Mutation Research, 145 (1985) 107–112
DNA Repair Reports
Elsevier

MTR 06093

Role of the *umuC* gene in postreplication repair in UV-irradiated *Escherichia coli* K-12 *uvrB*

Tzu-chien V. Wang and Kendric C. Smith

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

(Received 8 October 1984)
(Revision received 4 December 1984)
(Accepted 6 December 1984)

Summary

The role of the *umuC* gene product in postreplication repair was studied in UV-irradiated *Escherichia coli* K-12 *uvrB* cells. A mutation at *umuC* increased the UV radiation sensitivities of *uvrB*, *uvrB recF*, *uvrB recB*, and *uvrB recF recB* cells; it also increased the deficiencies in the repair of DNA daughter-strand gaps in these strains, but it did not affect the repair of DNA double-strand breaks that arose from unrepaired DNA daughter-strand gaps. We suggest that the *umuC* gene product is involved in a minor system for the repair of DNA daughter-strand gaps, possibly the repair of overlapping DNA daughter-strand gaps.

The major DNA 'dark' repair system operating in excision-repair-deficient *uvrABC* cells of *Escherichia coli* after UV irradiation is postreplication repair (Howard-Flanders, 1968). Studies on the genetic control of postreplication repair have indicated the existence of two major, independent pathways for postreplication repair, i.e., the *recF*-dependent and the *recB*-dependent pathways (Rothman et al., 1975; Wang and Smith, 1981). The *recF*-dependent pathway is largely responsible for the repair of DNA daughter-strand gaps that are formed in newly synthesized DNA after UV irradiation (Ganesan and Seawell, 1975; Kato, 1977; Rothman and Clark, 1977; Wang and Smith, 1983). The *recB*-dependent pathway plays little role in the repair of DNA daughter-strand gaps, but has recently been shown to be required for the repair of DNA double-strand breaks that arise from unrepaired DNA daughter-strand gaps (Wang and Smith, 1983). In addition to the two major pathways mentioned above, a minor, *recF recB*-independent pathway for the repair of DNA daughter-strand gaps has been observed (Wang and Smith, 1983; Sharma and Smith, submitted).

Among a number of genes that are thought to affect postreplication repair (see Wang and Smith, 1981), the *umuC* gene is unique in that a mutation in this locus specifically eliminates UV-radiation mutagenesis and Weigle reactivation of UV-irradiated bacteriophages, but does not affect the induction of other 'SOS' responses (Kato and Shinoura, 1977). This specificity makes *umuC* mutants ideal candidates for the study of postreplication repair process(es) that lead to UV radiation mutagenesis. The *umuC* gene codes for a 45 000 M_r protein (Elledge and Walker, 1983), and is one of the loci that are inducible by DNA damage, and are regulated by the $recA^+$ and $lexA^+$ gene products (Bagg et al., 1981). However, the biochemical function of the *umuC* gene product in UV-radiation mutagenesis is not known. Kato (1977) observed that a *uvrA umuC* strain repairs DNA daughter-strand gaps as efficiently as its parental *uvrA* strain, following a UV-radiation fluence of 2 J/m^2, sug-

gesting that there is no major deficiency in gap-filling repair in *umuC* mutants. However, it is not known if the *umuC* gene product is involved in the *recB*-dependent pathway of postreplication repair or in the minor, *recF recB*-independent repair of DNA daughter-strand gaps. We have investigated these possibilities.

Materials and methods

Bacterial strains and media. The bacterial strains used are listed in Table 1. The transduction technique used in strain construction was similar to that described by Miller (1972). Supplemented minimal medium (SMM) and DTM buffer have been described (Wang and Smith, 1982).

Irradiation. The source (254 nm) and measurement of fluence rate for UV irradiation have been described (Wang and Smith, 1981). For survival studies, cultures were grown in SMM and UV irradiated as previously described (Wang and Smith, 1983). Survivors were determined by assaying colony-forming units on SMM agar.

DNA-repair studies. Cells were grown exponentially at 37°C in SMM (containing thymine at 2 μg/ml) until they reached an optical density at 650 nm (OD_{650}) of 0.1–0.2 (Zeiss PMQ II spectrophotometer). The cultures were UV irradiated and pulse-labeled with [*Me*-^{3}H]thymidine (64 Ci/mmole; Amersham Corp.) as described previously (Wang and Smith, 1983). The fate of DNA newly synthesized after UV irradiation was followed during repair incubation by sedimentation analysis on both alkaline and neutral sucrose gradients, as previously described (Wang and Smith, 1983). Sedimentation data obtained from alkaline sucrose gradients were used to determine the relative proficiency of cells to repair DNA daughter-strand gaps. Neutral sucrose gradients were used to study the ability of cells to repair the DNA double-strand breaks that arise from unrepaired DNA daughter-strand gaps.

