Spontaneous Mutation in the *Escherichia coli lacI* Gene

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ABSTRACT

To gain more detailed insight into the nature and mechanisms of spontaneous mutations, we undertook a DNA sequence analysis of a large collection of spontaneous mutations in the N-terminal region of the *Escherichia coli lacI* gene. This region of circa 210 base pairs is the target for dominant *lacI* mutations (i^{-d}) and is suitable for studies of mutational specificity since it contains a relatively high density of detectable mutable sites. Among 414 independent i^{-d} mutants, 70.8% were base substitutions, 17.2% deletions, 7.7% additions and 4.3% single-base frameshifts. The base substitutions were both transitions (60%) and transversions (40%), the largest single group being G·C→A·T (47% of base substitutions). All four transversions were observed. Among the 71 deletions, a hotspot (37 mutants) was present: an 87-bp deletion presumably directed by an 8-bp repeated sequence at its endpoints. The remaining 34 deletions were distributed among 29 different mutations, either flanked (13/34) or not flanked (21/34) by repeated sequences. The 32 additions comprised 29 different events, with only two containing a direct repeat at the endpoints. The single-base frameshifts were the loss of a single base from either repeated (67%) or nonrepeated (33%) bases. A comparison with the spectrum obtained previously in strains defective in DNA mismatch correction (*mutH*, *mutL*, *mutS* strains) yielded information about the apparent efficiency of mismatch repair. The overall effect was 260-fold but varied substantially among different classes of mutations. An interesting asymmetry was uncovered for the two types of transitions, A·T→G·C and G·C→A·T being reduced by mismatch repair 1340- and 190-fold, respectively. Explanations for this asymmetry and its possible implications for the origins of spontaneous mutations are discussed.

THE mechanisms by which spontaneous mutations occur are of considerable interest. Their understanding is important for diverse subjects such as evolution, causes of disease (birth defects, cancer), and establishing a framework for more completely understanding other forms of mutagenesis. Typically, spontaneous mutations occur at very low rates: about 10^{-10} mutations per base pair per cell division in both pro- and eukaryotic organisms (DRAKE 1969). In order to achieve this low rate, cells must employ numerous mutation-avoidance strategies. These strategies include a high accuracy of DNA replication and the maintenance of the DNA in an error-free state by eliminating DNA-damaging agents or repairing DNA damage once it has occurred. Most of our knowledge on these cellular strategies has been derived from prokaryotic systems, in particular the bacterium *Escherichia coli* and its bacteriophages.

At the DNA replication step, several levels of fidelity have been uncovered. DNA polymerase III holoenzyme, the enzyme primarily responsible for the replication of the *E. coli* chromosome, has an error rate in the order of 10^{-6} to 10^{-7} per base pair (Fersht and KNILL-JONES 1981). This fidelity results from two separate steps: base selection at the insertion step and editing (or proofreading) by the 3′→5′ exonuclease associated with the DNA polymerase (LOEB and KUNKEL 1982; FERSHT and KNILL-JONES 1983). Following replication, the DNA is scrutinized by a mismatch-correction system encoded by the *mutH*, *mutL* and *mutS* genes (CLAVERYS and LACKS 1986; RADMAN and WAGNER 1986; MODRICH 1987). This system recognizes and corrects DNA mismatches that result from replication errors, distinguishing the correct from the incorrect half of the mismatch based on the undermethylation (at 5′ GATC sites) of the newly replicated strand. In addition to these generalized error prevention systems, more specialized systems aimed at a single class of mutations also operate at the DNA replication step, such as the *mutT* system, preventing A·T→·C·G transversions (SCHAAPER and DUNN 1987a; AKIYAMA *et al.* 1989) or the *mutY* system (NGIEM *et al.* 1988) repairing G·C→T·A transversions. Despite these error-avoidance systems, DNA replicational errors may occasionally escape these controls and contribute to spontaneous mutation.

Spontaneous mutations may also result from unrepaired DNA damage. DNA is subject to hydrolysis reactions (such as deamination, depurination, depyrimidination, strand breaking) which occur at physiologically significant rates (SHAPIRO 1981; SINGER and GRUNBERGER 1983). The many specific DNA repair

systems that exist for such damages evidence the threat of these reactions (LINDAHL 1982). Alkylation or other modification of the DNA by cellular components is another potential source of mutagenic damage (REBECK and SAMSON 1991). Intracellular reactions producing active oxygen species have been indicated as major sources of DNA damage (AMES and SAUL 1986) and as contributors to spontaneous mutation (SAUL and AMES 1986; STORTZ *et al.* 1987).

It is likely that spontaneous mutation consists of multiple components, each perhaps characterized by its own distinct pattern of mutations. The study of the specificity of mutation may therefore be a useful tool in dissecting these components. With this goal in mind, we have investigated the specificity of spontaneous mutation in the *E. coli lacI* gene. The *lacI* gene encodes the repressor of the *lac* operon and *lacI*$^-$ mutants (carrying a defective repressor) express the *lac* operon constitutively (MILLER 1978). These mutants can be readily selected on media containing the noninducing sugar phenyl-β-D-galactoside (P-gal) as sole carbon source. Since no constraints are placed on the specific nature of the mutations (base substitutions, deletions, etc.), the system is useful in defining the specificity of mutation.

