Mutator Gene of Escherichia coli B

ELI C. SIEGEL* AND VERNON BRYSON

Institute of Microbiology, Rutgers, The State University, New Brunswick, New Jersey 08903

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An azaserine-resistant derivative of Escherichia coli B/UV, AZA/R1, was found to carry a mutator gene. This gene, designated mutS1, was mapped by means of conjugation and Plkc-mediated transduction. The mutS1 gene was cotransduced with argB at a frequency of 2.4%; the gene order in this region of the chromosome is thy argB mutS1. To determine whether a relationship commonly exists between azaserine resistance and the mutator property, 12 additional azaserine-resistant derivatives of B/UV were developed and tested for the mutator phenotype. None of the twelve was a mutator strain. The level of azaserine resistance was not increased over that of the recipient parent when mutS1 was transduced to an azaserine-susceptible strain. Reversion studies indicated that mutS1 induced adenosine-ribosylthymine to guanosine-cytidine and guanosine-cytidine to adenosine-ribosylthymine transitions. Because such mutational changes are suppressible with deoxynucleosides when induced by base analogues, an attempt was made to suppress the mutator activity of mutS1 by the addition of deoxyribonucleosides to the medium.

No suppression was found. Recombinants were prepared containing mutS1 and the Treffers mutator gene of E. coli K-12. The effect of the mutator genes appears to be additive.

Mutator genes have been found in three laboratory strains of Escherichia coli (15, 35, 39). The genetic locations of two of these, the Treffers mutator gene of K-12, and ast, the mutator gene of the Harvard strain, have been determined (36, 42). The type of mutational change induced by the Treffers mutator has been identified by Yanofsky, Cox, and Horn (41) as an adenine-thymine to cytosine-guanine transversion (AT → CG).

In the present study, the new mutator strain found by us (35) is further characterized and genetically analyzed.

Materials and Methods

Bacterial strains. Table 1 lists the bacterial strains used. Where no source is given, the strain was either from The Institute of Microbiology or derived by us.

Media. The following media were used: nutrient broth, containing 0.8% Difco Nutrient Broth and 0.5% NaCl (with 1.5% Difco agar for solid medium); L broth, soft agar, and base layer agar (27); minimal medium A, solidified with 1.0% agar (9); Brom Thymol Blue (BTB)-sugar-agar (6); eosin-methylene blue (EMB)-sugar-agar (2.7% BBL Levine Eosin

1 Based on a thesis submitted by Eli C. Siegel to Rutgers, The State University, in partial fulfillment of the requirements for the Ph.D. degree.

2 Present address: Department of Biochemistry, Albert Einstein College of Medicine, Yeshiva University, Bronx, N.Y. 10461.

Methylene Blue Agar and 1.0% sugar). Saline diluent: 0.85% NaCl. Amino acids and thymine were added when necessary to the minimal medium to give a final concentration of 50 μg/ml. Thiamine hydrochloride was used at a concentration of 1 μg/ml. Vitamin Free Casamino Acids (Difco) was added to the minimal medium at a concentration of 50 μg/ml when E. coli strain JE1025 (22) was used. All percentages are w/v.

Bacteriophage. T2 was obtained from A. D. Hershey. Other phages were obtained from the Institute of Microbiology. E. coli B/UV was host for the T phages. Conventional phage techniques as described by Adams (1) were employed.

Culture conditions. All incubator or rotary-shaker temperatures were 37 °C unless otherwise stated. To obtain a stationary-phase culture, broth was incubated at 37 °C to a cell density of 10^8 ml^-1. To determine survival, a 10^8 cfu/ml dilution of the culture was plated onto nutrient broth and onto nutrient agar and incubated at 37 °C. The viable count of both cultures was then determined.

Determination of ultraviolet (UV) resistance. The UV source was a reflector containing two General Electric 15-w mercury vapor lamps, calibrated with a General Electric germicidal intensity meter (model UV 480). The dose rate was 4.1 ergs per mm² per sec. Precautions were taken to prevent photoreactivation. To determine survival, an 18-hr nutrient-broth culture was diluted 1,000-fold in saline, and 5 ml of this dilution was placed in a watch glass and continually agitated by a magnetic stirrer during irradiation. After each
Table 1. Bacterial strains used

