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DNA sequence analysis of spontaneous and γ-radiation (anoxic)-induced *lacI*^{d} mutations in *Escherichia coli umuC122*::Tn*5*: differential requirement for *umuC* at G · C vs. A · T sites and for the production of transversions vs. transitions

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Abstract

Escherichia coli umuC122::Tn*5* cells were γ-irradiated (^{137}Cs, 750 Gy, under N_2), and *lac*-constitutive mutants were produced at 36% of the wild-type level (the *umuC* strain was not deficient in spontaneous mutagenesis, and the mutational spectrum determined by sequencing 263 spontaneous *lacI*^{d} mutations was very similar to that for the wild-type strain). The specific nature of the *umuC* strain's partial radiation mutability was determined by sequencing 325 radiation-induced *lacI*^{d} mutations. The yields of radiation-induced mutation classes in the *umuC* strain (as a percentage of the wild-type yield) were: 80% for A · T → G · C transitions, 70% for multi-base additions, 60% for single-base deletions, 53% for A · T → C · G transversions, 36% for G · C → A · T transitions, 25% for multi-base deletions, 21% for A · T → T · A transversions, 11% for G · C → C · G transversions, 9% for G · C → T · A transversions, and 0% for multiple mutations. Based on these deficiencies and other factors, it is concluded that the *umuC* strain is near-normal for A · T → G · C transitions, single-base deletions and possibly A · T → C · G transversions; is generally deficient for mutagenesis at G · C sites and for transversions, and is grossly deficient in multiple mutations. Damage at G · C sites seems more difficult for translesion DNA synthesis to bypass than damage at A · T sites, and especially when trying to produce a transversion. The yield of G · C → A · T transitions in the *umuC* strain (36% of the wild-type level) argues that abasic sites are involved in no more than 64% of γ-radiation-induced base substitutions in the wild-type strain. Altogether, these data suggest that the UmuC and UmuD′ proteins *facilitate*, rather than being absolutely required for, translesion DNA synthesis; with the degree of facilitation being dependent both on the nature of the noncoding DNA damage, i.e., at G · C vs. A · T sites, and on the nature of the misincorporated base, i.e., whether it induces transversions or transitions.

Keywords: *umuC*; γ-Radiation mutagenesis; Spontaneous mutagenesis; *lacI*^{d}; i^{-d}; Mutational spectrum

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1. Introduction

Noncoding DNA damage, such as a UV radiation-induced pyrimidine dimer, normally blocks the contiguous progression of the replication complex, and reinitiation of DNA synthesis beyond the damage site results in the formation of a daughter-strand gap (Rupp and Howard-Flanders, 1968). The induction of a process called error-prone repair (or perhaps more accurately, translesion DNA synthesis) allows continued strand elongation across the damaged template region, and mutations are induced because of the noncoding nature of the DNA damage (Witkin, 1967; reviewed in Echols and Goodman, 1990). Kato and Shinoura (1977) and Steinborn (1978) isolated *umuC* and *umuD* mutants (Shinagawa et al., 1983) based on their lack of UV radiation mutability. The *umuC* mutant is unable to produce mutations in single-stranded bacteriophage genomes containing apurinic sites (Schaaper et al., 1982), and it is deficient in many other types of targeted mutagenesis as well (reviewed in Walker, 1984). Further evidence of the role of the UmuC and cleaved/activated UmuD (i.e., UmuD′) proteins (Burckhardt et al., 1988; Nohmi et al., 1988; Shinagawa et al., 1988) in translesion DNA synthesis is their requirement along with RecA protein and DNA polymerase III for the in vitro replication of a DNA template containing abasic sites (Rajagopalan et al., 1992). The present work investigates the specific nature of *umuC*-dependent and *umuC*-independent γ-radiation mutagenesis with the hope of gaining further insight into the mechanism of translesion synthesis.

Although *umuC* mutants were isolated on the basis of their UV radiation nonmutability, and this phenotype has been verified with several different assays (reviewed in Sargentini and Smith, 1984), there are certain instances where UV radiation mutagenesis can be produced in a *umuC* mutant. About 25% of the normal yield of *lacI* mutants can be obtained by culturing UV-irradiated *umuC* cells on complete minimal medium (Christensen et al., 1988). A similar fraction of the normal mutagenesis can be obtained with UV-irradiated *umuC* cells, when the cells are photoreactivated at some time after the UV irradiation (Bridges and Woodgate, 1984), or with UV-irradiated bacteriophage S13 plated on *umuC* cells, which are then subjected to an analogous photoreactivation protocol (Tessman, 1985). Thus, UV radiation mutagenesis occurs in *umuC* cells under some conditions, but not others.

On the other hand, γ-radiation (Steinborn, 1978; Sargentini and Smith, 1984), methyl methanesulfonate (Schendel and Defais, 1980), and streptozotocin (Fram et al., 1986) produce significant *recA*-dependent mutagenesis in *umuC* cells with the same mutation assays and conditions under which UV radiation is nonmutagenic. The nature of γ-radiation (anoxic) mutagenesis in a *umuC* strain has been examined with a crude, indirect sequencing assay (Sargentini and Smith, 1989), and the results were that G · C → A · T and A · T → G · C transitions (measured at two sites each) were produced at the wild-type rate in the *umuC* strain, and that poorly defined transversions (measured at five sites) were produced at a much lower rate than in the wild-type strain. These results suggest that the *umuC*-dependent and *umuC*-independent mutagenic mechanisms produce different kinds and distributions of mutations (i.e., mutational spectra). The present study was initiated: (i) to confirm the transition (*umuC*-independent) vs. transversion (*umuC*-dependent) hypothesis through the use of a large number of scorable mutation sites; (ii) to investigate the specific nature of the *umuC*-dependent transversion mutagenesis, a point which was ambiguous in the indirect sequencing analysis; and (iii) to analyze types of mutagenesis (i.e., additions, deletions, etc.) that were not assayable in the earlier study.

