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## SHORT COMMUNICATIONS

# A mutation (*radA100*) in *Escherichia coli* that selectively sensitizes cells grown in rich medium to X- or U.V.-radiation, or methyl methanesulphonate

WILLIAM P. DIVER†, NEIL J. SARGENTINI‡, and KENDRIC C. SMITH

Department of Radiology, Stanford University School of Medicine, Stanford, California 94305, U.S.A.

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## 1. Introduction

A major factor in the rapid accumulation of knowledge on the repair of radiation-damaged DNA has been the availability of DNA repair-deficient mutants of *Escherichia coli* (reviewed in Hanawalt *et al.* 1979). In order to further our studies on the repair of X-ray-induced DNA damage, we have undertaken a programme of isolating new X-ray-sensitive mutants of *E. coli*. This report describes the isolation and characterization of one such mutant, and the map location of its mutation (*radA100*).

## 2. Materials and methods

### 2.1. *Bacterial strains*

The strains of *E. coli* used are listed in table 1. Transductions and conjugations were accomplished generally as described by Miller (1972).

### 2.2. *Media*

SMM was a 0·4 per cent glucose-salts medium (Ganesan and Smith 1968), supplemented with required L-amino acids at 1 mM and thiamine·HCl at 0·5 $\mu$g/ml. SMM was solidified by adding Noble agar (Difco) at 1·6 per cent. YENB was yeast extract (Difco) at 0·75 per cent and nutrient broth (Difco) at 0·8 per cent. YENB agar was yeast extract at 0·75 per cent and nutrient agar (Difco) at 2·3 per cent. PB was $Na_2HPO_4$ at 5·83 g/l and $KH_2PO_4$ at 3·53 g/l, pH 7·0. LBP was tryptone (Difco) at 1 per cent, yeast extract (Difco) at 0·5 per cent, NaCl at 1 per cent, and 0·01 M PB. LBG was LBP without phosphate, but containing glucose at 0·2 per cent. Selection media used for transductions and conjugations were SMM lacking the appropriate amino acid or SMM containing 0·4 per cent L-arabinose in place of glucose.

### 2.3. *Procedures*

The culturing of cells, irradiation, methyl methanesulphonate (MMS) treatment, survival determination and measurement of DNA single-strand breaks are described in Sargentini *et al.* (1982). The technique for mutagenesis with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine is described in Sargentini and Smith (1982).

† Present address: Department of Bacteriology, The Medical School, University of Bristol, Bristol BS8 1TD, England.

‡ Offprint requests to N. J. Sargentini.

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| Stanford radiology number | Genotype† | Source or derivation‡ |
|---|---|---|
| SR36 | F⁻ *argE3 his-4 leuB6 proA2 thr-1 ara-14 galK2 lacY1 mtl-1 xyl-5 thi-1 tsx-33 rpsL31 supE44* $\lambda^-$ | AB1157, A. J. Clark |
| SR191 | F⁺? *metE thyA36 deoC(2?) lacZ53 rpsL151* $\lambda^-$ | DY98 (Youngs and Smith 1973) |
| SR248 | F⁻ *leuB19 metE70 thyA36 deoC(2?) lacZ53 malB45 rha-5 bioA2 rpsL151* $\lambda^-$ | KH21, R. B. Helling |
| SR327 | As SR36, but Δ(*xth-pnc*) | BW9109, B. Weiss |
| SR359 | Hfr Cavalli *metA mel* | S108, R. Schmitt |
| SR380 | Hfr R4 *argF58 argI61 serB28 thr-25 purA54 tonA49* | PCO950, ECGSC |
| SR702 | As SR36, but *thr*⁺ *radA100* | SR36 × P1kc·SR791, select Thr⁺ |
| SR707 | F⁻ *argF58 his-4 thr-25 serB28 galK2 lacY1 mtl-1 xyl-5 thi-1 tsx-33 tonA49 rpsL31* $\lambda^-$ | SR36 × SR380, select Pro⁺ |
| SR749 | Same as SR36 | AB1157, ECGSC |
| SR750 | F⁻ *argE3 his-4 leuB6 metE70 proA2 thr-1 ara-14 galK2 lacY1 mtl-1 rha-6 xyl-5 thi-1 tsx-33 rpsL31 supE44 xthA14* $\lambda^-$ | NH5016, ECGSC (described in Ljungquist *et al.* 1976) |
| SR774 | F⁻ *argF58 his-4 galK2 lacY1 mtl-1 xyl-5 thi-1 tsx-33 tonA49 rpsL31 radA100* $\lambda^-$ | SR707 × P1kc·SR702, select Thr⁺ |
| SR776 | As SR749, but *thr*⁺, *radA100* | SR749 × P1kc·SR774, select Thr⁺ |
| SR777 | As SR749, but *thr*⁺ | Same as SR776 |
| SR791 | As SR750, but *radA100* | SR317, MNNG |
| SR890 | As SR248, but Thy⁺ | SR248 × P1kc·SR327, select Thy⁺ |
| SR891 | F⁻ *metE70 thr-25 deoC(2?) lacZ53 malB45 rha-5 bioA2 rpsL151* $\lambda^-$ | SR890 × SR380, select Leu⁺ |

