DNA repair: **How yeast repairs radical damage**
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Cloning of the *OGG1* gene from *Saccharomyces cerevisiae* has revealed that DNA glycosylases are not necessarily conserved throughout phylogeny, yet there is a DNA-repair protein superfamily with a wide substrate specificity found from bacteria to man.

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All organisms must contend with the cellular damage caused by reactive oxygen species and free radicals produced by ionizing radiation and other free radical generators. One line of defense is the removal or inactivation of these highly reactive species. A second line of defense is the repair of the damage inflicted on a number of cellular targets. Damaged DNA must be repaired to prevent lethal and mutagenic events. Guanine is a prime site for oxidative attack and the resulting product, 8-oxoguanine (^oG), is highly mutagenic because of its propensity to mispair with adenine during replication. Studies with bacteria have revealed a complex system that both repairs such lesions in DNA and sanitizes the nucleotide precursor pool, where d^{o}GTP can accumulate and be incorporated into DNA by replicative polymerases. Now work on the budding yeast *Saccharomyces cerevisiae* has shown that eukaryotes repair ^oG using DNA glycosylases that are unrelated to their bacterial equivalents but that are members of a conserved DNA-repair protein superfamily.

Repair of ^oG in bacteria

Extensive studies carried out with *Escherichia coli* have characterized the ^oG repair enzymes and have also revealed, through genetic studies, the importance of this pathway for preventing mutagenesis during oxidative stress. Mutagenesis can occur *via* two routes. ^oG can be formed directly in DNA and will mispair with dAMP when the DNA is replicated. Replicative polymerases show a much higher incorporation rate of dAMP opposite a ^oG residue than do repair polymerases, which tend to insert dCMP [1]. Thus, replication of damaged DNA is mutagenic and leads to G:C→T:A transversions. Oxidative attack on dGTP creates d^{o}GTP, which can be inserted into newly synthesized DNA by replicative polymerases. This damaged triphosphate is incorporated opposite A and C at approximately equal frequencies, giving rise to A:T→C:G transversions [2].

Figure 1
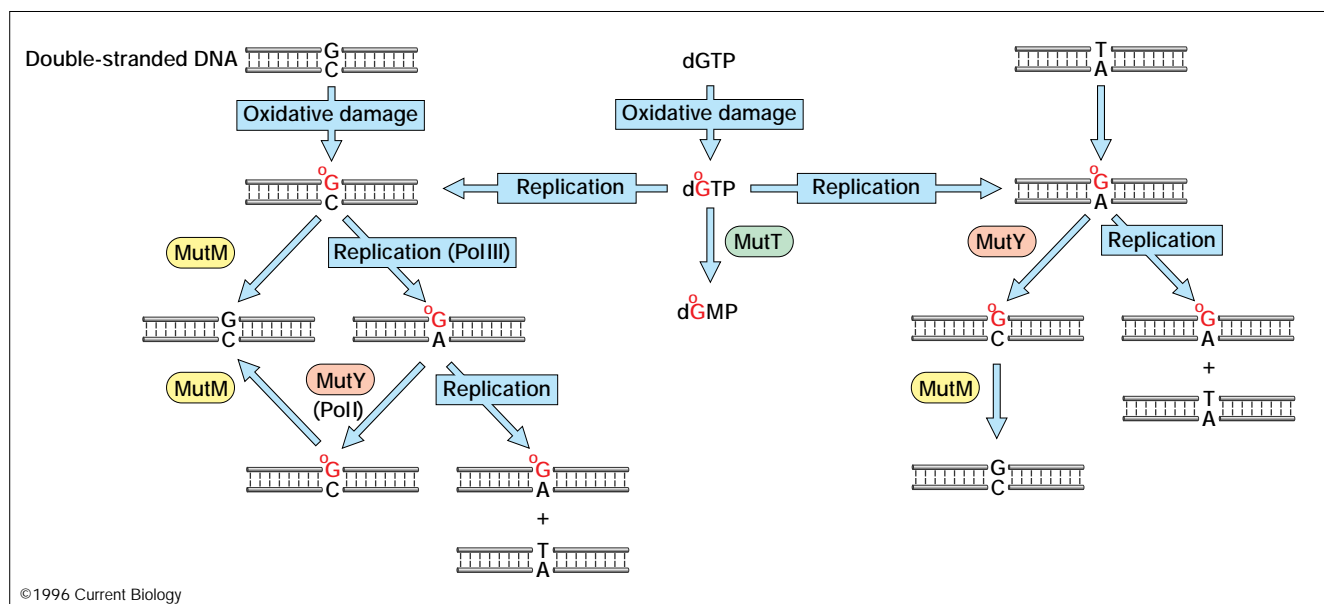
E. coli pathways leading to the repair of ^oG lesions in DNA or the removal of d^{o}GTP from the dNTP pool. Repair of ^oG eliminates the potential for G:C→A:T transversions. Removal of d^{o}GTP eliminates the potential for A:T→G:C transversions. The action of MutM and MutY proteins is mutagenic once d^{o}GTP is incorporated into DNA, as A:T→G:C transversions are fixed by these enzymes.

