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PROTECTION OF *ESCHERICHIA COLI* CELLS AGAINST THE LETHAL EFFECTS OF ULTRAVIOLET AND X IRRADIATION BY PRIOR X IRRADIATION: A GENETIC AND PHYSIOLOGICAL STUDY

KENDRIC C. SMITH and KLAUS D. MARTIGNONI*

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

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Abstract—When log phase cells of wild-type *E. coli* K-12 were maintained in growth medium after X irradiation, they became progressively more resistant to a subsequent exposure to UV or X radiation. The time to achieve maximum resistance was about 60 min. The *uvrB*, *uvrD*, *polA* and certain *exrA* strains (W3110 background) also demonstrated this X ray-induced resistance to subsequent UV or X irradiation but *recA*, *recB*, *lex* (AB1157 or W3110 backgrounds) and other *exrA* strains (AB1157 background) did not. The resistance induced in wild-type, *uvrB* and *uvrD* cells was characterized by the production or enhancement of a shoulder on the survival curves obtained for the second irradiation, while the resistance induced in the W3110 *exrA* strains was expressed only as a change in slope. The induction of resistance in the W3110 *exrA* strain was not inhibited by the presence of chloramphenicol, but that in the wild-type cells appeared to be. The production or enhancement of a shoulder on the survival curves of the rec^+ lex^+ exr^+ cells is consistent with the concept of the radiation induction of repair enzymes. Alternative explanations, however, are discussed.

INTRODUCTION

Ultraviolet-irradiated bacteriophage shows a higher survival if grown on host cells that have also received a small amount of UV radiation (for a review of earlier work, see Rupert and Harm, 1966). This phenomenon, called UV reactivation, is absent in *recA* and *exr* mutants but present in *recB* (Kerr and Hart, 1972), *uvr* (Radman and Devoret, 1971) and *polA* (Fauquet and Defais, 1972) mutants. UV reactivation has been observed for *Herpes* virus in human tumor cells (HeLa) but not in human embryonic lung culture (Lytle *et al.*, 1974), and in rat mammary tumor cells but not in normal rat embryo cells (Hellman *et al.*, 1974).

It has been suggested that the basis of UV reactivation is the radiation induction of repair enzymes that can only occur in $recA^+$ lex^+ strains (Defais *et al.*, 1971; Witkin and George, 1973; Witkin, 1974). However, Kerr and Hart (1972) do not favor an enzyme induction hypothesis, rather they suggest that the $recA^+$ and exr^+ gene products are required to stabilize UV-induced lesions prior to repair.

The rec^+ lex^+ exr^+ genes have been linked with other radiation-inducible responses such as induction of prophage and filament formation (Witkin, 1967), reactivation of irradiated phage (George *et al.*, 1974), error-prone repair (Witkin, 1974), and inhibition of post-irradiation DNA degradation (Pollard *et al.*, 1974).

*Present address: Strahlenbiologisches Institut der Universität München, *8 München 2*, Bavariaring 19, W. Germany.

The X ray reactivation of UV-irradiated phage has also been observed (W. Harm, 1962, unpublished observations cited in Rupert and Harm, 1966; Ono and Shimazu, 1966) but has not been as extensively studied as has UV reactivation. More recently the X ray-enhanced reactivation of a UV-irradiated mammalian virus (*Herpes simplex*) has been reported (Bockstahler and Lytle, 1971; Hellman *et al.*, 1974).

The sparing effect of dose fractionation in mammalian cells using X irradiation has been known for some years (for a review, see Elkind and Sinclair, 1965) but the molecular basis of this effect is still largely unknown. A similar effect has been found for UV-irradiated mammalian cells, but the kinetics were much slower than for fractionated doses of X radiation (Todd *et al.*, 1969). A similar split-dose recovery phenomenon has been observed in yeast for both electrons and UV radiation (Kiefer, 1971). Calkins (1973) has interpreted the "humped" UV and X radiation dose–response curves for protozoans (i.e. where a small dose of radiation is more lethal than a large dose) as indicating the radiation induction of repair capacity.