† Genotype nomenclature is that used by Bachmann and Low (1980). Strains SR317, SR702, and SR791 seem to possess a defect, mapping near *serB*, that does not allow them to grow at 42°C.

‡ ECGSC is the *E. coli* Genetic Stock Center. MNNG indicates that the strain was mutagenized with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. Thr⁺, Pro⁺, Thy⁺, and Leu⁺ indicate that threonine, proline, thymine or leucine, respectively, were no longer required for growth.

Table 1. Strains of *E. coli* K-12 used.

## 3. Results

The rationale for mutagenizing an *xthA* strain (SR750) in our search for new radiation sensitive mutants has been presented by Sargentini and Smith (1982). Strain SR791 is a mutant of strain SR750 that was originally selected for its ionizing radiation sensitivity relative to its parent (figure 1). Note that strain SR749 is the wild-type ancestor of strain SR750 (*xthA14*).

Mapping of the radiation-sensitivity marker(s) in strain SR791 began with mating it with an Hfr Cavalli derivative (SR359). After selecting for various donor markers, the exconjugants were screened for gamma-radiation sensitivity (stationary phase,

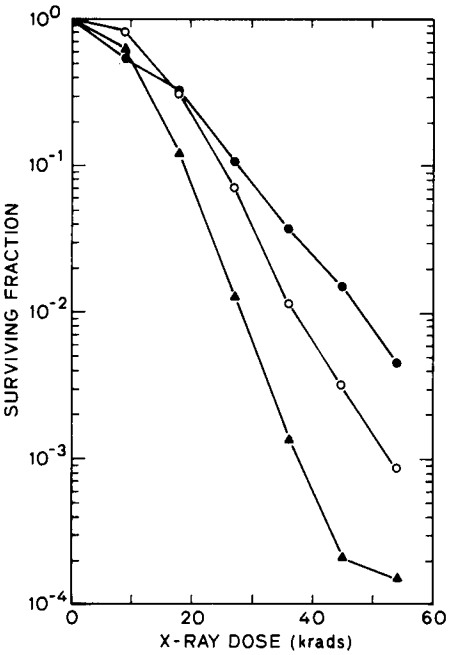


Figure 1. X-ray survival of wild-type, *xthA*, and *xthA radA* derivatives of *E. coli* K-12. Cells were grown to logarithmic phase in YENB, X-irradiated (50 kVp) in buffer, and plated on YENB. Symbols: wild type (SR749) (●), *xthA14* (SR750) (○), and *xthA14 radA100* (SR791) (▲). Data are the means of three experiments per point.

LBG-grown cells, 40 krad, YENB plates; data not shown). Since 73 of 100 exconjugants selected for ability to utilize arabinose ($Ara^+$) also showed wild-type radiation resistance, a radiation-sensitizing mutation in strain SR791 appeared to be closely linked with the *ara* locus at about 1 min on the linkage map for the *E. coli* K-12 chromosome (Bachmann and Low 1980). This mutation was designated *radA100* (for *rad*iation resistance).

