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Analysis of Spontaneous Base Substitutions Generated in Mismatch-Repair-Deficient Strains of *Escherichia coli*

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We used the *lacI* system of *Escherichia coli* to examine the distribution of base substitution mutations occurring spontaneously in different mismatch-repair-deficient strains. The examination of almost 1,200 nonsense mutations generated in strains carrying the *mutS*, *mutH*, and *mutU* alleles confirmed that transitions are highly favored over transversions. The detailed analysis of relative mutation rates at different sites revealed that the pattern of hot spots and cold spots is strikingly similar in each of the three strain backgrounds, strongly supporting the notions that the products of the three genes are part of the same system and that in the absence of any of the components the entire system fails to function. The distribution of mutations occurring in the absence of mismatch repair defined a pronounced topography of the *lacI* gene. There was no obvious correlation of the hot spots or cold spots with either nearest-neighbor sequences or A · T richness of the immediate surrounding sequence.

Spontaneous mutations in *Escherichia coli* are as rare as 10^{-10} to 10^{-9} per base pair per cell per generation (8). This level of accuracy is achieved by a combination of editing and repair (Fig. 1). Initial nucleotide insertion results in a frequency of base misincorporation of 10^{-5} to 10^{-4} per base pair per cell per generation (3). The epsilon subunit of DNA polymerase III provides an editing function which may increase fidelity to approximately 10^{-8} to 10^{-7} (3, 22). A postreplication mismatch repair system (14, 21, 23, 24) provides further precision so that single-base-pair substitutions are observed at 10^{-10} to 10^{-9}. The latter system presumably detects certain mismatched bases, discriminates the parent strand from the newly synthesized strand by recognizing unmethylated GATC sequences in heteroduplex DNA, and then excises the mismatched base together with neighboring bases from the newly synthesized strand. The resulting gaps are filled in by repair synthesis and ligase. In addition to the *dam* locus that encodes adenine methylase, four mutator loci (*mutH*, *mutS*, *mutL*, and *mutU*; 1) have been implicated in the mismatch repair pathway (1, 19, 20). Only the enzyme encoded by *mutU* (previously termed *uvrD*) has been identified; it was indicated to be helicase II (13). Genetic studies with mutators associated with the mismatch repair pathway have shown that transitions and certain frameshifts are preferentially generated, although transversions are also increased at a low level (4, 8, 10, 11). We undertook a more detailed study of the site specificity of mutations generated in different mutator strains. In the work reported here, we examined spontaneous base substitutions that lead to *lacI* nonsense mutations in strains carrying *mutU*, *mutH*, and *mutS* alleles. The *lacI* system facilitates the analysis of large numbers of mutations. The basic aspects of this system (described in detail in references 6 and 20) are as follows. All of the nonsense mutations derived from the wild type on an F′ *lac proB* episome via a single base change are known, as are the respective sequence changes that generate each nonsense mutation. The mutations are recognized by genetic methods, because each mutation has a characteristic map position and response to nonsense suppressors. This group of 87 mutations (UAG, UAA, and UGA) at 74 different positions consists of 30 G · C → A · T transitions, 25 G · C → T · A transversions, 21 A · T → T · A transversions, 6 A · T → C · G transversions, and 5 G · C → C · G transversions. Therefore, nonsense mutations, which often constitute close to 25% of the detectable *lacI* mutations, can be easily assigned to one of the 87 known mutations. The mutations are then grouped according to the base substitution involved, and the occurrences at each site are displayed in a histogram. This procedure demonstrates the specificity of a mutagenic process as a virtual spectrum of relative frequencies across many different sites. The advantage of this system is that it allows the rapid determination of the sequence changes by genetic methods without biochemical sequencing of the DNA. Thus, large number of mutations can be analyzed, allowing statistically significant determinations of mutational hot spots and cold spots. Also, because all possible mutational sites are known, we can attach importance to sites where no mutations are detected in a given experiment.

