JOURNAL OF BACTERIOLOGY, May 1983, p. 743–747
0021-9193/83/050743-05$02.00/0

Vol. 154, No. 2

New Mutation (*mmrA1*) in *Escherichia coli* K-12 That Affects Minimal Medium Recovery and Postreplication Repair After UV Irradiation

RAKESH C. SHARMA, NEIL J. SARGENTINI, AND KENDRIC C. SMITH*

Department of Radiology, Stanford University School of Medicine, Stanford, California 94305

Received 13 December 1982/Accepted 1 March 1983

After UV irradiation, *Escherichia coli uvrA* mutant cells show higher survival on minimal than on rich growth medium, i.e., they show minimal-medium recovery. This effect of rich growth medium on survival is not observed in a *uvrA* mutant carrying an *mmrA1* mutation, and the *uvrA mmrA* strain showed the same survival rate on minimal and rich growth media as the *uvrA* strain did on minimal medium plates. The *mmrA1* mutation was isolated as a hidden mutation from a *uvrA polA* mutant strain and shown to map at 84.3 min on the *E. coli* K-12 linkage map. In contrast to the *uvrA* strain, the repair of DNA daughter strand gaps was not inhibited in the *uvrA mmrA* strain by rich growth medium after irradiation. However, the *uvrA* and *uvrA mmrA* strains were similar in their ability to repair DNA when compared in minimal medium. These data are consistent with the idea that the *mmr* gene product is not involved directly in the repair of UV radiation-induced DNA damage, but rather allows rich growth medium to inhibit a portion of postreplication repair.

In excision repair-deficient strains of *Escherichia coli* K-12, pyrimidine dimers induced by UV radiation lead to the formation of daughter strand gaps (DSGs) in the DNA synthesized after irradiation. During subsequent incubation in growth medium, these gaps are filled by a postreplication repair process (10).

The survival of UV-irradiated cells of *uvrA*, *uvrB*, and *uvrC* mutants of *E. coli* is higher when the cells are plated on minimal rather than rich medium (3, 4, 12). This phenomenon is called minimal-medium recovery (MMR) (4, 5, 12, 14) and is due to the partial inhibition of postreplication repair by rich growth medium (12). In *uvrA* or *uvrB* mutants, MMR is largely blocked by *recA* or *lexA* mutations and partially blocked by *recB*, *uvrD*, or *recF* mutations, but a *polA* mutation has no effect on MMR (12). However, we recently observed that a *uvrA6 polA1* mutant (strain SR142) constructed by Monk et al. (9) did not show MMR. This raised the possibility that strain SR142 carries a hidden mutation that affects MMR. We report on a new mutation, *mmrA1*, which was isolated from strain SR142 and which affects MMR and postreplication repair.

MATERIALS AND METHODS

Bacterial strains. The derivatives of *E. coli* K-12 used in this study are listed in Table 1. Transduction was carried out by the method described by Miller (8). All strains were tested for P1 lysogeny.

Growth media and culture conditions. Minimal medium (MM) was a salts buffer (DTM) (3) containing 0.4% glucose, thymine (10 μg/ml), thiamine hydrochloride (0.5 μg/ml), D-biotin (1 μg/ml), and, when required, each amino acid at 1 mM. Minimal plating medium was MM solidified with 1.6% (wt/vol) Noble agar (Difco Laboratories). The rich plating medium (YENB agar) was yeast extract (0.75%) and nutrient agar (2.3%); liquid YENB was yeast extract (0.75%) and nutrient broth (0.8%) (all from Difco).

Logarithmic-phase bacteria were obtained by diluting 0.2 ml of an overnight MM culture into 10 ml of prewarmed MM and shaking the cultures at 37°C until an optical density at 650 nm (OD_{650}) of 0.4 (~3×10^8 cells per ml; Zeiss PMQ II spectrophotometer) was obtained. Cells were harvested by centrifugation, washed twice with 0.067 M sodium-potassium phosphate buffer (PB), pH 7.0 (16), and suspended in PB at an OD_{650} of 0.1 (or in DTM at an OD_{650} of 0.4 for DSG repair experiments).

UV irradiation. The UV source was a General Electric 8-W germicidal lamp (emitting mainly at 254 nm). The methods for UV irradiation, correction for sample absorption, and dosimetry were those previously described (11). All experiments were carried out under yellow light to prevent photoreactivation.

