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NOTES

Activity of the *Escherichia coli mutT* Mutator Allele in an Anaerobic Environment

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Mutation frequencies for an *Escherichia coli mutT* strain were measured in both aerobic and anaerobic environments. When cells were grown in a rich medium (L broth), mutation frequencies were similar in both aerobic and anaerobic conditions. In contrast, when grown in a minimal medium, *mutT* anaerobic mutation frequencies were reduced dramatically compared with aerobic values, which were similar to L broth frequencies. L broth *mutT* cultures treated with a commercial enzyme complex that reduces free oxygen in the medium also showed strongly reduced anaerobic mutation frequencies. These results indicate that the biological role of the MutT protein is to prevent oxidative damage from becoming mutagenic.

Although the *mutT* mutator allele was the first to be described in *Escherichia coli* (25), its mechanism of activity has remained unclear until recently. An early key to the nature of *mutT* activity was the observation that this mutator causes exclusively A · T→C · G transversions (27). These transversions arise through A · G mispairings rather than T · C intermediates (2, 23). The MutT protein catalyzes the hydrolysis of nucleoside triphosphates with a preference for dGTP (5, 6), and it was suggested that the biological role of MutT might be to hydrolyze dGTP before it could mispair with a template adenine during DNA replication (5). Recently, Maki and Sekiguchi (17) showed that 8-oxo-7,8-dihydro-2′-dGTP (8-oxodGTP) is more readily hydrolyzed to the monophosphate than dGTP and hypothesized that the MutT protein might act to remove 8-oxodGTP from the nucleotide pool during DNA replication because of its increased tendency to mispair with template adenine compared with that of dGTP (8, 16, 17, 22).

8-OxodGTP may be produced in the cell from dGTP by ionizing radiation and oxygen radical-producing chemicals (13–15) as well as from physiological and biochemical processes occurring during normal cellular metabolism (4). It is widely accepted that active partial reduction products of oxygen, i.e., superoxide, hydrogen peroxide, and hydroxyl radical, produced during aerobic respiration, are capable of directly (hydroxyl radical) or indirectly (superoxide radical and hydrogen peroxide) forming oxidative products with DNA and precursor molecules (3, 10, 11). 8-oxodGTP may be among the most abundant of these oxidative products in both bacteria and animals (21, 24). *mutT* homologs have been found in other bacteria (7, 12, 18), and a similar nucleoside triphosphatase activity has been found in human cells (20), suggesting that misincorporation of 8-oxodGTP may be a common problem.

If MutT does function to remove 8-oxodGTP from nucleotide pools, it might be expected that *mutT* strains would show lower mutation frequencies in an anaerobic environment than in an aerobic environment. We tested this prediction by measuring *mutT* mutation frequencies inside an anaerobic chamber (Anaerobe Systems, San Jose, Calif.). Anaerobic experiments were performed in an atmosphere of 85% nitrogen, 10% hydrogen, and 5% carbon dioxide, and a passbox was used to anaerobically transfer supplies and equipment into the chamber.

Cells were grown in L broth supplemented with glucose (1% tryptone, 0.5% yeast extract, 0.1% glucose, and 0.5% NaCl) or in minimal medium (0.2% glucose, 1 μg of thiamine HCl per ml, and Vogel and Bonner salts [26]) supplemented with the amino acids leucine, arginine, histidine, threonine, and tryptophan, each at a concentration of 50 μg/ml. Mutations to nalidixic acid resistance (Nal^r), streptomycin resistance (Str^r), and rifampin resistance (Rif^r) were selected on L-broth agar (L broth with 1.5% agar) plates containing 50 μg of nalidixic acid per ml, 150 μg of streptomycin per ml, and 100 μg of rifampin per ml, respectively. Liquid media were autoclaved and taken directly into the chamber and kept for at least 5 days before use. For some anaerobic experiments, media were autoclaved and then immediately transferred via hosing to a medium-preparatory anaerobic chamber in which plates were poured to ensure that the solid media be as reduced as possible. The plates were then transferred via a passbox to the chamber where the experiments were done. Preliminary results indicated that the mutation frequencies obtained with media made this way were similar to values obtained with plates poured under aerobic conditions on the bench and then placed in the anaerobic chamber at least 24 h before use. Subsequent anaerobic experiments used media prepared the latter way.