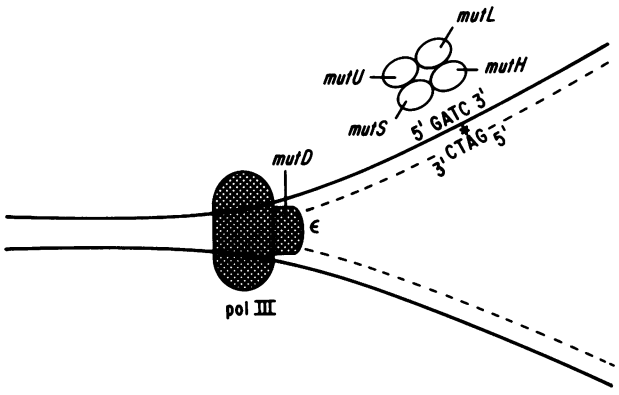

FIG. 1. A schematic view of DNA replication. Replication error is corrected by the epsilon subunit of the polymerase, as well as by the mismatch repair system, which is dependent on hemimethylated GATC sequences in heteroduplex DNA for strand discrimination. Strains lacking either the epsilon-subunit editing function or one of the mismatch repair functions have been identified as mutators, as indicated here.

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TABLE 1. Number of occurrences of spontaneous transitions at each site in the different mutator strains

G · C → A · T transition site	No. of occurrences for strain carrying[a]:								
	mutS3			*mutU4*			*mutH34*		
	a	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*
Ochre									
O9	4	5	6	0	1	6	5	6	10
O10	3	7	11	4	4	9	9	11	19
O11	0	0	1	1	2	6	3	3	4
O13	2	3	4	1	2	4	4	6	10
O17	0	0	0	0	0	3	1	1	2
O21	21	35	58	19	23	41	31	36	65
O24	0	0	4	3	3	9	2	2	8
O27	4	7	14	7	9	19	4	5	10
O28	3	9	19	18	20	33	11	15	26
O29	4	6	10	4	6	10	7	7	14
O34	16	29	48	21	29	50	16	18	37
O35	12	19	31	19	23	40	14	16	29
Amber									
A5	12	24	34	1	4	4	1	6	6
A6	5	9	20	1	3	4	2	7	11
A9	9	19	32	5	13	21	7	9	9
A15	2	3	6	0	0	0	1	4	4
A16	8	13	23	0	4	5	0	0	0
A19	5	8	12	0	2	2	0	1	2
A21	10	14	25	1	4	6	1	2	2
A23	2	2	3	0	1	3	2	2	3
A24	3	7	10	1	2	3	0	2	3
A26	2	4	12	5	11	14	0	1	1
A31	0	2	6	1	2	3	2	3	3
A33	2	4	6	1	2	2	1	2	2
A34	5	12	19	1	5	6	0	0	0
A35	2	10	15	3	6	10	1	1	1
UGA									
U1	0	1	1	0	1	1	3	5	5
U5	19	46	46	8	39	39	16	30	30

[a] Columns: *a*, the collection of the first nonsense mutation from each culture, whether it was UAA, UAG, or UGA; *b*, the collection of the first ochre mutation from each culture within the ochre group, the first amber mutation from each culture within the amber group, and the first UGA mutation from each culture within the UGA group; *c*, the collection in which for the ochre group several ochres were taken from each culture and recurrences of the same mutation from the same culture were discarded. A similar procedure was followed for the amber and UGA groups.

