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mutM, a Second Mutator Locus in *Escherichia coli* That Generates G · C → T · A Transversions

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We used strains carrying specific *lacZ* alleles to identify a new mutator locus in *Escherichia coli* which generates only G · C → T · A transversions among base substitutions. The locus, *mutM*, mapped near the *cysE* locus, which is at 81 min on the genetic map.

The pathways that lead to spontaneous base substitution mutations have not been completely defined. Although we know that replication errors and spontaneous lesions are potential sources of mutation, it is not certain which is the principal cause of each of the different spontaneous base substitutions that are observed. In particular, the pathways that lead to transversion mutations are still somewhat obscure. It is not clear how transversions occur as replication errors (see, for instance, reference 18) and how many repair systems exist which can efficiently correct these errors. The methyl-directed mismatch repair system encoded by the *Escherichia coli mutHLS* and *uvrD* genes (11, 14) can correct mispairings that lead to both transitions and most transversions (4–6, 17). Yet, strains lacking this system display more greatly enhanced levels of transitions than transversions (2, 8, 15, 16). This might simply be a reflection of the levels of each type of mispair generated during replication, or it might signal the existence of additional repair systems. Is there a system that specifically corrects mispairings that lead to transversions?

We have recently described a mutator locus in *E. coli*, *mutY*, which results in G · C → T · A transversions (13). Shin-San et al. (17) and Lu and Change (10) have described a DNA mismatch correction activity in vitro that specifically repairs G · A mispairs, resulting in G · C base pairs, and which is independent of the *mutHLS*-dependent pathway. Strains which carry *mutY* lose the *mutHLS*-independent repair activity specific for G · A mispairs (K. G. Au, P. Modrich, M. Cabrera, and J. H. Miller, unpublished results), demonstrating that this activity represents a repair system that functions in vivo as a mechanism for avoiding G · C → T · A transversions. These results also demonstrate the value of studying mutators as a way of elucidating mutagenic pathways.

Detection of new mutator strains. We looked for additional mutator strains that might have defects in the same or different pathways responsible for transversions. Here we describe a second locus, *mutM*, involved in the avoidance of G · C → T · A transversions. We detected this locus by mutagenizing a strain, CC104 (13), that carries a specific mutation in *lacZ* and then screening for colonies with an increased reversion rate to Lac$^+$. The mutation carried in strain CC104, which changes the active-site glutamic acid residue at position 461 in beta-galactosidase to alanine, resulting in a defective enzyme is shown in Fig. 1. Only the G · C → T · A change can restore the glutamic acid residue, which is essential for sufficient beta-galactosidase activity to allow growth on lactose (C. G. Cupples and J. H. Miller, Genetics, in press). Revertants to Lac$^+$ are detected by papillation on indicator media, as described previously (13). Mutator strains that revert CC104 are seen as colonies that have a significantly increased rate of papilla formation. We screened 5×10^4 colonies after mutagenesis with ethyl methanesulfonate (13) and generated 100 mutator strains. We concentrated on the 24 mutators with high enough levels of mutagenesis, determined by scoring Rif^r and Lac$^+$ colonies, to map easily.

Of the known mutator strains, only *mutD* and *mutY* strains revert CC104. Therefore, we rapidly mapped each of the 24 mutators against two Hfr strains which donated the wild-type allele for either the *mutY* or *mutD* locus at high efficiency closely linked to an antibiotic resistance marker (Fig. 2). Strains carrying *mutD* were converted to the wild type 75% of the time with EG3033, an HfrC strain carrying a Tet^r marker in the *car* locus, and strains carrying *mutY* were converted to the wild type greater than 95% of the time with a PK191 derivative, an Hfr strain carrying a Tet^r marker in the *nupG* locus. Of the 24 strains, 14 appeared to carry *mutY* and 8 carried *mutD*. Two of the mutator strains mapped apart from either the *mutD* or *mutY* locus and defined a new locus, which we term *mutM*.

Mapping the *mutM* locus. We used several Hfr strains to pinpoint the location of the *mutM* locus to the 80- to 82-min region of the *E. coli* genetic map. These strains are depicted in Fig. 2. A cross with Hfr strain CGSC 6754 allowed selection for Tet^r as a proximal marker and scoring for the inheritance of *metB*, *bgl*, and *mutM*. This placed *mutM* just counterclockwise to the *bgl* locus, which is at 83 min. P1 linkage experiments showed no cotransduction with markers

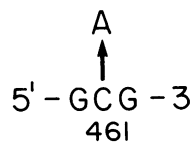

FIG. 1. Mutation in *lacZ* that reverts only via the G · C → T · A transversion. An alteration at the coding position specifying residue 461 in beta-galactosidase results in an alanine-for-glutamic acid exchange that renders the cell Lac$^-$. Only restoration of the glutamic acid, which requires a G · C → T · A transversion, can restore the Lac$^+$ phenotype.

