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Fundamental and Molecular Mechanisms of Mutagenesis

DNA sequence analysis of γ-radiation (anoxic)-induced and spontaneous *lacI*^{d} mutations in *Escherichia coli* K-12

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Abstract

An extensive spectrum of ionizing radiation mutagenesis was determined by sequencing 318 ^{137}Cs γ-radiation (anoxic)-induced episomal *lacI*^{d} mutations in *Escherichia coli* strain NR9102. The most commonly found radiation-induced mutations were base substitutions (44% transversions and 41% transitions). The radiation-induced spectrum consisted of: 23% G·C → A·T, 18% A·T → G·C, 17% G·C → T·A, 14% G·C → C·G, 8% A·T → T·A, 6% A·T → C·G, 8% single-base deletions, 5% multiple mutations, 3% multi-base deletions, and essentially no single- or multi-base additions. This spectrum compared better with spectra for other systems obtained by in vivo irradiation than with one obtained by in vitro irradiation. Multiple mutations, which were unique to the radiation-induced spectrum, generally consisted of one active and one closely linked silent mutation, and are suggested to result from an altered replication complex of reduced fidelity. Mutation rates were 4.1×10^{-8} *lac*-constitutive mutations/gene/Gy and 1.2×10^{-10} base substitutions/base pair/Gy. Thirty-two percent more radiation-induced mutations occurred at G·C vs. A·T base pairs. A strand asymmetry was noted for G·C → C·G and A·T → T·A transversions. A nearest-neighbor analysis showed that C (vs. A, G, or T), on either side of the mutation site, substantially enhanced most types of base substitutions. Similarly, G and C flanked both sides of single-base deletion sites twice as frequently as would be expected from the base composition of the mutation target. For comparative purposes, we sequenced 411 spontaneous *lac*-constitutive mutants of which 269 were *lacI*^{d} mutants, and there was good agreement between these and previously published mutational spectra. The spontaneous and radiation-induced mutational spectra differed substantially for virtually every class of mutation. For example, the set of spontaneous dominant *lac*-constitutive mutations contained many more mutations that did not map in the normal region for *lacI*^{d} mutations (i.e., 35% vs. 3%) and were presumed to be *lacO*-constitutive mutations. A sampling of these presumptive *lacO*^{c} mutations was also sequenced: 17/22 (spontaneous) and 1/9 (radiation) were found to be *lacO*^{c} long deletions, one from each set were base substitutions, and the remaining mutations showed the wild-type *lacO* sequence. Like the radiation-induced spectrum, the spontaneous spectrum showed enhanced mutagenesis at G·C sites, strand asymmetry, and enhanced mutagenesis when G or C were the nearest neighbors.

Key words: Gamma-radiation mutagenesis; Spontaneous mutagenesis; *LacI*^{d}; *i*$^{-d}$; Mutational spectrum

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1. Introduction

A major obstacle to understanding the molecular basis of mutagenesis by ionizing radiation, the first known mutagen (Muller, 1927), has been the huge number of different types of DNA damage produced by ionizing radiation (e.g., reviewed in Hutchinson, 1985). It follows that a valuable tool in understanding ionizing radiation mutagenesis would be knowledge of the complete mutation spectrum, which is the basis of this study.

One approach to identifying which types of ionizing radiation-induced DNA damage are mutagenic has been to irradiate naked DNA in vitro under conditions that favor certain subsets of damage, and to introduce these damaged templates into cells, thereby inducing mutations that can easily be recovered and analyzed by DNA sequencing. Such studies help in sorting out which types of DNA damage have a mutagenic potential, and ultimately can lead to plasmid studies involving pure ionizing radiation-induced damage, such as have been used in showing the importance of 8-hydroxyguanine in the induction of G · C → T · A mutations (Wood et al., 1990), and 5,6-dihydroxy-5,6-dihydrothymine in the induction of A · T → G · C mutations (Basu et al., 1989).

While these in vitro plasmid studies are able to address questions in a less complex fashion and they facilitate data gathering and analysis, it may be preferable to use in vivo (or endogenous) models for chromosomal DNA damage induction, repair and mutation induction. Since mutations on the bacterial chromosome are somewhat difficult to recover and sequence, Jeffrey H. Miller and co-workers developed the *lacI* mutation assay employing the *Escherichia coli* F'*lac* episome (e.g., reviewed in Miller, 1983; Glickman, 1990). F'*lac* shows the same supercoiled folding as the bacterial chromosome, its initiation of replication is under the same stringent control, and it exists in the same intracellular environment (Kline and Miller, 1975).

A major improvement over the original *lacI* assay involves the use of *lacI*-dominant (i.e., $lacI^{d}$ or i^{-d}) forward mutations. This assay developed by Schaaper and Dunn (1987, 1991) employs a 212-bp target (Schaaper et al., 1987) and not only detects deletions, insertions, and duplications, but can also detect at least 148 different base substitutions occurring at 85 sites in the *lacI* gene, with each of the six possible types of base substitutions having about the same chance of being scored (e.g., Zielenska et al., 1993).

Previous studies on the γ-radiation mutational spectrum, using in vivo irradiation, were not able to detect A · T → G · C transitions, deletions, additions, or multiple mutations (Glickman et al., 1980; Ise et al., 1984), or they were based on small numbers of mutations (e.g., Tindall et al., 1988; Miles and Meuth, 1989). Also, these latter studies do not appear to have attempted to control the level of O_2 present during irradiation (O_2 is consumed by cell metabolism and by radiation action), which is a concern since O_2 plays a major role in DNA damage induction and mutagenesis (e.g., von Sonntag, 1987; Sargentini and Smith, 1989).

The present study analyzes ^{137}Cs γ-radiation mutagenesis in repair-proficient logarithmic-phase cells growing in rich medium (growth conditions that maximize DNA repair capacity; Sargentini et al., 1983), and its goal is to produce an extensive and reliable radiation-induced mutational spectrum to serve as a reference point for future studies on chemical and biological factors controlling radiation mutagenesis. The cells used in this study were irradiated under N_2 to completely avoid the O_2 effect, but a future study will examine the effect of O_2 on ionizing radiation mutagenesis.

2. Materials and methods

Bacterial strains

Mutations were collected in this study in *E. coli* K-12 strain NR9102 (*lacI204* and promoter mutations i^{Q} (*lacI*) and L8 (*lacZ*)) (Schaaper and Dunn, 1991). Strains CHS52 and S90C (Schaaper and Dunn, 1987) were used to test *lacI* mutations for dominance, while NR9099 and the bacteriophage M13 derivative, mRS81 (Schaaper et al., 1985), were used for the in vivo cloning of *lacI* mutations. All strains and phage were kindly provided by Roel M. Schaaper.

Mutation terminology

To standardize terminology and avoid confusion, additional nomenclature for describing various *lac* operon mutations was developed: *lac*^{c} mutants (called i^- in some studies) constitutively express the *lac* operon (Miller, 1972); dominant *lac*^{c} mutations (called i^{-d} in some studies) are generally located either in the N-terminal (or early) part of the *lacI* gene, i.e., *lacI*^{d} (or i^{-d}) mutations, or in the *lac* operator region between the *lacI* and *lacZ* genes, i.e., *lacO*^{c} (or o^c) mutations (Schaaper and Dunn, 1987); presumptive *lacO*^{c} mutations are those shown by sequencing to not involve positions 26–249 of the *lacI* gene, and may include atypical *lacI*^{d} mutations unless involvement of the *lacO* region has been confirmed.