TABLE 2. Number of occurrences of spontaneous transversions at each site in the different mutator strains

Transversion type and site	No. of occurrences for strain carrying[a]:								
	mutS3			*mutU4*			*mutH34*		
	a	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*
G · C → T · A									
Ochre									
O3	0	0	0	0	0	1	0	0	0
O6	0	0	0	0	0	0	0	0	0
O7	0	0	0	1	1	2	2	2	2
O8	0	0	0	0	0	0	0	0	0
O14	0	0	0	0	0	1	0	0	0
O15	0	0	0	0	0	0	0	0	0
O19	0	0	0	0	0	0	0	0	0
O20	0	1	3	0	0	1	0	0	0
O25	0	0	0	0	0	1	0	0	2
O26	0	0	0	0	0	0	1	2	3
O30	0	0	0	0	0	0	0	0	0
O31	0	0	0	0	0	1	0	0	0
O32	0	0	0	0	0	0	0	1	1
O36	0	0	0	0	0	0	0	0	0
Amber									
A1	1	2	2	0	0	0	0	0	0
A2	0	0	0	0	0	0	0	0	0
A8	1	1	1	0	0	0	0	0	0
A10	0	0	0	0	0	0	0	0	0
A12	0	0	0	0	1	1	0	0	0
A13	0	0	1	0	0	0	0	1	1
A17	0	0	0	0	0	0	1	1	1
A20	0	0	0	0	0	0	0	0	0
A25	0	1	1	1	1	1	0	0	0
A27	1	1	1	0	0	0	0	1	2
A28	0	0	0	1	1	1	0	0	0
A29	0	0	0	1	1	1	0	0	0
UGA									
U2	0	0	0	0	0	0	0	0	0
Ochre									
O1	0	0	0	0	0	0	0	0	0
O2	0	0	0	0	0	0	0	0	0
O4	0	0	0	0	0	0	0	0	0
O5	0	0	0	0	0	0	0	0	0
O12	0	0	0	0	0	0	1	1	1
O16	0	0	0	0	0	0	0	0	1
O18	0	0	0	0	0	0	0	0	1
O22	0	1	1	0	0	0	0	1	1
O23	0	0	0	0	0	0	0	0	0
O33	0	0	0	0	0	0	0	0	0
Y3	0	0	0	0	0	0	0	0	0
Y4	0	0	0	0	0	0	0	0	0
Y6	0	0	0	0	0	0	0	0	0
Y8	0	0	0	0	0	0	0	0	0
Amber									
A11	0	0	0	0	0	0	0	0	0
A18	0	0	0	0	0	0	0	0	0
A32	0	0	0	0	0	0	0	0	0
A36	0	0	0	0	0	0	0	0	0
X9	0	0	0	0	0	0	0	0	0
UGA									
U3	0	0	0	0	0	0	0	0	0
U8	0	0	0	0	0	0	0	0	0

MATERIALS AND METHODS

Strains. *E. coli* KL838, KL839, KL862, and KL864 were donated by K. B. Low. KL838 is F⁻ *hisF818 leu-3 lacZ498 str-143*. KL839, KL862, and KL864 are identical to KL838 except that they carry *mutS3*, *mutH34*, and *mutU4*, respectively. These were converted to derivatives carrying a *lac proB* deletion by crossing with strain CSH63 (16), selecting for Leu⁺ Val^r colonies, and scoring for Pro⁻, as described previously (5). The F′ *lac proB* episome from strain GM1 (5, 18), which carries the *lacI* gene, was transferred to each derivative. These strains were named PM1, PM2, PM3, and PM4 for the respective derivatives of KL838, KL839, KL864, and KL862. All other strains used in the analysis have been described previously (5, 6).

Characterization of mutations. All manipulations with the *lacI* system have been described in detail (5, 6). All mutations shown in Tables 1 and 2 are of independent origin. Columns *c* of these tables represent a collection in which several nonsense mutations from each culture were collected and recurrences of the same mutation were discarded. This

Continued on following page

TABLE 2.—*Continued*

Transversion type and site	No. of occurrences for strain carrying[a]:								
	mutS3			*mutU4*			*mutH34*		
	a	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*
A · T → C · G									
Amber									
A3	0	0	0	0	0	0	0	0	0
A4	0	0	0	0	0	0	0	0	0
A14	0	0	1	0	0	0	0	0	0
A22	2	1	1	0	0	0	0	0	0
A30	0	0	0	0	0	0	0	0	0

[a] For identification of columns *a*, *b*, and *c*, see Table 1, footnote *a*.

can reduce the magnitude of some of the biggest hot spots, but the distortion is small unless a hot spot represents a very large fraction of the mutations, which was not the case. Corrections can be made for these effects, but they were not necessary here. A comparison of columns *c* and *b* revealed whether there were any significant distortions, since column *b* was tabulated by taking only the first amber (or first ochre) mutation from each culture. The profiles were similar for each column, in particular for the ochre mutations, which were more prevalent.

