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Much of spontaneous mutagenesis in *Escherichia coli* is due to error-prone DNA repair: implications for spontaneous carcinogenesis

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Abstract

The role of DNA repair genes *(uvrA, uvrB, uvrD, recA, recB, lexA,* and *umuC)* in spontaneous mutagenesis was examined in *Escherichia coli*. The spontaneous mutation rate per bacterium per cell division (μ) was determined for the reversion of UAA *(his-4* and *trpE65),* UAG *(lacZ53),* and frameshift *(trpE9777)* mutations, and for the occurrence of forward mutations to valine resistance. Rich growth medium enhanced μ in a wild-type strain but not in a *uvrB5* strain. In minimal growth medium, the *uvrA* and *uvrB* strains had the largest μ (1.9–6.2-fold greater than that for isogenic wild-type strains, depending on the mutation assay). The *uvrB* strains carrying *lexA, recA, umuC,* or both the *uvrD* and *rec B* mutations (in combination), i.e., mutations that inhibit error-prone DNA repair, had the lowest μ values (~10-fold less than the *uvrB* strain). The *recA* and *lexA* mutations also reduced μ (by ~2-fold) in uvr^+ strains. The genetic control of the error prone repair-dependent sector of spontaneous mutagenesis was shown to be qualitatively similar to the genetic control for u.v. radiation mutagenesis. The *umuC* mutation, which drastically reduced spontaneous mutagenesis, had no effect on genetic recombination. It is proposed that the low level of spontaneous mutagenesis observed in the *recA, lexA, umuC,* and the *uvrD recB* strains is due to errors made during DNA replication, while the enhanced level of spontaneous mutagenesis observed in the wild type, and especially in the *uvrA* and *uvrB* strains, is due to excisable lesions that are produced in the DNA by normal metabolic reactions, and that such unexcised lesions induce mutations via error-prone DNA repair. These results are discussed in terms of their relevance to spontaneous carcinogenesis.

Introduction

Spontaneous mutations are alterations in the chromosomal DNA that occur by unknown mechanisms. However, many possible mechanisms for "spontaneous" mutagenesis have been considered (e.g., 1,2). Such mutagenic mechanisms can be grouped into categories involving DNA replication, recombination, and repair. A role for DNA replication in spontaneous mutagenesis was indicated by Speyer *et al.* (3) and Drake *et al.* (4) in their descriptions of bacteriophage T4 mutator and antimutator DNA polymerase mutants. Konrad (5) has isolated a mutator DNA polymerase mutant of *Escherichia coli*. Other mutagenic mechanisms that might be placed in this category are tautomeric shifts involving nucleic acid bases (6,7), and inefficient mismatch repair (8).

With regard to recombinational mechanisms for spontaneous mutagenesis, Kondo *et al.* (9) showed that a *recA* mutation reduces spontaneous mutagenesis by ~50% in *E. coli*. Since the *recA* gene controls conjugational and transductional recombination (10,11), it was suggested (9) that much of spontaneous mutagenesis is due to recombination errors. However, since the *recA* gene also controls error-prone DNA repair (e.g., *recA* mutants are nonmutable by u.v. radiation) (for review see 12), the data of Kondo *et al.* (9) are also consistent with an error-prone repair deficiency being the basis for the lower spontaneous mutagenesis in *recA* mutants.

The third category of possible mechanisms involved in spontaneous mutagenesis would include mutagenic (error-prone) repair involving DNA lesions induced by normal metabolism (e.g., xanthine catabolism yields free radical species that could produce ionizing radiation-type lesions in DNA (13)), and DNA lesions induced by unknown environmental factors (e.g., background radiation, mutagenic contaminants in growth medium, etc.).

The involvement of excisable DNA lesions in spontaneous mutagenesis has been examined by assessing the role of nucleotide excision repair in spontaneous mutagenesis. Some workers (9,14,15) have not been able to detect any involvement of excision repair in spontaneous mutagenesis in *E.coli,* while others (16,17) have seen such an involvement in studies on *Salmonella typhimurium*. However, in the latter case, the strains that had a higher level of spontaneous mutagenesis were deficient in genes in addition to *uvrB* because a deletion covering the *chl* and *uvrB* genes was used. Similarly, the role of excision repair in spontaneous mutagenesis has been examined in lower eukaryotes. The *rad3* strain of *Saccharomyces cerevisiae* had a higher spontaneous mutability (18—20), but three other excision-repair deficient strains did not (19). The *uvs-3* strain (reduced rate of excision repair) of *Neurospora crassa* also had a higher spontaneous mutability, but the *uvs-2* and *upr-1* strains (excision-repair deficient) did not (21). However, these data for lower eukaryotes are not easily interpretable because the molecular nature of their excision repair defects is not known (e.g., 20). Similar direct correlations between excision-repair deficiency

*Abbreviations: MM, minimal medium, SMM, supplemented minimal medium; PB, phosphate buffer; CFU, colony-forming units.

Table I

Strains of *E. coli* used[a].