<table>
<thead>
<tr>
<th>Strain</th>
<th>Significant characteristics</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Derivatives of E. coli B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B/UV</td>
<td>malB⁻ val⁺ uv⁺</td>
<td></td>
</tr>
<tr>
<td>AZA/R₁</td>
<td>malB⁻ val⁺ mutSI uv⁺</td>
<td></td>
</tr>
<tr>
<td>A104</td>
<td>thr⁻ leu⁺ his⁺ lac⁻ malB⁻ mutSI</td>
<td></td>
</tr>
<tr>
<td>A105</td>
<td>thr⁻ leu⁺ his⁺ lac⁻ malB⁻ xyl⁻ mutSI</td>
<td></td>
</tr>
<tr>
<td>A106</td>
<td>thr⁻ leu⁺ his⁺ lys⁻ lac⁺ malB⁺ xyl⁺</td>
<td></td>
</tr>
<tr>
<td>A his-l⁻9</td>
<td>9 independent his⁺ derivatives of AZA/R₁</td>
<td></td>
</tr>
<tr>
<td>A his-3NM</td>
<td>his⁺ mutSI⁺ malB⁺</td>
<td></td>
</tr>
<tr>
<td>B(K)5</td>
<td>leu⁺ his⁺ lys⁺ lac⁺ xyl⁺ malB⁻ mutSI</td>
<td></td>
</tr>
<tr>
<td>B(K)5 Mu</td>
<td>leu⁺ lys⁻ lac⁺ xyl⁺ malB⁻ mutSI</td>
<td></td>
</tr>
<tr>
<td>B(K)5 NM</td>
<td>leu⁺ lys⁻ lac⁺ xyl⁺ malB⁻ mutS⁺</td>
<td></td>
</tr>
<tr>
<td>B(W)</td>
<td>uv⁺</td>
<td></td>
</tr>
<tr>
<td>B/r (W)</td>
<td>uv⁺</td>
<td></td>
</tr>
<tr>
<td>Derivatives of E. coli K-12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>W3780 (met⁻)</td>
<td>Hfr H met⁺ str⁻ λ⁻</td>
<td>Lederberg</td>
</tr>
<tr>
<td>AB259 (met⁻)</td>
<td>Hfr H met⁺ thi⁺ λ⁻</td>
<td>Adelberg, met⁺ induced with EMS</td>
</tr>
<tr>
<td>Hfr Mu trp⁻</td>
<td>Hfr C trp⁺ mutTI</td>
<td></td>
</tr>
<tr>
<td>JE1025</td>
<td>thy⁻ arg⁻ str⁺ gal⁻</td>
<td>Ishibashi</td>
</tr>
</tbody>
</table>

* Symbols: +, wild type or, in case of λ, lysogenic; -, inability to synthesize or utilize or, in case of λ, nonlysogenic; †, resistance; ‡, sensitivity; Hfr, high-frequency donor; arg, arginine; gal, galactose; his, histidine; lac, lactose; leu, leucine; lyr, lysine; mal, maltose; met, methionine; mutSI. Treffers K-12 mutator gene; mutSI, mutator gene of AZA/R₁; str, streptomycin; thi, thiamine; thr, threonine; trp, tryptophan; uv, ultraviolet irradiation; val, valine; xyl, xylose.

Dose increment, a sample was removed and assayed on nutrient agar for colony count. The rapid UV method of Greenberg and Woody-Karrer (17) was also used.

Selection of mutants. All mutations occurring in derivatives of AZA/R₁, with the exception of xyl⁺, were of spontaneous origin; xyl⁻ was induced with ethyl methanesulfonate (EMS) by the procedure of Schwartz (34). Auxotrophic mutants were selected by the penicillin method (16). The fermentation mutants were detected by plating on EMB or BTB media containing a particular sugar.

Matings. Samples (1 ml) of stationary-phase nutrient-broth cultures of the donor and recipient strains were separately inoculated into 9 ml of nutrient broth and incubated on a rotary shaker for 2 hr. Then, 0.2 ml of the donor culture was added to 2 ml of the recipient culture in a tube and incubated with gentle shaking. Alternatively, 0.4 ml of the donor culture was mixed with 4.0 ml of the recipient in a 125-ml Erlenmeyer flask and incubated at 37°C without shaking. Donor and recipient cultures contained approximately 5 × 10⁶ cells per ml when mixed. The mating mixture was incubated for 60 or 90 min and then plated at appropriate dilutions on the selective media.

Interrupted matings were used to determine whether certain thr⁻ recombinants from K-12 × B crosses still restricted K-12 deoxyribonucleic acid (DNA); 0.9 ml of a late-log-phase culture of the recipient to be tested and 0.1 ml of a late-log-phase culture of the donor K-12 Hfr were mixed in a screw-top tube and incubated for 30 min on a roller drum revolving at 1 rev/min. The tube contents were then blended for 1 min on a Vortex Junior Mixer, and 0.5 ml of male-specific phage f2 (about 10⁹ phage per ml) was added to kill the Hfr cells. The mixture was incubated for 20 min and then plated on selective media. If selection is for a marker close to the origin of the Hfr, restricting and nonrestricting females can be distinguished by the number of recombinants formed. In all experiments, known restricting and nonrestricting females were used as controls.

