JOURNAL OF BACTERIOLOGY, May 1996, p. 2709–2711 Vol. 178, No. 9
0021-9193/96/$04.00+0

NOTES

Elevated Mutation Rate in *mutT* Bacteria during Starvation: Evidence for DNA Turnover?

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Received 4 December 1995/Accepted 20 February 1996

The rate of appearance of prototrophic revertants when *Escherichia coli tyrA14* (ochre) or *trpA23* bacteria were incubated on plates lacking the required amino acid was greatly elevated when the organisms also carried a *mutT* mutation. One possible explanation for this result is that the amount of DNA replication or turnover under these conditions is much greater than has been previously recognized.

Most bacteria live the greater part of their lives waiting for their next meal to come along. In this resting state their metabolism is very different from that of growing bacteria (22). Many years ago, Ryan and his colleagues showed that mutations could arise in such bacteria, even in the absence of cell division (19, 21). More recently, it has become apparent that the mutations that are seen tend to be those that are being selected for (6, 13). This has been termed selection-induced or adaptive mutation and has been reviewed by Foster (8), who emphasizes the point made originally by Ryan et al. (20) that the rate at which mutations arise in resting cells appears to be much greater than that expected if the fidelity of their residual DNA synthesis is as high as it is in growing cells.

Recent research has suggested solutions to this paradox for two rather special systems. In the FC40 system, in which an episomal *lacI33* allele reverts adaptively to lac^+ by single base deletions, adaptive mutation is dependent upon the expression of conjugal functions, and it is suggested that conjugational DNA replication of the episome provides the enhanced opportunity for adaptive mutations to arise (10, 12, 18). For chromosomal base pair substitution mutations, DNA damage, such as the formation of 7,8-dihydro-8-oxoguanine (8-oxoG) on the transcribed strand, can in principle give rise to adaptive mutations without prior DNA synthesis (2–4). Nevertheless, if chromosomal DNA synthesis occurred under starvation conditions, it could provide an opportunity for adaptive mutations to arise both as errors during replication and as a result of replication of DNA containing damaged bases.

Various types of oxidizing species are believed to react with dGTP to give 7,8-dihydro-8-oxo-dGTP (8-oxo-dGTP), which may be incorporated during DNA replication with roughly equal efficiencies opposite both cytosine and adenine residues (16, 23). The latter misincorporation would potentially lead to an A-to-C transversion during a subsequent replication. To prevent incorporation of 8-oxo-dGTP, bacteria contain the MutT protein, which hydrolyses 8-oxo-dGTP to the nucleoside diphosphate, thus removing it from the triphosphate pool (16). Bacteria lacking MutT activity have a high rate of spontaneous mutation from A to C (27). This paper describes a study of mutation under starvation conditions in bacteria containing a *mutT* mutation, which provides an opportunity for 8-oxo-dGTP to be incorporated should any DNA synthesis occur.

Escherichia coli WU3610 has an ochre mutation in the *tyrA* gene. During fast logarithmic growth, this organism mutates to tyrosine independence by base substitution events of all types. When a *mutT* mutation is present, one sees only fast-growing mutants which have been shown to be true revertants at the ochre site, which is composed of three A · T base pairs (Fig. 1a). Since in starved stationary-phase mut^+ bacteria these revertants at A · T sites do not arise in significant numbers even after incubation of 30 times as many bacteria for 2 weeks on tyrosine selection plates (3), the mutator effect of *mutT* in resting cells must be at least several thousandfold. The *trpA23* missense mutation in IC3126 is another site where reversions can arise by a variety of mutational events (27). IC3126 showed little mutation to prototrophy under starvation conditions, but when *mutT* had been introduced there was a large mutator effect (Fig. 1b). Phenotypic typing of the mutants by the methods of Urios et al. (26) showed that all changes occurred at A · T base pairs. It is clear that 8-oxo-dGTP is highly mutagenic to starved *mutT* cells.