Strain designation	Relevant genotype	Other genotype	Source or derivation[b]
SR47	*recA56*	Hfr KL16 *ilv thr thi rpsE*	JC5088, J.Foulds
SR96	+	Hfr H *thyA deo thi*	Hfr H # 1, F.Bonhoeffer
SR113	*uvrB5*	F$^-$ *argE3 his-4 leuB6 proA2 thr-1 ara-14 galK2 lacY1 mtl-1 xyl-5 thi-1 tsx-33 rpsL32* λ$^-$	AB1885, S.Linn
SR114	*uvrA6*	F$^-$ *argE3 his-4 leuB6 proA2 thr-1 ara-14 galK2 lacY1 mtl-1 xyl-5 thi-1 tsx-33 rpsL31* λ$^-$	AB1884, S.Linn
SR192	*lexA101*	F$^+$?*metE thyA36 deo(C2?) lacZ53 rpsL151* λ$^-$	DY99 (reference 23)
SR248	+	F$^-$ *leuB19 metE70 thyA36 deo(C2?) lacZ53 malB45 rha-5 bioA2 rpsL151* λ$^-$	KH21, R.B.Helling
SR250	*uvrB5*	F$^-$ *leuB19 metE70 thyA36 deo(C2?) lacZ53 rha-5 rpsL151* λ$^-$	DY145 (reference 23)
SR251	*uvrB5 lexA101*	Same as SR250	DY146 (Same as SR250)
SR256	*uvrB5 recA56*	F$^-$ *leuB19 metE70 deo(C2?) lacZ53 rha-5 rpsL151* λ$^-$	DY155 (reference 24)
SR257	*uvrB5 recB21*	F$^-$ *leuB19 metE70 deo(C2?) lacZ53 rha-5 rpsL151* λ$^-$	DY157 (reference 24)
SR260	*Δ(uvrB-chlA)*	F$^-$ *leuB19 metE70 thyA36 deo(C2?) lacZ53 malB45 rha-5 rpsL151* λ$^-$	SR248 x P1·SR291, select Bio^{+c}
SR287	*uvrB5 uvrD3*	F$^-$ *leuB19 deo(C2?) lacZ53 rha-5 rpsL151* λ$^-$	DY196 (reference 25)
SR288	*uvrB5 uvrD3 recB21*	Same as SR287	DY197 (Same as SR287)
SR291	*Δ(uvrB-chlA)*	F$^-$ *his*	SA420, A.Campbell
SR349	*uvrA6*	F$^-$ *leuB19 metE70 thyA36 deo(C2?) lacZ53 rha-5 bioA2 rpsL151* λ$^-$	SR248 x P1·SR114, select, Mal^{+c}
SR352	*sulA1*	*trpE65*	WP2, E.M.Witkin
SR353	*sulA1 uvrA155*	*trpE65*	WP2$_s$, E.M.Witkin
SR359	+	Hfr 1 *metA mel*	S108, R.Schmitt
SR559	+	Same as SR260	Same as SR260
SR629	+	Same as SR349	Same as SR349
SR669	*recA56*	HfrPO45 *ilv-318 thr-300 sr1A300*::Tn*10*(Tc^r) *rpsE300*	JC10240, A.J.Clark
SR687	+	F$^-$ *leuB19 metE70 thyA36 deo(C2?) lacZ53 rha-5 bioA2 rpsL151* λ$^-$	SR248 x Plkc·SR192, select Mal$^+$
SR688	*lexA101*	Same as SR687	Same as SR687
SR709	*sul uvrA155 lexA102 rnmA201*	*trpE65 thyA malB*$^+$	MV1, E.M.Witkin
SR710	+	F$^-$ *metB1 lacY1 malA1 thi hemA8 rpsL134*	S729, T.Kato
SR711	+	F$^-$ *his-4 proA2 purB15 galK2 lacY1 mtl-1 xyl-1 thi-1 supE44* λ$^-$	AB470, T.Kato
SR712	*Δ(uvrB301) umuC36*	F$^-$ *argE3 his-4 leuB6 proA2 thr-1 ara-14 galK2 lacY1 mtl-1 xyl-5 thi-1 tsx-33 rpsL31 supE44 Δ(uvrB301 chl bio phr pgi)*	TK501, T.Kato
SR715	+	F$^-$ *trpC22*::Tn*10*(Tc^r) *tna* λ$^-$	W3110 *trpC22*, C.Yanofsky
SR716	+	F$^-$ *trpE9777*(fs)	W3110 *trpE9777*, C.Yanofsky
SR720	*uvrB5*	F$^-$ *leuB19 metE70 trpC22*::Tn*10*(Tc^r) *thyA36 deo(C2?) lacZ53 rha-5 rpsL151* λ$^-$	SR250 x P1::Tn*9*cts·SR715, select Tc^r
SR739	*uvrB5*	F$^-$ *leuB19 metE70 hemA8 thyA36 deo(C2?) lacZ53 rha-5 rpsL151* λ$^-$	SR720 x P1::Tn*9*cts·SR710, select Trp$^+$
SR740	*uvrB5*	F$^-$ *leuB19 metE70 thyA36 deo(C2?) purB15 lacZ53 rha-5 rpsL151* λ$^-$	SR739 x P1::Tn*9*cts·SR711, select Hem$^+$
SR741	*uvrB5*	F$^-$ *leuB19 metE70 thyA36 deo(C2?) lacZ53 rha-5 rpsL151* λ$^-$	SR740 x P1::Tn*9*cts·SR712, select Pur$^+$
SR742	*uvrB5 umuC36*	Same as SR741	Same as SR741
SR744	+	F$^-$ *leuB19 metE70 trpC22*::Tn*10*(Tc^r) *thyA36 deo(C2?) lacZ53 malB45 rha-5 bioA2 rpsL151* λ$^-$	SR248 x P1::Tn*9*cts·SR715, select Tc^r
SR745	+	F$^-$ *leuB19 metE70 trpE65 thyA36 deo(C2?) lacZ53 malB45 rha-5 bioA2 rpsL151* λ$^-$	SR744 x P1::Tn*9*cts·SR353, select Ant$^+$
SR746	+	F$^-$ *leuB19 metE70 trpE65 thyA36 deo(C2?) lacZ53 malB45 rha-5 rpsL151* λ$^-$	SR745 x P1::Tn*9*cts·SR250, select Bio$^+$
SR747	*uvrB5*	Same as SR746	Same as SR746
SR749	+	F$^-$ *argE3 his-4 leuB6 proA2 thr-1 ara-14 galK2 lacY1 mtl-1 xyl-5 thi-1 tsx-33 rpsL31 supE44* λ$^-$	AB1157, Coli Genetic Stock Center
SR819	+	F$^-$ *leuB19 metE70 thyA36 deo(C2?) lacZ53 rha-5 bioA2 rpsL151* λ$^-$	SR248 x P1::Tn*9*cts·SR709, select Mal$^+$

