B41

Cellular Responses to DNA Damage, pages 409–416

Escherichia coli K-12 *radC102*: ISOLATION, CHARACTERIZATION AND INTERACTION WITH DIFFERENT MUTATIONS[1]

Israel Felzenszwalb [2], Neil J. Sargentini and Kendric C. Smith

Stanford University School of Medicine
Department of Radiology
Stanford, California 94305

A new radiation-sensitive mutant (*radC102*) was isolated in *Escherichia coli* K-12. The *radC* gene is located at 81.0 min on the linkage map. The *radC* mutant showed normal radiation-induced DNA degradation, host cell reactivation and gamma radiation mutagenesis, but was partially deficient in UV radiation mutagenesis and recombination ability. The *radC* gene is suggested to play a role in *recA* gene-dependent DNA repair after X irradiation, and in postreplication repair after UV irradiation, since the *radC* mutation did not sensitize a *recA* strain, but did sensitize a *polA* strain to X and UV radiation, and a *uvrA* strain to UV radiation.

INTRODUCTION

Recognizing that the rapid accumulation of knowledge concerning DNA repair has come from studying DNA repair-

[1]This work was supported by Public Health Service research grant CA-06437 and research program grant CA-10372 from the National Cancer Institute. One of us (I.F.) was supported by funds from the International Atomic Energy Agency, BRA/8101 and the Conselho Nacional de Desenvolimento Cientifico e Tecnologico, 200018-81BF.

[2]On leave from: Instituto de Biologia, Universidade do Estado do Rio de Janeiro, 20551 Rio de Janeiro, Brazil.

deficient mutants of Escherichia coli, our laboratory has embarked on a program to isolate new X-ray sensitive mutants of E. coli in the hope of discovering new pathways of DNA repair and of better characterizing known pathways. These mutants have been designated rad (for radiation resistance). Two of these mutations have been characterized; radA100, mapping at 99.6 min (1), and radB101, mapping at 56.5 min (2). The present work characterizes a third mutant strain, radC102.

ISOLATION AND CHARACTERIZATION

The xthA strain, SR750, was mutagenized with N-methyl-N'-nitro-N-nitrosoguanidine (2), and a radiation-sensitive derivative was isolated (SR941). Hfr and preliminary transductional mapping indicated that a radiation-sensitizing mutation was located near the cysE and pyrE loci. Based on these data, strain SR1179 (cysE pyrE) was transduced with P1vira propagated on strain SR941. The cotransduction frequencies obtained with the pyrE (81%) and cysE (65%) markers were used to calculate that the radiation resistance gene (called radC) is at 81.0 min on the linkage map (3).

The radC mutation (called radC102) was removed from the xthA background by transducing it into a pyrE strain to produce two new strains, SR1186 (wild type) and SR1187 (radC). Relative to the wild-type strain, the radC mutant was sensitive to X rays (FIG. 1a) and to UV radiation (FIG. 1b) when grown in and plated on rich medium, but was not sensitive to X rays (FIG. 1a), and showed less sensitization to UV radiation (FIG. 1b) if grown in and plated on minimal medium. It was also observed that the radC mutation sensitizes cells slightly more to O_2-dependent lesions than to O_2-independent lesions produced in DNA by X-rays (FIG. 1a).

Since DNA repair mechanisms can exhibit both error-free and error-prone modes (4,5), the radC mutant was tested for gamma and UV radiation mutagenesis. The radC mutant was normal for gamma radiation mutagenesis whether irradiated under oxic or anoxic conditions (data not shown), but showed less UV radiation mutagenesis than the wild-type strain (TABLE 1).

