The Effect of Growth Conditions on Inducible, recA-Dependent Resistance to X Rays in Escherichia coli

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Escherichia coli cells grown to logarithmic phase in, and plated on, rich medium (yeast extract–nutrient broth) were more resistant to X rays, ultraviolet (uv) radiation, and methyl methanesulfonate (MMS) than cells grown in, and plated on, minimal medium. We have called this enhanced survival capability medium-dependent resistance (MDR). The magnitude of MDR observed after anoxic X irradiation was greater than that observed after anoxic X irradiation, UV irradiation, or MMS treatment. MDR was not observed in stationary-phase cells with X or UV radiation. MDR was associated with an increased ability to repair X-ray-induced DNA single-strand breaks, and with reduced X-ray-induced DNA degradation and protein synthesis retardation. Postirradiation protein synthesis was concluded to be critical in allowing the high X-ray survival associated with MDR, because of the large radiosensitization caused by a postirradiation growth medium shift down or treatment with rifampicin (RIF). recA protein must be at least one of the proteins whose synthesis is critical to MDR, as judged by the absence of MDR or a RIF effect in X-irradiated recA and lexA mutants. The results with X-irradiated temperature-conditional recA cells suggest that it is only after cells have been damaged that the recA gene plays a role in MDR.

INTRODUCTION

Logarithmic-phase Escherichia coli cells have been reported to be more resistant to 60Co γ radiation when grown in rich medium (yeast extract–nutrient broth–glucose) than when grown in glucose minimal medium (1). We will refer to such enhanced survival capability as medium-dependent resistance (MDR). MDR became of interest to us when we isolated a new mutant whose only known defect is a partial deficiency in MDR; i.e., compared to a wild-type strain, it is radiation sensitive when grown to logarithmic phase in rich medium, but not when grown in minimal medium. This mutant will be described in a separate report. The purpose of this study was to further characterize the phenomenon of MDR and to gain some insight into its mechanism.

MATERIALS AND METHODS

Bacterial strains. The strains of E. coli used are listed in Table I. Transduction was mediated by bacteriophage P1::Tn9ets (Tn9 confers chloramphenicol resistance to lysogens) and was accomplished generally as described by Miller (4).

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### TABLE I

**Strains of E. coli Used**

<table>
<thead>
<tr>
<th>Stanford radiology number</th>
<th>Genotype</th>
<th>Source or derivation</th>
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| SR38                     | F^- argE3 his-4 leuB6 proA2 thr-1 ara-14  
                          |                      |
|                          | galK2 lacY1 mil-1 xyl-5 thi-1 tss-33 
                          |                      |
|                          | rpsL31 supE44 recA13 λ             | AB2463, A. J. Clark  |
| SR749                    | Same as SR38, but recA*             | AB1157, ECGSC        |
| SR759                    | trpE65 srlA300::Tn10(Tc') sulA1  
                          | (3)                   |
|                          | recA200(Ts)                          |                      |
| SR777                    | Same as SR38, but Thr* recA*        | SR749 × P1kc·SR774,  
                          | select Thr*            |
|                          |                                        |                      |
| SR818                    | Same as SR38, but Arg* recA* malB  
                          | PAM5743, J. Donch    |
|                          | lexA1                                |                      |
| SR1115                   | Same as SR38, but srlA4300::Tn10(Tc')  
                          | SR749 × P1::Tn9cets·SR759,  
                          | select Tc              |
|                          | recA200(Ts)                          |                      |

*All strains are derivatives of strain K-12 except SR759, which is a derivative of strain B/r. Genotype nomenclature is that used by Bachmann and Low (2). Thr* indicates that threonine is no longer required for growth. Ts indicates a temperature-conditional mutation. Tc* indicates that isolates were tetracycline resistant. ECGSC is the E. coli Genetic Stock Center. Strain SR774 will be described in a separate publication.*