Table I *(continued)*

Strain designation	Relevant genotype	Other genotype	Source or derivation[b]
SR820	*uvrA155*	Same as SR819	Same as SR819
SR866	+	F⁻ *leuB19 metE70 trpE9777 thyA36 deo(C2?) lacZ53 malB45 rha-5 bioA2 rpsL151* λ⁻	SR744 x P1::Tn*9*cts·SR716, select Ant⁺
SR867	+	F⁻ *leuB19 metE70 trpE9777 thyA36 deo(C2?) lacZ53 malB45 rha-5 rpsL151* λ⁻	SR866 x P1::Tn*9*cts·SR250, select Bio⁺
SR868	*uvrB5*	Same as SR867	Same as SR867
SR871	*recA56*	F⁻ *leuB19 metE70 thyA36 deo(C2?) lacZ53 malB45 rha-5 srlA300*::Tn*10*(Tc^r) *bioA2 rpsL151* λ⁻	SR248 x P1::Tn*9*cts·SR669, select Tc^r
SR922	+	F⁻ *leuB19 metE70 deo(C2?) lacZ53 malB45 rha-5 bioA2 rpsL151* λ⁻	SR248 x SR47, select Thy⁺
SR923	*recA56*	Same as SR922	Same as SR922

[a]Genotype nomenclature is that used by Bachmann and Low (26). [b]"Same as..." is used to indicate that, that strain is a cotransductant with the indicated strain. Bio⁺, Thy⁺, Hem⁺, Trp⁺, and Pur⁺ indicate that isolates no longer required biotin, thymine, δ-aminolevulinic acid, tryptophan, and adenine, respectively. Mal⁺ indicates that the isolates could use maltose as a sole carbon and energy source. Ant⁺ indicates that isolates could use anthranilic acid to satisfy their tryptophan requirement. Tc^r indicates that isolates were tetracycline resistant. [c]Strains constructed by D.A.Youngs.

and higher spontaneous mutability, in other less well-studied organisms, have been reviewed by Drake (2).

Since a clear understanding of the basis of spontaneous mutagenesis is not only valuable in itself but also for its implications towards understanding spontaneous carcinogenesis, we have used DNA repair mutations, many of which are well-defined as to their effect at the molecular level on DNA repair (for review see 22), to examine and clarify the role of DNA nucleotide excision repair, error-prone repair, and genetic recombination in spontaneous mutagenesis in *E. coli*.

Materials and Methods

Bacterial strains

The *E. coli* strains used in this study are listed in Table I. The transductions used bacteriophage P1 or P1 :: Tn *9* (Cm^r)cts. Lysogenizations and transductions were performed generally as described by Miller (27).

Media

Minimal medium (MM)* is 0.4% glucose-salts medium (28). Supplemented minimal medium (SMM) in most experiments was MM supplemented with thiamine·HCl at 0.5 μg/ml, D-biotin at 1 μg/ml, thymine at 10 μg/ml, and L-leucine and L-methionine at 1 mM. L-Tryptophan at 1 mM was added to this SMM for strains requiring tryptophan. SMM for strains SR113 and SR749 was MM supplemented instead with thiamine·HCl at 0.5 μg/ml and L-arginine, L-histidine, L-leucine, L-proline, and L-threonine; all at 1 mM. Noble agar (Difco) was added at 1.6% to prepare SMM agar, because less pure grades of agar have been shown to inhibit the *recA* gene-dependent pathway of excision repair (29). All plating media were dispensed at 27 ml/plate. LBt medium is LB medium (27) supplemented with thymine at 10 μg/ml. YENB is yeast extract (Difco) at 0.75% and nutrient broth (Difco) at 0.8%. YENB agar is yeast extract at 0.75% and nutrient agar (Difco) at 2.3%. Glu-0, glu-300, glu-600, glu-900, glu-1200, and glu-2000 are SMM agar media modified to contain lactose at 0.4% and glucose at 0, 300, 600, 900, 1200, and 2000 μg/ml, respectively. Valine agar is SMM agar supplemented with L-valine at 40 μg/ml. Trp-0 and his-0 are SMM agar lacking tryptophan and histidine, respectively. The phosphate buffer (PB) is Na_2HPO_4 at 5.83 g/l and KH_2PO_4 at 3.53 g/l, pH 7.0.

Mutation assays

Incubations were performed in the dark, and all plating was performed under General Electric "gold" fluorescent lights. For each figure or table of data shown, all of the strains presented were tested as a complete set under the same experimental conditions. Two mutation assay protocols were used.

