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INDUCIBLE POSTREPLICATION REPAIR IS RESPONSIBLE FOR MINIMAL MEDIUM RECOVERY IN UV-IRRADIATED *Escherichia coli* K-12

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Abstract—Ultraviolet (UV)-irradiated *Escherichia coli* K-12 *uvrA* cells showed higher survival if plated on minimal growth medium rather than on rich growth medium, i.e., they showed minimal medium recovery (MMR). A 2-hour treatment of UV-irradiated cells with rifampicin inhibited the subsequent expression of MMR, and produced a large reduction in survival. We have recently isolated a new mutant (*mmrA1*) that does not show MMR. The *mmrA* mutation protected UV-irradiated *uvrA* cells from the effect of rich growth medium on survival, but not from the effect of rifampicin on survival. DNA daughter-strand gap (DSG) repair in UV-irradiated (4 J/m^2) *uvrA* cells was inhibited to the same degree whether rich growth medium was added immediately after irradiation or after 10 min of postirradiation incubation in minimal growth medium. However, chloramphenicol added immediately after irradiation greatly reduced this repair; there was less reduction if it was added 10 min after UV irradiation. These findings suggest that MMR is an inducible repair phenomenon, and that rich growth medium inhibits this repair process itself rather than its induction.

INTRODUCTION

Ultraviolet (UV)-irradiated *uvrA*, *uvrB* and *uvrC* strains of *Escherichia coli* K-12 show a higher survival if assayed on minimal growth medium rather than on rich growth medium (Ganesan and Smith, 1968a,b; Sharma *et al.*, 1982). This higher survival on minimal growth medium is known as minimal medium recovery (MMR)* (Ganesan and Smith, 1968b, 1970; Smith, 1971), and is correlated with the partial inhibition of postreplication repair (i.e., the repair of DNA daughter-strand gaps (DSG) formed after UV irradiation) by rich growth medium (Sharma *et al.*, 1982).

In *uvrA* or *uvrB* strains of *E. coli*, MMR is controlled by the *recA* and *lexA* genes (Ganesan and Smith, 1970; Sharma *et al.*, 1982). Since the *recA* and *lexA* genes are also known to control the inducibility of various radiation-induced phenomena (Witkin, 1976; Little and Mount, 1982), it suggests that MMR is an inducible repair process. Rich growth medium may either prevent the induction of this repair process, or it may inhibit the DNA repair process itself after it has been induced. The work reported here supports the latter hypothesis.

MATERIALS AND METHODS

Bacterial strains. The strains of *E. coli* K-12 used in this study were SR1097 (*uvrA6 thyA36 deo(C2?) lacZ53 rha-5 leuB19 bioA2 rpsL151* F$^-$ λ$^-$) and SR1098 (*uvrA6 mmrA1 thyA36 deo(C2?) lacZ53 rha-5 leuB19 bioA2 rpsL151* F$^-$ λ$^-$) (Sharma *et al.*, 1983).

Media and growth conditions. The minimal medium (MM) is a salts buffer (DTM) (Ganesan and Smith, 1968a) supplemented with 0.4% glucose, 0.5 μg/mℓ of thiamine hydrochloride, 10 μg/mℓ of thymine, 1 μg/mℓ of D-biotin, and 1 m*M* leucine. Minimal plating medium was MM solidified with 1.6% (wt/vol.) Difco Noble agar. Rich plating medium (YENB) was Difco yeast extract (0.75%) and Difco nutrient agar (2.3%). Liquid YENB was Difco yeast extract (0.75%) and Difco nutrient broth (0.8%). Rifampicin (RIF) solution was prepared fresh by dissolving RIF in dimethylsulfoxide (DMSO) at 1 mg/mℓ and diluting 50-fold into MM containing twice the normal concentration of organic supplements. DMSO, itself, had no effect on the survival of unirradiated or irradiated cells (data not shown).

Cells were grown in MM, in a shaking waterbath at 37°C, to an optical density at 650 nm (OD_{650}) of 0.4 (~3×10^8 cells/mℓ; Zeiss PMQ II spectrophotometer), collected by centrifugation, washed with 0.067 *M* NaK-phosphate buffer (PB) (Wang and Smith, 1981), and resuspended in PB at an OD_{650} of 0.1 (or resuspended in DTM at an OD_{650} of 0.4 for DSG repair experiments).

UV irradiation and survival determination. The UV radiation (254 nm) source was a General Electric 8-W germicidal lamp. The cultures were irradiated at room temperature in uncovered Petri dishes on a rotary shaker. The methods for UV irradiation, correction for sample absorption, and dosimetry have been described (Sargentini and Smith, 1979). All experiments were done under yellow light to prevent photoreactivation. For survival determination, UV-irradiated bacteria were diluted in PB and plated on MM and YENB plates. Incubation was for 1–3 days at 37°C.

