Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 7022–7025, August 1992
Biochemistry

Evidence that MutY and MutM combine to prevent mutations by an oxidatively damaged form of guanine in DNA

(8-oxo-7,8-dihydroguanine/8-hydroxyguanine/DNA glycosylase/Fpg protein/mismatch repair)

MARK LEO MICHAELS*, CHRISTINA CRUZ*, ARTHUR P. GROLLMAN†, AND JEFFREY H. MILLER*‡

*Department of Microbiology and Molecular Genetics and the Molecular Biology Institute, University of California, Los Angeles, CA 90024; and †Department of Pharmacological Sciences, State University of New York, Stony Brook, NY 11794-8651

Communicated by Charles Yanofsky, May 6, 1992 (received for review January 30, 1992)

ABSTRACT **It has been previously shown both *in vivo* and *in vitro* that DNA synthesis past an oxidatively damaged form of guanine, 7,8-dihydro-8-oxoguanine (8-oxoG), can result in the misincorporation of adenine (A) opposite the 8-oxodG. In this study we show that MutY glycosylase is active on a site-specific, oxidatively damaged A/8-oxoG mispair and that it removes the undamaged adenine from this mispair. Strains that lack active MutY protein have elevated rates of G·C → T·A transversions. We find that the mutator phenotype of a *mutY* strain can be fully complemented by overexpressing MutM protein (Fpg protein) from a plasmid clone. The MutM protein removes 8-oxoG lesions from DNA. In addition, we have isolated a strain with a chromosomal mutation that suppresses the *mutY* phenotype and found that this suppressor also overexpresses MutM. Finally, a *mutY mutM* double mutant has a 25- to 75-fold higher mutation rate than either mutator alone. The data strongly suggest that MutY is part of an intricate repair system directed against 8-oxoG lesions in nucleic acids and that the primary function of MutY *in vivo* is the removal of adenines that are misincorporated opposite 8-oxoG lesions during DNA synthesis.**

All organisms that use molecular oxygen need to defend themselves from the reactive byproducts of oxygen metabolism. Reactive oxygen species such as superoxide, hydrogen peroxide, and hydroxyl radical can be produced by the incomplete reduction of oxygen during aerobic metabolism (1). These oxidants can also be generated from lipid peroxidation, from phagocytic cells, and by exposure to radiation (1). Although organisms have developed systems to control active oxygen species, those that escape the primary defenses can damage nucleic acids and other cellular macromolecules.

Oxidative damage to DNA has been estimated at 10^4 lesions per cell per day in humans and an order of magnitude higher in rodents (2). Not surprisingly, organisms have developed a second line of defense to repair the oxidative damage to DNA. One of the lesions that is actively removed is 7,8-dihydro-8-oxoguanine (8-oxoG, also termed 8-hydroxyguanine). The lesion is removed by the glycosylase/apurinic endonuclease activity of the MutM protein [Fpg protein or 8-oxoG glycosylase] (3, 4). Recently, it has been shown that DNA synthesis past 8-oxoG can result in the misincorporation of adenine opposite the damaged guanine (5–7) and that inactivation of the *mutM* (*fpg*) gene leads specifically to G·C → T·A transversions (4, 8).

In this paper we present evidence that *Escherichia coli* has, at least in the case of the 8-oxoG lesion, a third line of defense against oxidative damage to DNA. Unlike the first two forms of protection, which target the active oxygen species or the damage it inflicts on nucleic acids, this activity leads to the correction of errors that are induced by replication of templates containing 8-oxoG lesions. We find that MutY protein, originally identified as an adenine glycosylase active on A/G mispairs (9), can also remove the undamaged adenine from A/8-oxoG mispairs. Strains that lack active MutY protein have elevated rates of G·C → T·A transversions (10). Overexpressing the MutM protein from a plasmid clone can completely complement the mutator phenotype of a *mutY* strain. We have isolated a strain with a chromosomal mutation that suppresses *mutY* and found that it has 15-fold greater MutM activity than the parental strain. Finally, a *mutM mutY* double mutant shows a 20-fold higher rate of G·C → T·A transversions than the sum of G·C → T·A transversions in either mutant alone. These observations suggest that the primary function of MutY glycosylase *in vivo* is the removal of adenines that have been misincorporated opposite 8-oxoG lesions during DNA replication, and that MutY and MutM are part of a multiple line of defenses against oxidative damage to DNA.