Plate method. Cells were grown overnight at 37°C in SMM with shaking, and diluted 1:50 into fresh, warm medium and grown to an optical density at 650 nm (OD_{650}) (Zeiss PMQII spectrophotometer) of 0.5 (this corresponds to $0.7-2.5 \times 10^8$ colony-forming units (CFU) per ml, depending on the strain). Cells were harvested by centrifugation (6 min at 6,000 x *g*), washed (once) and resuspended in PB to an OD_{650} of 0.200 ± 0.005. Cells (0.1 ml portions) were spread on five glu-0, glu-300, and glu-600 plates (in some experiments glu-900 and glu-1200 plates were also inoculated). Cell samples were also diluted in PB and spread on glu-300 plates to determine the CFU/ml. After 24 h (when glucose-dependent growth had ceased; data not shown) two plates for each glucose concentration (except glu-0) were flooded with 2.5 ml of PB. The cells were scraped off of the agar plates with a glass spreader rod, and the two suspensions were combined. The two plates were each rinsed with another 2.5 ml of PB and the combined washings (10 ml) were titered for cells/ml with a Coulter Counter, model F (Coulter Electronics, Inc.). The remaining mutant assay plates were incubated an additional two days at 37°C before counting Lac⁺

colonies. The *mutation rate per bacterium per cell division,* μ, was calculated according to Kondo (30) as follows: $\mu = [(M - M_0)/(N' - N_0)]$, where M_0 (the average number of Lac$^+$ clones on glu-0 plates) and M (the average number of Lac$^+$ clones on e.g., glu-300 plates) are the number of initial and final Lac$^+$ clones, respectively, on the mutant-selection plates, and N_0 is the number of initial CFU per plate, and N' is the total number of cells per plate (Coulter Counter) at 24 h. (Note: at 24 h the lactose-dependent growth is insignificant relative to the glucose-dependent growth).

Tube method. Cells were grown overnight at 37°C in SMM, with shaking, and diluted 10^{-2} into PB. These cells were further diluted 10^{-3} into SMM and dispensed into 31 tubes (2 ml each). The SMM suspension was titered for CFU (on glu-300 plates when Lac reversion was studied, on YENB plates for YENB-grown cells and on SMM plates in all other cases) and the overnight culture was titered for mutants on mutant assay plates (i.e., glu-0, trp-0, his-0, or valine plates, as appropriate). The tube cultures were incubated at 37°C, without agitation, in darkness for 24–30 h. Then each culture was vortexed for 5 s and plated on mutant assay plates. Ten cultures were also titered for CFU. Valine and SMM plates were incubated for 2 days at 37°C; YENB plates for 1 day; and other mutant assay plates for 3 days. μ was calculated according to Kondo (30) as follows: $M = (\mu N/\ln 2)\ln(\mu CN)$, where N is the average number of CFU in the cultures after 24–30 h, C is the number of cultures used (i.e., 31), and M is the mean number of mutants per culture after 24–30 h. μ is determined by testing values of μ until the right side of the equation approximates M. For mutagenesis to valine resistance, μ was determined from the median value of valine-resistant mutants per culture (r_0) according to Lea and Coulson (31). r_0 is used to determine m (the mean number of mutations per culture) by use of the equation, $(r_0/m) - \ln m = 1.24$. μ is then determined from the equation $\mu = m/(N - N_0)$, where N_0 and N are the initial and final CFU per culture, respectively.

U.v. radiation mutagenesis

Logarithmic phase cells were prepared as described under "plate method" (above). U.v. irradiation was performed and mutagenesis calculated as described previously (25,32). Cells were u.v. irradiated, concentrated (if required), and plated (0.2 ml) on lactose plates containing a small supplement of glucose to allow mutation fixation and expression. The composition of the mutant-selection plates was designated to allow maximum mutation fixation and expression (25). Viability was also determined on the mutant-selection plates.

Recombination experiments

Donor (Hfr) and recipient (F$^-$) strains were cultured overnight in LBt at 37°C with shaking. Cultures were diluted 1:100 into fresh, warm medium and cultured to an OD_{650} of ~0.3. A 0.25 ml sample of donor culture was added to 5 ml of recipient culture and the mixture was slowly shaken for 45 min at 37°C. The mixture was diluted 10-fold in PB and vortexed rapidly for 10 s before plating further dilutions on leu-0 (i.e., SMM agar deficient in leucine) or glu-0 plates containing streptomycin sulfate at 200 μg/ml. Lac$^+$ or Leu$^+$ colonies were counted after 3 days at 37°C.

Results

The spontaneous rate of *lacZ53* (UAG) reversion was determined with the plate method in several *uvr*$^-$ strains as well as, in most cases, the isogenic *uvr*$^+$ strains. The *uvrA* and *uvrB* mutants had mutation rates approximately 3–5-fold greater than that for *uvr*$^+$ strains (Figure 1). Assays for mutations at other loci were used to verify the enhanced spontaneous mutagenesis in *uvr*$^-$ strains. Depending on the assay system, mutagenesis was enhanced 1.9–6.2-fold by the presence of a *uvrA* or *uvrB* mutation (Table II). The rate of spontaneous mutations for valine resistance was found to vary between experiments by as much as 100-fold in strains derived from strain KH21, nevertheless, the *uvrB* strain always had a higher μ than the *uvr*$^+$ strain. Because of this variability, median values (for five experiments) are presented for the spontaneous mutation rates derived from the occurrence of valine resistance in KH21 strains (Table II).

We tested the possibility that our stock cultures had accumulated fluorescent light-induced excisable mutagenic lesions. Strains SR248 *(uvr*$^+$*)* and SR250 *(uvrB5)* were cloned and grown for ~50 generations in darkness or under gold lights (for brief periods) before performing a spontaneous mutagenesis experiment (tube method, Lac reversion). The mutation rates determined under these conditions were the same as those determined for strains that occasionally experienced white fluorescent light (data not shown).

