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Escherichia coli radC Is Deficient in the *recA*-Dependent Repair of X-Ray-Induced DNA Strand Breaks

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Escherichia coli K-12 cells incubated in buffer can repair most of their X-ray-induced DNA single-strand breaks, but additional single-strand breaks are repaired when the cells are incubated in growth medium. While the *radC102* mutant was proficient at repairing DNA single-strand breaks in buffer (*polA*-dependent repair), it was partially deficient in repairing the additional single-strand breaks (or alkali–labile lesions) that the wild-type strain can repair in growth medium (*recA*-dependent repair), and this repair deficiency correlated with the X-ray survival deficiency of the *radC* strain. In studies using neutral sucrose gradients, the *radC* strain consistently showed a small deficiency in rejoining X-ray-induced DNA double-strand breaks, and it was deficient in restoring the normal sedimentation characteristics of the repaired DNA.

Our laboratory is isolating new radiation-sensitive mutants of *Escherichia coli* K-12 in the hope of discovering new pathways of DNA repair and of better characterizing known pathways. Thus far, three mutants have been described: *radA100* (*1*), *radB101* (*2*), and most recently, *radC102,* which maps at 81.0 min on the *E. coli* linkage map (*3*).

The *radC* mutation sensitizes cells to uv radiation, but unlike most DNA repair mutations, it only sensitizes to X radiation when the cells are grown to logarithmic phase in rich medium (*3*), i.e., when a *radC* mutation partially inhibits medium-dependent resistance, an inducible repair process that shows a selectivity for ionizing radiation-induced DNA damage (*4*). For cells grown to logarithmic phase in rich medium, the *radC* mutant was normal for γ-radiation mutagenesis but showed less uv-radiation mutagenesis than the wild-type strain; it showed wild-type amounts of X- and uv-radiation-induced DNA degradation and of host cell reactivation of γ- and uv-irradiated bacteriophage λ and was about 60% deficient in recombination ability. The *radC* mutation did not sensitize a *recA* strain, but did sensitize a *radA* and a *polA* strain to X and uv radiation and a *uvrA* strain to uv radiation. Based upon these data, it was suggested that the *radC* gene product plays a role in the growth-medium-de-

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pendent, *recA* gene-dependent repair of DNA single-strand breaks after X irradiation and in postreplication repair after uv irradiation (*3*).

In the present work, we have investigated the ability of X-irradiated *radC* cells to repair DNA single-strand breaks (SSB) in buffer (*polA*-dependent repair) and in growth medium (*recA*-dependent repair) and their ability to repair DNA double-strand breaks (DSB) in growth medium.

MATERIALS AND METHODS

Bacterial strains. The strains of *E. coli* K-12 used in this study, SR1269 and SR1271, have been described (*3*).

Media. YENB was yeast extract (Difco) at 0.75% and nutrient broth (Difco) at 0.8%. [^{3}H]YENB was YENB containing [*methyl*-^{3}H]thymidine at 20 μCi/ml (New England Nuclear, 79.2 Ci/mmol). Phosphate buffer has been described (*1*).

Assays. Logarithmic-phase, YENB-grown cells were X-irradiated and assayed for survival, nonrepaired SSB (alkaline sucrose gradients), and nonrepaired DSB (neutral sucrose gradients) as previously described (*5*). Briefly, the DNA strand-break assays were as follows. The X-irradiated cells were diluted 20-fold into buffer or growth medium and incubated at 37°C for 30 or 120 min, respectively, to allow for the completion of repair. The cells were then diluted to about 8×10^6 cells per milliliter with homologous medium, converted to spheroplasts, and layered onto alkaline sucrose gradients for the assay of nonrepaired SSB. For the assay of nonrepaired DSB, the X-irradiated cells were incubated in growth medium for 120 min before being diluted (as above), converted to spheroplasts, and layered onto neutral sucrose gradients for sedimentation analysis.

