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Photochemistry and Photobiology Vol. 39, No. 6, pp. 793–797, 1984
Printed in Great Britain.

0031-8655/84 $03.00+0.00

RICH GROWTH MEDIUM ENHANCES ULTRAVIOLET RADIATION SENSITIVITY AND INHIBITS CELL DIVISION IN *ssb* MUTANTS OF *Escherichia coli* K-12

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(*Received* 27 *October* 1983; *accepted* 22 *December* 1983)

Abstract—DNA single-strand binding protein mutants (*ssb*) of *Escherichia coli* K-12 exhibit much greater UV radiation sensitivity when plated on rich medium (RM) than when plated on minimal medium (MM). However, when comparing UV-irradiated *ssb-113* cells (previously known as *exrB* and *lexC113*) incubated in RM *vs* MM, no difference was observed in DNA degradation or in the repair of incision breaks that arose during excision repair. Although UV-irradiated *ssb-113* cells incubated in RM did resume DNA synthesis slightly sooner than those incubated in MM, and there was a small increase in the production of DNA double-strand breaks in these cells, the most dramatic difference noted, however, was the much enhanced filamentation of irradiated cells incubated in RM *vs* MM. Therefore, we suggest that most of the RM-enhanced UV radiation sensitivity in *ssb-113* cells is due to an inhibitory effect of RM on cell division rather than on DNA repair.

INTRODUCTION

Some strains of *E. coli* exhibit a higher survival if UV-irradiated cells are assayed for colony-forming ability on minimal medium (MM)† rather than on rich medium (RM). This phenomenon, originally observed in *E. coli* B (Roberts and Aldous, 1949; Alper and Gillies, 1960), has been subsequently observed in a number of mutant strains of *E. coli* K-12, including *lon* (Howard-Flanders *et al.*, 1964), *uvrA*, *uvrB*, *uvrC*, (Ganesan and Smith, 1968), *ruvB* (Otsuji *et al.*, 1974) and *rer* (Srivastava, 1976). Under certain conditions, such as starving cells for amino acids prior to UV irradiation, this phenomenon can also be observed in *recA*, *recB* and *recC* strains of *E. coli* K-12 (Ganesan and Smith, 1968, 1970).

Although very little is known about the molecular basis for this plating medium effect on cell survival, it appears that the ability of cells to repair radiation-induced DNA damage and/or to grow and divide after irradiation may be different in different growth media. In the case of *lon* strains, RM is known to induce long nonseptate filaments even in unirradiated cells (Adler and Hardigree, 1964; Gayda *et al.*, 1976). On the other hand, RM was shown to inhibit a small portion of postreplication repair in UV-irradiated *uvrA* and *uvrB* cells (Sharma *et al.*, 1982).

We report here that strains deficient in DNA single-strand binding protein (*ssb*) also exhibit a higher survival if UV-irradiated cells are plated on MM *vs* RM. The effects of postirradiation growth media on the repair of UV radiation-induced DNA damage and on cell division were investigated in *ssb* mutants of *E. coli* K-12. The major effect of RM was the inhibition of cell division in UV-irradiated *ssb* cells.

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†*Abbreviations:* CAA, casamino acids; MM, minimal medium; RM, rich medium.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used are listed in Table 1. The transduction technique used was similar to that of Miller (1972). The cotransfer of the *ssb* mutation was identified by the failure of Mal$^+$ transductants to grow at 42°C.

Media. The minimal medium (MM) is a salts buffer (DTM) (Ganesan and Smith, 1968) supplemented with glucose at 0.4%, thiamine·HCl at 0.5 μg/mℓ, biotin at 1 μg/mℓ, thymine at 2μg/mℓ and L-leucine and L-methionine at 1 mM. Minimal plating medium was MM (containing thymine at 10 μg/mℓ) solidified with 1.6% Difco Noble agar. The rich plating medium was either YENB agar (7.5 g Difco yeast extract, 23 g Difco nutrient agar per liter of H_2O) or MM agar supplemented with Difco Casamino Acids (CAA, vitamin assay grade) at 2 mg/mℓ.