Nal^r, Str^r, and Rif^r mutation frequencies were measured by growing five cultures of a *mutT* strain (KD1088; *mutT thr leu trpA58 his arg*) to saturation (24 to 48 h) in minimal medium anaerobically at 37°C. The cultures were diluted in the chamber, and four new cultures, two of L broth and two of minimal medium, were started from each original culture with small inocula of a few hundred cells (a total of 20 cultures). Half of all L broth and minimal medium cultures were removed from the chamber and grown to saturation aerobically (24 h) in a shaking water bath at 37°C. These cultures were the aerobic controls. The remaining cultures were kept in the chamber and grown to saturation at 37°C. The cultures were diluted as appropriate and plated on selective media for Nal^r, Str^r, and

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TABLE 1. Aerobic and anaerobic mutation frequencies for *mutT* cultures grown in L broth and minimal medium

Strain	Culture condition	Medium[a]	No. of mutants/10^8 cells[b]		
			Rif^r	Nal^r	Sm^r
mutT	Aerobic	LB	22.3	42.6	41.3
		MM	35.9	27.4	47.5
	Anaerobic	LB	17.3 (1.3)[c]	18.6 (2.3)	29.2 (1.4)
		MM	0.16 (224)	0.24 (114)	0.18 (264)
mutT$^+$	Aerobic	LB	2.70	0.36	0.03
		MM	7.25	0.25	0.01
	Anaerobic	LB	0.19	<0.1	<0.1

[a] LB, L broth; MM, minimal medium.
[b] Aerobic titers for saturated *mutT* and *mutT*$^+$ cultures in both L broth and minimal medium ranged from 1.1×10^9 to 2.3×10^9 cells per ml, while anaerobic titers in L broth and minimal medium usually ranged from 7.4×10^8 to 3.4×10^9 cells per ml. Occasional cultures whose titers were below these values were discarded.
[c] Values in parentheses are the ratios of aerobic/anaerobic mutation frequencies.

Rif^r mutants and on L broth plates to determine the titer for total cells. The Rif^r-selective plates and L broth plates for total cells were incubated for 2 days while the Nal^r- and Str^r-selective plates were incubated for 3 days at 37°C. The anaerobic culture plates were then removed from the chamber, all plates were counted, and mutation frequencies were calculated by dividing the average number of mutant colonies by the average total number of cells. Mutation frequencies were also calculated for an isogenic *mutT*$^+$ strain.

Mutation frequencies for *mutT* and *mutT*$^+$ strains grown in L broth and minimal medium under aerobic and anaerobic conditions are shown in Table 1. Aerobic *mutT* mutation frequencies are high for both L broth and minimal medium cultures and indicate that the mutator activity of *mutT* is not medium dependent under aerobic conditions. *mutT* cultures grown in minimal medium in the anaerobic chamber show dramatically reduced mutation frequencies for all three markers scored. These values are close to wild-type (*mutT*$^+$) spontaneous frequencies. We conclude that most of the *mutT* mutator activity is oxygen dependent in minimal medium and indicates that the biological role of *mutT* is to prevent some form(s) of oxidative damage from being mutagenic, as suggested by Maki and Sekiguchi (17).

mutT cultures grown anaerobically in L broth show a different pattern of mutator activity. Here, strong mutator activity is clearly displayed for all markers, with anaerobic values only slightly lower than aerobic frequencies, a result observed by others (19). To determine if a more reduced L broth medium would affect anaerobic *mutT* frequencies, we added a commercial enzyme additive, Oxyrase (Oxyrase, Inc., Mansfield, Ohio), to L broth and then started *mutT* anaerobic cultures. Oxyrase is a sterile suspension of *E. coli* membrane fractions that contains the cytochrome-based electron transport system which apparently acts to reduce free oxygen to water by transferring hydrogen from donors in the medium (1). The results are shown in Table 2. This time, L broth *mutT* mutation frequencies are severely reduced to levels similar to those of minimal medium cultures. We interpret these results as showing that *mutT* mutator activity in anaerobic L-broth cultures is mediated by oxidative damage, widely assumed to be 8-oxodGTP (17, 19), that is prevented by the addition of Oxyrase. Whether this occurs by the enzymatic reduction of free oxygen in the L broth or by some other activity present in the membrane fractions is not clear.

