could lead to the growth of cells that would otherwise remain quiescent and thus lead to mutation due to the damaged DNA in a process termed retromutagenesis (Bregeon and Doetsch 2011). Such a process has been difficult to observe experimentally.

Most oxidative damage to DNA is expected to produce single point mutations. We previously developed a reversion assay with six different base substitutions in an essential codon of the *Saccharomyces cerevisiae TRP5* gene, yielding a mutagenesis assay with extremely low background and specificity for point mutations (Williams *et al.* 2005). Our interest in developing this assay stemmed from prior results with a similar assay system, using mutations in the *CYC1* gene (Hampsey 1991), that demonstrated an extremely high-base-pair mutation rate in the absence of MMR, due at least in part to oxidative damage (Earley and Crouse 1998). Reversion analysis of *cyc1* strains depends on restoration of mitochondrial function, and we found as we continued to use those strains that MMR mutants, in particular, were extremely unstable, giving rise to many derivatives that did not revert, presumably due to loss of functional mitochondrial DNA (results not shown). We report here that the *trp5* reversion rates of all strains are greatly increased by oxidative damage and that half of the strains demonstrate a synergistic increase in reversion rates with oxidative damage and the loss of MMR, although the absolute values in those cases are much lower than with the *cyc1* strains. By employing *ogg1* derivatives of the *trp5-A149C* strains, we are able to study mutagenesis specifically due to the formation of 8-oxoG in DNA. We find that the mutation rates due to 8-oxoG are strongly suppressed by MMR, but substantially decreased in the presence of Pol η, a translesion DNA polymerase known for accurate replication of 8-oxoG (Haracska *et al.* 2000; Yuan *et al.* 2000 ; De Padula *et al.* 2004; Carlson and Washington 2005). We further find evidence that template switching is important for avoiding mutagenesis due to 8-oxoG, as inactivation of either *RAD5* or *MMS2* significantly increases reversion rates. In addition, we observe increased numbers of revertants when *ogg1 trp5–A149C* strains remain on selective plates for extended periods, consistent with 8-oxoG-induced retromutagenesis.

Materials and Methods

Yeast strains

All strains used were derivatives of the previously published collection of *trp5* mutants (Williams *et al.* 2005). Gene deletions were created by one-step disruption with PCR-generated fragments. In general, gene deletions were made from a PCR fragment generated from the collection of yeast gene deletions (Winzeler *et al.* 1999). *sod1* strains were grown in anaerobic chambers except for growth immediately before transformation or for reversion analysis. In addition, *sod1* strains were routinely checked for a Lys- phenotype in aerobic growth to test for the presence of suppressors (Gralla and Valentine 1991). A complete list of strains is given in Supporting Information, Table S1.

Reversion analysis

Reversion analysis was performed by growth of parallel cultures inoculated with equal amounts of a dilute culture of cells (Rosche and Foster 2000), followed by plating each culture separately on SD–Trp medium (Sherman 2002). In practice, 3 μl of an overnight culture of a given strain was diluted into 110 ml of YPAD (Sherman 2002), and usually 12 cultures of 5 ml were incubated for 48 hr at 30° and then plated on SD–Trp, with each culture being split between two plates. In addition, before plating, the number of total viable cells was determined in 3 cultures by dilution and plating on YPAD.

Determination of reversion rates

Reversion rates were determined by fluctuation analysis using a maximum-likelihood method for analysis (Rosche and Foster 2000; Foster 2006). Computations were performed using the program Salvador, which calculates both m, the mean number of mutations occurring in a culture, and 95% confidence intervals from the fluctuation data (Zheng 2002, 2005, 2007). The mutation rate was then obtained by dividing m by the average number of total cells in the cultures.

Results

Oxidative damage increases mutation of all base pairs

Spontaneous reversion rates of our *trp5* strains were measured and, as shown in Figure 1, are extremely low in strains that are otherwise wild type. This low reversion rate is consistent with the very specific nature of the reversion assay in which each strain is able to revert to Trp+ function via only one specific base pair change (Williams *et al.* 2005). In contrast to results with the *cyc1* reversion assay (Earley and Crouse 1998), elimination of MutSα function (*msh6* strains) resulted in modest, at best, increases in mutation rates (Figure 1 and Table 1). Oxidative damage in the strains was increased by deleting the *SOD1* gene encoding superoxide dismutase (Gralla and Valentine 1991) and the reversion rates of those strains, with and without MMR, were determined (Figure 1 and Table 1).

It is apparent from this analysis that the mutation rates of all base pairs are increased substantially with increased oxidative damage, but that the effect of MMR is quite variable, depending on the particular base pair mutation involved. In some cases, such as the *trp5–G148C* and *trp5–A149G* strains, MMR has little effect on *sod1*-induced mutations, whereas in other strains such as *trp5–G148A*, *trp5–A149C*, and *trp5–A149T*, the effect of MMR is synergistic with oxidative damage.

Mutations due to 8-oxoG

One of the problems with the above analysis is that although the base pair change in each strain is known, the exact insult to the DNA causing the mutation is not. The *trp5–A149C* mutation reverts via a CG → AT mutation, which is a hallmark of oxidative damage, as an 8-oxoG frequently mispairs with an A, leading to a CG → AT transversion. One could still not ascribe all *trp5–A149C* reversion events to misreplication of a damaged guanine, but increased mutagenesis in an *ogg1* background should all be due to the 8-oxoG, as Ogg1 is quite specific for removal of an 8-oxoG opposite C (Nash *et al.* 1996; van der Kemp *et al.* 1996). The impact and specificity of an *ogg1* mutation is seen in the reversion analysis of Figure 2. In the *trp5–A149C* background, the reversion rate of an *ogg1* mutation is ~20× that of wild type and an *msh6 ogg1* strain is eightfold greater than the *ogg1*. In the *trp5–A149T* background, which would revert via a TA → AT transversion, however, neither an *ogg1* nor *msh6 ogg1* strain has a reversion rate significantly greater than wild type. Therefore in the *trp5–A149C ogg1* background, it is likely that essentially all mutation events can be ascribed to the misreplication of an 8-oxoG.

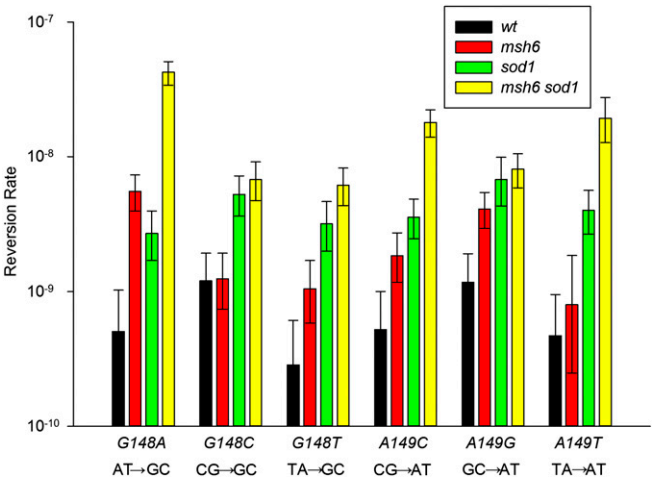

Figure 1 Spontaneous reversion rates of *trp5* strains. Reversion rates and 95% confidence intervals for strains of the indicated *trp5* mutation and genotype, all in the F orientation, are shown. The particular base pair change required for reversion of each strain is also shown.