RESULTS

Nonsense mutations in *lacI*. Strains PM2, PM3, and PM4, carrying an F′ *lac proB* episome and the alleles *mutS3*, *mutU4*, and *mutH34*, respectively (see Materials and Methods), were grown overnight in LB medium and plated on Pgal minimal plates to select for *lacI*$^-$ colonies (18). These were found at 10- to 20-fold-higher levels than were the wild-type controls, strains PM1 and GM1, at approximately 4×10^{-5} in the population. Approximately 15% of the *lacI* mutations detected in the repair-deficient background were nonsense mutations, compared with 1 to 2% of the nonsense mutations in the wild-type controls. Therefore, the overall increase in nonsense mutations, which result from base substitutions, was 100- to 200-fold. This increase was even higher at specific sites (see below). Most of the nonsense mutations detected in the mutator strains were ochre mutations. We analyzed 1,184 independently occurring mutations. The distribution of the *lacI* nonsense mutations is shown in Tables 1 and 2. (UGA sites U4, U6, U9, and U10 do not appear in the tables because the mutants carrying these alleles were not detected in the experiments.) The transition events are recorded in Table 1, and the transversion events from the same experiments are represented in Table 2. There are three column designations in each of these tables. Because columns *a* tabulate the first nonsense mutation collected from each culture without regard to type (UAG, UAA, or UGA), one can compare the UAA (ochre), UAG (amber), and UGA values against one another in columns *a* of Tables 1 and 2. To obtain a large number of independent mutations, we collected from each strain one amber mutation from the amber group, one ochre mutation from the ochre group, and one UGA mutation from the UGA group. These numbers are shown in column *b*. The ochre mutation values can be directly compared with one another in this column, and the amber values can be directly compared with one another, but the ochre and amber values cannot be directly compared with one another as they can be in column *a*. To increase the size of the nonsense collection even further, several amber, ochre, and UGA mutations

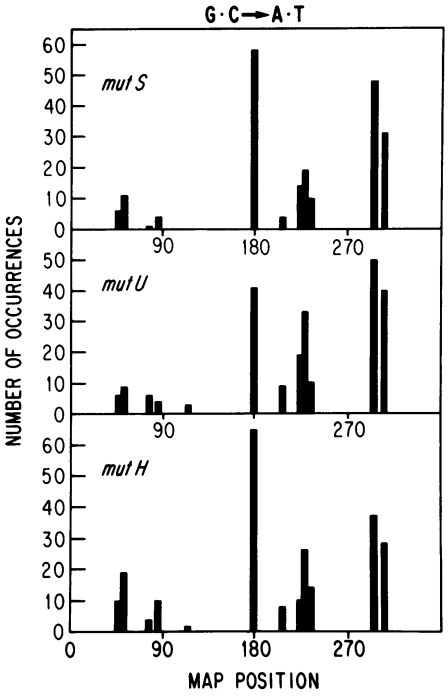

FIG. 2. The distribution of G · C → A · T transitions that yield UAA mutations in each of three mutator strains. The peak heights correspond to the number of occurrences of independent mutations. These data are taken from columns *c* of Table 1. The numbers on the horizontal axis represent the map positions in terms of the corresponding amino acid residue in the *lac* repressor.

from each culture were saved and characterized for presentation in column *c*. Recurrences of any mutation within a culture were not retained for the column *c* data. Therefore, all mutations retained in this collection were of independent origin. Although the resulting variety of mutations might dampen somewhat the magnitude of the largest hot spots, considerations mentioned in the Materials and Methods section argue that this is of little consequence. As in column *b*, the entries in column *c* can be directly compared only within the same grouping (e.g., ochre values can be compared only with other ochre values).