**Media.** SMM was a 0.4% glucose–salts medium (5), supplemented with L-arginine, L-histidine, L-leucine, L-proline, and L-threonine (all at 1 mM), and thiamine·HCl at 0.5 μg/ml. YENB was yeast extract (Difco) at 0.75% and nutrient broth (Difco) at 0.8%. YENBG was YENB containing glucose at 0.4%. Cells were plated either on YENB agar [yeast extract (Difco) at 0.75% and nutrient agar (Difco) at 2.3%] or on SMM media solidified with Noble agar (Difco) at 1.6%. Phosphate buffer (PB) was Na₂HPO₄ at 5.83 g/liter and KH₂PO₄ at 3.53 g/liter, pH 7.0. Phosphate-buffered saline (PBS) was 0.05 M KH₂PO₄ and 0.1 M NaCl, adjusted to pH 7.3 with NaOH. Rifampicin (RIF, Sigma), an inhibitor of RNA synthesis, was prepared fresh for each experiment by dissolving it in dimethylsulfoxide (DMSO) at 10 mg/ml, followed by a 100-fold dilution in the appropriate growth medium. Methyl methanesulfonate (MMS, Aldrich Chemical Co.) was dissolved in DMSO at 2.5 M. DMSO, at the concentration used, had no detectable effects on unirradiated or irradiated cells (data not shown).

**Preparation of cells.** Cells were grown to stationary phase in YENB and diluted 100-fold into either SMM, YENB, or YENBG for overnight growth (~15 hr) to prepare stationary-phase cells. Logarithmic-phase cells were prepared by diluting stationary-phase cells, 1:100 (for SMM-grown cells) or 1:500 (for YENB-grown cells), into the homologous medium. Cells were filter-harvested (Millipore HAWP, 0.45-μm pore size) at an optical density at 650 nm (OD₆₅₀) of 0.5 (Zeiss PMQ II spectrophotometer), which was reached after four or more cell population doublings. These cells were washed and resuspended in PB at an OD₆₅₀ of 0.05, i.e., ~5 × 10⁷ colony-
forming units (CFU) per milliliter for logarithmic-phase SMM-grown or stationary-phase YENB-grown cells, or \( \sim 1 \times 10^7 \) CFU/ml for logarithmic-phase YENB-grown or stationary-phase YENBG-grown cells.

**Irradiation.** X irradiation was accomplished using the twin-tube 50-kVp X-ray unit described by Loevinger and Huismans (6). The X-ray tubes were operated at 48 and 50 mA with 0.27 mm Al added filtration. The cells in PB (3.5 ml) were irradiated in a Plexiglas vessel (0.8-mm-thick walls). The dose rate was 7.1 krad/min as determined by a ferrous sulfate method (7). Cells were bubbled with air for 5 min or with N\(_2\) for 15 min (for anoxic irradiation) before irradiation. Gassing continued during the irradiation. Ultraviolet irradiation, using an 8-W General Electric germicidal lamp (G8T5) emitting primarily at 254 nm, was accomplished as described previously (8). No correction for cell masking was required at the cell concentration used. The fluence rate was \(~0.8\) J m\(^{-2}\) sec\(^{-1}\), as determined with an International Light germicidal photometer (IL-254).

**MMS treatment.** Logarithmic-phase cells were washed and resuspended in PBS at an OD\(_{550}\) of 0.05. These cells (24.5 ml at 37°C) were added to 0.5 ml of MMS (final concentration, 0.05 \( M \)) and the mixture was slowly shaken at 37°C. Samples were withdrawn and diluted 10-fold or more with PBS containing 0.1% sodium thiosulfate before plating. The 10-fold dilution into thiosulfate solution effectively neutralized the unreacted MMS (data not shown).

**Survival determination.** Colony-forming units per milliliter were determined by diluting cells appropriately in PB, plating them in duplicate or in triplicate on an appropriate medium, incubating them at 37°C (unless specified) for 24 to 48 hr, and then counting colonies.

