Mutation Research, 211 (1989) 193–203
Elsevier

MTR 04719

Mutational spectrum analysis of *umuC*-independent and *umuC*-dependent γ-radiation mutagenesis in *Escherichia coli*

Neil J. Sargentini and Kendric C. Smith

Department of Radiation Oncology, Stanford University School of Medicine, Stanford, CA 94305 (U.S.A.)

(Received 5 July 1988)
(Revision received 30 August 1988)
(Accepted 7 October 1988)

Keywords: *umuC*; *recA*; Gamma mutagenesis; Mutational spectra

Summary

γ-Radiation mutagenesis (oxic versus anoxic) was examined in wild-type, *umuC* and *recA* strains of *Escherichia coli* K-12. Mutagenesis [*argE3*(Oc) → Arg$^+$] was blocked in a *Δ(recA-srlR)306* strain at the same doses that induced mutations in *umuC122*::Tn*5* and wild-type strains, indicating that both *umuC*-independent and *umuC*-dependent mechanisms function within *recA*-dependent misrepair. Analyses of various suppressor and back mutations that result in *argE3* and *hisG4* ochre reversion and an analysis of *trpE9777* (+1 frameshift) reversion were performed on *umuC* and wild-type cells irradiated in the presence and absence of oxygen. While the *umuC* strain showed the γ-radiation induction of base substitution and frameshifts when irradiated in the absence of oxygen, the *umuC* mutation blocked all oxygen-dependent base-substitution mutagenesis, but not all oxygen-dependent frameshift mutagenesis. For anoxically irradiated cells, the yields of GC → AT [i.e., at the *supB* and *supE*(Oc) loci] and AT → GC transitions (i.e., at the *argE3* and *hisG4* loci) were essentially *umuC* independent, while the yields of (AT or GC) → TA transversions (i.e., at the *supC*, *supL*, *supM*, *supN* and *supX* loci) were heavily *umuC* dependent. These data suggest new concepts about the nature of the DNA lesions and the mutagenic mechanisms that lead to γ-radiation mutagenesis.

Kato and Shinoura (1977) and Steinborn (1978) isolated *umuC* and *umuD* mutants of *Escherichia coli* (Shinagawa et al., 1983) by their lack of mutability by 4-nitroquinoline 1-oxide or UV radiation. Kato and Nakano (1981) reported that the *umuC* mutant was also deficient in γ-radiation mutability. However, we showed that γ-radiation mutagenesis, but not UV-radiation mutagenesis, could be detected in a *umuC122*::Tn*5* strain with certain mutation assays (Sargentini and Smith, 1984). That is, about 30% of the wild-type level of γ-radiation mutagenesis was detected after 10 krad in *umuC* cells by the *argE3*(ochre) → Arg$^+$ and *trpE9777*(+1 frameshift) → Trp$^+$ reversion assay and by the assay for the production of rifampicin-resistant mutants, but no γ-radiation mutagenesis was detected in *umuC* cells with the assay for the production of spectinomycin-resistant mutants. In this study, we have used several

Correspondence: Dr. Neil J. Sargentini, Department of Radiation Oncology, Stanford University School of Medicine, Stanford, CA 94305 (U.S.A.). Tel.: (415) 723-5602.

approaches to gain a better understanding of why γ-radiation mutability is only partially dependent upon the *umuC* gene.

Early studies on the effect of *recA* mutations on radiation and chemical mutagenesis suggested two mechanisms of mutagenesis in *E. coli*: misreplication and misrepair (e.g., Kondo, 1968; Ishii and Kondo, 1975). Misreplication mutagenesis has been defined operationally as *recA*-independent mutagenesis, and is produced by agents (e.g., ethyl methanesulfonate) that alter the coding properties of the DNA template (e.g., Miller, 1983). Conversely, misrepair [or error-prone or SOS repair (Witkin, 1976)] mutagenesis has been synonymous with *recA*-dependent mutagenesis, and is produced by agents (e.g., UV radiation and 4-nitroquinoline 1-oxide) that produce noncoding sites in the template. The *recA* gene is thought to control misrepair largely through its role in the induction of the *umuDC* operon (Bagg et al., 1981) and in the processing of the *umuD* gene product (Nohmi et al., 1988; Burckhardt et al., 1988; Shinagawa et al., 1988). Since the *recA* mutant shows no γ-radiation mutagenesis (Kondo, 1968; Ishii and Kondo, 1975), it seems incongruous that the *umuC* mutant should show partial γ-radiation mutagenesis. However, the data for the *recA* and *umuC* strains were obtained with different mutation assays. Therefore, the *recA* strain needs to be tested with the same mutation assays and the same radiation doses that show partial γ-radiation mutability in the *umuC* strain before one can conclude that all of γ-radiation mutagenesis is the result of misrepair, and that the *recA* gene controls both *umuC*-dependent and *umuC*-independent mutagenesis.

Additional clues concerning the partial dependence of γ-radiation mutagenesis on the *umuC* gene can be derived from an analysis of the oxygen effect on γ-radiation mutagenesis. While irradiation in the presence of oxygen (versus anoxic irradiation) produced a huge enhancement of γ-radiation mutagenesis as measured by a *pur* → Pur^+ assay (Anderson, 1951), it had only a moderate effect on *trpE65* → Trp^+ reversion (Bridges, 1963; Deering, 1963), and no effect on an assay for the production of streptomycin-independent mutants (Anderson, 1951). Bleichrodt and Verheij (1974) have also shown a mutation-assay-dependent variability in the oxygen effect on γ-radiation mutagenesis in bacteriophage ΦX174. These data suggest that the 'oxygen effect' on γ-radiation mutagenesis is not universal, but depends on the nature of the mutation site(s) that is relevant to the mutation assay. Since there appear to be *umuC*-independent and *umuC*-dependent mechanisms for γ-radiation mutagenesis, it seems possible that only one of these mechanisms might show the oxygen effect.