Specificity of base substitutions. Base substitutions occurring in the mismatch-repair-deficient strains were primarily transitions (Tables 1 and 2). Of the mutations examined, totaling column *c* entries, we found that only 39 of 1,184 (3.3%) of the mutations were transversions. Moreover, these were scattered over many sites, with no one transversion site being particularly well induced. Similarly low percentages were obtained by totaling column *a* or *b* for each of the three mutator strains.

Distribution of transition mutations. We compared the distribution of transition mutations at the 28 sites used here to score the G · C → A · T substitution. Ochre mutations were the most prevalent among the nonsense sites, which was particularly evident for the strains carrying the *mutU4* and *mutH34* alleles in these experiments (Table 1). Therefore, comparing the ochre mutation distributions in the three strain backgrounds was more informative than comparing other mutation distributions, since the large number of ochre mutations characterized yielded the most statistically significant data. The ochre mutations from column *c* of Table 1 are shown in Fig. 2. Between 200 and 230 independently occur-

TABLE 3. Characteristics of surrounding sequences of detected mutations

Site	Surrounding sequence[a]	No. of A · T/8 base pairs	No. of A · T/10 base pairs
O9	5'T G G C A C A A C A A3'	4	6
O10	5'C A C A A C A A C T G3'	5	6
O11	5'C G T C G C A A A T T3'	5	6
O13	5'C C G A T C A A C T G3'	5	5
O17	5'T C G C G C A A C G C3'	2	3
O21	5'A C C A G C A A A T C3'	5	6
O24	5'G C A A T C A A A T T3'	7	8
O27	5'G T T T T C A A C A A3'	7	8
O28	5'T T C A A C A A A C C3'	6	7
O29	5'C C A T G C A A A T G3'	6	6
O34	5'G G G G C C A A A C C3'	3	3
O35	5'T G C T G C A A C T C3'	4	5
A5	5'C T T A T C A G A C C3'	6	6
A6	5'T G A A C C A G G C C3'	3	4
A9	5'G C A A A C A G T C G3'	5	5
A15	5'A T G A C C A G G A T3'	4	6
A16	5'C T G A C C A G A C A3'	4	5
A19	5'G T C A C C A G C A A3'	4	5
A21	5'T A T G C C A G C C A3'	3	5
A23	5'A A A T T C A G C C G3'	5	6
A24	5'C A C T C C A G T C G3'	4	4
A26	5'A C G A T C A G A T G3'	4	5
A31	5'T C A A A C A G G A T3'	5	7
A33	5'T C T C T C A G G G C3'	3	4
A34	5'G G G C C C A G G C G3'	1	1
A35	5'G C A A T C A G C T G3'	4	5
U1	5'T A G A A C G A A G C3'	5	6
U5	5'T T A T G C C A G C C3'	4	5

[a] Hyphens are omitted for clarity. The sequence on only one DNA strand is shown.

ring mutations from each strain are shown. Certain mutations recurred often in these spectra and represent hot spots for the mutators, whereas other sites were poorly represented. The most striking aspect of Fig. 2, however, is that the spectra for the three strains are virtually superimposable. In other words, the patterns of hot spots and cold spots were the same for the set of transition sites that result in ochre mutations via a G · C → A · T transition. The amber mutations were so poorly induced in at least two of the strain backgrounds in these experiments that similar comparisons are difficult to make. Of the two UGA sites examined, U5 was very well induced in all three strains, but U1 was not.