Radiation sensitive mutants can exhibit a deficiency in recombination ability (6), and show abnormal radiation-induced DNA degradation (7,8). The recombination ability

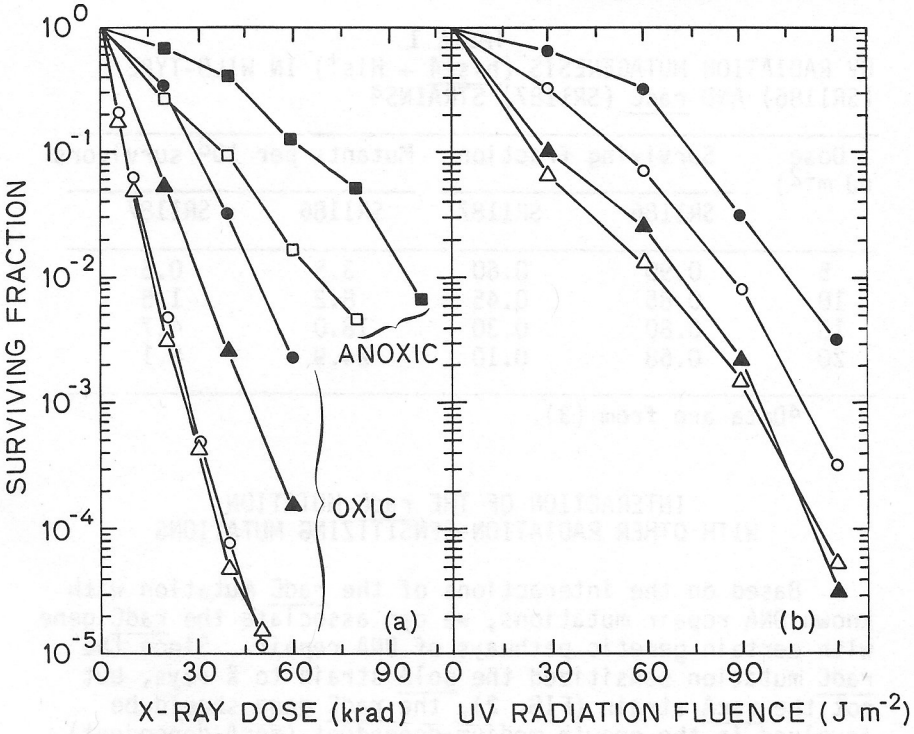

FIGURE 1. Radiation survival of wild-type and radC cotransductant strains of E. coli. Cells grown to logarithmic phase in yeast extract-nutrient broth (YENB) or glucose-salts medium, supplemented with required amino acids and thiamine (SMM) were treated with X (a) or UV (b) radiation before plating on YENB or SMM, respectively. X-irradiation was either oxic (air) or anoxic (N_2). Symbols: wild-type on YENB (●,■), SMM (o); radC on YENB (▲, □), SMM (△). Data are from (3).

of the radC mutant and its isogenic wild-type strain was measured by conjugation. Two Hfr strains were used and 5 recombinant markers were selected. The radC mutant was found to be 60% deficient in recombination ability regardless of which of the 5 markers was selected (data not shown). DNA degradation in UV or gamma irradiated radC cells was the same as in similarly irradiated wild-type cells (data not shown).

TABLE 1
UV RADIATION MUTAGENESIS (*his-4* → His^+) IN WILD-TYPE (SR1186) AND *radC* (SR1187) STRAINS[a]

Dose ($J\ m^{-2}$)	Surviving fraction		Mutants per 10^8 survivors	
	SR1186	SR1187	SR1186	SR1187
5	0.90	0.60	3.5	0.5
10	0.85	0.45	8.2	1.5
15	0.80	0.30	18.0	4.7
20	0.68	0.10	24.9	8.1

[a]Data are from (3).

INTERACTION OF THE *radC* MUTATION WITH OTHER RADIATION-SENSITIZING MUTATIONS

Based on the interactions of the *radC* mutation with known DNA repair mutations, we can associate the *radC* gene with certain genetic pathways of DNA repair. Since the *radC* mutation sensitized the *polA* strain to X rays, but not the *recA* strain (FIG. 2), the *radC* gene should be involved in the growth medium-dependent (*recA*-dependent) repair of DNA single-strand breaks produced by ionizing radiation (9). Since the *radC* mutation sensitized the *uvrA* strain to UV radiation (FIG. 2) and caused no deficiency in host cell reactivation (data not shown), the *radC* gene product must be involved in postreplication repair. The recombination deficiency described before is consistent with this proposition. The *radC* mutant could also be deficient in UV radiation-induced long patch excision repair, since all of the mutations that inhibit postreplication repair have also been shown (when tested) to inhibit long patch excision repair.

