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RADIATION RESEARCH **104,** 109–115 (1985)

Growth-Medium-Dependent Repair of DNA Single-Strand and Double-Strand Breaks in X-Irradiated *Escherichia coli*[1]

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SARGENTINI, N. J., AND SMITH, K. C. Growth-Medium-Dependent Repair of DNA Single-Strand and Double-Strand Breaks in X-Irradiated *Escherichia coli*. *Radiat. Res.* **104,** 109–115 (1985)

The X-ray resistance of logarithmic phase cells of *Escherichia coli* K-12 is enhanced threefold by growth in rich medium versus minimal medium (N. J. Sargentini, W. P. Diver, and K. C. Smith, *Radiat. Res.* **93,** 364–380, 1983). In this work, X-ray-induced DNA strand breaks were assayed by sedimentation in alkaline and neutral sucrose gradients to correlate the enhanced survival of rich-medium-grown cells with an enhanced capacity for DNA repair. While rich-medium-grown cells showed no enhanced capacity for repairing DNA single-strand breaks in buffer, i.e., fast, *polA*-dependent repair, they did show an enhanced capacity to repair both single-strand and double-strand breaks in growth medium, i.e., slow, *recA*-dependent repair. This enhanced capacity for DNA repair in rich-medium-grown cells was inhibited by rifampicin post-treatment, indicating the requirement for *de novo* RNA synthesis. Kinetic studies indicated that the repair of DNA double-strand breaks was a complex process. Relative to the sedimentation rate in neutral sucrose gradients of nonirradiated DNA, the sedimentation rate of X-irradiated DNA first changed from slow to very fast. Based on alkaline sucrose gradient sedimentation studies, all the strand breaks had been repaired during the formation of the very fast sedimenting DNA. With continued incubation, the sedimentation rate of the DNA on neutral sucrose gradients decreased to the normal rate.

INTRODUCTION

When logarithmic phase *Escherichia coli* K-12 cells are grown in and plated on rich medium, they are threefold more resistant to X rays than are cells grown in and plated on minimal medium (MM), i.e., they show growth-medium-dependent resistance. This enhanced resistance is *recA*-dependent and is prevented by a postirradiation treatment with rifampicin (*1*).

Two processes are known to function in the repair of the X-ray-induced DNA single-strand breaks (SSB) that are detected on alkaline sucrose gradients (*2, 3*). One process (called Type II repair) is fast (repairs half the damage in 1–2 min at room temperature), is largely *polA* dependent, and functions in buffer-held cells, i.e., it is growth-medium-independent. The other process (called Type III repair) is slow (repairs

[1] This paper is dedicated to the memory of Dr. Henry S. Kaplan (deceased: February 4, 1984), who pioneered the study of the repair of DNA double-strand breaks and who made major contributions to the study of the repair of DNA single-strand breaks.

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0033-7587/85 $3.00

the damage in 40–60 min at 37°C), is *recA* dependent, and does not function in buffer-held cells, i.e., it is growth-medium-dependent.

The repair of X-ray-induced DNA double-strand breaks (DSB) is also *recA*-dependent (*4*), and unrepaired DSB are an important cause of cell lethality after X irradiation (*5, 6*).

Since the phenomenon of medium-dependent resistance, the growth-medium-dependent repair of SSB, and the repair of DSB are all dependent on a functional *recA* gene, one can predict that the enhanced X-ray-resistance of rich-medium-grown cells should correlate with enhanced capacities for the repair of X-ray-induced DSB and for the repair of X-ray-induced SSB by the slow, *recA*-dependent, growth-medium-dependent process but not with the fast, *polA*-dependent, growth-medium-independent process. Our work confirms these predictions. In addition, our results provide additional information on the complex nature of the repair of X-ray-induced DSB.

MATERIALS AND METHODS

Bacteria. The DNA repair proficient *E. coli* K-12 strain SR749 (also known as AB1157) has been described (*1*).

