

# Mutator Gene of *Escherichia coli* B<sup>1</sup>

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An azaserine-resistant derivative of *Escherichia coli* B/UV, AZA/R, was found to carry a mutator gene. This gene, designated *mutS1*, was mapped by means of conjugation and P1kc-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 1.5% agar (9); Brom Thymol Blue (BTB)-sugar-agar (6); eosin-methylene blue (EMB)-sugar-agar (2.7% BBL Levine Eosin

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, a needle was touched to growth on a stock slant and used to inoculate 5 or 10 ml of nutrient broth. The culture was incubated on a rotary shaker for 18 hr. The viable count of such cultures was  $2 \times 10^8$  to  $4 \times 10^9$  cells per ml. Complete-medium plates were incubated for 1 day, and minimal-medium plates usually for 2 days.

**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 mm<sup>2</sup> 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 put in a watch glass and continually agitated by a magnetic stirrer during irradiation. After each

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TABLE 1. *Bacterial strains used*<sup>a</sup>

Strain	Significant characteristics	Source
<i>Derivatives of E. coli B</i>		
B/UV	<i>malB<sup>-</sup> val<sup>+</sup> uv<sup>r</sup></i>	—
AZA/R <sub>1</sub>	<i>malB<sup>-</sup> val<sup>+</sup> mutS1 uv<sup>r</sup></i>	Azaserine-resistant derivative of B/UV
A104	<i>thr<sup>-</sup> leu<sup>-</sup> his<sup>-</sup> lac<sup>-</sup> malB<sup>-</sup> mutS1</i>	Derived from AZA/R <sub>1</sub>
A105	<i>thr<sup>-</sup> leu<sup>-</sup> his<sup>-</sup> lac<sup>-</sup> malB<sup>-</sup> xyl<sup>-</sup> mutS1</i>	Derived from A104
A106	<i>thr<sup>-</sup> leu<sup>-</sup> his<sup>-</sup> lys<sup>-</sup> lac<sup>-</sup> malB<sup>-</sup> xyl<sup>-</sup> mutS1</i>	Derived from A105
A <i>his-1-9</i>	9 independent <i>his<sup>-</sup></i> derivatives of AZA/R <sub>1</sub>	—
A <i>his-3</i> NM	<i>his<sup>-</sup> mutS<sup>+</sup> malB<sup>+</sup></i>	<i>malB<sup>+</sup> met<sup>+</sup></i> nonmutator (NM) recombinant from AB259 ( <i>met<sup>-</sup></i> ) × A <i>his-3</i>
B(K)5	<i>leu<sup>-</sup> his<sup>-</sup> lys<sup>-</sup> lac<sup>-</sup> xyl<sup>-</sup> malB<sup>-</sup> mutS1</i>	<i>thr<sup>+</sup> met<sup>+</sup></i> recombinant from AB259 ( <i>met<sup>-</sup></i> ) × A106
B(K)5 Mu	<i>leu<sup>-</sup> lys<sup>-</sup> lac<sup>-</sup> xyl<sup>-</sup> malB<sup>-</sup> mutS1</i>	<i>his<sup>+</sup> met<sup>+</sup></i> recombinant from AB259 ( <i>met<sup>-</sup></i> ) × B(K)5
B(K)5 NM	<i>leu<sup>-</sup> lys<sup>-</sup> lac<sup>-</sup> xyl<sup>-</sup> malB<sup>-</sup> mutS<sup>+</sup></i>	<i>his<sup>+</sup> met<sup>+</sup></i> nonmutator recombinant from AB259 ( <i>met<sup>-</sup></i> ) × B(K)5
B(W)	<i>uv<sup>s</sup></i>	Witkin
B/r (W)	<i>uv<sup>r</sup></i>	Witkin
<i>Derivatives of E. coli K-12</i>		
W3780	Hfr H <i>met<sup>-</sup> str<sup>r</sup> λ<sup>-</sup></i>	Lederberg
AB259 ( <i>met<sup>-</sup></i> )	Hfr H <i>met<sup>-</sup> thi<sup>-</sup> λ<sup>-</sup></i>	Adelberg, <i>met<sup>-</sup></i> induced with EMS
Hfr Mu <i>trp<sup>-</sup></i>	Hfr C <i>trp<sup>-</sup> mutT1</i>	—
JE1025	<i>thy<sup>-</sup> argB<sup>-</sup> str<sup>r</sup> gal<sup>-</sup></i>	Ishibashi

