Mutation Research, 166 (1986) 23–28
DNA Repair Reports
Elsevier

MTR 06156

Repair of DNA double-strand breaks in UV-irradiated *Escherichia coli uvrB recF* cells is inhibited by rich growth medium

Rakesh C. Sharma and Kendric C. Smith

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

(Received 11 September 1985)
(Revision received 19 December 1985)
(Accepted 20 December 1985)

Summary

Ultraviolet (UV)-irradiated *uvrB recF* and *uvrB recB* cells of *Escherichia coli* K-12 showed similar radiation sensitivities when plated on minimal growth medium (MM), however, the *uvrB recF* cells were much more UV radiation-sensitive than the *uvrB recB* cells when plated on rich growth medium. Sedimentation analysis of the DNA from UV-irradiated *uvrB recF* cells suggests that the rich medium killing of *uvrB recF* cells is due to the inhibition of the repair of UV-radiation-induced DNA double-strand breaks, i.e., the killing is due to the inhibition of the *recB*-dependent pathway of postreplication repair. Furthermore, we demonstrated that the DNA double-strand breaks that were formed in UV-irradiated *uvrB recA200*(Ts) cells incubated at 42°C in rich growth medium were not repaired whether the medium during subsequent repair incubation at 30°C was MM or rich growth medium, while DNA double-strand breaks that were formed in MM at 42°C could be repaired in MM or in rich growth medium at 30°C. How the absence of an abrupt slowing of DNA synthesis when UV-irradiated cells are held in rich growth medium (Sharma and Smith, 1985b) may prevent the repair of these DNA double-strand breaks is discussed.

UV-irradiated *Escherichia coli* K-12 *uvrA*, *uvrB* and *uvrC* cells show higher survival if plated on minimal growth medium (MM) rather than on rich growth medium [e.g., yeast extract-nutrient broth (Ganesan and Smith, 1968a; Sharma et al., 1982), or MM plus Casamino Acids (Sharma et al., 1982)]. This phenomenon has been referred to as 'minimal medium recovery' (MMR) (Ganesan and Smith, 1968b; Smith, 1971). The lethal effect of rich growth medium on UV-irradiated *uvrA(B)* cells has been correlated with the inhibition of the repair of DNA daughter-strand gaps (Sharma et al., 1982, 1983). The repair process involved in MMR is largely inducible (Sharma and Smith, 1983), and requires functional *recA*, *lexA* and *recB* genes; the *recF* gene plays a minor role in MMR (Sharma et al., 1982). However, all of these genes play major roles in postreplication repair (Wang and Smith, 1983).

Recent genetic and molecular studies suggest the existence of two major pathways of postreplication repair (both require a functional *recA* gene) (Rothman et al., 1975; Smith and Meun, 1970; Wang and Smith, 1981, 1983). One pathway is dependent on a functional *recF* gene, and repairs the DNA daughter-strand gaps formed after UV irradiation (Ganesan and Seawell, 1975; Rothman and Clark, 1977; Kato, 1977; Wang and Smith, 1983). The second pathway requires a functional *recB* gene, and repairs the DNA double-strand

breaks that are assumed to be formed at unrepaired DNA daughter-strand gaps (Wang and Smith, 1983).

The *recB* and *recF* pathways appear to be equally important for cell survival if UV-irradiated cells are plated on MM (Rothman et al., 1975; Wang and Smith, 1983), however, after UV irradiation, *uvrB recF* cells are much more sensitive to rich medium killing than are *uvrB recB* cells, i.e., the *uvrB recF* strain shows much more MMR than does the *uvrB recB* strain. These results suggest that rich growth medium inhibits the *recB*-dependent pathway of postreplication repair much more than it inhibits the *recF*-dependent pathway of postreplication repair. This notion was tested in this work.

Materials and methods

Bacterial strains, media and cultures

The strains of *E. coli* K-12 used in this study are listed in Table 1.

The minimal growth medium (MM), salts buffer (DTM), and minimal plating medium have been described (Sharma and Smith, 1985a). The rich plating medium (YENB plates) was Difco yeast extract (0.75%) plus Difco nutrient agar (2.3%). Liquid YENB was Difco yeast extract (0.75%) and Difco nutrient broth (0.8%). Phosphate buffer (PB) was Na_2HPO_4 (5.83 g/l) and KH_2PO_4 (3.53 g/l), pH 7.0.