Media

YENB was 0.75% Bacto yeast extract (Difco) and 0.8% Bacto nutrient broth (Difco). LB was 1% Bacto tryptone (Difco), 0.5% yeast extract and 1% NaCl. GMM was a minimal medium containing Vogel-Bonner medium E (Maron and Ames, 1983), D-glucose at 0.4%, and thiamine · HCl at 0.5 μg/ml. PB was Na_2HPO_4 at 5.83 g/l and KH_2PO_4 at 3.53 g/l, pH 7.0. YENB agar was YENB solidified with Bacto agar (Difco) at 1.5%. F-top agar contained agar and NaCl, each at 0.8%. G-agar was GMM containing agar at 1.5%. S-agar was G-agar containing streptomycin sulfate at 200 μg/ml. X-agar was G-agar containing glucose at 0.2% and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal, Gold Bio-Technology) at 40 μg/ml (diluted from a 20 mg/ml stock in dimethyl sulfoxide). P-agar was G-agar with phenyl-β-D-galactoside (Sigma) at 750 μg/ml in place of glucose.

Inoculum preparation

Fifty-two GMM cultures of strain NR9102 were grown overnight from individual clones. After plating samples on P-agar to determine the frequency of spontaneous *lac*^{c} mutants (data not shown), the five samples with the lowest mutant frequency (about 40 *lac*^{c} mutants per 10^8 cells) were combined and frozen (at −70°C) in the presence of 15% glycerol. This mixture was used to start cultures for the radiation mutagenesis experiment and insure a low background of *lac*^{c} mutants.

Mutagenesis

The pooled inoculum culture was thawed, diluted 100-fold into three 400-ml volumes of warm YENB in 2-l flasks, and shaken (200 rpm) at 37°C until the cultures exhibited an OD_{650} of 0.4 (mid-log phase). The triplicate cultures were combined, centrifuged ($5000 \times g$), washed twice and resuspended in PB at an OD_{650} of 5 (i.e., 1.5×10^9 colony-forming units per ml). Cells were bubbled in a 30-ml Corex glass tube with nitrogen (99.99%) for 10 min before and during ^{137}Cs γ-irradiation with 750 Gy (75 krad) at 36.6 Gy/min as described previously (Sargentini and Smith, 1983). Afterwards, cells were plated on YENB agar to determine survival, and they were diluted 100-fold (irradiated) or 2000-fold (nonirradiated) into YENB, to yield about 1×10^6 colony-forming units (CFU) per ml. Cells diluted in YENB were dispensed under a laminar flow hood (within 15 min) into racks of 96 1.2-ml sterile polypropylene tubes (0.5 ml/tube) using a Varistaltic AL dispensing pump (Manostat) fitted with an 8-jet manifold (Wheaton). After 16 h of incubation at 37°C, 0.3 ml of 18.6% dimethyl sulfoxide (Fisher Scientific, ACS grade) was pumped into each of the cultures, and the tubes were capped and frozen at −70°C for subsequent analysis.

Mutant selection

Irradiated and control cultures were thawed at 37°C, diluted 10–200-fold in PB, and 0.2-ml volumes were spread onto P-agar, which selects for cells that are constitutively derepressed for β-galactosidase production (Miller, 1972). After incubation at 37°C for 2 days, *lac*^{c} mutants were purified by patching 26–100 colonies per culture onto P-agar, and incubating them overnight. (When selecting clones for patching, very small colonies (i.e., diameters less than 1/3 that of the most common colony size on any given plate) were avoided because they were considered to be mutants that had arisen spontaneously on the plate; these small colonies tended to increase in number if incubation was extended beyond 2 days.) The clone patches on the P-agar plate were then transferred sequentially with one velvet pad

to two S-agar plates that had been spread with 0.3 ml of fresh overnight LB culture, the first plate with CHS52 and the second plate with S90C. Following overnight incubation, the recombinant colonies derived from CHS52 were replica-transferred onto X-agar, and all plates were incubated one more night. Then, two independent recombinant colonies (A and B) derived from S90C (which corresponded to dark-blue CHS52-derived growth on the X-agar plate) were transferred to an S-agar plate for storage. Such S90C derivatives carry F'*lac* possessing a dominant *lac*^{c} mutation.

Cloning mutations

The dominant *lac*^{c} 'A' colonies described above were inoculated into YENB. Following overnight incubation, 0.025 ml of culture was added to 1 ml of LB containing phage mRS81 at 5×10^5 plaque-forming units per ml, and incubated for 4 h. After centrifugation, a sample of supernatant containing phage was plated with 0.1 ml of a saturated LB culture of strain NR9099 (F' *Δ(lacI-lacZ)*M15/*Δ(lac-pro) recA56*) in F-top agar on an X-agar plate. Glycerol was added at 15% to the remainder of each overnight YENB culture, and the mixtures were frozen for future reference. Phage mRS81 was developed by Schaaper et al. (1985) to facilitate the in vivo cloning of *lacI* mutations into a single-stranded viral genome. In this case, a single blue plaque carrying the 'A' colony dominant *lac*^{c} mutation was punched from the lawn of colorless plaques. If after two tries, no blue plaque could be found using 'A' cells, then mRS81 was plated once on 'B' cells to try to get a blue plaque. For radiation-induced mutants, the first plating of bacteriophage on 'A' cells always yielded blue plaques. For spontaneous mutants, the first plating on 'A' cells was about 50% successful; the second plating was about 25% successful; and the first and only plating on 'B' cells was about 50% successful. Phage from the first successful plating yielding a blue plaque on either A or B cells, but not both, were plated with 0.1 ml of a saturated LB culture of NR9099 cells in F-top agar on X-agar. From this second plating, a single, blue plaque was punched and stored in LB for subsequent propagation, DNA isolation and sequencing.

Sequencing mutations

Bacteriophage DNA was isolated as described in Sambrook et al. (1989) with minor modifications. Phage were propagated in LB, precipitated with polyethylene glycol, and resuspended in TE (10 mM Tris, 1 mM EDTA, pH 8). Phage DNA was extracted twice with phenol–chloroform and once with chloroform. DNA was then precipitated with sodium acetate–ethanol, washed twice with 70% ethanol and dried. Purified DNA was sequenced using Sequenase Version 2.0 (United States Biochemical), [α-^{35}S]dATP (DuPont NEN) and the Sanger dideoxy-chain termination method according to instructions provided with the DNA sequencing kit (United States Biochemical). All compressions and most frameshifts and multiple mutations were also sequenced with the dITP procedure described in the sequencing kit. Our standard primer (Genosys Biotechnologies) was TGATCGGCGCGAGATTTAA-3' (3' end at position 276 of *lacI*). Electrophoresis generally followed Sambrook et al. (1989) and employed Long Ranger acrylamide (AT Biochem), an STS-45 sequencer (International Biotechnologies), a single loading per template, 70 W constant power (with 2200 V and 100 mA maxima), and running times of about 2 h. Sequences were read from autoradiographs (Fuji RX) using a Hitachi digitizer with DNASIS software, and covered positions 26–249 in the *lacI* gene. Some DNA samples, for which no mutation was found using the *lacI* primer, were sequenced using a *lacZ* primer, TTCCCAGTCACGACGTTGTA-3' (3' end at position 35 of *lacZ*), and analysis covered from position 1010 in the *lacI* gene through the *lac* operator (*lacO*) region to position 17 in the *lacZ* gene. Mutated sequences were compared with the wild-type sequence described jointly by Farabaugh (1978), Reznikoff and Abelson (1978), and Kalnins et al. (1983).

