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Spectra of spontaneous mutations in *Escherichia coli* strains defective in mismatch correction: The nature of *in vivo* DNA replication errors

(*mutH, mutL, mutS* strains/*lacI* gene/base substitutions/frameshifts/nearest-neighbor effects)

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ABSTRACT **We have determined the DNA sequence changes in 487 spontaneous mutations in the N-terminal part of the *lacI* gene in *mutH, mutL*, and *mutS* strains of *Escherichia coli*. These strains display elevated spontaneous mutation rates because of a deficiency in the process of postreplicative mismatch correction. As a consequence the mutational spectra reveal the nature of spontaneous DNA replication errors. The spectra consist of base substitutions (75%) and single-base deletions (25%). Among the base substitutions, transitions (both A·T→G·C and G·C→A·T) are strongly favored over transversions (96% versus 4%). Large site-to-site differences are observed among identical base substitutions, presumably reflecting the modulating effects of neighboring bases. The single-base-deletion spectrum is dominated by a large hotspot at a run of adjacent identical base pairs, implying a Streisinger-slippage mechanism. The data, when compared to a previously determined wild-type spectrum, also provide information on the specificity of the mismatch repair system.**

Escherichia coli strains that carry a mutation in the *mutH, mutL*, or *mutS* genes display elevated spontaneous mutation rates because they are defective in methylation-instructed DNA mismatch correction (1–8). This system is thought to follow the replication fork, scrutinizing the newly replicated DNA for mismatches resulting from errors of DNA replication. The mismatches are then corrected using the undermethylation (at GATC sites) of the newly synthesized strand to distinguish the correct from the incorrect half of the mismatch.

Here, we have made use of the mismatch-repair-deficient strains to further investigate the mechanisms of mutation in *E. coli*. We previously reported in detail on the DNA sequence changes in a large collection of spontaneous mutants in the *lacI* gene in a wild-type strain (9). Diverse mutational classes were seen, presumably resulting from different mutational mechanisms. We now report on the DNA sequence changes in 487 mutants in *mutH, mutL*, and *mutS* strains. Because errors of DNA replication in these strains are no longer corrected, the data provide us with an intimate view on the nature of *in vivo* DNA replication errors. The data, in conjunction with the wild-type data, also allow an estimation of the efficiency of the mismatch-repair system for several mutational classes.

MATERIALS AND METHODS

Bacterial Strains. *Escherichia coli* strains NR3835 (*ara, thi, trpE9777,* Δ*prolac,* F′*prolac*), NR3939 (*ara, thi, mutH101,* Δ*prolac,* F′*prolac*), NR3940 (*ara, thi, trpE9777, mutL101,* Δ*prolac,* F′*prolac*), and NR3996 (*ara, thi, trpE9777, mutS-101,* Δ*prolac,* F′*prolac*), all derivatives of strain GM1 (10), were obtained from B. W. Glickman (York University, Toronto). The isolation and characterization of the mutator alleles has been described (2). The F′*prolac* carries the I^Q(*lacI*) and L8(*lacZ*) promoter mutations. Strains CSH51, CSH52, and S90C have been described (10, 11).

Media. Luria broth (LB) and minimal media were used as described (9). P-gal plates, used for the selection of *lacI*$^-$ mutants, are minimal media-containing plates that contain phenyl β-D-galactoside (P-gal; 75 μg/ml) instead of glucose as a carbon source.

Selection of *lacI*$^-$ Mutants. Overnight cultures of strains NR3939, NR3940, and NR3996 were diluted with fresh LB broth to a concentration of about 300 cells per ml after which 0.2-ml aliquots of the diluted cultures were distributed to a total of 400 wells per strain using 96-well microtiter dishes. After overnight growth at 37°C, *lacI*$^-$ mutants were obtained by spreading 1-μl aliquots from each well on quarter sections of P-gal plates. The average mutant frequency was determined by plating appropriate dilutions of the first 10 wells of each strain on both LB and P-gal plates. *lacI*$^-$ mutants were picked (one from each culture to ensure independence) and placed onto a grid on P-gal plates for replica mating (F′ transfer) into strains CSH51 and CSH52. In these backgrounds, i^{-d} mutations on F′*lac* can be detected by their dominance over *lacI*$^+$ residing on the chromosome (blue conjugants on X-gal minimal plates) (11, 12). F′*prolac* carrying i^{-d} mutations were then transferred by replica-mating into strain S90C for transfer of the *lacI*$^-$ mutation, by homologous recombination, to single-stranded phage mRS81 for DNA sequence analysis (9, 13).