Logarithmic-phase cultures were obtained by diluting (100-fold) a fresh overnight culture into MM, and incubating the diluted cultures in a shaking waterbath at 37°C [or at 30°C for the *uvrB recA200*(Ts) strain] until they reached an optical density at 650 nm (OD_{650}) of 0.4 (Zeiss PMQ II spectrophotometer). The cultures were diluted with MM to an OD_{650} of 0.1, and UV irradiated while in MM.

UV-irradiation

The source and method for UV-irradiation (254 nm) have been described (Sharma and Smith, 1983). For survival determination, irradiated and nonirradiated cell suspensions were diluted in PB and plated on MM and YENB plates. Incubation was for 1–3 days at 37°C. All experiments were done under yellow light to prevent photoreactivation.

Measurement of DNA repair

The methods used for alkaline and neutral sucrose gradient sedimentation were similar to those previously described (Sharma and Smith, 1983; Wang and Smith, 1983), except that the cells were UV-irradiated while in medium, and were shaken for 120 min at 37°C to allow the completion of repair.

The following protocol was used for the *uvrB recA200*(Ts) strain. Cells were grown as described above, adjusted to an OD_{650} of 0.1, and shaken for 60 min at 42°C. Then, the culture was readjusted to an OD_{650} of 0.1 (the media and dishes were preequilibrated to 42°C), UV-irradiated and shaken for 5 min at 42°C before pulse labeling

TABLE 1

LIST OF STRAINS OF *E. coli* K-12 [a]

Strain No.	Relevant genotype	Other characteristics	Source or reference
SR305	Δ(*uvrB-chlA*) *recF*143	*thyA36 deo*(C2?) *leuB19 lacZ53 malB45 rha-5 rpsL151*	D.A. Youngs
SR839	Δ(*uvrB-chlA*) *recA*56	Same as SR305, except also *srlA300*::Tn*10*	M. Tang
SR840	Δ(*uvrB-chlA*) *recB*21 *recF*143	*thyA deo* (C2?) *leuB19 lacZ53 malB45 rha-5 rpsL151*	M. Tang
SR1160	Δ(*uvrB-chlA*) *recB*21	Same as SR840	Wang and Smith, 1983
SR1347	Δ(*uvrB-chlA*) *recA*200(Ts)	Same as SR839	T.V. Wang

[a] Genotype symbols are those used by Bachmann (1983). All strains are F^- and λ^-.

with [*Me*-^{3}H]thymidine at 25 μCi/ml (80.2 Ci/mmole, New England Nuclear) for 10 min at 42°C. After pulse-labeling, the cultures (nonirradiated and UV-irradiated) were collected by filtration, washed with DTM and resuspended in MM. To resuspend the cells in rich growth medium, 1 vol. of 5 × YENB was added to 4 vol. of the culture in MM. The cells in MM and in rich growth medium were incubated for 100 min at 42°C to allow the maximum production of DNA double-strand breaks, and then were shifted to 30°C for 120 min to allow repair to occur. The cells were converted to spheroplasts and layered on top of gradients [4.8 ml of 5 to 20% sucrose containing 0.1% (vol./vol.) Triton X-100]. The layered gradients were held for at least 120 min in the dark at room temperature, and then were centrifuged at 3700 rpm for 40 h at 20°C. The number-average molecular weight (M_n) was calculated as described by Bonura et al. (1975) using [^{14}C]thymine-labeled bacteriophage T2 DNA as a molecular weight marker. The M_n values were used to calculate the number of DNA double-strand breaks (DSB) per double-strand genome (DSG) using the equation: $DSB/DSG = (M_{n(0)}/M_{n(uv)} - 1)\ (2.8 \times 10^9/M_{n(0)})$. Where $M_{n(0)}$ and $M_{n(uv)}$ represent the number-average molecular weight of DNA from unirradiated and UV-irradiated cells, respectively. A value of 2.8×10^9 dalton was used for the molecular weight of the *E. coli* genome (Cairns, 1963).