We have developed a method to recover mutant *lacI* genes from F′*lac* (on which they originate) onto a single-stranded phage vector to facilitate DNA sequencing (SCHAAPER, DANFORTH and GLICKMAN 1985) allowing the DNA sequence analysis of large collections of mutants (*e.g.*, SCHAAPER and DUNN 1987b; SCHAAPER 1988). A previous study on the specificity of spontaneous mutation in the *lacI* gene (SCHAAPER, DANFORTH and GLICKMAN 1986) was limited by the small number of mutations that was sequenced and the presence of one large hotspot (117/174) that dominated the spectrum. In the present study, we have sequenced a much larger collection of spontaneous mutants (414) and have concentrated on mutations in the N-terminal part of the gene (nucleotides 30–242, approximately) which encodes the operator-binding domain of the repressor (MILLER 1984). This i^{-d} target does not include the hotspot site and is particularly useful since, in contrast to the remainder of the *lacI* gene, it is highly responsive to amino acid substitutions and is large enough to reveal many frameshift, deletion and duplication mutations.

MATERIALS AND METHODS

Strains: *E. coli* strain NR9102 (*ara*, *thi*, Δ*prolac*, F′*prolac*) was used for the collection of spontaneous *lacI*$^-$ mutations. This strain is identical to GM1 (COULONDRE and MILLER 1977) except that the F′*prolac*, on which the mutations arise, carries two silent base substitutions in the *lacI* gene (C→A at position 622, G→A at position 628) which were introduced by site-specific mutagenesis (R. M. SCHAAPER, unpublished data). The resulting F′ has been termed F′128-27; the new *lacI* allele, *lacI204* (consultation with B. BACHMANN, *E. coli* Genetic Stock Center, Yale University). The F′ also carries the i^Q(*lacI*) and L8 (*lacZ*) promoter mutations (MILLER 1978) to facilitate the selection of *lacI*$^-$ mutations. Strains CSH52, S90C and NR9099, used in the recovery and sequencing of the mutant *lacI* genes, have been described (SCHAAPER, DANFORTH and GLICKMAN 1985; SCHAAPER and DUNN 1987b).

Media: LB broth was as described by MILLER (1972). P-gal plates contained 1 × VB salts (VOGEL and BONNER 1956), 750 μg/ml P-gal and 1 μg/ml thiamine. Minimal-streptomycin plates plates contained 1 × VB salts, 1 μg/ml thiamine, 0.2% glucose and 200 μg/ml streptomycin. Minimal X-gal plates contained 1 × VB salts, 40 μg/ml 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal), 1 μg/ml thiamine and 0.2% glucose.

Selection of mutants: In three separate experiments, a total of 4600 independent cultures (200 μl in LB broth) of strain NR9102 were grown in wells of 96-well-microtiter dishes at 37°, shaking on a rotating platform. Each well was started from a small number of cells (10–50) to avoid the collection of preexisting mutants. After growth overnight, 20 cultures were used to determine the *lacI*$^-$ frequency by plating appropriate dilutions on minimal-glucose plates (to determine the cell titer) and Pgal plates (to determine the number of *lacI*$^-$ mutants per culture). For the remaining cultures, small aliquots (10–25 μl) were spread on quarter sections of Pgal plates. After incubation for 48–72 hr at 37°, one *lacI*$^-$ mutant was picked randomly from each quarter-plate and gridded on P-gal plates. After overnight growth, the grids were replica-mated into strains CSH52 and S90C on minimal-streptomycin plates, which, after overnight growth, were further replicated onto minimal X-gal plates. On these plates, CSH52 derivatives with an F′*lac* containing a dominant mutation produce blue colonies, whereas those with a recessive mutation remain white (SCHAAPER and DUNN 1987b). In addition to dominant *lacI* mutations (termed i^{-d}), also *lacO* mutations are scored (these express the *lac* operon constitutively because of a mutation in the *lac* operator). Since the first of the three experiments revealed a large contribution of *lacO* mutations [mostly a T→C base substitution at position +6 of *lacO* (SCHAAPER, DANFORTH and GLICKMAN 1986)], in the subsequent experiments the *lacO* mutations were removed by genetic mapping (SCHMEISSNER, GANEM and MILLER 1977). The S90C conjugants containing the i^{-d} mutations were collected and used for DNA sequencing as described below.

DNA sequencing: S90C derivatives with an F′*lac* episome containing an i^{-d} mutation were infected with single-stranded phage vector mRS81 (SCHAAPER, DANFORTH and GLICKMAN 1985) for *in vivo* recombinational transfer of their *lacI* gene as described (SCHAAPER, DANFORTH and GLICKMAN 1985, 1986). DNA of the recombinant *lacI*$^-$ phage was isolated and sequenced by the dideoxy-chain-termination method (SANGER, NICKLEN and COULSON 1977) using oligonucleotide primers as described (SCHAAPER, DANFORTH and GLICKMAN 1986). The DNA sequence was compared to that of the wild-type *lacI* gene reported by FARABAUGH (1978).