Viability determination. Irradiated and control bacteria were diluted in PB, spread on YENB and MM plates, and incubated at 37°C for 1 to 2 days before survivors were scored.

Measurement of DSG repair. After UV irradiation, 1-ml samples of cells in DTM were pulse-labeled for 10 min by the addition of 1 ml of MM containing twice the normal concentration of organic supplements (but thymine at 4 μg/ml) and 50 μCi of [*methyl*-^{3}H]thymi-

TABLE 1. List of strains of *E. coli*[a]

Strain	Relevant genotype	Other characteristics	Source, derivation, reference
SR142	*uvrA6 polA1 mmrA1*[b]	*thyA36 deo(C2?) lacZ53 rha-5 rpsL151*	J. D. Gross (strain JG136)
SR143	*uvrA6*	Same as SR142	J. D. Gross (strain JG137)
SR349	*uvrA6*	*thyA36 deo(C2?) lacZ53 rha-5 leuB19 metE70 bioA2 rpsL151*	20
SR960	Wild type	*thyA deo ilvA700*::Tn*5*(Kn^r) λ^r	K. J. Shaw (strain CBK007)
SR1097	*uvrA6*	*thyA36 deo(C2?) lacZ53 rha-5 leuB19 bioA2 rpsL151*	P1 *vir* · SR142 × SR349; Met^{+c}
SR1098	*uvrA6 mmrA1*	Same as SR1097	Same as SR1097
SR1178	*uvrA6*	*thyA36 deo(C2?) lacZ53 rha-5 ilvA700*::Tn*5*(Kn^r) *leuB19 metE70 bioA2 rpsL151*	P1::Tn*9* *cts* · SR960 × SR349; Kn^r [d]

[a] Genotype symbols are those used by Bachmann and Low (1). All strains are F$^-$ and λ^-.
[b] Deduced from present work.
[c] Met$^+$, Selection for methionine prototrophy.
[d] Kn^r, Selection for kanamycin resistance.

dine (45 Ci/mmol; Amersham Corp.) per ml. A 1-ml sample of the pulse-labeled culture was collected by filtration (HAWG, 0.45-μm pore size; Millipore Corp.), washed, and suspended in 0.7 ml of MM. The remaining 1-ml sample was collected and suspended in 0.7 ml of YENB. After the suspensions had been shaken for 80 min at 37°C, 0.2-ml samples were mixed with 0.26 ml of ice-cold spheroplasting solution, containing 0.06 ml of 30% sucrose in 0.6 M Tris (pH 8.1), 0.1 ml of 32 mM EDT, and 0.1 ml of 0.1% lysozyme (Worthington Diagnostics) (modified from procedure in reference 15). After 10 to 15 min on ice, a 50-μl sample containing ~3 × 10^6 cells was layered on top of each gradient (5 to 20% sucrose in 0.1 N NaOH, 4.8 ml, capped with 0.2 ml of 0.5 N NaOH). After being held at room temperature for ~50 min, the gradients were centrifuged at 10,000 rpm for 16 h at 20°C in SW50.1 rotors in Beckman model L2 and L5-50 ultracentrifuges. [^{14}C]thymine-labeled bacteriophage T2 DNA was used as a molecular weight marker. The methods for processing the gradients and calculating the number-average molecular weight (M_n) have been described (7, 18, 19).

RESULTS

Figure 1A shows MMR in a *urvA* mutant (strain SR143); the F_{10} (UV radiation fluence to yield 10% survival) for YENB-plated cells was 2.5-fold less than it was for MM-plated cells. Our earlier study (12) showed that MMR was not affected by a *polA1* mutation; however, when we tested strain SR142 (*uvrA6 polA1*), it unexpectedly did not show MMR (Fig. 1B); i.e., after UV irradiation, the survival of MM-grown cells was the same on both MM and YENB plates. This result raised the possibility of an unknown mutation in strain SR142 that prevented it from showing MMR.