Transduction. P1kc-transducing-lysates were prepared according to Curtiss et al. (8). The recipient was grown in L broth containing 2.5 × 10⁻⁸ M CaCl₂ to log phase (about 3 × 10⁸ cells per ml). P1kc prepared on AZA/R₁ was then added to give a multiplicity of infection of 7 to 11. After 20 min of further
incubation, samples were plated undiluted, and di-
luted 10-fold, on the selective medium.

Unselected marker analysis. Recombinants from
transduction and mating experiments were purified
by single-colony isolation on the same selective me-
dium used for the original isolation. The purified
colonies were then replicated to test for unselected
markers.

Several tests were used to determine the mutator
character of the recombinants. When W3780 was
used as the donor, the resulting purified recombinants
were inoculated into 2 ml of nutrient broth and grown
for 18 hr with aeration; 0.01-ml samples of these cul-
tures were spread with a Breeding loop on one-quarter
of a nutrient agar plate previously spread with 10^9 bac-
teriophage T1. The plates were incubated for 1 day.
In control experiments, cultures of the mutSl recipi-
ent gave about 300 T1+ colonies per 0.01 ml, and the
donor, W3780, yielded an average of 5 per 0.01 ml.
Recombinants, however, often gave intermediate re-
sults and had to be retested several times. Therefore, a
Str+ donor, AB259 (mut-), was used, and 0.1-ml
samples of 2-ml cultures prepared as above were
plated on nutrient agar containing 150 μg (per ml) of
streptomycin. After 2 days of incubation, mutSl con-
taining recombinants gave 40 to 80 colonies per plate,
and nonmutator, mutSt, recombinants yielded an
average of 1 colony per five plates.

B/K5; a mutSl recipient used in several matings,
grew slowly in nutrient broth, and, therefore, L broth
was used in the mutator test. L-broth cultures of mutSt
recombinants formed about 30 Str+ colonies per plate.
Strains carrying mutSt gave an average of one colony
per five plates.

Recombinants giving more than 10 Str+ colonies
per plate were classified as carrying mutSl. Those
recombinants that grew more slowly than the donor
were considered nonmutator. If the initial cul-
ture tested gave 1 to 10 colonies, a new culture
was made from the recombinant and tested. Some K-12 ×
B/K5 recombinants repeatedly formed only 2 to 10
colonies per plate. These were mutator, and the low
frequency of colonies was attributable to the cul-
tures not growing to full titer, i.e., 2 × 10^6 cells per ml.

Determination of the mutator character of JE1025
transductants was made with the T1 mutator test and
by determining the frequency of sodium azide resis-
tance. Loopfuls, about 10^-2 ml, of the same broth cul-
tures used for the T1 mutator test were streaked on
nutrient agar containing 125 μg of sodium azide per
ml. After 24 hr of incubation, streaks from mutSt-
containing cultures gave 50 to 100 Azr+ colonies. The
nonmutators formed 0 to 5.

Reversion studies. Minimal medium A-agar, sup-
plemented with all required growth factors except that
of the mutant studied plus 100 μg of nutrient-broth
powder per liter, was used to select revertants. All
revertant plates were incubated for 3 days. Large colo-
nies of wild-type appearance were considered true
revertants. All tests were repeated at least twice; in
each case, two plates for the treated and two plates
for the control were used. The following procedures
were used with the different mutants.

EMS-0.1 ml of an 18-hr nutrient broth culture
(about 2 × 10^8 cells) was either plated with 2.5 ml of
melted 0.7% agar or spread on the selective medium.
A sterile 12.7-mm disc of absorbent paper was put in the
center of the plate and moistened with 0.03 ml of EMS
(Eastman Organic Chemicals). The control plate was
made in the same way, except that no disc or EMS was
used.

2-Aminopurine (AP), used as 2-aminopurine nitrate
(Calbiochem), was dissolved in distilled water to a
concentration of 2 mg/ml. This solution was sterilized
by passage through a Millipore HA filter. The stock
solution was stored up to 1 week in the cold. AP stock
solution (0.5 ml) was added to 4.5 ml of nutrient broth,
giving a final concentration of 200 μg/ml. For the con-
trol, 0.5 ml of sterile distilled water was added to 4.5
ml of nutrient broth. Into each tube, 0.05 ml of an 18-
hr nutrient broth culture of the mutant studied was
inoculated. The tubes were incubated for 5 hr with
aeration, and then 0.1-ml samples from each tube were
plated on the selective medium. The AP and control
tubes were assayed for total viable cells. The control
cultures had 2 × 10^8 to 1 × 10^9 cells per ml. The AP
cultures had 50 to 90% of the cell number of the con-
trol cultures.