RESULTS

Spontaneous *lacI* mutants were isolated in strain NR9102. This strain is identical to strains used previously in studies with the *lacI* gene as mutational target, except that we have introduced, by site-specific mutagenesis (R. M. SCHAAPER, unpublished data), two

TABLE 1

Nature of spontaneous i^{-d} mutations

Mutational class		Number of occurrences	(%)
Base-pair substitutions			293 (70.8)
Transitions:	A·T → G·C	38	
	G·C → A·T	137	
		175	
Transversions:	G·C → T·A	23	
	G·C → C·G	12	
	A·T → C·G	48	
	A·T → T·A	35	
		118	
Single-base frameshifts			18 (4.3)
Deletions			71 (17.2)
Additions			32 (7.7)
Total			414 (100)

silent base substitutions (*lacI204*) that abolish the spontaneous frameshift hotspot. This hotspot represents the loss or gain of the 4-base sequence TGGC (or CTGG) at positions 620–632 (5′-CTGGCTGGCTGGC-3′) and normally comprises close to 70% of all the spontaneous *lacI* mutations (FARABAUGH *et al.* 1978; SCHAAPER, DANFORTH and GLICKMAN 1986). Strain NR9102 was therefore constructed to avoid the repeated collection of the hotspot mutations and facilitate the analysis of the non-hotspot mutations. In NR9102, spontaneous *lacI* mutants arise at an average frequency of 1.3×10^{-6}, compared to 3.5×10^{-6} for the original strain. This 2.8-fold difference is as expected from the loss of the hotspot.

To analyze the specificity of mutation, we focused on the subclass of the dominant (or i^{-d}) mutations, which are located in the N-terminal portion of the gene (operator-binding domain), roughly nucleotides 30–242 (FARABAUGH 1978). Dominance results from the tetrameric nature of the repressor: monomers defective in DNA binding still aggregate through their attachment site in the C terminus, but the resulting mixed multimers are inactive. The N terminus contains a high density of detectably mutable sites (at least 115 different base-pair substitution pathways have been detected in our laboratory; see also MILLER 1984) and is therefore particularly useful for the study of the specificity of mutation. The strategy of studying i^{-d} mutations has already been used in two previous studies that investigated mutagenesis in *E. coli* mutator strains (SCHAAPER and DUNN 1987b; SCHAAPER 1988).

The test for dominance (see MATERIALS AND METHODS) on 4663 independent spontaneous mutations arising in strain NR9102 showed 40% to be dominant. Of these, about one fourth were i^{-d}, the remainder operator constitutive (*lacO*) mutants (see MATERIALS AND METHODS). Thus, the frequency for spontaneous i^{-d} mutations was 1.1×10^{-7}. The i^{-d} mutations were transferred from F′*lac* to single-stranded phage mRS81 by *in vivo* recombination (SCHAAPER, DANFORTH and GLICKMAN 1985) and sequenced. A total of 414 mutant sequences were obtained, the results of which are summarized in Table 1.

Base substitutions are by far the most frequent mutation (70.8%), followed by deletions (17.2%), duplications (7.7%) and single-base frameshifts (4.3%). Among the 293 base substitutions, all six possible pathways are observed. Both transitions (purine·pyrimidine → purine·pyrimidine changes) and transversions (purine·pyrimidine → pyrimidine·purine changes) contribute significantly (about 60% and 40%, respectively). Among the transitions, G·C→A·T were decidedly more frequent than A·T→G·C. Among the transversions, all four pathways occur, with G·C→C·G transversions being the least frequent. Overall, among the transversions, A·T base pairs appear more mutable than G·C pairs. The complete spectrum of the i^{-d} base substitutions is presented in Figure 1A.

The single-base frameshift mutations (4.3%) (Table 2) consisted exclusively of single-base losses. The scoring of i^{-d} mutations does not allow the detection of most (+1) frameshift mutations (CALOS and MILLER 1981) presumably because they do not allow the translational reinitiation that produces the negatively complementing C-terminal fragment in case of the (−1) mutations. The present data therefore cannot be used to estimate the relative prevalence of (+1) and (−1) frameshift mutations. Nevertheless, in the one study using the entire *lacI* gene as a target (SCHAAPER, DANFORTH and GLICKMAN 1986), among five single-base frameshift mutations, four were a deletion and one an addition mutation. The frameshift mutations occur both at runs of identical bases (where slippage may occur) and at non-runs: 6/18 occurred at a non-repeated base, 9/18 at a 2-base sequence, 1/18 at a 3-base sequence, and 2/18 at a 5-base sequence. As pointed out for previous observations for single-base frameshift mutations (SCHAAPER, DANFORTH and GLICKMAN 1986), some of the events may be templated by sequences nearby. For example, the loss of C from CC at positions 84–85 (Figure 1) is precisely predicted by a seven-base direct-repeat sequence 30 nucleotides downstream (114–120). Likewise, the loss of T from position 183 is precisely templated by a seven-base direct-repeat sequence 17 nucleotides downstream (197–203).

