Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 9211–9215, December 1990
Genetics

mutA and *mutC*: Two mutator loci in *Escherichia coli* that stimulate transversions

(mismatch repair/spontaneous mutations/*lacZ*/*lacI*/*mutY*)

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Communicated by Maurice S. Fox, August 20, 1990

ABSTRACT **Two transversion-specific mutator loci, *mutA* and *mutC*, were identified in *Escherichia coli*. Mutators with high rates of A·T → T·A transversions were identified using a screening technique that relied upon the reversion of an altered *lacZ* gene back to wild-type via a specific A·T → T·A transversion. Among the mutators collected, one class mapped to a previously unidentified locus that we designate *mutA*. Analysis of reverse mutations in *lacZ* and forward nonsense mutations in *lacI* showed that the *mutA* strain has higher levels of A·T → T·A and G·C → T·A transversions, and to a lesser degree A·T → C·G transversions. The *mutA* locus maps very near to, but is separable from, *mutL*, at about 95 min on the *E. coli* chromosome. Both its mutagenic specificity and complementation experiments confirmed that *mutA* is distinct from *mutL* and from a nearby mutator locus, *miaA*. The phenotype of a *mutA mutL* double mutator strain suggests that the *mutA* gene product prevents some replication errors. Another mutator, designated *mutC*, maps very near *uvrC*, at 42 min, but is distinguishable from *uvrC*, which has no mutator effects. The specificity of reversion of *lacZ* mutations in a *mutC* strain is identical to that in a *mutA* strain. Also, the behavior of a *mutC mutS* double mutant is identical to that of a *mutA mutL* double mutant. It is likely that *mutA* and *mutC* are components of the same error-avoidance system.**

Errors induced during DNA replication can result in base substitutions. Mismatch repair directed by *dam* methylation and requiring DNA helicase, single-stranded DNA-binding protein, and the products of the *mutH*, *mutL*, and *mutS* genes is believed to correct the bulk of base substitutions in *Escherichia coli* (1). Strains that are defective in methyl-directed mismatch repair show a strong bias of transitions over transversions (2–5). Although this bias may reflect the level of each type of mutation generated during replication (6), it might be attributable to the greater repair capability of the mismatch repair system to correct transition-type mismatches (7–10). Evidence has emerged for other mechanisms for mismatch repair that may operate in addition to the methyl-directed system (11–13).

A genetic screening technique that facilitates the detection of strains having high spontaneous rates of specific transversions has been developed in this laboratory (11). With this system, two additional mutator genes have been discovered, *mutY* and *mutM* (11, 12). Strains defective in these gene products show a high specificity for the generation of G·C → T·A transversions. *In vitro* evidence demonstrates that *mutY* codes for an adenine glycosylase that excises the A from a G–A mispair (14). This repair activity is methyl-independent and specifically directs G–A → G·C mismatch correction (15).

We now report the discovery of two more mutator loci in *E. coli*, *mutA* and *mutC*. Both mutators are specific for A·T → T·A and G·C → T·A transversions, and to a lesser extent A·T → C·G transversions, in an otherwise wild-type background. In a mismatch repair-deficient background, *mutA* and *mutC* also stimulate A·T → G·C transitions at the one site examined.

MATERIALS AND METHODS

Bacterial Strains. Mutators were selected in strain CC205, which contains an F′*lac proAB* episome carrying *lacI378* and *lacZ4615*. The chromosome of CC205 is *ara* Δ(*gpt–lac*)*5 rpsL*. The CC101–106 series (16, 17) is similar to CC205 but lacks the *rpsL* mutation and each carries a different *lacZ*⁻ allele. Hfr strain CSH63 (18) is a Hayes Hfr derivative, and strain CGSC6754 is an HfrP4X derivative carrying *zje*::Tn*10* at ≈94.5 min that was constructed by B. Wanner (BW6156) and generously supplied by B. Bachmann (Yale University School of Medicine). Markers used for P1 mapping were carried in strains ES4 (*purA*) and MM294a (*mutL*::Tn*10*), generously provided by E. Siegel (Tufts University). Plasmid pGW1842, constructed by G. Walker, carries the *Salmonella typhimurium mutL* gene and was provided by E. Siegel.