**Measurement of protein synthesis.** Overnight SMM and YENB cultures were pelleted (6000g, 6 min), washed, and resuspended in PB (25 vol for SMM-grown cells, 125 vol for YENB-grown cells). These cell suspensions were then diluted 20-fold into SMM (modified to contain L-leucine at 20 \( \mu \)g/ml) or into YENB, respectively; both media contained L-[\( 3,4,5-\text{H} \)]leucine at 2 \( \mu \)Ci/ml (New England Nuclear, 110 Ci/mmole). Cells were grown with aeration at 37°C for four or more population doublings until an OD\(_{550}\) of 0.5 was reached. \( [\text{\textsuperscript{3}H}] \)leucine incorporation had been proportional to OD\(_{550}\) increase for two population doublings at this time (data not shown). The cells were then filter-harvested, washed, resuspended in PB at an OD\(_{550}\) of 1.0, and X-irradiated (oxic). Both nonirradiated and X-irradiated cells were then diluted 20-fold into the same radioactive medium as above, and again incubated at 37°C with aeration. Both before and after irradiation, 0.2-ml samples of culture were periodically removed and added to 2-ml portions of ice-cold 10% trichloroacetic acid (TCA). The TCA-precipitated samples were held on ice until the end of the experiment. They were then filter-harvested (Millipore EHWP, 0.5-\( \mu \)m pore size; presoaked in leucine at 0.1 \( M \)), washed with three 5-ml portions of ice-cold 0.1% TCA, dried, and placed in Omnifluor (New England Nuclear), and their radioactivity determined with a liquid scintillation spectrometer.

**Measurement of DNA degradation.** DNA was labeled by growing cells at 37°C for at least four generations in SMM and YENB containing [methyl-\( \text{\textsuperscript{3}H} \)]thymidine at 10 \( \mu \)Ci/ml (Amersham, 47.5 Ci/mmole); SMM also contained deoxyguanosine \( \cdot \) H\(_2\)O at 250 \( \mu \)g/ml. Cells were then filter-harvested, washed, resuspended in homologous non-
radioactive medium at 37°C, and incubated for at least one generation time. Cells were then filter-harvested, washed, resuspended in PB at an OD_{650} of 0.05, X-irradiated, and diluted 1:20 into homologous nonradioactive growth medium. After 0 or 180 min of incubation at 37°C, 0.5-ml samples were added to 2-ml volumes of ice-cold 10% TCA, and then processed as described above for samples assayed for protein synthesis (except that the EHWP filters were presoaked in thymine at 1 mg/ml).

Measurement of DNA single-strand breaks. DNA was labeled by preparing logarithmic-phase cells as in a survival experiment except that the SMM and YENB contained [methyl-3H]thymidine at 10 μCi/ml (Amersham, 47.5 Ci/m mole); SMM also contained deoxyguanosine·H₂O at 300 μg/ml. Radioactive thymidine incorporation was proportional to culture OD_{650} increase for two population doublings preceding cell harvest (at an OD_{650} of 0.5) (data not shown). After cells were harvested from the radioactive medium and resuspended in PB at an OD_{650} of 1.0, they were X-irradiated. The irradiated cell suspension, 0.25 ml, was added to 4.75 ml of appropriate medium and incubated at 37°C in a shaking water bath. After various times of incubation, 0.3-ml samples were added to ice-cold spheroplasting solution containing 0.05 ml of 32 mM disodium ethylenedinitrilotetraacetate·H₂O, 0.03 ml of 30% sucrose in 0.6 M tris-(hydroxymethyl)aminomethane (pH 8.1), and 0.08 ml of lysozyme (Worthington Biochemical Corp.) at 1 mg/ml (9). After incubating the cells in ice-cold spheroplasting solution for 15 min, a 0.05-ml sample (<10⁷ cells) was layered onto a 0.2-ml, 0.5 N NaOH cap atop a 4.8-ml linear gradient of 5–20% sucrose in 0.1 N NaOH. After 60 min or longer, the lysed cells were sedimented for 16 hr at 10,000 rpm in a Beckman Model L or L5 ultracentrifuge at 20°C (SW 50.1 rotors). Then the cellulose nitrate tubes (Beckman) were punctured at the bottom and the gradients were pumped in discrete fractions onto Whatman No. 17 paper (cut in strips, 1.25 × 57 cm). The strips were washed twice in 5% TCA and once in 95% ethanol (10-min cycles) and dried before being cut into about 30 1.25 × 1.75 cm fractions. Radioactivity per fraction was determined as above.