The deletions and additions (size two or larger) observed in this collection are listed in Tables 3 and 4, respectively. Not all deletions and duplications in the N-terminal region of the *lacI* gene may be scored as an i^{-d} mutation. The same mechanism that permits the scoring of the (−1) but not (+1) single-base frameshifts as i^{-d} mutations (CALOS and MILLER 1981), presumably places some restriction on the detection of

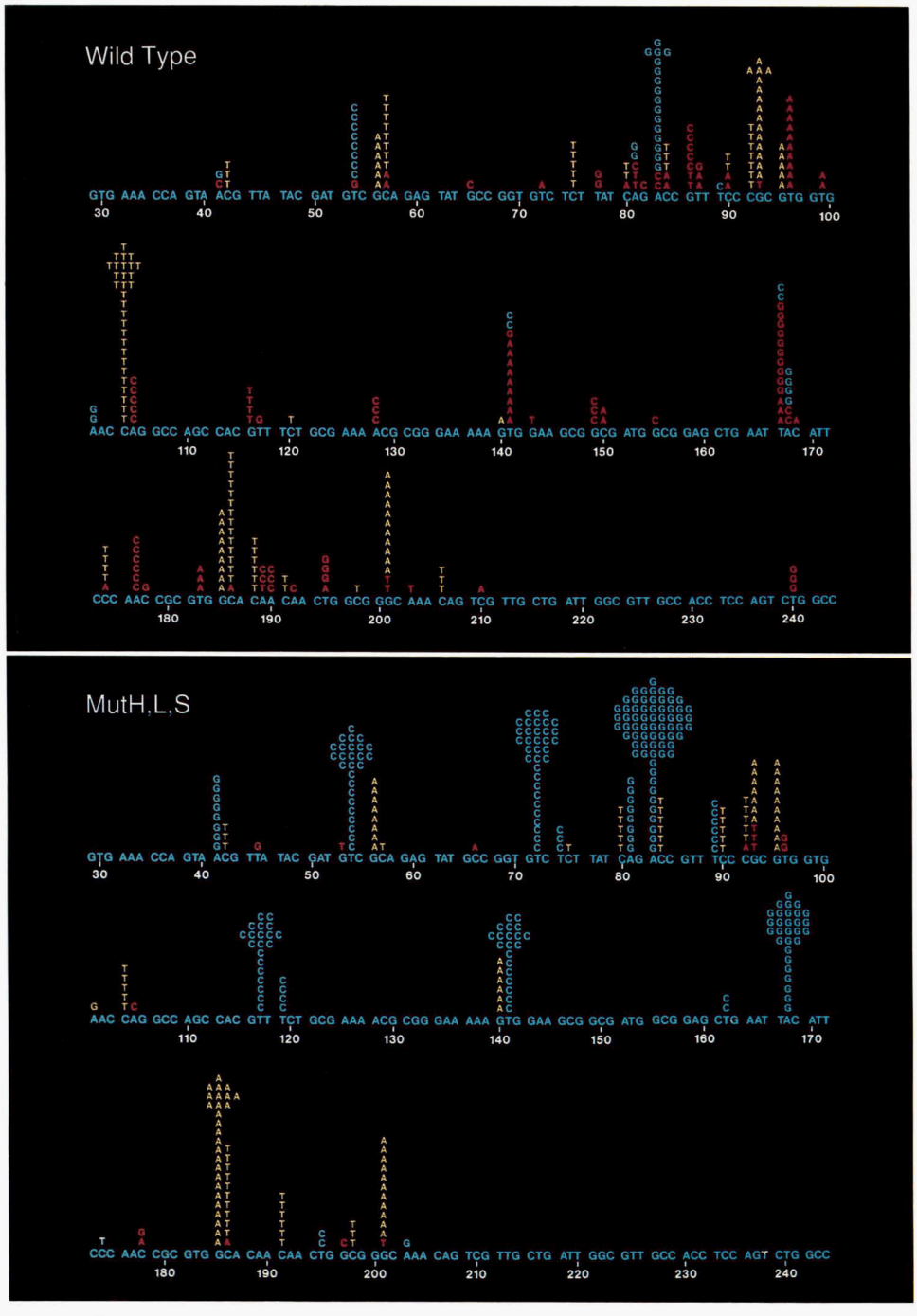

FIGURE 1.—Spectrum of i^{-d} base substitutions in NR9102 (wild type) (1A, top) and mismatch-repair deficient strains (*mutH,L,S*) (1B, bottom). The latter spectrum is a combined *mutH*, *mutL* and *mutS* spectrum taken from SCHAAPER and DUNN (1987b). Blue indicates A·T→G·C transitions, yellow G·C→A·T transitions, and red transversions.

deletions and duplications as dominant mutations. As can be seen from Tables 3 and 4, deletions were detected (with only one exception) of size $3n$ or $(3n + 1)$, but not $(3n - 1)$, and additions were detected of size $3n$ or $(3n - 1)$, but not $(3n + 1)$ [$n = 0, 1, 2, \ldots$]. Thus, deletions and additions are detected that either cause no change in the overall reading frame or produce a net (-1) shift, as in the case of the single-base frameshifts. Therefore, two-thirds of all possible deletion and addition events appear observable as i^{-d} mutations, and the present data are expected to only slightly underestimate the general contribution of deletions and additions.