2. Materials and methods

Bacterial strains

E. coli K-12 strain NR9102 (*lacI204* and promoter mutations i^{O} (*lacI*) and L8 (*lacZ*)) has been described (Schaaper and Dunn, 1991). Strain SR2412 was derived from strain NR9102 by transduction of the *umuC122*::Tn*5* allele from strain SR1165 (Sargentini and Smith, 1984). The

Umu$^-$ phenotype was confirmed by an assay for UV radiation mutagenesis, i.e., at 60 J/m^2 strain SR2412 yielded about 500-fold fewer rifampicin-resistant mutants than its parent, NR9102. Strains NR9102 and SR2412 are the wild-type and *umuC* strains, respectively, in which mutations were collected in this study.

Mutation terminology

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) (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.

Media, inocula preparation, mutagenesis, mutant selection, and mutation cloning and sequencing

These procedures have been described (Sargentini and Smith, 1994). Briefly, cells were grown in rich medium both before and after irradiation, *lac*^{c} mutants were then selected for their ability to utilize phenyl-β-D-galactoside, and were screened for dominant mutations. These mutations were cloned into the single-stranded bacteriophage mRS81 genome (converting plaque color from colorless to blue on X-gal medium) and sequenced by a dideoxy chain termination method.

3. Results

To analyze the mutagenic effect of ^{137}Cs γ-radiation on *E. coli* wild-type and *umuC* strains, cells were irradiated under N_2 with 750 Gy (the respective surviving fractions on rich plating media were 6% and 3.4%). After irradiation, cells were diluted into fresh growth medium and quickly dispensed into hundreds of tubes. After overnight incubation, the cultures were frozen for storage. A 45-tube sampling of cultures inoculated with either nonirradiated or irradiated cells, respectively, yielded median mutant frequencies (*lac*^{c} mutants per 10^8 cells) of 150 and 9400 for wild-type cells, and 180 and 3500 for *umuC* cells. Thus, the *umuC* strain had a slightly higher spontaneous level of *lac*^{c} mutants, but it showed only 37% of the radiation mutagenesis detected in the wild-type strain (36%, if corrected for the spontaneous mutant frequencies).

Dominant *lacI* mutations (i.e., *lacI*^{d}) are a very useful component of the *lac*^{c} mutational spectrum because they can include all classes of mutations (except + 1 frameshifts; Calos and Miller, 1981), and they can reveal at least 148 different base substitutions at 85 sites (Zielenska et al., 1993). Because almost all *lacI*^{d} mutations map in the N-terminal part of the *lacI* gene (Schaaper and Dunn, 1987), the target to be sequenced comprises only 212 bases (Schaaper and Dunn, 1991) of the 1111-base *lacI* gene (Farabaugh, 1978). To employ this assay, one selects *lac*^{c} mutants, clones and sequences the dominant *lac*^{c} mutations, and analyzes those that are *lacI*^{d} mutations. In this study, about 20% of the *lac*^{c} mutant colonies tested from each culture showed a dominant phenotype, but only one of these mutations per culture was sequenced. As with the wild-type strain (Sargentini and Smith, 1994), only about half of the spontaneous mutations (vs. 95% of the radiation-induced mutations) could initially be transferred from the *umuC* strain into phage mRS81, and only about half of these mutations produced dark-blue plaques (vs. light-blue plaques), which were almost always seen for radiation-induced mutations. The mutations that were difficult to clone were most likely long deletions involving the *lacO* region based on the sequencing results for the occasional light-blue plaques that were obtained after repeated attempts to clone these mutations (Sargentini and Smith, 1994).

If the cloned dominant *lac*^{c} mutation induced any degree of blue color in the phage plaque on X-gal medium, an attempt was made to determine the molecular nature of the *lac* defect. Initially, all DNA samples were sequenced with a *lacI* primer (a 19-mer with its 3′ end complementary to base position 276; cf. Fig. 1), which facili-

tated the detection of mutations in the N-terminal part of the *lacI* gene. Mutations that were not detected in this round of sequencing were called presumptive *lacO*^{c} mutations. A sample of these mutations were subsequently sequenced with a *lacZ* primer, whereby one can normally read the 224-base sequence extending from base position 17 of the *lacZ* gene through the entire *lacO* region to base position 990 of the *lacI* gene. When tested by this second sequencing, only 2/62 of these mutations could be identified as the A·T → G·C transition at position + 6 in the *lacO* region, which has been described as the hotspot for *lacO*^{c} mutations (Schaaper et al., 1986; Schaaper and Dunn, 1991). Combining all data for the wild-type and *umuC* strains, 60% (37/62) of the sequenced presumptive *lacO*^{c} mutations were found to be long deletions fusing the distal part of the *lacI* gene to the distal part of the *lacO* region, which deletes the operator binding site for the *lac* repressor and produces a *lac*-constitutive phenotype, and the remaining, unmapped mutations (35%) are assumed to be atypical *lacI*^{d} mutations, which map between base positions 249 and 1010. The long *lacO* deletions found in the *umuC* strain (Table 1), like those found in the wild-type strain (Sargentini and Smith, 1994), do not involve the start of the *lacZ* translation sequence and tend to lack terminal direct sequence repeats, which is consistent with the class II deletions described by Schaaper et al. (1986). Since the present mutational spectrum analysis would be based primarily on the *lacI*^{d} component of *lac*^{c} mutations, no further analysis of presumptive *lacO*^{c} or atypical *lacI*^{d} mutations was attempted. Instead, work proceeded towards the goal of sequencing 250 γ-radiation-induced and 200 spontaneous *lacI*^{d} base substitutions for the *umuC* strain.

