Regulation of $\textit{mutY}$ and Nature of Mutator Mutations in
\textit{Escherichia coli} Populations under Nutrient Limitation

Lucinda Notley-McRobb, Rachel Pinto, Shona Seeto, and Thomas Ferenci*

Department of Microbiology G08, University of Sydney, New South Wales 2006, Australia

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Previous analysis of aerobic, glucose-limited continuous cultures of \textit{Escherichia coli} revealed that G:C-to-T:A
(G:C$\rightarrow$T:A) transversions were the most commonly occurring type of spontaneous mutation. One possible
explanation for the preponderance of these mutations was that nutrient limitation repressed MutY-dependent
DNA repair, resulting in increased proportions of G:C$\rightarrow$T:A transversions. The regulation of the $\textit{mutY}$-
dependent DNA repair system was therefore studied with a transcriptional $\textit{mutY}$-lacZ fusion recombined into the
chromosome. Expression from the $\textit{mutY}$ promoter was fourfold higher under aerobic conditions than under
anaerobic conditions. But $\textit{mutY}$ expression was higher in glucose- or ammonia-limited chemostats than in
nutrient-excess batch culture, so $\textit{mutY}$ was not downregulated by nutrient limitation. An alternative explana-
tion for the frequency of G:C$\rightarrow$T:A transversions was the common appearance of $\textit{mutY}$ mutator mutations in
the chemostat populations. Of 11 chemostat populations screened in detail, six contained mutators, and the
mutator mutation in four cultures was located in the region of $\textit{mutY}$ at 66 min on the chromosome. The
spectrum of mutations and rate of mutation in these isolates were fully consistent with a $\textit{mutY}$-deficiency in
each strain. Based on PCR analysis of the region within and around $\textit{mutY}$, isolates from three individual
populations contained deletions extending at least 2 kb upstream of $\textit{mutY}$ and more than 5 kb downstream. In
the fourth population, the deletion was even longer, extending at least 5 kb upstream and 5 kb downstream of
$\textit{mutY}$. The isolation of $\textit{mutY}$ mutator strains from four independent populations with extensive chromosomal
rearrangements suggests that $\textit{mutY}$ inactivation by deletion is a means of increasing mutation rates under
nutrient limitation and explains the observed frequency of G:C$\rightarrow$T:A mutations in glucose-limited chemostats.

There is an increasing awareness that mutational processes
in bacteria are under environmental control or at least suscepti-
tble to stress responses (3, 9, 12, 13, 35, 37). Most of the
studies on these influences used batch cultured bacteria or
colonies on plates with their constantly changing or spatially
nonuniform environments. The complexity of the stress, be it
starvation, slow growth or external challenge, can be simplified
by using a steady-state application of particular stresses as can
be achieved in chemostat culture (14). As an initial study in this
direction, the mutational changes in three genes were analyzed in
and $\textit{mutM}$ genes were identified and
consisted of mainly base changes under steady-state glucose
limitation. The majority of nucleotide substitutions were G:C-
to-T:A (G:C$\rightarrow$T:A) transversions in all three genes. This
communication addresses possible causes of the mutational spec-
trum observed under glucose limitation, since it differs markedly from the generally observed spectrum of spontane-
ous mutations in \textit{E. coli} (29, 34).

A clue as to the origin of $\textit{mutT}$, $\textit{mutL}$, and $\textit{mutM}$ mutations was that the spectrum is characteristic of mutator mutants lacking
the oxodG repair system (20). The genes $\textit{mutM}$ and $\textit{mutY}$ are
involved in this process, but $\textit{mutM}$ changes are only weakly
mutagenic (2). Hence the mutational spectrum under glucose
limitation was most likely a function of $\textit{mutY}$ regulation or mutation. A question addressed here is whether $\textit{mutY}$ regula-
tion is changed by growth conditions to reduce DNA repair. Alternatively, was a mutation in $\textit{mutY}$ or, less likely, $\textit{mutM}$
commonly found in populations growing under glucose-limited
conditions?