Among the 71 deletions (Table 3), 29 different ones were noted, ranging in size from 3 to 544 bp, with the majority (20/29) less than 50 bp in length and a median size of 18. One particular deletion (87 bp, nucleotides 91–177) represented more than half of all deletion mutations. A direct repeat of eight base pairs (5′-CCGCGTGG-3′) adjoined its endpoints, and

TABLE 2

Single-base frameshifts

Position	Mutation	Sequence	Occurrences
46	−A	T **A** T	1
53	−G	T **G** T	1
78	−A	T **A** T	1
84–85	−C	A **CC** G	2
101–102	−A	G **AA** C	1
108–109	−C	G **CC** A	1
135–139	−A	G **AAAAA** G	2
154–155	−G	T **GG** C	1
166–167	−T	A **TT** A	1
180	−G	C **G** C	1
183	−T	G **T** G	2
189–190	−A	C **AA** C	1
192–193	−A	C **AA** C	1
199–201	−G	C **GGG** C	1
241–242	−G	T **GG** C	1
Total			18

TABLE 3

Deletions

Position	Size (bp)	Repeat[a]	Occurrences
(−354)–190	544	CAACTGGC	1
(−142)–183	325	**GGC**(^{GTC}/—)ACAAC	1
30–46	17	T	1
38–49	12	G	1
41–113	73	ACGTT	1
42–53	12	—	1
47–178	132	—	1
48–50	3	A	1
49–66	18	**CG**^{A}/$_{G}$TGTC	1
59–61	3	—	1
61–70	10	GT	1
69–75	7	—	1
70–81	12	—	1
85–114	30	CGTTTC	1
90–107	18	**CC**^{C}/$_{A}$GC	1
91–177	87	CCGCGTGG	37
101–203	103	AAC	1
102–111	10	—	1
114–117	4	—	1
122–139	18	G	1
130–196	67	**GCGGG**^{A}/$_{C}$AAA	3
136–202	67	GCGGG^{A}/$_{C}$**AAA**	1
137–142	6	^{A}/$_{G}$AAG	2
140–142	3	G	1
143–172	30	—	1
190–210	21	—	2
201–212	12	—	1
253–474	222	—	1
261–522	262	—	3
Total			71

[a] Direct repeats at deletion endpoints. In cases of imperfect repeats, the subhomology that exactly predicts the deletion (assuming a misalignment mechanism) is indicated in **bold**.

the misalignment facilitated by this repeat is most likely responsible for its occurrence (FARABAUGH *et al.* 1978). Direct repeats of three bases or longer were observed for 11 of the 29 different deletions (Table 3). Only 4 of the 29 different deletions described here have been observed in previous studies. Interestingly, the two last mutations of Table 3 (four occurrences total) were found to be located entirely outside the normal i^{-d} region. How these deletions may exert their dominant phenotype is unknown.

TABLE 4

Additions

Position	Size (bp)	Repeat	Occurrences
41–69	24	—	1
58–117	60	—	1
69–70	2	GT	1
75–103	29	C	1
75–361	287	C	1
76–117	42	TT	1
76–195	120	—	1
78–80	3	A	2
78–86[a]	768	IS*1*	1
80–85	6	C	1
89–90	2	—	3
90–205	116	C	1
92–93	2	CG	1
93–305	213	GCGTGGTG	1
113–126	14	—	1
113–138	26	—	1
118–147	30	—	1
119–120[b]	4	TC	1
121–137	17	—	1
134–138	5	—	1
135–137	3	AA	1
137–448	312	A	1
138–179	42	—	1
169–303	135	CA	1
173–214	42	C	1
187–189	3	ACAAC	1
199–200	2	G	1
207–236	30	—	1
212–213	2	—	1
Total			32

[a] Insertion of insertion element IS*1* [orientation II (SCHAAPER, DANFORTH and GLICKMAN 1986)].
[b] Triplication.

The 32 additions (Table 4) represented 29 distinct events. Only two events were represented more than once. One mutation was the insertion of the 768-bp element IS*1*. The remaining mutations all represented direct, tandem duplications of an existing *lacI* sequence. The duplications ranged in size from 2 to 312 bp, with 75% being less than 50 bp and a median size of 24–26. One mutation was a triplication of an existing 2-base sequence (119–120). As in the case of the deletions, direct repeats were observed at the endpoints of some of the mutations (Table 4), providing a possible explanation for their mechanism of origin. However, an extensive repeat was observed for only two of the duplications. This difference between deletions and duplications with regard to the frequency and extent of direct repeats is consistent with previous observations (SCHAAPER, DANFORTH and GLICKMAN

1986) and may reflect the different pathways by which the two types of mutations may arise. None of these duplications has been observed before.