The spontaneous dominant *lac*^{c} mutational spectrum determined for the *umuC* strain is quite similar to that for the wild-type strain (Table 2). For more specific detail, Fig. 1 shows the sites where spontaneous *lacI*^{d} base substitutions occurred in the two strains. These spectra are also very similar, but if one arbitrarily scores differences that are greater than threefold, then the wild-type strain (vs. *umuC*) shows more occurrences of $G_{90} \rightarrow A$, $G_{186} \rightarrow A$, $G_{201} \rightarrow A$, $A_{183} \rightarrow T$, and $A_{195} \rightarrow T$ substitutions, while the *umuC* strain (vs. wild-type) shows more occurrences of $G_{104} \rightarrow A$, $G_{198} \rightarrow A$, $A_{74} \rightarrow G$, $A_{81} \rightarrow C$, $A_{96} \rightarrow C$, $G_{93} \rightarrow C$, and $G_{66} \rightarrow T$ (for this comparison, data from both strands were combined, so that all base substitutions could be categorized as either occurring at A or at G sites, and a subscript is used to denote the base-pair position). While

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

Mutant number	Size (bp)	Base positions deleted [b]	Directly repeated sequences [c]
Spontaneous			
6-407	640	*lacI*562–*lacO* + 6	–
6-394	546	*lacI*654–*lacO* + 4	–
6-293	448	*lacI*756–*lacO* + 8	–
6-412	295	*lacI*916–*lacO* + 15	–
6-459	217	*lacI*981–*lacO* + 2	T
6-485	200	*lacI*1016–*lacO* + 20	–
6-492	188	*lacI*1024–*lacO* + 16	CAAT
6-402	176	*lacI*1036–*lacO* + 16	C
6-409	147	*lacI*1066–*lacO* + 17	A
6-481	137	*lacI*1090–*lacO* + 31	–
6-418	118	*lacI*1082–*lacO* + 4	–
Radiation-induced			
9-123	553	*lacI*658–*lacO* + 15	–
9-085	381	*lacI*823–*lacO* + 8	–
9-128	380	*lacI*824–*lacO* + 8	GCGGATA
9-016	316	*lacI*884–*lacO* + 4	–
9-048	220	*lacI*981–*lacO* + 5	TG
9-039	165	*lacI*1051–*lacO* + 20	–
9-011	137	*lacI*1075–*lacO* + 16	–
9-054	132	*lacI*1072–*lacO* + 9	–

[a] The 11 spontaneous deletions described are from a sample of 16 presumptive *lacO*^{c} mutations, which also included five atypical *lacI*^{d} mutations. The entire set of radiation-induced presumptive *lacO*^{c} mutations (15) included the eight deletions shown, six atypical *lacI*^{d} mutations, and a single A·T → G·C mutation at the + 6 position of the *lacO* sequence.

[b] Bases deleted extend from the listed position in the *lacI* gene to the listed position in the *lacI* operator (*lacO*) sequence, with + 1 being the first base (Reznikoff and Abelson, 1978). The deletions in mutants 6-402 and 9-128 were seen previously among the spontaneous deletions from the wild-type strain (Sargentini and Smith, 1994).

[c] 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.

there are some differences in the mutational spectra, there does not seem to be a clear trend. In the same way, no clear trend is obvious when comparing these two strains for spontaneous single-base deletions (Table 3), multi-base deletions (Table 4), and additions (Table 5). These deletion and addition spectra seem to be unique for each strain except for the frequently found 87-base deletion (cf. Table 4 (this study) and Schaaper and Dunn, 1991).

On the other hand, the radiation-induced dominant *lac*^{c} mutational spectrum for the *umuC* strain differed substantially from its spontaneous spectrum for virtually every class of mutations (Table 2). The most obvious differences shown by irradiated vs. nonirradiated *umuC* cells, respectively, are the decreased percentages of long deletions (2% vs. 11%), and increased percentages of A · T → G · C transitions (39% vs. 5%) and single-base deletions (12% vs. 2%).

If one focuses on the radiation-induced base substitution hotspots in *umuC* cells that are not seen in the spontaneous spectrum, it becomes apparent that these are almost all A · T → G · C substitutions. That is, mutations at A_{41}, T_{54}, T_{72}, A_{81}, T_{89}, A_{101}, T_{117}, T_{162}, T_{167}, A_{168}, and A_{203} show 107 A · T → G · C substitutions vs. only eight in the spontaneous spectrum, and the only other hotspot found exclusively in the radiation-induced spectrum is the sixfold enhancement by radiation of the G · C → A · T transition at C_{186} (cf. Figs. 1 and 2). Clearly, the *umuC* strain is proficient in the radiation induction of A · T → G · C transitions.

Of course, the primary goal for this study was to compare radiation-induced mutational spectra for the wild-type and *umuC* strains. Although the radiation-induced base substitution spectra are similar for these two strains, transitions at A · T sites were induced 2.3-fold less frequently in the

Table 2
Spontaneous and γ-radiation-induced dominant *lac*^{c} mutations in *E. coli* wild-type (WT) and *umuC* strains [a]

Mutation class (*lacI*^{d}, except where noted)	Occurrences (percentages in parentheses)			
	Spontaneous		Radiation-induced	
	WT	*umuC*	WT	*umuC*
Transitions: A · T → G · C	22 (5)	20 (5)	56 (17)	131 (39)
G · C → A · T	82 (20)	65 (15)	73 (22)	74 (22)
Total	104 (25)	85 (20)	129 (39)	205 (60)
Transversions: G · C → T · A	31 (8)	30 (7)	53 (16)	13 (4)
G · C → C · G	16 (4)	22 (5)	44 (13)	14 (4)
A · T → C · G	23 (6)	40 (9)	19 (6)	29 (9)
A · T → T · A	29 (7)	13 (3)	24 (7)	15 (4)
Total	99 (24)	105 (25)	140 (43)	71 (21)
Total base substitutions	203 (49)	190 (45)	269 (82)	276 (81)
Multiple mutations [b]	0 (0)	2 (0)	15 (5)	0 (0)
Single-base deletions	10 (2)	10 (2)	25 (8)	41 (12)
Multi-base deletions	43 (10)	45 (11)	8 (2)	6 (2)
Multi-base additions	12 (3)	16 (4)	1 (0)	2 (1)
Total *lacI*^{d} mutations	269 [c] (65)	263 (62)	318 (97)	325 (96)
Presumptive *lacO*^{c} mutations [d]	142 (35)	161 (38)	9 (3)	15 (4)
Total dominant *lac*^{c} sequenced	411 (100)	424 (100)	327 (100)	340 (100)