Strain KL862 was supplied by K. B. Low (Yale University School of Medicine). It is *leu lacY his mutH rpsL* and was converted to *ara leu*⁺ Δ(*gpt–lac*)5 by crossing it with Hfr CSH63. After the introduction of the F′ *lac–proAB* episome from CC105, this strain (now termed MC90) was used to detect mutators. Strains CAG12156 (MG1655 *uvrC279*::Tn*10*) and CAG12073 (MG1655 *cycA30*::Tn*10*) were obtained from M. Singer and C. A. Gross (19). XA101 [*ara* Δ(*gpt–lac*)5 *supD gyrA metB argE*(am)*rpoB*] was used to donate the *supD* mutation in genetic crosses (20). Strains carrying *mutL*::Tn*10kan* or *mutS*::Tn*10kan* (C.C. and J.H.M., unpublished work) or *miaA*::Tn*5* (21) were employed for additional P1 mapping studies and strain constructions.

Mutagenesis and Selection of Mutants. Strain CC205 was mutagenized with ethyl methanesulfonate as described (20). After mutagenesis and outgrowth on LB medium overnight, cells were plated on glucose minimal medium containing phenyl β-D-galactoside (P-Gal, 0.5 mg/ml) and 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-Gal, 40 μg/ml). Plates were incubated at 37°C for 3–5 days. Each mutagenesis was monitored for killing and for the generation of rifampicin-resistant mutants.

Genetic Mapping. All Hfr crosses and P1 transductions were performed as described (18). The selected marker in the Hfr cross involving CSH63 was resistant to valine (40 μg/ml), whereas crosses with CGSC6754 and other Hfr strains were selected for tetracycline resistance provided by a Tn*10* insertion (*zje* at ≈94 min, in the case of CGSC6754). The *metB* marker in CGSC6754 was also scored in these crosses. P1 transductions involving *purA* were done in a strain similar to CC205 but lacking an episome. This strain was constructed

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Abbreviations: X-Gal, 5-bromo-4-chloro-3-indolyl β-D-galactoside; P-Gal, phenyl β-D-galactoside.

by transducing *purA* from an ES4 strain that carried a Tn*10* insertion (*zje*). A nonreverting isolate was obtained. Purified transductant colonies were then scored for additional markers on minimal glucose medium containing P-Gal and X-Gal.

All media and genetic manipulations, unless otherwise stated, are as described (18).

RESULTS

Selection for Mutator Strains. A previous report (17) described the effects of amino acid substitutions in *E. coli* β-galactosidase at residues involved in catalysis. A set of six strains carrying missense mutations at position 461 of *lacZ* on an F'*lac–proB* episome were all found to be Lac$^-$ even though they were constitutively expressed in a strain that lacked a functional repressor. Each strain reverts to Lac$^+$ specifically by one of the six transition or transversion base changes to restore the wild-type glutamic acid codon.

To search for additional transversion-specific mutators, we mutagenized CC205, a strain carrying a valine missense at position 461. Only an A·T → T·A transversion will restore the wild-type codon (Fig. 1). Mutators were identified by screening on a glucose minimal plate containing P-Gal and X-Gal. Cells plated on the medium grow until they exhaust the glucose. Then, only Lac$^+$ revertants within the colony can continue to grow, using P-Gal as the carbon source. These revertants are stained blue by the X-Gal and appear as blue microcolonies or papillae growing out of the surface of the white colony. In theory, lactose could be substituted for P-Gal as the carbon source. However, the papillae are more diffuse when lactose is used, making the screen less sensitive.

After mutagenesis with ethyl methanesulfonate, we screened about 10,000 colonies and found 34 strains with elevated levels of papillae formation. Fourteen of the strains had levels of mutagenesis that were too low to allow unambiguous mapping. The 20 remaining strains were rapidly mapped against an HfrH strain, CSH63, that donates the wild-type allele for *mutD* early. *mutD*, which encodes the proofreading subunit of DNA polymerase III, is the only known locus that frequently reverts strain CC205 by the specific A·T → T·A transversion. Strains carrying *mutD* alleles are converted to wild-type 70% of the time when CSH63 is used and resistant to valine (40 μg/ml) is the selected marker. Of the 20 strains, 18 appeared to carry *mutD* alleles and the other 2 mapped away from the *mutD* locus to a new site, designated *mutA*. The two *mutA* alleles resulted in identical mutator effects. All work reported here was carried out with one allele, *mutA1*.