Results and discussion

The UV-radiation survival of various mutant cells grown to logarithmic-phase in MM and plated on MM and rich growth medium are shown in Fig. 1. In agreement with earlier data (Rothman et al., 1975; Wang and Smith, 1981, 1983), *uvrB recB* and *uvrB recF* cells showed a similar UV-radiation survival when plated on MM, however, the *uvrB recF* strain was much more UV-radiation-sensitive than the *uvrB recB* strain if plated on rich growth medium. The latter result is in agreement with our earlier observation (Sharma et al., 1982) that the *uvrB recB* strain shows only a small amount of MMR.

Since the major repair system operating in *uvrB recB* cells is the *recF*-dependent repair of DNA daughter-strand gaps that are formed after UV-irradiation, we studied the effect of rich growth medium on the repair of DNA daughter-strand gaps in *uvrB recB* cells. Consistent with the survival data, rich growth medium showed only a small effect on the amount of DNA daughter-strand gap repair observed in UV-irradiated *uvrB recB* cells (data not shown). Taken together, these data suggest only a small role for the *recF* gene in MMR repair.

The UV-radiation survival of the *uvrB recF* strain was ~ 6-fold higher (based upon a comparison of the F_{10} values, i.e., the fluence to yield 10% survival) than that for the *uvrB recB recF* strain when plated on rich growth medium (Fig. 1). When plated on rich growth medium, the *uvrB recB recF* strain was as UV radiation sensitive as a *uvrB recA* strain (Fig. 1), which shows no MMR and no postreplication repair (Ganesan and Smith, 1970; Sharma and Smith, 1985a; Smith and Meun, 1970). These survival data suggest that, in the *uvrB*

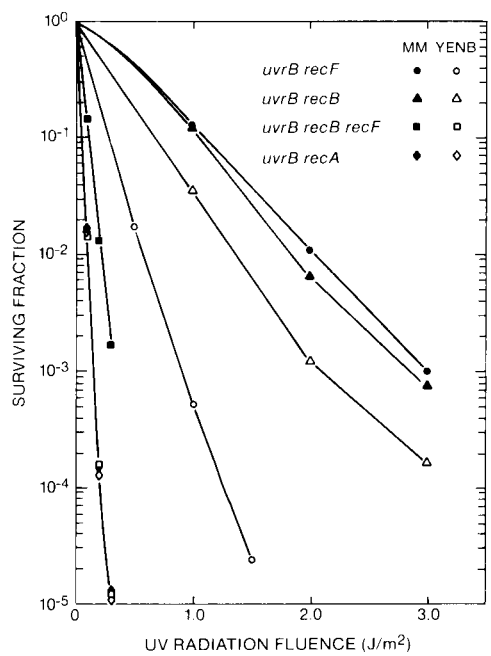

Fig. 1. Effect of plating medium on the UV-radiation survival of various DNA repair-deficient strains of *E. coli* K-12. Cells were grown to logarithmic phase in MM, UV irradiated (254 nm) in PB, and plated on MM (closed symbols) and on YENB (open symbols). Strains: *ΔuvrB recF143* (●, ○); *ΔuvrB recB21* (▲, △); *ΔuvrB recB21 recF143* (■, □); and *ΔuvrB recA56* (◆, ◇). Each data point represents the average of 2 Expts.

recF strain, rich growth medium may inhibit the *recB*-dependent pathway of postreplication repair. Therefore, we investigated the effect of rich growth medium on the *recB*-dependent pathway of postreplication repair, i.e., the repair of DNA double-strand breaks that apparently arise at unrepaired DNA daughter-strand gaps (Wang and Smith, 1983).

Based upon our survival studies (Fig. 1), and knowledge of the role of *recB* in postreplication repair (Wang and Smith, 1983), rich growth medium should inhibit the repair of DNA double-strand breaks in UV-irradiated *uvrB recF* cells. This inhibition could be due to one or more reasons: (i) more DNA double-strand breaks are produced when UV-irradiated *uvrB recF* cells are incubated in rich growth medium, (ii) the number of DNA double-strand breaks produced are the same in MM and rich growth medium, but their repair is inhibited by rich growth medium.