[a] WT data are from Sargentini and Smith (1994).
[b] Mutations consisting of base substitutions and/or single-base deletions. The sequences of these mutations (for the WT strain) are given in Sargentini and Smith (1994).
[c] This total includes a single, 9-base replacement mutation, which has not been categorized here.
[d] 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 noted in Table 1.

wild-type strain (vs. *umuC*), with positions A_{72}, A_{74}, A_{83}, A_{89}, A_{101}, A_{117}, and A_{168} showing a greater than threefold effect, while transversions at G · C sites were induced 3.6-fold more frequently in the wild-type strain (vs. *umuC*), with positions G_{56}, G_{86}, G_{93}, G_{116}, G_{132}, G_{134}, G_{180}, and G_{198} showing a greater than threefold effect (Fig. 2). Another major difference between the radiation-induced mutational spectra for the wild-type and *umuC* strains is the complete absence of multiple mutations in the latter (Table 2). Finally, the deletion and addition spectra show

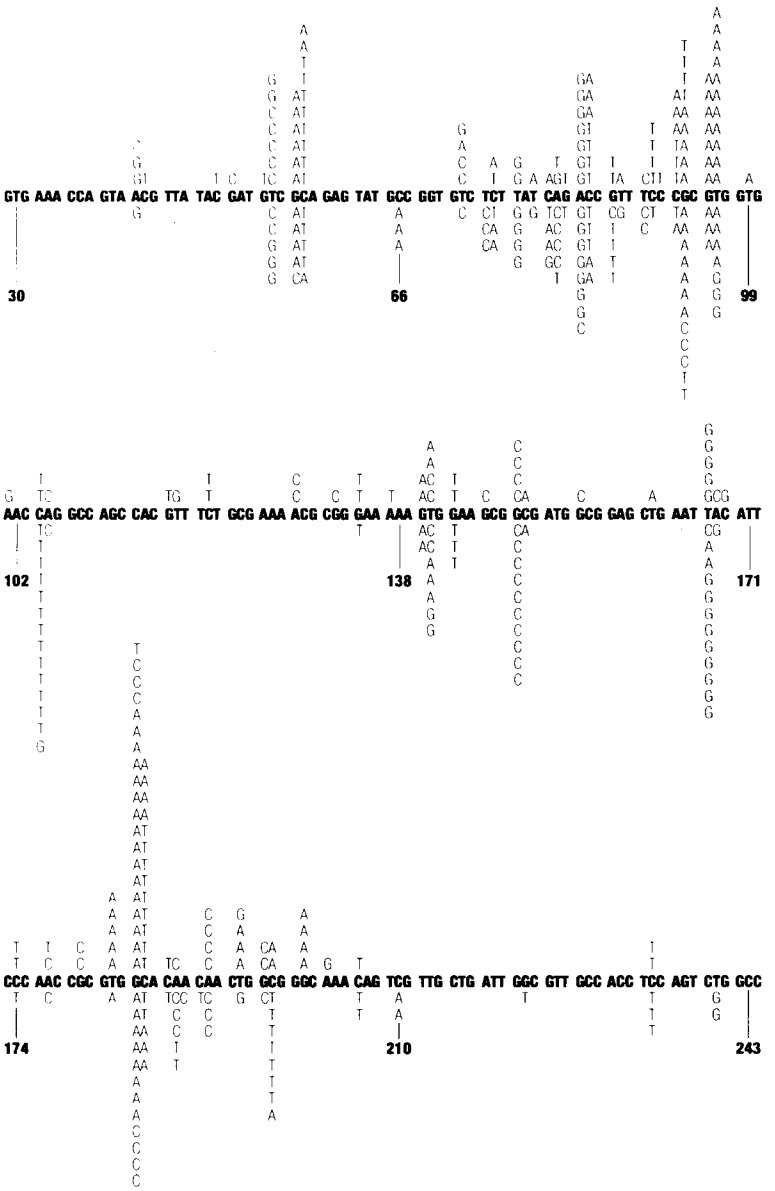

Fig. 1. Spontaneous *lacI*^{q} base substitutions in *Escherichia coli* wild-type and *umuC* strains. The three horizontal, numbered sequences (29–244) comprise the wild-type sequence (5′ → 3′) with substitutions found in the wild-type strain listed above and substitutions found in the *umuC* strain listed below. All mutations shown were sequenced from singly mutant strains.

Table 3
Spontaneous and γ-radiation-induced *lacI*[d] single-base deletions in *E. coli* wild-type (WT) and *umuC* strains [a]