Factors affecting 8-oxoG mutagenesis

A series of reversion experiments was performed with the *trp5–A149C* mutant gene in both orientations with respect to the *ARS306* origin of replication in a variety of different genotypes. The results are shown graphically in Figure 3, the reversion rates given numerically in Table S2, and comparisons of the reversion rates of various genotypes given in Table 2. Consistent with the results in Figure 2 with the *trp5–A149C* F strain, in both orientations of the *TRP5* gene, we find substantial increases in reversion rates in *ogg1* and *msh6 ogg1* genotypes. Pol η, the product of the *RAD30* gene, is responsible for accurate bypass of 8-oxoG (De Padula *et al.* 2004; Carlson and Washington 2005; Silverstein *et al.* 2010) and when *RAD30* is deleted, we see a two- to fourfold increase in reversion rate in both *ogg1* and *msh6 ogg1* strains (Figure 3 and Table 2). Inactivation of MMR and Pol η shows a synergistic effect in an *ogg1* background, as would be expected for the independent activities of MMR and Pol η in accurate replication of 8-oxoG (Mudrak *et al.* 2009).

In general, 8-oxoG has not been thought to affect replication, but we have found in work with oligonucleotides containing an 8-oxoG that this lesion can induce template switching (Rodriguez *et al.* 2013). Therefore we tested the role of template switching by deleting the *RAD5* or *MMS2* gene, either of which should inactivate template switching (Zhang and Lawrence 2005; Branzei *et al.* 2008; Minca and Kowalski 2010). In both cases, reversion rates were increased by approximately twofold in an *ogg1* background and four- to fivefold in an *ogg1 rad30* background (Figure 3 and Table 2).

ogg1 and transcriptional mutagenesis

In working with *ogg1* derivatives of the *trp5–A149C* mutation, we noted that new colonies arose when those strains

Table 1 Relative increases in *trp5* reversion rates

	G148A F	G148C F	G148T F	A149C F	A149G F	A149T F
wt	1.0	1.0	1.0	1.0	1.0	1.0
msh6	11.0*	1.0	3.7*	3.5*	3.5*	1.7
sod1	5.3*	4.4*	11.0*	6.8*	5.8*	8.5*
msh6 sod1	83.0*	5.6*	22.0*	35.0*	6.9*	41.0*

For each *trp5* mutant strain, the reversion rate of wild-type is set to 1.0 and the reversion rates of the indicated genotypes are compared to the wild-type rate. Differences are considered to be significant (*) if the 95% confidence intervals do not overlap.

were left on selection plates (SD–Trp) over extended periods of time. An example with *ogg1* and *msh6 ogg1* derivatives of *trp5–A149C* F is shown in Figure 4. We examined other *trp5–A149C ogg1* genotypes, including several with reversion rates considerably higher than the *msh6 ogg1* background, and all showed a similar pattern of colonies arising on plates over time (Figure S1, A–C). We wanted to determine if the phenomenon of increasing numbers of revertants on plates was a function of the *ogg1* mutation. We had tested various *msh6* strains, but the reversion rate in *msh6* strains is so low that one cannot obtain a reasonable number of revertants on one plate. We therefore treated *msh6* strains with H_2O_2 to increase the initial mutation rate and observed the resulting revertants over time. In contrast to the *ogg1* strains, no increase in revertants over time was seen (Figure S1D). One concern was that the late-arising phenotype of some revertants could be due to petite formation or an inherent slow-growth phenotype. On a plate containing *trp5–A149C ogg1* revertants that had appeared at various times, 28 of the smallest colonies were picked and assayed for respiratory competence; only 25% were petites. Colonies that arise late on a plate will in general be smaller than colonies that arise earlier, due to a shorter growth time. To determine if colony size was related to inherent growth rate, rather than time of appearance, we picked colonies of various sizes and then determined their rate of growth in rich medium. The results, shown in Figure S2, indicate that the growth rate of small *vs.* large colonies was not different.

When plated on such selection plates lacking tryptophan, Trp- cells remain viable for weeks, but do not grow for even one cell cycle in the absence of tryptophan (Rodriguez *et al.* 2012). Thus colonies that appear late must have gained the ability to produce tryptophan in order to form a colony, in a process that appears to be "adaptive mutation" (Rosenberg 2001; Foster 2004). As we discuss below, these late-arising colonies are likely the result of transcriptional mutagenesis followed by mutation, or retromutagenesis (Brégeon *et al.* 2003; Saxowsky and Doetsch 2006; Saxowsky *et al.* 2008).

Discussion

Oxidative damage, mutagenesis, and mismatch repair

Oxidative damage of DNA has long been known to be highly mutagenic, and as we demonstrated indirectly in yeast (Earley and Crouse 1998) and was shown specifically for

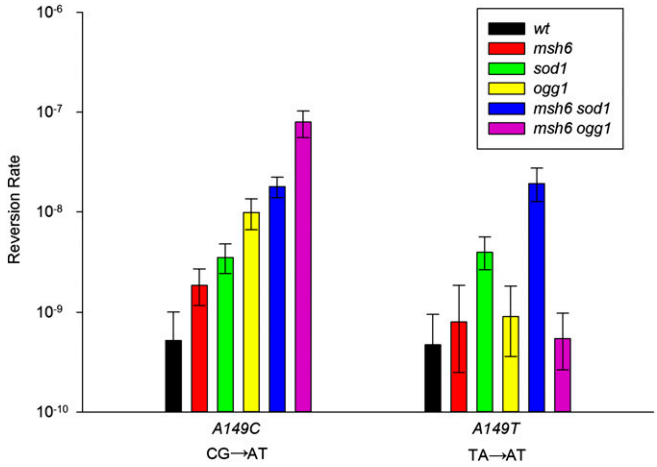

Figure 2 Spontaneous reversion analysis of *trp5* strains containing *ogg1* mutations. Reversion rates of *ogg1* strains of the indicated genotypes were determined; remaining data are from Figure 1. All strains are in F orientation; revertants were counted after 3 days on plates.

the *ogg1* mutation (Ni *et al.* 1999), MMR is very important in preventing mutation due to oxidative damage in yeast. Our original analysis of the effect of MMR on specific point mutations had measured reversion of mutations in one codon of the *CYC1* gene (Earley and Crouse 1998), whereas in the experiments here we measure reversion of mutations in one codon of the *TRP5* gene. Removal of MMR had drastically different effects in the two sets of strains, increasing reversion rates by up to orders of magnitude in the *cyc1* strains and only modestly in the *trp5* strains. We have subsequently found that the *cyc1* strains have extremely high levels of reactive oxygen species, likely accounting for much of the levels of mutagenesis seen in the absence of MMR (results not shown). In contrast, the *trp5* mutations are in an S288c background, which has a defect in Hap1, a transcription-factor-regulating response to oxygen and whose loss affects mitochondrial function, and alleles in other genes found to affect mitochondrial function (*MIP1*, *MRM1*, *ADE2*), likely resulting in levels of ROS below those of other strain backgrounds (Gaisne *et al.* 1999; Young and Court 2008). Therefore, much of the observed differences in reversion rates between the two sets of strains is likely due to endogenous levels of oxidative damage and the nature of the ROS.

In the experiments reported here, levels of endogenous ROS were substantially increased by deletion of the *SOD1* gene and those ROS levels were found to substantially increase the reversion rates of all six different mutations (Figure 1). However, for any of the strains, it is difficult to know what types of base damages are the ones likely causing the increased reversion rates. That is particularly true in considering that increased levels of ROS could also damage free nucleotides, leading to misincorporation during replication (Kamiya 2010). A variety of different structures are known to be caused by oxidative damage for all four DNA bases; their ultimate effect on mutation is a complicated process depending on their frequency of formation, miscoding

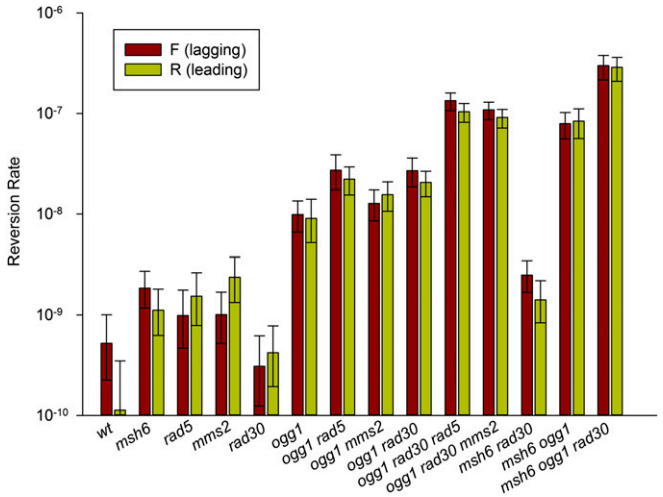

Figure 3 Spontaneous reversion analysis of *trp5–A149C* strains and the effect of 8-oxoG on reversion. Reversion rates of *trp5* strains of the indicated orientation relative to the *ARS306* origin of replication and with the indicated genotypes were determined and plotted as in Figure 1. In the F orientation, the presumed 8-oxoG would be replicated on the lagging strand.