Resistance determinations. The frequency of mu-
tation to phage or drug resistance was determined by
spreading 0.1-ml samples (undiluted for B/UV and
duly diluted 10^-2 or 10^-3 for AZA/R) on nutrient-
agar plates containing drug or previously spread with
10^6 or more T-odd phage. T-odd phage and bacteria
were mixed in soft agar (0.7%) and plated. Assays for
total viable cells were also performed.

Fluctuation test. The procedure was based on that
of Luria and Delbrück (28). Each of a series of tubes
containing 1 ml of nutrient broth was inoculated with
approximately 200 cells (0.1 ml of a 10^-4 dilution of a
stationary-phase culture). The tubes were incubated
for 18 hr with aeration, and a 0.1-ml sample from
each tube was spread on nutrient agar containing 1,000
μg (per ml) of streptomycin. Five tubes in each series
were combined and assayed for total viable cells. The
streptomycin plates in the first experiment were incu-
bated for 2 days at 37 C and for 3 days at room tem-
perature, and in the second experiment, for 5 days at
37 C. Mutation rates were determined from the
formula a = −(ln 2)(ln p)/N, where a is the muta-
tion rate, p is the fraction of plates with no Str+ col-
onies, and N is the average of cells per sample (30).

Azaserine gradient plates. The method of prepara-
tion of these plates was previously described (35).

RESULTS

Characteristics of B/UV and AZA/R. In a
previous publication (35), the parent of AZA/R,
was designated as B/r because the rapid UV test
(17) showed it to be radiosensitive. Survival after
UV irradiation for this strain of B/r, and for
B(W) and B/r(W), showed that B/r was inter-
mediate in resistance compared to B(W) and
B/r(W) (Table 2). Following the suggestion of
Adler and Haskins (2) that the designation B/r be
used only for strains having all the characteristics
of Wildin's original B/r, we have designated the
parent of AZA/R1 as B/UV. The increase in resistance to UV in AZA/R1 is attributed to the cross-resistance relationship between azaserine and UV (40).

Characteristic of B and its derivatives, B/UV and AZA/R1, are valine-resistant, nonmotile, susceptible to all seven Phages, and do not ferment maltose.

Table 3 gives mutation frequencies in B/UV and AZA/R1. Additional frequencies were given elsewhere (35). Table 4 presents the result of the fluctuation tests. AZA/R1 cultures yielded a variety of mutants at frequencies significantly higher than those given by the nonmutator parent.

Genetic analysis of AZA/R1. Derivatives of AZA/R1 were used as recipients in crosses with K-12 donors (Table 5). Jacob and Wollman (23) found that a marker which entered before the selected marker and was not closely linked to it appeared with a frequency of about 50%, or in some cases higher. Lower frequencies, such as reported here, are indicative of restriction of the K-12 donor DNA (5, 6).

The frequencies of appearance of unselected markers in crosses of W3780 × A104 and A105 gave a rough location for mutS1; mutS+ recombinants did not appear when selection was for thr+ leu+ met+ but were found when his+ was also selected. This indicated that mutS1 was between

<table>
<thead>
<tr>
<th>Strain</th>
<th>Expt</th>
<th>N</th>
<th>C</th>
<th>p∞</th>
<th>a</th>
</tr>
</thead>
<tbody>
<tr>
<td>B/UV</td>
<td>1</td>
<td>2.3 × 10^6</td>
<td>32</td>
<td>32/32</td>
<td>&lt;2 × 10^-10</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2.5 × 10^6</td>
<td>29</td>
<td>29/29</td>
<td>&lt;1 × 10^-10</td>
</tr>
<tr>
<td>AZA/R1</td>
<td>1</td>
<td>2.5 × 10^6</td>
<td>30</td>
<td>10/30</td>
<td>3 × 10^-8</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2.2 × 10^6</td>
<td>27</td>
<td>19/27</td>
<td>1 × 10^-8</td>
</tr>
</tbody>
</table>

* Abbreviations: N, average number of cells per sample; C, total number of cultures tested; p∞, fraction of plates with no Str+ colonies; a, mutation rate.

<table>
<thead>
<tr>
<th>Selected markers</th>
<th>Per cent recombination*</th>
<th>Total recombinants tested</th>
<th>Unselected markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>thr+ leu+ met+...</td>
<td>12</td>
<td>49</td>
<td>lac+ 8 16</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>his+ 1 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>mutS+ 0 &lt;2</td>
</tr>
<tr>
<td>thr+ leu+ his+ met+...</td>
<td>0.2</td>
<td>50</td>
<td>lac+ 17 34</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>mutS+ 2 4</td>
</tr>
<tr>
<td>his+ met+c...</td>
<td>0.2</td>
<td>125</td>
<td>thr+ leu+ 14 11</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>lac+ 8 6.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>mutS+ 6 4.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>str+ 2 1.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>xyl+ 0 &lt;0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>maltB+ 0 &lt;0.8</td>
</tr>
<tr>
<td>maltB+ met+c...</td>
<td>0.001</td>
<td>65</td>
<td>thr+ leu+ 20 30</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>lac+ 13 20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>his+ 16 25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>mutS+ 11 17</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>xyl+ 13 20</td>
</tr>
</tbody>
</table>

* Recombinants per 100 males in mating mixture.