<sup>a</sup> Symbols: +, wild type or, in case of  $\lambda$ , lysogenic; —, inability to synthesize or utilize or, in case of  $\lambda$ , nonlysogenic; <sup>r</sup>, resistance; <sup>s</sup>, sensitivity; Hfr, high-frequency donor; *arg*, arginine; *gal*, galactose; *his*, histidine; *lac*, lactose; *leu*, leucine; *lys*, lysine; *mal*, maltose; *met*, methionine; *mutT1*, Treffers K-12 mutator gene; *mutS1*, mutator gene of AZA/R<sub>1</sub>; *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 viable count. The rapid UV method of Greenberg and Woody-Karrer (17) was also used.

**Selection of mutants.** All mutations occurring in derivatives of AZA/R<sub>1</sub>, with the exception of *xyl<sup>-</sup>*, were of spontaneous origin; *xyl<sup>-</sup>* 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  $5 \times 10^8$  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<sup>+</sup>* 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  $10^{11}$  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  $2.5 \times 10^{-3}$  M CaCl<sub>2</sub> to log phase (about  $3 \times 10^8$  cells per ml). P1kc prepared on AZA/R<sub>1</sub> was then added to give a multiplicity of infection of 7 to 11. After 20 min of further

incubation, samples were plated undiluted, and diluted 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 medium 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 cultures were spread with a Breed loop on one-quarter of a nutrient agar plate previously spread with  $10^8$  bacteriophage T1. The plates were incubated for 1 day. In control experiments, cultures of the *mutS1* recipient gave about 300 T1<sup>r</sup> colonies per 0.01 ml, and the donor, W3780, yielded an average of 5 per 0.01 ml. Recombinants, however, often gave intermediate results and had to be retested several times. Therefore, a Str<sup>s</sup> donor, AB259 (*met*<sup>-</sup>), was used, and 0.1-ml samples of 2-ml cultures prepared as above were plated on nutrient agar containing 150  $\mu$ g (per ml) of streptomycin. After 2 days of incubation, *mutS1* containing recombinants gave 40 to 80 colonies per plate, and nonmutator, *mutS*<sup>+</sup>, recombinants yielded an average of 1 colony per five plates.

B(K)5, a *mutS1* recipient used in several matings, grew slowly in nutrient broth, and, therefore, L broth was used in the mutator test. L-broth cultures of *mutS1* recombinants formed about 30 Str<sup>r</sup> colonies per plate. Strains carrying *mutS*<sup>+</sup> gave an average of one colony per five plates.

Recombinants giving more than 10 Str<sup>r</sup> colonies per plate were classified as carrying *mutS1*. Those giving none were considered nonmutator. If the initial culture tested gave 1 to 10 colonies, a new culture was made from the recombinant and tested. Some K-12  $\times$  B(K)5 recombinants repeatedly formed only 2 to 10 colonies per plate. These were mutator, and the low frequency of Str<sup>r</sup> colonies was attributable to the cultures not growing to full titer, i.e.,  $2 \times 10^9$  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 resistance. Loopfuls, about  $10^{-3}$  ml, of the same broth cultures used for the T1 mutator test were streaked on nutrient agar containing 125  $\mu$ g of sodium azide per ml. After 24 hr of incubation, streaks from *mutS1*-containing cultures gave 50 to 100 Azi<sup>r</sup> colonies. The nonmutators formed 0 to 5.

*Reversion studies.* Minimal medium A-agar, supplemented with all required growth factors except that of the mutant studied plus 100 mg of nutrient-broth powder per liter, was used to select revertants. All revertant plates were incubated for 3 days. Large colonies 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 mutagens.

EMS-0.1 ml of an 18-hr nutrient broth culture (about  $2 \times 10^8$  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  $\mu$ g/ml. For the control, 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  $2 \times 10^8$  to  $1 \times 10^9$  cells per ml. The AP cultures had 50 to 90% of the cell number of the control cultures.

*Resistance determinations.* The frequency of mutation to phage or drug resistance was determined by spreading 0.1-ml samples (undiluted for B/UV and usually diluted  $10^{-1}$  or  $10^{-2}$  for AZA/R<sub>1</sub>) on nutrient-agar plates containing drug or previously spread with  $10^8$  or more T-odd phage. T-even 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  $10^{-6}$  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  $\mu$ g (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 incubated for 2 days at 37 C and for 3 days at room temperature, and in the second experiment, for 5 days at 37 C. Mutation rates were determined from the formula  $a = -(\ln 2) (\ln p_0) / N$ , where  $a$  is the mutation rate,  $p_0$  is the fraction of plates with no Str<sup>r</sup> colonies, and  $N$  is the average of cells per sample (30).