MATERIALS AND METHODS

Oligodeoxynucleotides. The 23-mer oligodeoxynucleotides, including the one containing the site-specific 8-oxoG lesion, were synthesized and purified as described (6, 11). The purified oligomers (4 pmol) were ^{32}P-labeled at the 5′ terminus with 10 units of T4 polynucleotide kinase in the presence of 7 pmol of [γ-^{32}P] ATP (6 Ci/μmol; New England Nuclear; 1 Ci = 37 GBq) for 10 min at 37°C. After heat inactivation at 90°C for 7 min, the labeled primers were annealed with an excess of unlabeled complementary oligodeoxynucleotide at 90°C in 50 mM NaCl for 2 min, cooled to room temperature, and then incubated on ice. The sequences of the oligodeoxynucleotides are shown below (nucleotides involved in mispair formation are underlined; GO, 8-oxoG).

(1) 5′-CTCTCCCTTCGOCTCCTTTCCTCT-3′

(2) 5′-CTCTCCCTTCGCTCCTTTCCTCT-3′

(3) 5′-AGAGGAAAGGAGAGAAGGGAGAG-3′

(4) 5′-AGAGGAAAGGAGCGAAGGGAGAG-3′

MutY Glycosylase Assay. Glycosylase reactions were carried out in a solution (10 μl) containing 20 mM Tris·HCl (pH 7.6), 0.5 μg of bovine serum albumin, 10 mM EDTA, 45 ng of MutY glycosylase [purified essentially as previously described (9)], and 20 fmol of 23-mer duplex with the indicated mispair. After incubation at 37°C for 30 min, reactions were terminated by the addition of 2 μl of 1 M NaOH and incubation

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: 8-oxoG, 7,8-dihydro-8-oxoguanine.

‡To whom reprint requests should be addressed.

at 90°C for 4 min. This also served to cleave any apurinic/apyrimidinic sites generated by the glycosylase. Four microliters of loading dye (95% formamide/20 mM EDTA/0.05% bromophenol blue/0.05% xylene cylanol FF) was added to the mix and aliquots were run in denaturing (7 M urea) 15% polyacrylamide gels.

Strains and Plasmids. *E. coli* strain GT100 is *ara*, Δ(*gpt–lac*)*5*, *rpsL*, [F'*lacI*^{q}*L8*, *proA*$^+$*B*$^+$]. Strain CC104 is *ara*, Δ(*gpt–lac*)*5*, [F'*lacI378*, *lacZ461*, *proA*$^+$*B*$^+$] (12). CSH115 is *ara*, Δ(*gpt–lac*)*5*, *rpsL*, *mutS*::mini-Tn*10* and CSH116 is *ara*, Δ(*gpt–lac*)*5*, *rpsL*, *mutD5 zae502*::Tn*10*; these strains are further described by Miller (13). Plasmid pKK223-3 was purchased from Pharmacia, and pKK-Fapy2 carries a wild-type *mutM* (*fpg*) gene (4). All media and genetic manipulations, unless otherwise stated, were as described (14).

Complementation Tests. Eight independent cultures containing plasmid pKK223-3 or pKK-Fapy2 in strains GT100, GT100 *mutY*::mini-Tn*10*, GT100 *mutM*, or GT100 *mutS*::mini-Tn*10* were grown overnight in LB medium supplemented with ampicillin (100 μg/ml), and 50-μl samples were plated onto LB with rifampicin (200 μg/ml). After overnight incubation at 37°C, Rif^r colonies were counted.