UV irradiation (254 nm) and assays for cell survival, DNA synthesis, and DNA degradation. These procedures were performed as previously described (Wang and Smith, 1982).

Analysis of DNA strand breaks. Cells were grown at 30°C for at least three generations in MM containing [methyl-^{3}H]thymine (30 Ci/mmol; Amersham Corp.) at 20 μCi/mℓ. When the culture reached an optical density at 650 nm of 0.2 (Zeiss PMQII spectrophotometer), the cells were harvested on Millipore HA membrane filters (0.45 μm pore size), washed with DTM, and resuspended at an optical density of 0.05 (~3 × 10^7 cells per mℓ) in MM. The resuspended cells were incubated at 30°C for 30 min before being UV-irradiated in the medium. After irradiation, half of the sample was supplemented with CAA at 2 mg/mℓ, and both halves were incubated. After different times of postirradiation incubation, samples were removed, treated with lysozyme, and layered onto alkaline or neutral sucrose gradients (5–20%, wt/vol). The composition of alkaline and neutral sucrose gradients and the centrifugation and processing of gradients were as previously described (Wang and Smith, 1983), except that Triton X-100 (0.1%) was included to facilitate the making of gradients when nonwetting polyallomer tubes (Beckman/Spinco) were

Table 1. *E. coli* strains used in this work

Stanford radiology No.	Genotype*	Source or derivation
SR248	F *leuB19 metE70 rha-5 bioA2 lacZ53 thyA36 deo(C2?) malB45 rpsL151* Su λ	R. B. Helling (KH21)
SR963	*ssb-113 lon sulA*	B. F. Johnson (PAM29)
SR995	F *ssb-1 thy mel rha*	J. W. Chase (KLC436)
SR996	Same as SR248 except Mal$^+$	P1::Tn*9*cts·SR963 × SR248 (select Mal$^+$)†
SR997	Same as SR248 except *ssb-113* Mal$^+$	P1::Tn*9*cts·SR963 × SR248 (select Mal$^+$)†
SR999	Same as SR248 except *ssb-1* Mal$^+$	P1::Tn*9*cts·SR995 × SR248 (select Mal$^+$)†

*Genotype symbols are those used by Bachmann and Low (1980). Strain SR963 is a derivative of *E. coli* B/r; all the remaining strains are derivatives of *E. coli* K-12. †Mal$^+$, cells can utilize maltose in place of glucose.

used. The calculations of molecular weights and determination of DNA strand breaks were as previously described (Wang and Smith, 1983).

RESULTS

We noted that the *ssb-113* (SR963) and *ssb-1* (SR995) strains exhibited a higher survival if UV-irradiated cells were plated on MM *vs* YENB agar (data not shown). To test whether this postirradiation growth medium effect on UV radiation sensitivity was associated with the *ssb* mutations, bacteriophage P1 lysates of strains SR963 (*ssb-113*) and SR995 (*ssb-1*) were used to transduce the *E. coli* K-12 wild-type strain SR248. The Mal$^+$ transductants that were temperature-resistant (two from each cross were tested), were also UV-radiation resistant and showed no effect of plating medium on cell survival, as was the case for the parental strain SR248. On the other hand, the temperature-sensitive Mal$^+$ transductants (i.e. *ssb* mutants) (four from each cross were tested) exhibited a higher survival if UV-irradiated cells were plated on MM *vs* YENB agar (representative transductant strains are shown in Fig. 1), establishing that a mutation at *ssb* renders cells susceptible to RM-enhanced radiation sensitivity. Figure 2 shows the effect of various plating media on the survival of UV-irradiated *ssb-113* cells. Supplementation of MM with CAA, or the 13 amino acids that are present in CAA (see Sharm and Smith, 1982), is sufficient to reduce cell survival to a level similar to that for cells plated on YENB agar. Furthermore, *ssb-113* cells grown in RM (YENB or MM + CAA) before UV-irradiation also exhibited enhanced radiation sensitivity when plated on RM *vs* MM (data not shown), suggesting that this RM-enhanced radiation sensitivity is not simply due to a shift-up in nutrition.

