Ultraviolet Sensitivity, Spontaneous Mutability and DNA Degradation in *Escherichia coli* Strains Carrying Mutations in *uvr* and *rec* Genes

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SUMMARY

Escherichia coli uvr502 bacteria have a higher spontaneous mutability and are 4.6 times more u.v.-sensitive than isogenic wild-type bacteria. Bacteria deficient in dimer excision (*uvrA6*) are 15 times more u.v.-sensitive than isogenic *uvrA*⁺ bacteria. Double mutant *uvrA6uvr502* bacteria are only 1.5 times more u.v.-sensitive than *uvrA6* bacteria and double mutant *uvr502recA56* bacteria were as u.v.-sensitive as *recA56* bacteria, suggesting that there exists a *uvrA*⁺- and *recA*⁺-dependent repair pathway which is blocked by the *uvr502* mutation. We suggest that the *uvr502* mutation affects primarily the excision repair pathway. Strict additivity of the *uvr502* and *recB21* mutations on u.v. sensitivity suggests that *recB*⁺-mediated repair acts in part of repair pathway different from excision repair. U.v.-induced DNA degradation in the *uvr502* bacteria is only slightly greater than that in *uvr*⁺ bacteria and the *uvr502* mutation does not affect the 'reckless' character of u.v.-induced DNA breakdown in the *recA56* bacteria. The *uvr502-uvrA6* and *uvr502recA56* or *uvr502recB21* double mutants have spontaneous mutation frequencies similar to that of the single *uvr502* strain.

INTRODUCTION

The rate of spontaneous mutability is determined genetically and mutator genes controlling this property in various bacterial and viral species have been described (e.g. Drake, 1969; Liberfarb & Bryson, 1970). In some cases the changes in spontaneous mutability were coupled with u.v. sensitivity (e.g. Smirnov, Filkova & Skavronskaya, 1972). These observations lead to the hypothesis that mechanisms of DNA repair are also involved in the determination of the spontaneous mutation rates. So far, however, unequivocal evidence that both mutator effect and u.v. sensitivity in bacteria are due to a single mutation have been presented only in few cases (Böhme, 1969; Smirnov *et al.* 1972; Siegel, 1973) and the mechanisms of action of the mutator genes as well as the modes of their involvement in DNA repair remain obscure.

We have found an increased frequency of spontaneous mutation in the u.v.-sensitive *Escherichia coli* mutant *uvr502* (Smirnov & Skavronskaya, 1968) and shown that both u.v. sensitivity and the mutator property of the mutant are recessive and determined by the single mutation *uvr502* (Smirnov *et al.* 1972) which has been mapped between *ilv* and *metE* loci on the *E. coli* genetic map (Smirnov & Skavronskaya, 1971). Similar results were obtained elsewhere (Mattern, 1971). The function affected in the *uvr502* mutant appears to be essential not only for maintenance of low mutability and DNA repair but also for the viability of *E. coli* lacking DNA polymerase I (Mattern, 1971; Smirnov *et al.* 1973).

In this paper we describe some properties of double mutants containing *uvr502* and other

Table 1. *Bacterial strains**

Strain	Sex	Relevant genetic character					Str.	Source
		<i>uvrA</i>	<i>uvr(502)</i>	<i>rec</i>	<i>thyA</i>	<i>metE</i>		
KS102	F ⁻	+	+	<i>A56</i>	+	—	R	This paper
KS105	F ⁻	+	+	+	+	—	R	
KS106	F ⁻	+	—	+	+	—	R	
KS107	F ⁻	+	—	<i>A56</i>	+	—	R	
KS112	F ⁻	+	+	+	—	—	R	
KS113	F ⁻	6	+	+	—	—	R	
KS114	F ⁻	+	—	+	—	—	R	
KS115	F ⁻	6	—	+	—	—	R	
KS129	Hfr	+	+	+	+	—	R	
KS130	Hfr	+	+	<i>B21</i>	+	—	R	
KS131	Hfr	+	—	+	+	—	R	
KS132	Hfr	+	—	<i>B21</i>	+	—	R	

* Nomenclature conforms in most ways to the recommendations of Demerec, Adelberg, Clark & Hartman (1966) except that the minus sign is used with the gene symbol to indicate a general mutant allele when the specific allele number is not required. The gene symbols are those recorded by Taylor (1970).

uvr and *rec* mutations in an attempt to determine the repair pathway affected by the *uvr502* mutation and the possible influence of mutations in *uvrA* or *rec* genes on the spontaneous mutability of the *uvr502* mutant.