*Azaserine gradient plates.* The method of preparation of these plates was previously described (35).

## RESULTS

*Characteristics of B/UV and AZA/R<sub>1</sub>.* In a previous publication (35), the parent of AZA/R<sub>1</sub> was designated as B/r because the rapid UV test (17) showed it to be radioresistant. Survival after UV irradiation for this strain of B/r, and for B(W) and B/r(W), showed that B/r was intermediate 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 Witkin's original B/r, we have designated the

parent of AZA/R<sub>1</sub> as B/UV. The increase in resistance to UV in AZA/R<sub>1</sub> is attributed to the cross-resistance relationship between azaserine and UV (40).

Characteristic of B and its derivatives, B/UV and AZA/R<sub>1</sub> are valine-resistant, nonmotile, susceptible to all seven T phages, and do not ferment maltose.

Table 3 gives mutation frequencies in B/UV and AZA/R<sub>1</sub>. Additional frequencies were given elsewhere (35). Table 4 presents the result of the fluctuation tests. AZA/R<sub>1</sub> cultures yielded a variety of mutants at frequencies significantly higher than those given by the nonmutator parent.

**Genetic analysis of AZA/R<sub>1</sub>.** Derivatives of AZA/R<sub>1</sub> 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*<sup>+</sup> recombinants did not appear when selection was for *thr*<sup>+</sup> *leu*<sup>+</sup> *met*<sup>+</sup> but were found when *his*<sup>+</sup> was also selected. This indicated that *mutS1* was between

TABLE 2. Percentage survival after UV irradiation

Dose	Strain			
	B(W)	B/UV	B/r (W)	AZA/R <sub>1</sub>
ergs/mm <sup>2</sup>				
150	5.4	66	86	90
300	1.3	36	62	71
600	—	9.0	37	30
900	—	0.86	4.6	>5

TABLE 3. Mutation frequencies in B/UV and AZA/R<sub>1</sub>

Nature of resistance mutation	Ratio of mutants to total cells <sup>a</sup>	
	B/UV	AZA/R <sub>1</sub>
Phage T2.....	<1 × 10 <sup>-9</sup>	2 × 10 <sup>-5</sup>
Phage T6.....	5 × 10 <sup>-7</sup>	1 × 10 <sup>-4</sup>
Na azide (125 μg/ml)....	6 × 10 <sup>-8</sup>	7 × 10 <sup>-5</sup>
Vancomycin (50 μg/ml)...	3 × 10 <sup>-7</sup>	2 × 10 <sup>-5</sup>
Streptomycin (150 μg/ml).....	<2 × 10 <sup>-9</sup>	2 × 10 <sup>-7</sup>

<sup>a</sup> The number of resistant cells varied in repeated experiments. The results of typical determinations are given.

TABLE 4. Rate of mutation to streptomycin resistance (1,000 μg/ml) in B/UV and AZA/R<sub>1</sub><sup>a</sup>

Strain	Expt	N	C	p <sub>0</sub>	a
B/UV	1	2.3 × 10 <sup>8</sup>	32	32/32	<2 × 10 <sup>-10</sup>
	2	2.5 × 10 <sup>8</sup>	29	29/29	<1 × 10 <sup>-10</sup>
AZA/R <sub>1</sub>	1	2.5 × 10 <sup>8</sup>	30	10/30	3 × 10 <sup>-9</sup>
	2	2.2 × 10 <sup>8</sup>	27	19/27	1 × 10 <sup>-9</sup>

<sup>a</sup> Abbreviations: N, average number of cells per sample; C, total number of cultures tested; p<sub>0</sub>, fraction of plates with no Str<sup>r</sup> colonies; a, mutation rate.