TABLE 1

E. coli K-12 STRAINS USED [a]

Stanford radiology No.	Relevant genotype	Source or derivation
SR772 [b]	*uvrB5 recA56*	N.J. Sargentini
SR1034 [b]	*uvrB5 umuC122*::Tn*5*	N.J. Sargentini
SR1040	*uvrB5*	This work
SR1041	*uvrB5 recF143*	This work
SR1042	*uvrB5 umuC36*	This work
SR1043	*uvrB5 umuC36 recF143*	This work
SR1202 [b]	*uvrB5 recB21*	This work
SR1203	*uvrB5 recF143 recB21*	This work
SR1204	*uvrB5 umuC36 recF143 recB21*	This work
SR1205 [b]	*uvrB5 umuC36 recB21*	This work
SR1358	*uvrB5 recF143 umuC122*::Tn*5*	This work
SR1361	*uvrB5 recF143 recB21 umuC122*::Tn*5*	This work

[a] These strains are derivatives of the R.B. Helling strain KH21. They are F$^-$ and λ$^-$, and carry *leuB19 thyA deo(C2?) lacZ53 rha-5 rpsL151*. Genotype symbols are those used by Bachmann (1983).

[b] These strains also carry *metE70*.

Results

Survival studies. The effect of the *umuC36* mutation on the UV-radiation sensitivity of *uvrB5* cells is shown in Fig. 1. A *umuC* mutation conferred a moderate increase in the UV-radiation sensitivity of *uvrB* cells, similar to that observed in the *uvrA* genetic background (Kato and Shinoura, 1977). The presence of the *umuC36* mutation further increased the UV radiation sensitivity of the *uvrB recF* and *uvrB recB* cells, and slightly increased the sensitivity of *uvrB recB recF* cells, but did not bring them to the same sensitivity as that of *uvrB recA* cells (Fig. 1). Substituting *umuC122*::Tn*5* for the *umuC36* allele yielded the same sensitizing effect in terms of UV-radiation survival (data not shown), although these two mutant alleles were reported to have different effects on UV radiation mutagenesis (Sargentini and Smith, 1984).

Effect of a umuC mutation on the repair of DNA daughter-strand gaps. Compared to *uvrB* cells, *uvrB umuC* cells showed little or no deficiency in the repair of DNA daughter-strand gaps at a UV-radiation fluence of 3 J/m^2; however, at fluences greater than 3 J/m^2, *uvrB umuC* cells accumulated more unrepaired DNA daughter-strand gaps than did *uvrB* cells (Fig. 2). The presence of a *umuC* mutation also decreased the ability of *uvrB recB*, *uvrB recF* and *uvrB recF recB*

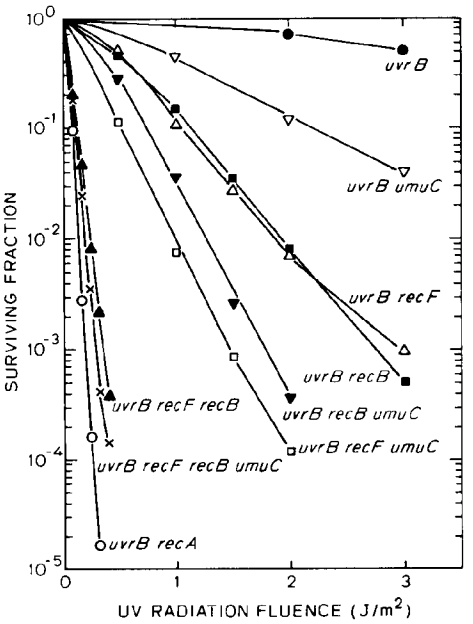

Fig. 1. Effects of a *umuC36* mutation on cell survival in UV-irradiated *uvrB* strains of *E. coli* K-12. All strains were grown and treated as described in Materials and Methods. Symbols: ●, *uvrB* (SR1040); △, *uvrB recF* (SR1041); ▽, *uvrB umuC* (SR1042); ■, *uvrB recB* (SR1202); ▼, *uvrB recB umuC* (SR1205); □, *uvrB recF umuC* (SR1043); ▲, *uvrB recF recB* (SR1203); ×, *uvrB recF recB umuC* (SR1204); and ○, *uvrB recA* (SR772).