When the UV-irradiated (5 J/m^2) *ssb-113* cells (SR997) were incubated in MM for varying periods of time before being plated on RM (MM + CAA) plates, the cells gradually became immune to the lethality caused by RM, and after 6 h at 30°C, the cells were no longer sensitive to RM-enhanced lethality. Conversely, if the irradiated cells were incubated in MM + CAA for varying periods of time before being plated on MM plates, the capacity of cells to form colonies on MM plates was slowly reduced, indicating that the RM had irreversibly impaired the capacity of the cells to form colonies (data not shown).

To probe why RM enhances the UV radiation sensitivity of *ssb-113* mutants, we investigated the effects of RM on DNA synthesis, DNA degradation, DNA repair, and cell division. The inclusion of CAA in MM had no effect on UV radiation-induced DNA degradation (data not shown); however, it allowed irradiated cells to resume DNA synthesis sooner than those incubated in MM (Fig. 3). The faster resumption of DNA synthesis in irradiated cells may increase the probability of producing DNA daughter-strand gaps during replication. Since survival studies have suggested that a mutation at *ssb* affects postreplication repair (Lieberman and Witkin, 1981; Whittier and Chase, 1981), any increase in the number of DNA daughter-strand gaps may lead to increased cell lethality through the conversion of DNA daughter-strand gaps to DNA double-strand breaks (Wang and Smith, 1982). We tested this hypothesis by determining the production of DNA double-strand breaks in UV-irradiated cells. Irradiated *ssb-113* cells incubated in RM consistently yielded slightly more DNA double-strand breaks than those incubated in MM (Table 2).

Based upon survival studies, it has been deduced that *ssb* mutants are not deficient in excision repair (Lieberman and Witkin, 1981; Whittier and Chase, 1981). To test whether RM may interfere with excision repair in *ssb* strains, we examined the ability of irradiated cells to repair incision breaks that arise

Table 2. Effects of casamino acids (CAA) on the accumulation of unrepaired DNA double-strand breaks and single-strand breaks in UV-irradiated *ssb-113* cells of *E. coli* K-12

UV radiation fluence (J/m^2)	*Number of unrepaired DNA double-strand breaks per genome		†Number of unrepaired DNA single-strand breaks per genome	
	MM	MM+CAA	MM	MM+CAA
2.5	0.90 ± 0.09	1.26 ± 0.03	‡ND	ND
5	1.27 ± 0.07	1.81 ± 0.16	ND	ND
10	ND	ND	2.88	2.40
20	ND	ND	5.29	4.06
30	ND	ND	6.27	6.16

*Determined 4 h after UV-irradiation. The number of unrepaired strand breaks was calculated with the formula

$$\left(\frac{M_c}{M_{uv}} - 1\right) \times \frac{2.8 \times 10^9}{M_c}$$

where M_c, M_{uv} are the number average molecular weights for unirradiated cells, and UV-irradiated cells, respectively. The value 2.8×10^9 is the molecular weight of *E. coli* genome. Data shown are the average of two separate experiments. †Determined 160 min after UV-irradiation. See above for calculation. Data shown are from a single experiment. ‡ND, not determined.

