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Role of the *radB* gene in postreplication repair in UV-irradiated *Escherichia coli uvrB*

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Summary

In UV-irradiated *Escherichia coli*, the *radB101* mutation sensitized *uvrB recF* cells 4-fold and *uvrB recB* cells 1.2-fold, but did not sensitize *uvrB recB recF* cells. The *radB* mutation had very little effect (1.2-fold or less) on the repair of UV radiation-induced DNA daughter-strand gaps in *uvrB* cells, but it did cause about a 3-fold deficiency in the repair of the DNA double-strand breaks that arise in association with nonrepaired daughter-strand gaps in UV-irradiated *uvrB recF* cells. Thus, the *radB* gene does not appear to be involved in the *recF*-dependent or *recF recB*-independent processes for the repair of DNA daughter-strand gaps, but is involved in the *recB*-dependent postreplication repair of DNA double-strand breaks.

The *radB101* mutation is similar to the *recB21* mutation in that it sensitizes *Escherichia coli* K-12 cells to γ-radiation slightly more than it sensitizes to UV radiation; all other mutations tested (*polA*, *recF*, *lexA*, *recA*, *uvrD*, and *uvrA*) sensitize cells much more to UV radiation (Sargentini and Smith, 1983). The *radB* cells are slightly deficient in recombination ability, and show a slight increase in radiation-induced DNA degradation. The fact that the *radB* mutation sensitizes a *uvrB* strain to UV radiation, but not a *recA* strain, suggests that the *radB* gene product plays a role in postreplication repair (Sargentini and Smith, 1983).

Postreplication repair in UV-irradiated *uvrA* or *uvrB* cells occurs by two major pathways, *recF*-dependent and *recB*-dependent (Rothman et al., 1975; Wang and Smith, 1981). The *recF*-dependent pathway is responsible for the majority of the repair of DNA daughter-strand gaps, i.e., the gaps that are formed when the replication fork encounters noncoding lesions in the parental strand (Ganesan and Seawell, 1975; Rothman and Clark, 1977; Kato, 1977; Wang and Smith, 1983). In contrast, the *recB*-dependent pathway is responsible for the repair and/or segregation of the DNA double-strand breaks that arise in association with nonrepaired daughter-strand gaps (Wang and Smith, 1983, 1986). These two pathways of postreplication repair are of about equal importance in terms of UV radiation survival (Wang and Smith, 1983).

Besides these two major pathways of postreplication repair, there is also a minor pathway (*recF recB*-independent) that repairs a small number of DNA daughter-strand gaps, and accounts for the fact that a *uvrB recF recB* strain is somewhat more resistant to UV radiation than is a *uvrB recA* strain (Wang and Smith, 1983; Sharma and Smith, 1985).

In order to understand how the *radB* gene

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functions in postreplication repair, we have determined whether the *radB* mutation sensitizes *uvrB recF*, *uvrB recB*, and *uvrB recF recB* cells to UV radiation, and whether the *radB* mutation affects the repair of DNA daughter-strand gaps and double-strand breaks in UV-irradiated cells.

Materials and methods

Bacteria and media

Strains used are listed in Table 1. Bacteriophage P1 transductions were performed generally as described by Miller (1972). SMM was a 0.4% glucose–salts medium (Ganesan and Smith, 1968), supplemented with thiamine · HCl at 0.5 μg/ml, thymine at 10 μg/ml, D-biotin at 1 μg/ml, and L-leucine and L-methionine at 1 mM. DTM was the salts portion of this medium. Cells were plated on SMM solidified with Noble agar (Difco) at 1.6%. PB was Na_2HPO_4 at 5.83 g/l and KH_2PO_4 at 3.53 g/l, pH 7.0.