RESULTS

Logarithmic-phase YENB-grown cells plated on YENB were more resistant to X rays (oxic) than were SMM-grown cells plated on SMM, i.e., the YENB-grown cells exhibited MDR (Fig. 1a). MDR was reduced for anoxically X-irradiated cells (Fig. 1b), uv-irradiated cells (Fig. 1c), and MMS-treated cells (Fig. 1d). MDR was not observed in stationary-phase cells with uv radiation (data not shown) or with X rays; the survival of X-irradiated stationary-phase YENB-grown cells approximated that for logarithmic-phase SMM-grown cells (cf. open symbols in Fig. 3).

When logarithmic-phase SMM-grown cells were plated on YENB, their X-ray survival was enhanced only slightly compared to that obtained on SMM plates (Fig. 2). However, when logarithmic-phase YENB-grown cells were plated on SMM (shift-

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Fig. 1. E. coli (SR777) survival after oxic X irradiation (a), anoxic X irradiation (N₂) (b), uv irradiation (c), or MMS treatment (d). Cells were grown to logarithmic phase in YENB (●) or in SMM (○) before treatment and plating on homologous medium. Data are the means of three experiments per point.
Fig. 2. Effect of a shift in the richness of the growth medium on the X-ray survival of *E. coli* (SR749). Cells were grown to logarithmic phase in YENB (solid symbols) or SMM (open symbols) before X irradiation (oxic) in buffer and plating on YENB (●, △) or SMM (▲, ○). Data are the means of three experiments per point.

down growth conditions), they exhibited an X-ray survival even lower than that seen with SMM-grown cells plated on SMM (Fig. 2), i.e., MDR was prevented. These results suggest that both preirradiation and postirradiation growth must be in YENB for MDR to be expressed.

Another radiation resistance phenomenon that is dependent on the preirradiation growth medium is glucose-induced resistance (GIR) (10). Hollaender *et al.* (11) noted that *E. coli* cells grown to stationary phase in glucose-enriched nutrient broth were more resistant to X rays than cells grown in broth without glucose. To test whether MDR is similar to GIR, cells grown to demonstrate MDR (i.e., logarithmic phase, YENB vs SMM) were compared for X-ray survival with cells grown to demonstrate GIR (i.e., stationary phase, YENBG vs YENB). The X-ray survival of cells exhibiting MDR and cells exhibiting GIR was quite similar (Fig. 3).

Cells exhibiting GIR are also able to initiate protein synthesis more rapidly after X irradiation (12). Because of the similarity between MDR and GIR (Fig. 3), we tested whether the growth conditions for MDR affected postirradiation protein synthesis. Cells grown in YENB before and after X irradiation with 10 krad synthesized protein at a rate identical to that of nonirradiated cells, while cells grown in SMM
Fig. 3. X-Ray survival of *E. coli* (SR749); MDR vs GIR. Cells were prepared to demonstrate MDR by growing them to logarithmic phase in YENB (●) or SMM (○) before X irradiation (oxic) in buffer and plating on homologous medium. Cells were prepared to demonstrate GIR by growing them to stationary phase in YENBG (▲) or YENB (▲) before X irradiation and plating on YENB medium. Data are the means of three experiments per point.

before and after the same X-ray dose showed a radiation-induced retardation of protein synthesis (Fig. 4). However, after equal-killing doses of X radiation, protein synthesis was affected similarly in both kinds of cells (cf. data for 10 krad, SMM, and 40 krad, YENB; Fig. 4).