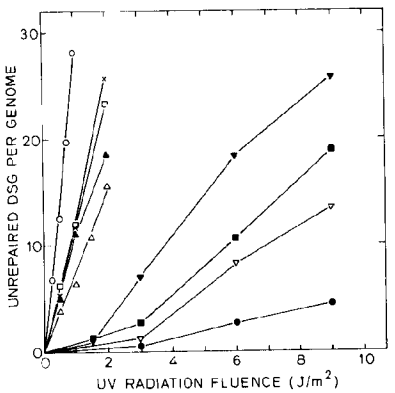

Fig. 2. Effects of a *umuC36* mutation on the repair of DNA daughter-strand gaps (DSG) in UV-irradiated *uvrB* strains of *E. coli* K-12. Symbols for the strains are the same as shown in the legend to Fig. 1.

cells to repair DNA daughter-strand gaps. Compared to 'repairless' *uvrB recA* cells, the *uvrB recB recF umuC* cells were able to perform a limited amount of repair of DNA daughter-strand gaps in minimal growth medium (Fig. 2), indicating that a *umuC* mutation did not completely inhibit the *recF recB*-independent repair of DNA daughter-strand gaps.

Effect of a umuC mutation on the repair of DNA double-strand breaks. In addition to the gap-filling repair mechanism of postreplication repair, a new mechanism, which is called 'sister duplex recombination', has been described (Wang and Smith, 1983). This sister duplex recombination process, which appears to be a type of recombination repair for DNA double-strand breaks, was originally observed in UV-irradiated *uvrB recF* cells, which are grossly deficient in daughter-strand gap-filling repair. Presumably, the accumulation of

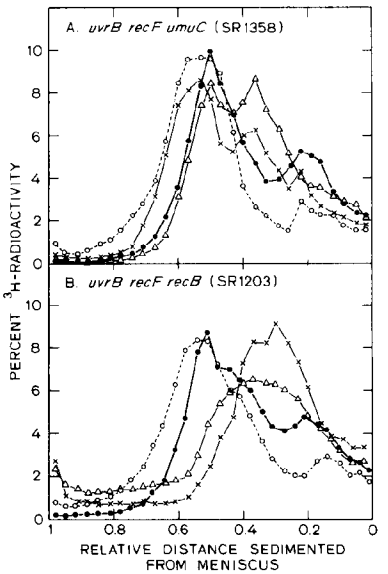

Fig. 3. Kinetics of the formation and repair of DNA double-strand breaks in UV-irradiated *uvrB recF umuC* (A) and *uvrB recF recB* (B) cells of *E. coli* K-12. The cells were UV irradiated with 0.5 J/m², and, after 10 min of postirradiation incubation at 37°C, were pulse-labeled with [³H]thymidine for 5 min. The fate of [³H]DNA was followed during subsequent repair incubation by sedimentation on neutral sucrose gradients. Symbols: unirradiated control (○); UV-irradiated with no repair incubation (●); UV-irradiated and incubated for 2 h (△) or 4 h (×).

unrepaired DNA daughter-strand gaps in *uvrB recF* cells leads to the formation of DNA double-strand breaks, which initiate a recombination repair process that is dependent on the *recB* and *recA* genes. To observe this process, the fate of DNA newly synthesized after UV irradiation was followed by sedimentation in neutral sucrose gradients. As shown in Fig. 3A, the newly synthesized DNA of UV-irradiated *uvrB recF umuC* cells sedimented more slowly than did the DNA from unirradiated control cells. After 2 h of repair incubation, the DNA from irradiated cells sedimented even more slowly, indicating an increase in the number of DNA double-strand breaks. However, after 4 h of incubation, a good fraction of the DNA from irradiated cells sedimented like the DNA from unirradiated cells, indicating that some DNA double-strand breaks were repaired. The kinetics for the formation and repair of DNA double-strand breaks in UV-irradiated *uvrB recF umuC* cells were similar to those for *ΔuvrB recF* (Wang and Smith, 1983) and *uvrB5 recF* cells (data not shown), indicating that the *umuC* mutation does not significantly affect the sister duplex recombination process. This result is in contrast to the inhibition of this repair process by a *recB* mutation (Fig. 3B).