TABLE 5. Genetic analysis of recombinants from matings of W3780 with AZA/R<sub>1</sub> derivatives

Selected markers	Per cent recombination <sup>a</sup>	Total recombinants tested	Unselected markers		
			Marker	No. found	Per cent of total
<i>thr</i> <sup>+</sup> <i>leu</i> <sup>+</sup> <i>met</i> <sup>+</sup> ....	12	49	<i>lac</i> <sup>+</sup>	8	16
			<i>his</i> <sup>+</sup>	1	2
			<i>mutS</i> <sup>+</sup>	0	<2
<i>thr</i> <sup>+</sup> <i>leu</i> <sup>+</sup> <i>his</i> <sup>+</sup> <i>met</i> <sup>+</sup> ....	0.2	50	<i>lac</i> <sup>+</sup>	17	34
			<i>mutS</i> <sup>+</sup>	2	4
<i>his</i> <sup>+</sup> <i>met</i> <sup>+</sup> ....	0.2	125	<i>thr</i> <sup>+</sup> <i>leu</i> <sup>+</sup>	14	11
			<i>lac</i> <sup>+</sup>	8	6.4
			<i>mutS</i> <sup>+</sup>	6	4.8
			<i>str</i> <sup>r</sup>	2	1.6
			<i>xyl</i> <sup>t</sup>	0	<0.8
			<i>malB</i> <sup>+</sup>	0	<0.8
<i>malB</i> <sup>+</sup> <i>met</i> <sup>+</sup> ....	0.001	65	<i>thr</i> <sup>+</sup> <i>leu</i> <sup>+</sup>	20	30
			<i>lac</i> <sup>+</sup>	13	20
			<i>his</i> <sup>+</sup>	16	25
			<i>mutS</i> <sup>+</sup>	11	17
			<i>xyl</i> <sup>t</sup>	13	20

<sup>a</sup> Recombinants per 100 males in mating mixture.

<sup>b</sup> Recipient A104.

<sup>c</sup> Recipient A105.

*lac* and *his*, or beyond *his* on the chromosome. When selection was for *his*<sup>+</sup> *met*<sup>+</sup> in the cross W3780 × A105, *mutS*<sup>+</sup> recombinants appeared with a frequency lower than *lac*<sup>+</sup>, which is proximal to *his*, but higher than *str*<sup>r</sup>, 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*<sup>-</sup> derivative of A105, designated

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*<sup>-</sup>) was crossed to A106, and *thr*<sup>+</sup> recombinants were selected. By use of the interrupted-mating procedure, six of the *thr*<sup>+</sup> recombinants were mated to AB259 (*met*<sup>-</sup>), and the frequency of *leu*<sup>+</sup> 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*<sup>-</sup>) × 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 *mutS1*.

The results of a typical cross between AB259 (*met*<sup>-</sup>) and B(K)5 are given in Table 6. Double selection, such as for *leu*<sup>+</sup> *lys*<sup>+</sup>, was used because *lys*<sup>+</sup> revertants were almost as frequent as *lys*<sup>+</sup> recombinants. Nonmutator recombinants appeared among *lys*<sup>+</sup> recombinants at a frequency of 46%. Judged from the frequency of appearance of other markers, this indicates close linkage between *lys* and *mutS1*.

TABLE 6. Genetic analysis of recombinants from the mating of AB259 (*met*<sup>-</sup>) × B(K)5

Selected markers	Per cent recombination	Total recombinants tested	Unselected markers		
			Marker	No. found	Per cent of total
<i>leu</i> <sup>+</sup> <i>met</i> <sup>+</sup> . . . .	13	46	<i>lac</i> <sup>+</sup>	5	11
			<i>his</i> <sup>+</sup>	0	<2.2
			<i>lys</i> <sup>+</sup>	0	<2.2
			<i>mutS</i> <sup>+</sup>	0	<2.2
			<i>xyl</i> <sup>+</sup>	0	<2.2
<i>leu</i> <sup>+</sup> <i>his</i> <sup>+</sup> <i>met</i> <sup>+</sup> . . . .	0.04	39	<i>lac</i> <sup>+</sup>	3	7.7
			<i>lys</i> <sup>+</sup>	1	2.6
			<i>mutS</i> <sup>+</sup>	3	7.7
			<i>xyl</i> <sup>+</sup>	0	<2.6
<i>leu</i> <sup>+</sup> <i>lys</i> <sup>+</sup> <i>met</i> <sup>+</sup>	0.03	41	<i>lac</i> <sup>+</sup>	4	9.8
			<i>his</i> <sup>+</sup>	6	15
			<i>mutS</i> <sup>+</sup>	19	46
			<i>xyl</i> <sup>+</sup>	2	4.9