Strains SR142 (Mmr$^-$) and SR143 (Mmr$^+$) were constructed by Monk et al. (9) using phage P1 propagated on strain p3478 (*polA1*) (6). Since strains SR142 and SR143 are cotransductants that had been selected for methionine prototrophy, we tested whether a mutation affecting MMR (which we shall call *mmrA1*) was linked to

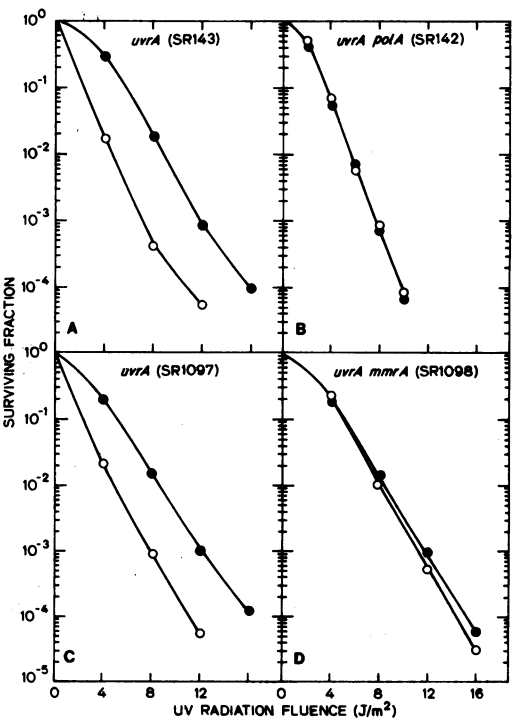

FIG. 1. Effect of plating medium on UV radiation survival for *uvrA* mutants of *E. coli* K-12. Cells grown in MM to logarithmic phase were UV irradiated (254 nm) in PB, diluted in PB, and plated on MM (●) or YENB (○). (A) Strain SR143 (*uvrA6*); (B) strain SR142 (*uvrA6 polA1*); (C) strain SR1097 (*uvrA6*); (D) strain SR1098 (*uvrA6 mmrA1*). Results are the average of two experiments.

the *metE* locus in strain SR142. A *uvrA metE* mutant (strain SR349) was transduced to Met^+ with phage P1 *vir* propagated on strain SR142, and the cloned transductants were screened for the Mmr phenotype. This allowed the construction of strains SR1097 (*uvrA6*) and SR1098 (*uvrA6 mmrA1*) and suggested that the *mmr* and *metE* loci were linked.

The *mmr* locus was mapped by transducing an *ilvA700*::Tn*5* mutation into the *uvrA metE* mutant (strain SR349) to construct strain SR1178 (*uvrA metE ilvA*). This strain was transduced to methionine or isoleucine-valine prototrophy by using phage P1 *vira* propagated on strain SR1098 (*uvrA6 mmrA1*), and the cloned transductants were screened for nonselected phenotypes. The results (Table 2) were most consistent with the *mmr* locus being at 84.3 min on the linkage map of *E. coli* K-12 (1), between the *ilvA* and *metE* loci. This map location was further supported by the higher transduction frequency obtained for the *mmr* mutation when both flanking markers (Met^+ and Ilv^+) were transduced (Table 2) and by the fact that when strain SR349 (*uvrA*) was transduced to Met^+ with phage P1 *vir* propagated on strain SR142 (*uvrA6 polA1 mmr*), 4 of 21 *uvrA6 polA1* transductants also received the *mmr* mutation (data not shown). These latter data (for the frequency of cotransduction of the *polA* and *mmr* genes) were most consistent with the *mmr* locus being on the same side of the *ilvA* locus (84.1 min) as the *metE* (85.0 min) and *polA* (86.1 min) loci.

The survival curves for the *uvrA* (strain SR1097) and *uvrA mmrA* (strain SR1098) cotransductants are also shown in Fig. 1. The F_{10} for *uvrA* mutant cells plated on YENB was 2.2-fold less than it was for cells plated on MM (Fig. 1C. The *uvrA mmrA* mutant showed the same survival on MM and YENB plates as the *uvrA* strain showed on MM plates (Fig. 1D).

Rich growth medium (e.g., YENB) has been shown to partially inhibit the repair of DNA DSGs in a *uvrB* mutant of *E. coli* K-12 (12). We tested whether the presence of the *mmr* mutation could eliminate this inhibition of repair by YENB by irradiating cells of MM-grown *uvrA* and *uvrA mmrA* mutants with UV, labeling them with [^{3}H]thymidine for 10 min, then switching them to either nonradioactive MM or YENB, and shaking them for 80 min at 37°C. The *uvrA* mutant cells incubated in MM showed complete repair of DSGs up to ~4 J/m^2 and then accumulated unrepaired DSGs as a linear function of UV radiation fluence (Fig. 2A). YENB-incubated *uvrA* mutant cells did not show this complete repair after low fluences of UV radiation and accumulated unrepaired DSGs in a linear manner over the entire fluence range (Fig. 2A). In contrast, the *uvrA mmrA* mutant cells showed