Finally, one complexity with many mutation assays, and especially with nonsense-reversion assays (e.g., Kato et al., 1980), is that more than one base change can lead to the same phenotypic reversion. Therefore, the partial requirement for the *umuC* gene in γ-radiation mutagenesis may be interpreted as either a partial *umuC* dependence at each specific mutation site, or as the net effect of an 'all-or-none' *umuC* dependence at several mutation sites that are simultaneously scored in one mutation assay. Since no γ-radiation mutagenesis has been observed in the *umuC* strain with certain mutation assays (e.g., resistance to spectinomycin; Sargentini and Smith, 1984), we favor the all-or-none requirement for the *umuC* gene in γ-radiation mutagenesis. Therefore, if the total *umuC* dependence of the γ-radiation induction of spectinomycin-resistant mutants is not an unusual case, we would expect that analyses of mutagenesis at several sites ought to show a total *umuC* dependence at some base-pair sites and no *umuC* dependence at other base-pair sites.

To increase our understanding of the partial requirement for the *umuC* gene in γ-radiation mutagenesis, we have attempted to detect γ-radiation mutagenesis in *recA* cells with a mutation assay that has previously been used to demonstrate γ-radiation mutagenesis in *umuC* cells, and we have employed a mutational spectrum analysis (modified from that described by Shinoura et al., 1983a) to determine the requirement of the *umuC* gene in oxic versus anoxic γ-radiation mutagenesis at several specific base-pair targets.

Materials and methods

Bacteria and bacteriophage

Bacterial strains used are listed in Table 1. Bacteriophage P1 transductions were performed

TABLE 1

E. coli K-12 STRAINS USED

Stanford Radiology No.	Genotype [a]	Source, reference or derivation [b]
SR710	*metB1 lacY1 malA1 thi hemA8 rpsL134* $F^- \lambda^-$	S729, T. Kato
SR749	*argE3 hisG4 leuB6 Δ(gpt-proA)62 thr-1 thi-1 ara-14 galK2 lacY1 mtl-1 xyl-5 tsx-33 rfbD1 mgl-51 kdgK51 rpsL31 supE44 rac* $F^- \lambda^-$	AB1157, B.J. Bachmann
SR1018	*umuC122*::Tn*5*, otherwise as SR749	GW2100, G.C. Walker
SR1165	*umuC122*::Tn*5*, otherwise as SR749	Sargentini and Smith, 1984
SR1285	*trpE9777*, otherwise as SR749	Sargentini and Smith, 1987
SR1314	*umuC122*::Tn*5 trpE9777*, otherwise as SR749	SR1285 × P1::Tn*9 cts* · SR1018, Kn^r
SR1438	*Δ(recA-srlR)306 srlR301*::Tn*10* (λ *recA*$^+$ *att*$^+$ *int*$^+$ *cI*$^+$), otherwise as SR749	EST945, E.S. Tessman
SR1914	Arg^+ *metB1*, otherwise as SR749	SR749 × P1::Tn*9 cts* · SR710, Arg^+
SR2151	Hfr Hayes *thi-1 lacI22 lacZ13*(Oc) *gluU69*(*supB*) *relA1 spoT1*	CA165, B.J. Bachmann
SR2153	*thi-1 ara-13 lacI22*(Oc) *malA1 mtl-2 xyl-7 supV rpsL135* $F^- \lambda$?	2320R8, A.J. Clark
SR2154	Hfr P02A *phoA5*(Oc) *supC47 rel-1 tonA22* tto(T2r)?	H12R7a, A.J. Clark
SR2155	Hfr AB312 *argH1 ilvD188*(Oc) *purF1 xyl-7 rfbD1 glnV44*(*supE*) *lysT102*(*supL2*)	AB2300, B.J. Bachmann
SR2156	*thi-1 supQ80 relA1 spoT1* λ^-	Hfr3000, B.J. Bachmann
SR2157	*hisG4*(Oc) *ilvD145 metE46 Δ(gpt-proA)62 trp-3*(Oc) *thi-1 ara-9 galK2 lacY1*(or *Z4*) *malT1*(λ^r) *mtl-1 ton-1 tsx-3 rpsL8*(or *9*) *supE44 supH11* $F^- \lambda^-$	AB2294, B.J. Bachmann
SR2159	Hfr AB312 *argH1 ilvD188*(Oc) *purF1 xyl-7 supE44 supO1*	AB2275, B.J. Bachmann
SR2160	Hfr Cavalli *phoA6*(Oc) *pit-10 lysT46*(*supG*) *tonA22 ompF627 relA1 spoT1* $T2^r$	U11R1d, B.J. Bachmann
SR2161	*hisG4*(Oc) *ilvD188*(Oc) *metE46 trp-3*(Oc) *thi-1 ara-9 lacZ13*(Oc) *malA1*(λ^r) *mtl-1 ton-1*? *tsx-3*? *rpsL8*(or *9*) *tyrU20*(*supM*) $F^- \lambda^-$	AB2577, B.J. Bachmann
SR2162	Hfr AB312 *argH1 ilvD188*(Oc) *purF1 xyl-7 supE44 supN23*	AB2547, B.J. Bachmann
SR2290	*trpE9777 srlR301*::Tn*10*, otherwise as SR749	SR1285 × P1 *vir*a · SR1438 [c], Tc^r
SR2291	*Δ(recA-srlR)306 trpE9777 srlR301*::Tn*10*, otherwise as SR749	Same as for SR2290

[a] Genotype nomenclature is that used by Bachmann (1983).

[b] Kn^r and Tc^r indicate resistance to kanamycin and tetracycline, respectively.

[c] P1 *vir*a was obtained from A.J. Clark (University of California at Berkeley) and is a reisolate of P1 *vir*.

generally as described by Miller (1972). Bacteriophage T4 strains were kindly provided by A. Templin (University of California at Berkeley).

Media

YENB was yeast extract (Difco) at 0.75% and nutrient broth (Difco) at 0.8%. λ-Broth, L-broth top agar, and L-broth agar plates have been described (Kato et al., 1977). Arg-0 was a 0.4% glucose-salts medium (Ganesan and Smith, 1968), supplemented with histidine, leucine, proline, threonine and tryptophan (if required) all at 1 mM, thiamine · HCl at 0.5 μg/ml, and Bacto agar (Difco) at 1.5%, and was dispensed at 27 ml/petri dish. His-0 and Trp-0 plates were prepared as Arg-0 plates, but contained 1 mM arginine in place of histidine or tryptophan, respectively. Arg-1.5, His-1.5, and Trp-1 plates were Arg-0, His-0, and Trp-0 plates that also contained YENB at 1.5%, 1.5%, or 1% (v/v), respectively. PB was

Na_2HPO_4 at 5.83 g/l and KH_2PO_4 at 3.53 g/l, pH 7.0.