DISCUSSION

The purpose of this study was to delineate in a comprehensive manner the nature of spontaneous mutations in *E. coli*. Using the approximately 210 base-pair target of the dominant *lacI* mutations (i^{-d}), it was shown that spontaneous mutation is comprised of four different classes (Table 1). The largest component was base substitutions (70.8%), followed by deletions (17.2%), additions (7.7%) and single-base frameshifts (4.3%). It is our expectation that this spectrum will serve several useful purposes, such as (i) provide insight into the nature of spontaneous mutations (ii) serve as a baseline for spectra of induced mutations and (iii) serve as a tool for the genetic dissection of the components of spontaneous mutation (see below).

A previous study using the entire *lacI* gene (1100 bp) as a target (SCHAAPER, DANFORTH and GLICKMAN 1986) was limited by the fact that the spectrum was dominated by the *lacI* frameshift hotspot (67% of all mutations) located in the middle of the gene. The frameshift hotspot (the loss or gain of a 4-bp sequence) results from a unique DNA sequence containing multiple direct and inverted repeats (FARABAUGH *et al.* 1978; SCHAAPER, DANFORTH and GLICKMAN 1986) and may not be generally representative of spontaneous mutation. Of the non-hotspot mutations, 37% were deletions, 34% base substitutions, and 29% others (frameshifts, duplications and IS*1* insertions). Thus, some differences are apparent when comparing the entire *lacI* gene to its N-terminal region. For example, the ratio of base substitutions to deletions was about 1:1 for the entire *lacI* gene, but about 4:1 for the N terminus. This observation illustrates the important point that the mutational specificity must always be viewed within the context of the size and function of the mutational target used. With respect to the *lac* repressor, the remainder of the gene (beyond the N terminus) is rather insensitive to base substitutions (particularly when using the i^q promoter which causes overproduction of *lac* repressor). For example, in a study of ultraviolet light-induced mutagenesis using the entire gene as a target (SCHAAPER, DUNN and GLICKMAN 1987), some 250 base substitutions were obtained of which 45% were located in the N-terminal region. Thus, taking into account the relative size of the two targets, the middle and C-terminal part of the repressor underrepresents base substitutions by about 5-fold. While it may be impossible to provide an absolute summation of the nature of spontaneous mutations, we believe that the data of Table 1 provide a generally representative description of spontaneous mutation for cases where amino acid changes can be readily scored. That spontaneous mutation may possess a preponderance of base substitutions has also been pointed out by SARGENTINI and SMITH (1985) on the basis of data of HARTMAN *et al.* (1971).

The spectrum presented here represents a first, but essential step in the elucidation of the origins of spontaneous mutation. To gain further insight into the pathways responsible for preventing or causing mutations, one may analyze the specificity of mutagenesis in *E. coli* mutator strains. The spectrum of mutations in a strain with a defect in a known mutation-avoidance pathway provides information on the type of mutations that are generated in such a strain but that are normally corrected, or avoided, in a wild-type strain. One may then consider the possibility that some, or perhaps all, mutations of this type in a wild-type strain result from this same pathway. An example is the spectrum of mutations in strains defective in postreplicative mismatch repair (SCHAAPER and DUNN 1987b), which revealed that primary DNA replication errors were mostly transition base substitutions. Alternatively, a mutator phenotype may result from activation of an error-prone pathway. MILLER and LOW (1984) investigated the specificity of mutation in strains constitutively induced for the SOS response and noted a spectrum dominated by G·C→T·A and A·T→T·A transversions. This might allow for the speculative conclusion that transversions of this type in a wild-type strain are likewise a consequence of SOS processing, possibly of spontaneous lesions. The approach using mutator strains, while yielding highly useful information, must be taken with care, however, and not without considering the generation of similar mutations by competing pathways.

We have previously reported a spectrum of 487 spontaneous mutations in *E. coli mutH*, *mutL* and *mutS* strains deficient in methylation-instructed mismatch repair (SCHAAPER and DUNN 1987b). Below, we describe a comparison of the current spectrum with that in the mismatch-repair defective strains. This allows a detailed analysis of the efficiency and specificity of this repair pathway, and offers some possible insights into the origins of spontaneous mutations as well.