Recipieint A104.

Recipient A105.

lac and his, or beyond his on the chromosome. When selection was for his+ met+c in the cross W3780 × A105, mutS+ recombinants appeared with a frequency lower than lac+, which is proximal to his, but higher than str+, which is distal. These results suggested that mutS1 was located between his and str. The locus lys is at this approximate location on the chromosome (38). Therefore, a lys+ derivative of A105, designated

<table>
<thead>
<tr>
<th>Table 3. Mutation frequencies in B/UV and AZA/R1.</th>
<th>Rate of mutation to streptomycin resistance (1,000 µg/ml) in B/UV and AZA/R1 derivatives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nature of resistance mutation</td>
<td>Ratio of mutants to total cells*</td>
</tr>
<tr>
<td>B/UV</td>
<td>AZA/R1</td>
</tr>
<tr>
<td>Phage T2</td>
<td>&lt;1 × 10^-3</td>
</tr>
<tr>
<td>Phage T6</td>
<td>5 × 10^-4</td>
</tr>
<tr>
<td>Na azide (125 µg/ml)</td>
<td>6 × 10^-4</td>
</tr>
<tr>
<td>Vancomycin (50 µg/ml)</td>
<td>3 × 10^-7</td>
</tr>
<tr>
<td>Streptomycin (150 µg/ml)</td>
<td>&lt;2 × 10^-4</td>
</tr>
</tbody>
</table>

* The number of resistant cells varied in repeated experiments. The results of typical determinations are given.
A106, was selected and used in additional matings.

A locus (or two closely linked loci) controlling restriction and modification is located on the E. coli chromosome near thr and to its left. If this locus in B is replaced by the corresponding K-12 locus, the B recipient will no longer restrict K-12 DNA (5). AB259 ($met^-$) was crossed to A106, and $thr^+$ recombinants were selected. By use of the interrupted-mating procedure, six of the $thr^+$ recombinants were mated to AB259 ($met^-$), and the frequency of $leu^+$ recombinants (the gene nearest the origin for which the strains were appropriately marked) was determined. One of the six, designated B(K)5, yielded as many recombinants as did a K-12 recipient. However, when the frequencies of unselected markers in an AB259 ($met^-$) \times B(K)5 cross were examined, B(K)5 still appeared to restrict. No explanation is offered for the behavior of B(K)5, which, although restricting K-12 DNA, yielded more recombinants than did its parent, A106; B(K)5 was therefore used for further mapping of mutSI.

The results of a typical cross between AB259 ($met^-$) and B(K)5 are given in Table 6. Double selection, such as for $leu^+$ $lys^+$, was used because $lys^+$ revertants were almost as frequent as $lys^+$ recombinants. Nonmutator recombinants appeared among $lys^+$ recombinants at a frequency of 46%. Judged from the frequency of appearance of other markers, this indicates close linkage between $lys$ and mutSI.

**Table 6. Genetic analysis of recombinants from the mating of AB259 ($met^-$) \times B(K)5**

<table>
<thead>
<tr>
<th>Selected markers</th>
<th>Per cent recombination</th>
<th>Total recombinants tested</th>
<th>Unselected markers</th>
<th>Marker</th>
<th>No. found</th>
<th>Per cent of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>$leu^+$ $met^-$, . . .</td>
<td>13</td>
<td>46</td>
<td>5</td>
<td>11</td>
<td>$lac^+$</td>
<td>$his^+$</td>
</tr>
<tr>
<td>$leu^+$ $his^+$ $met^-$, . . .</td>
<td>0.04</td>
<td>39</td>
<td>3</td>
<td>7.7</td>
<td>$lac^+$</td>
<td>$lys^+$</td>
</tr>
<tr>
<td>$leu^+$ $lys^+$ $met^-$</td>
<td>0.03</td>
<td>41</td>
<td>4</td>
<td>9.8</td>
<td>$lac^+$</td>
<td>$his^+$</td>
</tr>
</tbody>
</table>

An attempt was made to cotransduce $lys^+$ and mutSI. A Plkc lysate was prepared on AZA/R1, and a $lys^-$ mutSI recombinant from AB259 ($met^-$) $\times$ B(K)5, designated B(K)5 NM, was used as the recipient. A total of 107 $lys^-$ transductants were tested for mutSI with the streptomycin test. None had received mutSI. The genes thy and argB are linked to $lys$, the order being $lys$ thy argB (13). The same Plkc lysate was used to transduce thy+ and argB+ to JE1025; thy+ and argB+ transductants were selected and tested for mutSI; mutSI was found when selection was for argB+, but not thy+, although one argB+ mutSI transductant was also thy+ (Table 7). The gene order in this region is considered to be $lys$ thy argB mutSI.