Table 2 Relative increases in reversion rates for selected *trp5-A149C* genotypes

	Reversion Ratio	
Genotypes compared	F	R
msh6/wt	3.7*	10.0*
ogg1/wt	19.0*	830.0*
msh6 ogg1/wt	150.0*	760*
ogg1 rad30/ogg1	2.7*	2.3*
msh6 ogg1/ogg1	8.1*	9.2*
msh6 ogg1 rad30/ogg1	30.0*	32.0*
msh6 ogg1 rad30/msh6 ogg1	3.8*	3.5*
ogg1 rad5/ogg1	2.7*	2.4*
ogg1 mms2/ogg1	1.3	1.8
ogg1 rad5/ogg1 mms2	2.1*	1.4
ogg1 rad30 rad5/ogg1 rad30	5.2*	5.2*
ogg1 rad30 mms2/ogg1 rad30	4.1*	4.4*
ogg1 rad30 rad5/ogg1 rad30 mms2	1.3	1.2

For the indicated genotypes and *TRP5* orientation, the ratio of reversion rates given in Table S1 are shown. The difference is judged to be significant (*) if the 95% confidence intervals do not overlap.

potential, stability in the cell, and endogenous repair processes (Dizdaroglu 2005). The increase in reversion rate in the *trp5–A149C* strains due to oxidative damage is most likely a result of the formation of 8-oxoG, in spite of the presence of Ogg1 in the cell, although other oxidative products of G could also contribute to the same mutation (Neeley and Essigmann 2006; Dizdaroglu 2012). The increased reversion rates in the *trp5–A149G* strains (GC → AT transition) is likely due to oxidized cytosines resulting in C → T transitions (Kreutzer and Essigmann 1998). The increase in CG → GC transversions in the *trp5*–G148C strains could be due to oxidized guanines (Neeley and Essigmann 2006) or 5-OH-C (Dizdaroglu 2012). The mutations observed in the *trp5–G148A*, *trp5–G148T*, and *trp5–A149T* strains could be due to oxidized A (Dizdaroglu 2012); another possibility for the *trp5*–G148A would be a T → C transition due to a thymine glycol perhaps further converted to a urea species (McNulty *et al.* 1998). Thus all of the mutations we observe can be explained by known oxidative products, but which of the possible products are most important in leading to the mutations is for the most part not known. These experiments do demonstrate that increased oxidative damage can, in fact, lead to significant numbers of all types of mutations. We find here that MMR has a variable role in preventing mutations due to the oxidative damage, with loss of MMR showing synergistic increases in half of the strains and little increased effect over the *sod1* mutation in the others (Figure 1 and Table 1).

The advantage of a reversion assay such as the one used here is that it is possible to observe events that would be relatively rare in an entire spectrum of mutations. For example, the CG → GC transversions observed in the *msh6 trp5–G148C* strain are relatively infrequent compared to the reversion rates observed in other *msh6* strains, but can still be reliably measured. A disadvantage of reversion assays is that one observes mutation within only one sequence context. The dependence of mutation rate on sequence context is well known; the effect of sequence surrounding a mispair on MMR recognition has been well studied, for example, Marsischky and Kolodner (1999). In spite of that concern, our results clearly demonstrate two major points: increased levels of ROS can lead to all types of base pair changes, and the effect of MMR in preventing mutation due to oxidatively damaged bases is quite variable. It is important to realize that the reversion rates measure only the mispairs that *escape* cellular repair pathways. The reversion rates in *sod1* strains are increased over wild-type strains, but the increase in reversion rates in the absence of MMR demonstrates that in many cases much of the potential mutagenesis due to increased ROS was concealed by the presence of MMR. This point becomes even clearer with the study of mutagenesis due to 8-oxoG.

Analysis of defined oxidative damage: 8-oxoG

The base pair specificity of our *trp5* reversion assay allowed us to monitor only reversion events due to CG → AT mutations in the *trp5*–A149C strains. Such mutations are a signature of oxidative damage due to mutagenic replication of 8-oxoG. Elimination of *OGG1* would be expected to increase only CG → AT mutations and in addition only those CG → AT mutations due to formation of 8-oxoG; as expected we observed a large increase in the reversion rate of the *trp5–A149C* strain, but not of another *ogg1* strain (Figure 2). Additionally, there was a synergistic increase in the reversion rate of the *msh6 ogg1* strain compared to either single mutant (Table 2). The reversion rate of the *trp5–A149C ogg1* strain is approximately the same as the *trp5–A149C sod1* strain (Figure 2). Given the sensitivity of guanine to oxidative damage, there should be much more 8-oxoG formed in a *sod1* strain than in an *ogg1* strain; the high reversion rate observed in the *ogg1* strain is an indication that much of the 8-oxoG in the *sod1* strain is being

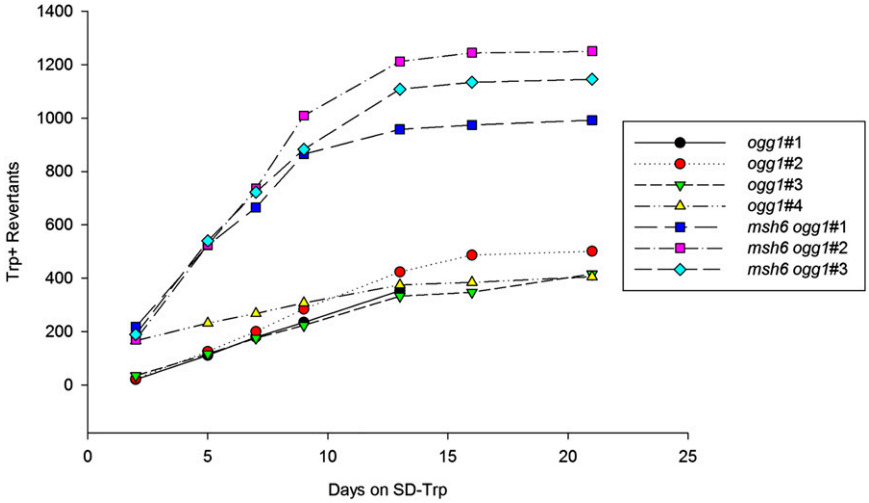

Figure 4 Trp+ revertants as a function of time on selection plates. o*gg1* and *msh6 ogg1* derivatives of *trp5–A149C F* strains were plated on SD–Trp media and colonies counted on the indicated days after plating.

repaired by the Ogg1 present in the cell, and a reminder, as noted above, that the reversion rates we measure are due to damage that has escaped the various repair mechanisms active in the cell. Similarly, the reversion rate of the *trp5–A149C ogg1* strain is much lower than that of the *trp5–A149C ogg1 msh6* strain, an indication that many of the 8-oxoG-A mispairs formed in the *ogg1* strain are repaired by MMR. Because we expect nearly all of the increased reversion events in the *trp5–A149C ogg1* strain to be due to 8-oxoG-A mispairs, which is a substrate for MMR, the increased reversion rate of the *ogg1* strain must be due to overwhelming the MMR system.