MutM Protein Assay. Crude extracts were obtained by growing Sup17 [a strain carrying a suppressor of *mutY* (C.C., M.L.M., and J.H.M., unpublished work)] in LB medium. Cells (30 OD_{600} units) were collected by centrifugation, resuspended in 2 ml 50 mM Tris·HCl, pH 7.5/20 mM β-mercaptoethanol/1 mM phenylmethylsulfonyl fluoride, and frozen at −70°C. The solution was thawed and sonicated on a Fisher Sonic Dismembrator model 300 on maximum output for two 30-sec bursts. The cell debris was removed by centrifugation (27,000 × *g*) for 20 min at 4°C. The supernatant was taken and glycerol was added to a concentration of 5% (vol/vol). The protein content of the extracts was measured with the BioRad protein assay kit using bovine serum albumin as the standard. Various amounts of extract were incubated with 20 fmol of 23-mer duplex containing a C/8-oxoG pair with a ^{32}P label in the 8-oxoG-containing strand (oligonucleotides 4 and 1; see above). The reaction was incubated at 37°C for 30 min in 10 μl of 20 mM Tris·HCl, pH 7.6/10 mM EDTA containing bovine serum albumin at 50 μg/ml. The reaction was stopped by the addition of 3 μl of loading dye and by heating at 100°C for 2 min. An aliquot of the reaction was loaded onto a denaturing 15% polyacrylamide gel. Autoradiographs were quantitated by measuring transmittance of substrate and product bands with a Bio-Rad 620 video densitometer.

Mutational Specificity Tests. Four or more independent cultures of CC104 and its derivatives were grown overnight, and 50 μl was plated onto LB with rifampicin (100 μg/ml) and 100 μl onto minimal lactose medium. After overnight incubation at 37°C, Rif^r colonies were counted. Lac$^+$ colonies were counted after 2 days of incubation at 37°C. For the double mutant (*mutY mutM*), 10^{-2} dilutions were done before plating. Lac$^+$ colonies were somewhat difficult to count for the double mutant due to Lac$^+$ colonies arising on the plate during incubation. Incubation times were reduced to combat this problem. This problem does not occur with the Rif^r mutants.

RESULTS

MutY Glycosylase Activity on A/8-oxoG Mispairs. Duplexes (23-mers) containing an undamaged A/G mispair or a site-specific A/8-oxoG mispair were tested as substrates for MutY glycosylase. The glycosylase was active on the duplex containing the A/8-oxoG mispair and removed the undamaged adenine from the duplex (Fig. 1, lane 7). MutY acted strictly as a glycosylase in this reaction—no endonuclease activity was detected. As previously observed (9), MutY was also active on the undamaged adenine of an A/G mispair and did not cause strand cleavage at the apurinic site (lane 5).

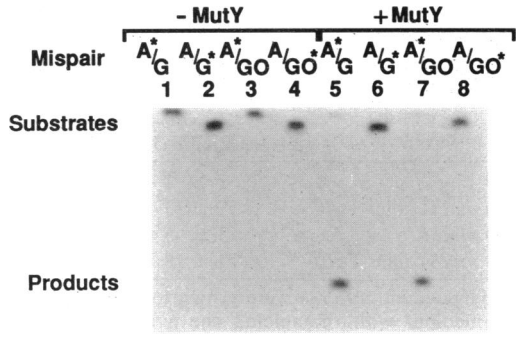

FIG. 1. Activity of MutY glycosylase on mispairs containing a site-specific 8-oxoG lesion. Glycosylase reactions were carried out as described in *Materials and Methods*. Blank reaction mixtures (lanes 1–4) contained everything except the glycosylase. Reactions were terminated by the addition of 2 μl of 1 M NaOH and incubation at 90°C for 4 min. This also served to cleave any apurinic sites generated by the glycosylase. The progress of the glycosylase reaction can therefore be monitored by a change in migration of the cleaved products. The A oligomer is sequence no. 3 (see *Materials and Methods*); the G and GO (8-oxoG) oligomers are nos. 2 and 1, respectively. The star indicates which strand in the duplex is ^{32}P-labeled.

All of the other possible combinations of mispairs with 8-oxoG were tested as potential substrates for MutY glycosylase. MutY does not remove cytosine, guanine, or thymine opposite 8-oxoG, nor does it remove 8-oxoG from any of the damaged duplexes (data not shown). MutY was active only on the A/8-oxoG and A/G mispairs.