An attempt was made to cotransduce *lys*<sup>+</sup> and *mutS1*. A P1kc lysate was prepared on AZA/R<sub>1</sub>, and a *lys*<sup>-</sup> *mutS*<sup>+</sup> recombinant from AB259 (*met*<sup>-</sup>) × B(K)5, designated B(K)5 NM, was used as the recipient. A total of 107 *lys*<sup>-</sup> transductants were tested for *mutS1* with the streptomycin test. None had received *mutS1*. The genes *thy* and *argB* are linked to *lys*, the order being *lys thy argB* (13). The same P1kc lysate was used to transduce *thy*<sup>+</sup> and *argB*<sup>+</sup> to JE1025; *thy*<sup>+</sup> and *argB*<sup>+</sup> transductants were selected and tested for *mutS1*; *mutS1* was found when selection was for *argB*<sup>+</sup>, but not *thy*<sup>+</sup>, although one *argB*<sup>+</sup> *mutS1* transductant was also *thy*<sup>+</sup> (Table 7). The gene order in this region is considered to be *lys thy argB mutS1*.

*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/R<sub>1</sub>. 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 μg/ml, and each tube was inoculated with 200 AZA/R<sub>1</sub> 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 μg/ml, whereas AZA/R<sub>1</sub> is not inhibited by a concentration of 200 μg/ml. The level of azaserine susceptibility of *argB*<sup>+</sup> transductants considered to carry *mutS1* or *mutS*<sup>+</sup> was

TABLE 7. Linked transduction of *mutS1* and *argB*<sup>+</sup>

Selected marker	No. of transductants tested for unselected markers	Transductants containing unselected marker (%)		
		<i>thy</i> <sup>+</sup>	<i>argB</i> <sup>+</sup>	<i>mutS1</i>
<i>argB</i> <sup>+</sup>	210	23	—	2.4 <sup>a</sup>
<i>thy</i> <sup>+</sup>	102	—	7.8	<0.98

<sup>a</sup> One of five *mutS1* transductants found was *thy*<sup>+</sup>.

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/R<sub>1</sub> are genetically distinct.

**Reversion studies.** To study reversion of auxotrophs presumably induced by *mutSI*, nine spontaneous histidine auxotrophs of AZA/R<sub>1</sub> 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*<sup>-</sup> strains were crossed to AB259 (*met*<sup>-</sup>), and *malB*<sup>+</sup> *met*<sup>+</sup> 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*<sup>-</sup> mutation was analyzed in strain A104. The reversion of *lys*<sup>-</sup> and *leu*<sup>-</sup>, 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 *mutS*<sup>+</sup> recombinants from the cross AB259 (*met*<sup>-</sup>) × B(K)5 were used to study the reversion of the *his*<sup>-</sup> 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*<sup>-</sup>) 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 *mutT1* (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 *mutT1* and *mutSI* acting together.** Treffers' mutator, *mutT1*, and *mutSI* were distinguishable by the frequency and pattern of appearance of Str<sup>r</sup> mutants induced by them. In the streptomycin mutator test, *mutT1* cultures yielded 100 to 200 Str<sup>r</sup> colonies per plate after 24

TABLE 8. Spontaneous and mutagen-induced reversion of *mutSI*-induced auxotrophs<sup>a</sup>

Class	Mutant	Spontaneous revertants per plate	Revertants per plate <sup>b</sup>			
			Control	EMS	Control	AP
I	A <i>his-1</i>	390-410	407	620	—	—
	A <i>his-2</i>	80-160	121	>1,000	83	835
	A <i>his-4</i>	22-55	22	69	—	—
	A <i>his-5</i>	130-200	133	281	—	—
	A <i>his-3</i>	70-100	79	153	76	118
	A <i>his-3</i> NM	0-1	1	48	0	11
	<i>thr</i> <sup>-</sup>	45-90	64	204	51	95
	<i>leu</i> <sup>-</sup>	250-360	360	1,050	261	468
	<i>leu</i> <sup>-</sup> NM	1-4	2	141	4	99
	<i>lys</i> <sup>-</sup>	300-580	576	ca.2,000	516	686
	<i>lys</i> <sup>-</sup> NM	1-4	1	386	2	288
	<i>his</i> <sup>-</sup>	100-110	108	153	107	275
	<i>his</i> <sup>-</sup> NM	0-1	1	22	1	150
	II	A <i>his-7</i>	50-70	50	69	—
A <i>his-8</i>		5-24	24	21	—	—
A <i>his-9</i>		ca.500	ca.400	ca.400	—	—

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

<sup>b</sup> 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; *mutS1* 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 *mutS1* and *mutT1* cultures were at the same cell density when plated, approximately  $2 \times 10^9$  cells per ml. Because of this difference in the effect of the two genes, it was thought that a recombinant containing both *mutT1* and *mutS1* could be detected.