The specificity of the *trp5* reversion assay allows us to examine other components of pathways dealing with oxidative damage. One example is the translesion DNA polymerase Pol η, a product of the *RAD30* gene. Pol η is necessary for accurate bypass of 8-oxoG (Haracska *et al.* 2000; Yuan *et al.* 2000; De Padula *et al.* 2004; Carlson and Washington 2005) and thus one would expect to observe an increased reversion rate in the *trp5–A149C* strain in the absence of Pol η. However, the importance of overlapping repair functions is observed in the *trp5–A149C rad30* strains, as the reversion rates are not significantly different from wild-type rates (Figure 3). The increase in reversion rates of *ogg1* strains over wild-type and of *ogg1 msh6* strains over *msh6* strains is an indication that there is insufficient Pol η present for replication of all 8-oxoG present in the absence of Ogg1 protein. Table 2 shows that deletion of *RAD30* in *ogg1* strains increases reversion rates by 2.7- and 2.3-fold in F and R strains, deleting *MSH6* increases reversion rates by 8.1- and 9.2-fold, and deletion of both increases reversion rates by 30- and 32-fold, respectively. This synergistic increase in reversion rates is consistent with the results of Mudrak *et al.* (2009), who showed synergism of Pol η and MMR.

As mentioned above, we found evidence in other experiments that 8-oxoG could induce template switching (Rodriguez *et al.* 2013). Both the *RAD5* and *MMS2* genes are necessary for template switching (Zhang and Lawrence 2005; Branzei *et al.* 2008; Minca and Kowalski 2010). As can be seen both in Figure 3 and Table 2, loss of either *MMS2* or *RAD5* in either *ogg1* or *ogg1 rad30* strains leads to significant increases in reversion rates. Template switching is an error-free method of bypassing lesions, and these experiments indicate that the absence of template switching leads to increased mutagenesis due to the presence of 8-oxoG. Template switching appears to be more important for fidelity in *ogg1 rad30* strains, in which 8-oxoG replication is less accurate, as the increase due to loss of either *MMS2* or *RAD5* is twofold greater than the loss in *ogg1* strains.

Retromutagenesis in ogg1 strains

When we plated *trp5–A149C ogg1* and *ogg1 msh6* strains on Trp- medium, we were surprised to see colonies continue to arise even after 10 days on plates (Figure 4). Late-arising revertants were also observed in other *ogg1* backgrounds of the *trp5–A149C* strains (Figure S1), but were not inherently slow growing (Figure S2). If the late-arising colonies were due to some inherent property of the *ogg1* mutation, one would expect other *ogg1* strains to also display late-arising colonies, but that was not seen in other of our *trp5* strains such as the *trp5–A149T ogg1* and *trp5–A149T ogg1 msh6* strains, for example. The late-arising phenotype appeared to be associated with the persistence of the 8-oxoG, for strains treated with H_2O_2 displayed increased initial reversion rates, but did not show any delayed appearance on plates (Figure S1D). Another possible explanation would be that the late-arising colonies are the result of low levels of replication and resulting mutation. As we have demonstrated previously (Rodriguez *et al.* 2012) the Trp- selection for these strains is extremely tight and cells plated on Trp- medium do not even undergo one round of replication although they remain viable on the selection plates for up to 2 weeks. Although Ogg1 is the main repair mechanism for 8-oxoG-C pairs, any type of repair mechanism would presumably target the removal of the damaged base and thus prevent reversion. We have shown that MMR acting outside of the context of replication can result in nondirected repair

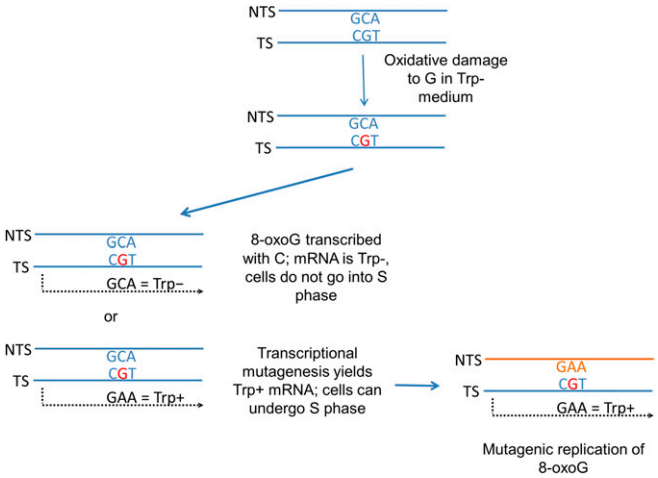

Figure 5 Model for 8-oxoG-induced retromutagenesis. The mutant codon in the *trp5–A149C* strain is shown. If the indicated G is oxidized while cells are in a nongrowth phase on SD–Trp plates, cells cannot grow due to lack of tryptophan. However, transcriptional mutagenesis of the 8-oxoG will result in Trp+ mRNA being produced, and after sufficient tryptophan is made, the cells will be able to undergo replication. If, during replication, an A is replicated opposite the 8-oxoG, a mutant, Trp+ strain, will be formed in a process known as retromutagenesis.

of mismatches (Rodriguez *et al.* 2012), but in this case MMR is not responsible for the late-arising colonies as there are substantially more late-arising revertants in the absence of MMR (Figure 4). The presence of the 8-oxoG on the transcribed strand also appears to be important. We demonstrated that we could induce reversion of *trp5–G148T* strains by electroporating 8-oxoGTP into cells (Rodriguez *et al.* 2012); in that case reversion was due to incorporation of the 8-oxoG into the nontranscribed strand. MMR-defective cells plated on Trp- plates that were not given sufficient time for a subsequent cell division after electroporation with 8-oxoGTP showed few revertants and did not accumulate any revertants over time on the plates (Rodriguez *et al.* 2012).

It has previously been shown that 8-oxoG can be mistranscribed by RNA polymerase resulting in a phenomenon known as transcriptional mutagenesis (Brégeon *et al.* 2003; Saxowsky and Doetsch 2006; Saxowsky *et al.* 2008). As illustrated in Figure 5, the target G in the *trp5–A149C* strain is on the transcribed strand; if the G at position 149 is an 8-oxoG and is transcribed with an A, the resulting mRNA will be Trp+ and when enough Trp+ mRNA accumulates, the strain would be able to begin growth. Such growth itself would not be sufficient to cause a mutation. However, misreplication of the 8-oxoG in a subsequent round of replication would produce the needed mutation. Given that these late-arising mutations depend on mistranscription before replication, the process has been called retromutagenesis (Doetsch 2002; Bregeon and Doetsch 2011). In this case, transcriptional mutagenesis would lead to the production of some wild-type Trp mRNA, which could then allow sufficient cell growth to allow a round of replication, leading to a permanent DNA change. The particular attributes of our assay have allowed us to demonstrate that retromutagenesis can be a significant cause of mutation in certain circumstances.

The advantage of a reversion assay compared to a forward mutation assay is that specific types of mutational events can be studied. In the experiments reported here, we increased ROS in the cell by inactivating *SOD1*, thus indicating both the importance of Sod1 in removing superoxide and the large increase in cellular oxidative damage in its absence. The reversion rates of all possible base–base mutations were increased in *sod1* and *sod1 msh6* strains, but the reversion rates differed by over an order of magnitude (Figure 1). Although the experiments shown in Figure 1 confirmed that MMR is important in preventing mutation due to oxidative damage, it is not possible to say exactly what damage is causing the increased reversion rates even when the base pair change is known. Elimination of *OGG1* specifically increases only the incidence of 8-oxoG opposite C, and when replicated, that mispair leads to CG → AT transversions. Our studies confirm not only that Pol η is important for accurate replication, but that it is independent of MMR. In addition, a role for template switching in accurate bypass has been observed. The particular characteristics of our assay system allow us to observe one of the first examples of retromutagenesis in a natural system.

Acknowledgments

We thank Paul Doetsch for helpful comments on the manuscript. This work was supported by National Institutes of Health grant R01 GM80754.