TABLE 2. Anaerobic mutation frequencies for *mutT* cultures grown in L broth and L broth supplemented with Oxyrase

Medium[a]	No. of mutants/10^8 cells[b]	
	Nal^r	Sm^r
LB	22.9	58.4
LB + Oxyrase	0.13	0.42

[a] LB, L broth.
[b] Results are an average of two independent cultures.

Our results directly demonstrate the potential for high rates of mutagenesis, specifically A · T→C · G transversions, that can occur through oxidative damage leading to A · G mispairing with a defective *mutT* gene. It has been shown in vivo that DNA polymerase III proofreading removes some *mutT*-caused A · G mispairings (9), and in vitro studies (22) have shown that the T4 polymerase proofreading prevents A · 8-oxodGMP mispairings. Thus, at least two mechanisms limit the stable incorporation of 8-oxodGTP at the replication fork, and other correction mechanisms exist to remove 8-oxodG from chromosomal DNA (19). What proportion of spontaneous A · T→C · G transversions found in wild-type cells are the result of A · 8-oxodGMP mispairings that survived the arsenal of repair and prevention mechanisms and became fixed as mutations remains unknown.

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REFERENCES

1. **Adler, H. I., and W. D. Crow.** 1981. A novel approach to the growth of anaerobic microorganisms. Biotechnol. Bioeng. Symp. **11**:533–540.
2. **Akiyama, M., H. Maki, M. Sekiguchi, and T. Horiuchi.** 1989. A specific role of MutT protein: to prevent dG/dA mispairing in DNA replication. Proc. Natl. Acad. Sci. USA **86**:3949–3952.
3. **Ames, B. N.** 1989. Endogenous DNA damage as related to cancer and aging. Mutat. Res. **214**:41–46.
4. **Bessho, T., K. Tano, H. Kasai, and S. Nishimura.** 1992. Deficiency of 8-hydroxyguanine DNA endonuclease activity and accumulation of the 8-hydroxyguanine in mutator mutant (*mutM*) of *Escherichia coli*. Biochem. Biophys. Res. Commun. **188**:372–378.
5. **Bhatnagar, S. K., L. C. Bullions, and M. J. Bessman.** 1988. Studies on the mutator gene, *mutT*, of *Escherichia coli*. J. Biol. Chem. **263**: 8953–8957.
6. **Bhatnagar, S. K., L. C. Bullions, and M. J. Bessman.** 1991. Characterization of the *mutT* nucleotide triphosphatase of *Escherichia coli*. J. Biol. Chem. **266**:9050–9054.
7. **Bullions, L. C., V. Mejean, J. P. Claverys, and M. J. Bessman.** 1993. Purification and characterization of the *S. pneumoniae mutX* protein, a homolog of *E. coli mutT*. FASEB J. **7**:1290.
8. **Cheng, K. C., D. S. Cahill, H. Kasai, S. Nishimura, and L. A. Loeb.** 1992. 8-Hydroxyguanine, an abundant form of oxidative DNA damage, causes G→T and A→C substitutions. J. Biol. Chem. **267**: 166–172.
9. **Fowler, R. G., M. V. Amutan, and R. J. Isbell.** 1992. The interaction of the *Escherichia coli mutD* and *mutT* pathways in the prevention of A · T→C · G transversions. Mutat. Res. **284**:307–319.
10. **Halliwell, B., and O. I. Aruoma.** 1991. DNA damage by oxygen-derived species: its mechanism and measurement in mammalian species. FEBS Lett. **281**:9–19.