It should be recalled that if rec^+ exr^+ strains of *E. coli* K-12 are irradiated with both UV and X radiation with little or no time separation between the two irradiations, a synergistic effect on killing is observed (Martignoni and Smith, 1973, and references cited therein). It has been shown for *E. coli* K-12 that prior UV irradiation specifically blocks the growth medium-dependent (Type III) repair of X ray-induced DNA single-strand breaks (Martignoni and Smith, 1973). However, if certain strains of *E. coli* are permit-

Table 1. *Escherichia coli* K-12 strains used

Strain			
Stanford No.	Source No.	Relevant genotype	Other markers
SR247	AB2497	wild-type	*thr leu arg his thi pro thy ara lac gal mtl xyl str tsx*
SR204	—	wild-type	*thy metE lac str* (W3110)
SR22B	AB2499	*uvrB5*	same as SR247
—	DY184	*uvrD3*	*thy lac str* (W3110)
SR144	JG138	*polA1*	*thy rha lac str* (W3110)
SR188	DY95	*exrA*	*thy rha lac str* (W3110)
SR192	DY99	*exrA*	*thy metE lac str* (W3110)
SR269	PAM5831	*exrA*	*thr his malB str* (originally from AB1157 and B_{s-2})
SR283	DY180	*exrA uvrB5*	*leuB thy rha lac str* (W3110)
SR194	DY101	*exrA polA1*	*thy metE lac str* (W3110)
SR239	DY126	*exrA recB21*	*metE lac str* (W3110)
SR14	B_{s-1}	*exrA uvrB*	
SR15	B_{s-1}	*exrA uvrB*	*thy*
SR203	AB2494	*lex*	*thi met his pro thr leu thy mtl xyl gal lac str tsx su*$^+$
SR205	—	*lex*	*thy metE lac str* (W3110)
SR63	—	*recA13*	same as SR247 except *thy*$^+$
SR70	JC2926	*recA13*	same as SR247 except *thy*$^+$
SR261	AB2487	*recA13*	same as SR247 (from Yale Culture Collection)
SR47	JC5088	*recA56*	*Hfr KL16 thr ilv thi spc*
SR176	MM450	*recA56*	*rha lac str* (W3110)
SR78	—	*recB21*	same as SR247
SR255	DY151	*recB21*	same as SR247 except *thy*$^+$

ted to incubate in complete growth medium between the two irradiations, there is not only a loss of the synergistic effect of the radiations but also an absolute gain in resistance to the second irradiation. The present report documents, for *E. coli*, the genetic control and physiological requirements for the X ray-induction of resistance to a subsequent exposure to UV or X radiations.†

MATERIALS AND METHODS

Bacterial strains. The characteristics of the strains used are listed in Table 1.

Experimental conditions. Exponential phase cells grown in supplemented minimal medium (SMM) (Ganesan and Smith, 1968) were irradiated at room temperature with 50 kVp X rays (Martignoni and Smith, 1973) in various media and incubation was continued (in various media) at 37°C. For the UV radiation studies, samples of the X-irradiated cells were taken at different times and diluted 1:10 in 0.067 *M* sodium–potassium phosphate buffer, pH 7. One portion was plated on SMM agar (1.6% Difco Noble agar) for viable counts, and another portion was UV irradiated (8-W General Electric germicidal lamp emitting primarily at 254 nm; the incident fluence rate was determined with an International Light germicidal photometer, Model IL-254) in an open glass Petri dish on a rotary shaker, and then plated. For the split dose X ray experiments, the second dose was also delivered with the cells in SMM. Samples were then diluted in buffer and plated.

RESULTS

X ray–UV radiation split dose experiments. When *E. coli* K-12 wild-type, *polA*, *uvrD* or *uvrB* (Fig. 1)

†A preliminary account of some of these data was presented at an international symposium entitled *New Trends in Photobiology, Rio de Janeiro, 15–20 July 1973* (*Smith and Martignoni*, 1973).