RESULTS

Forward *lacI* Mutant Frequencies. To study the specificity of mutation in *mutH, mutL*, and *mutS* strains we used the *E. coli lacI* gene (coding for the repressor of the *lac* operon) residing on F′*lac*. Forward mutants carrying defective repressors (*lacI*$^-$ mutants) can be easily selected (10–12). Because no restraints are placed on the specific nature of the mutations (base substitutions, deletions, etc.) this system has been useful in defining the specificity of mutation (9, 14). The *lacI*$^-$ mutation frequencies that we obtained are given in Table 1. Consistent with the general mutator property of the *mut* strains, their frequencies of *lacI*$^-$ mutants were increased over the frequency in the wild-type strain. The increase (16- to 20-fold) is moderate, but it should be noted that the spectrum of *lacI*$^-$ mutants in a wild-type strain contains a large hotspot (70%) of four-base-pair frameshift

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Abbreviation: i^{-d} mutation, *lacI*$^-$ mutation that is dominant over *lacI*$^+$; P-gal plates, plates that contain phenyl-β-D-galactoside as a carbon source.

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Table 1. Mutation frequencies

Strain	Frequency × 10^6	
	lacI$^-$*	i^{-d}
NR3939 (*mutH*)	53	24
NR3940 (*mutL*)	65	27
NR3996 (*mutS*)	69	27
NR3835 (*mut*$^+$)	3.4	0.10†

*Includes O^c mutants (which express the *lac* operon constitutively due to a defect in the *lac* operator). These contributed about 3% and 10–15% to the frequency in mutator and wild-type strains, respectively.
†Estimated from ref. 9 and unpublished data.

mutations (9, 15) as well as several other classes of mutation that may not be affected by mismatch repair.

To facilitate sequencing, we concentrated on *lacI*$^-$ mutants located in the "early," N-terminal part of the gene. This region (approximately nucleotides 30–240 or amino acid residues 1–70) encodes the operator-binding domain of the repressor and is useful for mutational studies because it contains a higher density of detectable sites than the rest of the gene (12, 16). Mutations in the early part of the gene are often referred to as i^{-d} because they are dominant over *lacI*$^+$. This is a consequence of the tetrameric nature of the *lac* repressor: repressors with defective operator binding still aggregate into tetramers through a site in the C-terminal domain, but the resulting, mixed *lacI*$^+$/*lacI*$^-$ tetramers are defective (16, 17). The use of i^{-d} mutants to obtain a representative spectrum of mutations is justified by the good correlation between early and dominant mutations [almost all early mutations are i^{-d}, and virtually all i^{-d} mutations map early (12, 17)]. Our results (this paper and unpublished results) indicate that mutations are obtained throughout the early region and include base substitutions, frameshifts, deletions, and duplications.

The frequencies of the i^{-d} mutations (as determined by the test for dominance) are also given in Table 1. A relatively high percentage of the mutants in the *mutH*, *mutL*, or *mutS* strains belongs to this category (≈45%). Because in wild-type strains a much smaller percentage is i^{-d} (ref. 9 and unpublished data), the mutator effect is now increased to 240- to 270-fold, values in good agreement with the range of effects generally observed (1, 2, 8).

DNA Sequence Analysis. A total of 487 independent i^{-d} mutants were obtained: 177 for *mutH*, 157 for *mutL*, and 153 for *mutS*. The mutant genes were transferred to the single-stranded phage vector mRS81 by recombination and sequenced. The results are presented in Table 2. The mutations were of only two kinds: base substitutions and frameshifts. This is true for each of the three strains, although the ratio of base substitutions to frameshifts varies somewhat from strain to strain. A detailed description of all mutations is given in Tables 3, 4, and 5.

Base Substitutions. The base substitutions produced by *mutH*, *mutL*, and *mutS* strains (Table 3) are, in the large majority, transitions. This is most clearly seen when they are summarized by class, as is done in Table 4. Transitions outweigh transversions about 96% to 4%. Interestingly, among the transitions, A·T→G·C are more frequent than G·C→A·T. These results apply equally to *mutH*, *mutL*, and *mutS* strains. When comparing individual sites (Table 3), this similarity generally holds, although some sites (for instance, positions 72 and 117) do seem different.