Media. MM was a 0.4% glucose–salts medium (*7*), supplemented with L-arginine, L-histidine, L-leucine, L-proline, and L-threonine (all at 1 m*M*), and thiamine · HCl at 0.5 μg/ml. YENB was yeast extract (Difco) at 0.75% and nutrient broth (Difco) at 0.8%. ^{3}H-MM was MM containing [*methyl*-^{3}H]thymidine (New England Nuclear, 79.2 Ci/mmole) at 10 μCi/ml and 2'-deoxyguanosine (Sigma) at 1 m*M*. ^{3}H-YENB was YENB containing [*methyl*-^{3}H]thymidine at 20 μCi/ml. The amounts of radioactivity in the two media were chosen to obtain similar amounts of ^{3}H- incorporation per unit of cell mass. Phosphate buffer and rifampicin preparation have been described (*1*). Phosphate–EDTA (*8*) was 0.05 *M* Na_2HPO_4 and 0.1 *M* disodium ethylenedinitrilotetraacetate (EDTA), pH 8.0.

Assay for DNA single-strand breaks after repair. Logarithmic-phase cells were prepared as previously described (*1*), except that they were harvested at an optical density at 650 nm of 0.4 (Zeiss PMQ II spectrophotometer) and resuspended at an OD_{650} of 1.0. Cells were irradiated [50 kVp, with aeration, as previously described (*1*)] in buffer at room temperature, diluted 20-fold into buffer or growth medium, and shaken at 37°C. The OD_{650} of the culture was maintained at 0.05–0.4 by dilution. After incubation, cells were generally collected on membrane filters, washed with buffer, and resuspended in Tris–EDTA (0.07 *M* Tris[2-amino-2(hydroxymethyl)-1,3-propanediol] and 0.017 *M* EDTA, pH 7.6) at an OD_{650} of 0.2 ($\leq 1.5 \times 10^7$ CFU/ml). For results shown in Fig. 3, after incubation the cells were not resuspended in Tris–EDTA but were simply diluted to an OD_{650} of 0.05 with homologous medium. In either case, a 0.2-ml sample of cells was added to 0.2 ml of ice-cold lysozyme (Worthington Biochemical Corp.) at 400 μg/ml in Tris–EDTA to convert the cells to spheroplasts. After 10 min on ice, 0.1 ml of spheroplast suspension was layered onto 4.8-ml linear alkaline gradients (sucrose at 5–20%, NaOH at 0.1 *N*, Triton X-100 at 0.1%), each possessing a 0.1-ml cap of 0.5 *N* NaOH. After standing for more than 60 min at room temperature, the alkaline gradients were centrifuged (Beckman SW 50.1 rotors) at 10,000 rpm for 16 hr at 20°C. Methods for gradient fractionation and the determination of trichloroacetic acid-insoluble radioactivity were described previously (*1*).

Assay for DNA double-strand breaks. Unless otherwise noted, the procedures were as described above. After repair incubation, the cells were diluted with homologous medium to an OD_{650} of 0.05 ($\leq 4 \times 10^6$ CFU/ml) and then converted to spheroplasts. The spheroplasts were layered onto neutral sucrose gradients [as in Ref. (*6*), but containing Triton X-100 at 0.1%]. After standing for more than 90 min at room temperature, neutral gradients were centrifuged at 3700 rpm for 40 hr at 20°C.

Assay for the initial yield of DNA single-strand breaks. Unless otherwise noted, the procedures were as described above for single-strand breaks. To inhibit enzymatic DNA repair, cells were irradiated in phosphate–EDTA at 0°C, sampled with ice-cold pipets, diluted 20-fold into ice-cold phosphate–EDTA, and immediately layered onto linear alkaline sucrose gradients containing a 0.1-ml cap of Sarkosyl solution [0.5% Sarkosyl NL30 (Geigy), 0.5 *N* NaOH, and 0.10 m*M* EDTA] (*8*).

Calculation of molecular weight. Both number-average and weight-average methods (*9*) were used to calculate molecular weights. Number-average calculations were used for the DNA profiles for nonirradiated

cells and for DNA profiles for irradiated cells that closely resembled the profiles for nonirradiated cells. Otherwise, one-half the weight-average value was taken as equivalent to the number-average molecular weight. [2-^{14}C]Thymine-labeled bacteriophage T2 were used as the molecular weight standard (*9*). The calculation for the number of strand breaks per genome has been described (*10*), except that 2.8×10^9 was used here as the molecular weight for a single *E. coli* chromosome (*11*).