To test the importance of postirradiation RNA and protein synthesis for the expression of MDR, YENB-grown and SMM-grown cells were treated with RIF after being X-irradiated [X rays are known to induce the synthesis of *recA* protein (13–17)]. YENB-grown cells were sensitized to X rays by RIF more than were SMM-grown cells (Fig. 5). These results are consistent with the hypothesis that the expression of MDR requires an inducible phenomenon. If this is true, then by incubating X-irradiated YENB-grown cells in YENB before challenging them with RIF or shift-down treatments one should be able to demonstrate a progressive loss of sensitivity to such treatments. YENB-grown cells incubated in YENB after X irradiation became immune to RIF in 20 min, while more than 60 min of incubation were required for the development of immunity to the shift-down treatment (Fig. 6). The cells that had become immune to RIF (e.g., at 20 min) were still sensitive to a shift-down treatment even after the incubation in the YENB–RIF medium (cf. ■ and ♦ in Fig. 6).
Fig. 4. Effect of X rays on protein synthesis in E. coli (SR749). Cells were grown in media containing [3H]leucine for at least four population doublings before being prepared for X irradiation (oxic) and post-irradiation incubation at 37°C in homologous, radioactive (same specific activity) medium. X-Ray doses for YENB-grown (solid symbols) or SMM-grown (open symbols) cells were: 0 (●, ○), 10 (▲, △), and 40 (●) krad. Data shown are TCA-insoluble radioactivity per milliliter of culture from one of two experiments with similar results. The arrows indicate when the cultures were harvested for X irradiation.

Since the recA and lexA genes control many radiation-induced phenomena (18), we tested whether they also control MDR. MDR, following X irradiation, was not observed in recA or lexA mutants (Figs. 7a, b). Furthermore, YENB-grown recA and lexA cells were not sensitized to X rays by a postirradiation treatment with RIF (data not shown), suggesting that the recA and lexA genes are involved in the postirradiation induction of functions that are important to MDR. To test whether the recA gene also played a role in MDR before the YENB-grown cells were X-irradiated, a temperature-conditional recA strain (SR1115) was used. Thus, by growing strain SR1115 at 40°C in the appropriate medium, X-irradiating it, and plating it at 30°C, one would have a recA strain before X irradiation, but a RecA+ strain after X irradiation. The result was that the cells grown at 40°C and those grown at 30°C exhibited MDR when plated at 30°C (Fig. 8), indicating that functional recA protein was not required prior to X irradiation. Growth at 40°C was sufficient to elicit the RecA− phenotype, as evidenced by the recA-like survival of cells grown and plated at 40°C (cf. Figs. 7a and 8).
Fig. 5. Effect of rifampicin (RIF) on the survival of X-irradiated E. coli (SR749). Cells were grown to logarithmic phase in YENB (solid symbols) or SMM (open symbols) before X irradiation in buffer at an OD_{600} of 1.0 (oxic) and plating on homologous medium (●, ○). Some X-irradiated cells were diluted 20-fold into homologous medium containing RIF at 100 µg/ml (●, △) and incubated 30 min at 37°C before being plated on homologous medium. Data are from one of two experiments with similar results.

An additional characteristic of MDR is that YENB-grown cells show much less X-ray-induced DNA degradation than do SMM-grown cells (Fig. 9). Finally, experiments were performed in order to see if MDR could be correlated with enhanced DNA repair. YENB-grown cells showed more repair of X-ray-induced DNA single-strand breaks (and alkali-labile lesions) than did SMM-grown cells after 120 min of incubation in the homologous medium at 37°C, i.e., the DNA profiles for irradiated YENB-grown cells resembled, with incubation, the DNA profile for nonirradiated cells (Fig. 10a), while the DNA profiles for irradiated SMM-grown cells, with incubation, assumed a gradient position intermediate between that for DNA from irradiated cells without incubation and that for DNA from nonirradiated cells (Fig. 10b).