METHODS

Bacterial strains. The strains used are described in the Table 1 or in the text.

Media. Bacteria were grown in L-broth (Difco tryptone, 10 g; Difco yeast extract, 5 g; NaCl, 5 g; distilled water to 1 l) supplemented with 2.5×10^{-3} M-CaCl₂. Viable counts after u.v. irradiation were performed on 1.5 % (w/v) Difco nutrient agar plates (NA plates). In the experiments with DNA degradation bacteria were grown in glucose salts medium A (Davis & Mingioli, 1950) supplemented with 0.25 % (w/v) Difco Casamino acids (CAA medium). Glucose salts medium A supplemented with appropriate amino acids and solidified with 1.5 % (w/v) Bactoagar Difco (A agar) was used also as selective plating medium for Hfr × F⁻ crosses, P1 transduction, and determination of spontaneous mutability. Amino acids and thymine were added to the medium at a final concentration of 20 µg/ml, and streptomycin at 200 µg/ml.

Conjugation. Exponential-phase Hfr donor bacteria grown in Penassay broth (Difco) without shaking and F⁻ recipients grown in the same medium with shaking were mixed in appropriate volumes to obtain a final ratio of donors to recipients of approximately 1:10 and incubated 40 min at 37 °C. Diluted samples were spread on to selective plates containing streptomycin and incubated at 37 °C for 48 h.

Transduction was carried out as described previously (Smirnov *et al.* 1972).

Selection of Val^r mutants was performed on A agar supplemented with 100 µg/ml of DL-valine as described previously (Smirnov *et al.* 1972).

U.v. irradiation. Stationary phase cultures of the strains KS112 to KS115 and KS129 to KS132 or logarithmic cultures of the strains KS102, KS105, KS106 and KS107 grown in L-broth were washed twice, resuspended in physiological saline to a density of 10⁷ bacteria/ml and 5 ml of suspension was irradiated with 254 nm of light from a BUV-15 germicidal lamp in the Petri dish (90 mm diam.) with constant stirring. An incident u.v. exposure rate of 1 erg/mm²/s was used for u.v.-sensitive strains and of 3.6 ergs/mm²/s for u.v.-resistant strains.

Gamma-ray treatment. To measure gamma-ray sensitivity suspensions containing 10^7 bacteria/ml were spotted on to the surface of NA and irradiated with 25 krad in a ^{60}Co source. Strains resistant to gamma-rays gave confluent growth in the drop area after 24 h of incubation at 37 °C while gamma-ray-sensitive bacteria failed to grow after irradiation.

Measurement of DNA degradation. Cultures grown overnight in CAA medium were washed once, resuspended to a density of 10^7 bacteria/ml in fresh CAA medium supplemented with 10 $\mu\text{Ci/ml}$ (9 $\mu\text{g/ml}$) of [^3H -methyl]thymidine, 5 μg unlabelled thymidine/ml and 100 μg deoxyadenosine/ml and incubated at 37 °C with shaking. After 2 h the bacteria were washed by centrifugation, resuspended in 10 ml of fresh prewarmed CAA medium lacking glucose to a density of 10^7 bacteria/ml and irradiated with u.v. light. After irradiation glucose (to 0.4 %, w/v) was added, the cultures were incubated at 37 °C with shaking and 1 ml samples were pipetted at intervals into 1 ml of ice-cold 10 % trichloroacetic acid (TCA). After standing in ice for 20 h the samples were filtered through nitrocellulose filters, Hufs (Chemapol CSSR, Prague) washed twice with 4 ml of ice-cold 5 % TCA, placed in scintillation vials and dried prior to the addition of scintillation liquid (4 g PPO, 0.1 g POPOP, toluene to 1 l). Samples were counted in an Intertechnique Scintillation Counter SL 40. The loss of cold TCA precipitable radioactivity during post-irradiation incubation from the cultures labelled by [^3H]thymidine was used as the measure of DNA breakdown.

RESULTS

Construction of bacterial strains

Sets of strains, isogenic except for *uvrA6*, *uvr502* or *recB21* mutations or approximately isogenic except for *uvr502* or *recA56* alleles were constructed in four similar but not identical genetic backgrounds.