Measurement of DNA daughter-strand gap repair. MM containing twice the normal concentration of organic supplements (but thymine at 4 μg/mℓ) and 50 μCi/mℓ of [methyl-^{3}H]-thymidine (45 Ci/mmol, Amersham Corp.) was added to an equal volume of UV-irradiated cells in DTM, and the mixture was shaken at 37°C for 10 min. This pulse-labeled culture was collected by filtration (Millipore HAWG, 0.45 μm pore size), washed and resuspended in an

*Abbreviation: CAP, chloramphenicol; DMSO, dimethylsulfoxide; DSG, daughter-strand gap; MM, minimal medium; MMR, minimal medium recovery; OD_{650}, optical density at 650 nm; PB, phosphate buffer; RIF, rifampicin; YENB, yeast extract-nutrient broth.

equal volume of MM. After shaking the cells for 80 min at 37°C, they were converted to spheroplasts and subjected to alkaline sucrose gradient centrifugation. The methods for producing spheroplasts (Sharma *et al.*, 1983) and for alkaline sucrose gradient centrifugation have been described (Hamelin *et al.*, 1976; Youngs and Smith, 1976b).

RESULTS AND DISCUSSION

In order to investigate whether MMR is an inducible DNA repair phenomenon, we have temporarily blocked the synthesis of RNA in UV-irradiated *E. coli uvrA* (SR1097) cells by treating them with RIF at 10 μg/mℓ for 2 h (this treatment had no effect on the viability of unirradiated cells) before diluting and plating them on minimal and rich growth medium (YENB). This treatment with RIF largely eliminated MMR, i.e., the survival of RIF-treated cells was only slightly less when plated on YENB versus MM (compare with the curves for cells not treated with RIF; Fig. 1a). This result suggests that MMR is an inducible DNA repair phenomenon. Since the survival of the cells treated with RIF was less than for cells blocked in MMR (i.e., cells not treated with RIF but plated on YENB), it suggests that some additional DNA repair not associated with MMR is also inducible in *uvrA* cells.

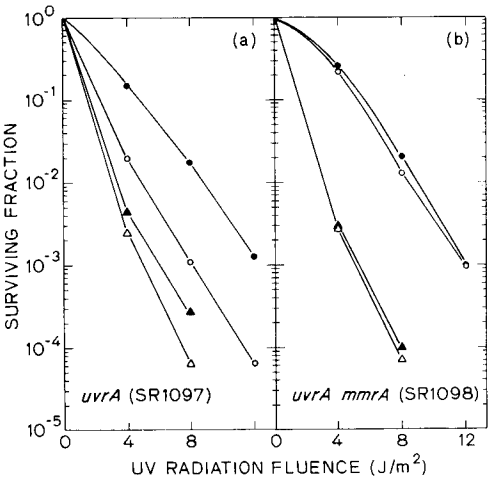

Figure 1. Effect of rifampicin (RIF) on minimal medium recovery in UV-irradiated *E. coli uvrA* cells. Cells were grown to logarithmic phase in MM and then were UV irradiated in DTM. Immediately after irradiation, untreated cells were plated on MM (●) and on YENB (○). Some cells were diluted 2-fold with MM containing twice the normal concentration of organic supplements and RIF (final concentration, 10 μg/mℓ), and were incubated for 2 h at 37°C before being plated on MM (▲) and on YENB (△). (a) *uvrA6* (SR1097), (b) *uvrA6 mmrA1* (SR1098). Results are the average of two experiments.

We have isolated a new mutation (*mmrA1*) that blocks the inhibitory action of rich growth medium on survival and DNA repair after UV irradiation (Sharma *et al.*, 1983). The above experiments were repeated using the *uvrA mmrA* strain (SR1098) that does not show MMR (i.e., the survival of this strain is the same on MM or YENB plates). When treated with RIF after UV irradiation, this strain also showed a big reduction in survival, but little or no effect of plating medium (Fig. 1b), consistent with this strain being Mmr⁻.

Since the *uvrA mmrA* strain appeared to be as inducible as the *uvrA* strain (i.e., the survival of the *uvrA mmrA* strain on MM both before and after treatment with RIF was the same as for the *uvrA* strain; cf., Fig. 1a and 1b), and the *uvrA mmrA* strain showed no reduction in survival by YENB, we suggest that the effect of rich growth medium on the MMR phenomenon is not due to its inhibition of the induction of DNA repair proteins, but rather, is due to the inhibition of the repair process itself after it has been induced.