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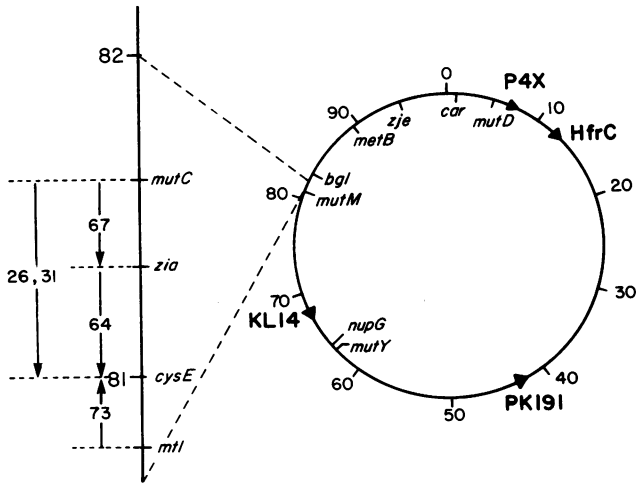

FIG. 2. Mapping the *mutM* locus. The positions of Hfr origins (9) and relevant genetic loci (1, 13) are shown. Procedures were as described in the text and in reference 13. The *mtl* mutation was from strain AB1157 (CGSC 1157 from B. Bachmann), the *cysE* mutation was from strain CGSC 5042 (B. Bachmann), and the *zia* mutation was from CK1606 (C. A. Kumamoto).

at 83 min (Table 1) but did show cotransduction with the *cysE* locus at 81 min. Three-factor crosses employing a Tn*10* element, *zia* (7), established the order shown in Fig. 2 (left portion), with *mutC* clockwise to *cysE*.

Specificity of the mutator character of *mutM*. We examined mutations generated by *mutM* with both the *lacZ* and *lacI* systems. The response of each of six strains containing different alterations at the coding position in *lacZ* for amino acid 461 in beta-galactosidase is shown in Table 1. Each strain reverts to Lac^+ via only one of the six possible base substitutions. It can be seen that only the G · C → T · A transversion was stimulated by the presence of the *mutM* allele. (Cultures of *mutM* strains have severalfold lower levels of mutants than do cultures of *mutY* strains.)

We also used the *lacI* system (3) to examine the distribution of nonsense mutations stimulated by the presence of the *mutM* allele. A severalfold increase of $LacI^-$ mutants was seen in *mutM* strains. Among mutations in *lacI*, 16% were nonsense mutations in a *mutM* strain, compared with 1 to 2% in a wild-type background. Therefore, nonsense mutations were increased 25- to 30-fold. The wild-type and the *mutM*-induced nonsense mutations are shown in Fig. 3 and 4.

TABLE 1. Mutational specificity of *mutM*[a]

Strain donating F′ *lac proB*	Base substitution	Lac^+ colonies/10^8 cells	
		Control	*mutM*
CC101	A · T → C · G	0	0
CC102	G · C → A · T	6	7
CC103	G · C → C · G	0	0
CC104	G · C → T · A	4	55
CC105	A · T → T · A	0	0
CC106	A · T → G · C	0	0

[a] Six strains (CC101 to CC106) carrying different *lacZ* mutations on F′ *lac proB* episomes were used to donate the episomes to both wild-type and *mutM* strains. The number of Lac^+ revertants in overnight cultures of each strain was monitored. Each value represents the average of several determinations.