Discussion

The generally accepted model for UV-radiation mutagenesis is that mutations arise from the inducible 'error-prone' repair of DNA damage (Witkin, 1976; Walker, 1984). Since the *umuC* mutation blocks UV-radiation mutagenesis (Kato and Shinoura, 1977), and is under the control of the *recA lexA* genes (Bagg et al., 1981), the *umuC* gene product should be one of the best candidates for a protein that plays a key mechanistic role in error-prone DNA repair. In the present work, we have attempted to explore the function of the *umuC* gene product in efror-prone DNA repair. Our survival studies indicate that the *umuC* mutation further sensitizes *uvrB, uvrB recF, uvrB recB,* and *uvrB recB recF* cells to UV irradiation (Fig. 1). An analysis of these survival curves (see Wang and Smith, 1981) indicates that the *umuC* mutation interacts mostly additively with both the *recF* and *recB* mutations in terms of radiation sensitization, while the *recF* mutation interacts synergistically with the *recB* mutation. An additive interaction between two repair functions (processes) suggests that the two repair functions act on different types of DNA damage; i.e., the substrates for the *umuC*-dependent repair and for the *recF recB*-dependent repair are different. One possible difference in the substrates for postreplication repair may be the physical nature of the DNA daughter-strand gaps. Some DNA daughter-strand gaps on one DNA duplex may overlap with DNA daughter-strand gaps on the other DNA duplex. Overlapping daughter-strand gaps may constitute a special obstacle for repair, and have been proposed to serve as inducing signals for the 'SOS' response, and as substrates for inducible, error-prone DNA repair (Sedgwick, 1975, 1976). According to such a hypothesis, it is likely that nonoverlapping DNA daughter-strand gaps are the substrates for *recB recF*-dependent repair, while overlapping DNA daughter-strand gaps are the substrates for *umuC*-dependent repair.

Our DNA-repair studies indicate that a *umuC* mutation does not significantly affect the repair of DNA double-strand breaks that arise from unrepaired DNA daughter-strand gaps in UV-irradiated (0.5 J/m^2) *uvrB recF* cells (i.e., the *recB*-dependent pathway of postreplication repair). Consistent with the notion that the *umuC* mutation does not affect sister duplex recombination repair, we observed that *uvrB umuC* (SR1042) cells are as proficient as *uvrB* (SR1040) and *uvrB recF* (SR1041) cells in repairing DNA double-strand breaks produced by γ-irradiation (4 krad), while *uvrB recB* (SR1202) cells showed no evidence of repairing these double-strand breaks (data not shown). On the other hand, a mutation at *umuC* decreases the efficiency of gap-filling repair in *uvrB, uvrB recB, uvrB recF,* and *uvrB recB recF* cells. A *umuC* mutation does not completely inhibit the *recF recB*-independent gap-filling repair process, since *uvrB recB recF umuC* cells can still perform a limited amount of repair of DNA daughter-strand gaps (Fig. 2). In general, these repair studies correlate with the survival studies, and suggest that the *umuC* gene product is involved in a minor system for the repair of DNA daughter-strand gaps, possibly the repair of overlapping DNA daughter-strand gaps, as discussed above.

TABLE 2

COMPARISON BETWEEN THE THEORETICAL FREQUENCY OF OVERLAPPING DNA DAUGHTER-STRAND GAPS AND THE MEAN LETHAL HITS IN UV-IRRADIATED *E. coli uvrB*

UV radiation fluences (J/m^2)	Number of overlapping DNA daughter-strand gaps [a]	Mean lethal hits ($-\ln$ of surviving fraction)
3	3.3	0.6
6	13.3	1.5
9	29.8	2.4

[a] Assuming a random distribution of pyrimidine dimers in *E. coli* DNA after UV irradiation, the frequency of forming overlapping DNA daughter-strand gaps is equal to $n^2 \cdot g/m$ (Sedgwick, 1975), where g is the mean length of the gaps in nucleotides and is taken as 1000 nucleotides (Iyer and Rupp, 1971) in this calculation, m is the number of nucleotides per genome (a value of 8.48×10^6 is used), and n is the initial frequency of DNA daughter-strand gaps per genome, and is assumed to be equivalent to the frequency of pyrimidine dimers in parental DNA. A value of 56 pyrimidine dimers per J/m^2 (Unrau et al., 1973) was used for n.