3. Results

Irradiation (750 Gy) of strain NR9102 yielded 6% survival and increased the mutant frequency

63-fold over the spontaneous level (i.e., 9400 vs. 150 *lac*^{c} mutants/10^8 cells). Assuming that the starter culture mutant frequency did not change much over the 3–4 cell doublings involved in the preparation of log-phase cells prior to irradiation, one can calculate that the 0.5-ml tube cultures of nonirradiated cells contained, on average, 0.15 preexisting spontaneous *lac*^{c} mutants, while tubes of irradiated cells contained 0.009 preexisting spontaneous mutants.

The N-terminal part of the *lacI* gene, where most *lacI*^{d} mutations occur, has been touted as a very sensitive, informational and economical mutation assay target (Schaaper and Dunn, 1987, 1991). To employ this assay, one selects *lac*^{c} mutants, and then sorts out the *lacI*^{d} mutations (see Methods), which are more valuable for sequencing and analysis. In this study, 16% (nonirradiated) to 31% (irradiated) of the 26–51 *lac*^{c} mutant colonies tested from each culture showed a dominant phenotype. The mutation from only one mutant per culture was transferred into phage mRS81, thereby producing blue plaques, in contrast to the colorless plaques typical of mRS81. This procedure revealed additional differences between the spontaneous and radiation-induced mutant sets. In contrast to the radiation-induced dominant *lac*^{c} mutants, which always yielded blue plaques (often 200/plate), only 58% of the spontaneous dominant *lac*^{c} mutants produced any blue plaques and half of those mutants (vs. 6% of the radiation-induced mutants) produced plaques that were very light in color. Since about 10% of the mutations associated with light blue plaques were found by sequencing to be *lacI*^{d} mutations, we did not screen out mutations on the basis of plaque color.

At any rate, if a bacterial mutant showed a dominant *lac*^{c} phenotype and if this mutation could be transferred to the phage genome producing any sort of blue plaque, we proceeded to determine the molecular nature of the mutation. Initially, all DNA samples were sequenced with the *lacI* primer, which allowed the detection of mutations involving positions 26–249 of the *lacI* gene, i.e., typical *lacI*^{d} mutations. Some mutants that showed the wild-type sequence over positions 26–249 were sequenced with the *lacZ* primer, which allows the detection of *lacO* mutations. Of all nine radiation-induced presumptive *lacO*^{c} mutants and a sample of 22 spontaneous presumptive *lacO*^{c} mutants, only one mutant (spontaneous) showed the A · T → G · C mutation previously reported as a *lacO*^{c} hotspot (Schaaper and Dunn, 1987, 1991). While one radiation-induced mutant carried a G · C → A · T transition at position 1019 in the *lacI* gene, most of the spontaneous mutants (17/22 or 77%) and one of the nine radiation-induced mutants showed large deletions fusing the latter part of the *lacI* gene to the end of the *lacO* sequence, which inactivates *lac* operator function (Table 1). Mutations were not detected in four spontaneous and seven radiation-induced mutants with either of the two sequencing protocols used, and these are pre-

Table 1
Spontaneous and γ-radiation-induced deletion mutations involving the *lac* operator (*lacO*) region in *E. coli*

Mutant number(s)	Size (bp)	Base positions deleted [a]	Directly repeated sequences [b]
Spontaneous			
1-484	830	*lacI*369–*lacO* +13	T
1-418	782	*lacI*422–*lacO* +8	G
1-353	689	*lacI*522–*lacO* +15	AC
1-463	685	*lacI*524–*lacO* +13	–
1-450	590	*lacI*607–*lacO* +1	–
1-443 and 1-580	380	*lacI*824–*lacO* +8	GCGGATA
1-421	323	*lacI*876–*lacO* +3	T
1-416	322	*lacI*894–*lacO* +20	T
1-409	270	*lacI*930–*lacO* +4	–
1-519	189	*lacI*1027–*lacO* +20	T
1-348	176	*lacI*1036–*lacO* +16	C
1-568	171	*lacI*954–*lacO* −72	A
1-367	165	*lacI*1052–*lacO* +21	T
1-433	129	*lacI*1085–*lacO* +18	–
1-361	113	*lacI*1096–*lacO* +13	–
1-521	28	*lacI* −11–*lacO* +17	A
Radiation-induced			
4-130	384	*lacI*818–*lacO* +6	G

[a] Bases deleted extend from the listed position in the *lacI* gene to the listed position in the *lacO* sequence with +1 being the first base (Reznikoff and Abelson, 1978).
[b] Bases listed start at the first base at one of the ends of the deleted DNA and match the first base on the distal side of the novel joint formed by the deletion; '–' indicates none detected.

Table 2
Spontaneous and γ-radiation-induced dominant *lac*^{c} mutations in *E. coli*