Both $\textit{mutM}$ and $\textit{mutY}$ genes are part of complex operons
(11), but no physiological data were available on expression of
$\textit{mutY}$ until recently (10), and few data on $\textit{mutM}$ were available
(16). $\textit{mutY}$ was shown to be the first gene in an operon and to
be regulated by aerobic stress, but not by oxygen stress regulators
(10, 11). Also, the effect of $\textit{mutM}$ and $\textit{mutY}$ on stationary-
phase, starvation-associated mutation indicated that $\textit{mutY}$, but
not $\textit{mutM}$, mutants increase mutation rates under these condi-
tions (2). The regulation of these genes by steady-state nu-
trient limitation was not previously studied, and to observe
whether control of $\textit{mutY}$ was responsible for the spectrum of
mutations in chemostat cultures, the expression of $\textit{mutY}$
needed to be studied under steady-state nutrient limitation.
Given the lack of information on $\textit{mutY}$ regulation, other
growth conditions were also investigated. In particular, $\textit{mutY}$
repair is potentially important in repair of oxidative DNA
damage (20), so the effect of $\textit{O}_2$ stress on $\textit{mutY}$ expression was also assessed.

The alternative explanation for the mutational spectra was
that $\textit{mutY}$ mutator mutations spread in glucose-limited popu-
lations and bias the type of DNA changes in these populations.
The advantage of mutators in adapting to chemostat condi-
tions is well-known (5). Mutator mutations have been observed
in long-term recycled batch cultures (31, 32) and occur at a
finite level in natural bacterial populations (15). Mutator mu-
tations are coenriched by selection pressure for advantageous
mutations (18), which are common in nutrient-limited popu-
errors were generated during cloning or amplification.

chosen recombinant was verified by the method of Powell et al. (27) and desig-
nated strain BW3500. The promoter insert was fully sequenced to ensure no
insertion in single copy in the lambda attachment site in strain MC4100. Insertion in single copy in the

TABLE 1. Strains and plasmids used in this study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>Reference or source</th>
</tr>
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<tbody>
<tr>
<td>AT2446</td>
<td>Hfr recA metC69 thi</td>
<td>Coli genetic stock center</td>
</tr>
<tr>
<td>BW2952</td>
<td>MC4100 F(lac-proAB) hsd5 argF-lac Z-proAB::Tn10</td>
<td>27</td>
</tr>
<tr>
<td>BW3143</td>
<td>BW2952 mglA::Tn10</td>
<td>23</td>
</tr>
<tr>
<td>BW3185</td>
<td>MC4100 mutS srZ::Tn10 (from chemostat isolate)</td>
<td>25</td>
</tr>
<tr>
<td>21Qa1 (BW3490)</td>
<td>BW2952 mutY 3490 + unknown</td>
<td>Chemostat 2 isolate</td>
</tr>
<tr>
<td>L30a1 (BW3489)</td>
<td>BW3143 mutY 3489 + unknown</td>
<td>Chemostat L3 isolate</td>
</tr>
<tr>
<td>10U6 (BW3488)</td>
<td>BW2952 mutY 3488 + unknown</td>
<td>Chemostat C isolate</td>
</tr>
<tr>
<td>C7F1 (BW3487)</td>
<td>BW2952 mutY 3487 + unknown</td>
<td>This study</td>
</tr>
<tr>
<td>BW3500</td>
<td>MC4100 katt[mutY-lacZ::Tn10]</td>
<td>This study</td>
</tr>
<tr>
<td>BW3509</td>
<td>BW3500 galf::Tn10</td>
<td>This study</td>
</tr>
<tr>
<td>BW3510</td>
<td>BW3500 galf::Tn10 mutY 3490</td>
<td>This study</td>
</tr>
<tr>
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<td>MC4100 galf::Tn10</td>
<td>This study</td>
</tr>
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<td>BW2952 mutY::Tn10</td>
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<td>BW3527</td>
<td>BW3500 mutY::Tn10</td>
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<tr>
<td>DY330</td>
<td>W3110 ΔlacU16 gal490 ΔcII857 Δ(cro-bioA)</td>
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<tr>
<td>JM2053</td>
<td>HisC galf::Tn10 mglP psmM psgC-G con pSF his gndR thyA ivs nag</td>
<td>P. J. F. Henderson</td>
</tr>
<tr>
<td>MC100</td>
<td>F' araD139 Δ(argF-lacU169 ppsM150 lacO1 RE1 lacI1 ppsF25 fht18051 rbsR</td>
<td>This study</td>
</tr>
<tr>
<td>NM522</td>
<td>F' supE thi Δ(lac-proAB) his5 (r m) [F' proAB' lacPZAM15]</td>
<td>Promega Corp.</td>
</tr>
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<td>pAM2555</td>
<td>pRS415 cut with SmaI containing 825-bp PCR fragment amplified from MC4100 and</td>
<td>2</td>
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<tr>
<td>pTTY</td>
<td>Intact mutY cloned in pTrc99A</td>
<td></td>
</tr>
<tr>
<td>pRS415</td>
<td>Vector for gene fusions; tet' on bla T14 'trp-lacZYA (Ap')</td>
<td>30</td>
</tr>
</tbody>
</table>