TABLE 2. Calculation of the *mmr* gene chromosome map position from cotransduction frequency data

Selected phenotype (no. of transductants)[a]	Nonselected phenotypes detected[b] (no. of transductants)					Cotransduction of *mmr* with selected marker[c] (%)	Distance between *mmr* and selected marker[d] (min)	Map position of selected marker[e] (min)	Calculated position of *mmr*[f] (min)
	Mmr^+	Mmr^-	Ilv^+ Mmr^+	Ilv^+ Mmr^-	Met^+ Mmr^+	Met^+ Mmr^-			
Ilv^+ (152)	36	116							
Met^+ (152)	99	53	12	45	4	10	76	0.17	84.1	84.3
							35	0.59	85.0	84.4

[a] Strain SR1178 (*metE70 ilvA700*::Tn*5*) was transduced (P1 *vira* · SR1098 [*mmrA1*]) to isoleucine-valine (Ilv^+) or methionine (Met^+) prototrophy.

[b] The ratio of the surviving fraction of transductants on MM plates to that on YENB plates was used to determine the Mmr phenotype. Our scoring procedure was validated by producing full survival curves on selected transductants.

[c] Calculated by dividing the number of Mmr^- transductants by the total number and multiplying by 100.

[d] The distances listed were calculated by the method of Wu (17) as equal to $L(1\text{-}F^{1/3})$, where L is the length of the DNA carried by the transducing bacteriophage, i.e., 2.0 min (2), and F is the frequency of cotransduction divided by 100.

[e] Map positions are from Bachmann and Low (1).

[f] Map positions were calculated by adding or subtracting the distance between the *mmr* locus and the selected marker to or from the map position of the selected marker.

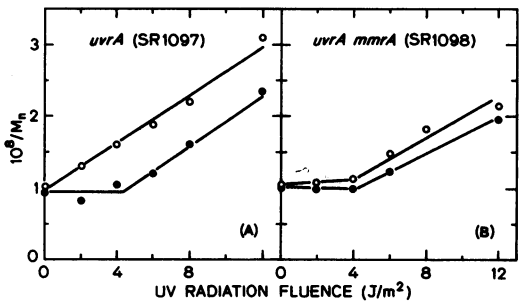

FIG. 2. Effect of culture medium on the repair of UV radiation-induced DSGs in *E. coli* strains SR1097 (*uvrA*) and SR1098 (*uvrA mmr*). After UV irradiation, the cells were pulse-labeled with [^{3}H]thymidine for 10 min and shaken in MM (●) or YENB (○) for 80 min at 37°C before being assayed for the number of unrepaired DNA DSGs. The reciprocal of the number-average molecular weight (M_n) (which is related to the number of DSGs remaining unrepaired) is plotted as a function of UV radiation fluence. (A) Strain SR1097 (*uvrA6*); (B) strain SR1098 (*uvrA6 mmrA1*).

essentially the same amount of repair in both MM and YENB (Fig. 2B).

DISCUSSION

In trying to understand why one *uvrA polA* mutant (strain SR142) did not show MMR (although other such mutants do [12]), we discovered a mutation affecting MMR in this strain. This mutation, which we call *mmrA1*, mapped at 84.3 min on the linkage map for *E. coli* K-12. This map position is close to that for the *uvrD* gene (84.6 min [1]). However, in contrast to the *mmr* mutation, *uvrD* mutations (e.g., *uvrD3*) have little effect on MMR (12), and they are known to make excision repair-deficient strains sensitive to UV radiation (13, 18). Therefore, we conclude that *mmrA1* is not an allele of the *uvrD* gene.

We transduced the *mmrA* mutation into another *E. coli* K-12 *uvrA* mutant strain and showed that it still blocked the effect of rich growth medium on survival and postreplication repair. Thus, the *mmr* gene seems to play a role in the process by which rich medium causes an inhibition of the filling of DSGs. This appears to be a unique way of regulating MMR. Other mutations that reduce or block MMR (e.g., *recB*, *recA*, *lexA*) are thought to do so by inactivating the specific DSG-filling process that would be affected by rich growth medium.