Preparation and irradiation of cells

Logarithmic-phase cells were prepared by diluting (1 : 500) an overnight culture into YENB and shaking at 37 °C until an optical density (OD) at 650 nm of 0.4 was attained. Cultures were pelleted by centrifugation (6 min, 6000 × *g*), washed twice, and resuspended in PB at an OD of 5, which corresponded to approximately 1×10^9 colony-forming units (CFU)/ml. Gamma (^{137}Cs) irradiation procedures have been described (Sargentini and Smith, 1983). Cells were bubbled with air or N_2, before (3 or 10 min, respectively) and during the irradiation.

Mutation assays

Mutant-selection plates, with or without broth supplementation, were spread with 0.2-ml volumes of undiluted cells to score the expression of mutants, while diluted cells were spread on broth-supplemented plates to determine viability. [The broth supplementation serves not only to allow a limited amount of cell growth to allow mutation fixation and expression, but it also prevents the shift-down killing that would be observed if these broth-grown γ-irradiated cells were plated on minimal medium (Sargentini et al., 1983).] The mutation assays were based on the reversion of either the *argE3* or *hisG4* ochre mutations (independent selections) (Kato and Shinoura, 1977) or the reversion of the sequenced *trpE9777*(+1 frameshift) mutation (Bronson and Yanofsky, 1974).

Indirect sequence determination of Arg$^+$ and His$^+$ mutants

This is modified from procedures for His$^+$ mutants described earlier (Kato et al., 1980; Shinoura et al., 1983a). Independent Arg$^+$ or His$^+$ mutants were transferred (patched) from the mutant-selection plates to Arg-0 or His-0 plates, respectively. After 2 days of growth at 37 °C, Arg$^+$ or His$^+$ cells from the patches were inoculated into 2-ml volumes of λ-broth, which were incubated for about 16 h at 37 °C with aeration. 0.1-ml samples of the λ-broth cultures were mixed with 2.5-ml portions of L-broth top agar and poured over L-broth agar plates. The plates were stored at room temperature for up to 2 h, and then 1.5-μl volumes of λ-broth containing bacteriophage T4 (1×10^7 plaque-forming units/ml) were spotted onto the soft agar surface; a set

TABLE 2

NUCLEOTIDE SEQUENCE CHANGE ASSOCIATED WITH THE Arg$^+$ OR His$^+$ PHENOTYPE OF REVERTANTS OF *E. coli* AB1157(*argE3 hisG4*)

Lysis of Arg$^+$ or His$^+$ cells by bacteriophage T4 strains [a]						Site of mutation [b]	Mutation [b]
WT	B17	NG19	oc427	ps292	ps205		
+	+	−	+	+	+	*supB*	GC → AT
+	−	−	+	+	+	*supE*(Oc)	GC → AT
+	+	−	−	−	−	*argE3* or *hisG4*	AT → GC
+	+	+	+	+	−	*supC, M*	(GC or AT) → TA
+	+	−	+	+	−	*supL, N*	AT → TA
+	+	−	+	−	−	*supX*	(GC or AT) → TA

[a] Lysis (+) or nonlysis (−) was determined by placing 1.5×10^4 PFU on a soft-agar lawn of Arg$^+$ or His$^+$ cells and incubating overnight. The T4 strains used were wild-type (WT), amber (B17 and NG19), and ochre (oc427, ps292, ps205).

[b] Suppressors were named by matching the T4 sensitivity pattern of an Arg$^+$ transductant of the AB1157 recipient strain (SR749) with the pattern of the donor strain [*supB* (SR2151), *supL* (SR2155), *supN* (SR2162), *supM* (SR2161), *supC* (SR2154), *supE*(Oc) (SR2153)], which carried a known suppressor mutation. We did not test a known *supX* strain, but interpreted the T4 sensitivity pattern from data presented in Kato et al. (1980). The *supG* (SR2160) and *supO* (SR2159) mutants gave the same T4 sensitivity pattern as the *supL* and *supC* mutants, respectively. Since these pairs of suppressor genes also show the same map locations, respectively (Bachmann, 1983), we assume that they are the same genes. The *supH* (SR2157) and *supQ* (SR2156) mutations were also tested, but did not seem to suppress the *argE3* mutation.

[c] See Table 3.

TABLE 3

ASSOCIATION OF SUPPRESSOR AND BACK MUTATIONS WITH DNA SEQUENCE CHANGES

Codon Recognized by the tRNA	Suppressor recognizing UAA [a]	Mutation consistent with change in codon recognition
CAA	*supB* (*glnU*)	GC → AT
AAA	*supL, N* (*lysT, V*)	AT → TA
GAA	*supX*? (*glt*)	GC → TA
UUA	*supX*? (*leu*)	AT → TA
UCA	*supX*? (*ser*)	GC → TA
UGA	None	-
UAU	*supM* (*tyrU*)	AT → TA
UAC	*supC* (*tyrT*)	GC → TA
UAG	*supE*(Oc) (*glnV*)	GC → AT

Back mutations: *argE3* → *arg*$^+$, *hisG4* → *his*$^+$
Codon change: AT → GC [b]

[a] Although the *supX* mutation (Kato et al., 1980) has not yet been mapped, it will probably be found to occur in either the *glt*, *leu* or *ser* genes; this putative tRNA mutation should not be confused with the *supX* mutations that occur in the *topA* gene (e.g., Bachmann, 1983). The tRNA genes in parentheses are according to Bachmann (1983) and Uemura et al. (1985).

[b] Shinoura et al. (1983a) concluded that ochre back mutants represent transitions because N^4-hydroxycytidine, which produces only AT → GC transitions, was only able to induce back mutants of *argE3*(Oc) and *hisG4*(Oc).

of 6 T4 strains were spotted per plate. After the spots had dried (~ 15 min), the plates were inverted and incubated overnight at 37 °C. The patterns of bacteriophage sensitivity and their meanings are given in Tables 2 and 3. To test the validity of the designation of Arg$^+$ back mutants by this procedure, bacteriophage P1 *vir*a was propagated on 98 Arg$^+$ mutants that were also to be characterized by their pattern of sensitivity to the bacteriophage T4 strains. The P1 lysates were used to transduce a *metB*(Arg$^+$) strain (SR1914) to Met$^+$, of which 20 transductants per lysate were then tested for a requirement for arginine (Arg$^-$). The ability to donate Arg$^-$ means that the P1 was grown on a suppressor mutant; alternately, the inability to donate Arg$^-$ suggests that the P1 was grown on a back mutant, i.e., it carried an intact *argE* gene. The results were that 65 of 71 (92%) of the suppressor mutants that were indicated by the T4 test were confirmed by the P1 test, and 26 of 27 (96%) of the back mutants that were indicated by the T4 test were confirmed by the P1 test. Please note that we have previously considered that the Arg$^+$ mutants that were still His$^-$ must be back mutants (Sargentini and Smith, 1984). We now know from our T4 test that some of these putative back mutants were probably *supX* suppressor mutants.