Hfr Mu *trp*<sup>-</sup>, a *mutT1* strain, was mated with A105, and *leu*<sup>+</sup> *trp*<sup>+</sup> recombinants were selected; *mutT1* 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 *mutT1*. Of 50 recombinants, 19 had received *mutT1* (Table 9). None of the donor markers beyond *thr* (*malB*<sup>+</sup>, *xyl*<sup>+</sup>, or *his*<sup>+</sup>) was found in the recombinants, so all could be assumed to retain *mutS1*. The 31 recombinants containing *mutS1* but not *mutT1* resembled the *mutS1* 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 mutator recombinants formed an average of 203 Str<sup>r</sup> colonies after 48 hr. This value is lower than the parental (Hfr Mu *trp*<sup>-</sup>), 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 *mutS1* alone. This indicated that *mutT1* could function in the presence of *mutS1*. The average increment for these 19 recombinants was more than double that found in the *mutT1* parent, indicating that *mutS1* was active when together with *mutT1*. The effect of the two mutator genes appears to be additive. Certainly, no marked synergism or antagonism was present.

#### DISCUSSION

Strains of *E. coli* with increased rates of mutation have been described by Gundersen et al. (19) and Jyssum (24). In a mutable strain of *Salmonella typhimurium*, as observed by Dawson and Smith-Keary (10), the basis of genetic instability is episomal. Reserving the term "mutator" for specific genes that accelerate mutation rates, the map position of such genes may be compared.

The mutator of AZA/R<sub>1</sub> (*mutS1*) has a genetic location distinct from other known mutators in bacteria (Fig. 1). Treffers' mutator (*mutT1*) is closely linked to *leu* (36). The Goldstein and Smoot mutator (*ast*) is between the locus for T1, 5 resistance and *pro* (42), and the mutator gene of *S. typhimurium* LT-7, characterized by Miyake (29), is cotransducible with *purA* according to

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

Mutator gene	No. of parental colonies or recombinants tested <sup>a</sup>	Total colonies <sup>b</sup> per plate	Increment <sup>b,c</sup>
Parents			
<i>mutT1</i>	9	224 ± 32	20 ± 5.7
<i>mutS1</i>	11	77 ± 23	67 ± 20
Recombinants			
<i>mutS1</i>	31	69 ± 15	55 ± 11
<i>mutT1</i> + <i>mutS1</i>	19	203 ± 59	44 ± 14

<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.

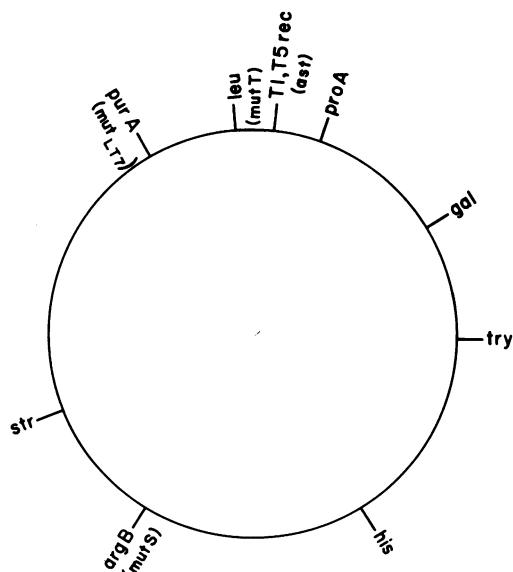


FIG. 1. Chromosomal locations of the mutator genes of *Escherichia coli* and *Salmonella typhimurium* LT7. The map is not meant to indicate on which side of the reference marker a mutator gene is located.