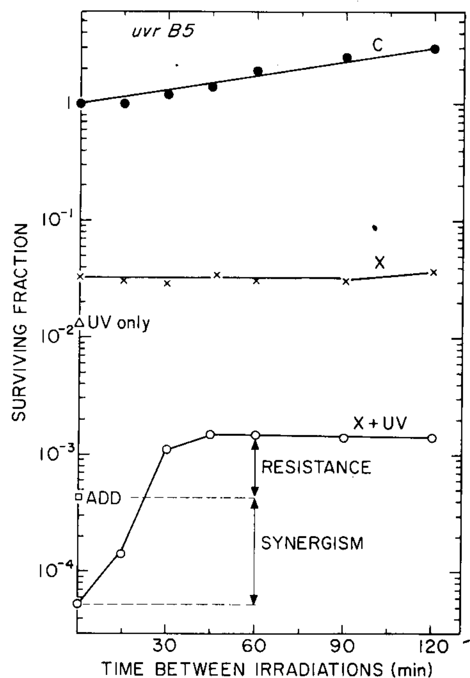

Figure 1. X ray-induced resistance to UV irradiation in *E. coli* K-12 *uvrB5* (SR22B). Log phase cells were X-irradiated in complete growth medium with 15 krad, and then incubated for various periods of time at 37°C in complete growth medium before one sample was diluted into buffer and plated. A second sample was irradiated with 10 J m^{-2} of UV radiation (254 nm) and plated. C, unirradiated control culture. X, culture was X irradiated and plated at the times indicated. X + UV, culture was X irradiated and then UV irradiated at the times indicated. △ UV only, survival after UV irradiation only (plated immediately). □ ADD, calculated summation of the separate UV and X ray effects on viability.

strains were X irradiated and then incubated at 37°C in SMM medium, they became progressively more resistant to a subsequent exposure to UV radiation. The maximum resistance to UV radiation was achieved 45–60 min after X irradiation. These experiments are complicated by the synergistic effect on survival of combined UV and X irradiation at time zero, and the total increase in resistance must be corrected for synergism. For the wild-type strain, the maximum protection against UV irradiation was obtained with a priming dose of X rays of about 15 krad (data not shown). The effect of this enhanced resistance on the shape of the full survival curve is shown in Fig. 2 for the wild-type strain. There was both an enhancement of the shoulder and a slight reduction in the final slope of the survival curve.

In the wild-type, *polA* and *uvrB* (Fig. 1) strains the induced resistance did not seem to decay with time. This was also true for experiments run on the wild-type strain for as long as 480 min (data not shown). Since a decay in induced resistance might be masked in these experiments by cell division, we performed full survival curves on the wild-type strain at various times after the inducing irradiation, and found that the X ray-induced resistance to UV radiation was still maximal after 360 min of incubation (data not shown).

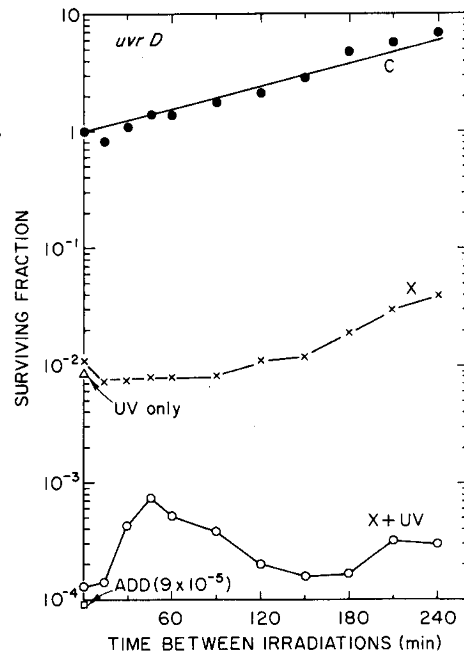

Figure 3. X Ray-induced resistance to UV irradiation in *E. coli* K-12 *uvrD3* (DY184). Log phase cells were treated as described in the legend to Fig. 1, except that the X ray dose was 10 krad and the UV fluence was 6 J m^{-2}.