Table 5 summarizes, side-by-side, the spectra of spontaneous mutations in wild-type and mismatch-repair deficient strains and calculates a frequency ratio for each of the classes. This ratio represents the extent to which mismatch-repair reduces the contribution of each individual mutational class. The ratio does not necessarily reflect the absolute efficiency of the *mutHLS* system for each class because mutations might also arise from sources that are not subject to mismatch correction. Thus, the ratios must be considered minimum values. Figure 1 provides the complete base-

TABLE 5

Spontaneous mutations in wild-type and *mutH, L* and *S* strains

Mutation	Number of mutants		Frequency ratio
	mut$^+$	*mutH,L,S*	
Base-pair substitutions	293	365	275
Transitions	175	350	440
A·T → G·C	38	231	1340
G·C → A·T	137	119	190
Transversions	118	15	28
Frameshifts	18	122	1500
Deletions	71	0	<3
Additions	32	0	<7
Total	414	487	260

The mutational data are from Table 1 for the wild-type strain, and from SCHAAPER and DUNN (1987b) for the mismatch-repair-deficient strains. The data for *mutH*, *mutL* and *mutS* strains are combined since the individual spectra are very similar. The frequency ratios are calculated taking into account the mutation frequencies in the two strains, which differ by a factor of 260 (1.0×10^{-7} and 2.6×10^{-5} for i^{-d} mutations in wild-type and mutator strains, respectively). The ratio for base substitutions, for example, is derived as follows: [(365/487)/(293/414)] × 260 = 275. No calculations were made for individual transversions because of their limited number in the mutator strains.

substitution spectra in the two backgrounds.

The overall effect of mismatch repair for the i^{-d} target is 260-fold. This value may have general significance, since it represents an analysis comprising 901 defined mutations at a large number of base-pair sites. The value fits in the range of previous estimates derived from mostly reversion studies (COX, DEGNEN and SCHEPPE 1972; GLICKMAN and RADMAN 1980). The correction factor for base substitutions is 275-fold, very similar to the overall value. Frameshift mutations are also efficiently corrected, 1500-fold. [This number reflects, in part, the very efficient correction of one particular frameshift mutation (-A at 135–139) which comprised 69% (84/122) of the frameshifts in the *mutH*, *mutL* and *mutS* strains (SCHAAPER and DUNN 1987b) but only 11% (2/18) in the wild-type strain (Table 2). The correction factor for the remaining frameshift mutations is 470-fold.] On the other hand, deletions and duplications seem poorly corrected and may, in fact, not be subject to this type of repair at all.

Different base substitutions are corrected with different efficiencies. For instance, transitions appear well corrected (440-fold), transversions much less so (28-fold). This difference between transitions and transversions is consistent with data from reversion systems (CHOY and FOWLER 1985) and direct measurements using heteroduplex molecules *in vivo* (KRAMER, KRAMER and FRITZ 1984; DOHET, WAGNER and RADMAN 1985) and *in vitro* (SU *et al.* 1986; LAHUE, AU and MODRICH 1989).

An interesting aspect of the data in Table 5 is the apparent asymmetry between A·T→G·C and G·C→A·T transitions. While the wild-type spectrum shows an excess of G·C→A·T transitions, the mutator spectrum shows the reverse. As a result, the apparent correction factor for A·T→G·C is much higher (1340-fold) than for G·C→A·T (190-fold). In a first analysis, this is unexpected because the replicational intermediates that lead to these two transitions are identical, a G·T or a C·A mismatch, with the only difference being the inversion of the mismatch with regard to template and primer strands. Direct measurements of mismatch correction, using DNA heteroduplex molecules, have provided no indication that G·T mismatches are corrected differently from T·G mismatches (or A·C differently from C·A) (KRAMER, KRAMER and FRITZ 1984; DOHET, WAGNER and RADMAN 1985; JONES, WAGNER and RADMAN 1987; SU *et al.* 1986). We can envision three possible explanations for the discrepancy.

First, it is possible that A·T→G·C and G·C→A·T transitions are actually created by separate mispairing pathways, *i.e.*, one transition might proceed predominantly via G·T mispairing and the other predominantly via A·C mispairing, and these two mispairs might subsequently be corrected *in vivo* with different efficiencies. It is worth noting that for several DNA polymerases examined *in vitro* (KUNKEL and ALEXANDER 1986; TINDALL and KUNKEL 1988; MENDELMAN *et al.* 1989; BEBENEK *et al.* 1990) some interesting (and opposite) directionalities have emerged for the mispairings leading to A·T→G·C or G·C→A·T transitions. For A·T→G·C transitions, T·G pairings (template base stated first) are significantly more frequent than A·C, but for G·C→A·T transitions, C·A appear more frequent than G·T. While this information is intriguing and consistent with the suggested possibility, this kind of data has not yet been obtained for *E. coli* DNA polymerase III holoenzyme or any enzyme containing a proofreading exonuclease. It should also be noted that experiments with heteroduplex DNA either *in vivo* or *in vitro* have not revealed any large differences between the correction of G·T *vs.* A·C mispairs.