Subsequent mapping of the *radA* locus was performed using bacteriophage P1kc-mediated transduction. Strain SR791 (*xthA14 radA100*) was transduced (P1kc·SR191) to $Ara^+$ or to leucine or threonine prototrophy ($Leu^+$ or $Thr^+$, respectively). None of 50 $Ara^+$ or 50 $Leu^+$ transductants became gamma-radiation resistant, but 25 of 50 $Thr^+$ transductants did (data not shown). This was consistent with the *radA* locus being located between 99 and 1 min (i.e., a 2 map-unit interval) on the *E. coli* chromosome linkage map.

As a purification procedure, the *radA100* mutation was transduced along with $Thr^+$ into a wild-type strain (SR36, $xth^+$) to yield strain SR702 (*radA100*). The mutation was then further mapped by transduction (P1kc·SR702) using a *thr-1 serB* wild-type strain (SR707) as the recipient. When transductants from this cross were selected for threonine prototrophy, 66 of 77 (86 per cent) became $Ser^+$ (prototrophic for serine) while only 35 of 77 (44 per cent) became gamma-radiation sensitive (data not shown). These linkage data, in addition to the fact that all 35 of the transductants that incorporated the donor gamma-radiation sensitivity character also incorporated the $Ser^+$ donor character, indicated that the most likely map order was: *thr serB radA*. At this time, it was noted that strain SR702 (*radA100*) was unable to grow well

| Deo$^+$ transductant phenotype† | | | Fraction of total Deo$^+$ transductants |
|---|---|---|---|
| Deo | RadA | Thr | |
| +(D)‡ | +(R) | −(R) | 13/44 |
| +(D) | −(D) | −(R) | 10/44 |
| +(D) | −(D) | +(D) | 19/44 |
| +(D) | +(R) | +(D) | 2/44 |

† Deo: ability (+/−) to utilize thymidine (0·1 per cent) as a sole carbon source; RadA: ability of stationary phase cells grown in YENB+1 per cent glucose and 0·01 M $NaH_2PO_4$ (adjusted to pH 7·4) and diluted 100-fold in PB to survive 65 krad of $^{137}Cs$ gamma radiation (when plating 0·05 ml of undiluted PB suspension on YENB; + = ~1200 colonies, − = ~265 colonies); Thr: ability (+/−) to grow on minimal medium not containing threonine.

‡ D = donor phenotype, R = recipient phenotype.

Table 2. Phenotype of Deo$^+$ transductants from SR891 × P1::Tn*9cts*·SR776. Recipient, SR891 [*deoC(2?) radA*$^+$ *thr-25*]; Donor, SR776 (*deoC*$^+$ *radA100 thr*$^+$).

at 42°C and, in fact, this was also a characteristic of the mutagenized strain, SR750 (data not shown). Furthermore, the temperature-sensitive defect seemed to map near *serB* (data not shown).

One Thr$^+$ transductant of SR707 (described above) that incorporated the *radA100* allele, but not the temperature-sensitive defect (strain SR774), was used as a donor for the *radA100* allele in making strains SR776 (*radA100*) and SR777 (*radA*$^+$ cotransductant with SR776). Strain SR776 was then used as a donor to further define the *radA100* map position and to check that the presence of the temperature-sensitive defect in the previous experiments did not affect the mapping results. The data in table 2, in conjunction with those described above, are most consistent with the map order: *thr serB radA deoC*.

Strains SR777 and SR776 (*radA100*) were compared for sensitivity to DNA-damaging agents using both YENB-grown and SMM-grown cells in order to test for the role of the *radA* gene in medium-dependent resistance (MDR)(Sargentini *et al.* 1982), i.e., the *radA* mutation would sensitize the rich medium-grown logarithmic phase cells more than the minimal medium-grown logarithmic phase cells. The *radA100* mutation conferred sensitivity to X-radiation under air (figure 2 (*a*)) and $N_2$ (data not shown) for logarithmic phase cells grown in and plated on YENB, but not for cells grown in and plated on SMM. The oxygen enhancement ratio (calculated here as the dose killing 98 per cent of the cells ($D_2$) irradiated under $N_2$ divided by the $D_2$ for the cells irradiated under air) for both the *radA* and the wild-type strains was 2·3 for YENB-grown cells and 3·0 for SMM-grown cells (data not shown). The *radA* mutant was also sensitive to U.V. radiation (figure 2 (*b*)) and MMS (figure 2 (*c*)) when grown in and plated on YENB, but not when grown in and plated on SMM; the YENB-grown *radA* cells showed survival levels similar to those for SMM-grown cells.