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 mutant, 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 *mutS1* 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 deoxynucleosides in suppressing the mutator effect produced by AZA/R<sub>1</sub> 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 Mn<sup>2+</sup>, 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 *mutS1* is found. The UV resistance of the original mutator strain, AZA/R<sub>1</sub>, 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 *mutS1*-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 *mutS1* differs from *mutT1* not only in its genetic location but also in phenotype; *mutT1* strains yield more Str<sup>r</sup> colonies per plate in the streptomycin mutator test than do *mutS1* strains. Also, *mutT1* does not increase the frequency of mutation to T2 resistance (39; Siegel, Ph.D. Thesis), whereas *mutS1* does. A possible explanation of this observation is offered: *mutT1* induces the transversion AT → CG (41), and the evidence suggests that *mutS1* 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 *mutS1* 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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#### LITERATURE CITED

- ADAMS, M. H. 1959. Bacteriophages. Interscience Publishers, Inc., New York.
- ADLER, H. I., AND S. D. HASKINS. 1960. Heterogeneity of cultures of *Escherichia coli* strain B/r. *Nature* **188**:249-251.
- APOSHION, H. V., AND A. KORNBERG. 1962. Enzymatic synthesis of deoxyribonucleic acid. IX. The polymerase formed after T2 bacteriophage infection of *Escherichia coli*. *J. Biol. Chem.* **237**:519-525.
- BERG, P., H. FANCHER, AND M. CHAMBERLIN. 1963. The synthesis of mixed polynucleotides containing ribo- and deoxyribonucleotides by purified preparations of DNA polymerase from *Escherichia coli*, p. 467-483. In H. J. Vogel, V. Bryson, and J. O. Lampen [ed.], Informa-