Table 2. Distribution of 487 sequenced i^{-d} mutations

	mutH	*mutL*	*mutS*	Total
Base substitutions	130	132	103	365
Frameshifts	47	25	50	122
Total	177	157	153	487

Table 3. Base substitutions

Nucleotide	Type	Occurrences		
		mutH	*mutL*	*mutS*
41	A·T→G·C	1	1	6
42	G·C→A·T	2	1	0
45	A·T→C·G	1	0	0
53	G·C→T·A	0	1	0
54	A·T→G·C	5	13	8
56	G·C→A·T	0	5	3
57	G·C→A·T	1	0	0
66	G·C→T·A	0	0	1
72	A·T→G·C	17	16	1
74	A·T→G·C	0	3	0
75	G·C→A·T	0	0	1
80	G·C→A·T	0	3	2
81	A·T→G·C	4	0	4
83	A·T→G·C	27	19	21
84	G·C→A·T	3	1	2
89	A·T→G·C	3	2	1
90	G·C→A·T	3	1	1
92	G·C→T·A	0	0	1
92	G·C→A·T	1	3	1
93	G·C→T·A	2	0	1
93	G·C→A·T	2	0	5
95	G·C→A·T	2	7	1
96	A·T→C·G	0	0	2
101	A·T→G·C	1	0	0
104	G·C→A·T	1	3	1
105	A·T→C·G	1	0	0
117	A·T→G·C	6	14	0
119	A·T→G·C	0	2	2
140	G·C→A·T	5	1	0
141	A·T→G·C	7	6	7
162	A·T→G·C	1	1	0
168	A·T→G·C	13	11	5
174	G·C→A·T	1	0	0
178	G·C→C·G	0	0	1
178	G·C→T·A	0	0	1
185	G·C→A·T	7	7	12
186	G·C→A·T	5	4	1
186	G·C→T·A	0	0	1
191	G·C→A·T	3	1	2
195	A·T→G·C	0	1	1
197	G·C→C·G	0	0	1
198	G·C→A·T	1	2	0
201	G·C→A·T	3	2	6
201	G·C→T·A	1	0	0
203	A·T→G·C	0	1	0
	Total	130	132	103

Frameshifts. All frameshift mutations represented the loss of a single base. Table 5 shows that the abundance of frameshift mutations is to a very large extent due to a single hotspot, nucleotides 135–139, a run of five adjacent A·T base pairs (see also Fig. 2). A minor hotspot is observed at

Table 4. Specificity of base substitutions

Substitution	*mutH*	*mutL*	*mutS*	Total
G·C→A·T	40	41	38	119
A·T→G·C	85	90	56	231
G·C→T·A	3	1	5	9
A·T→T·A	0	0	0	0
G·C→C·G	0	0	2	2
A·T→C·G	2	0	2	4
Total	130	132	103	365

Table 5. Frameshift mutations

Nucleotide(s)	Mutation	*mutH*	*mutL*	*mutS*
76–77	−T	2	1	1
79	−T	1	1	0
87–89	−T	1	2	0
90–92	−C	0	0	1
108–109	−C	1	0	0
115	−C	0	0	1
116	−G	0	0	1
125–128	−A	1	3	4
131	−C	0	0	1
132–134	−G	1	1	1
135–139	−A	36	15	33
154–155	−G	1	0	0
157–158	−G	0	0	1
171–172	−T	0	0	1
173–175	−C	1	0	0
176–177	−A	0	0	1
184–185	−G	0	0	1
189–190	−A	1	0	0
199–201	−G	1	1	2
207	−A	0	0	1
225–226	−T	0	1	0
	Total	47	25	50

nucleotides 125–128, a run of four adjacent A·T base pairs. No +1 frameshifts were observed in the spectra. This does not imply that they do not occur in the mutator strains. Rather, +1 frameshifts, in contrast to −1 frameshifts, do not yield a dominant phenotype (18).

DISCUSSION

In this paper we present the spectra of spontaneous mutations as they occur in *mutH*, *mutL*, and *mutS* strains of *E. coli*. The spectra in the three strains were remarkably similar, although some differences were observed as well; the latter were mostly quantitative in nature. The *mutS* spectrum may serve as an example. This spectrum shows a somewhat reduced ratio of base substitutions to frameshifts (Table 2), a relatively higher number of transversions, and a less pronounced excess of A·T→G·C over G·C→A·T mutations (Table 4). Several of these differences may be of only marginal statistical significance. However, one observation seems significant: the reduced occurrence of the A·T→G·C transitions at nucleotides 72 and 117 (Table 3). We have currently no direct explanation for the particular behavior of these sites. It may be that the *mutS* allele used in our study is somewhat leaky and might still be partially effective at these sites. The *mutS* gene product has been shown to bind directly to the mismatches, with a different affinity for different mismatches (19).