RESULTS AND DISCUSSION

Town *et al.* (*6*) differentiated the repair of X-ray-induced SSB in *E. coli* (assayed with alkaline sucrose gradients) into a *polA*-dependent process that repairs SSB rapidly during postirradiation incubation in buffer and a *recA*-dependent process that repairs SSB more slowly and requires postirradiation incubation in growth medium. When X-irradiated wild-type and *radC* cells were assayed for their ability to perform the rapid repair of SSB in buffer, no significant difference could be detected (open symbols, Fig. 1a). This finding is in agreement with the fact that the *radC* mutation sensitizes a *polA* strain to killing by X rays (*3*).

However, the *radC* mutant did show a deficiency in the slow, growth-medium-dependent, *recA*-dependent repair of SSB (closed symbols, Fig. 1a). The involvement of the *radC* gene in the *recA*-dependent repair of X-ray-induced SSB is consistent with the fact that the *radC* mutation does not sensitize a *recA* strain to killing by X rays (*3*). Also, the *radC* deficiency in the repair of SSB in growth medium compares favorably with the X-ray-survival deficiency seen in the *radC* mutant. That is, the enhanced resistance of the wild-type strain relative to the *radC* strain was 1.69 for survival (ratio of doses at a surviving fraction of 0.01; Fig. 1b) and 1.36 for the repair of SSB (ratio of doses to leave 20 nonrepaired SSB; closed symbols, Fig. 1a).

The growth-medium-dependent repair of SSB and the repair of DSB share many common characteristics, and it has been proposed that they are mostly the same process (e.g., (*5*)). Thus one can predict from the preceding data that the increased X-ray sensitivity seen in the *radC* mutant results from a deficiency in the repair of DSB.

We attempted to confirm that the *radC* mutant was deficient in the repair of X-ray-induced DSB using a neutral sucrose gradient sedimentation technique. In the

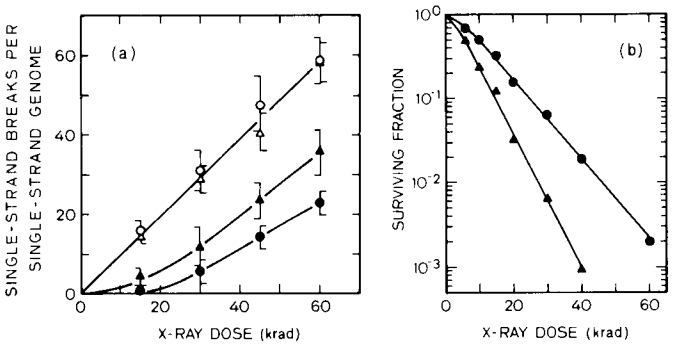

FIG. 1. Effect of the *radC* mutation on the repair of X-ray-induced DNA single-strand breaks and X-ray survival in *E. coli*. (a) X-Irradiated cells were incubated at 37°C for 30 min in buffer (open symbols) or for 120 min in growth medium (closed symbols) and then assayed for the number of nonrepaired DNA single-strand breaks per single-strand genome. (b) X-Irradiated cells were plated on YENB agar to determine the surviving fraction. (○, ●) Wild type (SR1269), and (△, ▲) *radC* (SR1271). The points are the means of data from quadruplicate experiments.

dose range of 1–4 krad, both the wild-type and *radC* strains showed the induction of about 1 DSB per genome per krad, and after 120 min of repair incubation in YENB the DNA of both strains sedimented like that from nonirradiated cells; i.e., DSB could no longer be detected (data not shown). Using this same protocol, a *recA* strain, which is known to be deficient in the repair of DSB (*8, 9*), showed even more DSB after incubation in YENB than were initially induced by the radiation (data not shown). Thus, at low X-ray doses, where one can quantitate strand breaks using neutral sucrose gradients, we did not observe in the *radC* strain a deficiency in the repair of DSB.