The wild-type and *radA100* strains were also compared for their ability to demonstrate glucose-induced resistance (GIR) (Friesen *et al.* 1970), i.e., a glucose supplement enhanced the survival of rich medium-grown stationary phase wild-type cells. The results for X-irradiated stationary phase LBP+glucose-grown versus LBP-grown cells (figure 3) were qualitatively similar to those for X-irradiated

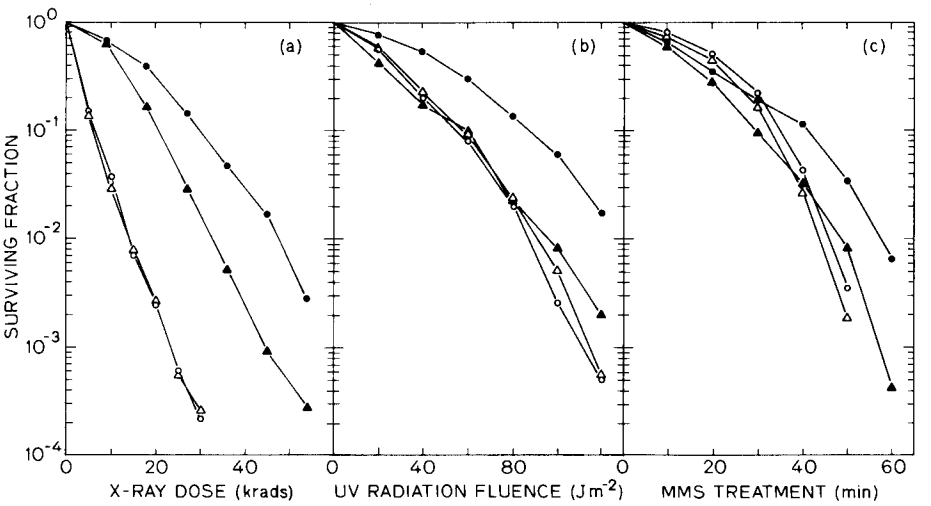


Figure 2. Medium-dependent resistance of logarithmic phase wild-type and *radA* derivatives of *E. coli* K-12 after treatment with: (*a*) 50 kVp X-rays, (*b*) 254 nm U.V.-radiation, or (*c*) 0·05 M methyl methanesulphonate. Cells were grown in SMM (open symbols) or YENB (closed symbols), treated in buffer, and plated on SMM or YENB, respectively. Symbols: wild type (SR777) (○, ●), *radA100* (SR776) (△, ▲). Data are the means of three experiments per point.

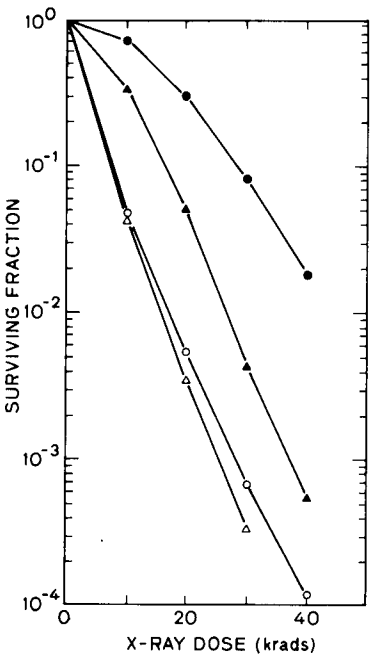


Figure 3. Glucose-induced resistance to X-radiation of stationary phase wild-type and *radA* derivatives of *E. coli* K-12. Cells were grown in LBP with (closed symbols) or without (open symbols) 1 per cent glucose, X-irradiated (50 kVp) in buffer, and plated on YENB. Symbols: wild type (SR777) (●, ○), *radA100* (SR776) (▲, △). Data are the means of two experiments per point.