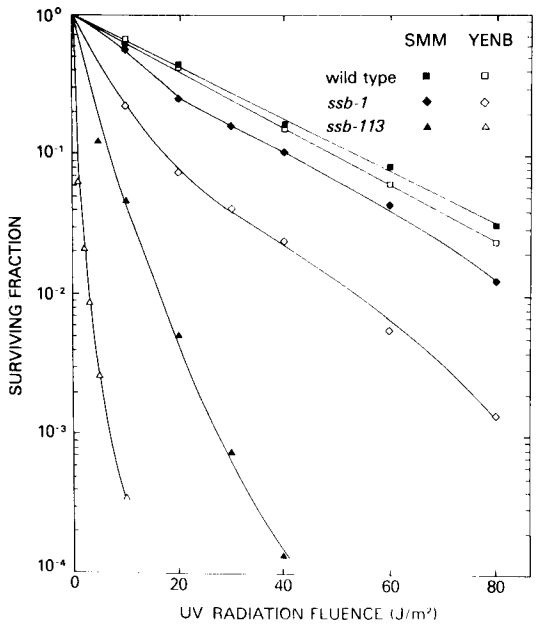

Figure 1. Effect of plating media on the colony-forming ability of UV-irradiated cells of *E. coli* K-12. Cells were grown in MM to logarithmic phase, UV-irradiated and plated on MM (filled symbols) or on YENB (open symbols) for the determination of colony-forming units. All points are the average of at least two experiments. Symbols: wild-type (SR996) (■, □); *ssb-1* (SR999) (◆, ◇); *ssb-113* (SR997) (▲, △).

during the excision repair process (Youngs *et al.*, 1974). No difference was observed in the number of single-strand breaks remaining in the DNA of irradiated *ssb-113* cells incubated in RM and MM

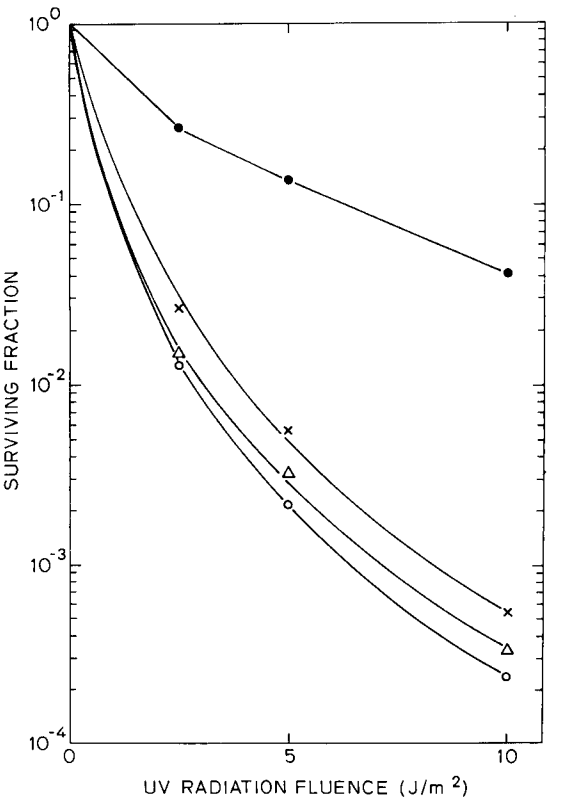

Figure 2. Effect of different rich plating media on the colony-forming ability of UV-irradiated *ssb-113* (SR997) cells of *E. coli* K-12. All points are the average of two experiments. Symbols: MM, ●; MM + CAA, △; MM + 13 amino acids, ×; YENB, ○. The 13 amino acids added to MM are those that are present in CAA (see Sharma and Smith, 1982).