Bacterial strains. All bacterial strains used in this study are derivatives of E. coli K-12 and are shown in Table 1. P1 transduction was carried out using P1 cml cI9000 according to the method of Miller (19). A P1 lysate made on strain JM2053, containing galf::Tn10, was used to transduce selected chemostat isolates with increased mutation rates. For each chemostat isolate, a P1 lysate was made of a tetracycline-resistant transductant that still had an elevated mutation rate. Then, the mutation causing the elevated mutation rate was then cotransduced into strain NM522, and those forming blue colonies on X-Gal (5-bromo-4-chloro-3-indolyl-beta-D-glucuronic acid) plates were digested with EcoRI to test whether the insert was ligated in the correct orientation. The fusion construct was then recombined into the complementary phage λRS45 (30) before insertion at the lambda attachment site in strain MC4100. Insertion in single copy in the chosen recombinant was verified by the method of Powell et al. (27) and designated strain BW3500. The promoter insert was fully sequenced to ensure no errors were generated during cloning or amplification.

MATERIALS AND METHODS

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Construction of a mutT::Tn10 insertion. A mutT::Tn10 insertion was constructed by the method of Yu et al. (38). Primers F1Tn10F (5' GCCAACAAC TACCGTACCGCGATGGCGGCTGACATCGTGTAGCC-3') and R2Tn10R (5' ATCTTGACTTCTGGTACCTACGGAAACAGAGGG TCATTATTTCCG-3') were designed with tet homology at the 3' ends to amplify the tetracycline resistance cassette from strain BW3143 (mgl::Tn10) and with sequences homologous to the mutT region at the 5' ends. PCR was carried out under standard conditions with primer annealing at 58°C and extension for 2 min. The 2.075-bp product was purified using Wizard PCR prep DNA purification kit (Promega Corp., Sydney, Australia) and electroporated into DY330 as per the method of Yu et al. (38). Recombination in the DY330 background resulted in deletion of the entire mutT gene, including 203 bp upstream and 54 bp downstream, and replacement with the tet resistance cassette to form strain BW3523. A P1 lysate was then made of this strain and used to transduce BW2952 and BW3500, selecting for tetracycline resistance, to give strains BW3526 and BW3527, respectively.

Culture conditions. The basal salts medium used in all experiments was minimal medium A (MMA) (19) supplemented with glucose, lactate, or glycolate as specified for each experiment. Batch cultures contained 0.4% (wt/vol) sugar, unless otherwise specified, and were harvested during mid-exponential growth as defined in the individual experiments. TA plates contained NaCl (5 g/liter), tryptone (10 g/liter), and 1.5% (wt/vol) agar. The expression of the mutT-lacZ fusion in strain BW3500 was measured during growth in minimal medium containing 0.02% (wt/vol) glucose with vigorous shaking at 37°C. Pararquat (5 μM), H₂O₂ (0.0025% [vol/vol]), and novobiocin (20 μg/ml) were added to the culture medium, and expression was measured at early exponential phase. Anaerobic cultures were grown in filled bottles without shaking.

