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NOTES

The *Escherichia coli* Methyl-Directed Mismatch Repair System Repairs Base Pairs Containing Oxidative Lesions

Jennifer Wyrzykowski and Michael R. Volkert*

Department of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester, Massachusetts 01655

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A major role of the methyl-directed mismatch repair (MMR) system of *Escherichia coli* is to repair postreplicative errors. In this report, we provide evidence that MMR also acts on oxidized DNA, preventing mutagenesis. When cells deficient in MMR are grown anaerobically, spontaneous mutation frequencies are reduced compared with those of the same cells grown aerobically. In addition, we show that a *dam* mutant has an increased sensitivity to hydrogen peroxide treatment that can be suppressed by mutations that inactivate MMR. In a *dam* mutant, MMR is not targeted to newly replicated DNA strands and therefore mismatches are converted to single- and double-strand DNA breaks. Thus, base pairs containing oxidized bases will be converted to strand breaks if they are repaired by MMR. This is demonstrated by the increased peroxide sensitivity of a *dam* mutant and the finding that the sensitivity can be suppressed by mutations inactivating MMR. We demonstrate further that this repair activity results from MMR recognition of base pairs containing 8-oxoguanine (8-oxoG) based on the finding that overexpression of the MutM oxidative repair protein, which repairs 8-oxoG, can suppress the *mutH*-dependent increase in transversion mutations. These findings demonstrate that MMR has the ability to prevent oxidative mutagenesis either by removing 8-oxoG directly or by removing adenine misincorporated opposite 8-oxoG or both.

In *Escherichia coli*, the methyl-directed mismatch repair (MMR) system is initiated after replication and one of its primary functions is to remove base-base mismatches or small insertion-deletion loops generated by misincorporation or strand slippage during replication of DNA (10). MMR has been conserved from prokaryotes to eukaryotes and has been shown to function in homologous and homeologous recombination and transcription-coupled repair and to act on base pairs containing lesions (5, 6, 8, 15, 20, 28). In humans, defects in MMR result in elevated spontaneous mutation rates and microsatellite instability, leading to an increased predisposition to certain cancers (19).

Reactive oxygen species are considered to be a major threat to the integrity of DNA, as well as that of proteins, lipids, and carbohydrates (1, 14). In aerobically growing cells, reactive oxygen species are produced as by-products of normal metabolic pathways and have been shown to contribute to human diseases including cancer, cardiovascular disease, immune system decline, brain dysfunction, and cataracts (1). Some of these by-products include singlet oxygen ($^1O_2$), peroxide radicals ($\cdot O_2$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($\cdot$OH) (1, 7). Although H_2O_2 is relatively stable, it can rapidly react with Fe^{2+} to produce highly reactive $\cdot$OH radicals in a process described by the Fenton reaction (11, 12). This $\cdot$OH radical can then react with DNA to produce a variety of DNA lesions. Reactions with guanine lead to 7,8-dihydro-8-oxoguanine (8-oxoG), which is the most common lesion produced (27).

In *E. coli*, several enzymes are involved in processing oxidative DNA damage due to 8-oxoG. One enzyme is the MutM glycosylase, or formamidopyrimidine-DNA glycosylase, encoded by the *mutM* gene (17). This enzyme functions to remove 8-oxoG lesions found in DNA (22). If MutM removes the lesion prior to replication, then the base excision repair pathway can restore the original G·C base pair (18). If the lesion is not removed prior to replication, then this will result in either another C·8-oxoG pair, which is subject to another attempt at repair by MutM, or misincorporation of adenine opposite the 8-oxoG lesion, leading to GC → TA transversions (3, 17). Another enzyme involved in removal of oxidative damage due to 8-oxoG is MutY, encoded by the *mutY* gene (18). Whereas the MutM protein removes 8-oxoG lesions from DNA, the MutY protein removes the adenine base from the A·8-oxoG mispair (16). Once the misincorporated adenine is removed, MutM can then make another attempt at repair. Together, these enzymes function to reduce the GC → TA transversions most commonly associated with 8-oxoG.