The similarities between the three strains as observed in this study are extensive and include: (*i*) the frequencies with which the mutations occurred, (*ii*) the composition of the spectrum: base substitutions and frameshifts, with a preference for the former, (*iii*) the strong preference of transitions over transversions, and (*iv*) the preference of A·T→G·C over G·C→A·T. The similarities extend further to many of the individual sites within the base substitutions and frameshifts (Tables 3 and 5). The similarity is consistent with the view that the products of the *mutH*, *mutL*, and *mutS* genes work concertedly or in complex (3, 7) and that abolishing one of the subactivities leads to a virtually complete loss of the repair capacity.

A spectrum of *lacI* nonsense mutations in strains defective in mismatch correction has already been described (20). The use of nonsense mutations has as a limitation that many kinds of mutations, including A·T→G·C transitions and frameshift mutations, which comprise the two most frequent events in the present study, cannot be observed. Nevertheless, for several findings the studies are in good agreement. For example, an equally moderate mutator effect was observed for the overall *lacI*$^-$ mutants (20-fold), which was raised substantially for the subgroup of the nonsense mutations (100- to 200-fold). Second, spectra of nonsense mutations obtained in *mutH*, *mutS*, and *mutU* strains were very similar amongst each other. Third, nonsense mutations generated by G·C→A·T transitions strongly outnumbered those produced by transversions.

In the following we will analyze the *mutH*, *mutL*, and *mutS* data in the light of (*i*) the nature of DNA replication errors in *E. coli* and (*ii*) the specificity of the mismatch correction system. Because the three spectra were very similar, we will use their combined data, as represented in Figs. 1 and 2.

DNA Replication Errors. In this section we make the assumption that the spectrum of spontaneous mutations in the *mutH*, *mutL*, and *mutS* strains may be considered as the spectrum of *in vivo* DNA replication errors. This seems reasonable based on our present knowledge of mutagenesis in *E. coli*, although the possibility that DNA replication errors are subject to another, as yet undiscovered, postreplicative correction process cannot be entirely excluded. We conclude then that uncorrected DNA replication errors are almost exclusively base substitutions and single-base frameshifts. The ratio of these two kinds of errors, as observed for the present target, is ≈3:1. Because about 70% of the frameshifts occurred at a single hotspot site, this ratio may represent an overestimation of the general contribution of frameshifts.

The base substitutions are not distributed randomly but exhibit a distinct pattern of hot and cold spots (Fig. 1). This is true for both A·T→G·C and G·C→A·T changes. The frequency differences between individual sites amount to 10- to 100-fold. Site-to-site differences for mutational markers have long been known. The present data demonstrate that such differences may already originate in the process of DNA replication, presumably through the effects of the neighboring sequences. DNA replication errors are the combined result of misinsertion by the DNA polymerase and editing by the proofreading function. The two steps are likely to have their own dependence on the local DNA sequence, and the resulting pattern may be complex. An analysis limited to the immediately adjacent 5′ and 3′ bases revealed no striking correlations for the G·C→A·T transitions. For the A·T→G·C transitions, however, we noted a definite sequence preference. Normalized to template-T residues, A·T→G·C changes seem to prefer a 5′-adjacent G: at 5′-GT-3′ sequences an average of 29 occurrences was seen (positions 41, 54, 72, 83, 117, 141, and 168) compared to only 3.8 for 5′-TT-3′ (88, 101, 119, 167, and 203) and 2.4 for 5′-CT-3′ (74, 81, 162, and 195); no 5′-AT-3′ sites were seen. No particular preference is apparent at the 3′-side. An analysis of the frequencies in terms of the G/C content of the surrounding area (several bases on either side), which proved useful for the case of the misincorporation of 2-aminopurine in place of adenine (21), did not yield any striking correlations (results not shown). It is likely that an understanding of the DNA-sequence dependence of replication errors will require a fuller knowledge of the nearest-neighbor-interaction (i.e., stacking) energies between adjacent base pairs. Although these energies have been determined for DNA doublets containing normal base pairs (22–24), those for doublets containing incorrect pairs are not yet available. It is hoped that the present data will be useful for future analyses of this kind.