Results

γ-Radiation mutagenesis was assayed in wild-type (*umuC*$^+$ *recA*$^+$), *umuC122*::Tn*5*, and Δ(*recA-srlR*)*306* strains of *E. coli* K-12 using the *argE3*(Oc) → Arg$^+$ mutation assay, and using radiation doses selected such that the *recA* strain would show greater than 20% survival. The results in Table 4 (especially note the 8th and 10th columns) indicate that the *recA* gene is absolutely required for oxic and anoxic γ-radiation mutagenesis, as measured by the same ochre reversion assay that shows only a partial requirement for the *umuC* gene. The less sensitive *trpE9777*(+1 Fs) → Trp$^+$ mutation assay also seemed to show less γ-radiation mutagenesis in the *recA* strain versus the *umuC* and wild-type strains, but the differences in mutagenesis were not statistically significant at doses ⩽ 5 krad (data not shown).

When the *umuC* strain was tested for mutagenesis over a wider γ-radiation dose range than used in Table 4, it showed less γ-radiation mutagenesis than wild-type cells, whether irradiated oxically or

TABLE 4

γ-RADIATION MUTAGENESIS [*argE3*(Oc) → Arg$^+$] OF *E. coli* K-12 STRAINS

Irradiation gassing conditions	γ-radiation dose (krad)	Relevant genotype [a]	M_t [b]	M_{po} [c]	M_o [d]	Surviving fraction	M_x [e]	Viable cells/plate	Mutants/10^8 viable cells ±1 SD [f]
Air	2.5	WT	42	14	3	0.94	28	2.8×10^8	10±5
		umuC	28	10	3	1.04	18	2.7×10^8	7±3
		recA	11	14	2	0.21	−1	3.4×10^7	−3±4
N_2	2.5	WT	52	13	3	0.94	39	2.8×10^8	14±1
		umuC	32	10	3	0.97	22	2.6×10^8	9±3
		recA	15	13	2	0.64	3	9.6×10^7	2±2
	5.0	WT	72	13	3	1.02	59	3.0×10^8	20±2
		umuC	51	10	3	0.99	41	2.7×10^8	17±8
		recA	11	13	2	0.28	−1	4.2×10^7	−2±6

[a] The wild-type strain (WT) is SR2290, a cotransductant of the *recA* strain (SR2291). The *umuC* strain is a kanamycin-resistant (*umuC122*::Tn*5*) derivative of the parent of SR2290.

[b] M_t is the average number of mutant colonies arising from irradiated cells per Arg-1.5 plate; 8 plates for *recA*, 4 plates for wild-type and *umuC* strains; 3–4 Expts./strain.

[c] M_{po} is as M_t but is for nonirradiated cells.

[d] M_o is as M_{po} but is for Arg-0 plates.

[e] M_x is equal to $M_t - M_{po}M_o(1 -$ surviving fraction).

[f] The mutant frequency is equal to $(M_x)(1\times10^8)$/(viable cells/plate); see Sargentini and Smith (1980) for details. (In brief, the frequency of radiation-induced mutants has been corrected for spontaneous 'plate' mutants, and for the death of pre-existing spontaneous mutants.) These data are averages of the calculated mutant frequencies from the individual experiments rather than a direct calculation of the averaged data in this table.

anoxically (Fig. 1a–c). The *umuC* strain did not show the oxygen enhancement of γ-radiation-induced base substitution that was seen in the wild-type strain (Fig. 1a and b); however, both strains showed the oxygen enhancement of radiation-induced frameshift reversion (Fig. 1c) and cell killing (Fig. 1d).

Reversion of *argE3* and *hisG4* ochre mutations can be accomplished at the ochre site (a back mutation) or by a second mutation at any one of several sites (suppressor mutations; Kato et al., 1980). With this complexity of mutation sites in mind, one can imagine that the roles of oxygen and of the *umuC* gene in γ-radiation mutagenesis might be dependent on the specific base pairs involved in mutagenesis at each site. To test this point, spectral analyses of γ-radiation-induced ochre reversions in the wild-type and *umuC* strains were performed. We have modified the procedure of Shinoura et al. (1983a) (as described in the Materials and methods section) to make it somewhat more convenient and also applicable to *argE3* → Arg$^+$ reversion. We prefer Arg$^+$ mutants for the spectral analysis of mutagenesis because 6 kinds of Arg$^+$ mutants versus 2 kinds of His$^+$ mutants were identified in γ-irradiated cells (Table 5). The results of the analyses of the Arg$^+$ and His$^+$ mutants that were produced in the wild-type and *umuC* strains, either spontaneously or when γ-irradiated (30 krad) oxically or anoxically, are given in Table 5. Ratios of these data that define the relative effect of the presence of oxygen during irradiation for either wild-type or *umuC* cells, and the relative effect of the *umuC* mutation for either oxic or anoxic irradiation mutagenesis are listed in the lower portion of Table 5. These data indicate that the presence of oxygen during the γ-irradiation of wild-type cells either had no effect or it enhanced mutagenesis up to 9-fold depending upon the specific mutation scored; no oxygen effect on mutagenesis was observed in *umuC* cells. For anoxically irradiated cells, the requirement for the *umuC* gene in γ-radiation mutagenesis was very small at the *supB, supE*(Oc), *argE3,* and *hisG4* sites (where transition mutations should be produced), while the requirement for the *umuC* gene was very large at the *supC* or *M, supL* or *N,* and *supX* sites (where transversion mutations