In conclusion, the *mmr* gene does not seem to be involved directly in the repair of DNA lesions since the *mmrA1* mutation does not affect the survival of UV-irradiated cells on MM. However, the presence of this mutation helps the cells in repairing UV radiation-induced damage when they are incubated in rich growth medium. Further studies are underway to explicate the role of the *mmr* gene in MMR at the molecular level.

ACKNOWLEDGMENTS

We thank Israel Felzenszwalb and Tzu-chien V. Wang for helpful discussions.

This work was supported by Public Health Service research grant CA-02896 and research program grant CA-10372 from the National Cancer Institute, DHHS.

LITERATURE CITED

1. **Bachmann, B. J., and K. B. Low.** 1980. Linkage map of *Escherichia coli* K-12, edition 6. Microbiol. Rev. **44**:1–56.
2. **Bachmann, B. J., K. B. Low, and A. L. Taylor.** 1976. Recalibrated linkage map of *Escherichia coli* K-12. Bacteriol. Rev. **40**:116–167.
3. **Ganesan, A. K., and K. C. Smith.** 1968. Dark recovery processes in *Escherichia coli* irradiated with ultraviolet light. I. Effect of *rec*$^-$ mutations on liquid holding recovery. J. Bacteriol. **96**:365–373.
4. **Ganesan, A. K., and K. C. Smith.** 1968. Recovery of recombination deficient mutants of *Escherichia coli* K-12 from ultraviolet irradiation. Cold Spring Harbor Symp. Quant. Biol. **33**:235–242.
5. **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.
6. **Gross, J., and M. Gross.** 1969. Genetic analysis of an *E. coli* strain with a mutation affecting DNA polymerase. Nature (London) **224**:1166–1168.
7. **Hamelin, C., D. A. Youngs, and K. C. Smith.** 1976. Role of deoxyribonucleic acid polymerase III in the repair of single-strand breaks produced in *Escherichia coli* deoxyribonucleic acid by gamma radiation. J. Bacteriol. **127**:1307–1314.
8. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
9. **Monk, M., M. Peacey, and J. D. Gross.** 1971. Repair of damage induced by ultraviolet light in DNA polymerase-defective *Escherichia coli* cells. J. Mol. Biol. **58**:623–630.
10. **Rupp, W. D., and P. Howard-Flanders.** 1968. Discontinuities in the DNA synthesized in an excision-defective strain of *Escherichia coli* following ultraviolet irradiation. J. Mol. Biol. **31**:291–304.
11. **Sargentini, N. J., and K. C. Smith.** 1979. Multiple, independent components of ultraviolet mutagenesis in *Escherichia coli* K-12 *uvrB5*. J. Bacteriol. **140**:436–444.
12. **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.
13. **Smirnov, G. B., E. V. Filkova, and A. G. Skavronskaya.** 1973. Ultraviolet sensitivity, spontaneous mutability and DNA degradation in *Escherichia coli* strains carrying mutations in *uvr* and *rec* genes. J. Gen. Microbiol. **76**:407–416.
14. **Smith, K. C.** 1971. The roles of genetic recombination and DNA polymerase in the repair of damaged DNA. Photophysiology **6**:209–278.
15. **Smith, K. C., and D. H. C. Meun.** 1970. Repair of radiation-induced damage in *Escherichia coli*. I. Effect of *rec* mutations on post-replication repair of damage due to ultraviolet radiation. J. Mol. Biol. **51**:459–472.
16. **Wang, T. V., and K. C. Smith.** 1981. Effect of *recB21*, *uvrD3*, *lexA101* and *recF143* mutations on ultraviolet

radiation sensitivity and genetic recombination in *uvrB* strains of *Escherichia coli* K-12. Mol. Gen. Genet. **183**:37–44.
17. **Wu, T. T.** 1966. A model for three-point analysis of random general transduction. Genetics **54**:405–410.
18. **Youngs, D. A., and K. C. Smith.** 1976. Genetic control of multiple pathways of post-replicational repair in *uvrB* strains of *Escherichia coli* K-12. J. Bacteriol. **125**:102–110.
19. **Youngs, D. A., and K. C. Smith.** 1976. Single-strand breaks in the DNA of the *uvrA* and *uvrB* strains of *Escherichia coli* K-12 after ultraviolet irradiation. Photochem. Photobiol. **24**:533–541.
20. **Youngs, D. A., and K. C. Smith.** 1978. Genetic location of the *phr* gene of *Escherichia coli* K-12. Mutat. Res. **51**:133–137.