Literature Cited

Beard, W. A., V. K. Batra, and S. H. Wilson, 2010 DNA polymerase structure-based insight on the mutagenic properties of 8-oxoguanine. Mutat. Res. 703: 18–23.

Bjelland, S., and E. Seeberg, 2003 Mutagenicity, toxicity and repair of DNA base damage induced by oxidation. Mutat. Res. 531: 37–80.

Boiteux, S., L. Gellon, and N. Guibourt, 2002 Repair of 8-oxoguanine in *Saccharomyces cerevisiae*: interplay of DNA repair and replication mechanisms. Free Radic. Biol. Med. 32: 1244–1253.

Branzei, D., F. Vanoli, and M. Foiani, 2008 SUMOylation regulates Rad18-mediated template switch. Nature 456: 915–920.

Brégeon, D., and P. W. Doetsch, 2011 Transcriptional mutagenesis: causes and involvement in tumour development. Nat. Rev. Cancer 11: 218–227.

Brégeon, D., Z. A. Doddridge, H. J. You, B. Weiss, and P. W. Doetsch, 2003 Transcriptional mutagenesis induced by uracil and 8-oxoguanine in *Escherichia coli*. Mol. Cell 12: 959–970.

Burhans, W. C., and M. Weinberger, 2007 DNA replication stress, genome instability and aging. Nucleic Acids Res. 35: 7545–7556.

Carlson, K. D., and A. T. Washington, 2005 Mechanism of efficient and accurate nucleotide incorporation opposite 7,8-dihydro-8-oxoguanine by *Saccharomyces cerevisiae* DNA polymerase η. Mol. Cell. Biol. 25: 2169–2176.

Chang, D. J., and K. A. Cimprich, 2009 DNA damage tolerance: when it's OK to make mistakes. Nat. Chem. Biol. 5: 82–90.

Chen, Y. H., and D. F. Bogenhagen, 1993 Effects of DNA lesions on transcription elongation by T7 RNA polymerase. J. Biol. Chem. 268: 5849–5855.

Colussi, C., E. Parlanti, P. Degan, G. Aquilina, D. Barnes *et al.*, 2002 The mammalian mismatch repair pathway removes DNA 8-oxodGMP incorporated from the oxidized dNTP pool. Curr. Biol. 12: 912–918.

De Padula, M., G. Slezak, P. A. van der Kemp, and S. Boiteux, 2004 The post-replication repair *RAD18* and *RAD6* genes are involved in the prevention of spontaneous mutations caused by 7,8-dihydro-8-oxoguanine in *Saccharomyces cerevisiae*. Nucleic Acids Res. 32: 5003–5010.

D'Errico, M., E. Parlanti, and E. Dogliotti, 2008 Mechanism of oxidative DNA damage repair and relevance to human pathology. Mutat. Res. 659: 4–14.

Dizdaroglu, M., 2005 Base-excision repair of oxidative DNA damage by DNA glycosylases. Mutat. Res. 591: 45–59.

Dizdaroglu, M., 2012 Oxidatively induced DNA damage: mechanisms, repair and disease. Cancer Lett. 327: 26–47.

Doetsch, P. W., 2002 Translesion synthesis by RNA polymerases: occurrence and biological implications for transcriptional mutagenesis. Mutat. Res. 510: 131–140.

Earley, M. C., and G. F. Crouse, 1998 The role of mismatch repair in the prevention of base pair mutations in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA 95: 15487–15491.

Evans, M. D., M. Dizdaroglu, and M. S. Cooke, 2004 Oxidative DNA damage and disease: induction, repair and significance. Mutat. Res. 567: 1–61.

Fishel, M. L., M. R. Vasko, and M. R. Kelley, 2007 DNA repair in neurons: So if they don't divide what's to repair? Mutat. Res. 614: 24–36.

Foster, P. L., 2004 Adaptive mutation in *Escherichia coli*. J. Bacteriol. 186: 4846–4852.

Foster, P. L., 2006 Methods for determining spontaneous mutation rates. Methods Enzymol. 409: 195–213.

Gaisne, M., A. M. Becam, J. Verdiere, and C. J. Herbert, 1999 A 'natural' mutation in *Saccharomyces cerevisiae* strains derived from S288c affects the complex regulatory gene *HAP1* (*CYP1*). Curr. Genet. 36: 195–200.

Gralla, E. B., and J. S. Valentine, 1991 Null mutants of *Saccharomyces cerevisiae* Cu,Zn superoxide dismutase: characterization and spontaneous mutation rates. J. Bacteriol. 173: 5918–5920.

Gredilla, R., V. A. Bohr, and T. Stevnsner, 2010 Mitochondrial DNA repair and association with aging: an update. Exp. Gerontol. 45: 478–488.

Gu, Y. S., A. Parker, T. M. Wilson, H. B. Bai, D. Y. Chang *et al.*, 2002 Human MutY homolog, a DNA glycosylase involved in base excision repair, physically and functionally interacts with mismatch repair proteins human MutS homolog 2/human MutS homolog 6. J. Biol. Chem. 277: 11135–11142.

Hampsey, M., 1991 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 128: 59–67.

Haracska, L., S. L. Yu, R. E. Johnson, L. Prakash, and S. Prakash, 2000 Efficient and accurate replication in the presence of 7,8-dihydro-8-oxoguanine by DNA polymerase η. Nat. Genet. 25: 458–461.

Huang, M. E., and R. D. Kolodner, 2005 A biological network in *Saccharomyces cerevisiae* prevents the deleterious effects of endogenous oxidative DNA damage. Mol. Cell 17: 709–720.

Imlay, J. A., 2003 Pathways of oxidative damage. Annu. Rev. Microbiol. 57: 395–418.

Imlay, J. A., 2008 Cellular defenses against superoxide and hydrogen peroxide. Annu. Rev. Biochem. 77: 755–776.

Jeppesen, D. K., V. A. Bohr, and T. Stevnsner, 2011 DNA repair deficiency in neurodegeneration. Prog. Neurobiol. 94: 166–200.

Kamiya, H., 2010 Mutagenicity of oxidized DNA precursors in living cells: roles of nucleotide pool sanitization and DNA repair enzymes, and translesion synthesis DNA polymerases. Mutat. Res. 703: 32–36.

Karthikeyan, G., and M. A. Resnick, 2005 Impact of mitochondria on nuclear genome stability. DNA Repair 4: 141–148.

Kim, J., K. Chandrasekaran, and F. Morgan, 2006 Mitochondrial dysfunction, persistently elevated levels of reactive oxygen species and radiation-induced genomic instability: a review. Mutagenesis 21: 361–367.

Klungland, A., and S. Bjelland, 2007 Oxidative damage to purines in DNA: role of mammalian Ogg1. DNA Repair 6: 481–488.

Kreutzer, D. A., and J. M. Essigmann, 1998 Oxidized, deaminated cytosines are a source of C → T transitions in vivo. Proc. Natl. Acad. Sci. USA 95: 3578–3582.

Kryston, T. B., A. B. Georgiev, P. Pissis, and A. G. Georgakilas, 2011 Role of oxidative stress and DNA damage in human carcinogenesis. Mutat. Res. 711: 193–201.

Lambert, A. J., and M. D. Brand, 2009 Reactive oxygen species production by mitochondria. Methods Mol. Biol. 554: 165–181.

Li, X., and W. D. Heyer, 2008 Homologous recombination in DNA repair and DNA damage tolerance. Cell Res. 18: 99–113.

Linford, N. J., S. E. Schriner, and P. S. Rabinovitch, 2006 Oxidative damage and aging: spotlight on mitochondria. Cancer Res. 66: 2497–2499.

Liu, Y., and S. H. Wilson, 2012 DNA base excision repair: a mechanism of trinucleotide repeat expansion. Trends Biochem. Sci. 37: 162–172.

Longo, V. D., E. B. Gralla, and J. S. Valentine, 1996 Superoxide dismutase activity is essential for stationary phase survival in *Saccharomyces cerevisiae*. mitochondrial production of toxic oxygen species *in vivo*. J. Biol. Chem. 271: 12275–12280.