Biochemical and genetic studies in *E. coli* have converged in a most satisfying way to give us a fairly complete view of the repair of oxidative damage to guanine [3–6] (Fig. 1). Genetic studies have defined a number of mutator genes, three of which show a mutational specificity consistent with the transversions expected from oxidative damage to guanine, either in DNA or the nucleotide precursor pool. These three genes, *mutT, mutM* and *mutY*, have been shown to code for enzymes which were identified on the basis of their ability to act upon damaged guanine in DNA and in deoxyribonucleoside triphosphates.

The product of *mutT* is a nucleoside triphosphatase that can hydrolyze d^{o}GTP and eliminate it from the nucleoside precursor pool. Mutants carrying *mutT* exhibit an approximately 1000-fold increase in A:T→C:G transversions. The product of the *mutM* gene is a DNA glycosylase/AP (apurinic/apyrimidinic) lyase which removes ^{o}G from a ^{o}G:C base pair, the primary damage formed in DNA by oxidative attack. DNA glycosylases are enzymes which cleave the glycosyl bond of a nucleotide subunit in DNA, releasing a free base (Fig. 2). The enzyme also has an additional activity, the AP lyase activity, which promotes a β,δ elimination event resulting in a one nucleotide gap in DNA bordered on both sides by phosphoryl groups (Fig. 2). Mutants deficient in this glycosylase/lyase activity show slightly enhanced mutation frequencies.

The product of the *mutY* gene is a ^{o}G:A mismatch-specific adenine-DNA glycosylase, which functions to counter the mutagenic potential of the ^{o}G:A base pairs that arise when damaged DNA is replicated before the *mutM* gene product has functioned. The removal of ^{o}G, which is the damaged base, at this point would be counterproductive, as it would leave an A in the DNA to be repaired to an A:T base pair, thus fixing a G:C→T:A transversion. Instead, the A is removed and the ^{o}G lesion serves as the template for a repair polymerase, which usually inserts a C opposite the ^{o}G, creating a ^{o}G:C base pair which can then be processed by the *mutM* gene

product to yield the original G:C base pair. Mutants deficient in the *mutY* gene product show a slightly enhanced mutation frequency; however, when *mutY* and *mutM* mutations are combined, the double mutant shows an extremely high mutation rate, consistent with the idea that the two gene products work synergistically to counter the mutagenic potential of ^{o}G in DNA.

Repair of ^{o}G in yeast

The enzymes involved in the repair of oxidized guanines in yeast are the focus of two recent papers [7,8] that point