Recently, mismatch correction has been implicated in the repair of oxidatively damaged bases, possibly due to 8-oxoG. In a recent report by DeWeese et al. (5), mouse embryonic stem cells deficient in MMR were shown to display increased levels of 8-oxoG after exposure to low-level radiation compared with those in wild-type cells. Earley and Crouse (6) have determined that *Saccharomyces cerevisiae* cells deficient in MMR

* Corresponding author. Mailing address: Department of Molecular Genetics and Microbiology, University of Massachusetts Medical School, 55 Lake Ave. North, Worcester, MA 01655. Phone: (508) 856-2314. Fax: (508) 856-5920. E-mail: Michael.Volkert@umassmed.edu.

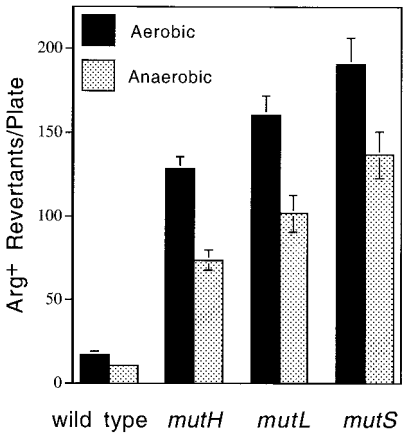

FIG. 1. Reduction of spontaneous mutagenesis in anaerobically growing MMR-deficient strains. Overnight cultures grown in Luria broth were plated onto semienriched (ESEM) plates (24) for selection of Arg^+ revertants and incubated aerobically at 37°C for 2 days and anaerobically at 37°C for 5 days. The Arg^+ revertants were counted. Data shown are averages of 19 independent experiments; error bars indicate the standard errors of the means.

that are grown anaerobically display a reduction in reversion rates, presumably due to 8-oxoG, and Ni et al. (20) have shown that the MutS homologs MSH2 and MSH6 function to remove adenine misincorporated opposite 8-oxoG. In *E. coli*, the overexpression of MutS protein was shown to reduce GC → TA transversions, suggesting the ability to correct A·8-oxoG mismatches (28), and human homologs of the MutS protein have been shown to bind to mismatched 8-oxoG lesions (15). In this study, we show that the MMR system of *E. coli* acts on DNA containing oxidative damage. In addition, we show that overexpression of MutM in a MutH-deficient strain reduces the rate of the GC → TA transversions most commonly associated with 8-oxoG. Therefore, the results presented here, along with previously reported results, indicate that the MMR system of *E. coli* functions to recognize and repair oxidative damage due to 8-oxoG.

Reduction of spontaneous mutagenesis in anaerobically growing MMR-deficient strains. Since oxidative mutagenesis results from the production of reactive oxygen species, which are by-products of normal metabolic pathways in aerobically growing cells, we investigated whether growing cells anaerobically would reduce the mutation frequency of MMR-deficient strains. Figure 1 presents a comparison of mutagenesis in wild-type strains and MMR-deficient strains grown aerobically or anaerobically. The strains used in this experiment (Table 1) are Arg^- and grow to a constant level until the limited amount of arginine is exhausted, forming a lawn of approximately 5×10^9 cells per plate. Arg^+ revertants continue to grow, forming colonies on the lawn of Arg^- mutants, and are counted after incubation (25). Strains carrying a mutation in *mutH*, *mutL*, or *mutS* that are grown aerobically show an increased reversion frequency compared with that of the wild type. When these strains are grown anaerobically, the spontaneous mutation rate is decreased by roughly 30 to 40%. This indicates that some of the increase in mutations seen in *mutH*, *mutL*, or *mutS* strains can be reduced by limiting oxygen levels and implies that MMR may function to prevent oxidative mutagenesis. These results are similar to those of Earley and Crouse (6), who examined the effect of anaerobiosis on mutagenesis in MMR-deficient strains of *S. cerevisiae*, but must be taken with caution as factors other than oxidative damage may affect mutagenesis when cells grown with and without oxygen are compared.

***dam* mutation results in increased sensitivity to oxidative DNA damage.** To determine whether MMR repairs mismatches of bases containing oxidative lesions, a *dam* mutant, which lacks the ability to methylate DNA, was examined for increased sensitivity. A *dam* mutant strain retains the ability to carry out MMR; however, without methylation of DNA, MutH