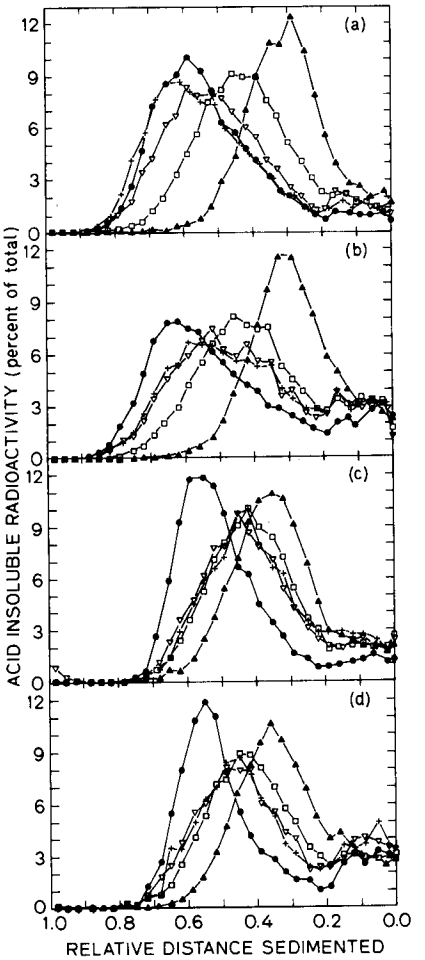


Figure 4. Repair of X-ray-induced DNA single-strand breaks in wild-type and *radA* derivatives of *E. coli* K-12. Cells were grown to logarithmic phase in YENB ((*a*) wild type (SR777), (*b*) *radA100* (SR776)) or SMM ((*c*) wild type (SR777), (*d*) *radA100* (SR776)), containing [methyl-$^3$H]thymidine before being X-irradiated (30 krad) in buffer, and incubated at 37°C in nonradioactive homologous medium. Before being sampled (constant volume) for the determination of unrepaired DNA single-strand breaks, nonirradiated cells were incubated for 120 min (●). Irradiated cells were incubated for (▲) 0, (□) 30, (▽) 90 or (+) 120 min. The 0 min incubation data are for cells diluted into post-treatment media, and then mixed and sampled within 10 s. Total radioactivity (counts per min) per akaline sucrose gradient for the 120 min samples were: (*a*) 0 krad, 4747; 30 krad, 3895 (*b*) 0 krad, 4804; 30 krad, 3623 (*c*) 0 krad, 3704; 30 krad, 842 (*d*) 0 krad, 2251; 30 krad, 980. Data are from one of two experiments with similar results.

logarithmic phase YENB-grown versus SMM-grown cells (c.f. figures 2 (*a*) and 3) indicating a role for the *radA* gene in GIR.

The results for the X-ray survival of logarithmic phase cells were correlated with a defect in the repair of X-ray-induced DNA single-strand breaks. That is, when the wild-type and *radA* strains were grown in YENB before and after X-irradiation with 30 krad, the wild-type strain showed complete repair, while the *radA* mutant showed

incomplete repair (c.f. figure 4 (*a*) and (*b*)). When these strains were grown in SMM before and after X-irradiation, neither strain showed complete repair, and the extent of repair appeared to be similar for both strains (c.f. figures 4 (*c*) and (*d*)).

## 4. Discussion

The *radA100* mutation sensitized the wild-type strain 1·5-fold, while it sensitized the *xthA14* strain 1·3-fold (calculated as the ratio of $D_2$ values) (c.f. figures 1 and 2 *a*)). Thus, there turned out to be no distinct advantage in isolating the *radA* mutation in the *xthA* strain as opposed to a wild-type strain.

The *radA100* mutation sensitized logarithmic phase YENB-grown wild-type cells 1·5-fold to X-rays (figure 2 (*a*)), 1·5-fold to U.V-radiation (figure 2 (*b*)), and 1·2-fold to MMS (figure 2 (*c*)). However, little or no sensitization by the *radA* mutation was observed for logarithmic phase SMM-grown cells (figures 2 (*a*), (*b*) and (*c*)). Such data indicate that the *radA* mutant is deficient in MDR, or more precisely, *recA lexA*-dependent inducible DNA repair (Sargentini *et al.* 1982). This deficiency in MDR is consistent with the *radA* strain's deficiency in GIR (figure 3), since MDR and GIR have been shown to be similar phenomena (Sargentini *et al.* 1982).