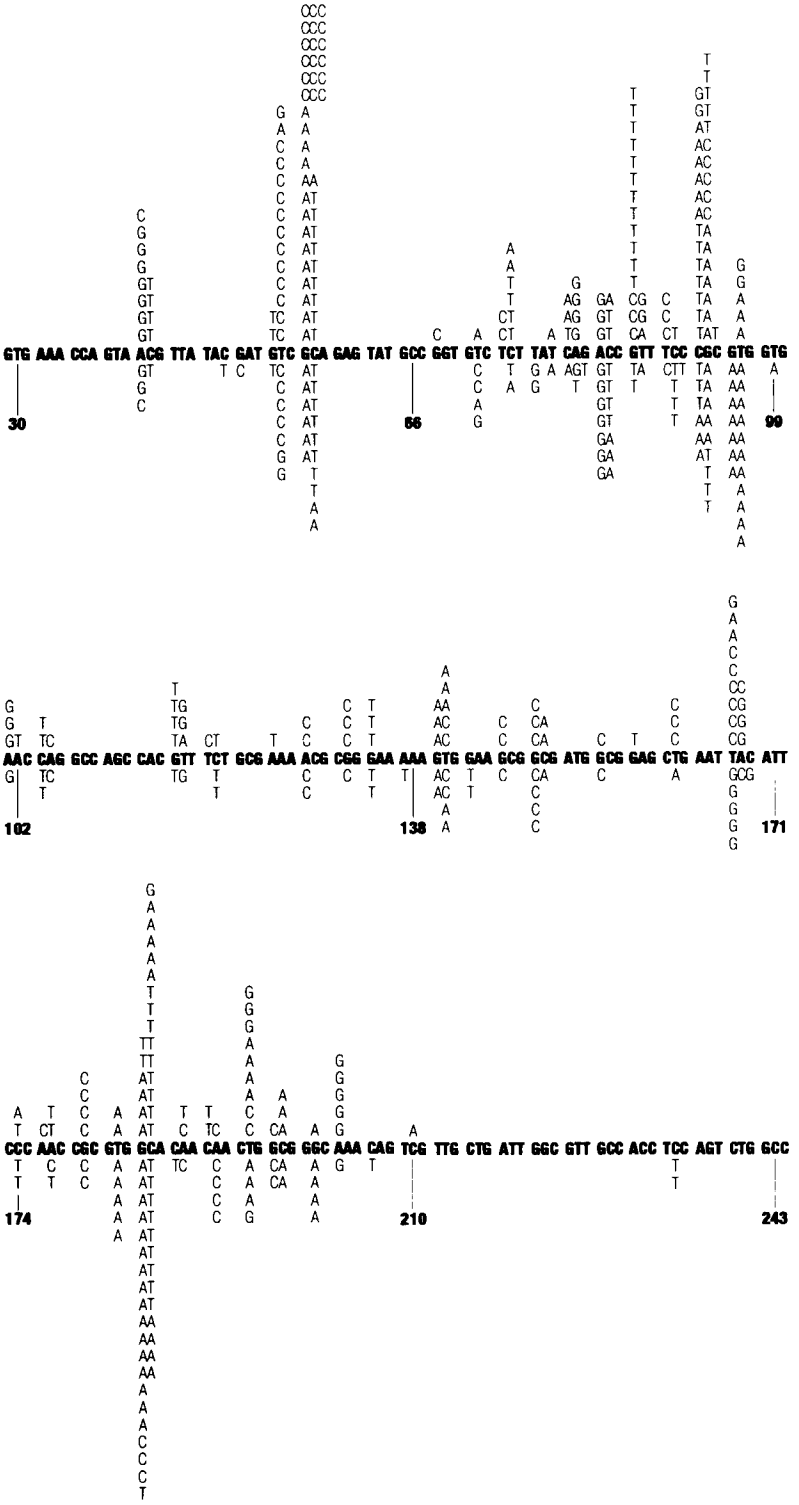
Mutational class (*lacI*^{d}, except where noted)	Spontaneous			Radiation-induced		
	Occurrences	% Base substitutions	% All mutations	Occurrences	% Base substitutions	% All mutations
Transitions: A·T → G·C	22	11	5	56	21	17
G·C → A·T	82	40	20	73	27	22
Total	104	51	25	129	48	39
Transversions: G·C → T·A	31	15	8	53	20	16
G·C → C·G	16	8	4	44	16	13
A·T → C·G	23	11	6	19	7	6
A·T → T·A	29	14	7	24	9	7
Total	99	49	24	140	52	43
Total base substitutions	203	100	49	269	100	82
Multiple mutations [a]	0	–	0	15	–	5
Single-base deletions	10	–	2	25	–	8
Multi-base deletions	43	–	10	8	–	2
Multi-base additions	12	–	3	1	–	0
Total *lacI*^{d} mutations	269 [c]	–	65	318	–	97
Presumptive *lacO*^{c} mutations [b]	142	–	35	9	–	3
Total dominant *lac*^{c} sequenced	411	–	100	327	–	100

[a] Mutations consisting of multiple-base substitutions and/or single-base deletions. Components of these mutations were not included in the single mutation categories.
[b] *Sequencing did not reveal a mutation between positions 26 and 249 in the lacI gene. Most of these mutations are likely to be long* deletions involving the *lac* operator, as described in Table 1.
[c] This total includes a single, 9-base replacement mutation (to be described in Fig. 2), which has not been categorized here.

sumed to carry atypical *lacI*^{d} mutations. Since our spectral analysis would be based primarily on typical *lacI*^{d} mutations, we attempted no further analysis of these presumptive *lacO*^{c} or atypical *lacI*^{d} mutations, and instead worked towards a sequencing endpoint of at least 250 radiation-induced and 200 spontaneous *lacI*^{d} base substitutions.

The radiation-induced dominant *lac*^{c} mutational spectrum was different from the spontaneous spectrum for virtually every class of mutations (Table 2). The most obvious difference was the lower production of multi-base deletions by radiation (vs. spontaneous), which was seen both for the *lacI*^{d} multi-base deletions (2% vs. 10%, respectively), and for the calculated multi-base deletion component of the presumptive *lacO*^{c} mutations (calculated using the 77% value determined above), i.e., 2% vs. 27%, respectively (data not shown). Similarly, radiation (vs. spontaneous) yielded much fewer multi-base additions (none vs. 3%) and atypical *lacI*^{d} mutations (1% vs. 8%, calculated as above for presumptive *lacO*^{c} mutations). Conversely, radiation induced a higher yield (vs. spontaneous) of multiple mutations (5% vs. none), single-base deletions (8% vs. 2%), A·T → G·C transitions (17% vs. 5%), G·C → T·A (16% vs. 8%) and G·C → C·G (13% vs. 4%) transversions (Table 2). While the relative yield of radiation-induced base substitutions (82%) was greater than in the spontaneous spectrum (49%, Table 2), the sites where mutations occurred were

Fig. 1. Spontaneous and γ-radiation-induced *lacI*^{d} base substitutions in *Escherichia coli* NR9102. The three horizontal, numbered sequences (29–244) comprise the wild-type sequence (5′ → 3′), with γ-radiation-induced substitutions listed above and spontaneous substitutions listed below. All mutations shown were sequenced from singly mutant strains, i.e., no components of multiple mutations are shown here.


GTG AAA CCA GTA ACG TTA TAC GAT GTC GCA GAG TAT GCC GGT GTC TCT TAT CAG ACC GTT TCC CGC GTG GTG
30
66
99
AAC CAG GCC AGC CAC GTT TCT GCG AAA ACG CGG GAA AAA GTG GAA GCG GCG ATG GCG GAG CTG AAT TAC ATT
102
138
171
CCC AAC CGC GTG GCA CAA CTG GCG GGC AAA CAG TCG TTG CTG ATT GGC GTT GCC ACC TCC AGT CTG GCC
174
210
243

mostly similar for the spontaneous and radiation *lacI*[d] data sets (Fig. 1). That is, 51 sites showed both spontaneous and radiation-induced mutations, while only 13 sites were unique to the spontaneous spectrum and 10 sites were unique to the radiation-induced spectrum; moreover, almost all of these unique sites yielded only one or two base substitutions (Fig. 1). (Note that the base substitutions involved in multiple mutations (Table 2) were not included in Fig. 1, since many were unusual and may have resulted from a special mechanism (see Discussion).)