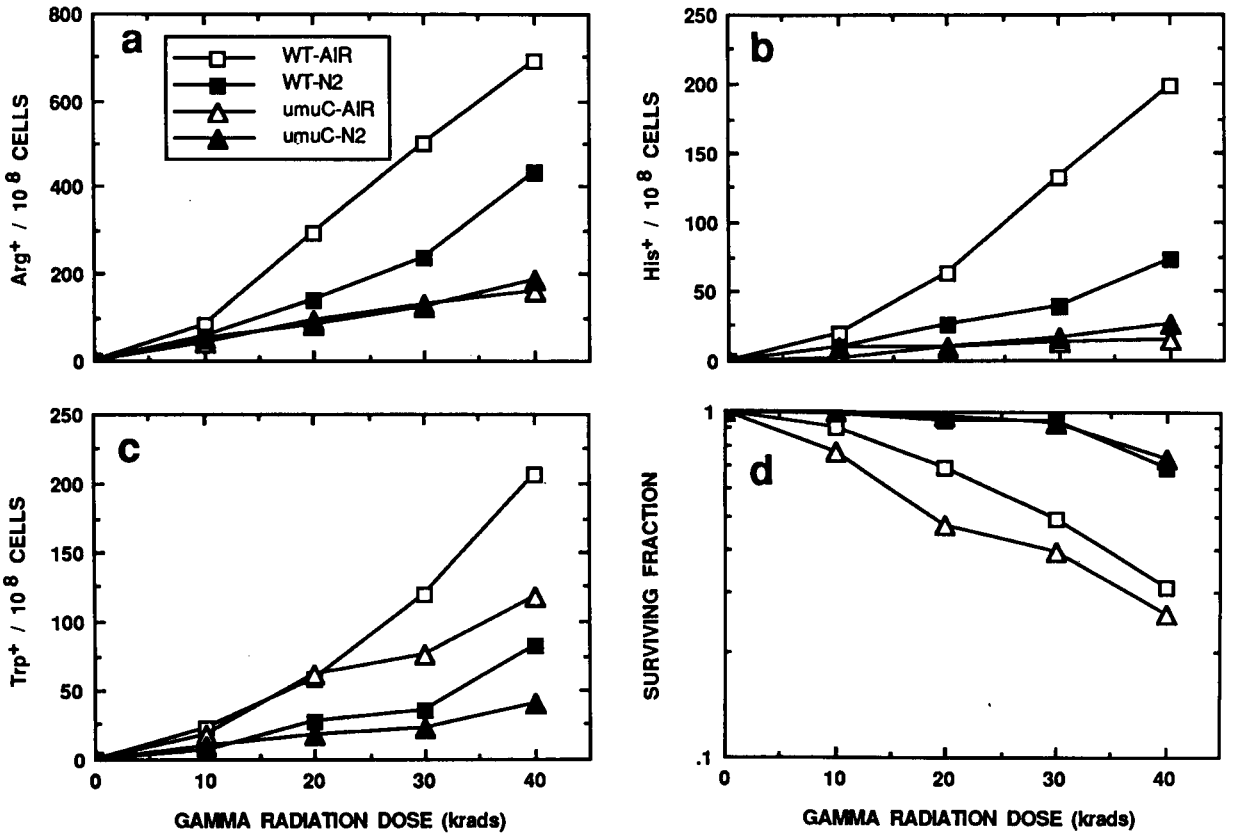

Fig. 1. γ-Radiation mutagenesis and survival for *E. coli umuC*$^+$ (□, ■) and *umuC122*::Tn*5* (△, ▲) cells, irradiated in the presence (open symbols) or absence (closed symbols) of O_2. Mutagenesis was assayed by reversion assays: *argE3*(Oc) → Arg$^+$ (a); *hisG4*(Oc) → His$^+$ (b); and *trpE9777*(+1 Fs) → Trp$^+$ (c). Survival (d) was determined by plating diluted cells on mutant-selection plates. Data are the means from 3 Expts./strain.

TABLE 5

EFFECTS OF THE *umuC* MUTATION AND OXIC IRRADIATION CONDITIONS ON γ-RADIATION MUTAGENESIS OF *E. coli*

Comparison of mutant frequencies	Relevant genotype [a]	Irradiation gassing condition	Mutants/10^8 cells induced by 30 krad [b]							
			supB (Arg$^+$)	*supE*(Oc) (Arg$^+$)	*argE3* (Arg$^+$)	*hisG4* (His$^+$)	*supC, M* (Arg$^+$)	*supL, N* (Arg$^+$)	*supL, N* (His$^+$)	*supX* (Arg$^+$)
	WT	Air	49	34	119	88	95	80	44	125
		N_2	35	10	70	35	36	18	5	66
	umuC	Air	31	8	62	12	3	2	2	21
		N_2	29	10	56	16	2	2	1	23
WT: Air/N_2			1	3	2	2	3	4	9	2
umuC: Air/N_2			1	1	1	1	1	1	2	1
Air: WT/*umuC*			2	4	2	7	32	40	22	6
N_2: WT/*umuC*			1	1	1	2	18	9	5	3
Putative base changes			GC → AT		AT → GC		(AT or GC) → TA			
			Transitions				Transversions			

[a] The wild-type (WT) strain was SR749; the *umuC* strain was SR1165, a transductant derived from SR749.

[b] The types of mutants (e.g., *supB*) and their base substitutions were determined as described in Methods. The number of each type of mutant was determined in a sample from each of the M_o, M_{po} and M_t categories, and these are listed in Table 6. Fractional values were calculated and applied to the 'mean total mutants per plate' (Table 6), to determine M_o, M_{po}, and M_t values for each type of mutant (e.g., *supB*). These data and other values in Table 6 were used to calculate (as in Table 4) the induced mutant frequencies shown here.