Deletion site (in bold) and flanking bases [b]	Occurrences [c]			
	Spontaneous		Radiation	
	WT	*umuC*	WT	*umuC*
T_{39}**AAC**$_{42}$	–	–	–	1
C_{80}**AG**$_{82}$	–	–	1	–
G_{100}**AAC**$_{103}$	–	–	1	1
G_{134}**AAAAAG**$_{140}$	2	2	1	3
G_{151}**AT**$_{153}$	–	2	1	–
C_{186}**AC**$_{188}$	–	–	–	1
C_{188}**AAC**$_{191}$	1	3	1	–
C_{202}**AAAC**$_{206}$	–	–	1	1
C_{206}**AG**$_{208}$	–	–	1	–
G_{43}**TTA**$_{46}$	–	–	–	1
A_{51}**TG**$_{53}$	–	–	–	1
G_{61}**TA**$_{63}$	–	–	1	–
A_{63}**TG**$_{65}$	–	–	–	1
G_{86}**TTTC**$_{90}$	–	–	1	2
C_{161}**TG**$_{163}$	–	–	1	–
A_{170}**TTC**$_{173}$	–	1	–	2
G_{182}**TG**$_{184}$	–	–	–	1
G_{211}**TTG**$_{214}$	–	–	1	2
C_{42}**GT**$_{44}$	–	–	–	1
C_{55}**GC**$_{57}$	–	–	–	1
A_{60}**GT**$_{62}$	–	–	1	–
C_{67}**GGT**$_{70}$	–	–	1	–
A_{105}**GGC**$_{108}$	–	–	–	2
C_{131}**GGGA**$_{135}$	–	1	–	2
C_{147}**GGC**$_{150}$	2	–	3	3
C_{150}**GA**$_{152}$	–	–	1	–
T_{153}**GGC**$_{156}$	–	–	–	1
C_{156}**GGA**$_{159}$	1	–	–	–
T_{183}**GGC**$_{186}$	–	1	–	–
C_{198}**GGGC**$_{202}$	–	–	1	5
T_{54}**CG**$_{56}$	–	–	1	–
G_{56}**CA**$_{58}$	–	–	–	1
G_{65}**CCG**$_{68}$	1	–	2	1
A_{83}**CCG**$_{86}$	–	–	–	1
T_{89}**CCCG**$_{93}$	1	–	1	2
G_{93}**CG**$_{95}$	–	–	–	1
G_{107}**CCA**$_{110}$	–	–	–	1
A_{114}**CG**$_{116}$	–	–	–	1
G_{122}**CG**$_{124}$	–	–	–	1
G_{130}**CG**$_{132}$	1	–	–	–
G_{149}**CG**$_{151}$	–	–	1	–
T_{172}**CCCA**$_{176}$	–	–	1	–
A_{177}**CCG**$_{180}$	–	–	1	–
G_{185}**CA**$_{187}$	1	–	–	–

[a] WT data are from Sargentini and Smith (1994).
[b] When more than one base is listed in bold type, it is not clear which base was deleted. Subscript values indicate the position of the base in the *lacI* gene.
[c] – indicates none detected.

a similar degree of randomness for the two strains (Tables 3–5), except for the common 87-base deletion (Table 4), i.e., the *umuC* strain shows no particular deficiency for deletions or additions.

4. Discussion

Spontaneous mutational spectra

The wild-type spontaneous mutational spectrum has already been discussed in detail (Schaaper and Dunn, 1991; Sargentini and Smith, 1994). The effect of the *umuC* mutation on spontaneous mutagenesis in a *uvrA* strain has been determined by Christensen et al. (1988). Since the *uvrA* mutation produces a mutator phenotype, which the *umuC* mutation abolishes (Sargentini and Smith, 1981), the effect of the *umuC* mutation on spontaneous mutagenesis was determined here to provide a more valid baseline for the radiation data set. The *umuC* mutation had no negative effect on the yield of spontaneous *lac*^{c} mutations (see Results), which is consistent with other mutation studies comparing *uvr*$^+$ cells with and without the *umuC* mutation (Steinborn, 1978; Kato and Nakano, 1981). In fact, the only notable differences in the mutational spectra for the *umuC* and wild-type strains were the 1.5-fold increase in A · T → C · G transversions and the twofold decrease in A · T → T · A transversions (Table 2; the few specific base sites that showed greater than threefold differences were noted in the Results section). The most important point relevant to the discussion of the radiation-induced mutational spectrum in the *umuC* strain is that it showed no deficiency in spontaneous transversions at G · C sites (Table 2).

Radiation-induced mutational spectra

Since nearly equal numbers of mutations were sequenced for the wild-type and partially mutable *umuC* strains, the classes of mutations that are not *umuC*-dependent exhibit a greater prominence in the mutational spectrum for the *umuC* strain than in the wild-type strain, i.e., the *umuC* strain appears hypermutable for single-base deletions, A · T → G · C transitions, and perhaps for A · T → C · G transversions (Table 2). Table 6 compares the wild-type and *umuC* radiation-in-

duced mutational spectra in a more valid manner, i.e., the *umuC* data were multiplied by 327/340 (to normalize them to the wild-type sample size) and by 0.37 (to reflect the actual mutant yield relative to that for the wild-type strain). In Table 6, the *umuC*/wild-type ratio of mutational occurrences approaches 1.0 for mutation classes that are *umuC*-independent, or zero for mutation classes that are *umuC*-dependent. Further discussion on the effect of the *umuC* mutation on radiation-induced mutational spectra will refer to Table 6, rather than Table 2.

umuC-Dependent mutagenesis

Based on the relative frequency of base substitutions occurring at 74 sites (Fig. 2), this work has

Table 4
Spontaneous and γ-radiation-induced *lacI*[d] multi-base deletion mutations in *E. coli* wild-type (WT) and *umuC* strains [a]

Size (bp)	Base positions deleted	Directly repeated sequences [b]	Occurrences [c]			
			Spontaneous		Radiation	
			WT	*umuC*	WT	*umuC*
> 93	< 29–120	–	1	–	–	–
87	91–177 [d]	CCGCGTGG	29	30	3	2
67	130–196 [d]	GCGGG	3	1	–	1
63	151–213	G	1	–	–	–
63	126–188	AA	–	1	–	–
57	123–179	–	–	1	–	–
54	160–213	GCTGA	–	2	–	–
39	90–128	C	–	1	–	–
30	43–72	–	1	–	–	–
> 25	< 29–53	unknown	–	–	–	1
19	47–65 [d]	–	–	1	–	–
18	49–66	CG	–	–	–	1
16	104–119	C	1	–	–	–
15	189–203	AACA	1	–	–	–
13	175–187	CAAC	–	1	–	–
12	43–54	–	–	1	–	–
12	55–56	CG	–	1	–	–
12	161–172	C	–	1	–	–
10	31–40	–	1	–	–	–
9	146–154	GCGG	1	–	–	–
9	159–167	A	1	–	–	–
9	117–125	–	–	1	–	–
6	137–142 [d]	–	1	–	–	–
6	190–195	–	1	–	–	–
6	74–79	–	–	1	–	–
3	143–145	G	–	1	–	–
3	46–48	–	–	1	–	–
3	154–156	GG	–	–	1	–
3	165–167	A	–	–	1	–
3	187–189	ACA	1	–	–	–
3	186–188	–	–	–	1	–
3	188–190	CAA	–	–	1	–
3	189–191	AAC	–	–	1	–
3	68–70	G	–	–	–	1