DISCUSSION

We examined the base substitution spectra of mismatch-repair-deficient strains by using the *lacI* system of *E. coli* to monitor nonsense mutations. Our object was to use the detailed "fingerprint" of relative frequencies at many sites to compare different mutator strains. From the analysis of almost 1,200 independently occurring mutations, it was clear that transitions were the predominant event, since approximately 97% of the mutations were G · C → A · T transitions. This finding is in agreement with genetic studies that show a strong preference for transitions among base substitution mutations in mismatch-repair-deficient strains of *E. coli* (4, 8, 14) and *Pneumococcus* sp. (for a review, see reference 10) and with transformation experiments in *E. coli* (9, 12, 23) that show preferential repair of transition mismatches by the mismatch repair system. The *lacI* nonsense system does not score A · T → G · C transitions, although it is clear that these mutations are also well stimulated in mismatch-repair-deficient strains (4, 8, 11). The results shown here do not contradict studies which show that transversions are also stimulated (4) but simply confirm that the absolute level of transitions was much higher.

Although there are some suggestions that certain mutation rates vary in different mismatch-repair-deficient strains (see for instance the amber mutation data in Table 1 and reference 15), examination of the most statistically significant data (the ochre transition sites) shows that the profiles of hot spots and cold spots for the ochre transition sites were identical for three mutator strains, carrying *mutU*, *mutH*, and *mutS* alleles, that have been implicated in mismatch repair. This profile is not inconsistent with that reported for *lacI* ochres in a *dam* background (11). These results support the idea that the products of the three respective genes are involved in the same mismatch repair system and that the elimination of one component eliminates the entire system. The mutations detected in a strain lacking the mismatch repair system represent the replication errors that have slipped through the editing function and which are normally corrected by mismatch repair. The pattern of hot spots and cold spots defines the topography of the gene (2). The topography is a function of the state of repair in the cell,

TABLE 4. Effect of nearest neighbor

5' Nearest neighbor and site	No. of occurrences[a]	
	a	*b*
G		
O11	4	11
O17	1	4
O21	71	164
O29	15	34
O35	45	95
U5	43	0
A		
O9	9	22
O10	16	39
O28	32	77
A9	21	0
A31	3	0
U1	3	0
T		
O13	7	18
O24	5	21
O27	15	43
A5	14	0
A23	4	0
A33	4	0
A26	7	0
A35	6	0
C		
O34	53	135
A6	8	0
A15	3	0
A16	8	0
A19	5	0
A21	12	0
A24	4	0
A34	6	0

[a] No. of occurrences, the sum for each of the three mutator strains. Column *a* here represents the sum of columns *a* in Table 1; column *b* represents the sum of columns *c* in Table 1.

since it is dramatically different in mismatch-repair-deficient strains from the topography of a wild-type strain (6). This finding was supported by the virtual absence from the forward mutation spectra of transversions and of hot spots at 5-methylcytosines (Tables 1 and 2), both of which are characteristics of the wild-type spectrum (6, 7; Leong and Miller, unpublished data). Can we correlate mutation rates with aspects of the surrounding DNA sequence which might influence polymerase error rates? The DNA sequences surrounding each of the transition sites examined in this study are given in Table 3, which also indicates the degree of A · T richness for surrounding sequences of 8 and 10 base pairs. Some of the values derived from Table 1 are compared in Table 4 with the 5′ nearest neighbor of the C residue undergoing transition. (Because of the nature of the genetic code, the 3′ base is always A for the amber and ochre codons, so only the 5′ base varies in these cases.) There is no clear correlation between the nearest neighbors and the relative mutation rates. The values in Table 4 represent the sum of mutations in all three mutator strains, but the same result can be reached by considering individually any of the sets of data in Table 1. Also, a comparison of the relative mutation rates with the A · T richness (Table 3) does not reveal any obvious correlation. Therefore, further work is needed to define the aspects of the DNA sequence which are responsible for the large variance in mutation rates in mismatch-repair-deficient strains. It would be of great interest to analyze the *lacI* mutational spectrum in a *mutD*-carrying strain that lacks the editing function as well as in a double mutant that lacks both the editing function and the mismatch repair system (for instance, a *mutD mutH* strain). Such experiments are in progress.

ACKNOWLEDGMENTS

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