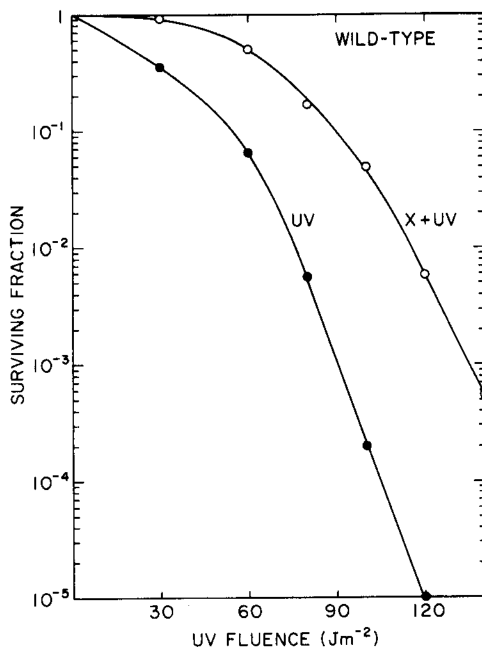

Figure 2. Survival curves for UV irradiated *E. coli* K-12 wild-type (SR247) with and without prior X irradiation. Log phase cells were X irradiated (15 krad) in complete growth medium (survival = 2.8×10^{-2}) and then incubated at 37°C in complete growth medium for 60 min (no change in survival) before being diluted into buffer and then exposed to various fluences of UV radiation. ●, UV irradiation only. ○, X irradiation plus incubation plus UV irradiation normalized to the survival of the sample exposed only to X radiation plus 60 min incubation as 1.0. The results are the average of two experiments.

With the *uvrD* strain, however, there was a clear variation in the amount of resistance as a function of the time of incubation (Fig. 3). A small amount of resistance to the second irradiation was even seen at time zero. Then, after approximately a 15 min lag, there was a rapid build-up of resistance to a maximum at 45 min followed by a gradual return to the time zero resistance by about 150 min, after which time cell division resumed. These temporal changes in sensitivity to a fixed dose of radiation were also expressed as changes in both the slope and shoulder of the UV survival curves (Fig. 4).

The *recA13*, *recA56*, *recB21* (Fig. 5), *exrA* (except in the W3110 background) and *lex* strains showed little or no radiation-induced resistance (Table 2). The W3110 *exrA* strains showed a response similar to that described in Fig. 1 for the *uvrB* strain, except that there was no synergism, and it took longer (90–120 min) to achieve maximum resistance. The enhanced resistance of the W3110 *exrA* strain was dependent upon functional $uvrB^+$ and $recB^+$ genes, but not on the $polA^+$ gene (Table 2). The enhanced resistance was not accompanied by the formation of a shoulder on the UV survival curve, but only by a reduction in slope (Fig. 6).

The *exrA* mutation used in most experiments was transduced from *E. coli* B_{S-1} to *E. coli* K-12 W3110 (Youngs and Bernstein, 1973). An *exrA* mutation transduced from *E. coli* B_{S-2} into the AB1157 background (SR269) did not show X ray induced resistance to UV radiation (Table 2). When SR269 was transduced to mal^+, still no inducible resistance was