The abundance of frameshift mutations in the spectrum (25% of the total) is in large measure due to a single hotspot site where about 70% of the mutations are found (see Fig. 2). The mechanism responsible for these mutations can be easily envisioned when viewing the DNA sequence at the site: a run

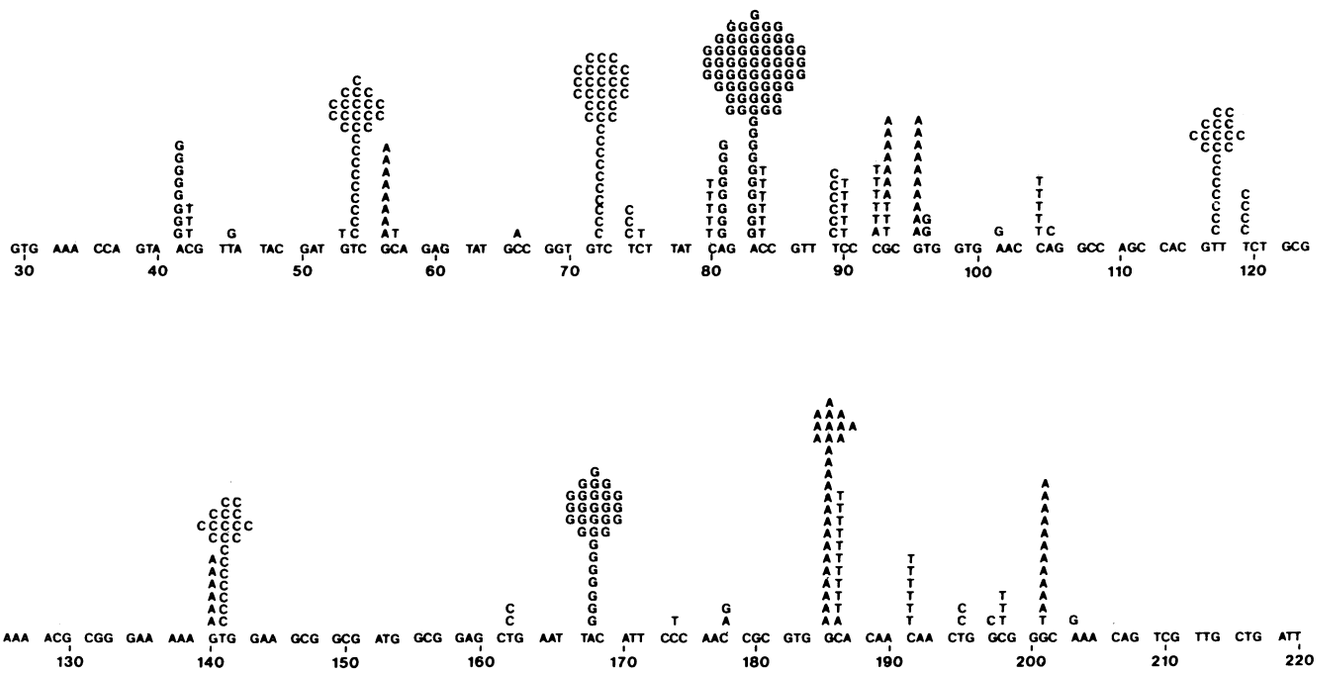

FIG. 1. Spectrum of 365 dominant spontaneous base substitutions produced by *E. coli mutH, mutL*, and *mutS* strains in the N-terminal part of the *lacI* gene. The figure contains the combined data from Table 3.

of five consecutive identical A·T base pairs. Slippage by DNA polymerase at repeated sequences during replication was early proposed as a mechanism for frameshift mutation in bacteriophage T4 (25, 26). Recent measurements with DNA polymerases *in vitro* have confirmed this propensity to produce frameshifts (predominantly −1 frameshifts) at repeated bases (27). From the present data it would appear that polymerase III holoenzyme *in vivo*, despite its complex structure and general accuracy, has remained highly prone to such slippage events (at least in comparison to base substitution errors). The general tendency of frameshift mutations to occur at repeated sequences is evident when viewing the entire collection of frameshift mutations (Fig. 2): 116 of the 122 occurred at a site of at least two adjacent identical bases.