Attempted suppression of mutator activity. Howard and Tessman (20) were able to suppress the mutagenic activity of base analogues with deoxyribonucleosides that are found in DNA. The deoxyribonucleosides of A, T, G, and C were tested with AZA/R1. The procedure was similar to the T1 or streptomycin mutator tests, except that the culture medium contained one of the four deoxyribonucleosides at a concentration of 200 $\mu$g/ml, and each tube was inoculated with 200 AZA/R1 cells. Eleven such tubes were prepared for each of the four deoxyribonucleosides. The control tubes contained only culture medium. The tubes were incubated for 18 hr with aeration, and the T1 and streptomycin mutator tests were performed. No suppression of mutator activity was observed.

Azaserine resistance and the mutator property. To determine whether a relationship frequently exists between azaserine resistance and the mutator property, 12 additional azaserine-resistant derivatives of B/UV were developed and tested. None of the 12 was a mutator strain.

JE1025, the recipient used in the transduction, is susceptible to azaserine. As determined with gradient plates, it is inhibited by an azaserine concentration of 16 $\mu$g/ml, whereas AZA/R1 is not inhibited by a concentration of 200 $\mu$g/ml. The level of azaserine susceptibility of argB+ transductants considered to carry mutSI or mutS+ was

**Table 7. Linked transduction of mutSI and argB+**

<table>
<thead>
<tr>
<th>Selected marker</th>
<th>No. of transductants tested for unselected markers</th>
<th>Transductants containing unselected marker (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>argB+ thy+</td>
<td>210</td>
<td>23</td>
</tr>
</tbody>
</table>

*One of five mutSI transductants found was thy*.
determined by means of gradient plates. The transductants, whether mutator or nonmutator, had the same azaserine susceptibility as the JE1025 parent. The results show that the azaserine resistance and the mutator property of AZA/R₁ are genetically distinct.

Reversion studies. To study reversion of auxotrophs presumably induced by mutSI, nine spontaneous histidine auxotrophs of AZA/R₁ were obtained with the penicillin treatment and designated A his-1 to A his-9. A his-6 was not used because it formed a very large number of partial revertants. Some of these his⁻ strains were crossed to AB259 (mer⁻), and maIB⁺ mer⁺ recombinants were selected and examined for the nonmutator phenotype. Because of restriction, nonmutator recombinants were found only in the cross with A his-3. The reversion of the thr⁻ mutation was analyzed in strain A104. The reversion of lys⁻ and leu⁻, both in the presence and absence of the mutator gene, were studied in B(K)5 Mu and B(K)5 NM, respectively. Two additional mutSI and mutSI⁺ recombinants from the cross AB259 (mer⁻) × B(K)5 were used to study the reversion of the his⁻ mutation in B(K)5 (Table 8).

The mutants were placed in two classes on the basis of the reversion studies. Class I mutants responded to EMS and, in those mutants tested, to AP. The response to AP was sometimes obscured by the large number of spontaneous revertants. Class I mutants reverted spontaneously at a moderately high frequency, ranging from 40 (for A his-4) to 600 (for lys⁻) revertants per plate. This is in contrast to the finding of Yanofsky, Cox, and Horn (41) that only 4 of 23 auxotrophic mutants isolated in a mutTI (Treffers mutator) strain reverted at a frequency which indicated a pronounced mutator effect. In all cases in which the mutator gene was replaced by its wild-type allele, the spontaneous reversion frequency decreased about 100-fold.

Mutants of class I were considered transitions, because a response to AP is considered indicative of this type of mutational change (14). These mutants were induced by mutSI and could be induced to revert by mutSI; therefore, the mutator gene is thought to affect the two types of transition, AT = GC.

Class II is a heterogeneous grouping. A his-7 yielded many partial revertants, which made it difficult to determine the magnitude of response to EMS. The high frequency of revertants formed by A his-9 also produced this difficulty. A his-8 did not respond to EMS.