Phenotypic expression of mutations resulting from incorporation of 8-oxo-dGTP should be rapid. The MutY protein, a DNA glycosylase which removes adenine residues opposite 8-oxoG moieties, is active in stationary-phase cells (5). MutY activity at an 8-oxoG · A mispair at the *tyrA* (ochre) or *trpA23* codons would have the effect of rapidly establishing an 8-oxoG · C base pair, which would confer a prototrophic genotype on the cell. Moreover, incorporation of 8-oxo-dGTP directly into the transcribed strand (as can happen at both the *tyrA14* and *trpA23* codons) would give rise to many transcripts with C instead of A (7). This would have the effect of making the cell phenotypically prototrophic. The resulting protein synthesis would trigger a round of DNA replication during which the mutation could become established on both strands, if this had not already been achieved by the MutY glycosylase.

Since 8-oxo-dGTP can lead to mutations only when it is incorporated into DNA, the rate of occurrence of mutations is dependent upon the amount of DNA synthesized, whether this synthesis be a rare complete chromosomal replication cycle occurring when the cell has scavenged enough resources to justify it or limited synthesis of particular DNA regions. I have determined the relative rates of mutation by 8-oxo-dGTP in growing and resting cells by using CM1325, a *mutT mutM*

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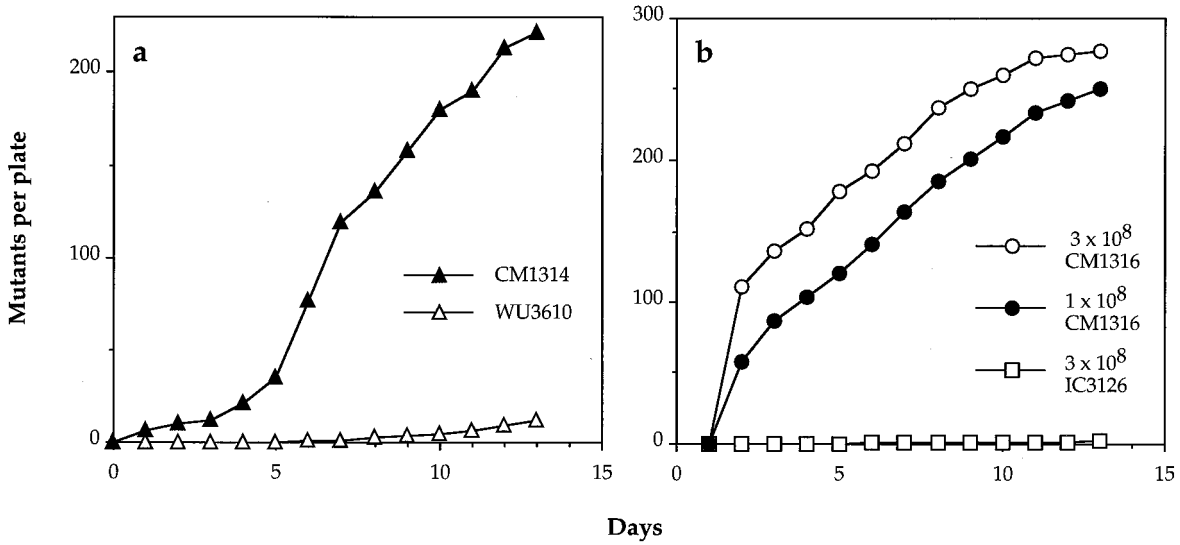

FIG. 1. (a) Appearance of prototrophic (Tyr^+) mutants during incubation of 10^7 bacteria (WU3610 *tyrA14 leu308* and a *mutT* derivative, CM1314) at 37°C on glucose minimal leucine plates. For details of the method, see reference 3. (Data are the means of three experiments.) (b) Appearance of Trp^+ mutants during incubation of IC3126 *trpA23* and its *mutT* derivative, CM1316, at 37°C on glucose minimal plates. The colonies appearing on day 2 are largely preexisting mutants carried over in the inoculum. The slope of the line after day 2 represents the rate of appearance of mutants in starved bacteria. (Data are the means of three experiments.)

derivative of WU3610. The MutM(Fpg) protein is a glycosylase which removes much of the 8-oxo-dGTP that has become incorporated opposite cytosine. Although it removes little of what has been incorporated opposite adenine (25), the *mutM* mutation was present to ensure that, once incorporated, 8-oxoG remained in the DNA.