Glucose-limited chemostats (80 ml) were set up as previously described (6). Monitoring of mutT-lacZ expression in strain BW3500 was studied in chemostats run for several days, limited for both glucose and nitrogen. Glucose-limiting chemostats had 0.02% (wt/vol) glucose in the feed medium and were run at dilution rates of 0.1, 0.3, and 0.6 h⁻¹. Nitrogen-limiting chemostats at a dilution rate of 0.3 h⁻¹ had reduced ammonium sulfate levels from 1 to 0.04 g/liter, and the glucose concentration increased to 0.2% (wt/vol) glucose with vigorous shaking at 37°C. Pararquat (5 μM), H₂O₂ (0.0025% [vol/vol]), and novobiocin (20 μg/ml) were added to the culture medium, and expression was measured at early exponential phase. Anaerobic cultures were grown in filled bottles without shaking.
DNA extraction and blotting analysis. Chromosomal DNA of the starter strain (BW2595) and the mutant Tn10-containing strain BW3143, and the four chemostat isolates was prepared as follows. A 10-mM aliquot of overnight culture grown on Luria broth was centrifuged at 3,120 × g for 10 min and resuspended in 5 ml of TE buffer, pH 8 (50 mM Tris, pH 8; 50 mM EDTA, pH 8). The suspension was frozen at −20°C for 30 min and then thawed at room temperature in the presence of lysozyme (1 mg/ml). Once thawed, the culture was placed on ice for 45 min. One milliliter of STE buffer (0.5% [wt/vol] sodium dodecyl sulfate; 50 mM Tris, pH 7.5; 0.4 M EDTA, pH 8; protease K, 1 mg/ml) was added before heating at 50°C for 60 min. DNA was extracted with an equal volume of Tris-buffered phenol (pH 8) and precipitated with 3 M sodium acetate and ethanol. The DNA precipitate was spooled out and dissolved in 5 ml of TE buffer, pH 7.5 (50 mM Tris, pH 7.5; 1 mM EDTA, pH 8) containing 100 μg of RNase per ml. The DNA was extracted once with an equal volume of chloroform before precipitation with sodium acetate and ethanol and then finally was dissolved in 2 ml of TE buffer, pH 7.5, and stored at 4°C. This DNA was used both in PCRs and for dot blot analysis. Equivalent loadings of chromosomal DNA (as determined by staining in an agarose gel) were boiled for 10 min before blotting directly onto a positively charged nylon membrane (Hybond-N+; Amersham Int. Plc.) using a Bio-Dot Slot format apparatus (Bio-Rad Laboratories, Richmond, Calif.). The membrane was air dried for 30 min and then baked at 120°C for 30 min. Prehybridization and hybridization reactions were carried out overnight at 50°C with digoxigenin (DIG)-dUTP-tailed probes including mutY-F2, mutY-F3, and mutY-F9. DIG-labeled dUTP was added to the 3′ end of each oligonucleotide probe using a DIG oligonucleotide labeling kit (Roche Diagnostics Australia, Pty. Ltd.) and the recommended protocol. As a positive control, the strains were probed with an oligonucleotide complementary to the housekeeping gene encoding β-galactosidase (eno: 5′-TGAACGACAAGCCCAGTACGACGAGG-3′) and mutY with digoxigenin (DIG)-dUTP-tailed probes including mutY-F2, mutY-F3, and mutY-F9. DIG-labeled dUTP was added to the 3′ end of each oligonucleotide probe using a DIG oligonucleotide labeling kit (Roche Diagnostics Australia, Pty. Ltd.) with the recommended protocol was used to detect DIG-labeled DNA.

RESULTS

Regulation of mutY expression. A transcriptional mutY-lacZ fusion was constructed, recombined in single copy into the chromosome, and grown under a variety of physiological conditions to test the range of variation in mutY expression. As shown in Fig. 1, growth phase controlled the mutY promoter. Expression was maximal approximating stationary phase in aerobic cultures but decreased in stationary phase. In confirmation of recent transcript data, aerobicism was another factor in mutY regulation (10). As shown in Table 2, the presence of O2

![Image](http://jb.asm.org/Downloaded from http://jb.asm.org/)