Lovell, M. A., and W. R. Markesbery, 2007 Oxidative DNA damage in mild cognitive impairment and late-stage Alzheimer's disease. Nucleic Acids Res. 35: 7497–7504.

Marsischky, G. T., and R. D. Kolodner, 1999 Biochemical characterization of the interaction between the *Saccharomyces cerevisiae* MSH2–MSH6 complex and mispaired bases in DNA. J. Biol. Chem. 274: 26668–26682.

Maynard, S., S. H. Schurman, C. Harboe, N. C. de Souza-Pinto, and V. A. Bohr, 2009 Base excision repair of oxidative DNA damage and association with cancer and aging. Carcinogen. 30: 2–10.

Mazurek, A., M. Berardini, and R. Fishel, 2002 Activation of human MutS homologs by 8-oxo-guanine DNA damage. J. Biol. Chem. 277: 8260–8266.

McNulty, J. M., B. Jerkovic, P. H. Bolton, and A. K. Basu, 1998 Replication inhibition and miscoding properties of DNA templates containing a site-specific cis-thymine glycol or urea residue. Chem. Res. Toxicol. 11: 666–673.

Melis, J. P., M. Luijten, L. H. Mullenders, and H. Van Steeg, 2011 The role of XPC: implications in cancer and oxidative DNA damage. Mutat. Res. 728: 107–117.

Minca, E. C., and D. Kowalski, 2010 Multiple Rad5 activities mediate sister chromatid recombination to bypass DNA damage at stalled replication forks. Mol. Cell 38: 649–661.

Mudrak, S. V., C. Welz-Voegele, and S. Jinks-Robertson, 2009 The polymerase η translesion synthesis DNA polymerase acts independently of the mismatch repair system to limit mutagenesis caused by 7,8-dihydro-8-oxoguanine in yeast. Mol. Cell. Biol. 29: 5316–5326.

Mure, K., and T. G. Rossman, 2001 Reduction of spontaneous mutagenesis in mismatch repair-deficient and proficient cells by dietary antioxidants. Mutat. Res. 480: 85–95.

Nakabeppu, Y., K. Sakumi, K. Sakamoto, D. Tsuchimoto, T. Tsuzuki *et al.*, 2006 Mutagenesis and carcinogenesis caused by the oxidation of nucleic acids. Biol. Chem. 387: 373–379.

Nash, H. M., S. D. Bruner, O. D. Scharer, T. Kawate, T. A. Addona *et al.*, 1996 Cloning of a yeast 8-oxoguanine DNA glycosylase reveals the existence of a base-excision DNA-repair protein superfamily. Curr. Biol. 6: 968–980.

Neeley, W. L., and J. M. Essigmann, 2006 Mechanisms of formation, genotoxicity, and mutation of guanine oxidation products. Chem. Res. Toxicol. 19: 491–505.

Ni, T. T., G. T. Marsischky, and R. D. Kolodner, 1999 MSH2 and MSH6 are required for removal of adenine misincorporated opposite 8-oxo-guanine in *S. cerevisiae*. Mol. Cell 4: 439–444.

Paz-Elizur, T., Z. Sevilya, Y. Leitner-Dagan, D. Elinger, L. C. Roisman *et al.*, 2008 DNA repair of oxidative DNA damage in human carcinogenesis: potential application for cancer risk assessment and prevention. Cancer Lett. 266: 60–72.

Rodriguez, G. P., N. V. Romanova, G. Bao, N. C. Rouf, Y. W. Kow *et al.*, 2012 Mismatch repair dependent mutagenesis in nondividing cells. Proc. Natl. Acad. Sci. USA 109: 6153–6158.

Rodriguez, G. P., J. B. Song, and G. F. Crouse, 2013 *In Vivo* bypass of 8-oxodG. PLoS Genet. 9: e1003682.

Rosche, W. A., and P. L. Foster, 2000 Determining mutation rates in bacterial populations. Methods 20: 4–17.

Rosenberg, S. M., 2001 Evolving responsively: adaptive mutation. Nat. Rev. Genet. 2: 504–515.

Russo, M. T., M. F. Blasi, F. Chiera, P. Fortini, P. Degan *et al.*, 2004 The oxidized deoxynucleoside triphosphate pool is a significant contributor to genetic instability in mismatch repair-deficient cells. Mol. Cell. Biol. 24: 465–474.

Saxowsky, T. T., and P. W. Doetsch, 2006 RNA polymerase encounters with DNA damage: Transcription-coupled repair or transcriptional mutagenesis? Chem. Rev. 106: 474–488.

Saxowsky, T. T., K. L. Meadows, A. Klungland, and P. W. Doetsch, 2008 8-Oxoguanine-mediated transcriptional mutagenesis causes Ras activation in mammalian cells. Proc. Natl. Acad. Sci. USA 105: 18877–18882.

Serra, V., T. Von Zglinicki, M. Lorenz, and G. Saretzki, 2003 Extracellular superoxide dismutase is a major antioxidant in human fibroblasts and slows telomere shortening. J. Biol. Chem. 278: 6824–6830.

Sherman, F., 2002 Getting started with yeast. Methods Enzymol. 350: 3–41.

Shin, C. Y., and M. S. Turker, 2002 A:T→G:C base pair substitutions occur at a higher rate than other substitution events in *Pms2* deficient mouse cells. DNA Repair 1: 995–1001.

Silverstein, T. D., R. Jain, R. E. Johnson, L. Prakash, S. Prakash *et al.*, 2010 Structural basis for error-free replication of oxidatively damaged DNA by yeast DNA polymerase η. Structure 18: 1463–1470.

Slupphaug, G., B. Kavli, and H. E. Krokan, 2003 The interacting pathways for prevention and repair of oxidative DNA damage. Mutat. Res. 531: 231–251.

Speakman, J. R., and C. Selman, 2011 The free-radical damage theory: accumulating evidence against a simple link of oxidative stress to ageing and lifespan. BioEssays 33: 255–259.

Stone, M. P., H. Huang, K. L. Brown, and G. Shanmugam, 2011 Chemistry and structural biology of DNA damage and biological consequences. Chem. Biodivers. 8: 1571–1615.

Trushina, E., and C. T. McMurray, 2007 Oxidative stress and mitochondrial dysfunction in neurodegenerative diseases. Neuroscience 145: 1233–1248.

Tsuzuki, T., Y. Nakatsu, and Y. Nakabeppu, 2007 Significance of error-avoiding mechanisms for oxidative DNA damage in carcinogenesis. Cancer Sci. 98: 465–470.

Tudek, B., A. Winczura, J. Janik, A. Siomek, M. Foksinski *et al.*, 2010 Involvement of oxidatively damaged DNA and repair in cancer development and aging. Am. J. Transl. Res. 2: 254–284.

van der Kemp, P. A., D. Thomas, R. Barbey, R. de Oliveira, and S. Boiteux, 1996 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*-methylformamidopyrimidine. Proc. Natl. Acad. Sci. USA 93: 5197–5202.

van Loon, B., E. Markkanen, and U. Hubscher, 2010 Oxygen as a friend and enemy: how to combat the mutational potential of 8-oxo-guanine. DNA Repair 9: 604–616.

Van Raamsdonk, J. M., and S. Hekimi, 2012 Superoxide dismutase is dispensable for normal animal lifespan. Proc. Natl. Acad. Sci. USA 109: 5785–5790.

Ventura, I., M. T. Russo, L. G. De, and M. Bignami, 2010 Oxidized purine nucleotides, genome instability and neurodegeneration. Mutat. Res. 703: 59–65.

Viswanathan, A., and P. W. Doetsch, 1998 Effects of nonbulky DNA base damages on *Escherichia coli* RNA polymerase-mediated elongation and promoter clearance. J. Biol. Chem. 273: 21276–21281.