[a] WT data are from Sargentini and Smith (1994).
[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.
[c] – indicates none detected.
[d] Mutations also reported by Schaaper and Dunn (1991).

confirmed and extended the hypothesis (Sargentini and Smith, 1989) that the *umuC*-independent and *umuC*-dependent mechanisms involved in γ-radiation mutagenesis differ in their mutagenic specificity. The radiation-induced mutational spectrum in the *umuC* strain is threefold (0.57/0.18) more deficient in transversions vs. transitions, and is deficient to the same degree (0.63/0.21) in base substitutions at G · C vs. A · T sites (Table 6). Thus, the *umuC* strain is most deficient (10-fold) in transversions at G · C sites. This finding that UmuC protein plays a much greater role in transversion (vs. transition) mutagenesis is in agreement with our limited, earlier study (Sargentini and Smith, 1984) and with several other findings: (1) The mutagenesis-enhancing plasmid pKM101, which carries the *umuDC*$^+$ analogs *mucAB*$^+$, shows a substantial preference for enhancing γ-radiation-induced transversions over transitions in *Salmonella typhimurium* (Eisenstadt et al., 1989). (2) Similar results were obtained for a battery of chemical mutagens with *E. coli* tester strains carrying either *mucAB*$^+$ or *umuDC*$^+$ on multicopy plasmids (Watanabe et

Table 5
Spontaneous and γ-radiation-induced *lacI*[d] addition mutations in *E. coli* wild-type (WT) and *umuC* strains [a]

Inserted bases or positions of duplicated bases (in parentheses), and flanking bases	Size (bp)	Directly repeated sequences [b]	Occurrences [c]			
			Spontaneous		Radiation	
			WT	*umuC*	WT	*umuC*
51(TG)52	2 [d]	TG	–	1	–	–
134(AA)135	2 [d]	AA	–	1	–	–
134(AAA)135 [e]	3	AAA	–	1	1	–
227(42–227)228	186	C	–	1	–	–
167(43–167)168	125 [d]	–	1	–	–	–
69(52–69)70	18	TGTC	1	–	–	–
177(91–177)178	87	CCGCGTGG	–	–	–	1
137(118–137)138	20 [d]	–	1	–	–	–
128(118–128)129	11 [d]	–	–	1	–	–
120(118–120)121	3	T	–	1	–	–
128(121–128)129	8 [d]	–	–	1	–	–
136(125–136)137	12	AAA	–	1	–	–
173(129–173)174	45	C	–	1	–	–
138(133–138)139	6	–	1	–	–	–
260(136–260)261	125 [d]	AAA	–	2	–	–
235(138–235)236	98 [d]	A	–	–	–	1
147(139–147)148	9	–	1	–	–	–
150(139–150)151	12	–	1	–	–	–
168(148–168)169	21	–	–	1	–	–
161(153–161)162	9	TG	1	1	–	–
165(159–164)166	6	–	1	–	–	–
284(165–284)285	120	–	–	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	–	–	–
> 284(191–> 284)	> 94	unknown	–	1	–	–
226(207–226)227	20 [d]	–	–	1	–	–

[a] WT data are from Sargentini and Smith (1994).
[b] 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.
[c] – indicates none detected.
[d] These additions induced −1 shifts in the reading frame; other additions are in-frame.
[e] A mutation also reported by Schaaper and Dunn (1991).