Preparation and irradiation of cells

Cells were grown overnight in SMM, diluted 1 : 50 with fresh SMM, but with thymine at 2 μg/ml, and they were shaken at 37°C to an optical density at 650 nm (OD_{650}) of 0.4 (Zeiss PMQ II spectrophotometer), which corresponded to 3×10^8 colony-forming units (CFU)/ml. Cells were harvested on membrane filters, washed and resuspended in DTM (PB for cell survival studies) at an OD_{650} of 0.2. UV irradiation (254 nm) was as previously described (Sargentini and Smith, 1979).

Alkaline sucrose gradients

After UV irradiation, cells were added to an equal volume of warm, concentrated medium to reproduce SMM, but with thymine at 2 μg/ml

TABLE 1

STRAINS OF *E. coli* K-12 USED

Stanford Radiology No.	Genotype [a]	Source or derivation [b]
SR255	*argE3 hisG4 leuB6 Δ(gpt-proA) 62 thr-1 deoB16 thi-1 ara-14 galK2 lacY1 mtl-1 xyl-5 tsx-33 rpsL31 supE44 recB21*	Youngs and Smith, 1976
SR256	*leuB19 metE70 deo(C2?) lacZ53 rha-5 rpsL151 uvrB5 recA56*	Youngs and Smith, 1976
SR772	Same as SR256, but *thyA*	SR256, Tmp^r
SR884	Same as SR255, but Mtl$^+$ Xyl$^+$ Sup$^+$ *tna300*::Tn*10 recF143*	JC12334, A.J. Clark
SR1110	*leuB19 metE70 thyA36 deo(C2?) lacZ53 rha-5 rpsL151 uvrB5*	Sargentini and Smith, 1983
SR1111	Same as SR1110, but *radB101*	Sargentini and Smith, 1983
SR1192	Same as SR1110, but Thy$^+$ *recB21*	SR1110 × P1::Tn*9*cts · SR255, Thy$^+$
SR1194	Same as SR1111, but Thy$^+$ *recB21*	SR1111 × P1::Tn*9*cts · SR255, Thy$^+$
SR1196	Same as SR1110, but *tnaA300*::Tn*10 recF143*	SR1110 × P1vir · SR884, Tc^r
SR1198	Same as SR1111, but *tnaA300*::Tn*10 recF143*	SR1111 × P1vir · SR884, Tc^r
SR1203	Same as SR1192, but Met$^+$ *thyA recF143*	Wang and Smith, 1985
SR1490	Same as SR1196, but Thy$^+$ *recB21*	SR1196 × P1::Tn*9*cts · SR255, Thy$^+$
SR1492	Same as SR1198, but Thy$^+$ *recB21*	SR1198 × P1vira · SR255, Thy$^+$

[a] Genotype nomenclature is that used by Bachmann (1983). All strains are F$^-$ and λ$^-$. Tn*10* is an inserted transposon carrying tetracycline resistance.

[b] Tmp^r indicates a trimethoprim-resistant isolate. Thy$^+$ indicates that transductants were prototrophic for thymine. Tc^r indicates that transductants were tetracycline resistant.

and [*Me*-^{3}H]thymidine (Amersham) at 20 μCi/ml. These cells were shaken for 10 min at 37°C, harvested on membrane filters, washed and resuspended in nonradioactive SMM, incubated an additional 2 h, and then 0.3 ml of cells was added to 0.16 ml of spheroplasting solution, before centrifugation and DNA sedimentation analysis as previously described (Sargentini and Smith, 1985). The assumption was made that the UV radiation-induced DNA single-strand breaks remaining after repair incubation constituted nonrepaired daughter-strand gaps.