DISCUSSION

In comparing the different survival curves, it is useful to consider the dose modification factor (DMF), i.e., the ratio of equal-killing doses. In this discussion, the DMF is calculated by dividing the $D_2$ (the dose killing 98% of the cells) for resistant
cells (e.g., YENB-grown) by the $D_2$ for sensitive cells (e.g., SMM-grown).] The DMF associated with the MDR for cells X-irradiated in the presence of oxygen (3.7) is somewhat larger than that for cells X-irradiated in the absence of oxygen (2.6) (data from Figs. 1a, b). This difference presumably reflects the greater sensitivity of SMM-grown cells to oxygen-dependent X-ray-induced lesions, because the oxygen enhancement ratio (calculated like the DMF for cells X-irradiated under $N_2$ vs air) for SMM-grown cells (3.1) is larger than that for YENB-grown cells (2.2) (data from Figs. 1a, b). Also, the DMF associated with the MDR for cells treated with X radiation, i.e., 2.6–3.7, are larger than those for cells treated with uv radiation (1.5) or MMS (1.4). These calculations suggest that the magnitude of MDR depends on the types of DNA lesions produced.

Phenomena that are jointly controlled by the recA and lexA genes (i.e., so-called SOS phenomena), such as radiation mutagenesis, W-reactivation, inactivation of bacteriophage $\lambda$ repressor, and the processes controlled by the din loci, have all been shown to be inducible processes (18–21). Since MDR is also absent in both recA and lexA mutants (Fig. 7), one can assume that it is also an inducible process.

One possible explanation for MDR is that cells growing in rich medium encounter a “signal” that induces them for the enhanced ability to survive DNA-damaging agents. If YENB-grown cells are preinduced for all needed repair functions, then they should not be sensitized to X rays by a postirradiation treatment with RIF. However, YENB-grown cells, as well as SMM-grown cells, were sensitized by RIF (the DMF were 3.1 and 1.7, respectively) (Fig. 5). By comparing the DMF associated with MDR...
in the presence of RIF and in the absence of RIF, one can calculate that \( \sim 60\% \) of MDR is inhibitable by RIF. If one assumes that all repair functions induced by X irradiation can be prevented by a 30-min postirradiation treatment with RIF, then a portion of MDR might be the result of a preirradiation induction effect for the cells grown in rich medium. Alternately, \( \sim 40\% \) of the postirradiation induction of functions needed for the expression of MDR may simply be delayed until the cells are returned to non-drug-containing growth medium. Consistent with this latter possibility is the observation that only \( \sim 25\% \) of MDR is inhibitable by a 30-min postirradiation treatment with 100 \( \mu g/ml \) of chloramphenicol (data not shown), an even more direct inhibitor of protein synthesis than RIF. Additionally, nonirradiated YENB-grown cells, relative to SMM-grown cells, did not seem to be induced for SOS functions because they showed no enhanced ability to reactivate X-irradiated bacteriophage \( \lambda \) [as would be expected from the notion of W-reactivation (22)] (data not shown).

Based on these arguments, we conclude that MDR is the result of the postirradiation induction of DNA repair functions. We assume that the sensitivity of MDR to RIF (Fig. 5) and shift-down (Fig. 2) postirradiation treatments reflects a need for the postirradiation induction of the \textit{recA} protein (and possibly other proteins) [a shift-
Fig. 8. Effect of pre- and post-X-irradiation growth conditions on the X-ray survival of *E. coli recA200(ts) (SR1115). Cells were grown to logarithmic phase in YENB (solid symbols) or SMM (open symbols) at 30 or 40°C. Cells were then incubated in buffer for 30 min at their original growth temperature, before X irradiation (oxic) and plating on homologous medium prewarmed at 30 or 40°C. Pre- and post-X-irradiation temperatures are indicated by the respective values adjacent to the survival curves. Data are from one of two experiments with similar results.

down treatment is known to block net RNA and protein synthesis for more than 1 hr (23)]. This assumption relative to the recA protein arises from the total dependence of MDR upon the recA gene after, but not before, X irradiation (the DMF associated with MDR in the temperature-conditional recA strain was 2.4 for cells grown at 40°C and 2.2 for cells grown at 30°C; data from Fig. 8), and the fact that the *lexA* gene, which regulates the inducible functions but not the constitutive functions of the recA gene (18, 24, 25), is also absolutely required.