Table 3
Spontaneous and γ-radiation-induced *lacI*[d] single-base deletion mutations in *E. coli*

Base position deleted [a]	Deletion site (in bold) and flanking bases	Occurrences [b]	
		Spontaneous	Radiation
$-A_{81}$	C_{80}**AG**$_{82}$	–	1
$-A_{101-102}$	G_{100}**AAC**$_{103}$	–	1
$-A_{135-139}$	G_{134}**AAAAAG**$_{140}$	2	1
$-A_{152}$	G_{151}**AT**$_{153}$	–	1
$-A_{189-190}$	C_{188}**AAC**$_{191}$	1	1
$-A_{203-205}$	C_{202}**AAAC**$_{206}$	–	1
$-A_{207}$	C_{206}**AG**$_{208}$	–	1
$-T_{62}$	G_{61}**TA**$_{63}$	–	1
$-T_{87-89}$	G_{86}**TTTC**$_{90}$	–	1
$-T_{162}$	C_{161}**TG**$_{163}$	–	1
$-T_{212-213}$	G_{211}**TTG**$_{214}$	–	1
$-G_{61}$	A_{60}**GT**$_{62}$	–	1
$-G_{68-69}$	C_{67}**GGT**$_{70}$	–	1
$-G_{148-149}$	C_{147}**GGC**$_{150}$	2	3
$-G_{151}$	C_{150}**GA**$_{152}$	–	1
$-G_{157-158}$	C_{156}**GGA**$_{159}$	1	–
$-G_{199-201}$	C_{198}**GGGC**$_{202}$	–	1
$-C_{55}$	T_{54}**CG**$_{56}$	–	1
$-C_{66-67}$	G_{65}**CCG**$_{68}$	1	2
$-C_{90-92}$	T_{89}**CCCG**$_{93}$	1	1
$-C_{131}$	G_{130}**CG**$_{132}$	1	–
$-C_{150}$	G_{149}**CG**$_{151}$	–	1
$-C_{173-175}$	T_{172}**CCCA**$_{176}$	–	1
$-C_{178-179}$	A_{177}**CCG**$_{180}$	–	1
$-C_{186}$	G_{185}**CA**$_{187}$	1	–

[a] When the deleted base is in a run of repeated bases, i.e., the bases listed in bold type under 'Deletion site', it is not clear which one of the repeated bases was deleted.
[b] '–' indicates none detected.

Table 4
Spontaneous and γ-radiation-induced *lacI*[d] multi-base deletion mutations in *E. coli*

Size (bp)	Base positions deleted	Directly repeated sequences [a]	Occurrences [b]	
			Spontaneous	Radiation
> 92	< 29–120	–	1	–
87	91–177 [c]	CCGCGTGG	29	3
67	130–196 [c]	GCGGG	3	–
63	151–213	G	1	–
30	43– 72	–	1	–
16	104–119	C	1	–
15	189–203	AACA	1	–
10	31– 40	–	1	–
9	146–154	GCGG	1	–
9	159–167	A	1	–
6	137–142 [c]	–	1	–
6	190–195	–	1	–
3	154–156	GG	–	1
3	165–167	A	–	1
3	186–188	–	–	1
3	187–189	ACA	1	–
3	188–190	CAA	–	1
3	189–191	AAC	–	1

[a] Bases listed start at the first base at one of the ends of the deleted DNA and match the first base on the distal side of the novel joint formed by the deletion.
[b] '–' indicates none detected.
[c] Mutations also reported by Schaaper and Dunn (1991).

Data for the specific nature of single-base deletions or frameshifts (Table 3), multi-base deletions (Table 4), and additions (Table 5) highlight the already noted large percent composition differences between radiation and spontaneous mutagenesis.

The analysis of multiple mutations (Table 6) shows 17 mutations (in bold type) that were not seen in the spontaneous or radiation-induced sets of single-base substitutions (Fig. 1) or single-base deletions (Table 3). However, one can note that in every pair or triplet of mutations, there is at least one mutation that had been recovered in the radiation set, and that occasionally the combination of two mutations caused a repressor-inactivating amino acid substitution.

A single, unique *lacI*[d] mutation found in the spontaneous set was included in the total listed in Table 2, but was not categorized. This consisted

Table 5
Spontaneous and γ-radiation-induced *lacI*^{d} addition mutations in *E. coli*

Inserted bases or positions of duplicated bases (in parentheses), and flanking bases	Size (bp)	Directly repeated sequences [a]	Occurrences [b]	
			Spontaneous	Radiation
134(AAA)135 [c]	3	AAA	–	1
167(43–167)168	125 [d]	–	1	–
69(52–69)70	18	TGTC	1	–
137(118–137)138	20 [d]	–	1	–
138(133–138)139	6	–	1	–
147(139–147)148	9	–	1	–
150(139–150)151	12	–	1	–
161(153–161)162	9	TG	1	–
165(159–164)166	6	–	1	–
245(168–245)246	78	–	1	–
234(169–234)235	66	CA	1	–
192(179–192)193	14 [d]	–	1	–
219(190–219)220	30	–	1	–

[a] Bases listed start at the first base of the duplicated sequence (the first position listed in parentheses) and match the base at the position listed after the parentheses. '–' indicates none detected.
[b] '–' indicates none detected.
[c] A mutation also reported by Schaaper and Dunn (1991).
[d] These additions induced −1 shifts in the reading frame; other additions are in-frame.

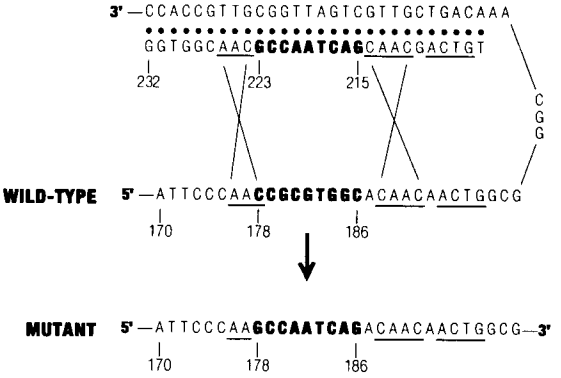


Fig. 2. Nonhomologous recombination is the likely mechanism of induction of the spontaneous 9-base replacement *lacI*^{d} mutation in strain 1-569.

of a 9-base replacement mutation, i.e., A_{177}(CCGCGTGGC)A_{187} was converted to A_{177}(GCCAATCAG)A_{187} causing four consecutive amino acid changes: AspN → Lys, Arg → Pro, Val → Leu, and Ala → Arg. This mutation almost certainly arose by a single recombination event because a copy of the 9-base insert and homologous flanking sequences occurs 28 bases away at positions 215–223 (Fig. 2).