Second, it is possible that in addition to the *mutHLS* system, some other postreplicative mismatch repair system might function to repress G·C→A·T transitions, particularly in the *mutHLS*$^-$ background. While this is largely speculative, one possible candidate for such a system might be the *mutY* (or *micA*) system. This system represents a methylation-independent postreplicative mismatch-repair activity that converts, unidirectionally, G·A replicational errors to G·C (RADICELLA, CLARK and FOX 1988; AU *et al.* 1988). Using bacteriophage lambda heteroduplexes RADICELLA, CLARK and FOX (1988) observed that, in addition to G·A mispairs, the *mutY* (or *micA*) system also corrected C·A mispairs (to yield C·G). Thus, to the

extent that G·C→A·T transitions are mediated by C·A mispairing, the *mutY* system might be antimutagenic for this transition. Furthermore, to the extent that A·T→G·C transitions are mediated by A·C mispairing, the *mutY* system might be mutagenic for this transition. We are currently investigating this possibility by analyzing the spectrum of mutations in a *mutLmicA* strain to see if the *micA* deficiency changes the ratio of A·T→G·C and G·C→A·T transitions in the *mutL* background.

Third, the G·C→A·T transitions observed in this study may actually be arising from sources not subject to correction by the *mutHLS* system. In order to be refractory to DNA mismatch repair, G·C→A·T transitions could have arisen, for example, during DNA repair (when DNA might be fully methylated) or as replication errors across modified or damaged bases. For example, deamination of cytosine, producing uracil, is a common DNA decay reaction (LINDAHL and NYBERG 1974; SHAPIRO 1981) and produces a G·C→A·T transition unless the uracil is removed by uracil-*N*-glycosylase prior to replication. *E. coli* strains defective in uracil-*N*-glycosylase display increased spontaneous mutation frequencies (DUNCAN and MILLER 1980). The importance of this pathway to spontaneous mutagenesis was demonstrated in a study of the *lacI* gene using nonsense mutations (COULONDRE *et al.* 1978): frequent G·C→A·T transitions were observed at CC*AGG sites, where the second (starred) cytosine is normally methylated (5-methylcytosine). In a non-methylating strain the hotspots were absent. Thus, the mutational hotspots likely resulted from the deamination of 5-methylcytosine yielding thymine, which is refractory to uracil-*N*-glycosylase repair. Interestingly, one CC*AGG site is present in the i^{-d} target (position 104, Figure 1) and, among the 137 G·C→A·T transitions, this site was the most frequent (29 occurrences, Figure 1A). Its correction factor was only 40-fold. Thus, small apparent correction factors may indeed signal a mutagenic mechanism related to DNA damage.

Possibilities other than cytosine deamination must be considered as well. Methylation of guanine (at the O^6 position) is a well known inducer of G·C→A·T transitions (LOECHLER, GREEN and ESSIGMANN 1984; BHANOT and RAY 1986) and the presence of low basal levels of methyltransferase in *E. coli* (LINDAHL 1982) may indicate that inadvertent methylations of this kind may occur (LINDAHL *et al.* 1982). Methylation of DNA by intracellular *S*-adenosylmethionine has been demonstrated and suggested as a potentially mutagenic pathway (BARROWS and MAGEE 1982; RYDBERG and LINDAHL 1982) as has alkylation via intracellular nitrosamines (TSIMIS and YAROSH 1990). A strain devoid of DNA methyltransferases was recently shown to possess a mutator activity (REBECK and SAMSON 1991).

DNA oxidation damage has also been implicated in recent years as a source of spontaneous mutation (AMES and SAUL 1986; STORTZ *et al.* 1987). Strains defective in certain defenses against oxidative stress display enhanced spontaneous mutation frequencies (FARR, D'ARI and TOUATI 1986; STORTZ *et al.* 1987; GREENBERG and DEMPLE 1988), and overexpression of some of these defenses may actually reduce them (STORTZ *et al.* 1987). The specificity of mutagenesis by oxidation damage is the subject of current interest. Reversion studies with *Salmonella typhimurium* have indicated a preponderance of mutagenesis at A·T sites, especially A·T→T·A transversions (STORTZ *et al.* 1987). However, other studies have indicated a broader specificity including significant contributions of G·C→A·T transitions (AYAKI, HIGO and YAMAMOTO 1986; MORAES *et al.* 1989; MCBRIDE, PRESTON and LOEB 1991; TKESHELASHVILI *et al.* 1991). In the *lacI* gene, mutagenesis resulting from X-rays or tritium decay, which may in part mimic oxidation mutagenesis, produced G·C→A·T transitions predominantly (ISE, KATO and GLICKMAN 1984).

In addition to studying mutagenesis in mutator strains, a second, more direct approach to investigate the sources of spontaneous mutation is to investigate the spectrum of mutations in *E. coli* antimutator strains (strains with a lower spontaneous mutation rate than the wild type). Antimutator phenotypes are presumably brought about by rendering error-avoidance pathways more efficient or error-prone pathways less efficient. If the precise mechanism of the affected pathway is known, then the mutations that are reduced or eliminated from the mutational spectrum can be directly assigned to a specific pathway in the wild-type strain. To date, only limited information is available on the pathways of *E. coli* antimutator strains (LYONS, SPEYER and SCHENDEL 1985; QUIÑONES and PIECHOCKI 1985). Current efforts in our laboratory are directed at obtaining antimutator strains specific for defined pathways so that we may approach the question of the origins of spontaneous mutation in this manner.

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