Since MDR appears to be associated with the postirradiation induction of macromolecular synthesis, one needs to consider why MDR shows the preirradiation requirement of growth in rich medium (Fig. 2). Stapleton and Fisher (12) correlated GIR with a higher cellular content of rRNA and 30S and 50S ribosomes. MDR, which is quantitatively similar to GIR for X-ray survival (Fig. 3), can also be assumed to show this correlation with protein-synthesizing capacity, because logarithmic-phase YENB-grown cells double in 26 min while SMM-grown cells take 61 min to double (data not shown). The fact that both GIR (12) and MDR (Fig. 4) are correlated with
Fig. 9. X-Ray-induced DNA degradation in E. coli (SR777). Cells were grown in SMM (△) or YENB (●) supplemented with [methyl-\(^3\)H]thymidine before being "chased" with nonradioactive medium, X-irradiated (oxic) in buffer, and incubated in SMM or YENB, respectively, for 180 min at 37°C. Data are the means of two experiments in which the TCA-insoluble counts per milliliter for the 180-min samples were divided by the counts per milliliter for nonincubated cells [mean 10-min counts were 15,055 (YENB) and 63,682 (SMM)].

smaller radiation effects on protein synthesis rate suggests that the requirement of preirradiation growth in rich medium for the expression of MDR (Fig. 2) may simply reflect the need for more protein-synthesizing components per unit of cell volume after irradiation. In fact, the density of ribosomes per cell increases with growth rate (23). Thus the destruction of a given amount of protein-synthesizing components per cell should have a smaller effect on the rate of protein synthesis for YENB-grown cells than for SMM-grown cells. This could explain the correlation between X-ray-induced killing and retardation of the rate of protein synthesis shown in Fig. 4. Additionally, since stalled DNA replication forks have been implicated in the production of a signal for the synthesis and/or activation of recA protein [reviewed in (18)], this signal would be expected to be produced earlier in YENB-grown cells than in SMM-grown cells containing the same number of DNA synthesis-blocking lesions (i.e., YENB-grown cells can be assumed to possess a greater number of replication forks per cell than SMM-grown cells).

In conclusion, preirradiation growth conditions that diminish the effects of radiation on the rate of synthesis of cellular proteins (i.e., rich medium for logarithmic-phase cells) allow the development of a recA \textit{lexA} gene-dependent radiation resistance that is correlated with increased DNA repair capability. The deleterious effects of a postirradiation growth medium shift-down procedure or of the inhibition of RNA synthesis with RIF on the survival of rich-medium-grown cells suggest that growth in rich medium does not itself induce repair proteins (which might be expected to function even under conditions where \textit{de novo} RNA and protein synthesis are blocked),
but rather logarithmic growth in rich medium simply provides a protein-synthesizing capability that is more resistant to damaging agents. At least one of the proteins involved in MDR (and presumably G+R) that must be synthesized rapidly after X irradiation to ensure maximum survival (and presumably after uv irradiation or MMS treatment as well) is the recA protein.

Since DNA repair is generally studied in logarithmic-phase minimal medium-grown cells (e.g., to facilitate labeling of the DNA), a portion of MDR may be a heretofore unstudied DNA repair system rather than just being the enhanced capacity of previously known systems [e.g., Type III repair (26)]. In support of the concept that at least some of MDR is the result of an unstudied DNA repair system, we have isolated a new mutation that sensitizes YENB-grown cells but not SMM-grown cells to X rays (27).
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