MDR after X-irradiation may be more complex than after U.V.-irradiation, because the *radA* mutant was only partially deficient in MDR after X-irradiation (figure 2 (*a*)), but it was completely deficient in MDR after U.V.-irradiation (figure 2 (*b*)). Thus, YENB-grown cells relative to SMM-grown cells seem to have more repair processes that can deal with X-ray-induced damage, but only one of these (*radA*-dependent) appears to deal with U.V. radiation-induced damage.

GIR is blocked by *recA*, *recB*, and *lexA* mutations (Friesen *et al.* 1970). These mutations, which are known to inhibit the repair of damaged DNA, also sensitize logarithmic phase SMM-grown cells to X- and U.V.-radiation (e.g., Van der Schueren *et al.* 1977, Tang and Smith 1980). Thus, *radA100* is a novel mutation in that it inhibits GIR (figure 3) and also MDR (figures 2 (*a*) and (*b*)), but it does not sensitize logarithmic phase SMM-grown cells to X- or U.V.-radiation (figures 2 (*a*) and (*b*)).

In conclusion, a new gene, *radA*, has been discovered in *E. coli* and shown to affect, in a novel way, cell survival after treatment with X- or U.V.-radiation or MMS, and DNA repair after X-irradiation. Also, the survival characteristics of the *radA* mutant suggest that there are two or more *recA lexA*-dependent, inducible DNA repair processes that are involved in X-ray survival for logarithmic phase rich-medium-grown and plated cells, but only one of these processes is involved in U.V.-radiation survival.

## 5. Summary

The *radA100* mutant was isolated from *Escherichia coli* K-12 after mutagenesis with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine and selection for gamma radiation sensitivity. The *radA* gene is located between the *serB* and *deoC* loci (at 99·6 min) on the *E. coli* K-12 linkage map. The *radA100* mutation sensitized stationary phase cells to X-rays if they had been grown in glucose-supplemented rich medium, but not if they had been grown in nonsupplemented rich medium (indicating a defect in glucose-induced resistance). Similarly, logarithmic phase cells were sensitized to X-rays, U.V. radiation and methyl methanesulphonate if they had been grown in rich medium, but not if they had been grown in minimal medium (indicating a defect

in medium-dependent resistance). Relative to the wild-type strain, the *radA100* mutant was deficient in the repair of X-ray-induced DNA single-strand breaks when grown to logarithmic phase in rich medium, but not when grown in minimal medium. This is a novel mutation amongst the known DNA repair defects in that it did not sensitize logarithmic phase cells, grown in minimal medium, to either X- or U.V.-radiation.

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**References**

BACHMANN, B. J., and LOW, K. B., 1980, *Microbiol. Rev.*, **44,** 1.

FRIESEN, B. S., IYER, P. S., BAPTIST, J. E., MEYN, R., and RODGERS, J.-M., 1970, *Int. J. Radiat. Biol.*, **18,** 159.

GANESAN, A. K., and SMITH, K. C., 1968, *J. Bacteriol.*, **96,** 365.

HANAWALT, P. C., COOPER, P. K., GANESAN, A. K., and SMITH, C. A., 1979, *A. Rev. Biochem.*, **48,** 783.

LJUNGQUIST, S., LINDAHL, T., and HOWARD-FLANDERS, P., 1976, *J. Bacteriol.*, **126,** 646.

MILLER, J. H., 1972, Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

SARGENTINI, N. J., DIVER, W. P., and SMITH, K. C., 1982, *Radiat Res.*, (in the press).

SARGENTINI, N. J., and SMITH, K. C., 1982, *Radiat. Res.*, (in the press).

VAN DER SCHUEREN, E., YOUNGS, D. A., and SMITH, K. C., 1977, *Int. J. Radiat. Biol.*, **31,** 507.

YOUNGS, D. A., and SMITH, K. C., 1973, *J. Bacteriol.*, **114,** 121.