The Specificity of Mismatch Correction. A comparison of the mutational spectra in strains deficient and proficient in mismatch repair provides a basis to deduce the specificity of the mismatch repair process itself. However, because spontaneous mutations in a wild-type strain may have resulted from sources other than DNA replication, only an apparent (or minimum) estimate for the correction efficiency can be obtained. Available data in the literature already show that mismatch correction acts effectively against transitions and frameshifts but less so against transversions. This is based on

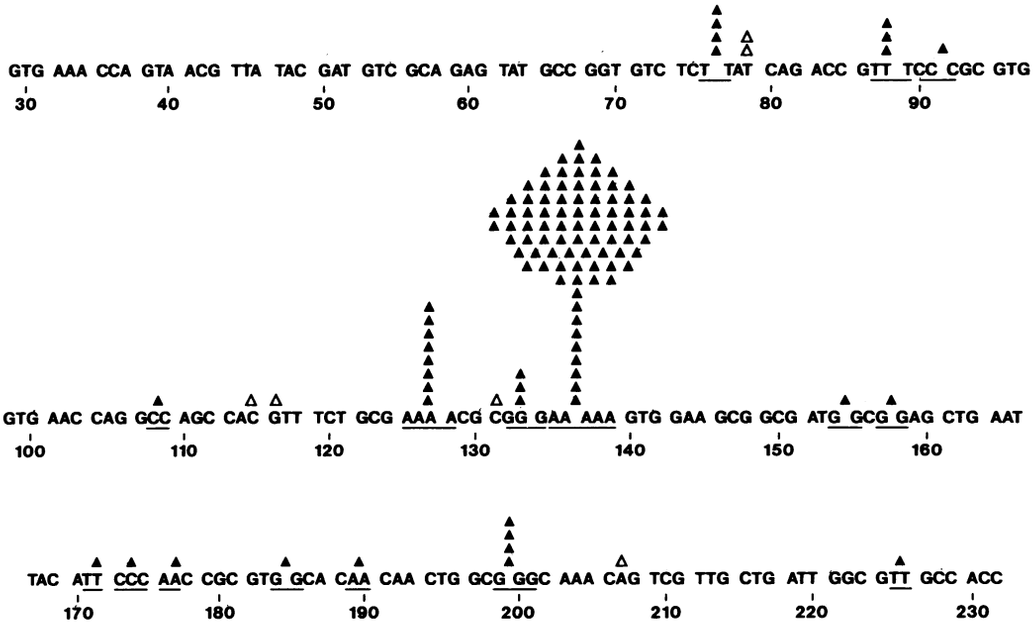

FIG. 2. Spectrum of 122 dominant frameshift mutations produced by *E. coli mutH, mutL*, and *mutS* strains in the N-terminal part of the *lacI* gene. The figure contains the combined data from Table 5. Closed triangles, frameshift mutations occurring at a site of at least two adjacent identical bases; open triangles, frameshift mutations at nonrepeated bases.

both mutational data (8, 28–30) and more direct measurements of the correction efficiency by transfection of heteroduplex phage molecules (31–33). In general, both approaches have shown the existence of substantial site-to-site variations, making it difficult to draw generalized conclusions. A spectrum of *lacI* mutations in a wild-type strain, parallel to the one presented in this paper, may allow the accurate estimation of the correction efficiency for the various mutational classes as well for a large number of individual sites within the classes. Using the data from a previous study employing the entire *lacI* gene (9, 34), some conclusions can already be drawn.

For base substitutions the overall correction factor is estimated to be ≈290-fold (i.e., $20 \times 10^{-6}/7 \times 10^{-8}$, the ratio of i^{-d} base-substitution frequencies in mutator and wild-type strains). However, this factor does not apply equally to all base substitutions. In the *mutH*, *mutL*, and *mutS* spectra transitions strongly outnumber transversions, whereas in the wild-type strain about equal numbers of the two were observed (9, 34). Consequently, the correction factors are widely different, 500- to 600-fold for the transitions and 30- to 40-fold for the transversions. These differential values are consistent with data obtained with the reversion of specific *trpA* alleles (8). Measurements of mismatch correction using heteroduplex bacteriophage molecules have also shown preferential correction of transition over transversion mismatches (31, 32).