Strain MC90 was used to detect additional mutators. This strain carries a mutation (*mutH*) that inactivates the mismatch repair system. We sought transversion mutators that might be detected only in the absence of mismatch repair. Also, the reduced viability of *mutH mutD* double mutants precludes *mutD* strains from passing through our genetic screen, facilitating the detection of new mutators. Six strains were detected and five of these carried mutations that mapped at the *mutA* locus (see below). The sixth strain carried a new mutator locus, termed *mutC*.

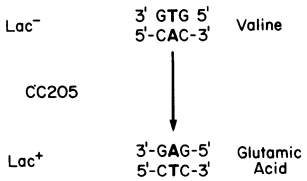

FIG. 1. Reversion of CC205. By site-directed mutagenesis, a valine missense mutation was introduced at codon 461 in the *lacZ* gene that renders it Lac$^-$ (16). β-Galactosidase activity can be restored only by an A·T → T·A transversion, which restores the wild-type glutamic acid codon (17).

Mapping the *mutA* Locus. We used several Hfr strains to approximate the position of *mutA* to 95–98 min on the *E. coli* genetic map (22). This placed *mutA* in the same region of the chromosome as *mutL*. We were able to distinguish between *mutA* and *mutL* by their phenotypes and by the properties of a double mutant we constructed. *mutA* strongly papillates in the strains that monitor A·T → T·A and G·C → T·A transversions, while *mutL* strongly papillates in the strain that scores for G·C → A·T transitions and weakly papillates in the strain that is sensitive to A·T → G·C transitions. *mutA mutL* double mutants papillate strongly in all four strain backgrounds. The distinct phenotype of the three strains facilitated more accurate mapping experiments. A series of two- and three-factor crosses were carried out with P1 to map the location of *mutA* relative to nearby markers (Fig. 2 and Table 1). The three loci *mutA*, *mutL*, and *miaA* map close together near 95 min. Physical mapping has established the order *mutL–miaA–purA* (21). Although the three-factor crosses do not permit a definitive ordering of *mutA* with respect to *mutL* and *miaA*, the data in Table 1 indicate that *mutA* maps on the *purA*-distal side of *mutL*.

Mapping the *mutC* Locus. Hfr crosses indicated that *mutC* mapped close to 42 min, and P1 cotransduction showed that *mutC* mapped extremely close to *uvrC* (98% cotransducible; see Fig. 2). It was not possible to order *mutC* and *uvrC* on the *E. coli* genetic map.

Mutagenesis Tests. Several tests were run to score for mutagenic potency. First, the generation of rifampicin-resistant cells in liquid culture was tested. The two *mutA* strains increased the number of rifampicin-resistant revertants by about 20-fold over the starting strain control and the *mutC* strain by about 10-fold. Second, we tested the mutators in the set of six *lacZ* missense strains that revert to the wild-type glutamate at codon 461 via one specific base change. By examining the level of papillation in both mutators, it is evident that A·T → T·A and G·C → T·A transversions are well stimulated and A·T → C·G transversions are moderately stimulated. The Lac$^+$ revertant frequencies re-

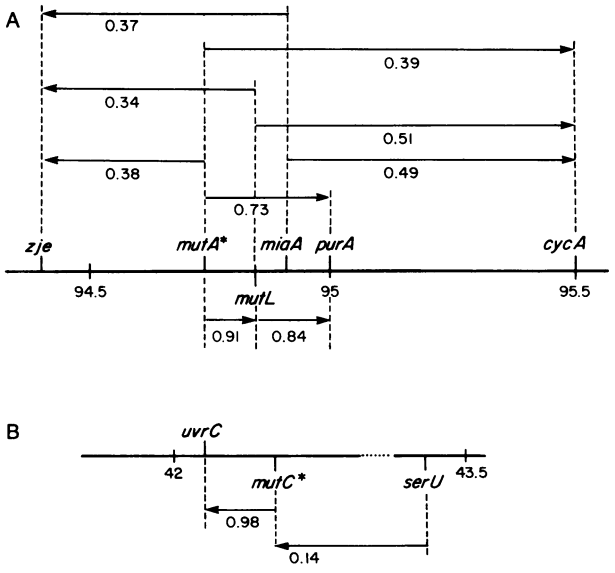

FIG. 2. Genetic mapping of *mutA* and *mutC*. P1 cotransduction frequencies are given with the arrow pointing toward the selected marker. Cotransduction frequencies can vary somewhat, depending on the exact markers used (see *Materials and Methods*). The values usually represent the average of several experiments. The map is not drawn to exact physical scale. The asterisks indicate that the ordering of *mutA* and *mutC* with respect to closely linked markers is tentative (see Table 1).