At higher doses of ionizing radiation, it becomes increasingly difficult to quantitate the repair of DSB due to the formation of DNA, during repair incubation, that sediments faster on neutral sucrose gradients than nonirradiated DNA (*5, 7, 9, 10*). The kinetics of this process for the wild-type strain incubated in YENB have been reported earlier (*5*). That is, immediately after irradiation (e.g., 15 krad), slow-sedimenting DNA is observed. After 30 min of repair incubation, perhaps half of the slow-sedimenting DNA has begun to sediment faster. After 90 min of repair incubation, radiation-induced slow-sedimenting DNA is no longer observed and a large amount of DNA sediments faster than nonirradiated DNA. [At this point, when a duplicate sample of cells is sedimented on an alkaline sucrose gradient, it sediments exactly like nonirradiated DNA, suggesting that most of the DSB have been repaired during the formation of fast-sedimenting DNA (*5*).] After 120 min of repair incubation, one observes the beginning of the conversion of fast-sedimenting DNA into a form that sediments like nonirradiated DNA.

Similar kinetics to those described above were observed for the disappearance of slow-sedimenting DNA and for the appearance and disappearance of fast-sedimenting DNA in the wild-type strain used in this work (Fig. 2a, and data not shown). However, the *radC* strain appeared to be different in two aspects of this process after 120 min of repair incubation. First, the *radC* strain consistently showed slightly more DNA near the top of the gradient than did the wild-type strain (e.g., Fig. 2a), which suggests

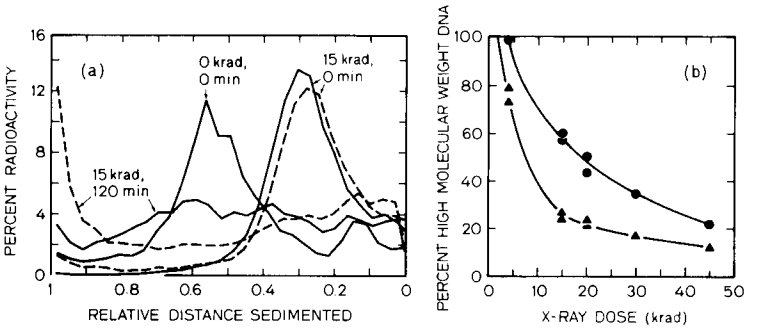

FIG. 2. Kinetics of repair of X-ray-induced DNA double-strand breaks in wild-type and *radC* strains of *E. coli*. (a) The DNA profiles are from neutral sucrose gradients for X-irradiated cells incubated in growth medium at 37°C (dose and incubation times indicated): (solid lines) wild type (SR1269), and (broken lines) *radC* (SR1271). Data are representative of duplicate experiments. (b) The percentage of the DNA that sedimented at a relative distance of 0.5–0.7 after 120 min of repair incubation [see (a)] was plotted as a percentage of the nonirradiated DNA that sedimented in that interval. Each point represents data from a single experiment of the type shown in (a). (●) Wild type (SR1269), and (▲) *radC* (SR1271).

a small deficiency in the rejoining of DSB. Second, the *radC* strain consistently appeared to be slower in the coversion of fast-sedimenting DNA to DNA that sediments like nonirradiated DNA (e.g., Fig. 2a). This latter difference in the two strains was quantitated for graded doses of X rays by comparing the percentage of X-irradiated DNA sedimenting like nonirradiated DNA after 120 min of repair incubation (Fig. 2b). After each dose of X rays, there was a lower percentage of high molecular weight DNA reformed in the *radC* strain relative to the wild-type strain.

The conversion of slow-sedimenting DNA to fast-sedimenting DNA seems to indicate the formation of a DNA structure that is part of the process for the repair of DSB. Certainly its presence in irradiated wild-type cells and its absence in irradiated *recA* cells (*9*) is consistent with this notion. This fast-sedimenting DNA has been suggested to be large, unresolved, DNA-recombination intermediates or DNA complexed with other cellular components (*7, 9, 10*), and there is some evidence in *Micrococcus radiodurans* (*7*) that it represents a DNA–membrane complex. Thus one interpretation of our data is that the *radC* strain maintains the fast-sedimenting DNA structure longer than the wild-type strain because it is less efficient than the wild-type strain in the rejoining of DSB. Alternately, the *radC* gene product may be directly involved in the conversion of fast-sedimenting DNA to normally sedimenting DNA.

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