The effect of growth medium on spontaneous muta-

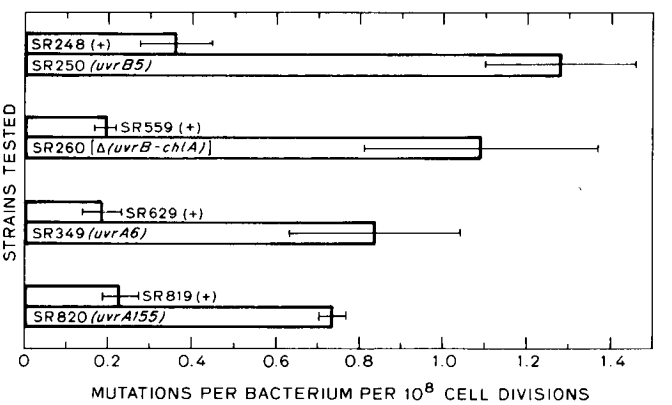

Fig. 1. Effect of *uvrA* and *uvrB* mutations on spontaneous mutagenesis (*lacZ53*→Lac$^+$) in *E. coli* K-12. The spontaneous mutation rates were determined by the plate method as described in **Materials and Methods.** Values given are the mean ± one standard deviation for data from three experiments per strain. In each experiment, data were averaged for mutagenesis occurring on glu-300, glu-600, glu-900, and glu-1200 plates. With the exception of strains SR248 and SR250, paired data are for cotransductants. All strains are closely related.

Table II

Spontaneous mutation rates of *uvr*$^+$ and *uvr*$^-$ strains of *Escherichia coli*

Strain background	Mutation assay system[a]	Repair marker (strain no.)[b]	μ[c] (per 10^8 divisions)		Enhancement of mean μ relative to *uvr*$^+$ strain
			experiment 1	experiment 2	
K-12 (AB1157)	*his-4* (UAA)→His$^+$	+ (SR749)	5.76	5.54	-
		uvrB5(SR113)	18.8	19.5	3.4
	Val^s→Val^r	+ (SR749)	112	117	-
		uvrB5(SR113)	936	248	5.2
K-12 (KH21)	Val^s→Val^r	+ (SR248)	3.72[d]	-	-
		uvrB5(SR250)	22.4[d]	-	6.0
	lacZ53 (UAA)→Lac$^+$	+ (SR248)	0.32 ± 0.17(11)[e]		-
		uvrB5(SR250)	1.95 ± 0.47(17)[e]		6.2
	trpE9777 (fs)→Trp$^+$	+ (SR867)	5.41	8.23	-
		uvrB5(SR868)	13.5	10.5	1.9
	trpE65 (UAA)→Trp$^+$	+ (SR746)	3.73	4.88	-
		uvrB5(SR747)	10.1	10.0	2.4
B/r (WP2)	*trpE65* (UAA)→Trp$^+$	+ (SR352)	2.92	2.59	-
		uvrA155(SR353)	5.33	6.16	2.1

[a] UAA = ochre nonsense mutation; UAG = amber nonsense mutation; fs = frameshift mutation. All assays used the tube method (see **Materials and Methods**). His, Lac, and Trp reversions (to prototrophy or to the ability to metabolize lactose, in the case of Lac$^+$) were determined by spreading 0.2 ml of cell culture on his-0, glu-0, or trp-0 plates (one per tube), respectively. Val^r mutations (conferring ability to grow in the presence of valine) were determined by spreading 0.1 ml of cells diluted 5-fold onto valine plates. [b]Strains SR867 and SR868, and SR746 and SR747 are cotransductants. Strains SR248 and SR250 are related by two transduction steps. Strains SR113 and SR353 are nitrous acid-induced mutants of strains SR749 and SR352, respectively (references 33,34; respectively). [c]μ = mutation rate per bacterium per cell division, and was calculated as described in **Materials and Methods** for the tube method. [d]Median value from five experiments. [e]Mean ± one standard deviation for a large number of experiments (see value in parentheses).

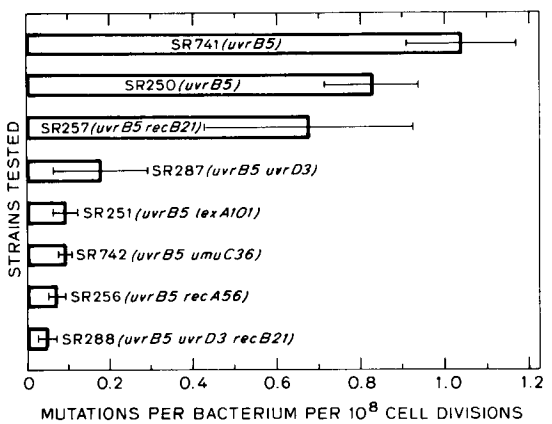

Fig. 2. Effect of *recA56, lexA101, umuC36, recB21* and *uvrD3* mutations on spontaneous mutagenesis (*lacZ53*→Lac$^+$) in *E. coli* K-12 *uvrB5*. The spontaneous mutation rates were determined by the plate method as described in **Materials and Methods.** Values given are the mean ± one standard deviation for data from four experiments per strain. In each experiment, data were averaged for mutagenesis occurring on glu-300 and glu-600 plates. Pairs of strains that are cotransductants are: SR250 and SR251, SR287 and SR288, and SR741 and SR742. All strains are closely related.

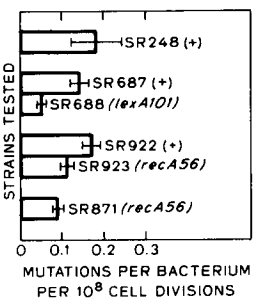

Fig. 3. Effect of *recA56* and *lexA101* mutations on spontaneous mutagenesis (*lacZ53*→Lac$^+$) in *E. coli* K-12. The spontaneous mutation rates were determined by the plate method as described in **Materials and Methods.** Values given are the mean ± one standard deviation for data from four experiments per strain. In each experiment, data were averaged for mutagenesis occurring on glu-300 and glu-600 plates. Pairs of strains that are cotransductants or coconjugants are: SR687 and SR688, and SR922 and SR923. All strains are closely related.

genesis (Lac reversion) was tested with the tube method (Table III). Strain SR248 *(uvr*$^+$*)* showed a higher mutation rate when grown in complex medium (YENB) than when grown in defined medium (SMM). The *uvrB5* strain (SR250) did not show this medium effect.