Table 1. Mapping *mutA* by three-factor crosses

Cross	Outside selected marker	No. of colonies examined	Recombinant class	No. found	Assigned order
(P1) *mutA mutL* + × + + *purA*	PurA$^+$	398	+ *mutL* +	42	*mutA–mutL–purA*
			mutA + +	2	
(P1) *zje* + + × + *mutA mutL*	Tet^r (*zje*)	177	*zje* + *mutL*	36	*zje–mutA–mutL*
			zje mutA +	0	
(P1) *zje mutA mutL* × + + +	Tet^r (*zje*)	470	*zje mutA* +	7	
			zje + *mutL*	11	
(P1) *mutA* + *cycA* × + *mutL* +	Tet^r (*cycA*)	100	+ + *cycA*	10	*(mutA mutL)–cycA*
			mutA mutL cycA	1	
(P1) + + *cycA* × *mutA mutL* +	Tet^r (*cycA*)	357	*mutA* + *cycA*	8	
			+ *mutL cycA*	25	
(P1) *mutA* + *cycA* × + *miaA* +	Tet^r (*cycA*)	184	+ + *cycA*	36	*mutA–miaA–cycA*
			mutA miaA cycA	0	
(P1) *zje mutA* + × + + *miaA*	Tet^r (*zje*)	150	*zje mutA miaA*	5	*zje–(mutA miaA)*
			zje + +	10	

A series of P1 three-factor crosses was conducted in which the outside marker was the selected marker, and *mutA* was scored, together with either *mutL* or *miaA*. For each cross, crossovers between the two unselected markers resulted in one of two possible recombinant types, depending on whether the crossover was accompanied by one additional or three additional crossovers. Both of these recombinant types are displayed for each cross, along with the number of each type detected. Reciprocal crosses are grouped together; as is often the case in crosses of this type, one of the reciprocal crosses will give a definitive ordering and the other will not. Therefore, although the crosses clearly point to the order *zje–mutA–mutL*, and *mutA–miaA–cycA*, this ordering must be considered tentative.

flect the papillation results (Table 2). Finally, the ability of the mutator to cause frameshift mutations was scored in strains where reversion to the Lac$^+$ phenotype could occur if there were +1 or −1 frameshifts in monotonous runs of A·T or G·C base pairs (23). No increase in frameshift mutations over the control was observed in any of the strains (data not shown).

***lacI* Nonsense Analysis.** One of the limitations of the rifampicin revertant test is the lack of specific information on the type of mutation leading to the reversion. While the *lacZ* missense revertant screen can give precise information, it is limited because it monitors only one site, which may be either "hot" or "cold" for mutagenesis. Therefore, to determine the mutagenic specificity of *mutA* in more detail, we used the *lacI* nonsense system, which monitors the generation of 90 UAG (amber), UAA (ochre), and UGA (opal) mutations at 78 different sites in the *lacI* gene (24).

Nearly 11% of all *lacI* mutations generated in *mutA* are nonsense mutations. Compared with a wild-type strain, nonsense mutations were increased 10- to 15-fold. The distribution of nonsense mutations in a wild-type strain is shown in Fig. 3. All types of spontaneous mutation are observed in the spectrum, with three hotspots for the G·C → A·T transition

Table 2. Mutational specificity of *mutA* and *mutC* in the *lacZ* reversion system

Strain	Reversion event	No. of Lac$^+$ revertants per 10^8 cells		
		+	*mutA*	*mutC*
CC101	A·T → C·G	0.85 ± 0.11	4.3 ± 0.69	5.6 ± 1.2
CC102	G·C → A·T	3.1 ± 1.6	2.4 ± 1.7	3.0 ± 0.43
CC103	G·C → C·G	≤0.1	≤0.1	≤0.1
CC104	G·C → T·A	6.2 ± 1.1	22 ± 3.8	32 ± 3.4
CC105	A·T → T·A	2.3 ± 0.65	18 ± 2.0	22 ± 3.8
CC106	A·T → G·C	0.5 ± 0.3	0.7 ± 0.2	0.3 ± 0.4