The relative activity of MutY glycosylase on an A/8-oxoG or A/G mispair duplex substrate was determined. MutY removed the undamaged adenine from the A/G and A/8-oxoG mispairs at approximately the same rate (Fig. 2). The glycosylase specific activity on these substrates was >26 nmol/hr per mg of protein. This is comparable to the glycosylase specific activity observed for purified MutY glycosylase on a phage f1-derived heteroduplex containing an A/G mispair (1.35 nmol/hr per mg; ref. 9).

Complementation Tests. Previous work has shown that *mutY* (10) and *mutM* (*fpg*) (8) have nearly identical mutation spectra *in vivo* and that both specifically increase G·C → T·A transversions. Therefore, we suspected that the two proteins could be part of a common repair pathway. The above biochemical assay suggested that 8-oxoG might be common to both repair proteins. In the proposed scheme, the MutM protein would remove 8-oxoG lesions in DNA, and MutY

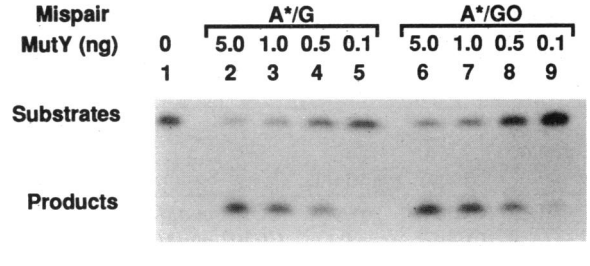

FIG. 2. Relative activity of MutY glycosylase on A/G and A/8-oxoG substrates. The blank reaction (lane 1) and glycosylase reactions (lanes 2–9) were performed on duplex 23-mers containing the indicated mismatch and analyzed as described in the legend to Fig. 1 except that the amount of MutY glycosylase was varied as shown in the figure. The A* oligomer in each duplex is no. 3 (*Materials and Methods*); the G and GO (8-oxoG) oligomers are nos. 2 and 1, respectively. The blank reaction (no glycosylase) was performed on A*/G.

Table 1. Complementation of a *mutY* strain with a MutM-overproducing plasmid

Strain	No. of Rif^r colonies per 10^8 cells	
	pKK223-3	pKK-Fapy2
GT100	1 ± 1	1 ± 1
GT100 *mutY*::mini-Tn*10*	57 ± 13	2 ± 1
GT100 *mutM*	13 ± 5	1 ± 1
GT100 *mutS*::mini-Tn*10*	71 ± 18	102 ± 21

Eight independent cultures were grown to saturation and plated onto LB medium with rifampicin. The average number of Rif^r colonies (±SD) per 10^8 cells is reported. Plasmid pKK223-3 is the vector and pKK-Fapy2 is a MutM overproducer (4).

glycosylase would remove any adenines that were misincorporated opposite 8-oxoG lesions that were not removed before replication.

The results of complementation tests support this theory. By overexpression of MutM protein in a *mutY* strain, the mutation rate of *mutY* was reduced to wild-type levels (Table 1). Overexpression of MutM protein in a mismatch repair-deficient strain (*mutS*) had no effect on its mutation rate. This control shows that the overproducer does not affect a mutator strain that causes mutations by an unrelated pathway. As expected, the clone complemented a *mutM* strain, and the vector alone had no effect on the mutation rate of any strain.

Characterization of a *mutM mutY* Double Mutant. Consistent with the idea that MutY glycosylase and the MutM protein protect the cell from the potentially mutagenic 8-oxoG lesion, a strain that lacks both defense systems has an extremely high rate of G·C → T·A transversions. We used P1 phage to transfer a *mutY*::mini-Tn*10* marker into a *mutM* strain and found that, like *mutM* and *mutY*, the double mutant was very specific for stimulating G·C → T·A transversions (Table 2). However, the mutation rate of the double mutant, as judged by both the generation of Rif^r colonies and the rate of G·C → T·A transversions in a Lac$^+$ reversion assay, is 20-fold higher than the sum of the separate mutators, suggesting that they are involved in a related repair system (Table 3).