Single-base frameshift mutations appear well corrected by the mismatch repair system because they are frequent in the mutator spectrum and rare in the wild-type spectrum. Although their low frequency in the wild-type spectrum currently precludes a reliable quantitative estimate, it appears that they are corrected at least as efficiently as are base substitutions. The frameshift hotspot at positions 135–139 (Fig. 2) might serve as an indicator for this phenomenon. This mutation was not observed in a wild-type spectrum among 176 total mutants (9), indicating a correction factor of 400-fold or greater. Efficient correction of frameshift mutations is consistent with earlier mutational data based on reversion studies (29) and direct measurements of mismatch correction using λ heteroduplexes (33).

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1. Cox, E. C. (1976) *Annu. Rev. Genet.* **10,** 135–156.
2. Glickman, B. W. & Radman, M. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 1063–1067.
3. Lu, A.-L., Clark, S. & Modrich, P. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 4639–4643.
4. Pukkila, P. J., Peterson, J., Herman, G., Modrich, P. & Meselson, M. (1983) *Genetics* **104,** 571–582.
5. Claverys, J.-P. & Lacks, S. A. (1986) *Microbiol. Rev.* **50,** 133–165.
6. Radman, M. & Wagner, R. (1986) *Annu. Rev. Genet.* **20,** 523–538.
7. Modrich, P. (1987) *Annu. Rev. Biochem.* **56,** 435–466.
8. Choy, H. E. & Fowler, R. G. (1985) *Mutat. Res.* **142,** 93–97.
9. Schaaper, R. M., Danforth, B. N. & Glickman, B. W. (1986) *J. Mol. Biol.* **189,** 273–284.
10. Miller, J. H., Ganem, D., Lu, P. & Schmitz, A. (1977) *J. Mol. Biol.* **109,** 275–301.
11. Miller, J. H. (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
12. Miller, J. H. & Schmeissner, U. (1979) *J. Mol. Biol.* **131,** 223–248.
13. Schaaper, R. M., Danforth, B. N. & Glickman, B. W. (1985) *Gene* **39,** 181–189.
14. Coulondre, C. & Miller, J. H. (1977) *J. Mol. Biol.* **117,** 577–606.
15. Farabaugh, P. J., Schmeissner, U., Hofer, M. & Miller, J. H. (1978) *J. Mol. Biol.* **126,** 847–863.
16. Miller, J. H. (1984) *J. Mol. Biol.* **180,** 205–212.
17. Müller-Hill, B. (1975) *Prog. Biophys. Mol. Biol.* **30,** 227–252.
18. Calos, M. P. & Miller, J. H. (1981) *J. Mol. Biol.* **153,** 39–66.
19. Su, S.-S. & Modrich, P. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 5057–5061.
20. Leong, P.-M., Hsia, H. C. & Miller, J. H. (1986) *J. Bacteriol.* **168,** 412–416.
21. Petrusca, J. & Goodman, M. F. (1985) *J. Biol. Chem.* **260,** 7533–7539.
22. Gotoh, O. & Tagashira, Y. (1981) *Biopolymers* **20,** 1033–1042.
23. Ornstein, R. L. & Fresco, J. R. (1983) *Biopolymers* **22,** 1979–2000.
24. Breslauer, K. J., Frank, R., Blöcker, H. & Marky, L. A. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 3746–3750.
25. Streisinger, G., Okada, Y., Emrich, J., Newton, J., Tsugita, A., Terzhagi, E. & Inouye, M. (1966) *Cold Spring Harbor Symp. Quant. Biol.* **31,** 77–84.
26. Streisinger, G. & (Emrich) Owen, J. (1985) *Genetics* **109,** 633–659.
27. Kunkel, T. A. (1986) *J. Biol. Chem.* **261,** 13581–13586.
28. Cox, E. C., Degnen, G. E. & Scheppe, M. L. (1972) *Genetics* **72,** 551–567.
29. Siegel, E. C. & Kamel, F. (1974) *J. Bacteriol.* **117,** 994–1001.
30. Glickman, B. W. (1979) *Mutat. Res.* **61,** 153–162.
31. Kramer, B., Kramer, W. & Fritz, H.-J. (1984) *Cell* **38,** 879–887.
32. Dohet, C., Wagner, R. & Radman, M. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 503–505.
33. Dohet, C., Wagner, R. & Radman, R. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 3395–3397.
34. Glickman, B. W., Fix, D. F., Yatagai, F., Burns, P. A. & Schaaper, R. M. (1986) in *Mechanisms of DNA Damage and Repair*, eds. Simic, M. G., Grossman, L. & Upton, A. C. (Plenum, New York), pp. 425–437.