Table 6
Identification of silent mutations among γ-radiation-induced multiple *lacI*^{d} mutations in *E. coli* [a]

Mutant No.	Mutations (amino acid substitutions in LacI protein) [a]	
	lacI^{d} active mutations	Silent or probably silent mutations
4-310	C → T_{42}, **G → T_{43}** (Thr → Ile)	
4-270	**−T_{52}** (truncation [b]), **A → C_{48}** (Tyr → Ser)	
4-106	G → A_{56}, C → T_{57} (Ala → Ile)	
4-249	G → A_{56}, C → T_{57} (Ala → Ile)	
4-187	C → A_{57}, **A → G_{58}** (Ala → Glu)	
4-241	C → A_{75} (Ser → Tyr)	**A → C_{137}** (Lys → GluN)
4-048	C → A_{92} (Arg → Ser)	**C → G_{91}** (Ser → Ser)
4-216	A → G_{168} (Tyr → Cys)	**G → T_{95}** (Val → Leu), **G → T_{98}** (Val → Leu)
4-142	C → T_{120} (Ser → Phe)	T → A_{96}, **G → T_{97}** (Val → Val)
4-198	A → C_{105}, **G → T_{106}** (GluN → Pro)	
4-209	T → A_{167} (Tyr → AspN)	**C → G_{108}** (Ala → Gly)
4-156	A → T_{125} (Lys → ochre)	**G → C_{122}**, **C → G_{123}** (Ala → Arg)
4-266	G → C_{132}, **G → T_{133}** (Arg → Pro)	
4-150	T → A_{141}, **G → T_{142}** (Val → Asp)	
4-366	**G_{149}(+C)C_{150}**, **−T_{153}** (Ala Met → Ala Glu)	

[a] Mutations in bold type were not seen in the spontaneous or radiation-induced data sets as single mutations (Fig. 1, Table 3 and Table 5).
[b] **−T_{52}** induces TGA nonsense triplets at positions 99, 162, and 216.

4. Discussion

Spontaneous mutations

The spontaneous *lac*^{c} frequency of 1.5×10^{-6} observed in this study compares well with the value of 1.3×10^{-6} obtained by Schaaper and Dunn (1991), and there is very good agreement between the two sets of *lacI*^{d} mutational spectra (Table 7). As in the data of Schaaper and Dunn (1991), we did not find mutations that produced +1 translational frameshifts, i.e., we only found single-base deletions (Table 2), or multi-base *lacI*^{d} deletions (Table 4) and additions (Table 5) that either produced −1 frameshifts or did not alter the reading frame (these results are consistent with the translation reinitiation model discussed by Calos and Miller, 1981). However, our single-base deletions occurred at sites distinct from those observed by Schaaper and Dunn (1991), except for the minor hotspots found in both studies. Consistent with the slipped-mispairing model for frameshift mutagenesis (Streisinger et al., 1966), both studies found almost all of the single-base deletions to occur in short runs of repeated bases. Long deletions showed the same hotspot, an 87-bp deletion comprising 67% of all deletions (Table 4) vs. 52% in the study of Schaaper and Dunn (1991). Our 12 addition-type mutations (Table 5) occurred at different sites than those of Schaaper and Dunn (1991), but showed the same lack of a hotspot and lack of obvious direct repeats that could be used to explain the mutagenic mechanism.

Table 7
Comparison of spontaneous *lacI*^{d} mutation data sets in *E. coli* strain NR9102

Mutational class		Percent occurrences [a]	
		This study	Schaaper and Dunn (1991)
Transitions:	A·T → G·C	8	9
	G·C → A·T	30	33
	Total	39	42
Transversions	G·C → T·A	12	6
	G·C → C·G	6	3
	A·T → C·G	9	12
	A·T → T·A	11	8
	Total	37	29
Total base substitutions		75	71
Multiple mutations		0	0
Single-base deletions		4	4
Multi-base deletions		16	17
Multi-base additions		4	8

[a] Total samples sequenced: 269 (data recalculated from Table 2, this study); 414 (Schaaper and Dunn, 1991).

The only apparent inconsistency between this study and that of Schaaper and Dunn (1991) involves the initial sorting out of spontaneous *lacI*^{d} mutations. They found 40% of their *lac*^{c} mutants to be dominant, while we report 16% (data not shown). Only 25% of their dominant *lac*^{c} mutations mapped in the N-terminal part of the *lacI* gene, while 65% of ours mapped in this region (Table 2). At any rate, one can calculate that both studies ended up with 10% of their *lac*^{c} mutants resulting from *lacI*^{d} mutations.

Schaaper and Dunn (1991) screened out 75% of their dominant *lac*^{c} mutations before sequencing, using a genetic test identifying the *lacO*^{c} region, because early experiments showed a 'substantial contribution' of these *lacO*^{c} mutations to be a T → C transition at the +6 base position in the *lac* operator region. We did not screen out putative *lacO*^{c} mutations, but instead sequenced all mutations within base positions 26–249 of the *lacI* gene. Then, a sampling of mutations not mapping in this N-terminal part of *lacI* were sequenced to verify that they were *lacO*^{c} mutations. Only one occurrence of the common transition reported by Schaaper et al. (1986) and Schaaper and Dunn (1991) was found in the 22 '*lacO*^{c}' mutations that we sequenced from the spontaneous data set (data not shown). On the other hand, 17 of these 22 sequencings identified long deletions involving the *lacO* region, and possessing the same 'unique characteristics' described by Schaaper et al. (1986) for class II deletions, i.e., they lacked terminal direct repeats and they did not reach the start of the *lacZ* translation sequence (Table 1). Sequencing over the *lacO* region of the four remaining mutants showed the wild-type sequence (data not shown), thus their mutations are assumed to lie in a 'later' portion of the *lacI* gene and represent atypical *lacI*^{d} mutations. The different '*lacO*^{c}'

Table 8
Comparison of *lacI*^{d} and previously published ionizing radiation mutational spectra

Mutational class	Percent occurrences					
	Irradiated in vivo					Irradiated in vitro
	lacI^{d} [a]	λ*cI* [b]	*aprt* [c]	*aprt* [d]	*aprt* [e]	*lacZ* [f]
Transitions A·T → G·C	18	15	7	6	5	0
G·C → A·T	23	21	7	19	19	7
Total	41	37	15	25	24	7
Transversions G·C → T·A	17	12	19	6	9	71
G·C → C·G	14	8	15	6	14	14
A·T → C·G	6	2	4	19	14	0
A·T → T·A	8	15	4	12	5	4
Total	44	37	41	44	43	89
Total base substitutions	85	73	56	69	67	96
Multiple mutations	5	6	11	0	0	0
Single-base deletions	8	13	11	6	14	4
Single-base additions	0	2	4	0	5	0
Multi-base deletions	3	0	19	25	9	0
Multi-base additions	0	3	0	0	5	0
Total sequences	318	52	27	16	21	28
Induced/spontaneous rates	94	20	10–20	13	3	7

[a] F′*lac*, *lacI*^{d}, *E. coli*, ^{137}Cs γ-radiation under N_2, recalculated from Table 2, this study.
[b] λ-prophage, *cI*, *E. coli*, ^{60}Co γ-radiation, Tindall et al., 1988.
[c] Chromosomal *aprt*, Chinese hamster ovary cells, ^{60}Co γ-radiation, Miles and Meuth, 1989.
[d] Chromosomal *aprt*, Chinese hamster ovary cells, ^{137}Cs γ-radiation, Grosovsky et al., 1988.
[e] Integrated shuttle vector *aprt*, Chinese hamster ovary cells, 180 kVp X-rays, Skandalis et al., 1992.
[f] Double-stranded M13mp10, *lacZ*, *E. coli*, ^{60}Co γ-radiation under N_2, Braun et al., 1993.

components in this study and that of Schaaper and Dunn (1991) may reflect a bias produced in our spontaneous mutation spectrum by the initial selection for a low background starter culture.