**TABLE 2. Expression of mutY-lacZ fusion under various growth conditions**

<table>
<thead>
<tr>
<th>Culture methoda</th>
<th>Limiting substrate</th>
<th>Dilution rate (h⁻¹)</th>
<th>Growth conditions</th>
<th>β-Galactosidaseb (Miller units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch</td>
<td></td>
<td>+/−</td>
<td>−/−</td>
<td>65 ± 10</td>
</tr>
<tr>
<td>Batch</td>
<td></td>
<td>+/−</td>
<td>+/−</td>
<td>78 ± 2</td>
</tr>
<tr>
<td>Batch</td>
<td></td>
<td>−/−</td>
<td>+/−</td>
<td>86 ± 5</td>
</tr>
<tr>
<td>Batch</td>
<td></td>
<td>−/−</td>
<td>−/−</td>
<td>108 ± 5</td>
</tr>
<tr>
<td>Batch</td>
<td></td>
<td>−/−</td>
<td>−/−</td>
<td>17 ± 1</td>
</tr>
<tr>
<td>Batch</td>
<td></td>
<td>+/−</td>
<td>−/−</td>
<td>36 ± 4</td>
</tr>
<tr>
<td>Chemostat Glucose</td>
<td>0.1</td>
<td>+/−</td>
<td>+/−</td>
<td>159 ± 2</td>
</tr>
<tr>
<td>Chemostat Glucose</td>
<td>0.3</td>
<td>+/−</td>
<td>+/−</td>
<td>131 ± 3</td>
</tr>
<tr>
<td>Chemostat Glucose</td>
<td>0.6</td>
<td>+/−</td>
<td>+/−</td>
<td>148 ± 8</td>
</tr>
<tr>
<td>Chemostat Nitrogen</td>
<td>0.3</td>
<td>+/−</td>
<td>+/−</td>
<td>178 ± 7</td>
</tr>
</tbody>
</table>

a Batch-grown cells were grown in 0.02% glucose–MMA until exponential phase (OD580 between 0.3 and 0.4) either aerobically by shaking vigorously at 300 rpm or anaerobically in filled bottles without shaking. Paraquat was added to cultures at a concentration of 5 μM and H2O2 at a concentration of 0.0025% (vol/vol). Novobiocin was added at a concentration of 20 μg/ml. Chemostat cultures were grown aerobically under nitrogen or glucose limitation at various dilution rates.

b The values reported are means and standard deviations measured from at least three independent cultures.
resulted in a four- to fivefold elevation of mutY fusion activity. The stimulation by O2 did not appear to be part of a stress response, given that neither superoxide (from added paraquat) or H2O2 affecting stress responses (36) stimulated mutY expression much above aerobic levels. The lack of target sequences in the mutY promoter for global stress regulators (11) and recent transcript data (10) were also consistent with the lack of superoxide or H2O2 effects. The aerobic-to-anaerobic transition is regulated by multiple transcriptional factors as well as DNA-structural changes in E. coli (28). This left open the possibility that mutY was regulated by the level of DNA supercoiling. Indeed, the extent of regulation of mutY in Fig. 1 and Table 2 resembled that of tonB, which is also influenced by aerobicis and growth phase in a similar manner (7). To test the possible influence of supercoiling, the effect of the DNA gyrase inhibitor novobiocin was tested as shown in Table 2. Reduced negative supercoiling associated with novobiocin resulted in increased mutY expression in both aerobic and anaerobic cultures, also by a similar margin as with tonB (7).

Aside from batch cultures, mutY expression was also monitored in continuous cultures limited in growth rate either by glucose or ammonia (N) limitation. As shown in Table 2, there was no difference between glucose and N limitation at a particular growth rate and no difference between different growth rates. These results point to lack of regulation by RpoS, cAMP, or ppGpp, all of which markedly change in concentration under the range of conditions analyzed (8). Importantly, the level of expression in nutrient-limited cultures was actually higher than that in nutrient-excess exponential batch cultures. The level of supercoiling in nutrient-limited cultures may be lower and potentially explains the higher expression of mutY under nutrient limitation. However, an obvious conclusion is that glucose-limited continuous cultures are unlikely to have a particular mutational spectrum due to increased mutation rates caused by downregulation of mutY.