Neutral sucrose gradients

After UV irradiation, cells were diluted as above and shaken for 10 min at 37°C before the [*Me*-^{3}H]thymidine was added to initiate a 5-min pulse-labeling period (Wang and Smith, 1983). Afterwards, cells were harvested on membrane filters, washed and resuspended in nonradioactive SMM, and shaken at 37°C for up to 6 h. Incubated cells were maintained at an OD_{650} of 0.05–0.4 by dilution, and were diluted to an OD_{650} of 0.05 at the time of adding cells to the spheroplasting solution (as above). After 15 min on ice, 0.1 ml of the spheroplast suspension was layered onto a neutral sucrose (5–20%) gradient (4.8 ml) (Bonura et al., 1975). After standing for at least 90 min at room temperature, the gradients were centrifuged at 3700 rpm for 40 h at 20°C. Gradient fractionation and processing were as above. The complex DNA gradient profiles (see Wang and Smith, 1983) did not allow us to quantitate the numbers of DNA strand breaks, rather, we analyzed these profiles as described by Wang and Smith (1983). For each set of gradients, i.e., made with the same gradient-maker apparatus and centrifuged together, two vertical lines were drawn on the DNA profile for nonirradiated cells. One line corresponded to a relative sedimentation distance of 0.82, while the other line corresponded to a value between 0.51 and 0.55, such that 36% of the total DNA profile was between the two lines. The percent high-molecular-weight DNA (e.g., Fig. 3a) was determined from the fraction of the DNA profile for UV-irradiated cells that was between the two lines determined for the DNA profile for the nonirradiated control cells. The percent reformation of high-molecular-weight DNA (Fig. 3b) was determined from kinetic data for the disappearance and reformation of high-molecular-weight DNA (e.g., Fig. 3a) using the formula $(B - A)$ divided by $(36\% - A)$, where A = the lowest value for the high-molecular-weight DNA in the kinetic experiment, B = the value for the high-molecular-weight DNA at 4.25 h in the kinetic experiment, 36% = the maximum value for high-molecular-weight DNA in the selected 'window' in the gradient profiles (see above).

Results

To predict the role of the *radB* gene in postreplication repair, the effect of the *radB* mutation on UV radiation survival was tested in *uvrB recF*, *uvrB recB*, and *uvrB recB recF* strains. The *radB* mutation sensitized the *uvrB recF* strain 4-fold (Fig. 1a) and the *uvrB recB* strain 1.2-fold (Fig. 1b), but did not sensitize the *uvrB recB recF* strain (Fig. 1c).

To determine the molecular nature of the *radB* deficiency in postreplication repair, the ability of *uvrB radB* cells to repair UV radiation-induced DNA daughter-strand gaps was measured with alkaline sucrose gradients. Relative to the *uvrB* strain, the *uvrB radB* strain showed no deficiency in repair below a UV radiation fluence of 8 J/m^2, and showed only a 1.2-fold deficiency (comparing UV radiation fluences at equal levels of repair) at higher fluences (Fig. 2).

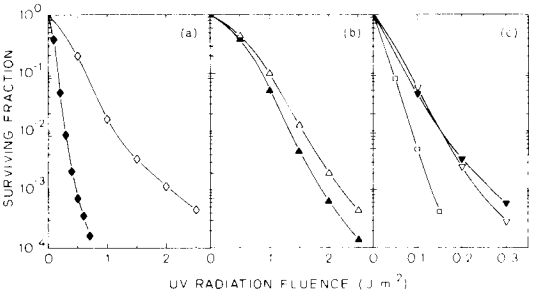

Fig. 1. Effect of the *radB* mutation on the UV radiation survival of *E. coli uvrB recF* (a), *uvrB recB* (b), and *uvrB recF recB* (c) strains. Cells were grown in SMM, UV irradiated and plated on SMM. Data are the means from quadruplicate experiments. ◇, SR1196 (*uvrB recF*); ◆, SR1198 (*uvrB recF radB*; △, SR1192 (*uvrB recB*); ▲, SR1194 (*uvrB recB radB*); ▽, SR1490 (*uvrB recF recB*); ▼, SR1492 (*uvrB recF recB radB*); □, SR256 (*uvrB recA*).