Figure 2

An abbreviated reaction mechanism for the yeast Ogg1 protein. The enzyme is shown acting on an oligonucleotide containing an ^{o}G residue. All DNA glycosylase/AP lyases use a similar reaction mechanism, which includes the formation of an *N*-acyl iminium ion intermediate (**1**). Normally these enzymes carry out a β- or a β,δ-elimination, leading to a strand break. Ogg1 is shown carrying out a β,δ-elimination, leading to a one nucleotide gap bordered by two phosphoryl groups. When the *N*-acyl iminium ion intermediate is intercepted by a strong reducing agent, such as sodium borohydride, a stable amine is formed (**2**) and the protein–DNA complex is trapped. This complex can then be run on a polyacrylamide gel and visualized by protein stain or autoradiography. This protocol allows for the determination of the number and molecular size of all DNA glycosylase/AP lyases in a crude cell extract.
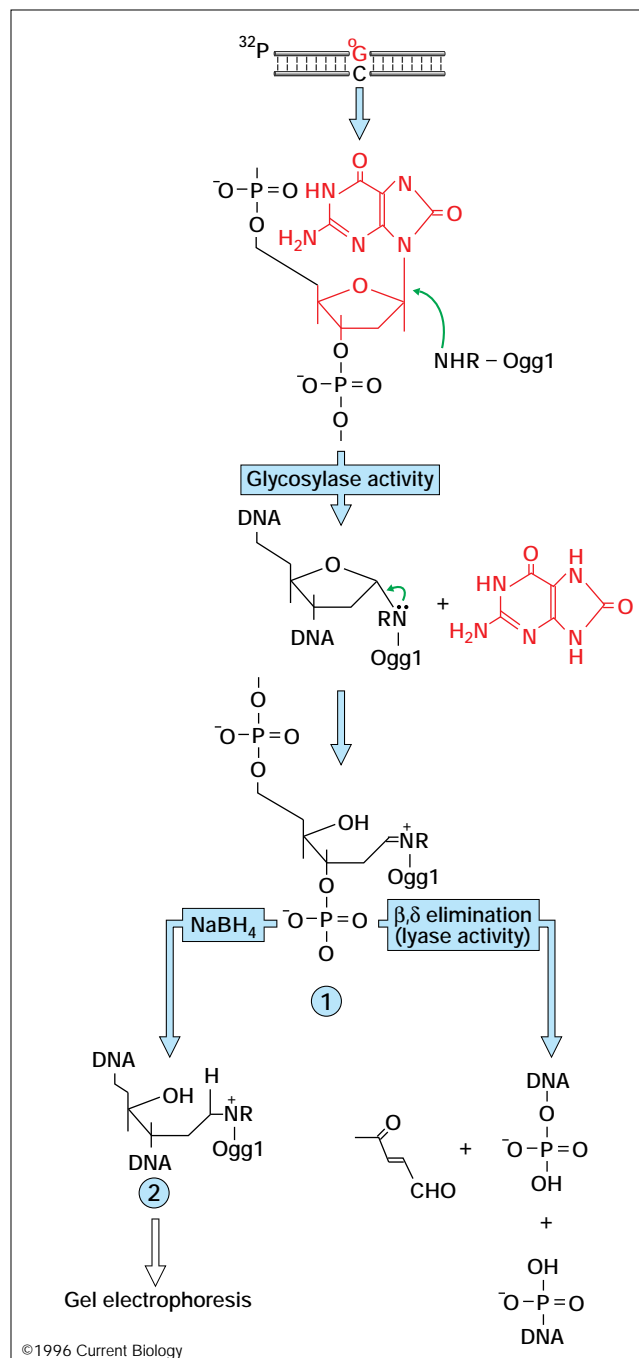
out several intriguing similarities and differences between this eukaryotic organism and the prokaryote *E. coli*. Two distinct approaches were used to identify enzymes from yeast that act at ^{o}G lesions in DNA. Using a genetic approach, van der Kamp *et al.* [7] cloned a gene encoding an ^{o}G DNA glycosylase from yeast. Using a biochemical approach, Nash *et al.* [8] identified a protein which acts upon ^{o}G-containing DNA; protein sequencing then allowed identification of the gene coding for this protein. Both groups identified the gene *OGG1* (named for its product 8-oxoguanine glycosylase).

In their genetic screen of a yeast genome library, van der Kamp *et al.* [7] looked for DNA able to suppress the excess G:C→T:A transversions in a *mutM mutY* double mutant of *E. coli*. They then expressed the cloned gene and characterized the encoded protein, Ogg1, as an 8-oxoguanine glycosylase. Ogg1 was also found to share with the *E. coli* MutM protein the ability to remove from DNA the ring fragmented form of guanine, 2,6-diamino-4-hydroxy-5-*N*-methylformamidopyrimidine, that is formed by oxidative stress. Both ^{o}G:C and ^{o}G:T base pairs serve as substrate for Ogg1, whereas ^{o}G:G is only slowly repaired and ^{o}G:A is not detectably repaired. These substrate specificities are similar to those of MutM, which repairs the ^{o}G:C lesion formed by primary attack on the DNA, but not the ^{o}G:A lesion that results from replication of the primary lesion and is the substrate of MutY. The sequence of Ogg1 shows no similarity to that of MutM. As Ogg1 is not homologous to MutM, and as no gene encoding a MutY-like protein was recovered from the genetic screen — either a MutY- or MutM-like activity would be expected to suppress the rate of G:C→T:A transversions in a *mutY mutM* double mutant — it is possible that yeast has a different strategy to that of *E. coli* for dealing with ^{o}G.

Nash *et al.* [8] used a biochemical approach to determine whether yeast cells contain an 8-oxoguanine glycosylase/AP lyase activity similar to that found in *E. coli*. Several groups have shown that glycosylase/AP lyases [9–13] share a common mechanism in which the enzyme forms an *N*-acyl iminium ion intermediate (**1** in Fig. 2) that results in a transient covalent attachment between protein and DNA. This transient intermediate can be reduced with borohydride to yield a stable amine (**2** in Fig. 2). This so called trapping assay results in a stable complex which can then be analyzed by gel electrophoresis. This protocol has been used to identify a mammalian homologue of *E. coli* endonuclease III in a partially purified enzyme preparation from calf thymus [13]. Nash *et al.* [8] have now demonstrated that this assay works in crude extracts, and used it to identify two 8-oxoguanine glycosylase/AP lyases in yeast. They have also shown that this assay can be used to determine substrate specificities for these enzymes directly in crude extracts by measuring the competition between oligonucleotides bearing various substrate base pairs.