To explore these two possibilities, we measured the effect of rich growth medium on the formation and repair of DNA double-strand breaks in UV-irradiated *uvrB recF* cells. After 2 h of postirradiation incubation, the formation of DNA double-strand breaks was observed for cells suspended either in MM or rich growth medium (Fig. 2). The number of DNA double-strand breaks at 2 h of postirradiation (0.25 J/m^2) incubation in MM and rich growth medium were 2 and 4 DNA double-strand breaks per double-strand genome, respectively. On further incubation in MM (4 h at 37°C), most of these DNA double-strand breaks were repaired (Fig. 2A). However, only about half of the [^{3}H]DNA returned to a high molecular weight [i.e., it sedimented under the peak of unirradiated DNA (Fig. 2B)] when the postirradiation incuba-

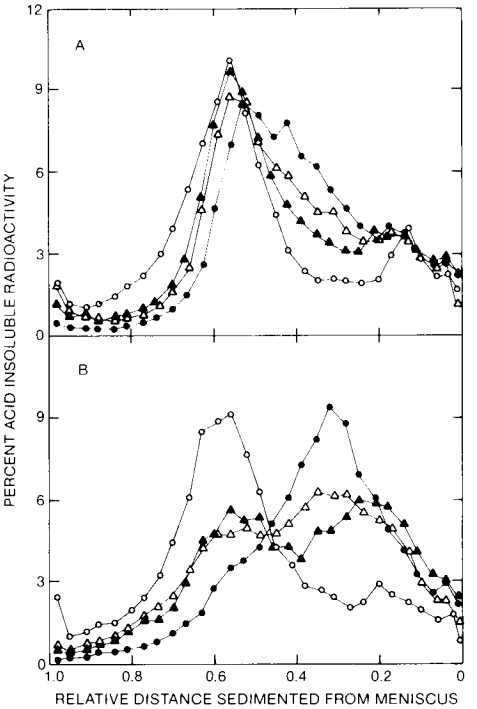

Fig. 2. Effect of rich growth medium on the formation and repair of DNA double-strand breaks in UV-irradiated (0.25 J/m^2) *ΔuvrB recF143* cells. Cells grown in MM were pulse-labeled with [^{3}H]thymidine for 10 min in MM, and then were allowed to repair either in MM (A), or in YENB (B). Symbols: unirradiated control (○); UV-irradiated cells after 2 h (●), 3 h (△), and 4 h (▲) of postirradiation incubation. The data are from a representative experiment.

TABLE 2

EFFECT OF RICH GROWTH MEDIUM ON THE REPAIR OF DNA DOUBLE-STRAND BREAKS IN UV-IRRADIATED *E. coli* K-12 *ΔuvrB recA200*(Ts) [a]

Medium during the formation of DNA DSB at 42°C [b]	Medium during the repair of DNA DSB at 30°C [c]	Did the repair of DSB occur?	Panels in Fig. 3
MM	MM	Yes	A (compare ● and ▲)
MM	RM	Yes	B (compare ● and △)
RM	RM	No	C (compare ○ and △)
RM	MM	No	D (compare ○ and ▲)

[a] Cells were UV-irradiated (0.5 J/m^2) and pulse-labeled with [^{3}H]thymidine in MM at 42°C.

[b] Incubated for 100 min to observe the maximum production of DNA double-strand breaks (DSB). Rich growth medium (RM) was yeast extract-nutrient broth.

[c] Incubated for 120 min to observe the maximum repair of DNA DSB.

tion was in rich growth medium, suggesting that the repair of DNA double-strand breaks under these conditions was incomplete.

The accumulation of more DNA double-strand breaks in rich growth medium than in MM after 2 h of postirradiation incubation (Fig. 2) suggests that rich growth medium enhances the formation of DNA double-strand breaks, however, since the kinetics reflect both the formation and repair of DNA double-strand breaks, an inhibition of repair by rich growth medium would yield the same result.

To study separately the formation and repair of DNA double-strand breaks, we have employed a *uvrB recA200*(Ts) strain. This strain is RecA$^-$ at 42°C (nonpermissive temperature) and RecA$^+$ at 30°C (permissive temperature) (Lloyd et al., 1974). After UV irradiation (0.5 J/m^2), *uvrB recA*(Ts) cells were pulse-labeled with [^{3}H]thymidine and incubated at 42°C (in MM or rich growth medium) to accumulate DNA double-strand breaks, and then the repair of these DNA double-strand breaks was followed by further incubation in MM or rich growth medium at 30°C. The results shown in Fig. 3, and summarized in Table 2, demonstrate that in *uvrB recA*(Ts) cells: (i) the numbers of DNA double-strand breaks formed during postirradiation incubation in MM or rich growth medium were the same (12 DNA double-strand breaks per genome), (ii) DNA double-strand breaks formed in rich growth medium were not repaired whether the cells were incubated in MM or rich growth medium, and (iii) DNA double-strand breaks formed in MM were repaired whether the cells were incubated in MM or rich growth medium.