RESULTS

Minimal-medium-grown and rich-medium (YENB)-grown cells were X-irradiated at 0°C in phosphate–EDTA (pH 8) to estimate the initial number of radiation-induced SSB (*8*). The initial yield was 5-6 SSB per single-strand genome per krad and was independent of the medium in which the cells were grown (○, ●; Fig. 1).

A large reduction in the number of SSB occurred when X-irradiated MM-grown and YENB-grown cells were incubated in buffer for 20 min at 37°C (△, ▲; Fig. 1). Kinetic experiments indicated that a 20-min repair incubation was more than sufficient for the completion of repair in buffer (data not shown). In the terminology of Town *et al.* (*2*), this phenomenon is Type II, or growth-medium-independent repair. When MM-grown cells are X-irradiated, diluted into MM instead of buffer, and incubated at 37°C for 120 min, one expects to see a small additional reduction in the number of unrepaired SSB compared to the number of unrepaired SSB that accumulate during incubation in buffer (*2*). This additional repair of SSB (after accounting for the repair in buffer) has been called Type III, or growth-medium-dependent repair (*2*). In our

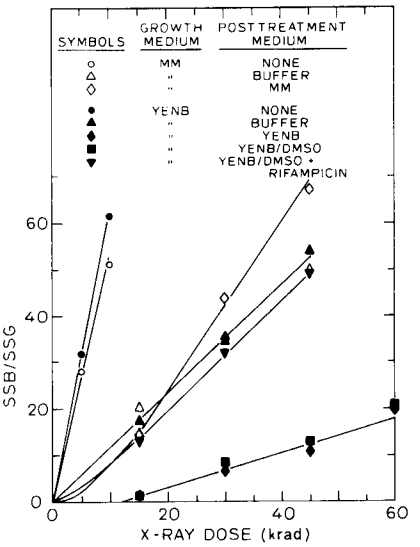

FIG. 1. Effect of growth medium and post-treatment on the repair of X-ray-induced DNA single-strand breaks (SSB) in *E. coli*. Cells were grown in minimal medium (MM) or yeast-extract nutrient-broth (YENB), irradiated (in phosphate–EDTA or buffer), and then assayed for the number of SSB per single-strand genome (SSG) either immediately (phosphate–EDTA irradiation), after 20 min of incubation at 37°C in buffer (buffer irradiation) to assay growth-medium-independent repair, or after 120 min of incubation at 37°C in growth medium (buffer irradiation) to assay growth-medium-dependent repair. Dimethyl sulfoxide (DMSO) was used to dissolve rifampicin and was present at 1% (v/v) in the specified media. Points are the means of data from triplicate experiments.

experiments for MM-grown cells, we observed growth-medium-dependent repair only after 3 and 6 krad (data not shown) and after 15 krad (◇, Fig. 1). At X-ray doses above 15 krad, even less repair of SSB was observed in MM than was observed in buffer (◇, Fig. 1). However, when YENB-grown cells were X-irradiated and diluted into YENB, a large reduction in the number of unrepaired SSB occurred after all doses tested (◆, Fig. 1). This growth-medium-dependent repair in YENB-grown cells required 60–90 min for completion (e.g., Fig. 3a) and was inhibited by the presence of rifampicin (▼, Fig. 1), suggesting the requirement for RNA synthesis.

The effect of growth medium on the repair of DSB was also tested. After an 8-krad dose, both YENB-grown and MM-grown cells showed slowly sedimenting DNA (0 min, Fig. 2). With repair incubation in homologous growth medium, the MM-grown cells were able to convert a small amount of slowly sedimenting DNA to fast-sedimenting DNA (Fig. 2a). This change in sedimentation speed of the DNA is consistent with the repair of DSB. After the same 8-krad dose, however, YENB-grown cells were able to convert a much larger amount of the slowly sedimenting DNA to fast-sedimenting DNA, in fact; the DNA sedimented even faster than the DNA from nonirradiated cells (Fig. 2b). Similar results to those in Fig. 2 were obtained with X-ray doses of 6 and 10 krad (data not shown).