Genes mutTI and mutSI acting together. Treffers' mutator, mutTI, and mutSI were distinguishable by the frequency and pattern of appearance of Str⁺ mutants induced by them. In the streptomycin mutator test, mutTI cultures yielded 100 to 200 Str⁺ colonies per plate after 24

<table>
<thead>
<tr>
<th>Class</th>
<th>Mutant</th>
<th>Spontaneous revertants per plate</th>
<th>Revertants per plate²</th>
<th>Str⁺ Control</th>
<th>EMS</th>
<th>Control</th>
<th>AP</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>A his-1</td>
<td>390-410</td>
<td>407</td>
<td>620</td>
<td>83</td>
<td>835</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A his-2</td>
<td>80-160</td>
<td>121</td>
<td>&gt;1,000</td>
<td>51</td>
<td>95</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A his-4</td>
<td>22-55</td>
<td>2</td>
<td>69</td>
<td>0</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A his-5</td>
<td>130-200</td>
<td>133</td>
<td>281</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A his-3</td>
<td>70-100</td>
<td>79</td>
<td>153</td>
<td>76</td>
<td>118</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A his-3 NM</td>
<td>0-1</td>
<td>1</td>
<td>48</td>
<td>0</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>thr⁻</td>
<td>45-90</td>
<td>64</td>
<td>204</td>
<td>51</td>
<td>95</td>
<td></td>
</tr>
<tr>
<td></td>
<td>leu⁻</td>
<td>230-360</td>
<td>360</td>
<td>1,050</td>
<td>261</td>
<td>468</td>
<td></td>
</tr>
<tr>
<td></td>
<td>leu⁺ NM</td>
<td>1-4</td>
<td>2</td>
<td>141</td>
<td>4</td>
<td>99</td>
<td></td>
</tr>
<tr>
<td></td>
<td>lys⁻</td>
<td>300-580</td>
<td>576</td>
<td>ca.2,000</td>
<td>516</td>
<td>686</td>
<td></td>
</tr>
<tr>
<td></td>
<td>lys⁺ NM</td>
<td>1-4</td>
<td>1</td>
<td>386</td>
<td>2</td>
<td>288</td>
<td></td>
</tr>
<tr>
<td></td>
<td>his⁻</td>
<td>100-110</td>
<td>108</td>
<td>153</td>
<td>107</td>
<td>275</td>
<td></td>
</tr>
<tr>
<td></td>
<td>his⁺ NM</td>
<td>0-1</td>
<td>1</td>
<td>22</td>
<td>1</td>
<td>150</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>A his-7</td>
<td>50-70</td>
<td>50</td>
<td>69</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A his-8</td>
<td>5-24</td>
<td>24</td>
<td>21</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A his-9</td>
<td>ca.500</td>
<td>ca.400</td>
<td>ca.400</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* All mutants are also mutSI unless followed by NM (nonmutator) indicating that mutSI was replaced by the wild-type allele.

¹ Results are given for a representative experiment with each mutant. Dashes represent no determination.
hr of incubation. When the plates were recounted after 48 hr, there was approximately a 10% increase in colonies; mutSI cultures, in comparison, formed only about 10 colonies per streptomycin plate after 24 hr, and the number increased to about 70 per plate after 48 hr. This is shown in Table 9 (parental values). Both mutSI and mutTI cultures were at the same cell density when plated, approximately 2 × 10^9 cells per ml. Because of this difference in the effect of the two genes, it was thought that a recombinant containing both mutTI and mutSI could be detected.

Hfr Mu trp-, a mutTI strain, was mated with A105, and leu+ trp+ recombinants were selected; mutTI is closely linked to leu, as indicated by cotransduction (Siegel, Ph.D. Thesis, Rutgers, The State University, New Brunswick, N.J., 1966). Cells from each purified recombinant were grown overnight in nutrient broth and plated on nutrient agar containing 200 μg of streptomycin per ml. The plates were scored after 24 and 48 hr. Recombinant cultures giving more than 50 colonies per plate after 24 hr of incubation were regarded as carrying mutTI. Of 50 recombinants, 19 had received mutTI (Table 9). None of the donor markers beyond thr (mutB+, xylB+, or his+) was found in the recombinants, so all could be assumed to retain mutSI. The 31 recombinants containing mutSI but not mutTI resembled the mutSI parent with respect to the total number of Str<sup>r</sup> colonies formed and the increment in colonies between 24 and 48 hr. The 19 double mutant recombinants formed an average of 203 Str<sup>r</sup> colonies after 48 hr. This value is lower than the parental (Hfr Mu trp-), but the standard deviation in both cases is very high because of the inherent fluctuation in mutant frequency. The average of 203 colonies per plate was significantly different from the value obtained with mutSI alone. This indicated that mutTI could function in the presence of mutSI. The average increment for these 19 recombinants was more than double that found in the mutTI parent, indicating that mutSI was active when together with mutTI. The effect of the two mutant genes appears to be additive. Certainly, no marked synergism or antagonism was present.