The importance of the repair system is illustrated by comparing the mutation rate of a *mutM mutY* double mutant to the mutation rates obtained when other well-characterized error-avoidance systems are disabled. Strains lacking the polymerase III editing subunit (*mutD*) or the methyl-directed mismatch repair system (*mutS*) are the most potent mutator strains in *E. coli*. As judged by the formation of Rif^r colonies, the *mutM mutY* double mutant is just as strong a mutator as *mutD* and about an order of magnitude stronger than a strain lacking the mismatch repair system (Table 3).

Characterization of a Suppressor of *mutY*. We have characterized a suppressor of *mutY* that maps near to but distinct from the *mutM* gene (C.C., M.L.M., and J.H.M., unpublished work). We suspected that this antimutator candidate, Sup17, might overexpress MutM protein based upon our previous complementation results. In fact, when the extracts were tested for glycosylase/apurinic endonuclease activity on a 23-mer duplex containing a site-specific C/8-oxoG pair, the Sup17 extract had 15-fold greater activity than the parent strain. These results will be described in detail elsewhere.

Table 2. Mutational specificity of *mutM mutY* strains

Reversion event	Strain	No. of Lac$^+$ revertants	Strain	No. of Lac$^+$ revertants
A·T → C·G	CC101	1	CC101 *mutM mutY*	1
G·C → A·T	CC102	2	CC102 *mutM mutY*	2
G·C → C·G	CC103	<1	CC103 *mutM mutY*	<1
G·C → T·A	CC104	2	CC104 *mutM mutY*	5300
A·T → T·A	CC105	1	CC105 *mutM mutY*	1
A·T → G·C	CC106	<1	CC106 *mutM mutY*	3

Four or more independent cultures of each strain were grown to saturation in LB medium and plated onto minimal lactose medium. Average number of Lac$^+$ colonies per 10^8 cells is recorded above. Strains in this table are streptomycin-resistant (*rpsL*) versions of the CC101-106 series (12).

Table 3. Mutation frequency of *mutM*, *mutY*, and *mutM mutY* strains

Strain	No. of Lac$^+$ revertants	No. of Rif^r colonies
CC104	3	5–10
CC104 *mutM*	25	151
CC104 *mutY*	62	290
CC104 *mutM mutY*	1900	8200
CSH115 (*mutS*)	ND	760
CSH116 (*mutD*)	ND	4900

Mutation frequency of the double mutant is compared with those of the separate *mutM* and *mutY* mutants as well as with the mutation frequency of strains lacking the polymerase III editing function (*mutD*) or the mismatch repair system (*mutS*). Cultures were grown to saturation in LB medium and plated onto minimal lactose medium and LB with rifampicin. Average numbers of Lac$^+$ and Rif^r colonies per 10^8 cells are recorded. ND, not determined.

DISCUSSION

The attack of reactive oxygen species on DNA poses a substantial threat to the cell. Endogenous oxidants generated by the incomplete reduction of oxygen or by lipid peroxidation can damage DNA and may be a major cause of the physiological changes associated with aging and cancer (2).

One of the products of oxidative attack on DNA is the 8-oxoG lesion. It has been determined that rat liver cells have a steady-state level of over 4×10^5 8-oxoG lesions per cell, yet it is estimated that 8-oxoG lesions may represent only 5% of the total oxidative damage to DNA (2). Even more significant is the finding that 8-oxoG lesions can lead to replication errors. *In vivo* and *in vitro* studies have shown that adenine is frequently misincorporated opposite oxidatively damaged guanine residues in DNA (5–7). In a *mutM* strain the protein that removes 8-oxoG lesions in DNA is not active (3, 4). The elevated level of G·C → T·A transversions in a *mutM* strain is thus due to the misinsertion of adenine residues opposite the accumulated 8-oxoG lesions in the parental strand during DNA replication. MutM (Fpg protein) can also excise formamidopyrimidine lesions in DNA (20).