Wallace, S. S., 2002 Biological consequences of free radical-damaged DNA bases. Free Radic. Biol. Med. 33: 1–14.

Williams, T.-M., R. M. Fabbri, J. W. Reeves, and G. F. Crouse, 2005 A new reversion assay for measuring all possible base pair substitutions in *Saccharomyces cerevisiae*. Genetics 170: 1423–1426.

Winzeler, E. A., D. D. Shoemaker, A. Astromoff, H. Liang, K. Anderson *et al.*, 1999 Functional characterization of the *S. cerevisiae* genome by gene deletion and parallel analysis. Science 285: 901–906.

Wyrzykowski, J., and M. R. Volkert, 2003 The *Escherichia coli* methyl-directed mismatch repair system repairs base pairs containing oxidative lesions. J. Bacteriol. 185: 1701–1704.

Young, M. J., and D. A. Court, 2008 Effects of the S288c genetic background and common auxotrophic markers on mitochondrial DNA function in *Saccharomyces cerevisiae*. Yeast 25: 903–912.

Yuan, F. H., Y. B. Zhang, D. K. Rajpal, X. H. Wu, D. Y. Guo *et al.*, 2000 Specificity of DNA lesion bypass by the yeast DNA polymerase η. J. Biol. Chem. 275: 8233–8239.

Zahn, K. E., S. S. Wallace, and S. Doublie, 2011 DNA polymerases provide a canon of strategies for translesion synthesis past oxidatively generated lesions. Curr. Opin. Struct. Biol. 21: 358–369.

Zhang, H., and C. W. Lawrence, 2005 The error-free component of the *RAD6/RAD18* DNA damage tolerance pathway of budding yeast employs sister-strand recombination. Proc. Natl. Acad. Sci. USA 102: 15954–15959.

Zheng, Q., 2002 Statistical and algorithmic methods for fluctuation analysis with SALVADOR as an implementation. Math. Biosci. 176: 237–252.

Zheng, Q., 2005 New algorithms for Luria–Delbruck fluctuation analysis. Math. Biosci. 196: 198–214.

Zheng, Q., 2007 On Haldane's formulation of Luria and Delbruck's mutation model. Math. Biosci. 209: 500–513.

Zlatanou, A., E. Despras, T. Braz-Petta, I. Boubakour-Azzouz, C. Pouvelle *et al.*, 2011 The hMsh2-hMsh6 complex acts in concert with monoubiquitinated PCNA and Pol η in response to oxidative DNA damage in human cells. Mol. Cell 43: 649–662.

Communicating editor: N. Hunter

GENETICS

Supporting Information
http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.113.153874/-/DC1

Oxidative Damage and Mutagenesis in *Saccharomyces cerevisiae*: Genetic Studies of Pathways Affecting Replication Fidelity of 8-Oxoguanine

Arthur H. Shockley, David W. Doo, Gina P. Rodriguez, and Gray F. Crouse

DOI: 10.1534/genetics.113.153874

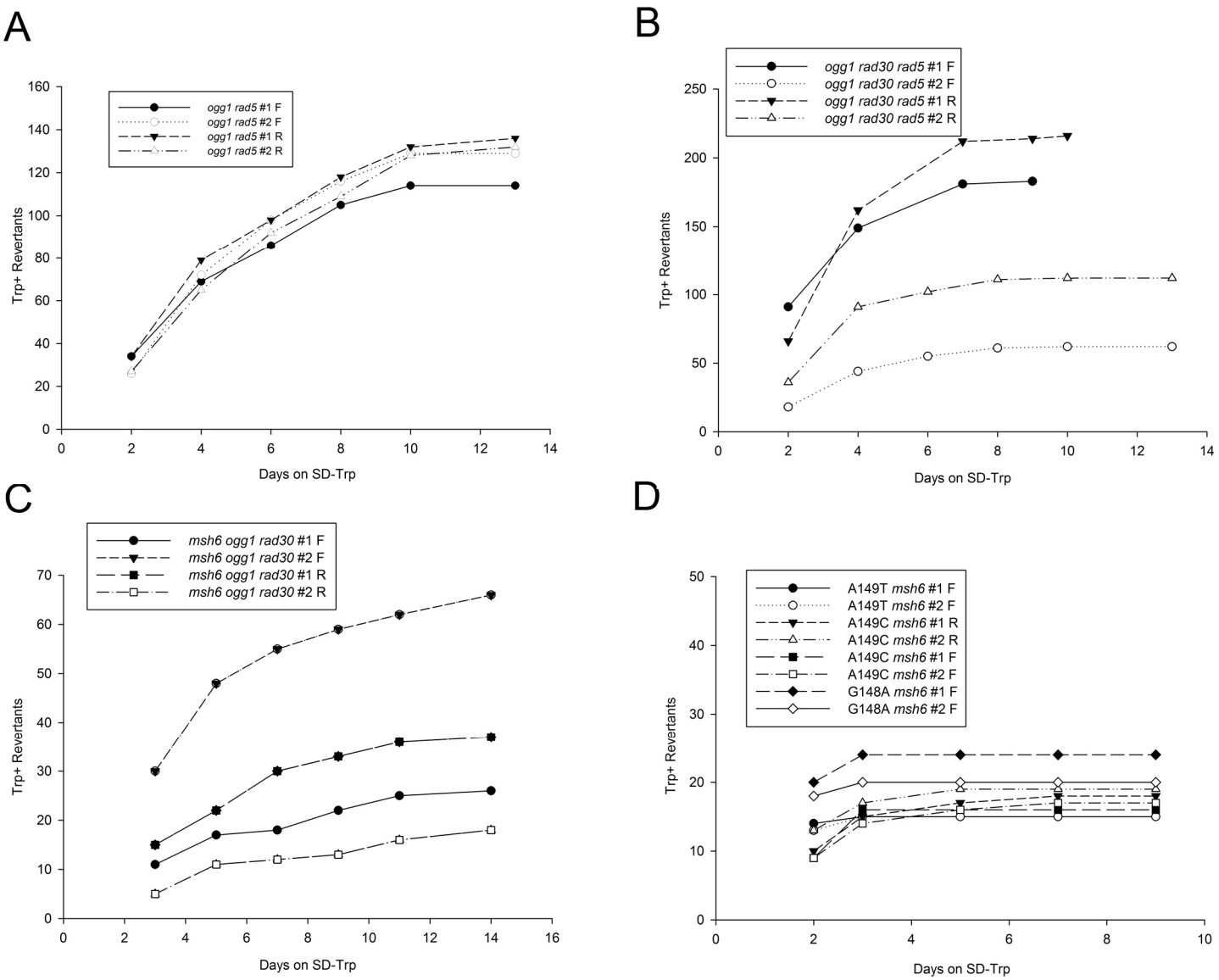

Figure S1 Trp+ revertants as a function of time on selection plates. (A-C) *trp5-A149C F* strains of the indicated genotypes were plated on SD-Trp media and colonies counted on the indicated days after plating. The absolute number of revertants on a particular plate cannot be compared across genotypes. (D) *msh6* strains with the indicated *TRP5* mutation and orientation were treated with 20mM H_2O_2 for 1 hr at 30°, plated on SD-Trp plates, and the number of revertants determined over time.