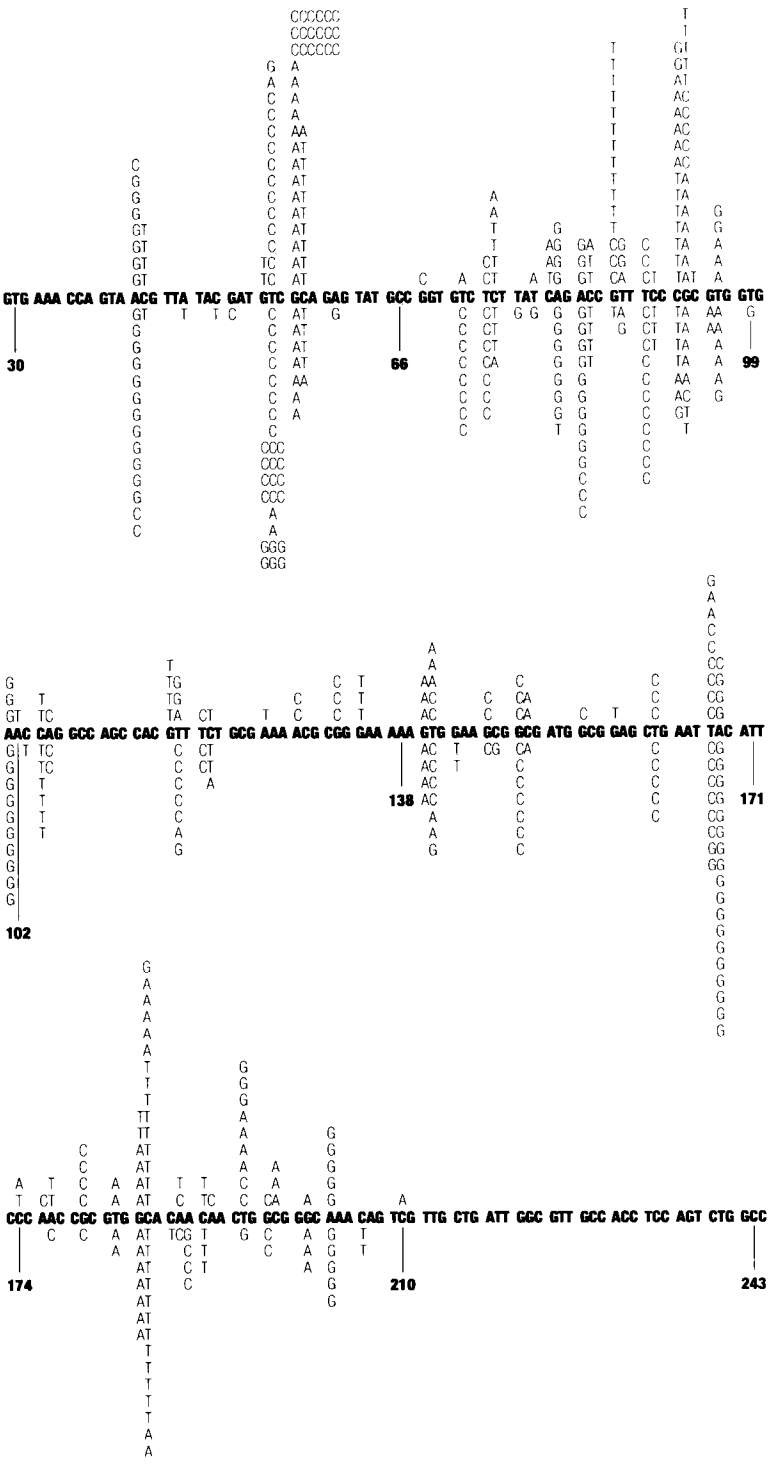

Fig. 2. Gamma-radiation-induced *lacI*^{d} base substitutions in *Escherichia coli* wild-type and *umuC* strains. The three horizontal, numbered sequences (29–244) comprise the wild-type sequence (5′ → 3′) with γ-radiation-induced substitutions found in the wild-type strain listed above and those found in the *umuC* strain listed below. All mutations shown were sequenced from singly mutant strains.

Table 6
Nature of γ-radiation-induced *lacI*^{d} mutations in *E. coli* (WT) and *umuC* strains after correcting *umuC* for sample size [a]

Mutation class (*lacI*^{d}, except where noted)	Occurrences		
	WT	*umuC* [b]	*umuC*/WT
Transitions: A·T → G·C	56	47	0.84
G·C → A·T	73	26	0.36
Total	129	73	0.57
Transversions: G·C → T·A	53	5	0.09
G·C → C·G	44	5	0.11
A·T → C·G	19	10	0.53
A·T → T·A	24	5	0.21
Total	140	25	0.18
Total base substitutions	269	98	0.36
Multiple mutations [c]	15	0	0.00
Single-base deletions	25	15	0.60
Multi-base deletions	8	2	0.25
Multi-base additions	1	0.7	0.70
Total *lacI*^{d} mutations	318	116	0.36
Presumptive *lacO*^{c} mutations [c]	9	5	0.56
Total dominant *lac*^{c} sequenced	327	121	0.37

[a] WT data are from Sargentini and Smith (1994).
[b] *umuC* data shown are the *umuC* γ-radiation-induced data of Table 2 multiplied by 0.356, i.e., (327/340)(0.37), to produce data that would be identical to those for the WT strain, if the *umuC* mutation did not affect that particular mutational class.
[c] See footnotes b and d, Table 2.

al., 1994). (3) Growth of the *recA441* strain at high temperature, which can be expected to induce the *umuDC* operon, preferentially enhanced the spontaneous frequency of transversions vs. transitions (Yatagai et al., 1991). (4) Methyl methanesulfonate mutagenesis, which is *recA*-dependent but only partially *umuC*-dependent, appears to show a much larger *umuC* dependence for transversions over transitions (Śledziewska-Gójska, 1993).

Todd and Schendel (1983) showed that the role of the *umuC* gene in chemical-induced mutagenesis increases as the alkylation damage becomes more bulky (perhaps helical distortion is a better term). Bridges and Woodgate (1984) used delayed photoreactivation experiments to support a two-stage model whereby nucleotides are first incorporated opposite noncoding lesions, and then UmuC protein becomes involved in the extension of DNA synthesis beyond the lesion. Presently, the UmuD′ and RecA proteins are also thought to be involved in this DNA synthesis extension function (Woodgate et al., 1989; Bates and Bridges, 1991). With reference to these models, our data suggest that γ-radiation-induced DNA damage at G·C sites may exhibit more distortion of the DNA helix than at A·T sites, and that this may make it more difficult to bypass or extend DNA synthesis beyond an unmatched base pair. Probably the major conclusions that should come from this work are that the UmuC and UmuD′ proteins *facilitate* rather than being absolutely required for translesion DNA synthesis (in agreement with the notion of Christensen et al., 1988), and that the requirement for this facilitation of translesion synthesis is strongly dependent on the nature of the lesion (lesions at G·C sites being much more dependent on *umuC* than lesions at A·T sites) and on the nature of the misincorporated base (with *umuC* playing a greater role when the damaged base and the misincorporated base are both either purines or pyrimidines, as is necessary to produce a transversion).

Apurinic/apyrimidinic or abasic sites (Ullrich and Hagen, 1971) are one of the numerous classes of DNA damage produced by ionizing radiation (reviewed in Hutchinson, 1985). Translesion DNA synthesis at abasic sites is strongly dependent on SOS induction (Schaaper and Loeb, 1981), and requires the *umuC* gene product (Schaaper et al., 1982). Since abasic sites are produced equally well at all base sites (reviewed in Hutchinson, 1985), one can calculate the maximum role of abasic sites in γ-radiation mutagenesis from the minimum *umuC* dependence found for G·C → A·T, A·T → T·A, and G·C → T·A base substitutions (the three possible types of abasic site mutagenesis, given a perfect 'A rule' (Strauss, 1991)). The *umuC*/wild-type ratios for G·C → A·T, A·T → T·A and G·C → T·A were 0.36, 0.21, and 0.09, respectively (Table 6). Since the *umuC* dependence was least for the G·C → A·T class, one can use its value (0.36) to derive the maximum role that abasic sites should play in any of the three classes of mutations. Thus, one can infer that no more than 64% of each of these

three classes of mutations arise from abasic sites. Mutagenic DNA damage other than abasic sites must be invoked to explain the *umuC* dependences above 64%, i.e., 79% and 91% for A · T → T · A and G · C → T · A, respectively.