TABLE 6

DATA USED TO CALCULATE THE INDUCED MUTANT FREQUENCIES PRESENTED IN TABLE 5

Strain [a]	Mutants selected	Irradiation gassing condition	Data term [b]	Cell surviving fraction after 30 krad	Mean viable cells spread per plate	Mean total mutants/plate	Total mutants classified [c]	Number of mutants/class [c]					
								Back	*supB*	*supE*(Oc)	*supC*	*supL*	*supX*
WT	Arg^+	–	M_o	–	–	6.3	150	27	20	4	6	70	23
		–	M_{po}	–	–	19.7	295	79	66	17	35	81	17
		Air	M_t	0.62	1.28×10^8	644.8	245	68	29	12	49	35	52
		N_2	M_t	0.96	2.13×10^8	515.0	241	80	36	9	36	19	61
	His^+	–	M_o	–	–	3.3	99	28	0	0	0	71	0
		–	M_{po}	–	–	11.5	277	176	0	0	0	101	0
		Air	M_t	0.62	1.28×10^8	177.2	251	168	0	0	0	83	0
		N_2	M_t	0.96	2.13×10^8	93.7	251	208	0	0	0	43	0
umuC	Arg^+	–	M_o	–	–	2.5	58	18	19	6	1	9	5
		–	M_{po}	–	–	11.7	271	93	86	20	19	11	42
		Air	M_t	0.50	1.03×10^8	140.5	244	119	60	15	6	3	41
		N_2	M_t	1.04	1.85×10^8	234.7	251	117	62	21	4	3	44
	His^+	–	M_o	–	–	1.2	24	23	0	0	0	1	0
		–	M_{po}	–	–	6.3	149	136	0	0	0	13	0
		Air	M_t	0.50	1.03×10^8	19.5	114	102	0	0	0	12	0
		N_2	M_t	1.04	1.85×10^8	36.5	199	190	0	0	0	9	0

[a] As in Table 5.

[b] Data terms are defined in Table 4.

[c] 'Total mutants' and 'mutants per class' are the sums from 3 Expts. and were classified by the indirect sequence determination procedure described in Methods.

should be produced). In addition, all of the mutant base pairs at the suppressor loci were AT, while the mutant base pairs at the back mutation sites were GC (Table 5).

Discussion

The concept has developed that the mechanism of mutagenesis (i.e., misreplication or misrepair) for any mutagen can be determined by testing whether its mutagenicity in *E. coli* depends upon the *recA* gene, i.e., misrepair is *recA* dependent but misreplication is not (Kondo, 1968; Ishii and Kondo, 1975; reviewed in Witkin, 1976). However, *recA* mutations affect many phenomena in addition to mutagenesis (reviewed in Walker, 1984). With the discovery of the *umuC* and *umuD* mutations (Kato and Shinoura, 1977; Steinborn, 1978; Shinagawa et al., 1983), which seem to only abolish misrepair, it has been considered preferable to test for *umuC* dependence rather than for *recA* dependence when one is trying to ascertain the basis of the mutagenicity of a new agent (e.g., Schendel and Defais, 1980; Shinoura et al., 1983b). However, this preference for a *umuC* test over a *recA* test may be leading to incorrect conclusions. Cases in point are the following: (i) The *recA* strain was not mutated by methyl methanesulfonate (Kondo et al., 1970; Walker, 1977), while the *umuC* strain showed 30% of the mutagenesis seen in the wild-type strain when assayed by reversion of the *argE3* mutation (Schendel and Defais, 1980). [N.B., A *umuC* strain did not show the methyl methanesulfonate-induced reversion of the *hisG4* mutation (Walker and Dobson, 1979).] (ii) The *recA* strain was not mutated (a rifampicin-resistance assay) by the alkylating agent, streptozotocin (a monofunctional nitrosourea), while the *umuC* strain showed the wild-type level of mutagenesis (Fram et al., 1986). (iii) UV-irradiated *recA* strains did not show the mutagenesis of nonirradiated bacteriophage λ (indirect mutagen-

esis), while *umuC* strains did show indirect mutagenesis (Maenhaut-Michel and Caillet-Fauquet, 1984; Wood and Hutchinson, 1984). (iv) The *recA* strain was not mutated by γ-radiation when assayed either by *arg*(Am) reversion or by assays for the production of large deletions (Kondo, 1968; Ishii and Kondo, 1975), or in the present work when assayed by *arg*(Oc) reversion (Table 4). However, the *umuC* strain showed mutagenesis after the same radiation doses and with the same *arg*(Oc) reversion assay that failed for the *recA* strain (Table 4). These results reaffirm that γ-radiation mutagenesis is totally dependent on misrepair, and they suggest that the *recA* gene controls mutagenesis via both *umuDC*-independent and *umuDC*-dependent mechanisms.

One might expect that different mutagenic mechanisms would be specific for different kinds of DNA lesions, and that a different spectrum of mutations would result from the action of each mechanism. Consistent with this notion, our data suggest some unifying concepts and predictions about the mechanisms for γ-radiation mutagenesis.

The additional mutagenic lesions that are produced in the presence of oxygen all require the *umuC* gene for the production of base substitutions. The types of alterations produced in pyrimidines by γ-irradiation are affected markedly by the presence of oxygen (Teoule, 1987). For example, 7 radiolytic products of thymine are produced in DNA only in the presence of oxygen: the hydroperoxides of thymine and their degradation products (e.g., urea), and 5-hydroxymethyl uracil (reviewed in Teoule, 1987). In fact, 5-hydroxymethyl-2′-deoxyuridine produces base substitutions at AT and GC sites when it is present in bacterial-culture media, and its mutagenicity depends on the presence of the *mucAB* genes, which are analogues of the *umuDC* genes (Shirname-More et al., 1987). Thus, 5-hydroxymethyl uracil seems to be one candidate for producing *umuC*-dependent, oxygen-dependent γ-radiation-induced base substitutions.

Our results also suggest that the yield of DNA lesions that cause *umuC*-independent mutagenesis is *not affected by oxygen* (Table 5). Among the thymine radiolysis products, only 5,6-dihydroxy-5,6-dihydrothymine (thymine glycol) is produced both in the presence and in the absence of oxygen (Teoule, 1987). However, this type of damage does not seem to be mutagenic even though it does block the replication fork (e.g., Laspia and Wallace, 1988). Another possibility is *trans*-5,6-dihydroxy-5,6-dihydrouracil (uracil glycol). This cytosine-derived base damage is associated with the production of C → T transitions (Ayaki et al., 1987), which we find to be *umuC*-independent in our anoxically irradiated cells (Table 5). Finally, the same kinds of purine radiolytic products are produced whether oxygen is present or not (R. Teoule, personal communication), which suggests that damaged purines must also be considered as a source of the oxygen-independent, *umuC*-independent mutagenesis that we have observed.