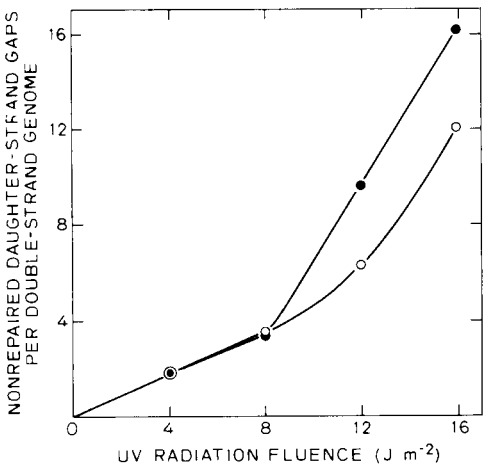

Fig. 2. Effect of the *radB* mutation on the accumulation of nonrepaired UV radiation-induced DNA daughter-strand gaps in *E. coli uvrB*. Cells were UV irradiated, pulse-labeled, then incubated for 120 min in nonradioactive medium before being assayed for nonrepaired daughter-strand gaps on alkaline sucrose gradients. Data are the means from triplicate experiments. ○, SR1110 (*uvrB*); ●, SR1111 (*uvrB radB*).

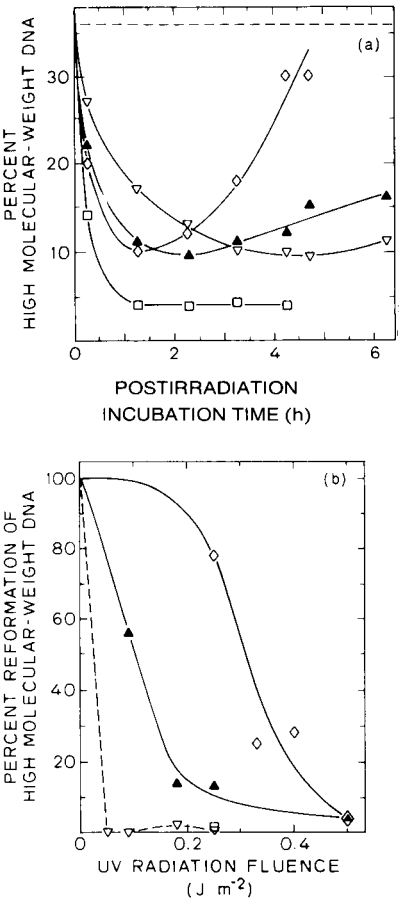

Fig. 3. Effect of *radB* and *recB* mutations on the production of DNA double-strand breaks and the reformation of high-molecular-weight DNA in UV-irradiated *E. coli uvrB recF*. Cells were UV irradiated, pulse-labeled, then incubated in nonradioactive medium before being assayed on neutral sucrose gradients for the percent high-molecular-weight DNA (see Materials and Methods). In a, all cells received 0.25 J/m^2, except the nonirradiated-control samples (------). Data are the means from 3–4 replicate experiments. In b, all UV-irradiated cells were incubated for 4.25 h before being assayed for the percent reformation of high-molecular-weight DNA (see Materials and Methods). Data are the means from 2–4 replicate experiments. ◇, SR1196 (*uvrB recF*); ▲, SR1198 (*uvrB recF radB*); ▽, SR1203 (*uvrB recF recB*); □, SR772 (*uvrB recA*).

We have used neutral sucrose gradient sedimentation to monitor the effect of the *radB* mutation on the repair of DNA double-strand breaks in UV-irradiated *uvrB recF* cells. A useful way to follow the kinetics of the formation of the double-strand breaks and the reformation of high-molecular-weight DNA, is to focus just on the high molecular-weight DNA (see Materials and Methods). As expected from a previous report (Wang and Smith, 1983), the *uvrB recF* strain was proficient in the reformation of high-molecular-weight DNA while the *uvrB recF recB* and *uvrB recA* strains were completely deficient (Fig. 3a). (As previously suggested (Wang and Smith, 1983), the *uvrB recA* strain produces more double-strand breaks than the *uvrB recF recB* strain, because it also lacks the *recF recB*-independent mode as well as the *recF*-dependent mode for the repair of daughter-strand gaps.) The *uvrB recF radB* strain produced double-strand breaks in a fashion like the *uvrB recF* strain, but it resembled the *uvrB recF recB* strain in not being able to reform a substantial amount of high-molecular-weight DNA after a UV radiation fluence of 0.25 J/m^2 (Fig. 3a).