Radiation-induced mutation rates

Analysis of our data yields a specific-gene mutation rate of 4.1×10^{-8} *lac*^{c} mutations/gene/Gy (correcting for spontaneous mutants and three copies of F′*lac* per cell, as per Glickman et al., 1980). This value compares favorably with 4.5×10^{-8}, which was reported earlier for the same gene (Glickman et al., 1980; with conversion of rad to Gy). Similarly, the rate of induced base substitutions is 1.2×10^{-10} base substitutions/base pair/Gy (calculated from 9.25×10^{-8} *lac*^{c} mutations/cell, 0.31 dominant *lac*^{c}/*lac*^{c}, 269/327 *lacI*^{d} base substitutions/dominant *lac*^{c}, 3 F′*lac*/cell, 85 mutable base pairs giving *lacI*^{d} mutations (Zielenska et al., 1993), and a dose of 750 Gy), which agrees with other values for bacteria, e.g., 2.2×10^{-10} (Glickman et al., 1980) and 1.3×10^{-10} (Tindall et al., 1988).

Radiation-induced mutational spectra

The *lacI*^{d} mutation assay used here detects all kinds of mutations except +1 frameshifts (discussed in Calos and Miller, 1981). Actually, one +1 frameshift was found, but it was compensated for by a nearby −1 frameshift (Table 6). Since radiation-induced +1 frameshifts seem to occur 1/3 to 1/7 as frequently as −1 frameshifts (Tindall et al., 1988), and −1 frameshifts comprised only 2.5% of our radiation-induced *lacI*^{d} mutations (Table 2), the effects of this omission on the percentages of the other classes of mutations seem negligible.

In Table 8, we compare our radiation-induced spectra with other comprehensive mutation spectra either obtained with anoxic irradiation (Braun

et al., 1993), or obtained under questionable oxic conditions (i.e., in the absence of constant aeration, and with metabolically active cells and long irradiation times, it may be more proper to consider irradiation conditions to be anoxic, rather than oxic). Mutation data from noncomprehensive assays were not included because the failure to detect some major classes of mutations has the effect of inflating the percentages of the mutations that are detected. Similarly, mutation spectra that contain large components of multiple mutations, which were not separated from their singly occurring mutations, were not included because multiple mutations may arise by mechanisms different than for single mutations (discussed below). The data in Table 8 show fairly good agreement between the two in vivo bacterial studies (this study and that of Tindall et al., 1988). When compared to these bacterial studies, the three in vivo *aprt* studies in Chinese hamster cells show more variation amongst themselves, and they show consistently lower yields of transitions and higher yields of multi-base deletions than the bacterial studies. The mutational spectrum derived by irradiating naked DNA in vitro stood apart from the other mutational spectra, i.e., no A · T → G · C transitions were produced, transitions and deletions were much less frequent, and transversions were less uniform. While naked DNA experiments play an important role in radiation research, it is not clear that they are good models for discerning the in vivo situation for mutagenesis.

Base substitutions

Radiation lethality (Kaplan and Zavarine, 1962) and chemistry (e.g., Fuciarelli et al., 1990) data suggest that ionizing radiation more efficiently produces radiation damage at G · C vs. A · T sites. The mutational target in the present study contains 109 G · C sites (67 non-wobble sites) vs. 85 A · T sites (63 non-wobble) between positions 41 and 234 of the *lacI* gene (Fig. 1). Taking these numbers of available sites into account, there were 32% more mutations at G · C vs. A · T sites, and 59% more if one considers just the non-wobble sites. The relative 'hotness' of G · C vs. A · T sites can be calculated by dividing the percentage of total mutations by the number of sites actually mutated, and shows that G · C sites were 54% hotter than A · T sites. This preference for ionizing radiation mutagenesis at G · C vs. A · T sites is in agreement with data from Glickman et al. (1980), Brandenburger et al. (1981), Ise et al. (1984), Ayaki et al. (1986), Hoebee et al. (1988, 1991), Takimoto et al. (1991), Sikpi et al. (1991), Waters et al. (1991), Jaberaboansari et al. (1991), Bertram and Hagen (1992), and all of the studies compared in Table 8, except that by Grosovsky et al. (1988).

Two types of radiation-induced G · C damage have been shown to be mutagenic: *trans*-5,6-dihydroxy-5,6-dihydrouracil (a cytosine deamination product) that leads to G · C → A · T transitions (Ayaki et al., 1986, 1987) and 7-hydro-8-oxoguanine (a damaged guanine) that leads to G · C → T · A transversions (Wood et al., 1990). In addition to a putative lower yield of A · T vs. G · C damage, A · T damage may simply be less mutagenic as suggested by the poor mutagenicity of *cis*-5,6-thymine glycol, which leads to T → C transitions (Basu et al., 1989), and the nonmutagenicity of 8-oxoadenine (Wood et al., 1992), an adenine product analogous to the mutagenic 7-hydro-8-oxoguanine product cited above.

The 'A rule' (reviewed in Strauss, 1991) is another mechanism that favors mutagenesis at G · C vs. A · T sites. This rule defines the high probability that DNA polymerase will insert adenine opposite noncoding lesions such as abasic sites, which are produced by ionizing radiation (e.g., Ullrich and Hagen, 1971) and during the repair of ionizing radiation damage (Breimer and Lindahl, 1985). Thus, abasic sites resulting from the loss of thymine are likely to be nonmutagenic vs. abasic sites resulting from the loss of the other three bases.

To facilitate a direct comparison of radiation-induced and spontaneous base substitution occurrences, the latter were multiplied by 1.33 to normalize their total number (203) to the total number of radiation-induced base substitutions (269). A comparison of the radiation-induced (Fig. 1) and normalized spontaneous base substitutions showed surprisingly few qualitative differences (comparison not shown). At G sites, A and T

substitutions were very similar in both spectra. C substitutions occurred at four radiation sites (positions 56, 86, 92, and 93) that were not seen in the spontaneous spectrum, while the reverse was true only at position 185 in the spontaneous spectrum (Fig. 1). At A sites, T substitutions were very similar in both spectra, but G was substituted at four radiation sites (162, 167, 168, and 195) that were not seen in the spontaneous spectrum, and the reverse was true only at position 72 in the spontaneous spectrum (Fig. 1). These radiation-induced A·T → G·C transitions may be associated with *cis*-5,6-thymine glycol damage (Basu et al., 1989), but there is not yet a good explanation for the radiation-induced G·C → C·G transversions noted above.

Transcribed vs. nontranscribed strands of DNA

Preferential repair of damage in the transcribed strand and a lower incidence of mutations in the nontranscribed strand have been reported for various mutagenic agents (Leadon and Cooper, 1993; reviewed in Bohr, 1991). While it is difficult to do such calculations without knowing the exact nature of the mutagenic damage, one can test whether the mutations shown in Fig. 1 occurring at A·T vs. G·C base pairs show a strand bias. In fact, while the occurrence of most radiation-induced mutations was very similar in the opposite strands, there were 10-fold more G·C → C·G and 2.4-fold more A·T → T·A transversions in the nontranscribed strand (i.e., the strand shown in Fig. 1) vs. the transcribed strand (this situation is not explainable by a differential in the number of mutated sites on the two strands (Fig. 1)). Finding similar biases among the spontaneous mutations argues either that these biases simply reflect the nonuniform distribution of mutable sites that can be scored well in the *lacI*^{d} mutation assay, or that there is a large similarity between ionizing radiation-induced and 'spontaneous' DNA base damage, a notion supported by the detection of radiation-type base damage in nonirradiated cells (e.g., Fuciarelli et al., 1990).