Since the DNA double-strand breaks that were formed when the cells were incubated in rich medium were not repairable regardless of the type of medium used during the repair phase, we suggest that the DNA double-strand breaks that were not repaired when the *uvrB recF* cells were switched to rich medium (Fig. 2) were probably the double-strand breaks that were formed after these cells were switched to rich growth medium.

To explain how the presence of rich growth medium may convert repairable DNA double-strand breaks to nonrepairable DNA double-strand breaks, we propose the following mechanism. Recently we have shown that the rich-medium killing of UV-irradiated *uvrA* cells correlates with the lack of an abrupt inhibition of DNA synthesis in rich growth medium. The abrupt inhibition of DNA synthesis that occurs immediately after UV-

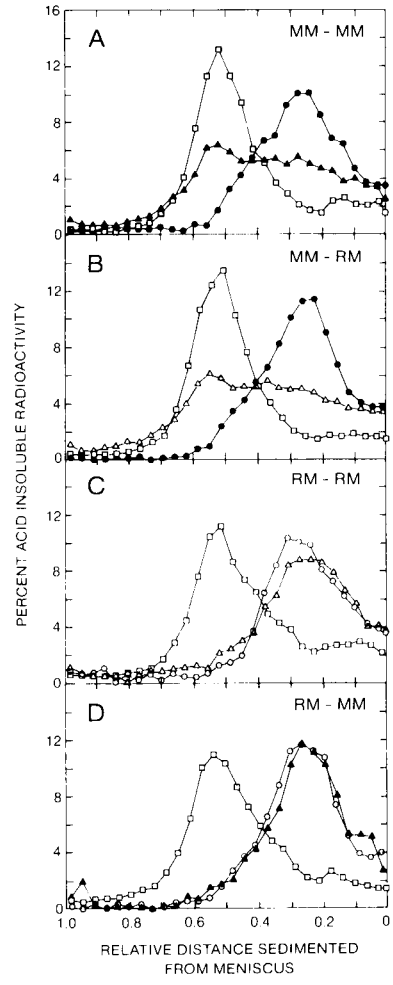

Fig. 3. Effect of postirradiation culture medium on the formation and repair of UV radiation-induced DNA double-strand breaks in a *ΔuvrB recA200*(Ts) strain of *E. coli* K-12. UV-irradiated (0.5 J/m^2) cells were pulse-labeled in MM with [^{3}H]thymidine for 10 min at 42°C, and shaken in MM (●) (A, B) or YENB (○) (C, D) for 100 min at 42°C to allow the formation of DNA double-strand breaks. The repair of these breaks was then followed either in MM (▲) (A, D) or YENB (△) (B, C) at 30°C for 120 min. Profiles shown for unirradiated cells (□) (A–D) are after 100 min of incubation at 42°C. Similar profiles were obtained for unirradiated cells immediately after pulse-labeling or after an additional 120 min of incubation at 30°C (data not shown). The data are from a representative experiment for each set of conditions.

irradiation in cells incubated in MM apparently favors the recombinational repair of DNA daughter-strand gaps (Sharma and Smith, 1985b). However, if DNA synthesis is not inhibited, the sister duplex containing a double-strand break may move further away from the adjacent sister duplex needed to initiate recombination in order to generate lesion free DNA, thereby converting repairable DNA double-strand breaks to nonrepairable DNA double-strand breaks without affecting the yield of double-strand breaks.

In summary, a major portion of the rich-medium killing of UV-irradiated *uvrB recF* cells is due to the inhibition of the *recB*-dependent pathway of postreplication repair, i.e., the repair of DNA double-strand breaks that arise at the sites of unrepaired DNA daughter-strand gaps.

Acknowledgements

We thank Drs. Neil J. Sargentini and Tzu-chien V. Wang for helpful discussions, and Carmencita Nicolas for skillful technical assistance.

This work was supported by Public Health Service Research grant CA-02896 from the National Cancer Institute, DHHS.