Construction of the *uvr502recA56* double mutant, its component single mutants and *uvr⁺recA⁺* strain. Strains KS102, KS105, KS106 and KS107 were constructed by conjugation as follows. Strain KS52F⁻*uvr⁺rec⁺thyA324argAlacY14rhametE*Str^r was crossed with the donor strain JC5088 (Hfr KL16) *recA56thr300ilv318spc300*Str^s and Thy⁺Arg⁺[Str^r] derivatives were selected. One of the u.v.-sensitive (UV^s) and gamma-ray-sensitive (X^s) derivatives obtained in the cross was designated KS102, and one of the u.v.-resistant (UV^r) and gamma-ray-resistant (X^r) derivatives was designated KS105.

Strain KS106 is an UV^sX^rThy⁺Arg⁺[Str^r] derivative and the strain KS107 is an UV^sX^sThy⁺Arg⁺[Str^r] derivative obtained in the cross of the *uvr502* strain KS103 (isogenic with KS52 except for the *uvr502* mutation) with the same donor strain JC5088. Proof of the presence of both *uvr502* and *recA56* mutations in the chromosome of the presumed double mutant KS107 was obtained by using this strain as transductional donor and the *ilv* strain MM300 and *cysC* strain JC2915 as recipients. Out of selected Ilv⁺ transductants of MM300 17 % were UV^sX^r and 6 % of selected Cys⁺ transductants of JC2915 were UV^sX^s. The co-transduction of the UV^s character with *ilv⁺* and the UV^sX^s character with *cysC⁺* confirms the presence of both *uvr502* and *recA56* mutations in the chromosome of KS107.

Construction of the *uvr502uvrA6* double mutant, its component single mutants and the *uvr(502)⁺uvrA⁺* strain. Strains KS112, KS113, KS114 and KS115 are isogenic except for the *uvrA6* or *uvr502* mutations or both. The *uvrA⁺uvr(502)⁺* strain KS112 and *uvrA6uvr(502)⁺* strain KS113 were constructed by co-transduction of *metE* and *ilv⁺* from the strain x478 into MM300 *uvr⁺ilvrhalacY14thyA*Str^r and MM302 *uvrA6* (which is isogenic to MM300 except for *uvrA6*) respectively.

The *uvrA⁺uvr502* strain KS114 and the *uvrA6uvr502* strain KS115 were obtained by

Fig. 1. U.V. sensitivity of the strains KS112 *uvrA*⁺*uvr*(502)⁺ (●), KS113 *uvrA6 uvr*(502)⁺ (■), KS114 *uvrA*⁺*uvr*(502) (▲) and KS115 *uvrA6 uvr*502 (○).

co-transduction of the *uvr502* and *metE* mutations with *ilv*⁺ from the *ilv*⁺*uvr502 metE* strain KS20 into the strains MM300 and MM302 respectively. The presence of the *uvr502* and *uvrA6* mutations in the chromosome of KS115 was confirmed by making this strain a P1 donor, crossing it with the *malB5* strain JG123 or *ilv* strain MM300 and testing Mal⁺ or Ilv⁺ transductants for u.v. sensitivity. Approximately half of Mal⁺ and 18 % of Ilv⁺ transductants obtained were UV^s.

Construction of the uvr502 recB21 double mutant, its component single mutants and the uvr⁺ recB⁺ strain. The strains KS129, KS130, KS131 and KS132 are isogenic except for the *uvr502* or *recB21* mutations or both. The strains KS129 and KS130 were obtained in a transductional cross where the *thyA*⁺*recB21 argA*⁺ strain AB2470 was used as P1 donor and the *uvr*⁺*thyA recB*⁺*argA* strain KS60 as a recipient. The majority of selected Thy⁺Arg⁺ transductants obtained in this cross were UV^s and X^s and one of them was designated KS130. The UV^rX^r Thy⁺Arg⁺ transductant selected was designated KS129. The strains KS131 and KS132 are Thy⁺Arg⁺ X^r and Thy⁺Arg⁺ X^s transductants respectively obtained in a cross of the *uvr502 argA recB*⁺*thyA* strain KS59 (isogenic with KS60 except *uvr502*) with the same P1 donor AB2470.