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant genotype	Description	Source or reference
AB1157 and its derivatives			
AB1157	Wild type	*argE3 hisG4 leu-6 proA2 thr-1 ara14 galK2 lacY1 mtl-1 xyl-5 rpsL31 supE44 tsx-6*	2
GM3819	*dam-16*::*kan*	*dam-16*::*kan* derivative of AB1157	M. Marinus[a]
GM5556	*dam-16 mutH464*	*dam-16*::*kan mutH464*::Tn*10* derivative of AB1157	M. Marinus[a]
GM7650	*dam-16 mutS215*	*dam-16*::*kan mutS215*::Tn*10* derivative of AB1157	M. Marinus[a]
MV1161 and its derivatives			
MV1161	Wild type	*rfa-550* derivative of AB1157	26
MV4478	*mutH472*::Tn*10*	*mutH* transductant of MV1161	This study
MV4479	*mutL218*::Tn*10*	*mutL* transductant of MV1161	This study
MV4480	*mutS215*::Tn*10*	*mutS* transductant of MV1161	This study
CC104 and its derivatives			
CC104	Wild type	*lacZ* CC104	4
MV6015	Wild type/pTrc99a	pTrc99a transformant of CC104	This study
MV6016	Wild type/p*mutM*	p*mutM* transformant of CC104	This study
MV4511	*mutH472*::Tn*10*	*mutH* transductant of CC104	This study
MV6017	*mutH472*::Tn*10*/pTrc99a	pTrc99a transformant of MV4511	This study
MV6018	*mutH472*::Tn*10*/p*mutM*	p*mutM* transformant of MV4511	This study
Plasmids			
pTrc99a		Vector	Pharmacia
p*mutM*		*mutM*$^+$ cloned into pTrc99a	Lab stock

[a] Full descriptions of these strains are available at the following website: http://users.umassmed.edu/martin.marinus/dstrains.html.

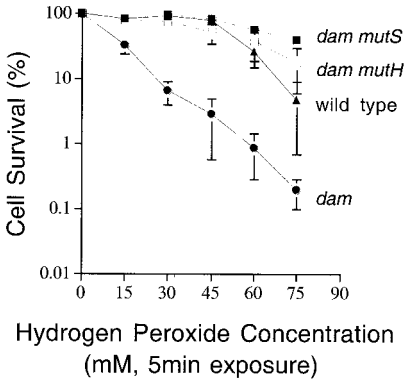

FIG. 2. MutHLS suppression of *dam*-mediated peroxide sensitivity. Frozen stocks were streaked onto Luria broth (LB) plates containing, when necessary, kanamycin (75 μg/ml) and/or tetracycline (15 μg/ml) and grown overnight at 30°C. Single colonies were then inoculated into LB medium containing the appropriate antibiotics along with 1× MOPS (morpholinepropanesulfonic acid) buffer and 2 mM IPTG (isopropyl-β-D-thiogalactopyranoside) when necessary. Cultures were grown with aeration to mid-log phase and then stored at 4°C overnight. Cultures were then diluted 1:50 in fresh Luria-Bertani medium containing the appropriate antibiotics, MOPS buffer, and IPTG when necessary and grown with aeration to mid-log phase. Cells were harvested and resuspended in 5 ml of 1× E salts (23). Samples were taken and treated with H_2O_2 at the indicated concentrations for 5 min at 37°C. After treatment, cells were diluted in 1× E salts, plated on LB plates containing the appropriate antibiotics, and incubated at 37°C overnight. Colonies were counted to determine the percentage of surviving cells. Data shown are averages of three independent experiments; error bars indicate the standard errors of the means.

cannot discriminate between parental and newly synthesized DNA strands and will therefore nick both strands at d(GATC) sequences in a *mutS*-dependent reaction (13). The predominant oxidative lesion, 8-oxoG, is readily bypassed by DNA polymerases and has little effect on lethality (9). In a *dam* mutant strain, base pairs containing 8-oxoG will be converted to single- and double-strand breaks if MMR recognizes base pairs containing oxidative lesions, which will increase sensitivity to hydrogen peroxide treatment. The data in Fig. 2 show this to be the case; the *dam* mutant strain is more sensitive to hydrogen peroxide treatment than is a wild-type strain. This result strongly suggests that MMR recognizes base pairs containing oxidative lesions.