Six episomes carrying *lacZ* mutations at codon 461 were donated from strains CC101–106 (17) to a *mutA* strain that had been cured of its original episome. The series of *lacZ* mutations each revert by a specific base substitution. The number of Lac$^+$ revertants in overnight cultures of each derivative was compared with control strains. Each number represents the average of several platings of 4–10 separate cultures. (The *mutC* locus has been separated from *mutH* in these experiments.)

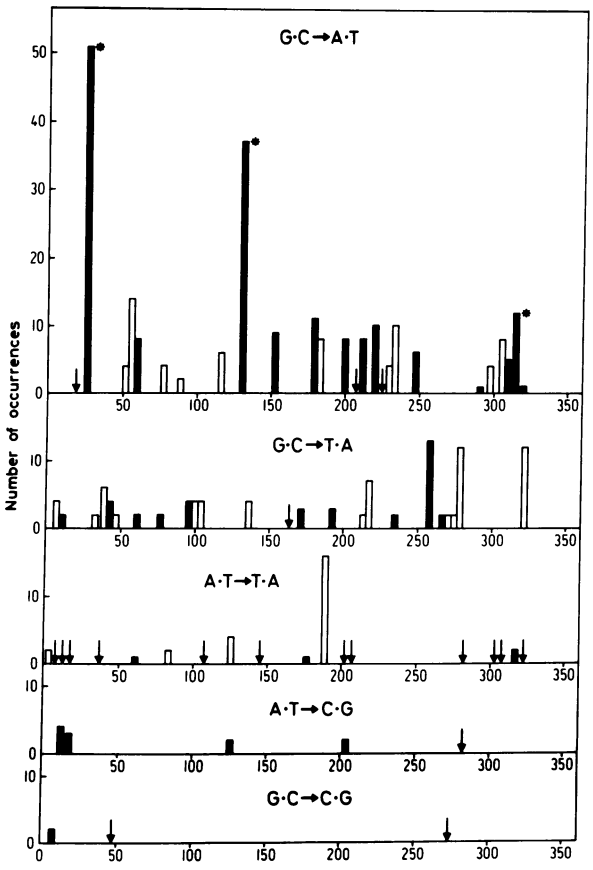

FIG. 3. Spontaneous *lacI* forward nonsense mutations in a wild-type (*mutA*$^+$) strain. The height of each bar represents the number of independent occurrences of amber (solid bars) and ochre (open bars) mutations in a collection of 306 nonsense mutations. (The ochre bar heights have been normalized to account for a smaller sample size.) Arrows indicate the positions of nonsense sites at which there were no occurrences in this collection. Asterisks indicate 5-methylcytosine residues. The position of sites in the *lacI* gene is indicated on the horizontal axis by the number of the corresponding amino acid in the *lac* repressor. (Redrawn from ref. 20.)

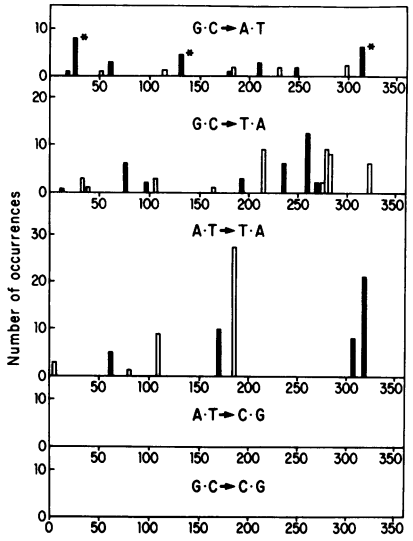

FIG. 4. Distribution of nonsense mutations in *lacI* in a *mutA* strain. The position and number of occurrences of 194 amber and ochre mutations are shown. As in Fig. 3, solid bars represent amber mutations and open bars represent ochre mutations. Bar heights reflect the exact number of mutations detected. Each mutation was of independent origin. See also the legend to Fig. 3.

at 5-methylcytosine residues. The distribution of nonsense mutations in a *mutA* background is dramatically different and a marked stimulation in A·T → T·A and G·C → T·A transversions relative to G·C → A·T transitions is observed (Fig. 4 and Table 3). Although the A·T → C·G transversion is detected in the *lacZ* reversion test, none of the five available sites that can be monitored in the *lacI* system was represented in the collection of nonsense mutations.