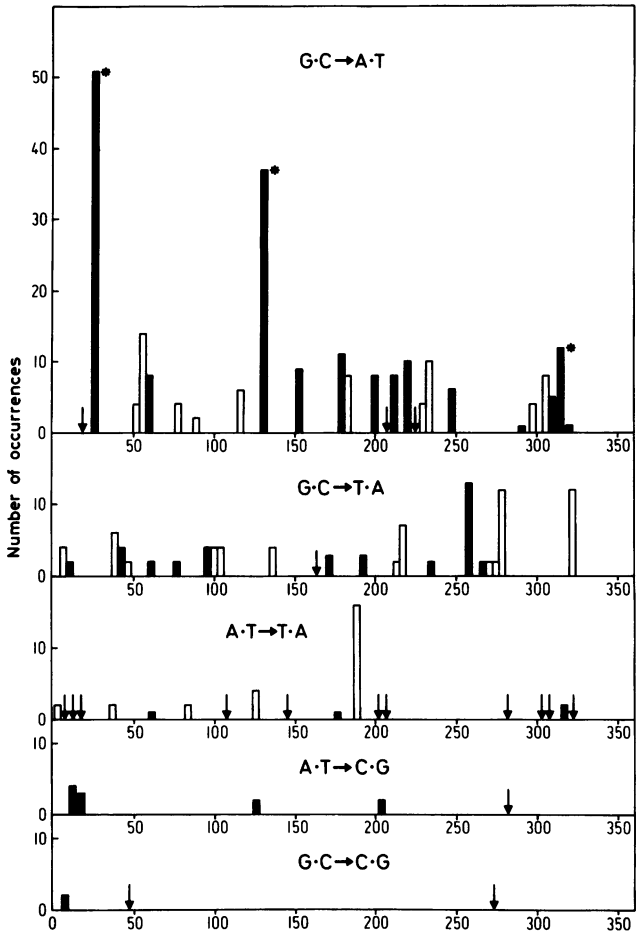

FIG. 3. Spontaneous amber (■) and ochre (□) mutations occurring in a wild-type (*mutM*$^+$) strain. The height of each bar represents the number of independent occurrences in a collection of 306 nonsense mutations. (The ochre bar heights have been normalized to account for a smaller sample size.) Arrows indicate the positions of nonsense sites at which there were no mutations in this collection. Asterisks indicate 5-methylcytosine residues. The positions of sites in the *lacI* gene are indicated on the horizontal axis by the numbers of the corresponding amino acids in the *lac* repressor. (redrawn from reference 3).

Whereas the spontaneous nonsense mutations in the wild-type strain resulted from many different base substitutions, with prominent hot spots at 5-methylcytosine residues, the nonsense mutations in the *mutM* background were almost exclusively due to G · C → T · A transversions. In fact, 282 of 299 (94.3%) nonsense mutations were derived from G · C → T · A transversions. The remainder were very probably due to the normal spontaneous background. The detailed spectra of the *mutM* (Fig. 4) and *mutY* (13) strains are somewhat similar, with some of the same hot spots, which is consistent with the possibility that the two loci are involved in the same pathway. Experiments are in progress to determine whether the *mutY*-dependent correction of G · A mismatches is abolished in *mutM* strains (K. G. Au, P. Modrich, M. Cabrera, and J. H. Miller, unpublished results). Future experiments are aimed at cloning both the *mutY* and *mutM* genes as a step in studying the proteins involved in the transversion avoidance pathway.

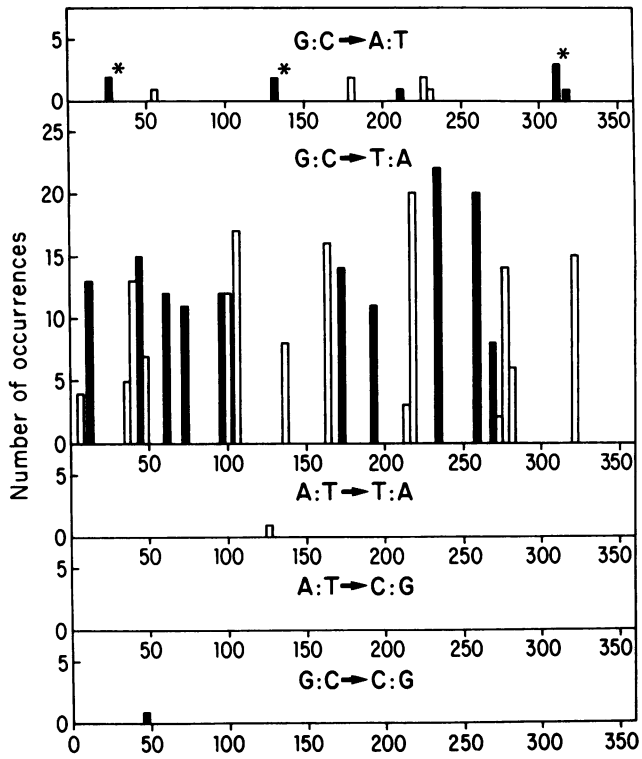

FIG. 4. Distribution of nonsense mutations in *lacI* in a *mutM* strain. The positions and number of occurrences of 299 amber and ochre mutations are shown. Symbols: ■, amber mutations; □, ochre mutations. Bar heights represent the exact numbers of mutations detected. Each mutation was of independent origin. The mutations were analyzed as described previously (8). See also the legend to Fig. 3.

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