Effects of the uvrA6, recA56 or recB21 mutations on u.v. sensitivity of the uvr502 strains

Fig. 1 shows u.v.-survival curves of the strains KS112 *uvrA*⁺*uvr*(502)⁺, KS113 *uvrA6 uvr*(502)⁺, KS114 *uvrA*⁺*uvr*502 and KS115 *uvrA6 uvr*502. The *uvr502* bacteria were about 4.6 times and the *uvrA6* bacteria about 15 times more u.v.-sensitive than wild-type bacteria. The *uvr502* strain was therefore about 3.3 times more u.v.-resistant than *uvrA6* strain. The *uvrA6 uvr*502 double mutant was only 1.5 times more u.v.-sensitive than the *uvrA6* single

Fig. 2. U.v. sensitivity of: (a) strains KS105 *uvr*⁺*recA*⁺ (●), KS106 *uvr502recA*⁺ (▲), KS102 *uvr*⁺*recA56* (■), KS107 *uvr502recA56* (○); (b) strains KS129 *uvr*⁺*recB*⁺ (●), KS130 *uvr*⁺*recB21* (■), KS131 *uvr502recB*⁺ (▲), KS132 *uvr502recB21* (○).

mutant. (Relative sensitivities were calculated as the ratios of the doses necessary to reduce survival by 63 % in the exponential regions of the curves.)

The above result is quite different from that demonstrating the strictly additive effects of the *uvrA6* and *recA* mutations on the u.v. sensitivity of *Escherichia coli* (Howard-Flanders & Boyce, 1966) and is similar to those showing the absence of additivity of the *uvrA6* and *resA* (Kato & Kondo, 1970) or *uvrA6* and *polA1* (Monk, Peacey & Gross, 1971) mutations. This suggested that the *uvr502* mutation, like *polA1* or *resA*, affects the excision repair pathway and not, perhaps, the repair pathway impaired in the *recA* mutants.

To verify this conclusion we studied the u.v. sensitivity of the double mutant *uvr502recA56* and of component single mutants and wild-type cells. Should the *uvr(502)*⁺ gene product act in the excision repair pathway then the *uvr502* mutation as well as *uvrA6* mutation ought to increase the u.v. sensitivity of the *recA*-strain. However we were surprised to find that *uvr502recA56* double mutant was no more u.v.-sensitive than the single *recA56* mutant (Fig. 2a). Thus the *uvr502* mutation affects some repair pathway which also requires both the *uvrA*⁺ and the *recA*⁺ genes.

Fig. 2(b) presents u.v.-survival curves of the strains isogenic except for the *uvr502* or *recB21* mutations or both. The double mutant *uvr502recB21* was much more sensitive than either the *uvr502* or *recB21* mutants, indicating an additivity of the *uvr502* and *recB21* mutations, i.e. *recB*⁺ coded products are required for a repair pathway different from that affected by the *uvr502* mutation.

Effect of the uvrA6, recA56 or recB21 mutations on spontaneous mutability of the uvr502 bacteria

Table 2 shows the spontaneous mutability of the *uvr502* mutants, wild-type bacteria and their *uvrA6*, *recA56* or *recB21* derivatives. The data (of this Table) show that the frequencies of Val^r mutants in populations of the *uvr502* strains were much higher than those of wild-type strains. The *uvrA6*, *recA56* or *recB21* single mutants did not differ in this respect from

Fig. 3. Loss of acid-insoluble radioactivity from DNA of: (a) strains KS129 *uvr*⁺ (●) and KS131 *uvr502* (▲) irradiated with 300 ergs/mm² of u.v. light; of (b) strains KS105 *uvr*⁺ *recA*⁺ (●), KS106 *uvr502 recA*⁺ (▲), KS102 *uvr*⁺ *recA56* (■) and KS107 *uvr502 recA56* (○) irradiated with 100 ergs/mm² of u.v. light.

Table 2. Mutant frequencies in the populations of the *uvrA6 uvr502*, *uvr502 recA56*, *uvr502 recB21* double mutants, their component single mutants and wild-type strains

Strain	Relative genotype	Val ^r mutant frequency
KS112	<i>uvrA</i> ⁺ <i>uvr(502)</i> ⁺	1.6×10^{-8}
KS113	<i>uvrA6 uvr(502)</i> ⁺	2×10^{-8}
KS114	<i>uvrA</i> ⁺ <i>uvr502</i>	5.3×10^{-6}
KS115	<i>uvrA6 uvr502</i>	4.8×10^{-6}
KS105	<i>uvr</i> ⁺ <i>recA</i> ⁺	8×10^{-9}
KS102	<i>uvr</i> ⁺ <i>recA56</i>	1.4×10^{-8}
KS106	<i>uvr502 recA</i> ⁺	10^{-6}
KS107	<i>uvr502 recA56</i>	3.1×10^{-6}
KS129	<i>uvr</i> ⁺ <i>recB</i> ⁺	10^{-8}
KS130	<i>uvr</i> ⁺ <i>recB21</i>	10^{-8}
KS131	<i>uvr502 recB</i> ⁺	2.3×10^{-6}
KS132	<i>uvr502 recB21</i>	5.4×10^{-6}

the corresponding *uvr*⁺ *rec*⁺ strains. The data presented in Table 2 show also that neither *uvrA6* nor *recA56* or *recB21* mutations reduced the mutator effect of the *uvr502* mutation, suggesting that neither *uvrA*⁺ nor *recA*⁺ or *recB*⁺ gene products are involved in the formation or repair of mistakes in DNA of the *uvr502* or *uvr(502)*⁺ bacteria.