Mutagenesis Tests in a Mismatch Repair-Deficient Background. As mentioned above, not only does *mutA* map near the mutL locus, but also the double mutant has a phenotype that is not simply a combination of the phenotypes of the separate mutators. Specifically, the double mutant has a far greater rate of papillation in the strain that monitors A·T → G·C transitions. To obtain a more accurate measure of the magnitude of the increase, we tested the mutants individually or in combination for their ability to revert several of the *lacZ* missense mutations (Table 4). The A·T → T·A and G·C → A·T base changes in the double mutant background are not significantly different from their levels in *mutA* or *mutL* backgrounds individually. However, A·T → G·C transitions are increased 120-fold over the *mutA* background and 12-fold over the *mutL* background. In a similar manner, *mutC* increases A·T → G·C transitions in a *mutS* background.

For further confirmation that *mutA* and *mutL* are indeed distinct loci, we introduced a plasmid, pGW1842, containing the *mutL* gene from *S. typhimurium* (25) into each of the strain backgrounds and repeated the reversion analysis. The plasmid complemented the *mutL* strain. Double mutants carrying the plasmid behaved essentially as *mutA* alone. The *mutA* strain was unaffected by the presence of the plasmid (Table 5).

Table 3. Distribution of nonsense mutations in a *mutA* strain

Substitution	No. of sites available	No. of sites found	Total occurrences
G·C → A·T	26	14	41
G·C → T·A	24	16	73
A·T → T·A	19	8	80
A·T → C·G	5	0	0
G·C → C·G	3	0	0

The A·T → G·C transitions cannot be monitored by the *lacI* nonsense system. Not surprisingly, when the double mutant was analyzed by the forward nonsense system, the spectrum looked much like those generated in *mutH* or *mutS* backgrounds (2) (data not shown).

DISCUSSION

A genetic screening technique that relies on the reversion of *lacZ* missense mutations to identify strains with elevated rates of base substitutions has been developed in this laboratory and has led to the discovery of two mutator loci, *mutY* and *mutM*, that result specifically in G·C → T·A transversions (11, 12). Here we describe two additional mutator loci, *mutA* and *mutC*, that specifically stimulate certain transversions. Analysis of the *mutA* and *mutC* strains in the *lacZ* reversion system (Table 1) reveals that A·T → T·A and G·C → T·A transversions, as well as A·T → C·G transversions, are specifically stimulated. Analysis of the *mutA* strain in the *lacI* forward nonsense system shows a marked stimulation in A·T → T·A and G·C → T·A transversions (Fig. 4 and Table 3), but none of the five sites that monitor A·T → C·G transversions was represented in the collection of nonsense mutations. The *lacI* system monitors an extensive array of base substitutions, providing a broad profile of the mutagenic specificity of *mutA*. Figs. 3 and 4 compare the wild-type and *mutA* nonsense spectra. Of 194 nonsense mutations from a *mutA* background, 79% arose from A·T → T·A or G·C → T·A transversions. In contrast, ≈68% of the spontaneous nonsense mutations are G·C → A·T transitions (20). The remaining mutations in the *mutA* spectrum are all G·C → A·T transitions and are most likely attributable to the background of spontaneous mutations.

The patterns of base changes that are stimulated in *mutA* and *mutC* are different from those of other described mutator loci, many of which predominantly stimulate transitions. The SOS mutator effect stimulates two of the same transversions as *mutA*. However, *lacI* analysis of the SOS mutator effect results in a very different mutagenic profile than *mutA*, with G·C → T·A base changes predominating, suggesting that these two systems are not related (26).

mutA maps near 95 min on the *E. coli* genetic map, very close (Fig. 2) to *mutL* and *miaA*, a locus reported to result in a low level of mutator activity (21). Several lines of evidence show that *mutA* is distinct from both *mutL* and *miaA*. (*i*) Their mutagenic specificity is vastly different. *mutL* stimu-