11. **Joenje, H.** 1989. Genetic toxicology of oxygen. Mutat. Res. **219:** 193–208.
12. **Kamath, A. V., and C. Yanofsky.** 1993. Sequence and characterization of *mutT* from *Proteus vulgaris*. Gene **134:**99–102.
13. **Kasai, H., P. F. Crain, Y. Kuchino, S. Nishimura, A. Ootsuyama, and H. Tanooka.** 1986. Formation of 8-hydroxyguanine moiety in cellular DNA by agents producing oxygen radicals and evidence for its repair. Carcinogenesis **7:**1849–1851.
14. **Kasai, H., and S. Nishimura.** 1984. Hydroxylation of deoxyguanosine at C-8 position by ascorbic acid and reducing agents. Nucleic Acids Res. **12:**2137–2145.
15. **Kasai, H., H. Tanooka, and S. Nishimura.** 1984. Formation of 8-hydroxyguanine residues by X-irradiation. Gann **75:**1037–1039.
16. **Kouchakdjian, M., V. Bodupudi, S. Shibutani, M. Eisenberg, F. Johnson, A. P. Grollman, and D. J. Patel.** 1991. NMR structural studies of the ionizing radiation adduct 7-hydro-8-oxodeoxyguanosine (8-Oxo-7H-dG) opposite deoxyadenosine in a DNA duplex. 8-Oxo-7H-dG (syn):dA(anti) alignment at lesion site. Biochemistry **30:**1403–1412.
17. **Maki, H., and M. Sekiguchi.** 1992. MutT protein specifically hydrolyzes a potent mutagenic substrate for DNA synthesis. Nature (London) **355:**273–275.
18. **Méjean, V., C. Salles, L. C. Bullions, M. J. Bessman, and J.-P. Claverys.** 1994. Characterization of the *mutX* gene of *Streptococcus pneumoniae* as a homologue of *Escherichia coli mutT*, and tentative definition of a catalytic domain of the dGTP pyrophosphohydrolases. Mol. Microbiol. **11:**323–330.
19. **Michaels, M. L., and J. H. Miller.** 1992. The GO system protects organisms from the mutagenic effect of the spontaneous lesion 8-hydroxyguanine (7,8-dihydro-8-oxoguanine). J. Bacteriol. **174:** 6321–6325.
20. **Mo, Y.-J., H. Maki, and M. Sekiguchi.** 1992. Hydrolytic elimination of a mutagenic nucleotide, 8-oxodGTP, by human 18-kilodalton protein: sanitation of nucleotide pool. Proc. Natl. Acad. Sci. USA **89:**11021–11025.
21. **Park, E. M., M. K. Shigenaga, P. Degan, T. S. Korn, J. W. Kitzler, C. M. Wehr, P. Kolachana, and B. N. Ames.** 1992. Assay of excised oxidative DNA lesions: isolation of 8-oxoguanine and its nucleoside derivatives from biological fluids with a monoclonal antibody column. Proc. Natl. Acad. Sci. USA **89:**3375–3379.
22. **Pavlov, Y. I., D. T. Minnick, S. Izuta, and T. A. Kunkel.** 1994. DNA replication fidelity with 8-oxodeoxyguanosine triphosphate. Biochemistry **33:**4695–4701.
23. **Schaaper, R. M., and R. L. Dunn.** 1987. *Escherichia coli mutT* mutator effect during *in vitro* DNA synthesis. J. Biol. Chem. **262:** 16267–16270.
24. **Shigenaga, M. K., C. J. Gimeno, and B. N. Ames.** 1989. Urinary 8-hydroxy-2′-deoxyguanosine as a biological marker of *in vivo* oxidative DNA damage. Proc. Natl. Acad. Sci. USA **86:**9697–9701.
25. **Treffers, H. P., V. Spinelli, and N. O. Belser.** 1954. A factor (or mutator gene) influencing mutation rates in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **40:**1064–1071.
26. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylornithinase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218:**97–106.
27. **Yanofsky, C., E. C. Cox, and V. Horn.** 1966. The unusual mutagenic specificity of an *E. coli* mutator gene. Proc. Natl. Acad. Sci. USA **55:**274–281.