Regardless of which DNA lesions are responsible for *umuC*-independent mutagenesis (transitions) in anoxically irradiated cells, we predict that another kind of lesion must be produced in anoxically irradiated cells to explain the *umuC*-dependent transversions that are produced. Apurinic/apyrimidinic site mutagenesis is *umuC* dependent (Schaaper et al., 1982). These lesions are produced directly in DNA by γ-irradiation (Ullrich and Hagen, 1971), and they are also transiently present during the repair of γ-radiation-induced base damage (e.g., Breimer and Lindahl, 1985). It would seem more than a coincidence that our spectral analysis shows that adenine is always part of the mutant base-pair for *umuC*-dependent anoxic γ-radiation mutagenesis (Table 5). It is known from studies on apurinic-site mutagenesis that the *umuC*-dependent mechanism shows a strong preference for inserting adenine when it encounters an apurinic/apyrimidinic site in the template strand (e.g., Kunkel, 1984). Further, if the lesion relevant to *umuC*-dependent anoxic γ-radiation mutagenesis is a damaged purine rather than an apurinic site, then the tendency for damaged purines to lead to transversions via SOS repair (Rabkin et al., 1983) provides an even better explanation for our data on *umuC*-dependent γ-radiation mutagenesis in anoxically irradiated cells.

Even though base-substitution and frameshift mutagenesis are similar in being totally *umuC* dependent in UV-irradiated cells, and in being only partially *umuC* dependent in γ-irradiated

cells (Sargentini and Smith, 1984), the *umuC* gene seems to play a different role in base-substitution versus frameshift mutagenesis. The *umuC* gene is required for the oxygen effect on base substitutions but not for the oxygen effect on frameshifts (Fig. 1a–c). Also, the UV-radiation induction of base substitutions, but not of frameshifts, is enhanced in *umuC* cells by a delayed photoreactivation procedure (Sargentini and Smith, 1987).

In support of the notion that there is a site-specific, all-or-none requirement for the *umuC* gene in γ-radiation mutagenesis, we found little or no requirement for the *umuC* gene at the *supB, supE*(Oc), *argE3* and *hisG4* sites, while there was a large requirement for the *umuC* gene at the *supC* or *M, supL* or *N*, and *supX* sites in anoxically irradiated cells (Table 5).

In conclusion, our data for anoxically irradiated cells support the hypothesis that γ-radiation produces two kinds of DNA lesions that require *recA*-dependent misrepair to induce mutations. For base-substitution mutagenesis, one kind of lesion requires the *umuC* gene and produces transversion mutations, while a second kind of lesion produces transition mutations and does not require the *umuC* gene. For cells irradiated in the presence of oxygen, there seems to be additional kinds of lesions whose mutagenic potential for base substitutions (but not frameshifts) is completely dependent on the *umuC* gene.

Acknowledgements

We are grateful to Dr. Tzu-chien V. Wang for valuable discussions, and to Cheryl A. Cheng for skillful technical assistance.

This investigation was supported by Public Health Service Grant CA-33738 from the National Cancer Institute, DHHS.

References

Anderson, E.H. (1951) The effect of oxygen on mutation induction by X-rays, Proc. Natl. Acad. Sci. (U.S.A.), 37, 340–349.

Ayaki, H., O. Yamamoto and S. Sawada (1987) Role of the main cytosine radiolytic product in ionizing radiation-induced mutagenesis, J. Radiat. Res., 28, 254–261.

Bachmann, B.J. (1983) Linkage map of *Escherichia coli* K-12, edition 7, Microbiol. Rev., 47, 180–230.

Bagg, A., C.J. Kenyon and G.C. Walker (1981) Inducibility of a gene product required for UV and chemical mutagenesis in *Escherichia coli,* Proc. Natl. Acad. Sci. (U.S.A.), 78, 5749–5753.

Bleichrodt, J.F., and W.S.D. Verheij (1974) Influence of oxygen on the induction of mutations in bacteriophage ΦX174 by ionizing radiation, 25, 505–512.

Breimer, L.H., and T.Lindahl (1985) Enzymatic excision of DNA bases damaged by exposure to ionizing radiation or oxidizing agents, Mutation Res., 150, 85–89.

Bridges, B.A. (1963) Effect of chemical modifiers on inactivation and mutation-induction by gamma radiation in *Escherichia coli,* J. Gen. Microbiol., 31, 405–412.

Bronson, M.J., and C. Yanofsky (1974) Characterization of mutations in the tryptophan operon of *Escherichia coli* by RNA nucleotide sequencing, J. Mol. Biol., 88, 913–916.

Burckhardt, S.E., R. Woodgate, R.H. Scheurermann and H. Echols (1988) UmuD mutagenesis protein of *Escherichia coli*: overproduction, purification and cleavage by RecA, Proc. Natl. Acad. Sci. (U.S.A.), 85, 1811–1815.

Deering, R.A. (1963) Mutation and killing of *Escherichia coli* WP-2 by accelerated heavy ions and other radiations, Radiat. Res., 19, 169–178.

Fram, R.J., J. Sullivan and M.G. Marinus (1986) Mutagenesis and repair of DNA damage caused by nitrogen mustard, *N,N'*-bis(2-chloroethyl)-*N*-nitrosourea (BCNU), streptozotocin, and mitomycin C in *E. coli,* Mutation Res., 166, 229–242.

Ganesan, A.K., and K.C. Smith (1968) Dark recovery processes in *Escherichia coli* irradiated with ultraviolet light, I. Effect of *rec*$^-$ mutations on liquid holding recovery, J. Bacteriol., 96, 365–373.

Ishii, Y., and S. Kondo (1975) Comparative analysis of deletion and base-change mutabilities of *Escherichia coli* B strains differing in DNA repair capacity (*wild type, uvrA*$^-$, *polA*$^-$, *recA*$^-$) by various mutagens, Mutation Res., 27, 27–44.

Kato, T., and E. Nakano (1981) Effects of the *umuC36* mutation on ultraviolet-radiation-induced base-change and frameshift mutations in *Escherichia coli,* Mutation Res., 83, 307–319.

Kato, T., and Y. Shinoura (1977) Isolation and characterization of mutants of *Escherichia coli* deficient in induction of mutations by ultraviolet light, Mol. Gen. Genet., 156, 121–131.

Kato, T., R.H. Rothman and A.J. Clark (1977) Analysis of the role of recombination and repair in mutagenesis of *Escherichia coli* by UV irradiation, Genetics, 87, 1–18.

Kato, T., Y. Shinoura, A. Templin and A.J. Clark (1980) Analysis of ultraviolet light-induced suppressor mutations in the strain of *Escherichia coli* K-12 AB1157: An implication for molecular mechanisms of UV mutagenesis, Mol. Gen. Genet., 180, 283–291.