A theoretical prediction for the formation of overlapping DNA daughter-strand gaps as a function of UV radiation fluence was derived according to Sedgwick (1975). Taking an average gap size of 1000 nucleotides (Iyer and Rupp, 1971), the number of overlapping DNA daughter-strand gaps produced in UV-irradiated *uvrB* cells is considerably greater than the number of mean lethal hits (i.e., $-\ln$ of the surviving fraction), suggesting that overlapping DNA daughter-strand gaps are repairable (Table 2). Although practically nothing is known about the mechanism or genetic control of the repair of overlapping DNA daughter-strand gaps, the proposed error-prone replication of damaged DNA (i.e., transdimer synthesis) (Caillet-Fauquet et al., 1977) represents one possible mechanism of action for the *umuC* gene product. An involvement of the *umuC* gene in error-prone replication has also been suggested (Kato and Shinoura, 1977; Defais, 1983). Alternatively, the *umuC* gene may be involved in the induction of genes that are involved in the repair of these gaps.

Recently, Bridges and Woodgate (1984) made an interesting observation that *umuC* strains are mutable by UV radiation, provided that the irradiated cells are photoreactivated after prolonged incubation in growth medium. These authors suggested that the *umuC* gene product may be required for strand elongation past a blocking lesion rather than being involved in the crucial misincorporation step in UV radiation mutagenesis. However, other interpretations of their interesting data are also possible.

In summary, the *umuC* gene product is required for the repair of a small number of DNA daughter-strand gaps. On the basis of survival studies, we suggest that the substrates for the *umuC*-dependent repair are different from those for *recF*-dependent gap-filling repair, and are possibly overlapping DNA daughter-strand gaps.

Acknowledgements

We are grateful to Carmencita E. Nicolas for skillful technical assistance, and to Drs. Neil J. Sargentini and Rakesh C. Sharma for valuable discussions.

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

References

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.

Bridges, B.A., and R. Woodgate (1984) Mutagenic repair in *Escherichia coli*, X. The *umuC* gene product may be required for replication past pyrimidine dimers but not for the coding error in UV-mutagenesis, Mol. Gen. Genet., 196, 364–366.

Caillet-Fauquet, P., M. Defais and M. Radman (1977) Molecular mechanisms of induced mutagenesis. Replication in vivo of bacteriophage ΦX174 single-stranded, ultraviolet light-irradiated DNA in intact and irradiated host cells, J. Mol. Biol., 117, 95–112.

Defais, M. (1983) Role of the *E. coli umuC* gene product in the repair of single-stranded DNA phage, Mol. Gen. Genet., 192, 509–511.

Elledge, S.J., and G.C. Walker (1983) Proteins required for ultraviolet light and chemical mutagenesis, Identification of the products of the *umuC* locus of *Escherichia coli*, J. Mol. Biol., 164, 175–192.

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.

Howard-Flanders, P. (1968) DNA repair, Annu. Rev. Biochem., 37, 175–200.

Iyer, V.N., and W.D. Rupp (1971) Usefulness of benzoylated naphthoylated DEAE-cellulose to distinguish and fractionate double-stranded DNA bearing different extents of single-stranded regions, Biochim. Biophys. Acta, 228, 117–126.

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.

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.

Miller, J.H. (1972) Experiments in molecular genetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

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.

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.

Sedgwick, S.G. (1975) Genetic and kinetic evidence for different types of post-replication repair in *Escherichia coli* B, J. Bacteriol., 123, 154–161.

Sedgwick, S.G. (1976) Misrepair of overlapping daughter strand gaps as a possible mechanism for UV induced mutagenesis in *uvr* strains of *Escherichia coli*: A general model for induced mutagenesis by misrepair (SOS repair) of closely spaced DNA lesions, Mutation Res., 41, 185–200.

Sharma, R.C., and K.C. Smith (1984) A minor pathway of postreplication repair in *Escherichia coli* is independent of the *recB* and *recF* genes, Mutation Res., in press.

Unrau, P., R. Wheatcraft, B. Cox and T. Olive (1973) The formation of pyrimidine dimers in the DNA of fungi and bacteria, Biochim. Biophys. Acta, 312, 626–632.

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

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*, Mol. Gen. Genet., 183, 37–44.

Wang, T.V., and K.C. Smith (1982) Effects of the *ssb-1* and *ssb-113* mutations on survival and DNA repair in UV-irradiated *uvrB* strains of *Escherichia coli* K-12, J. Bacteriol., 151, 186–192.

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.

Witkin, E.M. (1976) Ultraviolet mutagenesis and inducible DNA repair in *Escherichia coli*, Bacteriol. Rev., 41, 869–907.