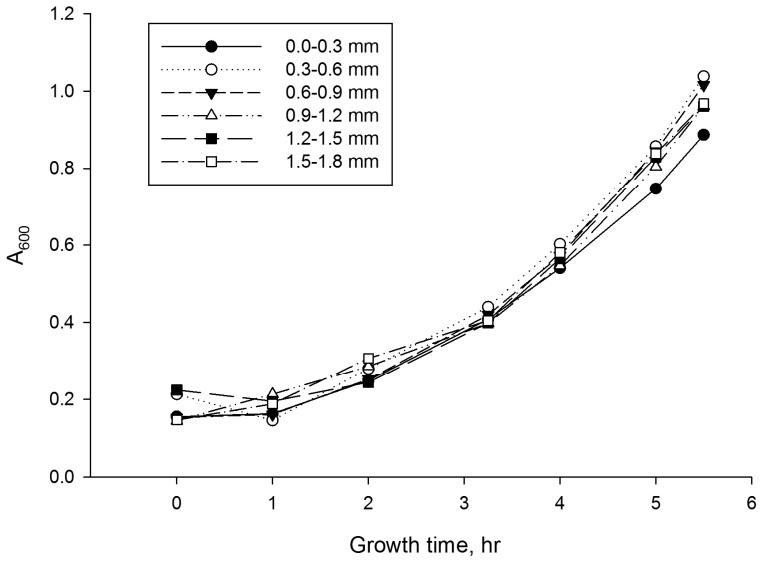

Figure S2 Growth rate of late-arising colonies. Cells from a *trp5-A149C ogg1 msh6* F strain were plated and revertants allowed to develop over a period of 2 weeks. Colony size was measured using a ProtoCOL 2 colony counter (Synbiosis) and colonies of the indicated sizes were picked and their growth rate measured in YPD as shown.

Table S1 Strain genotypes

Strain number	*TRP5* orientation	Relevant Genotype
GCY1675	F	*MATα his3Δ200 ura3-52 leu2Δ1 trp5-A149C*
GCY2038	F	GCY1675 *msh6Δ::kanMX*
GCY1984	F	GCY1675 *sod1Δ::kanMX*
GCY2868	F	GCY1675 *rad5Δ::his5MX*
GCY2869	F	GCY1675 *mms2Δ::his5MX*
GCY1925	F	GCY1675 *rad30Δ::kanMX*
GCY1709,1710	F	GCY1675 *ogg1Δ::kanMX*
GCY2162	F	GCY1675 *msh6Δ::kanMX sod1Δ::hygMX*
GCY2650	F	GCY1675 *ogg1Δ::kanMX rad5Δ::his5MX*
GCY2871	F	GCY1675 *ogg1Δ::kanMX mms2Δ::his5MX*
GCY2501	F	GCY1675 *ogg1Δ::kanMX rad30Δ::natMX*
GCY2461	F	GCY1675 *msh6Δ::hygMX rad30Δ::kanMX*
GCY2274	F	GCY1675 *msh6Δ::hygMX ogg1Δ::kanMX*
GCY2816	F	GCY1675 *ogg1Δ::kanMX rad30Δ::natMX rad5Δ::his5MX*
GCY2880	F	GCY1675 *ogg1Δ::kanMX rad30Δ::natMX mms2Δ::his5MX*
GCY2502	F	GCY1675 *msh6Δ::hygMX ogg1Δ::kanMX rad30Δ::natMX*
GCY1663	F	*MATα his3Δ200 ura3-52 leu2Δ1 trp5-A149G*
GCY2184	F	GCY1663 *msh6Δ::kanMX*
GCY1981	F	GCY1663 *sod1Δ::kanMX*
GCY2199	F	GCY1633 *msh6Δ::kanMX sod1Δ::hygMX*
GCY1903	F	*MATα his3Δ200 ura3-52 leu2Δ1 trp5A-149T*
GCY2031	F	GCY1903 *msh6Δ::kanMX*
GCY2192	F	GCY1903 *msh6Δ:: kanMX*
GCY1995	F	GCY1903 *sod1Δ::kanMX*
GCY2266	F	GCY1903 *ogg1Δ::kanMX*
GCY2235	F	GCY1903 *msh6Δ::kanMX sod1Δ::hygMX*
GCY2276	F	GCY1903 *msh6Δ::loxP ogg1Δ::kanMX*
GCY1868	F	*MATα his3Δ200 ura3-52 leu2Δ1 trp5-G148A*
GCY2037	F	GCY1868 *msh6Δ::kanMX*
GCY2178	F	GCY1868 *sod1Δ::hygMX*
GCY2161	F	GCY1868 *msh6Δ::kanMX sod1Δ::hygMX*
GCY1756	F	*MATα his3Δ200 ura3-52 leu2Δ1 trp5-G148C*
GCY2141	F	GCY1756 *msh6Δ::kanMX*
GCY2193	F	GCY1756 *sod1Δ::hygMX*
GCY2194	F	GCY1756 *msh6Δ::kanMX sod1Δ::hygMX*

GCY1862	F	*MATα his3Δ200 ura3-52 leu2Δ1 trp5-G148T*
GCY2036	F	GCY1862 *msh6Δ::kanMX*
GCY1989	F	GCY1862 *sod1Δ::kanMX*
GCY2160	F	GCY1862 *msh6Δ::kanMX sod1Δ::hygMX*
GCY1649	R	*MATα his3Δ200 ura3-52 leu2Δ1 trp5-A149C*
GCY2043	R	GCY1649 *msh6Δ::kanMX*
GCY2867	R	GCY1649 *rad5Δ::his5MX*
GCY2889	R	GCY1649 *mms2Δ::his5MX*
GCY2447	R	GCY1649 *rad30Δ::kanMX*
GCY1707,1708	R	GCY1649 *ogg1Δ::kanMX*
GCY2651	R	GCY1649 *ogg1Δ::kanMX rad5Δ::his5MX*
GCY2870	R	GCY1649 *ogg1Δ::kanMX mms2Δ::his5MX*
GCY2500	R	GCY1649 *ogg1Δ::kanMX rad30Δ::natMX*
GCY2285	R	GCY1649 *msh6Δ::loxP ogg1Δ::kanMX*
GCY2462	R	GCY1649 *msh6Δ::hygMX rad30Δ::kanMX*
GCY2815	R	GCY1649 *ogg1Δ::kanMX rad30Δ::natMX rad5Δ::his5MX*
GCY2879	R	GCY1649 *ogg1Δ::kanMX rad30Δ::natMX mms2Δ::his5MX*
GCY2503	R	GCY1649 *msh6Δ::loxP ogg1Δ::kanMX rad30Δ::natMX*

All strains are derivatives of *S. cerevisiae* GCY1487 (SJR828a) [*MATα his3Δ200 ura3-52 leu2* Δ1] and the description of the *trp5* mutations and orientation are contained in (Williams *et al.* 2005)

Williams, T.-M., R. M. Fabbri, J. W. Reeves, and G. F. Crouse, 2005 A new reversion assay for measuring all possible base pair substitutions in *Saccharomyces cerevisiae*. Genetics **170**: 1423-1426.

Table S2 Reversion rates of *trp5-A149C* strains

	F	R
wt	0.52 (0.22, 1.0)	0.11 (0.02, 0.35)
msh6	1.9 (1.2, 2.7)	1.1 (0.62, 1.8)
rad5	1.0 (0.47, 1.8)	1.5 (0.78, 2.6)
mms2	1.0 (0.52, 1.7)	2.4 (1.3, 3.7)
rad30	0.31 (0.12, 0.62)	0.42 (0.19, 0.78)
ogg1	9.9 (6.6, 13.)*	9.1 (5.2, 14.)*
ogg1 rad5	27. (18., 39.)*	22. (15., 30.)*
ogg1 mms2	13. (8.6., 17.)*	16. (10., 21.)*
ogg1 rad30	27. (19., 36.)*	21. (15., 27.)*
ogg1 rad30 rad5	140 (100, 160)	110 (82, 130)*
ogg1 rad30 mms2	110 (87., 130)	92. (72., 110.)
msh6 rad30	2.5 (1.7, 3.5)	1.4 (0.83, 2.2)
msh6 ogg1	80. (56., 100)*	84 (56, 110)*
msh6 ogg1 rad30	300 (220, 380)	290 (210, 360)

Reversion rates are those shown in Figure 3. All reversion rates have been multiplied by 1×10^{9}. Shown in parentheses are 95% confidence intervals. For some genotypes, multiple independent reversion experiments were performed, in which case the median result is indicated by *. In the F orientation, the presumed 8-oxoG would be replicated on the lagging strand.