The 8-oxoG lesion is not only present in chromosomal DNA but is also found in the nucleotide pool as 8-oxo-dGTP. This damaged nucleotide is potentially mutagenic, as 8-oxo-dGTP can be frequently misincorporated opposite template adenines (15). The MutT protein hydrolyzes 8-oxo-dGTP to 8-oxo-dGMP, thus eliminating the mutagenic substrate from the nucleotide pool (15). A strain that lacks active MutT protein has elevated levels of A·T → C·G transversions (16). These transversions are presumably due to the misincorporation of 8-oxo-dGTP opposite template adenines. Strains deficient in MutM or MutT protein have increased frequencies of opposite transversions. In a *mutM* strain, 8-oxoG lesions accumulate in the chromosome and lead to the misincorporation of adenine opposite template 8-oxoG lesions and produce G·C → T·A transversions, while in a *mutT* strain 8-oxo-dGTP nucleotides are misinserted opposite template adenines, resulting in A·T → C·G transversions.

Cells have multiple lines of defense against oxidative damage to DNA. The primary line of defense guards against the active oxygen species themselves. Oxidants can be

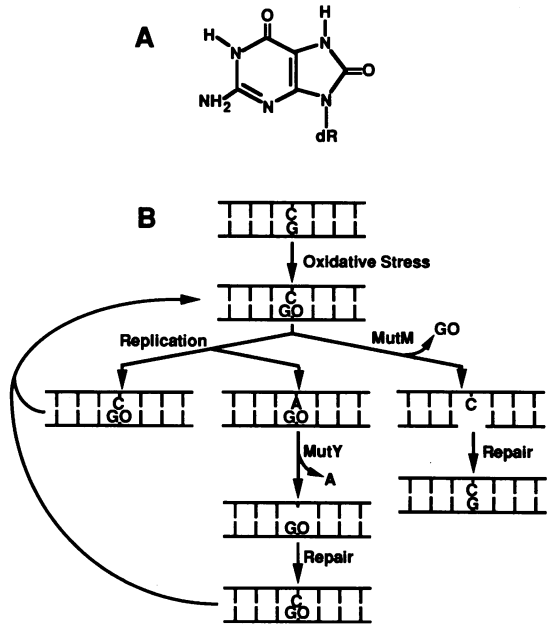

FIG. 3. Role of MutM and MutY in the 8-oxoG repair system. (*A*) Structure of the predominant tautomeric form of the 8-oxoG lesion. dR, deoxyribose. (*B*) Oxidative processes can lead to 8-oxoG lesions in DNA. The MutM protein removes 8-oxoG lesions and subsequent repair restores the original G·C base pair. If the 8-oxoG lesion (GO) is not removed before replication, translesion synthesis can be accurate or inaccurate. Accurate translesion synthesis results in a C·8-oxoG pair—a substrate for the MutM protein. However, inaccurate translesion synthesis leads to the misincorporation of dAMP opposite the 8-oxoG lesion (5–7). MutY glycosylase removes the misincorporated dA from the A/8-oxoG mispairs that result from error-prone replication past the 8-oxoG lesion. Repair polymerases are less error-prone during translesion synthesis (5) and can lead to a C·8-oxoG pair—a substrate for MutM.

eliminated by various enzymatic and nonenzymatic systems such as superoxide dismutase, catalase, ascorbic acid, and β-carotene (17). However, active oxygen species that escape these primary defenses can damage nucleic acids and other cellular macromolecules. The second line of defense works to remove the oxidative damage from nucleic acids. The MutM and MutT proteins are examples of this type of defense. Exonuclease III, endonuclease IV, and the excision nuclease and exonuclease activities associated with UvrAB are further examples of enzymes that can repair oxidized DNA (18–20).

The MutY protein represents a third level of protection against oxidative damage to DNA. Unlike the other defenses, which seek to neutralize the reactive oxygen species or repair the damage those species cause to nucleic acids, MutY glycosylase helps to correct the errors that result from the replication of DNA containing oxidative damage. It removes undamaged adenines that are misincorporated opposite template 8-oxoG lesions in DNA.