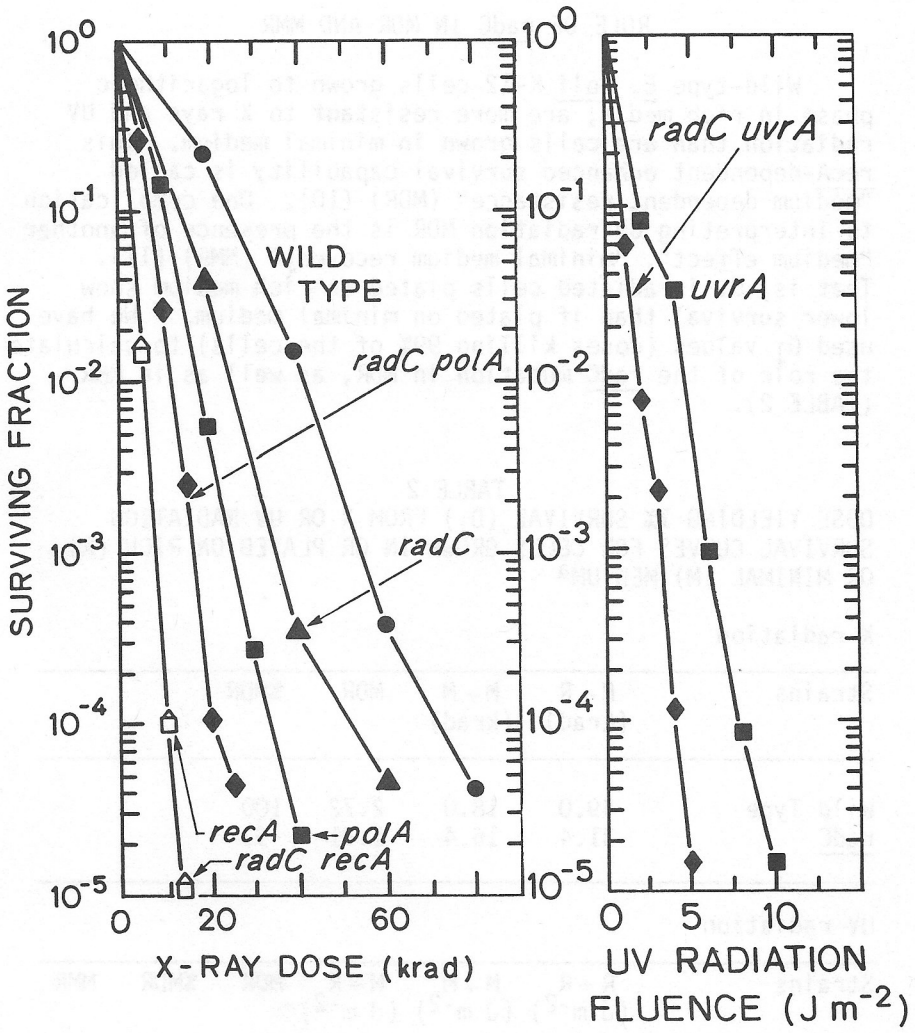

FIGURE 2. X-radiation and UV radiation survival of E. coli strains. Cells were grown to logarithmic phase in and plated on YENB. Data are from (3).

ROLE OF *radC* IN MDR AND MMR

Wild-type *E. coli* K-12 cells grown to logarithmic phase in rich media, are more resistant to X rays and UV radiation than are cells grown in minimal medium. This *recA*-dependent enhanced survival capability is called "medium dependent resistance" (MDR) (10). One complication to interpreting UV radiation MDR is the presence of another "medium effect", "minimal medium recovery" (MMR) (11). That is, UV irradiated cells plated on rich medium show lower survival than if plated on minimal medium. We have used D_1 values (doses killing 99% of the cells) to calculate the role of the *radC* mutation in MDR, as well as in MMR (TABLE 2).