Appearance of mutY mutator mutations in glucose-limited populations. In the absence of mutY downregulation, continuous cultures were analyzed for mutational changes affecting mutY. Eleven populations growing under glucose limitation at dilution rates of 0.3 or 0.6 h−1 were tested for mutators in daily samples of the cultures. Random colonies from each day sample (between 8 and 20 colonies tested) were screened for increased mutation frequencies on rifampin plates for RifR. Of the 11 independent chemostat populations growing and assessed daily for mutators in this way, 6 cultures had isolates with increased mutation rates. In these cultures, isolates with elevated mutation frequencies comprised 30 to 100% of the population in daily samples after approximately 100 to 150 generations of continuous culture. The appearance of mutators coincided with a 60- to 70-fold increase in the frequency of neutral T5-resistant mutants as well as the emergence of selectively favorable mutations (in mgl, mutT, mlc, and ptsG [17, 23, 24]) in a high percentage of the population (results not shown).

Of the six populations with mutators, two contained mutS mutations as determined previously (25). The mutS isolates exhibited an increase in mutation frequencies of more than 100-fold when cultured on Rif plates, whereas the other four populations contained isolates with lower mutation frequencies. The lower increase (approximately 40-fold) in mutation frequency in members of populations 10, 21, C7, and L3 was comparable with the increase in a mutY deletion strain (BW3526) and that found in mutY mutators in other studies (references 21 and 33 and results not shown). Mapping of the mutator mutation in an isolate of each of the four populations by P1 transduction located the mutations in the galP-metC region at 66 min on the chromosome of E. coli, consistent with mutY as the mutated locus. Sequencing of mutations in mgl and ptsG arising in these populations showed that the DNA changes were indeed transversions, characteristic of the mutY mutator genotype (Table 3) (21). Transfer of the mutation by P1 transduction with galP::Tn10 into a strain containing a mutY-lacZ fusion (BW3500) at the lambda attachment site (to create BW3510), showed there was no influence on mutY transcription in trans (Table 4); therefore, the mutation affected mutY directly and was not in a nearby regulator of mutY. A similar result was obtained with the mutY::Tn10 insertion on mutY transcription (Table 4), so mutY does not autoregulate its own expression. Finally, introduction of intact mutY on a plasmid, pTTY (2), reduced mutation rates back to the wild-type (WT) level for each of the four presumptive mutY isolates, but not for the mutS isolates (result not shown).

The nature of the mutator mutation in an example of each of the chemostat populations (isolate 21Qa1 from population 21;
isolate L3Oa1 from population L3; isolate 10U6 from population 10; and isolate C7F1 from population C7) was investigated for the mutator phenotype. As a control to ensure that the mutY+ allele was intact in these isolates, PCR failed to amplify any fragment in isolate 21Qa1, suggesting a deletion that extended at least beyond the mutY promoter (11). Much of the mRNA expression under aerobic conditions than under anaerobic growth conditions. The extent of the increase with the mutY promoter (11). Much of the mutY regulation described in Fig. 1 and Table 2 and in reference 10 can be rationalized on the basis of the aerobic-to-anaerobic transition affecting mutY promoter structure. The results with the gyrA210 promoter (mutY-flanking primers. Initially, a primer pair surrounding the mutY gene was used (mutY2 and mutY3) to amplify the region (Fig. 2A) in both the WT and the four mutant strains. The fragment could be amplified in the WT strain (BW2952), but no PCR fragment was amplified in any of the four mutator mutants. As there may have been a loss of one or both of the primer sites, more primer pairs were designed (as shown in Fig. 2A). A 4,222-bp fragment extending from primers mutY2 to mutY5 also gave no PCR product in each of the mutants (Fig. 2B). Primers were then used in pairs that amplified a wider region surrounding mutY.

Upstream of mutY, primers mutY7 to mutY9 amplified a 5,040-bp region in the WT and in three of the chemostat isolates: L3Oa1, 10U6, and C7F1. This suggests this region is still intact in these isolates. However, PCR failed to amplify any fragment in isolate 21Qa1, suggesting a deletion that extends at least beyond the mutY9 site 4.5 kb upstream of mutY.

Other pairs of primers included mutY7 to mutY11 (4,264-bp fragment), and mutY10 to mutY12 (5,534-bp fragment). There was no amplification using these primers in any of the isolates, suggesting rearrangement or loss of DNA downstream of mutY as well. As a control to ensure that the DNA prepared was intact, all isolates were subject to PCR using primers galSF1 and galSR2 amplifying the galS gene. All gave the correct amplification product (results not shown).