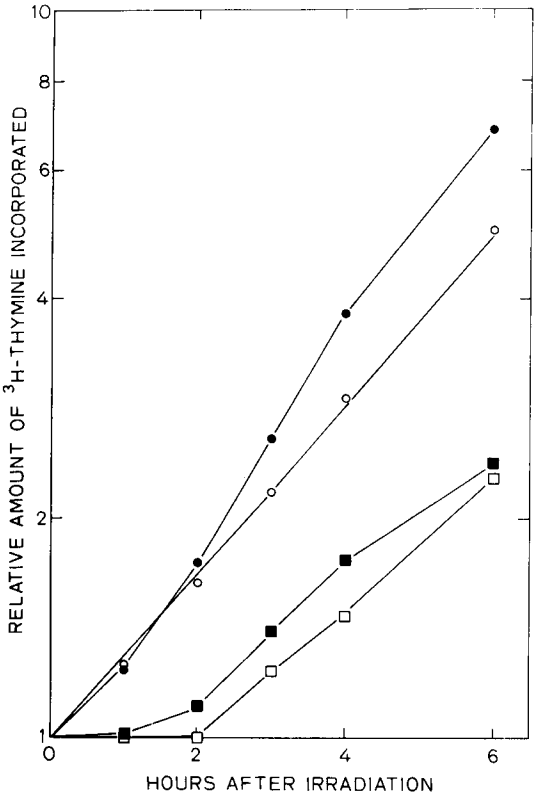

Figure 3. Effect of casamino acids on net DNA synthesis in UV-irradiated *ssb-113* (SR997) cells of *E. coli* K-12. Cultures were grown at 30°C in MM containing [^{3}H]thymine for at least four generations. After UV-irradiation, cells were incubated in the same MM containing [^{3}H]thymine with (filled symbols) or without (open symbols) the presence of 0.2% Casamino Acids. Symbols: unirradiated controls (○, ●); 5 J/m^2 (□, ■).

(Table 2). Similarly, the *ssb-113* cells were proficient in the ability to host-cell reactivate UV-irradiated lambda phage (see also Whittier and Chase, 1981) whether the assays were performed with RM or with MM (data not shown), suggesting that RM does not interfere with excision repair.

Finally, the effect of RM on UV-radiation-induced filament formation was examined under a microscope. The cells were UV irradiated (5 J/m^2) in MM at 6×10^7 cells/mℓ. After irradiation, half of the sample was supplemented with CAA at 2 mg/mℓ, and both halves were incubated at 30°C for 16 h. For unirradiated *ssb-113* (SR997) cells grown in MM, the majority of the cells were short rods, although there was a small fraction of cells (~ 0.4%) that appeared as long chains. Inclusion of CAA in the MM enhanced spontaneous filament formation, but the majority of the cells (~ 98.6%) still appeared as short rods. The most dramatic difference was noted for UV-irradiated cells incubated in MM (~ 5% as filaments) *vs* MM + CAA (~ 35% as filaments), indicating that RM enhances filamentation in irradiated *ssb-113* cells. Similar results were also obtained for UV-irradiated *ssb-1* (SR999) cells (data not shown). In contrast, very few filaments (<1%) were observed in UV-irradiated wild-type cells, whether they were grown in RM or MM.

DISCUSSION

Rich-medium-enhanced radiation sensitivity has been observed in a number of mutants of *E. coli*. The *lon* (Howard-Flanders *et al.*, 1964), *rer* (Srivastava, 1976) and *ruvB* (Otsuji *et al.*, 1976) mutants are UV-resistant, like wild-type cells, if the irradiated cells are plated on MM, but they are UV-radiation sensitive when plated on RM. These mutants are not known to have any defect in DNA repair. The enhanced radiation sensitivity of these mutants in RM, at least for the *lon* and *ruvB* mutants, is correlated with enhanced filament formation (Gayda *et al.*, 1976; Otsuji *et al.*, 1976). On the other hand, the RM-enhanced radiation sensitivity in excision-repair deficient *uvrA* and *uvrB* cells was reported to correlate with the inhibition of a small portion of *recA lexA*-dependent postreplication repair (Sharma *et al.*, 1982, 1983).

It is less clear why RM-enhanced radiation sensitivity also occurs in *recA*, *recB*, *recC* (Ganesan and Smith, 1968, 1970) and *recF* (unpublished data) mutants, which are known to be deficient to varying degrees in postreplication repair (see Wang and Smith, 1983). One possible explanation is that irradiated cells grown in RM may synthesize DNA faster and, therefore, may have less time to remove dimers by excision repair. This would lead to the formation of more DNA daughter-strand gaps, which are the substrates for postreplication repair. Since these mutants are deficient in postreplication repair, this may explain their enhanced radiation sensitivity in RM.