While 15 radiation-induced multiple mutations were found among the 318 *lacI*^{d} mutations collected in the wild-type strain (Sargentini and Smith, 1994), no multiple mutations were found among 325 *lacI*^{d} mutations in the *umuC* strain (Table 2). One possible explanation for this phenomenon is that the wild-type replication complex (involving intact UmuC and UmuD′ proteins) demonstrates reduced fidelity at DNA damage sites and is able to produce extra mutations, which are silent and would not have been selected if produced alone (Sargentini and Smith, 1994). Probably a more tenable explanation for the deficiency in multiple mutations in the *umuC* strain can be derived from the specific *umuC* deficiency at G · C sites. Almost every multiple mutation in the wild-type strain involves one or more transversions and/or transitions at G · C sites (Sargentini and Smith, 1994). If these mutations arise independently, then the overall probability of multiple mutations in the *umuC* strain may become vanishingly small (i.e., the product of the reduced probabilities of mutagenesis at each G · C site in the *umuC* strain).

umuC-Independent mutagenesis

The *umuC* gene does not seem to be required for radiation-induced A · T → G · C transitions, most single-base deletions, and probably most A · T → C · G transversions (Table 6). Similarly, streptozotocin mutagenesis, which is *recA*-dependent but *umuC*-independent (Fram et al., 1986), produces essentially only G · C → A · T transitions (Mack et al., 1988). If streptozotocin produced significant numbers of transversions in the wild-type strain, then one might also see a differential *umuC* dependence with this mutagen.

While the *umuC* strain appears hypermutable for single-base deletions or −1 frameshifts (Table 2), correction for sample size suggests that the *umuC* strain is only slightly deficient (Table 6), and, in fact, the same correction applied to the mutation site data (Table 3) yields *umuC*/wild-type ratios of 0.55 (A · T sites) vs. 0.64 (G · C sites). Thus if anything, single-base deletions occur more frequently at G · C vs. A · T sites in the *umuC* strain. More importantly, one should note that the radiation induction of single-base deletions is driven by the presence of adjacent G · C base pairs (Sargentini and Smith, 1994). This can be deduced by assuming that the flanking bases at a deletion site ought to consist of A · T pairs on both sides or G · C pairs on both sides, a quarter of the time in each case. In Table 3, deletions flanked by A · T pairs on both sides were observed in only 2/25 (wild-type) and 0/41 (*umuC*) of the occurrences. Conversely, deletions flanked on both sides by G · C pairs were observed in 16/25 (wild-type) and 23/41 (*umuC*) of the occurrences. Thus, one can conclude that the *umuC* mutant is not deficient in the single-base deletions that are induced by damage at the radiosensitive G · C base pairs.

Frameshift mutagenesis that is *recA*-dependent, but *umuC*-independent, has also been reported for *N*-acetoxy-*N*-2-acetylaminofluorene (AAF) mutagenesis (Koffel-Schwartz et al., 1984; Maenhaut-Michel et al., 1992). However, our results for γ-radiation mutagenesis are quite different. AAF frameshift mutagenesis within repetitive sequences (defined as a base repeated three or more times) is largely inhibited in the *umuC* strain (Koffel-Schwartz et al., 1984), while such mutagenesis was unaffected in our γ-radiation studies. That is, there were six occurrences in the wild-type strain vs. 5.3 in the *umuC* strain (after applying the 0.356 correction factor to the 15 occurrences actually recorded; cf. Tables 3 and 6). Similarly, −1 frameshifts, which are the only type of frameshift that can be detected with the *lacI*^{d} assay (Calos and Miller, 1981), were largely unaffected by the *umuC* mutation (Table 6 and discussed above), while this mutagenesis was strongly dependent on *umuC* in AAF-treated cells (Maenhaut-Michel et al., 1992). Thus, the role of *umuC* in single-base deletion mutagenesis seems to be strongly dependent on the nature of the DNA damage in the case of AAF vs. γ-radiation treatment. Since the misaligned pairing (or replication slippage) model of Streisinger (reviewed in Streisinger and Owen, 1985) is often

cited as the mechanism for frameshifts occurring within repetitive sequences, one can argue that our data suggest that the *umuC* gene may not be generally involved in replication slippage mutagenesis in γ-irradiated cells. However, the *umuC* gene does have a partial effect at specific frameshift sites within repetitive sequences (Sargentini and Smith, 1984), and the reversion of three repetitive sequence frameshift mutations in γ-irradiated cells was enhanced in the presence of plasmid pKM101 (Sargentini and Smith, 1987), which encodes the UmuD and C analogous proteins, MucA and B.

Conclusion

This study confirms our hypothesis (Sargentini and Smith, 1994) that the *umuC* mutation selectively inhibits γ-radiation-induced transversions vs. transitions, and also shows an enhanced deficiency in mutagenesis at G · C vs. A · T sites. These data suggest a modification in the two-stage model of Bridges and Woodgate (1984) for translesion DNA synthesis. That is, these data provide strong evidence that the role of the UmuC/D′ proteins in translesion DNA synthesis is not required, but varies depending on the site of the DNA damage, i.e., G · C vs. A · T, and on the nature of the misincorporated base, i.e., whether it induces a transversion or a transition. Since γ-radiation mutagenesis seems to be completely dependent on RecA protein (Kondo, 1968; Kondo et al., 1970; Sargentini and Smith, 1989), its role in the extension of DNA synthesis beyond a lesion in the template (Bates and Bridges, 1991) must be more fundamental than is the role for the UmuC/D′ proteins.

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