Nearest-neighbor analysis

As with the analysis of strand bias, to perform a nearest-neighbor analysis on base substitutions in a double-stranded target, one is limited to the assessment of what happens at G·C vs. A·T sites. To accomplish this for the data shown in Fig. 1, mutations at G sites in both DNA strands were combined and compared with mutations occurring at A sites in both strands, and the yield of each base substitution as affected by neighboring nucleotides was calculated. The left side of Table 9 shows the effect on radiation-induced and normalized spontaneous (discussed above) base substitutions caused by each of the four

Table 9
Nearest-neighbor nucleotide effects on spontaneous and γ-radiation-induced base substitutions

Base substitutions at position N	Number of base substitutions occurring at position N in the listed triplets: radiation-induced (and spontaneous) [a]							
	CNX or XNC	TNX or XNT	ANX or XNA	GNX or XNG	CNC	TNT	ANA	GNG
G → C	45 (20)	4 (1)	2 (3)	11 (15)	27 (3)	0 (1)	0 (0)	0 (0)
G → T	40 (27)	27 (19)	9 (9)	14 (15)	10 (9)	2 (1)	2 (3)	1 (0)
G → A	56 (80)	32 (48)	10 (15)	18 (51)	22 (15)	2 (3)	5 (4)	1 (4)
A → C	18 (21)	2 (11)	11 (23)	5 (7)	2 (0)	0 (0)	0 (0)	0 (0)
A → T	20 (36)	3 (1)	9 (4)	8 (9)	8 (25)	0 (0)	0 (1)	0 (0)
A → G	41 (27)	9 (0)	25 (7)	33 (23)	2 (3)	0 (0)	0 (0)	2 (0)

[a] 'X' represents A, G, C, or T. 'N' is G or A as specified in the first column. Radiation-induced data are derived from Fig. 1 by combining all like base substitutions at G (or at A) from both the transcribed and nontranscribed strands. The data for spontaneous base substitutions were prepared in the same way and then were increased 1.33-fold (i.e., 269/203, derived from Table 2) to normalize the total number of spontaneous base substitutions to the total number of radiation-induced substitutions and facilitate direct comparisons of both sets of data in this table.

nucleotides being on either or both sides of the mutated nucleotide, i.e., the sum of all possible effects for a particular neighboring nucleotide. With this calculation, there is a clear trend of C enhancing mutagenesis vs. A, G, or T (it was not useful to separate 5′ from 3′ data because there did not seem to be much difference, and because when testing A, T, or G data with C as the other nucleotide, C seemed to overwhelm the effect of the tested nucleotide (data not shown)). Perhaps a better way to analyze neighboring-nucleotide effects is to assess the case where both neighbors are the same, as in the right side of Table 9. While this interpretation suffers from the smaller amount of data available, one can see an unambiguous enhancement by C as the nearest neighbor for G → C, G → T, G → A, and A → T base substitutions in both the irradiated and spontaneous data sets. The nature of this effect is not understood, but hopefully will stimulate studies on comparative analyses of radiation damage within different nucleotide contexts. No mutagenesis-enhancing effect could be discerned by our analysis (not shown) of the data of Tindall et al. (1988), but their numbers of mutations are smaller, which reduces the confidence in such an analysis.

Single-base deletions

Consistent with the Streisinger model (Streisinger et al., 1966), 16/25 radiation-induced frameshifts occurred in runs of two or more bases (Table 3). In contrast to studies showing enhanced yields of frameshifts at sequences containing longer runs of repeated bases (Streisinger and Owen, 1985; Sargentini and Smith, 1987), this correlation was not observed in this study (Table 3). A further deviation from the Streisinger model are the nine of 25 frameshifts that occurred in the complete absence of a run of repeated bases (Table 3). These frameshifts may be explainable by the complex mechanisms described by Ripley (1982) and Bebenek and Kunkel (1990).

As with the analysis of base substitutions, more radiation-induced single-base deletions occurred at G · C vs. A · T sites (56% vs. 44%), but this seems to reflect the 56% G · C composition of the mutable target (positions 41–234, Fig. 1). On the other hand, 16 of 26 radiation-induced and seven of nine spontaneous single-base deletions occurred at sites flanked on both sides by G or C, where chance would predict eight of 26 and three of nine, respectively, i.e., a twofold enhancement of mutagenesis. This suggests that G or C damage plays an important role in inducing the deletion of adjacent bases.

Multi-base deletions and additions

The mechanism for long deletions is distinct from that for single-base deletions in that long deletions seem to require *recA*- and *recB*-dependent recombination (Sargentini and Smith, 1992). In line with this recombination model, four out of five of the radiation-induced deletion mutations involve directly repeated terminal sequences with the greatest yield associated with the longest direct repeat (Table 4). While the single radiation-induced *lacO* deletion does not have an impressive terminal repeat sequence (Table 1), it should probably not be considered as radiation-induced. That is, one can calculate that this class II deletion is likely to be a spontaneous mutation, since 1.8 (i.e., (327)(35%)(77%)(2%)) spontaneous *lacO* deletions are predicted to occur in the radiation set. The single radiation-induced addition mutation detected in this study (AAAAA → AAAAAAAA, Table 5) seems more likely to have resulted from a Streisinger slipped-mispairing mechanism than from a *recB*-dependent recombination mechanism even though it involves three bases.

Multiple mutations

The analysis of multiple mutations (Table 6) shows that 13 of 15 mutants possessed paired mutations separated by five or fewer nucleotides, which argues either for an altered, low-fidelity replication complex making unprovoked replication errors near a damage site, or for the occurrence of closely situated mutagenic lesions. Nine mutations are highly likely to be silent (Table 6), since the amino acid substitutions produced are similar in class to the original amino acids, and because these were not listed in a compilation of the amino acid substitutions that had been found to be associated with *lacI*^{d} mutations (Miller,

1984). If the nine 'silent' mutations found in 327 sequencings of the 224 base F'*lac* target from irradiated cells were independent of the other mutations found in those cells, then the mutation rate (5.5×10^{-8} mutations/base/Gy) for such silent mutations would be 550-fold greater than that calculated from the *lacI*^{d} data discussed earlier. Thus, it seems more likely that these 'silent' mutations are somehow linked to the selected mutations. Either the presence of one mutagenic lesion increases the likelihood of the formation of nearby lesions, or perhaps more likely, once a DNA polymerase has been altered or induced to perform translesion synthesis, simple replication errors are more likely to occur. This model is consistent with data for a *umuC* strain, which does not show multiple mutations under the same irradiation conditions (manuscript in preparation).

Conclusion

A mutational spectrum analysis has been produced for both spontaneous and γ-radiation (anoxic)-induced (in vivo) *lacI*^{d} mutations in *E. coli*. The present radiation-induced spectrum is much more extensive than those reported in previous studies, and will serve as a valuable baseline for further studies on the genetic and radiation chemical factors that affect γ-radiation-induced mutagenesis.

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