DNA degradation in u.v.-irradiated uvr502 and uvr⁺ strains and their *recA56* derivatives

It was suggested above that the absence of additivity of the *uvr502* and *recA56* mutations on the rate of u.v. killing observed in our experiments reflects the participation of the *recA*⁺ gene product in an excision repair pathway which is partly blocked by the *uvr502* mutation. Since the 'reckless' type of u.v.-induced DNA degradation was found to be a characteristic property of *recA* mutants (Howard-Flanders & Boyce, 1966; Horii & Suzuki, 1968) we measured the extent of u.v.-induced DNA degradation in *uvr502* and found that it only slightly exceeded that in the isogenic *uvr*⁺ bacteria (Fig. 3a).

To study the effect of the *uvr502* mutation on u.v.-induced DNA breakdown in *recA* bacteria DNA degradation was measured in the double mutant *uvr502recA56*, its single component mutants and wild-type bacteria (Fig. 3*b*). The results obtained show a similar extensive loss of acid-precipitable radioactivity from DNA of the u.v.-irradiated *recA56* mutant and of *uvr502recA56* double mutant, showing that the enzymatic DNA breakdown in u.v.-irradiated *recA* bacteria is not affected by the *uvr502* mutation.

DISCUSSION

The data presented in this paper show that the double *uvrA6uvr502* mutant is only slightly more u.v.-sensitive than the *uvrA6* single mutant. A possible interpretation is that the *uvr(502)⁺* gene is responsible for some step of excision repair taking place after the incision step. However, we have not observed any additive effect of *uvr502* and *recA56* mutations. We suppose therefore that there exists a *uvrA⁺recA⁺* dependent repair pathway which also requires the *uvr(502)⁺* gene product. The activity coded by the *uvrA⁺* gene determines the ability to perform excision repair (Boyce & Howard-Flanders, 1964) while the *recA⁺* gene product acts in postreplicative repair (Smith & Meun, 1970) and in excision repair sequence (Monk *et al.* 1971; Cooper & Hanawalt, 1972; Yonei & Nozu, 1972). Moreover recently Bridges (1972) has postulated the existence of a third dark repair process for u.v. damage, re-initiation recovery, which may also be dependent upon both *recA⁺* and *uvrA⁺* functions. However, we suggest that the *uvr502* mutation affects primarily the excision repair pathway. This suggestion was confirmed by the examination of the single-strand molecular-weight distribution of the parent and progeny DNA in u.v.-irradiated *uvr⁺* and *uvr502* strains (Sinzinis, Saenko & Smirnov, in preparation). The results obtained in these experiments show that the *uvr502* mutation greatly reduces the joining of breaks made by incision in parent DNA strands, but not the extent of postreplicative repair.

Thus the excision repair pathway seems to require both the *uvrA⁺* and *recA⁺* genes and is most probably affected by the *uvr502* mutation. We suggest that the *recA* and *uvr502* mutations partly block the sequential linear reactions or the same reaction (if combined in a complex) of a particular step of excision repair. Inability to join interruptions resulting from dimer excision have been recently demonstrated for a *recA* mutant (Yonei & Nozu, 1972). Because of the apparent role of the *recA⁺* gene in repair replication (Cooper & Hanawalt, 1972) we suggest that the *uvr502* and *recA* mutations affect the resynthesis step of excision repair, which precedes final joining of single strand interruptions.

The fact that the *uvr502* bacteria are less u.v.-sensitive than *uvrA6* indicates that the excision repair pathway is not completely blocked in the *uvr502* mutant. The possible reasons for this relative resistance are those suggested by Klein & Niebch (1971) and Monk *et al.* (1971) for relatively high u.v. resistance of the *polA1* mutant, namely that either *uvr502* is leaky or the *uvr(502)⁺* gene product as well as DNA polymerase I may be substituted by another enzyme. If the second is true it seems possible that DNA polymerase I may substitute for the *uvr(502)⁺* gene product since *uvr502* is lethal in bacteria lacking DNA polymerase I (Mattern, 1971; Smirnov *et al.* 1973) as also is *recA* (Gross, Grunstein & Witkin, 1971). Thus in at least one of their vital functions the *uvr(502)⁺* and *recA⁺* genes may be similar in that it may be carried out by DNA polymerase I in each case. The viability of the double mutant *uvr502recA56* and inviability of either *polArecA* (Gross *et al.* 1971) or *polAuvr502* (Mattern, 1971; Smirnov *et al.* 1973) double mutants indicate that the product of neither *uvr(502)⁺* nor *recA⁺* gene alone can substitute for DNA polymerase I, although the latter can substitute for both simultaneously.