- tional macromolecules. Academic Press, Inc., New York.
5. BOYER, H. 1964. Genetic control of restriction and modification in *Escherichia coli*. *J. Bacteriol.* **88**:1652-1660.
  6. COPELAND, J. C., AND V. BRYSON. 1966. Restriction in matings of *Escherichia coli* strain K-12 with strain B. *Genetics* **54**:441-452.
  7. COUGHLIN, C. A., AND E. A. ADELBERG. 1956. Bacterial mutation induced by thymine starvation. *Nature* **178**:531-532.
  8. CURTISS, R. III, L. J. CHARAMELLA, C. M. BERG, AND P. E. HARRIS. 1965. Kinetic and genetic analyses of D-cycloserine inhibition and resistance in *Escherichia coli*. *J. Bacteriol.* **90**:1238-1250.
  9. DAVIS, B. D., AND E. S. MINGIOLI. 1950. Mutants of *Escherichia coli* requiring methionine or vitamin B<sub>12</sub>. *J. Bacteriol.* **60**:17-28.
  10. DAWSON, G. W. P., AND P. F. SMITH-KEARY. 1963. Episomic control of mutation in *Salmonella typhimurium*. *Heredity* **18**:1-20.
  11. DEMEREC, M., AND U. FANO. 1945. Bacteriophage-resistant mutants in *Escherichia coli*. *Genetics* **30**:119-136.
  12. DEMEREC, M., AND J. HANSON. 1951. Mutagenic action of manganous chloride. Cold Spring Harbor Symp. Quant. Biol. **16**:215-228.
  13. EISENSTARK, A. 1965. Transduction of *Escherichia coli* genetic material by phage P22 in *Salmonella typhimurium* × *E. coli* hybrids. *Proc. Natl. Acad. Sci. U.S.A.* **54**:1557-1560.
  14. FREESE, E. 1959. The specific mutagenic effect of base analogues on phage T4. *J. Mol. Biol.* **1**:87-105.
  15. GOLDSTEIN, A., AND J. S. SMOOT. 1955. A strain of *Escherichia coli* with an unusually high rate of auxotrophic mutation. *J. Bacteriol.* **70**:588-595.
  16. GORINI, L., AND H. KAUFMAN. 1960. Selecting bacterial mutants by the penicillin method. *Science* **131**:604-605.
  17. GREENBERG, J., AND P. WOODY-KARRER. 1963. Radioresistant mutants of *Escherichia coli* B(ORNL). *Radiation Res.* **20**:350-363.
  18. GUNDERSEN, W. B. 1963. New type of streptomycin resistance resulting from action of the episomelike mutator factor in *Escherichia coli*. *J. Bacteriol.* **86**:510-516.
  19. GUNDERSEN, W. B., K. JYSSUM, AND S. LIE. 1962. Genetic instability with episome-mediated transfer in *Escherichia coli*. *J. Bacteriol.* **83**:616-623.
  20. HOWARD, B. D., AND I. TESSMAN. 1964. Identification of the altered bases in mutated single-stranded DNA. II. *In vivo* mutagenesis by 5-bromodeoxyuridine and 2-amino purine. *J. Mol. Biol.* **9**:364-371.
  21. HOWARD-FLANDERS, P., R. P. BOYCE, AND L. THERIOT. 1966. Three loci in *Escherichia coli* K-12 that control the excision of pyrimidine dimers and certain other mutagen products from DNA. *Genetics* **53**:1119-1136.
  22. ISHIBASHI, M., Y. SUGINO, AND Y. HIROTA. 1964. Chromosomal location of thymine and arginine genes in *Escherichia coli* and an F' incorporating them. *J. Bacteriol.* **87**:554-561.
  23. JACOB, F., AND E. L. WOLLMAN. 1961. Sexuality and the genetics of bacteria. Academic Press, Inc., New York.
  24. JYSSUM, K. 1960. Observations on two types of genetic instability in *Escherichia coli*. *Acta Pathol. Microbiol. Scand.* **48**:113-120.
  25. KIRCHNER, C. E. J. 1960. The effects of the mutator gene on molecular change and mutation in *Salmonella typhimurium*. *J. Mol. Biol.* **2**:331-338.
  26. KIRCHNER, C. E. J., AND M. J. RUDDEN. 1966. Location of a mutator gene in *Salmonella typhimurium* by cotransduction. *J. Bacteriol.* **92**:1453-1456.
  27. LENNOX, E. S. 1955. Transduction of linked genetic characters of the host by bacteriophage P1. *Virology* **1**:190-206.
  28. LURIA, S. E., AND M. DELBRÜCK. 1943. Mutations from virus sensitivity to virus resistance. *Genetics* **28**:491-511.
  29. MIYAKE, T. 1960. Mutator factor in *Salmonella typhimurium*. *Genetics* **45**:11-14.
  30. NEWCOMBE, H. B. 1948. Delayed phenotypic expression of spontaneous mutations in *Escherichia coli*. *Genetics* **33**:447-476.
  31. ORGEL, A., AND L. E. ORGEL. 1965. Induction of mutations in bacteriophage T4 with divalent manganese. *J. Mol. Biol.* **14**:453-457.
  32. PIERCE, B. L. S. 1966. The effect of a bacterial mutator gene upon mutation rates in bacteriophage T4. *Genetics* **54**:657-662.
  33. PUTTE, P. VAN DE, H. ZWENK, AND A. RÖRSCH. 1966. Properties of four mutants of *Escherichia coli* defective in genetic recombination. *Mutation Res.* **3**:381-392.
  34. SCHWARTZ, N. M. 1963. Nature of ethyl methane-sulfonate induced reversions of *lac*<sup>-</sup> mutants of *Escherichia coli*. *Genetics* **48**:1357-1375.
  35. SIEGEL, E. C., AND V. BRYSON. 1964. Selection of resistant strains of *Escherichia coli* by antibiotics and antibacterial agents: role of normal and mutator strains. *Antimicrobial Agents and Chemotherapy—1963*, p. 629-634.
  36. SKAAR, P. D. 1956. A binary mutability system in *Escherichia coli*. *Proc. Natl. Acad. Sci. U.S.A.* **42**:245-249.
  37. SPEYER, J. F. 1965. Mutagenic DNA polymerase. *Biochem. Biophys. Res. Commun.* **21**:6-8.
  38. TAYLOR, A. L., AND M. S. THOMAN. 1964. The genetic map of *Escherichia coli* K-12. *Genetics* **50**:659-677.
  39. 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. U.S.A.* **40**:1064-1071.
  40. WOODY-KARRER, P., AND J. GREENBERG. 1963. Resistance and cross resistance of *Escherichia coli* S mutants to the radiomimetic agent nitrofurazone. *J. Bacteriol.* **85**:1208-1216.
  41. YANOFSKY, C., E. C. COX, AND V. HORN. 1966. The unusual mutagenic specificity of an *E. coli*

- mutator gene. Proc. Natl. Acad. Sci. U.S. **55**:274-281.
42. ZAMENHOF, P. J. 1966. A genetic locus responsible for generalized high mutability in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S. **56**:845-852.
43. ZAMENHOF, S., L. H. HELDENMUTH, AND P. J. ZAMENHOF. 1966. Studies on mechanisms for the maintenance of constant mutability: mutability and the resistance to mutagens. Proc. Natl. Acad. Sci. U.S. **55**:50-58.