Experiments of the type described in Fig. 3a were run after a graded series of UV radiation fluences in order to compare the abilities of the several strains to reform high-molecular-weight DNA (Fig. 3b). These data substantiate the fact that the *radB* mutant is partially deficient (3-fold, measured at the 50% level of repair) in the postreplication repair of DNA double-strand breaks.

Discussion

We have used survival assays to predict the pathway(s) of postreplication repair in which the *radB* gene functions. Since the *radB* mutation sensitized the *uvrB recF* strain substantially more than it sensitized the *uvrB recB* strain (4-fold versus 1.2-fold; cf., Fig. 1a and b), the *radB* gene appears to function in the *recB*-dependent pathway and not in the *recF*-dependent pathway of postreplication repair. Similarly, the lack of sensitization of a *uvrB recF recB* strain (Fig. 1c) argues that the *radB* gene does not function in the *recF recB*-independent pathway of postreplication repair.

Consistent with the survival data, the *radB* mutation caused little or no deficiency in the repair of DNA daughter-strand gaps (Fig. 2), which is largely controlled by the *recF* gene (Ganesan and Seawell, 1975; Rothman and Clark, 1977; Kato, 1977; Wang and Smith, 1983). However, the *radB* mutation did cause a deficiency in the reformation of high-molecular-weight DNA (Fig. 3a and b), i.e., the *radB* mutation caused a deficiency in the postreplication repair of DNA double-strand breaks, which is controlled by the *recB* gene (Wang and Smith, 1983). The deficiency in the repair of double-strand breaks caused by the *radB* mutation in the *uvrB recF* strain was not quite as severe as that caused by the *recB* mutation (Fig. 3b), and probably explains the smaller sensitization to UV radiation caused by the *radB* mutation compared to the *recB* mutation in *uvrB recF* cells (cf., Fig. 1a and c).

It is relevant to note that the *recN* gene plays a role in the repair of ionizing radiation-induced DNA double-strand breaks (Picksley et al., 1984), and that it maps to essentially the same locus as the *radB* gene (Sargentini and Smith, 1983, and data not shown; Lloyd et al., 1983; Picksley et al., 1984). Thus, the *radB* and *recN* mutations may be alleles of the same gene.

According to the sister-duplex-recombination model for *recB*-dependent postreplication repair, a gene that functions in this process could be involved in the scission of DNA at a daughter-strand gap to form a double-strand break, or it could be involved in the recombination process that reforms high-molecular-weight DNA (Wang and Smith, 1983, 1986). Since the *recB* mutation did cause a slower rate of DNA double-strand break formation (Fig. 3a), this suggests that one of the functions of the *recB* gene could be a partial role in producing double-strand breaks at nonrepaired daughter-strand gaps. Such a role is consistent with the enzyme's known ability to degrade duplex DNA circles when they contain single-stranded gaps of 5 nucleotides or more, but not when they are closed or nicked (Karu et al., 1973). Since the rate of formation of DNA double-strand breaks at nonrepaired daughter-strand gaps did not seem to be altered by the *radB* mutation (Fig. 3a), the *radB* gene probably does not function in the formation of DNA double-strand breaks, but functions in their repair. Consistent with this conclusion and the sister-duplex-recombination model for postreplication repair is the fact that a *radB* strain shows a 60% deficiency in recombination (conjugation assay; Sargentini and Smith, 1983).

If the *radB* mutation is not leaky, our work suggests that the *radB* gene plays a partial role in the postreplication repair of DNA double-strand breaks in UV-irradiated cells.

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