The amount of DNA that sedimented faster than nonirradiated DNA increased in a radiation-dose-dependent manner. After X-ray doses of 1–4 krad, the slowly sedimenting DNA was converted directly to DNA sedimenting like nonirradiated DNA

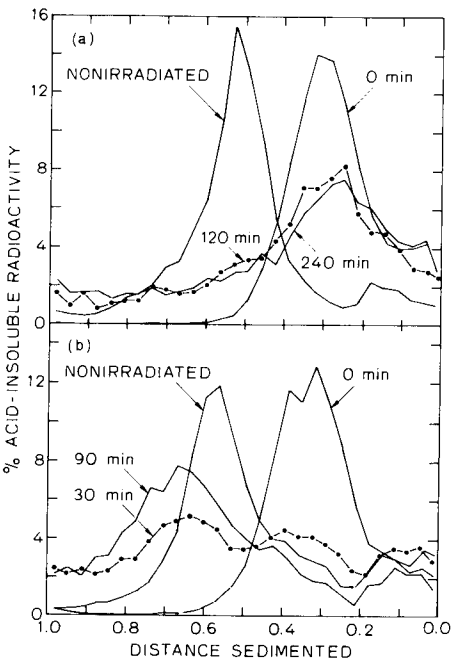

FIG. 2. Kinetics of the repair of X-ray-induced DNA double-strand breaks in *E. coli*. Cells were grown in minimal medium (a) or yeast-extract nutrient-broth (b) before irradiation (8 krad) and post-treatment in homologous medium at 37°C (incubation time is indicated). Cell survival: (a) 6%; (b) 70%. Data are from a single experiment, but similar data were obtained in other experiments using 6 and 10 krad. For the irradiated cells in (a), 60- and 180-min incubations gave results similar to those shown.

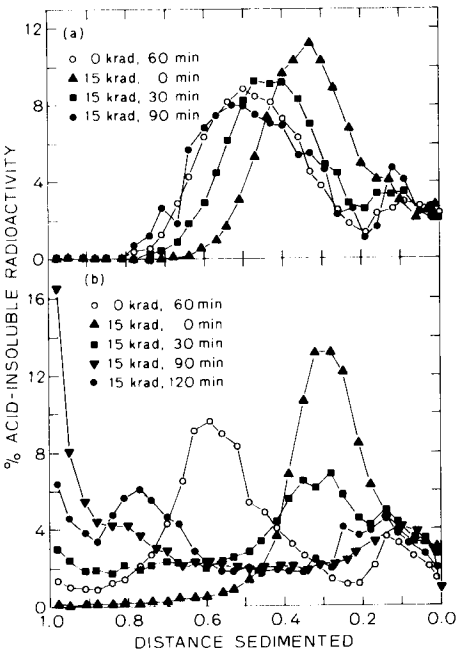

FIG. 3. Kinetics of the repair of DNA single- and double-strand breaks induced by 15 krad of X rays in *E. coli*. X-irradiated, yeast-extract nutrient-broth (YENB)-grown cells were incubated in YENB for the specified time, converted to spheroplasts, then layered onto alkaline (a) and neutral (b) sucrose gradients to assay DNA single- and double-strand breaks, respectively. Cell survival was 50%. Data are from one of two experiments with similar results. In (a), data for 15 krad, 120 min were omitted because they were similar to those for 15 krad, 90 min.

in less than 120 min of repair incubation (data not shown). After X-ray doses of 6–10 krad, the slowly sedimenting DNA was converted to DNA that sedimented somewhat faster than nonirradiated DNA (e.g., Fig. 2b). After 15 krad, the slowly sedimenting DNA in YENB-grown cells was converted during 90 min of repair incubation to DNA that sedimented to the bottom of the neutral gradient, but with further incubation this DNA showed evidence of returning to the sedimentation pattern of nonirradiated DNA (Fig. 3b). Since the irradiated cells continued to grow (doubling their OD_{650} in 50 min), the dilution of the radioactive DNA precluded repair-incubation times much longer than about 120 min. However, if we continued to label the DNA after X irradiation, the results suggested that both the preexisting and nascent DNA sedimented unusually fast for up to 5 hr of postirradiation incubation (data not shown).

The repair of SSB was monitored with alkaline gradients at the same time that the repair of DSB was monitored with neutral gradients. After 15 krad and 90 min of repair incubation, no unrepaired SSB could be detected on the alkaline gradients (Fig. 3a), suggesting that all the DSB had been repaired even though the DNA was sedimenting faster than nonirradiated DNA on the neutral gradients (Fig. 3b).