The misincorporation rate in growing cells on plates was measured according to the method of Newcombe (17). Bacteria (10^6) from an overnight culture of the *mutT mutM* strain CM1325 were plated on minimal agar containing 10 μg of leucine and 0.25 μg of tyrosine per ml. The bacteria grew until the limiting tyrosine was exhausted and the number of viable bacteria increased to 1.27×10^9, as determined by washing the cells off the minimal agar plates and plating them on L agar. Mutants that arose during growth on these plates formed visible colonies within 2 days. (Since the lawn of auxotrophic growth rapidly metabolizes the available leucine and probably much of the glucose, mutants arising among stationary-phase cells were not able to form visible colonies in this time.) The mutation rate under these conditions for mutations at the ochre site was estimated to be 4.44×10^{-7} per generation (mean of two experiments).

In resting cells under selection conditions, tyrosine-independent mutants of CM1325 appeared at a rate of roughly 30 per day when 10^7 bacteria were plated (Fig. 2a). Foster (8) has reviewed the limited data on DNA synthesis in resting cells and concluded that the rate lies in the range of 0.005 to 0.05 genomes per cell per day. If 8-oxo-dGTP is as mutagenic when

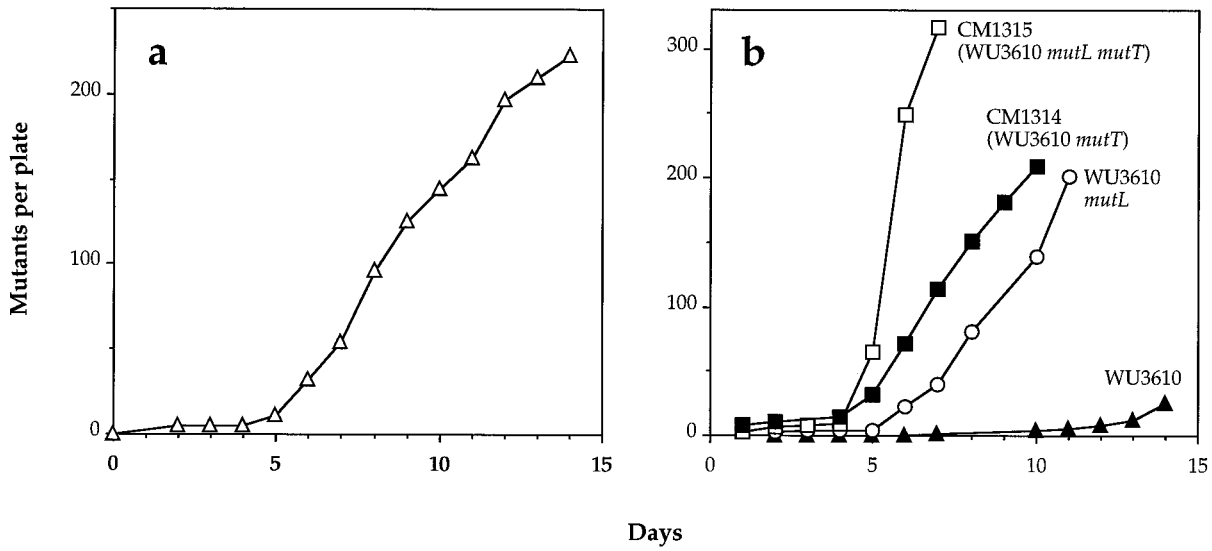

FIG. 2. (a) Appearance of Tyr^+ mutants during incubation at 37°C of 10^7 *mutT mutM* bacteria (strain CM1325, derived from WU3610) on glucose minimal leucine plates. (Data are the means of three experiments.) (b) Appearance of Tyr^+ mutants during incubation of 10^7 bacteria (WU3610 and its derivatives, CM1314 [*mutT*], WU3610 *mutL*, and CM1315 [*mutT mutL*]) at 37°C on glucose minimal leucine plates. (Data are the means of three experiments.)

it is incorporated in nongrowing cells as it is in growing cells, one would expect between 0.022 and 0.22 mutants on average to appear per day. How can this discrepancy of two to three orders of magnitude be explained?