References

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

Bonura, T., C.D. Town, K.C. Smith and H.S. Kaplan (1975) The influence of oxygen on the yield of DNA double-strand breaks in X-irradiated *Escherichia coli* K-12, Radiat. Res., 63, 567–577.

Cairns, J. (1963) The chromosome of *Escherichia coli*, Cold Spring Harbor Symp. Quant. Biol., 28, 43–46.

Ganesan, A.K., and P.C. Seawell (1975) The effect of *lexA* and *recF* mutations on post-replication repair and DNA synthesis in *Escherichia coli* K-12, Mol. Gen. Genet., 141, 189–205.

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

Ganesan, A.K., and K.C. Smith (1968b) Recovery of recombination deficient mutants of *Escherichia coli* K-12 from ultraviolet irradiation, Cold Spring Harbor Symp. Quant. Biol., 33, 235–242.

Ganesan, A.K., and K.C. Smith (1970) Dark recovery processes in *Escherichia coli* irradiated with ultraviolet light, III. Effect of *rec* mutations on recovery of excision-deficient mutants of *Escherichia coli* K-12, J. Bacteriol., 102, 404–410.

Kato, T. (1977) Effects of chloramphenicol and caffeine on postreplication repair in *uvrA umuC*$^-$ and *uvrA recF*$^-$ strains of *Escherichia coli* K-12, Mol. Gen. Genet., 156, 115–120.

Lloyd, R.G., B. Low, G.N. Godson and E.A. Brige (1974) Isolation and characterization of an *Escherichia coli* K-12 mutant with a temperature-sensitive RecA$^-$ phenotype, J. Bacteriol., 120, 407–415.

Rothman, R.H., and A.J. Clark (1977) The dependence of postreplication repair on *uvrB* in a *recF* mutant of *Escherichia coli* K-12, Mol. Gen. Genet., 155, 279–286.

Rothman, R.H., T. Kato and A.J. Clark (1975) The beginning of an investigation of the role of *recF* in the pathways of metabolism of ultraviolet-irradiated DNA in *Escherichia coli*, in: P.C. Hanawalt and R.B. Setlow (Eds.), Molecular Mechanisms for Repair of DNA, Plenum, New York, pp. 283–291.

Sharma, R.C., and K.C. Smith (1983) Inducible postreplication repair is responsible for minimal medium recovery in UV-irradiated *Escherichia coli* K-12, Photochem. Photobiol., 38, 301–303.

Sharma, R.C., and K.C. Smith (1985a) A minor pathway of postreplication repair in *Escherichia coli* is independent of the *recB*, *recC* and *recF* genes, Mutation Res., 146, 169–176.

Sharma, R.C., and K.C. Smith (1985b) A mechanism for rich-medium inhibition of the repair of daughter-strand gaps in the deoxyribonucleic acid of UV-irradiated *Escherichia coli* K12, Mutation Res., 146, 177–183.

Sharma, R.C., T.R. Barfknecht and K.C. Smith (1982) Postreplication repair in *uvrA* and *uvrB* strains of *Escherichia coli* K-12 is inhibited by rich growth medium, Photochem. Photobiol., 36, 307–311.

Sharma, R.C., N.J. Sargentini and K.C. Smith (1983) New mutation (*mmrA1*) in *Escherichia coli* K-12 that affects minimal medium recovery and postreplication repair after UV irradiation, J. Bacteriol., 154, 743–747.

Smith, K.C. (1971) The roles of genetic recombination and DNA polymerase in the repair of damaged DNA, Photophysiology, 6, 209–278.

Smith, K.C., and D.H.C. Meun (1970) Repair of radiation-induced damage in *Escherichia coli*, I. Effect of *rec* mutations on postreplication repair of damage due to ultraviolet radiation, J. Mol. Biol., 51, 459–472.

Wang, T.V., and K.C. Smith (1981) Effect of *recB21*, *uvrD3*, *lexA101* and *recF143* mutations on ultraviolet radiation sensitivity and genetic recombination in Δ*uvrB* strains of *Escherichia coli* K-12, Mol. Gen. Genet., 183, 37–44.

Wang, T.V., and K.C. Smith (1983) Mechanisms for the *recF*-dependent and *recB*-dependent pathways of postreplication repair in UV-irradiated *Escherichia coli uvrB*, J. Bacteriol., 156, 1093–1098.