Using this competition assay, Nash *et al.* [8] found that one of the activities from yeast preferred a ^{o}G:C substrate, whereas the other preferred a ^{o}G:G substrate. They also used the competition assay to determine if the activities bound strongly to non-cleavable substrates. Several damage-mimetic non-cleavable oligonucleotides have been analyzed and shown to bind AP lyases quite tightly. The ^{o}G:C-specific glycosylase from yeast binds tightly to an oligonucleotide containing a reduced AP site. A reduced-AP-site affinity column was then used to purify the yeast glycosylase so that a single band could be identified and microsequenced. Peptide sequences from this protein matched an open reading frame in the yeast genome identical to the *OGG1* gene identified by van der Kamp *et al.* [7]. The *OGG1* gene was cloned and its protein product overexpressed and purified for further study. This glycosylase shows the following cleavage specificity: ^{o}G:C > ^{o}G:T >> ^{o}G:G > ^{o}G:A, as does the *E. coli* MutM protein. A targeted disruption of the *OGG1* gene was created, and mutant cells shown to have only one crosslinking activity, corresponding to the enzyme that prefers the ^{o}G:G base pairs as substrates, but also works quite well on ^{o}G:A base pairs.

The pathway for the repair of oxidized guanines in yeast is partially illuminated by these two papers [7,8]. It is clear that yeast has an 8-oxoguanine glycosylase with a substrate specificity similar to that of *E. coli* MutM. It also appears that yeast has a second 8-oxoguanine glycosylase, which prefers ^{o}G:G and ^{o}G:A substrates. This second enzyme is a bit of a mystery, as ^{o}G:G base pairs are not expected to be physiologically relevant; ^{o}G:A base pairs do, however, occur *in vivo*. It may also be relevant that the second enzyme has a much higher activity on 2,6-diamino-4-hydroxy-5-*N*-methylformamidopyrimidine residues [14]. Yeast may thus have two separate enzymes to deal with oxidized guanines, whereas *E. coli* manages with MutM alone. It is also possible that yeast has evolved a different strategy to deal with ^{o}G. In *E. coli*, MutY protein is necessary because MutM does not appear to repair all lesions before replication. If MutM were more efficient, MutY would not be necessary, and its absence would eliminate the mutagenic potential when d^{o}GTP is incorporated during replication (Fig. 1). In fact, an enzyme that repairs the ^{o}G in a ^{o}G:A mismatch would be anti-mutagenic in the presence of highly efficient MutM (see Fig. 1).

It has not yet been demonstrated that *S. cerevisiae* has a MutY protein. There is no open reading frame in the yeast genome that codes for a protein homologous to MutY. As Ogg1 and MutM are not homologous, however, this observation may not be meaningful. Further genetic

and biochemical studies will be necessary to define fully the repair pathway for ^{o}G in *S. cerevisiae*.

Perspectives

The procedure outlined in the paper by Nash *et al.* [8] will surely be repeated many times in the near future, as it is an extremely powerful way to determine the number of glycosylase/AP lyases in any organism. The assay will work for any damaged base that can be introduced into an oligonucleotide, and it seems that clever organic chemists will soon synthesize a wide variety of phosphoramadites with such bases. Substrate specificities can be readily determined, and competition assays can also define the damage-mimetic non-cleavable substrate best suited for purification of the enzyme by affinity chromatography. Armed with a set of appropriate substrates and inhibitors, one can readily determine the repair capacity of any cell line and easily purify the enzymatic complement of that particular cell line.

A final observation made by Nash *et al.* [8] is also of great interest. They note that Ogg1 has a potential helix––hairpin–helix (HhH) motif. This motif was first recognized in endonuclease III and several of its homologues, which all are [4Fe–4S] cluster enzymes [15]. Recently, the crystal structure of 3-methyladenine DNA glycosylase II (AlkA) from *E. coli* was solved [16], and the protein was found to share a common core structure with endonuclease III, including the HhH motif but not the [4Fe–4S] cluster. Ogg1 appears also to share this common core structure. Thus, there appears to be a superfamily of DNA repair enzymes, based on the HhH motif and a common catalytic mechanism, present throughout phylogeny, from bacteria to man. So far this superfamily has close to twenty members, with a number of substrate specificities. Although the *E.coli* 8-oxoguanine glycosylase is a zinc-finger protein with no similarity to Ogg1, they have remarkably similar properties. It will be of great interest to see if there is also a MutM superfamily of repair enzymes and, if so, how its members are distributed throughout phylogeny.

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