Five possible explanations spring to mind. (i) There may be relatively more 8-oxo-dGTP in the precursor pool in stationary-phase than in growing *mutT* bacteria. There seems to be no obvious reason why the generation of oxidative species should be greater in stationary-phase than in growing bacteria; in fact, one might suppose that since their metabolic rate is reduced, there might be a reduced generation of such species, at least from metabolic sources. However, although the bacteria in this study were starved of a required amino acid, they were not starved of glucose, and it is possible that the metabolism of glucose yields more oxidative species when protein synthesis is inhibited. Fowler et al. (11) have reported that under anaerobic conditions the level of oxidative species, as reflected in the mutation rate of *mutT* bacteria, is influenced by the richness of the growth medium, illustrating that differences may occur under different nutrient conditions; however, it is not possible to extrapolate from these results to those reported here. While a difference in the relative level of 8-oxo-dGTP is possible (a subject worthy of study), it is unlikely that this would entirely explain the difference in the mutation rate. (ii) The rate of incorporation of 8-oxo-dGTP relative to that of dTTP may be greater in the type of DNA synthesis that occurs in nongrowing cells. (iii) There may be other, as-yet-unknown systems for preventing 8-oxo-dGTP mutagenicity that operate exclusively or predominantly in growing cells. (iv) The biochemical experiments may not reflect the situation in glucose minimal plates under amino acid selection conditions. It is true that none of the labelling experiments reviewed (8) were carried out on starvation plates. On the other hand, such experiments are almost impossible to perform on plates when using exogenous label because of the prototrophic bacteria growing on the plates. (v) A substantial amount of DNA synthesis may occur under the conditions of this study that would not be detectable when using exogenous labelled triphosphate precursors because the cells use endogenously generated precursors derived from the breakdown of RNA or DNA. This would be cryptic DNA synthesis and would, if breakdown of DNA were involved, represent DNA turnover. There exists a study, not reviewed by Foster (8), of such DNA turnover in bacteria held in buffer (24).

I am inclined to view explanations iv and v as being the most likely explanations for most of the observed discrepancy. That DNA synthesis goes on to a considerable extent under selection conditions is implicit in the observation that defects in the *mutHLS* mismatch correction genes cause a large increase in the rate of adaptive mutation (1, 9, 14, 15). Such an effect also implies that the DNA synthesized is hemimethylated. A *mutL* mutation caused an increase in the rate of appearance of Tyr^+ mutants in strain WU3610 that was similar in magnitude to that conferred by the *mutT* mutation (Fig. 2b). The fact that the mutator effect of *mutL* under starvation conditions is comparable to that during logarithmic growth does not suggest that there is any gross defect in methyl-directed mismatch correction during starvation; thus, the mutation rate of the *mutL* strain under starvation conditions implies either an increase in the polymerase error rate of several orders of magnitude or a rate of DNA synthesis similar to that which would account for the *mutT* mutator effect. In a double mutant, *mutT mutL*, the mutator effects appeared to be roughly additive, suggesting that the effects of the two deficiencies are independent and do not interact.

That there is DNA synthesis under the starvation conditions used here is indisputable, but further work is needed to establish its extent and whether it is responsible for the greatly elevated mutation rate observed. Future work should address the nature of the synthesis, the conditions under which it may occur, whether it is repair synthesis, whether it is associated with cell division, and whether there is turnover. If it is a general response to growth inhibition, it may have widespread implications for microbial ecology and evolution.

I thank M. Blanco for strain IC3126, R. Bockrath for WU3610 *mutL*, H. Maki for the *mutT*::Km^r mutation, P. Foster and M. H. L. Green for discussion, and A. R. Lehmann for a critical review of an early draft of the manuscript.

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