DISCUSSION

Previously, it was shown, using alkaline sucrose gradients, that rich-medium (YENB)-grown cells repaired more X-ray-induced SSB (30 krad) than minimal-medium-grown

cells (*1*). In this work, we tested whether this enhanced repair of SSB was growth-medium-independent or -dependent and whether the repair of X-ray-induced DSB was enhanced in rich-medium-grown cells. The initial yield of X-ray-induced SSB was similar for cells grown in either medium (Fig. 1). Similarly, the yield of unrepaired SSB *after* the completion of growth-medium-independent (buffer) repair was the same for YENB-grown and MM-grown cells (Fig. 1). Thus preirradiation growth in rich medium, a procedure that greatly enhances cell survival after X irradiation (*1*), did not enhance the capacity of cells to repair SSB in buffer beyond that seen in MM-grown cells.

In contrast, the growth-medium-dependent repair of SSB was greatly enhanced in YENB-grown cells relative to MM-grown cells (Fig. 1). For example, after an X-ray dose of 15 krad and 120 min of repair incubation, 14.6 SSB per single-strand genome remained unrepaired in MM-grown cells, while a dose of 51 krad was required to produce the same number of unrepaired SSB in YENB-grown cells (Fig. 1), yielding a dose modification factor of 3.4. This value should be compared with the dose modification factor of 2.7 for medium-dependent resistance obtained from a comparison of the X-ray doses that gave equal surviving fractions of 0.023 in MM-grown (15 krad) and YENB-grown (40.5 krad) SR749 cells [Fig. 3 in Ref. (*1*)]. Therefore, medium-dependent resistance appears to correlate well with the enhanced capacity for the growth-medium-dependent repair of SSB that is observed in YENB-grown cells relative to MM-grown cells.

The growth-medium-dependent repair of X-ray-induced SSB in YENB-grown cells was almost totally blocked by rifampicin treatment (Fig. 1), which suggests that this repair is inducible (i.e., dependent on *de novo* RNA and protein synthesis) and is consistent with similar results obtained after treatment with chloramphenicol (*12*). Note that medium-dependent resistance is also blocked by rifampicin treatment and is considered to be an inducible phenomenon (*1*).

Our inability to detect the repair of X-ray-induced DSB (after 5, 10, and 20 krad) in buffer during a 20-min postirradiation incubation at 37°C, although SSB were being repaired (data not shown), is consistent with the conclusion that the repair of DSB is inducible (*13*). In growth medium, where macromolecular synthesis could be induced, the repair of DSB was observed (Fig. 2), and it, like medium-dependent resistance, was greatly enhanced in YENB-grown cells versus MM-grown cells.

Even when the repair of DSB has been completed in YENB-grown cells (in less than 90 min), some further processing seems to be required before the DNA will sediment at the normal position in a neutral sucrose gradient (*4, 14, 15*). One explanation for the dose-dependent fast-sedimenting species is that after the DSB have been repaired, alkali-labile attachments to membrane or other cellular components persist which cause the DNA to sediment faster than normal in a neutral gradient (e.g., Fig. 3b).

Dugle *et al.* (*16*) concluded for Chinese hamster cells that true single-strand breaks (like the damage we see repaired in buffer) are completely repairable and that unrepaired single-strand breaks (as we see after the completion of repair in buffer) are all associated with DSB. Thus the most simple conclusion for *E. coli* is that the growth-medium-dependent repair of X-ray-induced SSB is essentially the repair of DSB and that it is the enhanced repair of DSB that results in the enhanced resistance to X rays when

comparing rich-medium-grown cells with minimal-medium-grown cells (i.e., medium-dependent resistance).

ACKNOWLEDGMENTS

We are grateful to Professor Israel Felzenszwalb, Dr. Rakesh C. Sharma, and Dr. Tzu-chien V. Wang, for much helpful criticism; to Cheryl A. Cheng for skillful technical assistance; and to Dr. Barbara J. Bachmann for strain AB1157. This investigation was supported by Public Health Service Grant CA-06437 from the National Cancer Institute, DHHS.

RECEIVED: April 29, 1985; REVISED: June 25, 1985

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