Table 4. Mutational specificity displayed by *mutA mutL* and *mutC mutS* strains

	No. of Lac$^+$ revertants per 10^8 cells					
	mutA/mutL experiments			*mutC/mutS* experiments		
Substitution	*mutA*	*mutL*	*mutA mutL*	*mutC*	*mutS*	*mutC mutS*
A·T → G·C	0.3 ± 0.4	31 ± 9.3	360 ± 73	0.7 ± 0.2	29 ± 6.9	220 ± 18
G·C → A·T	2.4 ± 1.7	200 ± 24	280 ± 40	—	—	—
A·T → T·A	22 ± 3.8	2.9 ± 0.29	18 ± 4.0	—	—	—

The number of Lac$^+$ revertants in overnight cultures of each derivative was compared with control strains. Each number represents the average of several platings of multiple cultures.

Table 5. Mutational specificity and complementation of *mutA*, *mutL*, and *mutA mutL*

Substitution	No. of Lac$^+$ colonies per 10^8 cells							
	+	pGW1842/ +	*mutA*	pGW1842/ *mutA*	*mutL*	pGW1842/ *mutL*	*mutA mutL*	pGW1842/ *mutA mutL*
A·T → G·C	0.5	1.0	0.7	0.3	31	2.1	360	4
G·C → A·T	7.2	5.2	2.4	1.8	200	7	280	15
A·T → T·A	0.9	0.9	18	29	2.9	1.8	—	—

Three episomes carrying *lacZ* mutations at codon 461 were donated to *mutA mutL* strains that either contained or lacked the *mutL* clone from plasmid pGW1842. The number of Lac$^+$ revertants in overnight cultures of each strain was determined after 37 hr of incubation on minimal lactose plates. Strains containing the plasmid were plated on medium supplemented with ampicillin (100 μg/ml). Each number represents the average of several determinations.

lates mainly transitions and frameshifts (2–5), whereas *mutA* stimulates three specific transversions and does not induce frameshifts. Also, *miaA* does not stimulate either A·T → T·A or A·T → C·G transversions in the *lacZ* system (data not shown). (*ii*) Double mutant strains carrying *mutA miaA* or *mutA mutL* and displaying the combined phenotypes of each respective single mutant could be constructed even though the *mutL* and *miaA* genes were transposon-interrupted and therefore not likely to be leaky. The double mutations were separable in P1 mapping experiments (Fig. 2 and Table 2). (*iii*) A *mutL*-containing plasmid complemented only *mutL*, not *mutA* (Table 5).

Interestingly, the phenotype of a *mutA mutL* or *mutC mutS* double mutant strain is not simply the addition of the two independent mutators. Instead, the double mutant has a much higher rate of A·T → G·C transitions in the *lacZ* reversion system. In a double mutant background, Lac$^+$ revertants are increased 12-fold over a *mutL* strain and 120-fold over a *mutA* strain. Since this base change cannot be monitored in the *lacI* nonsense system, the data on the cooperative interaction are limited to the analysis of a single site by the *lacZ* reversion system.

Because of their identical specificity and reaction in combination with mismatch repair-deficient backgrounds, *mutA* and *mutC* are probably part of the same error-avoidance system. They may be involved in one of several possible repair pathways. They may function at the level of replication. This would be consistent with the phenotype of the *mutA mutL* and *mutC mutS* double mutants, which show that A·T → G·C transitions are increased relative to the *mutL* strain or *mutS* strain alone. It implies that loss of the *mutA* protein can stimulate a variety of misincorporations, but those that lead to transitions can be processed by the methyl-directed mismatch repair system.

A previously characterized transversion-specific mutator, *mutT* (27), has also been shown to function during replication (28–31). This mutator, which generates A·T → C·G transversions, interacts with the replication complex to process A–G mispairs, possibly by preventing the incorporation of *syn*-guanine (32). *mutA* and *mutC* may provide a reciprocal function by preventing misincorporation of *syn*-adenine. Such a model would account for the two predominant transversions observed in this strain, A·T → T·A and G·C → T·A, but would not account for the properties of the double mutant, unless the incorporation of *syn*-guanine were also partly affected by the *mutA*- and *mutC*-encoded functions.