Several strains containing other DNA repair deficiencies, in addition to the *uvrB5* mutation, were assayed for their spontaneous mutation rates (Lac reversion) (Figure 2). The presence of the *recB21* mutation (strain SR257) only slightly lowered μ in the *uvrB5* background. The *uvrD3* mutation caused a large reduction in μ (~4-fold), and when combined with the *recB21* mutation (strain SR288) it resulted in the lowest μ value (~18-fold reduction) determined for the strains described in Figure 2. The presence of the *lexA101, umuC36,* or *recA56* mutations also resulted in very low μ values (9–18-fold lower than for the *uvrB* control strains). As in the *uvrB5* strains, the *recA56* and *lexA101* mutations also reduced μ in *uvr*$^+$ strains (~2-fold) (Figure 3).

Table III

Effect of growth medium on the spontaneous mutation rate for *lacZ53*→Lac$^+$ in *uvr*$^+$ and *uvrB5* strains of *Escherichia coli* K-12

Experiment no.	μ^a (per 10^8 divisions) for strain SR248 *(uvr$^+$)* grown in:		YENB effect[b] for *uvr*$^+$	μ^a (per 10^8 divisions) for strain SR250 *(uvrB5)* grown in:		YENB effect[b] for *uvrB5*
	SMM[c]	YENB[c]		SMM[c]	YENB[c]	
1	0.255	0.634	2.49	1.72	2.70	1.57
2	0.167	0.862	5.16	2.31	1.76	0.76
3	0.278	1.19	4.28	1.90	1.56	0.82
4	0.200	0.722	3.61	1.07	1.66	1.55
Mean	0.225	0.852	3.88	1.75	1.92	1.18

[a] μ = mutation rate per bacterium per cell division, determined using the tube method (see **Materials and Methods**); for each tube, 0.2 ml of cell culture was spread on a glu-0 plate (for the mutant assay). [b]YENB effect = μ for cells grown in YENB relative to μ for cells grown in SMM. [c]SMM = supplemented minimal medium; YENB = yeast extract-nutrient broth; the compositions of the media are described in **Materials and Methods.** Overnight cultures and viability testing used the homologous medium.

Table IV

Effect of DNA repair deficiencies on u.v. radiation mutagenesis (*lacZ53*→Lac$^+$) in *E. coli* K-12[a]

Strain	u.v. radiation fluence (J m^{-2})	Surviving fraction	Lac$^+$ mutants/ 10^8 survivors
SR687 (+)	0.50	1.02	1.7
SR250 *(uvrB)*	0.50	0.98	208.0
SR688 *(lexA)*	0.50	0.96	0.0
SR923 *(recA)*	0.50	0.54	0.0
SR250 *(uvrB)*	0.13	1.07	62.0
SR257 *(uvrB recB)*	0.13	0.86	53.0
SR287 *(uvrB uvrD)*	0.13	0.97	17.0
SR288 *(uvrB uvrD recB)*	0.13	0.69	2.8
SR742 *(uvrB umuC)*	0.13	1.00	1.6
SR251 *(uvrB lexA)*	0.13	0.71	0.2

[a]Strains were u.v. irradiated, concentrated (if needed), and plated (0.2 ml) on lactose plates containing a small supplement of glucose to allow mutation fixation and expression. Strains SR687, SR688 and SR923 were concentrated 100-fold and spread on glu-2000 plates (i.e., glucose at 2000 μg/ml). Strain SR250 (0.5 J m^{-2}) was spread without concentration on glu-300 plates. All other strains were concentrated 10-fold and spread on glu-1200 plates. U.v. radiation mutagenesis was calculated by taking into account preexisting spontaneous mutants and spontaneous mutants arising on the mutant-selection plates (25). The data for strains SR250, SR257, SR287, and SR288 are from Sargentini and Smith (25,32).

Table V

Effect of the *umuC36* mutation on recombination (conjugation assay) in *E. coli* K-12 *uvrB5*

Experiment no.	Recombinants per ml from conjugation mixtures[a]			
	SR96 (Leu$^+$) x		SR359 (Lac$^+$) x	
	SR741 *(uvrB)*	SR742 *(uvrB umuC)*	SR741 *(uvrB)*	SR742 *(uvrB umuC)*
1	1.74 x 10^5	1.34 x 10^5	NT[b]	NT[b]
2	8.4 x 10^4	8.2 x 10^4	8.8 x 10^3	1.58 x 10^4
3	6.1 x 10^4	6.5 x 10^4	2.0 x 10^3	2.8 x 10^3
4	2.13 x 10^5	2.42 x 10^5	4.5 x 10^3	4.5 x 10^3
5	4.04 x 10^4	3.72 x 10^4	8.9 x 10^3	1.45 x 10^4
6	3.34 x 10^4	3.80 x 10^4	7.0 x 10^3	1.30 x 10^4
Mean	1.01 x 10^5	1.00 x 10^5	6.2 x 10^3	1.01 x 10^4

[a] In each experiment, the Hfr (i.e., SR96 or SR359) was added to both recipient cultures, (i.e., SR741 and SR742) simultaneously. Details are described in **Materials and Methods.** In control experiments to measure the ability of the recipients to take up DNA (i.e., an F′ factor *(lac$^+$)* from *E. coli* K-12 strain JC2625), both strains were equally proficient. [b]Not tested.