Although MutY glycosylase can remove the undamaged A from both an A/8-oxoG mispair or an undamaged A/G mispair duplex in DNA (Fig. 1), our results suggest that its primary function *in vivo* is the removal of the A from the oxidatively damaged mispair, A/8-oxoG. First, overexpression of MutM protein, which removes 8-oxoG lesions, completely complements a *mutY* strain (Table 1). Similarly, a chromosomal suppressor of the mutator phenotype of *mutY* had 15-fold greater glycosylase/apurinic endonuclease activity on an 8-oxoG substrate than the parent strain. Finally, a *mutM mutY* double mutant has an extremely high G·C → T·A mutation rate (Tables 2 and 3). The mutation rate is about 20-fold higher than would be expected if the genes were involved in unrelated repair mechanisms. In fact, the mutation rate of the double mutant suggests that although the only mutagenic substrate this system handles is 8-oxoG, it is nonetheless one of the more important error-avoidance systems in *E. coli*.

The A/8-oxoG mispair represents an oxidatively damaged purine/purine mispair that is not efficiently removed by the proofreading function of polymerase III and appears to require a devoted repair system to prevent the oxidatively damaged guanine from introducing mutations into the chromosome. An intricate mechanism involving the MutM, MutT, and MutY proteins has evolved to protect the cell from the mutagenic effect of 8-oxoG (see Fig. 3).

We thank V. Bodepudi and R. Rieger for preparing the oligonucleotides containing the 8-oxoG lesion. We thank J. Tchou for helpful discussions and H. Hsiao for technical assistance. This work was supported by Grant GM32184 from the National Institutes of Health to J.H.M., Grant CA47995 from the National Cancer Institute to A.P.G., and a California Institute for Cancer Research Fellowship to M.L.M.

1. Halliwell, B. & Gutteridge, J. M. C. (1989) *Free Radicals in Biology and Medicine* (Clarendon, Oxford), 2nd Ed.
2. Fraga, C. G., Shigenaga, M. K., Park, J.-W., Degan, P. & Ames, B. N. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 4533–4537.
3. Tchou, J., Kasai, H., Shibutani, S., Chung, M.-H., Grollman, A. P. & Nishimura, S. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 4690–4694.
4. Michaels, M. L., Pham, L., Cruz, C. & Miller, J. H. (1991) *Nucleic Acids Res.* **19**, 3629–3632.
5. Shibutani, S., Takeshita, M. & Grollman, A. P. (1991) *Nature (London)* **349**, 431–434.
6. Moriya, M., Ou, C., Bodepudi, V., Johnson, F., Takeshita, M. & Grollman, A. P. (1991) *Mutat. Res.* **254**, 281–288.
7. Wood, M. L., Dizdaroglu, M., Gajewski, E. & Essigman, J. M. (1990) *Biochemistry* **29**, 7024–7032.
8. Cabrera, M., Nghiem, Y. & Miller, J. H. (1988) *J. Bacteriol.* **170**, 5405–5407.
9. Au, K. G., Clark, S., Miller, J. H. & Modrich, P. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 8877–8881.
10. Nghiem, Y., Cabrera, M., Cupples, C. G. & Miller, J. H. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 2709–2713.
11. Bodepudi, V., Iden, C. R. & Johnson, F. (1991) *Nucleotides Nucleosides* **10**, 755–761.
12. Cupples, C. G. & Miller, J. H. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 5345–5349.
13. Miller, J. H. (1992) *A Short Course in Bacterial Genetics* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
14. Miller, J. H. (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
15. Maki, H. & Sekiguchi, M. (1992) *Nature (London)* **355**, 273–275.
16. Yanofsky, C., Cox, E. C. & Horn, V. (1966) *Proc. Natl. Acad. Sci. USA* **55**, 274–281.
17. Degan, P., Shigenaga, M. K., Park, E.-M., Alperin, P. E. & Ames, B. N. (1991) *Carcinogenesis* **12**, 865–871.
18. Demple, B., Johnson, A. & Fung, D. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 7731–7735.
19. Lin, J. J. & Sancar, A. (1989) *Biochemistry* **28**, 7979–7984.
20. Czeczot, H., Tudek, B., Lambert, B., Laval, J. & Boiteux, S. (1991) *J. Bacteriol.* **173**, 3419–3424.