Our data establish that *ssb* mutants exhibit a higher survival if UV-irradiated cells are assayed for colony-forming ability on MM *vs* RM. Among the several parameters that might account for these results, we observed that RM greatly promotes filament formation in irradiated *ssb-113* cells (in agreement with an earlier observation by Greenberg *et al.*, 1974), and it slightly enhances the production of DNA double-strand breaks, but it does not affect excision repair (Table 2). Since the slight increase in DNA double-strand breaks observed for irradiated cells incubated in RM can only account for about 10–15% of the RM-enhanced cell lethality (calculated from comparing the number of DNA double-strand breaks *vs* the average number of lethal hits, −ln of the surviving fraction), this suggests that most of the RM-enhanced UV radiation sensitivity in *ssb-113* cells is due to the effect of RM on cell division rather than on DNA repair.

Division inhibition is one of the many inducible "SOS" responses that occur after perturbation of DNA metabolism (Witkin, 1976; Little and Mount, 1982). Although *ssb* mutants have been shown to be

deficient in the induction of some *recA lexA* regulated functions such as prophage induction (Vales *et al.*, 1980), Weigle reactivation and mutagenesis (Whittier and Chase, 1981), UV radiation mutagenesis (Lieberman and Witkin, 1981, 1983), and increased synthesis of RecA protein (Baluch *et al.*, 1980), they are not deficient in the inhibition of cell division after UV irradiation (unpublished data) and in the thermal induction of prophage and Weigle reactivation in *tif* cells (Whittier and Chase, 1983). The observation that *ssb* cells showed enhanced filamentation after UV irradiation, especially when grown in RM, suggested that a prolonged or irreversible inhibition of cell division had occurred in the irradiated cells. According to the current hypothesis of "SOS" induction, damage to DNA leads to the derepression of a set of genes including *sulA*, which codes for a cell division inhibitor (Gottesman *et al.*, 1981; Huisman and D'Ari, 1981). It is thought that the enhanced production of cell division inhibitors, such as the $sulA^+$ gene product, is responsible for the inhibition of cell division. Recently it was suggested that the *lon* gene codes for a protease that has activity towards the $sulA^+$ gene product; therefore, it can control cell division by regulating the level of $sulA^+$ protein in cells (Mizusawa and Gottesman, 1983). Although the DNA single-strand binding protein is not known to possess any protease activity itself, it might be required for the *lon* gene product to exert its proteolytic activity. A recent study indicates that DNA single-strand binding protein affects the proteolytic cleavage of phage λ repressor by RecA protein *in vitro* (Resnick and Sussman, 1982). Alternately, DNA single-strand binding protein may be involved in the *sulA sulB*-independent pathway of division inhibition that was suggested to result from the failure to terminate DNA replication (Burton and Holland, 1983). According to this hypothesis, the termination of a round of DNA replication is required to initiate cell division. It is possible that the *ssb* mutants, because of the defective DNA single-strand binding proteins they carry, somehow are not able to complete the round of replication after UV irradiation, and this results in an inhibition of cell division. At present, we favor this latter hypothesis to account for the inhibition of cell division observed in UV-irradiated *ssb* mutants, since a *ssb-113 sulA lon* mutant (SR963) in an *E. coli* B/r genetic background also exhibits the RM-enhanced lethality in irradiated cells (data not shown).

In summary, RM is lethal for UV-irradiated *ssb* strains, even if they were grown in RM before irradiation, and this enhanced lethality in RM is due mainly to an inhibition of cell division.

Acknowledgements—We are grateful to Carmencita E. Nicolas for skillful technical assistance, and to Professor Israel Felzenszwalb and 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.

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