TABLE 2
DOSE YIELDING 1% SURVIVAL (D_1) FROM X OR UV RADIATION SURVIVAL CURVES FOR CELLS GROWN IN OR PLATED ON RICH (R) OR MINIMAL (M) MEDIUM[a]

X radiation

Strains	R→R (krad)	M→M (krad)	MDR	%MDR
Wild Type	49.0	18.0	2.72	100
radC	31.4	16.4	1.91	53

UV radiation

Strains	R→R ($J\ m^{-2}$)	M→M ($J\ m^{-2}$)	M→R ($J\ m^{-2}$)	MDR	%MDR	MMR
Wild Type	104.5	87.6	86.2	1.19	100	1.02
radC	71.4	63.2	48.4	1.13	68[b]	1.31

[a]Data are from (3). Nomenclature: For example, M→R means cells grown in minimal medium and plated on rich medium. Calculations: MDR is the D_1 ratio R→R ÷ M→M. %MDR is the (MDR - 1) value for the strain divided by the (MDR - 1) value for the wild-type strain ; then multiply by 100. MMR is the

D_1 ratio M→M ÷ M→R. A value greater than 1.0 shows MMR.

[b]This value is difficult to interpret because the strain also shows MMR.

The radC mutant shows MMR. Thus, the UV-radiation MDR observed in the radC mutant is actually the result of an antagonistic interaction between the processes causing MMR and MDR. Therefore, UV-radiation MDR for the radC mutant, or for other mutants showing MMR, will be difficult to interpret. On the other hand, the X-ray MDR deficiency observed in the radC mutant (47%, TABLE 2) is suggestive that the mutant will be deficient in Type III repair of X-ray induced single-strand breaks (9).

REFERENCES

1. Diver WP, Sargentini NJ, Smith KC (1982). A mutation (radA100) in Escherichia coli that selectively sensitizes cells grown in rich medium to X- or U.V.-radiation, or methyl methanesulphonate. Int J Radiat Biol 42:339.
2. Sargentini NJ, Smith KC (1983). Characterization of an Escherichia coli mutant (radB101) sensitive to γ and UV radiation, and methyl methanesulfonate. Radiat Res 93:461.
3. Felzenszwalb I, Sargentini NJ, Smith KC (1983). Characterization of a new radiation-sensitive mutant, Escherichia coli K-12 radC102. Radiat Res (Submitted)
4. Witkin EM (1966). Radiation-induced mutations and their repair. Science 152:1324.
5. Witkin EM (1967). Mutation-proof and mutation-prone modes of survival in derivatives of Escherichia coli B differing in sensitivity to ultraviolet light. Brookhaven Symp Biol 20:17.
6. Clark AJ (1973). Recombination deficient mutants of E. coli and other bacteria. Annu Rev Genet 7:67.
7. Howard-Flanders P, Boyce RP (1966). DNA repair and genetic recombination: studies on mutants of Escherichia coli defective in these processes. Radiat Res Suppl 6:156.
8. Ogawa H, Shimada K, Tomizawa J (1968). Studies on radiation sensitive mutants of E. coli. I. Mutants defective in the repair synthesis. Mol Gen Genet 101:227.

9. Town CD, Smith KC, Kaplan HS (1973). Repair of X-ray damage to bacterial DNA. Curr Top Radiat Res Q 8:351.
10. Sargentini NJ, Diver WP, Smith KC (1983). The effect of growth conditions on inducible, recA-dependent resistance to X rays in Escherichia coli. Radiat Res 93:364.
11. Sharma RC, Barfknecht TR, Smith, KC (1982). Postreplication repair in uvrA and uvrB strains of Escherichia coli K-12 is inhibited by rich growth medium. Photochem Photobiol 36:307.