To test this hypothesis at the molecular level, we measured the repair of DNA DSG in UV-irradiated (4 J/m²) *uvrA* cells with the following posttreatments: (1) incubated at 37°C for 80 min in MM; (2) immediately after resuspension in MM, cells were diluted into YENB and incubated for 80 min; (3) immediately after resuspension in MM, chloramphenicol (CAP) was added and the cells were incubated for 80 min; (4) after 10 min in MM, YENB was added and incubation was continued for 70 min; or (5) after 10 min in MM, CAP was added and incubation was continued for 70 min. If YENB inhibits the repair process and not the induction of repair, then one would expect that the addition of YENB after 10 min of incubation in MM should show about the same amount of DSG repair inhibition as if YENB was added at time zero. This was found to be true (cf., ○, ●; Fig.2), i.e., the same DNA profiles were obtained whether YENB was added at time zero or after 10 min of incubation in MM. If CAP inhibits the induction of repair and not the repair process itself, then one would expect that the addition of CAP after 10 min should have less of an effect on the repair of DSG than if added at time zero. This was found to be true (cf., △, ▲; Fig.2). The CAP treatment data are in agreement with the results reported by Youngs and Smith (1976a) and Sedgwick (1975), who suggested that a portion of DNA DSG repair is inducible.

Similarly, if rich growth medium inhibits the induction of the repair responsible for MMR, then the prior induction of repair by a UV radiation primer-fluence should abolish MMR. However, we found no reduction in MMR in *uvrA* and *uvrB* strains exposed to 4 J/m² of UV radiation when the cells were first exposed to a UV radiation primer-fluence (0.2 and 0.4 J/m²), and incubated in growth medium for 60 min (data not shown). This result further strengthens the conclusion that the MMR phenomenon is due to the inhibition of induced repair by rich growth medium, rather than to the inhibition of its induction.

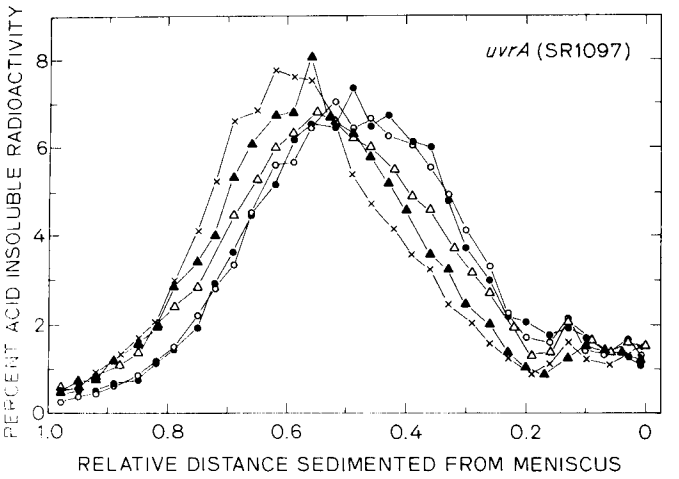

Figure 2. Effect of postirradiation treatments on the repair of DNA daughter-strand gaps in *E. coli uvrA* (SR1097) cells. UV-irradiated cells were pulse-labeled with [^{3}H]-thymidine, resuspended in MM and the sedimentation characteristics of their DNA were assayed as follows: (1) after incubating for 80 min in MM at 37°C (×); (2) immediately after resuspension in MM, 0.9 mℓ of cells were mixed with 0.1 mℓ of 10-fold concentrated YENB and incubated for 80 min (○); (3) immediately after resuspension in MM, cells were mixed with MM plus CAP (final concentration, 100 μg/mℓ) and incubated for 80 min (△); (4) after 10 min in MM, YENB was added [as in (2) above] and further incubated for 70 min (●); (5) after 10 min in MM, CAP was added [as in (3) above] and further incubated for 70 min (▲). The results are from a representative experiment.

The scheme shown in Fig. 3 summarizes the present understanding of MMR.

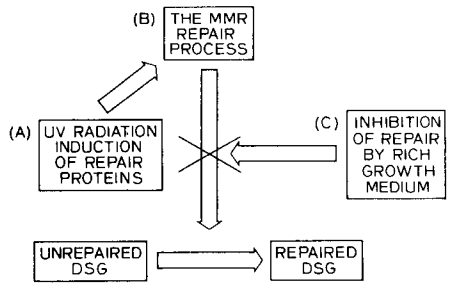

Figure 3. Scheme showing the present understanding of minimal medium recovery (MMR). (A) After UV irradiation, proteins required for MMR and the associated repair of DNA daughter-strand gaps (DSG) are induced. In the absence of functioning *recA* and *lexA* genes, which are required for the radiation induction of DNA repair proteins (Witkin, 1976; Little and Mount, 1982), MMR is absent (Sharma *et al.*, 1982). This induction process is also inhibited by treatment with rifampicin and chloramphenicol (present work). (B) Once induced, the MMR repair process can then function to repair DNA DSG. This process is under the control of the *recA*, *lexA* and *recB* genes; the *uvrD* and *recF* genes play a minor role, and the *polA* gene plays no role in MMR (Sharma *et al.*, 1982). (C) Rich growth medium, which can just be amino acid-enriched minimal growth medium, inhibits the MMR repair process (Sharma *et al.*, 1982), but not its induction (present work). The *mmrA1* mutation (Sharma *et al.*, 1983) prevents the inhibition of the MMR repair process by rich growth medium.

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