Kondo, S. (1968) Mutagenicity versus radiosensitivity in *Escherichia coli,* Proc. 12th Int. Congr. Genet., 2, 126–127.

Kondo, S., H. Ichikawa, K. Iwo and T. Kato (1970) Base-change mutagenesis and prophage induction in strains of *Escherichia coli* with different DNA repair capacities, Genetics, 66, 187–217.

Kunkel, T.A. (1984) Mutational specificity of depurination, Proc. Natl. Acad. Sci. (U.S.A.), 81, 1494–1498.

Laspia, M.F., and S.S. Wallace (1988) Excision repair of thymine glycols, urea residues, and apurinic sites in *Escherichia coli,* J. Bacteriol., 170, 3359–3366.

Maenhaut-Michel, G., and P. Caillet-Fauquet (1984) Effect of *umuC* mutations on targeted and untargeted ultraviolet mutagenesis in bacteriophage λ, J. Mol. Biol., 177, 181–187.

Miller, J.H. (1972) Experiments in Molecular Genetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Miller, J.H. (1983) Mutational specificity in bacteria, Annu. Rev. Genet., 17, 215–238.

Nohmi, T., J.R. Battista, L.A. Dodson and G.C. Walker (1988) RecA-mediated cleavage activates UmuD for mutagenesis: mechanistic relationship between transcriptional derepression and posttranslational activation, Proc. Natl. Acad. Sci. (U.S.A.), 85, 1816–1820.

Rabkin, S.D., P.D. Moore and B.S. Strauss (1983) In vitro bypass of UV-induced lesions by *Escherichia coli* DNA polymerase I: Specificity of nucleotide incorporation, Proc. Natl. Acad. Sci. (U.S.A.), 80, 1541–1545.

Sargentini, N.J., and K.C. Smith (1980) Involvement of genes *uvrD* and *recB* in separate mutagenic deoxyribonucleic acid repair pathways in *Escherichia coli* K-12 *uvrB5* and B/r *uvrA155,* J. Bacteriol., 143, 212–220.

Sargentini, N.J., and K.C. Smith (1983) Characterization of an *Escherichia coli* mutant (*radB101*) sensitive to γ and UV radiation, and methyl methanesulfonate, Radiat. Res., 93, 461–478.

Sargentini, N.J., and K.C. Smith (1984) *umuC*-dependent and *umuC*-independent γ- and UV-radiation mutagenesis in *Escherichia coli,* Mutation Res., 128, 1–9.

Sargentini, N.J., and K.C. Smith (1987) Ionizing and ultraviolet radiation-induced reversion of sequenced frameshift mutations in *Escherichia coli*: a new role for *umuDC* suggested by delayed photoreactivation, Mutation Res., 179, 55–63.

Sargentini, N.J., W.P. Diver and K.C. Smith (1983) The effect of growth conditions on inducible *recA*-dependent resistance to X rays in *Escherichia coli,* Radiat. Res., 93, 364–380.

Schaaper, R.M., B.W. Glickman and L.A. Loeb (1982) Mutagenesis resulting from depurination is an SOS process, Mutation Res., 106, 1–9.

Schendel, P.F., and M. Defais (1980) The role of *umuC* gene product in mutagenesis by simple alkylating agents, Mol. Gen. Genet., 177, 661–665.

Shinagawa, H., T. Kato, T. Ise, K. Makino and A. Nakata (1983) Cloning and characterization of the *umu* operon responsible for inducible mutagenesis in *Escherichia coli,* Gene, 23, 167–174.

Shinagawa, H., H. Iwasaki, T. Kato and A. Nakata (1988) RecA protein-dependent cleavage of UmuD protein and SOS mutagenesis, Proc. Natl. Acad. Sci. (U.S.A.), 85, 1806–1810.

Shinoura, Y., T. Kato and B.W. Glickman (1983a) A rapid and simple method for the determination of the base substitution and frameshift specificity of mutagens, Mutation Res., 111, 43–49.

Shinoura, Y., T. Ise, T. Kato and B.W. Glickman (1983b) *umuC*-mediated misrepair mutagenesis in *Escherichia coli*: extent and specificity of SOS mutagenesis, Mutation Res., 111, 51–59.

Shirname-More, L., T.G. Rossman, W. Troll, G.W. Teebor and K. Frenkel (1987) Genetic effects of 5-hydroxymethyl-2′-deoxyuridine, a product of ionizing radiation, Mutation Res., 178, 177–186.

Steinborn, G. (1978) *uvm* mutants of *Escherichia coli* K12 deficient in UV mutagenesis, I. Isolation of *uvm* mutants and their phenotypical characterization in DNA repair and mutagenesis, Mol. Gen. Genet., 165, 87–93.

Teoule, R. (1987) Radiation-induced DNA damage and its repair, Int. J. Radiat. Biol., 51, 573–589.

Uemura, H., S. Thorbjarnardottir, V. Gamulin, J. Yano, O.S. Andresson, D. Soll and G. Eggertsson (1985) *supN* Ochre suppressor gene in *Escherichia coli* codes for $tRNA^{Lys}$, J. Bacteriol., 163, 1288–1289.

Ullrich, M., and U. Hagen (1971) Base liberation and concomitant reactions in irradiated DNA solutions, Int. J. Radiat. Biol., 19, 507–517.

Walker, G.C. (1977) Plasmid (pKM101)-mediated enhancement of repair and mutagenesis: dependence on chromosomal genes in *Escherichia coli* K-12, Mol. Gen. Genet., 152, 93–103.

Walker, G.C. (1984) Mutagenesis and inducible responses to deoxyribonucleic acid damage in *Escherichia coli,* Microbiol. Rev., 48, 60–93.

Walker, G.C., and P.P. Dobson (1979) Mutagenesis and repair deficiencies of *Escherichia coli umuC* mutants are suppressed by the plasmid pKM101, Mol. Gen. Genet., 172, 17–24.

Witkin, E.M. (1976) Ultraviolet mutagenesis and inducible DNA repair in *Escherichia coli,* Bacteriol. Rev., 40, 869–907.

Wood, R.D., and F. Hutchinson (1984) Non-targeted mutagenesis of unirradiated lambda phage in *Escherichia coli* host cells irradiated with ultraviolet light, J. Mol. Biol., 173, 293–305.