Dot blotting using DIG-dUTP-labeled probes was able to confirm the deletion results. Primers mutY2 (300 bp upstream of mutY), mutY3 (mid-mutY), and mutY9 (approximately 5 kb downstream of mutY) were chosen and hybridized to DNA on membranes at 50°C (Fig. 2C). All three probes hybridized to the WT strain but not to any of the mutator mutants, while mutY2 and mutY9 hybridized to the second control strain (BW3526) containing the mutY::Tn10 insertion. The mutY3 probe did not react in this strain as this region is deleted and replaced with the Tn10 insertion. The positive control probe, eno, specific for the housekeeping gene enolase, located at 60 min on the chromosome, hybridized with all strains.

**DISCUSSION**

The role of mutY in E. coli is to contribute to repair of DNA damaged by reactive O2 species (20). Hence, it is not surprising that there was a higher level of mutY expression under aerobic conditions than under anaerobic growth conditions. The extent of the increase with the mutY fusion was in line with recent results with transcript analysis (10). Also confirming the results of Gifford et al., the lack of stimulation by the major O2 stresses through superoxide and peroxide is consistent with the lack of obvious binding sites for global regulators in the mutY promoter (11). Much of the mutY regulation described in Fig. 1 and Table 2 and in reference 10 can be rationalized on the basis of the aerobic-to-anaerobic transition affecting mutY promoter structure. The results with the gyrA210 promoter (mutY-flanking primers) and the influence of aerobicism on DNA supercoiling (7) suggest that the pattern of regulation of mutY has many similarities to the supercoiling-mediated control of tonB expression by aerobiosis and growth phase (7). The absence of regulation by growth rate under nutrient limitation also rules out mutY as being part of the RpoS-mediated general stress response or ppGpp-dependent regulation (8).

The expression of mutY under nutrient limitation ruled out the notion that the high frequency of G:C→T:A transversions under glucose limitation was due to reduced mutY expression. The earlier mutation data were more likely be due to the repeated occurrence of mutator mutations in long-term continuous culture populations. Evidence for the occurrence of mu-
tators was obtained in the populations 21, C7, 10, and L3 analyzed in detail. The appearance of mutators was accompanied by major population shifts as well as biased mutation rates. The appearance of mutY mutations in two of the chemostat populations coincided with the appearance of mgl and ptsG changes, with retransformations being the predominant class of mutation. Unfortunately, reanalysis of the earlier populations (23, 24) failed to find mutators in the stored weekly samples, but the 7-day intervals previously used could easily miss a transient mutator sweep that can appear and disappear within days (reference 25 and results not shown).

The mutY mutation present in isolates from each of the chemostat populations was stable and maintained for multiple generations after subculture. The PCR analysis of the mutations showed the mutations were caused by a substantial deletion in each case. The rearrangement extended at least 2 to 5 kb on one or more sides of the gene. Since the endpoints of the deletion were not sequenced, there is no clue as to the cause of the mutY mutations. Given the similarity of events in four independent populations, it would be interesting to determine if particular features of the surrounding genome contribute to these events. An inspection of the chromosomal region for insertion sequences, BIME sites, chi sites, or repeats did not reveal obvious features contributing to the results.

Our observations with experimental populations may well be mirrored in evolving natural populations. Loss of the mutY gene was evident in evolving Pseudomonas aeruginosa populations in cystic fibrosis patients, and two individual isolates from two individual patients both failed to amplify mutY (26). The extent of the deletions was not determined, but PCR primers surrounding mutY failed to amplify the gene. Perhaps the major mutator gene rearrangements we observe occur in Pseudomonas as well.

There is a curious similarity between these mutY deletions and the extensive rearrangement and/or deletion of the mutS region previously observed (15). In chemostat populations, the two independent mutS mutators (populations 20 and 26 [25]) are not fully characterized yet, but preliminary evidence also suggests major deletions. This leaves open the possibility that mutator deletions are significant in evolving populations, but the source of these deletions requires further definition. In any case, the mutY inactivation by a chromosomal rearrangement is a means of increasing mutation rates in times of nutrient limitation and results in the prevalence of G:C→T:A mutations in glucose-limited populations.

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REFERENCES