**TABLE 9. Number and increase in appearance of Str<sup>r</sup> colonies when parents and recombinants from Hfr Mu trp- × A104 were tested**

<table>
<thead>
<tr>
<th>Mutator gene</th>
<th>Normal colonies tested</th>
<th>Total colonies&lt;sup&gt;a&lt;/sup&gt; per plate</th>
<th>Increment&lt;sup&gt;b,c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parents</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mutTI</td>
<td>9</td>
<td>224 ± 32</td>
<td>20 ± 5.7</td>
</tr>
<tr>
<td>mutSI</td>
<td>11</td>
<td>77 ± 23</td>
<td>67 ± 20</td>
</tr>
<tr>
<td>Recombinants</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mutSI</td>
<td>31</td>
<td>69 ± 15</td>
<td>55 ± 11</td>
</tr>
<tr>
<td>mutTI + mutSI</td>
<td>19</td>
<td>203 ± 59</td>
<td>44 ± 14</td>
</tr>
</tbody>
</table>

<sup>a</sup> Streptomycin mutator test with streptomycin (200 μg/ml).

<sup>b</sup> Mean ± standard deviation.

<sup>c</sup> Increment = Str<sup>r</sup> colonies per plate at 48 hr minus number at 24 hr.

**FIG. 1. Chromosomal locations of the mutator genes of Escherichia coli and Salmonella typhimurium LT7. The map is not meant to indicate on which side of the reference marker a mutator gene is located.**
Kirchner and Rudden (26). This indicates that a mutation resulting in the mutator phenotype can occur in genes which differ in genetic location and presumably in function. There are several ways in which a mutator may act. Speyer (37) found a mutator effect associated with a temperature-sensitive mutant of the phage T4 DNA polymerase gene. Pierce (32) attempted to rule out mutT1 as producing a mutant DNA polymerase. She grew phage on Treffers’ mutator strain and its nonmutator parent, and observed an increase in mutation rate in phage grown on the mutator strain. Such an effect would not be found if Treffers’ mutator were a bacterial DNA polymerase, because the phage induces formation of its own DNA polymerase (3). However, Pierce used only two strains, 58-278 M*, the original Treffers strain, and 58-278, its nonmutator parent. The two strains were assumed to be “co-isogenic” except for the mutator, and therefore the observed effect was attributed to the mutator. To demonstrate this point conclusively, it would be necessary to determine phage mutation rates in a nonmutator strain, and then transfer to this strain Treffers’ mutator gene and observe an increase. It is not known whether mutSI affects phage mutation, leaving open the possibility that it is a mutation in the bacterial DNA polymerase.

Kirchner (25) suggested that an unusual mutagenic base may be formed in the mutator strain of S. typhimurium. Base synthesis may also be involved in the mutator effect if there is an insufficiency of a base. Thymine starvation, for example, is mutagenic as well as lethal (7). The ineffectiveness of deoxyribonucleosides in suppressing the mutator effect produced by AZA/R1 argues against insufficient synthesis of DNA precursor.

The mutator may alter the internal cellular environment so that errors in DNA replication occur. In vitro, in the presence of Mn²⁺, which is mutagenic for bacteria (12) and phage (31), purified DNA polymerase will incorporate both ribo- and deoxyribonucleotides into DNA (4).

Zamenhof, Heldenmuth, and Zamenhof (43) suggested that loss of the capacity to repair lesions normally occurring in DNA could result in the mutator phenotype. Mutants with increased susceptibility to UV irradiation resulting from defective repair mechanisms have been mapped in E. coli (21). Among such mutants is a recombination-deficient mutant, rec-36, which is located between his and str (33), the general region in which mutSI is found. The UV resistance of the original mutator strain, AZA/R1, was higher than that of its nonmutator parent, B/UV. This was attributed to the azaserine resistance, not the mutator property of the strain. To find out whether mutSI-carrying strains are defective in repair of lesions in the DNA, it will be necessary to perform UV survival determinations with the mutator and nonmutator transductants of JE1025.

The mutSI differs from mutT1 not only in its genetic location but also in phenotype; mutT1 strains yield more Str+ colonies per plate in the streptomycin mutator test than do mutSI strains. Also, mutT1 does not increase the frequency of mutation to T2 resistance (39; Siegel, Ph.D. Thesis), whereas mutSI does. A possible explanation of this observation is offered: mutT1 induces the transversion AT → CG (41), and the evidence suggests that mutSI induces AT → GC. The mutation to T2 resistance occurs at a low frequency in wild-type strains (10). The low frequency may indicate that only specific changes in a relatively small number of base pairs can result in T2 resistance. Conceivably, the AT → CG transversion is a mutational change which cannot result in T2 resistance, whereas a transition induced by mutSI can do so. Zamenhof (42) found that ast did not increase the rate of mutation to streptomycin-resistance in its original host, the Harvard strain, but did so when transferred to K-12. A possible explanation can be made by assuming that ast induces a specific type of mutational change which, because of differences in base pair sequence in the str region of the Harvard and K-12 strains, might result in a resistant phenotype in K-12 alone.

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