Another possibility is that *mutA* and *mutC* code for proteins that specifically recognize or excise mispairs. For instance, *mutY* codes for an adenine glycosylase that removes the A from a G–A mispair (14). The MutY glycosylase has homology to endonuclease III (33), an enzyme that recognizes and removes mispaired, damaged bases. Similarly, *mutA* and *mutC* may code for proteins that recognize certain base-pair mismatches and excise them.

We wish to thank Drs. B. Bachmann, K. B. Low, D. M. Connolly, and E. Siegel for bacterial strains and Drs. P. Modrich and M. Fox for helpful discussions. We thank Lucie Pham for expert technical assistance. This work was supported by a grant from the National Institutes of Health (GM32184).

1. Modrich, P. (1987) *Annu. Rev. Biochem.* **56,** 435–466.
2. Leong, P.-M., Hsia, H. C. & Miller, J. H. (1986) *J. Bacteriol.* **168,** 412–416.
3. Schaaper, R. M. & Dunn, R. L. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 6220–6224.
4. Rewiniski, C. & Marinus, M. G. (1987) *Nucleic Acids Res.* **15,** 8205–8215.
5. Choy, H. E. & Fowler, R. G. (1985) *Mutat. Res.* **142,** 93–97.
6. Ferscht, A. R. & Knill-Jones, J. W. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 4251–4255.
7. Su, S.-S., Lahue, R. S., Au, K. G. & Modrich, P. (1988) *J. Biol. Chem.* **263,** 6829–6835.
8. Kramer, B., Kramer, W. & Fritz, H.-J. (1984) *Cell* **38,** 879–887.
9. Dohet, C., Wagner, R. & Radman, M. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 503–505.
10. Jones, M., Wagner, R. & Radman, M. (1987) *Genetics* **115,** 605–610.
11. Nghiem, Y., Cabrera, M. & Miller, J. H. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 2709–2713.
12. Cabrera, M., Nghiem, Y. & Miller, J. H. (1989) *J. Bacteriol.* **170,** 5405–5407.
13. Radicella, J. P., Clark, E. A. & Fox, M. S. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 9674–9678.
14. Au, K. G., Clark, S., Miller, J. H. & Modrich, P. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 8877–8881.
15. Au, K. G., Cabrera, M., Miller, J. H. & Modrich, P. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 9163–9166.
16. Cupples, C. G. & Miller, J. H. (1988) *Genetics* **120,** 637–644.
17. Cupples, C. G. & Miller, J. H. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 5345–5349.
18. Miller, J. H. (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
19. Singer, M., Baker, T. A., Schnitzler, G., Dieschel, S. M., Goel, M., Dove, W., Jaacks, K. J., Grossman, A. D., Erickson, J. W. & Gross, C. A. (1989) *Microbiol. Rev.* **53,** 1–24.
20. Coulondre, C. & Miller, J. H. (1977) *J. Mol. Biol.* **117,** 525–567.
21. Connolly, D. M. & Winkler, M. E. (1989) *J. Bacteriol.* **171,** 3233–3246.
22. Bachmann, B. J. (1983) *Microbiol. Rev.* **47,** 180–230.
23. Cupples, C. G., Cabrera, M., Cruz, C. & Miller, J. H. (1990) *Genetics* **125,** 275–280.
24. Coulondre, C. & Miller, J. H. (1977) *J. Mol. Biol.* **117,** 577–606.
25. Pang, P. P., Lundberg, A. S. & Walker, G. C. (1985) *J. Bacteriol.* **163,** 1007–1015.
26. Miller, J. H. & Low, K. B. (1984) *Cell* **37,** 675–682.
27. Cox, E. C. (1976) *Annu. Rev. Genet.* **10,** 135–156.
28. Cox, E. C. (1973) *Genetics* **73,** Suppl., 67–80.
29. Schaaper, R. M. & Dunn, R. L. (1987) *J. Biol. Chem.* **262,** 16267–16270.
30. Bhatnagar, S. K. & Bessman, M. J. (1988) *J. Biol. Chem.* **263,** 8953–8957.
31. Akiyama, M., Maki, H., Sekiguchi, M. & Horiuchi, T. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 3949–3952.
32. Fresco, J. R. & Topal, M. D. (1976) *Nature (London)* **263,** 285–293.
33. Michaels, M. L., Pham, L., Nghiem, Y., Cruz, C. & Miller, J. H. (1990) *Nucleic Acids Res.* **18,** 3841–3846.