Some of the strains tested above were also tested for u.v. radiation mutability (Table IV) and recombination ability (Table V). The u.v. radiation mutability (measured with the Lac reversion assay) of a *uvrB* strain was ~100-fold greater than a wild-type strain, while *recA* and *lexA* mutants were nonmutable. In the *uvrB* background, u.v. radiation mutability was slightly reduced by a *recB* mutation, while a *uvrD* mutation reduced mutability ~3-fold. Much larger reductions in mutability were seen for *umuC, lexA,* or *uvrD recB* (in combination) mutants (Table IV). Recombination ability (in the *uvrB* background) was unaffected in the *umuC* mutant (Table V).

Discussion

Several workers have presented data on the role of nucleotide excision repair in spontaneous mutagenesis in bacteria, but their conclusions are in conflict. For example, Kondo *et al.* (9), Smirnov *et al.* (14), and Kato *et al.* (15) reported that *E. coli uvrA* and *uvrB* mutants show a normal spontaneous mutability. However, Ames (16) and Mortelmans and Stocker (17) noted that *S. typhimurium* strains, which carry deletions covering the *uvrB* and *chlA* loci, had a 3–5-fold higher level of spontaneous mutagenesis for missense or nonsense reversion (no effect was detected when frameshift rever-

sion was assayed; 17). They (16,17) suggested that the *uvrB* defect enhanced spontaneous mutagenesis.

We report here that *uvrA* and *uvrB* mutations enhance the spontaneous mutability of *E. coli* B/r and the AB1157 and KH21 derivatives of *E. coli* K-12 (Table II, Figure 1) for all of the mutation assay systems that we have tested (i.e., UAA (*his-4* and *trpE65*), UAG *(lacZ53),* and frameshift *(trpE9777)* reversion, and mutations to valine resistance), although the degree of enhancement varied (i.e., 1.9–6.2-fold) depending on the assay system used (Table II). It is important to note that we found that complex growth medium increased the spontaneous mutation rate for a uvr^+ strain, but not for a *uvrB* strain (Table III). The consequence was that the difference between the μ values for the uvr^+ and the *uvrB* strains was smaller when the cells were both grown in complex growth medium. The workers cited above, who did not detect the *uvr* effect on spontaneous mutagenesis (9,14,15), all used complex growth medium (i.e., nutrient broth or Casamino Acid-enriched minimal medium) for their spontaneous mutagenesis experiments. We suspect that the complex growth medium effect, which we have noted, obscured the *uvr* effect on spontaneous mutagenesis in their experiments by fortuitously raising the spontaneous mutation rate of their wild-type strains up to that of their uvr^- strains.

The complex growth medium effect noted above is quite similar to the well-known "broth effect" for u.v. radiation mutagenesis. That is, u.v. radiation mutagenesis, as measured by the reversion of a suppressible nonsense mutation (e.g., commonly, *trpE65*→Trp^+) (35,36), is enhanced (~10-fold) when irradiated cells are plated on nutrient broth or mutant-selection plates enriched with a mixture of amino acids (37). This broth effect also works for u.v. radiation-induced reversion of the *lacZ53* marker (i.e., the mutation assay used in Table III) (38). Enriched medium is believed to protect some mutagenic lesions from error-free excision repair, i.e., a process called "mutation frequency decline" (39). Thus, post-irradiation incubation in enriched medium yields enhanced u.v. radiation mutagenesis relative to posttreatment in minimal medium in wild-type cells, but not in *uvrA* cells (36,40).

Mutations that increase the mutation frequency of an organism are called mutator genes. *E. coli* K-12 has been well-studied in this context, and both major and minor mutator genes have been identified. The major mutator genes, i.e., *mutD, mutL, mutH (mutR), mutS, mutT, mutU (uvrE)* (for review see 41), and *dnaQ* (42) increase the spontaneous mutation rate by 100–100,000-fold. The minor mutator genes, e.g., *polA, polC* and *tif-1* (for review see 41) increase the spontaneous mutation rate 2–100-fold. Clearly, our data (Table II) suggest that *uvrA* and *uvrB* mutations belong in this latter class of mutator genes.

The class of major mutator genes are thought to cause mutations through replication errors coupled with mismatch repair. This type of mutagenesis is *recA* independent (for review see 41), and therefore does not fit into the realm of "SOS" or "error-prone" repair, which is *recA* and *lexA* dependent (for review see 12). As shown in Figure 2, the enhanced spontaneous mutagenesis in *uvrA* and *uvrB* strains is *recA* and *lexA* dependent, as well as being dependent on the *umuC, uvrD,* and *recB* (in combination with *uvrD*) genes; therefore it presumeably arises from noncoding lesions in DNA, and requires some sort of error-prone DNA repair system. It should be emphasized that the effect of the DNA repair deficiencies on the error-prone repair-dependent sector of spontaneous mutagenesis (studied here) closely resembles their effect on u.v. radiation mutagenesis (Table IV and references 25,43—47).

In uvr^+ strains, *lexA* (Figure 3) and *recA* (Figure 3 and references 9,44) mutations significantly reduce the level of spontaneous mutagenesis. Thus, error-prone DNA repair also causes much of the spontaneous mutagenesis seen in wild-type strains. We presume that the residual spontaneous mutagenesis observed in *recA* and *lexA* strains is due to errors in DNA replication, since these strains are nonmutable by u.v. radiation (Table IV and references 43–46), but mutations can be induced in a *recA* strain by a mutator mutation in *E. coli* DNA polymerase (5), by nucleic acid base analogues (e.g., 5-bromouracil; 48), and certain alkylating agents (e.g., ethyl methanesulfonate; 9,44).