The strict additivity of the effects of *uvr502* and *recB21* mutations on u.v. sensitivity suggests that *recB*⁺-mediated repair acts predominantly in a repair pathway different from excision repair. Hence the *uvr502* mutation affects some step of the excision repair pathway in which the *recA*⁺, but not the *recB*⁺ gene also operates.

Recently Siegel has isolated a new u.v.-sensitive mutator strain of which the mutation *mutUI* is recessive and mapped between *ilv* and *metE* loci (Siegel, 1973). Thus the *mutUI* mutation described by Siegel is very similar to the *uvr502* mutation. In addition, the absence of additive effects of the *uvrA*⁻ and *mutUI* mutations on the u.v. sensitivity (Siegel, 1973) suggests that the *mutUI* mutation as well as the *uvr502* mutation affects some step of excision repair. However, the double mutant *mutUIrecA* was more u.v.-sensitive than the single *recA* mutant. This implies that the *mutUI* affects a step of excision repair which is not defective in the *recA* mutant and therefore the function controlled by the *mutU*⁺ is somewhat different from that controlled by the *uvr(502)*⁺. Possibly *mutUI* and *uvr502* mutations affect the different subunits or activities of the same enzyme.

In both *polA* and *recA* bacteria irradiated with u.v. light the free ends of unrepaired single-strand nicks are liable to the action of exonucleases that cause an extensive DNA breakdown (Howard-Flanders & Boyce, 1966; Paterson, Boyle & Setlow, 1971). The *mutUI* mutation, which is very similar in genetic location and phenotypic effect to the *uvr502* mutation, also causes increases in the rate of u.v.-induced DNA degradation when compared with *mutU*⁺ (Siegel, personal communication). In contrast to these observations we have found that the extent of u.v.-induced DNA degradation in the *uvr502* bacteria is only slightly greater than that in *uvr*⁺ bacteria. The result may be explained by at least two assumptions: (i) free ends of nicks made by *uvrA*⁺*B*⁺ or *C*⁺ coded endonucleases in the absence of the *uvr(502)*⁺ gene product are different in chemical structure from those made in *uvr(502)*⁺ cells; or (ii) the *uvr(502)*⁺ gene product is responsible for the modification of the ends of breaks made by *uvrA*⁺*B*⁺ or *C*⁺ coded endonucleases. This modification might be necessary for the availability of the ends for hydrolysis and probably for resynthesis. However, in the double mutant *uvr502recA56* the rate and final extent of u.v.-induced DNA degradation was the same as in the *recA56* single mutant. It means that *recB*⁺*recC*⁺ coded nucleases responsible for DNA degradation in u.v.-irradiated *recA* bacteria (Willets & Clark, 1969) can also degrade DNA in the u.v.-irradiated *uvr502recA56* double mutant. Although the behaviour of the double mutant *uvr502recA56* does not confirm our assumptions it does not definitely disprove them because the absence of the *recA*⁺ gene product may significantly alter the correlation of enzyme activities or the structure of breaks in the *uvr502* mutant.

The increased rate of spontaneous mutagenesis (mutator effect) is another well-known property of the *uvr502* mutant. We have suggested that the *uvr(502)*⁺ gene product is required for fidelity of replication rather than for repair of replication errors (Smirnov, Filkova & Skavronskaya, manuscript in preparation). In an attempt to determine the possible component of replication complex which makes mistakes in the absence of *uvr(502)*⁺ gene product we have compared the mutant frequencies in populations of the *uvr502* mutant and its *recA*⁻, *recB*⁻ or *uvrA*⁻ derivatives. All studied double mutants showed the mutability within the range of the *uvr502* single mutant. The data show that neither *uvrA*⁺ nor *recA*⁺ or *recB*⁺ gene products are responsible for the mutator effect of the *uvr502* mutation. The same results were obtained in the experiments of Siegel with *mutUI* mutation (Siegel, 1973).

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