***dam*-mediated peroxide sensitivity requires MMR.** If *dam*-mediated peroxide sensitivity is due to conversion of oxidative lesions to strand breaks by MMR, then inactivation of MMR should prevent strand break formation and restore peroxide resistance in a *dam* mutant. Figure 2 demonstrates that *dam mutH* and *dam mutS* strains are much more resistant to hydrogen peroxide than the *dam* single mutant and are even slightly more resistant to hydrogen peroxide than is a wild-type strain. These results indicate that the peroxide sensitivity of the *dam* mutant is due to the action of MMR on DNA containing oxidized bases, and that *dam*-mediated peroxide sensitivity requires the action of *mutH* and *mutS*. Based on these results, we conclude that *mutS* recognizes base pairs containing oxidative lesions and triggers a *mutH*-dependent cleavage at GATC sites. The small but reproducible increase in resistance seen when the *mutH dam* and *mutS dam* strains are compared with the wild type suggests that conversion of nonlethal oxidative lesions to lethal strand breaks may also occur in the wild type, minimizing mutagenesis but increasing lethality.

Reduction of GC → TA transversions by overexpression of *mutM* in a *mutH* mutant strain. Since 8-oxoG lesions in DNA result in GC → TA transversions, we determined whether MMR acts specifically on 8-oxoG-containing base pairs by using the bacterial strain CC104. This strain carries a point mutation at the glutamic acid codon 461 located in the active site of the β-galactosidase enzyme that reverts only by GC → TA transversion (4). Therefore, if MMR prevents 8-oxoG-mediated mutagenesis, then CC104 strains deficient in MMR should show an elevated frequency of GC → TA transversions. Moreover, if MutM levels in wild-type strains are limiting, then it should be possible to reduce GC → TA transversions by increasing MutM protein levels. Figure 3 shows that overexpression of MutM from a strong promoter on a high-copy plasmid decreases GC → TA transversions in a wild-type strain. This indicates that most spontaneous GC → TA transversions result from oxidative damage that has escaped repair by the MutM system and that increasing levels of MutM can repair most of the residual damage, preventing mutagenesis. When GC → TA transversions are measured in a *mutH* strain, there is a substantial increase relative to those seen in the wild type. *mutM* overexpression in this strain reduces the transversion frequency to a level similar to that of the wild-type strain. This indicates that the *mutH*-dependent increase in GC → TA transversions is due to lesions that can be repaired by MutM.

A role for MMR in the repair of oxidative DNA damage was

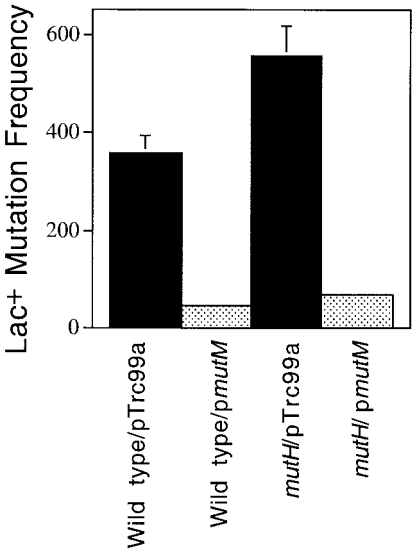

FIG. 3. Reduction of GC → TA transversions by overexpression of *mutM* in a *mutH* mutant strain. Lac^+ revertants were determined by using the strain CC104 developed by Cupples and Miller (4). Strains were grown overnight at 37°C with aeration in Luria-Bertani medium containing 200 μg of ampicillin/ml and then spread on Lac mutagenesis assay plates by using the plating medium of Cupples and Miller (4) with the addition of 50 μg of carbenicillin/ml to maintain plasmids. IPTG was used when necessary to induce expression of *mutM* on the plasmid pTrc99a. The plates were incubated at 37°C for 4 days, and then the Lac^+ revertants were counted. Mutation frequencies are expressed as *lacZ* revertants per 10^9 viable cells. Data shown are averages of 10 independent experiments; error bars indicate the standard errors of the means.

first identified in yeast (6), and it was suggested that the ability of this repair system to perform this function may have evolved because yeast lack MutY and MutT homologs. Organisms such as *E. coli* and mammals, which have both MutY and MutT, may not need to repair oxidized bases by the MMR system (15). Since we found that the action of MMR on oxidative DNA damage is influenced by the *dam* mutation, and the hemimethylated state required for MMR exists only transiently after replication in *E. coli* (21), then it is possible that the role of MMR is to immediately repair the products of misreplication past oxidative lesions. Furthermore, if it is found that MMR can also repair 8-oxoG itself, then MMR may also function to remove 8-oxoG incorporated by the replication machinery. Other repair systems acting on oxidized bases may function to repair the bulk of the oxidative lesions not closely associated with replication forks, repairing oxidized bases in fully methylated DNA.

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