Strains containing *recA* and/or *recB* mutations are known to produce nonviable cells (for review see 49). We determined this lethal sectoring phenomenon by comparing the ratios of viable cells (CFU/ml) to total cells (Coulter counts) for pairs of strains, which had been washed off of mutant-selection plates in a spontaneous mutagenesis experiment (plate method) (data not shown). In order to calculate μ, we divided the mutants, arising on the mutant-selection plates, by the total cells arising from growth on those plates (see **Materials and Methods**). A partial correction for lethal sectoring might be to reduce the "total cell" term by the fraction of nonviable cells in the calculation of μ. However, it is not clear how one should correct the mutant term in the calculation. That is, lethal sectoring may have an effect on the probability of an original mutant cell initiating a clone, but once the clone has divided a few times, lethal sectoring should not prevent it from eventually being scored. Nevertheless, if we made this simplistic correction (which was not done in any data presented earlier), the *uvrB recB* strain (SR257, 38% nonviable cells) would not have had a μ lower than that of the *uvrB* control strains. The *uvrB uvrD recB* strain (SR288) and the *uvrB lexA* strain (SR251) would show an ~18% increase in μ (i.e., a quantitative but not a qualitative change, relative to the control strains). No lethal sectoring was detected in the *lexA* (SR688), *uvrB umuC* (SR742), or *uvrB uvrD* (SR287) strains, thus no μ correction would be necessary. We did not test our *recA* strains but they generally should have about half as much lethal sectoring as the *recB* strains (49). Overall, we feel that a consideration of lethal sectoring does not

effect the conclusion that there is a large error-prone repair-dependent sector of spontaneous mutagenesis.

It does not seem likely that DNA recombination errors can be an important cause of spontaneous mutagenesis, as was proposed by Kondo *et al.* (9), because there seems to be no correlation between recombination ability and spontaneous mutability in *E. coli*. Kato *et al.* (15) showed that the *recB recF* double mutants, which showed virtually no *recA*-dependent recombination, were normally spontaneously mutable. We, on the other hand, found that the *umuC* mutation, which abolishes the error-prone repair-dependent sector of spontaneous mutagenesis, has no effect on recombination (Table V).

The most tenable conclusions from our data with different DNA repair-deficient mutants, are that mutagenic lesions of unknown source occur in the DNA of bacteria during a spontaneous mutagenesis experiment, that these lesions induce mutations as a result of error-prone repair, and that the mutagenic potential of these lesions is lower in nucleotide excision repair proficient cells. (Excision repair of u.v. radiation damage appears to be largely error-free (50,51).) In yeast, error-prone DNA repair also appears to play a role in spontaneous mutagenesis (51a).

Nishioka and Doudney (52) and Bridges and Mottershead (53) have presented data consistent with the presence of a constitutive level of error-prone repair that functions in *E. coli* B/r. We have demonstrated a one-hit, u.v. radiation-induced mutant frequency response (which we therefore assume to be constitutive) in *E. coli* K-12 (32). One explanation for the presence of a basal level of an error-prone repair process is that it is partially induced (or fully induced in part of the cell population) by the presence of the excisable lesions involved in spontaneous mutagenesis. Alternatively, one could surmise that we can only detect the error-prone repair-dependent sector of spontaneous mutagenesis because of a constitutive level of error-prone repair.

The nature of the putative spontaneous mutagenic lesions in *E. coli* can be inferred from the specificity of the u.v. endonuclease. The *uvrA* and *uvrB* strains (i.e., strains deficient in the u.v. endonuclease) are very sensitive to u.v. radiation (34,54), but show little sensitivity to ionizing radiation (55,56). Similarly, these strains are hypermutable by u.v. radiation (34,36,56–59) but not by ionizing radiation (55,56). These facts suggest that the lesions in DNA leading to "apparent" spontaneous mutagenesis are not produced by free radicals (i.e., characteristic of ionizing radiation) but may be produced by excited state reactions (i.e., characteristic of u.v. radiation). It is interesting to note that a model system exists for the metabolic production of excited state molecules that can damage DNA (60). We are currently attempting to test a hypothesis that normal metabolism is the cause of the excisable lesions discussed above, by identifying metabolic steps that lead to spontaneous mutagenesis, e.g., phenylalanine, but not tryptophan or xanthine, enhances the spontaneous mutation rate in *E. coli* K-12 *uvrB5* (unpublished observation).

We feel that the concept of metabolically-induced lesions being important in spontaneous mutagenesis may have important implications for understanding spontaneous carcinogenesis. Totter (61) has reported that, although environmental carcinogens are an important factor affecting the local cancer incidence rate, the world-wide cancer incidence rate appears to be largely independent of external environmental hazards. Since there is a high degree of correlation between mutagenesis and carcinogenesis (62), we speculate that metabolically-produced DNA lesions, which are subject to excision repair, may play a significant role in spontaneous carcinogenesis. While our results on spontaneous mutagenesis in no way lessens the importance of reducing the level of exposure of humans to environmental carcinogens, they do suggest that a significant level of mutagenic damage (and hence, carcinogenic damage) may be produced by normal metabolic processes within cells. If our results for *E. coli* are relevant to mammalian cells, they suggest that cells from individuals deficient in nucleotide excision repair (i.e., afflicted with the disease, xeroderma pigmentosum (for review see 63)) should show a higher than normal rate of spontaneous mutagenesis, and if the life span of these individuals can be extended by protecting them from the complications of exposure to sunlight, they should show a higher than normal incidence of internal organ cancer.

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