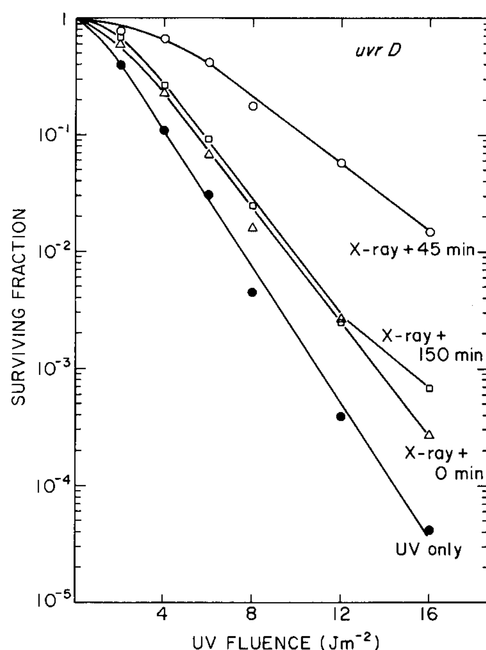

Figure 4. Survival curves for UV irradiated *E. coli* K-12 *uvrD3* (DY184) with or without prior X irradiation. Log phase cells at $\sim 2 \times 10^8$ per mℓ were X irradiated (10 krad) in complete growth medium (survival = $\sim 1 \times 10^{-2}$) and then incubated at 37°C in complete growth medium for 0, 45 or 150 min before being diluted 1:5 into buffer and then exposed to various fluences of UV radiation. ●, UV irradiation only. △, X irradiation plus no incubation plus UV irradiation. ○, X irradiation plus 45 min incubation plus UV irradiation. □, X irradiation plus 150 min incubation plus UV irradiation. The curves for cells that were X irradiated have been normalized to the survival of the sample exposed only to X radiation plus 0, 45 or 150 min incubation, respectively, as 1.0.

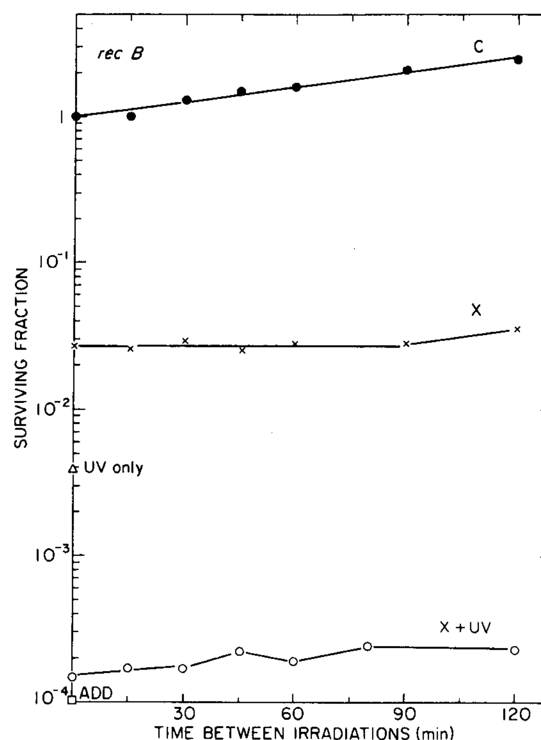

Figure 5. Lack of X ray-induced resistance to UV irradiation in *E. coli* K-12 *recB* (SR78). Log phase cells were treated as described in the legend to Fig. 1 except that the X ray dose was 5 krad, and the UV fluence was 50 J m^{-2}.

observed, but when it was transduced to *mal*$^+$ *exrA*$^+$, full wild-type resistance was observed (data not shown). When the *exrA* mutation was transduced from SR269 to a W3110 strain, X ray-induced resistance to UV irradiation was observed (data not shown).

The results for the various strains are summarized in Table 2.

X ray–X ray split-dose experiments. The shape of the kinetic experiments for the X–X split-dose experiments (Fig. 7, 8) were essentially the same as for the X-UV experiment shown in Fig. 1. These experiments were not complicated by the problem of radiation synergism. A summary of the results for the various strains is given in Table 3.

The maximum resistance was reached by about 60 min for the wild-type, *uvrB* and *polA* strains. This resistance was manifested in the *uvrB* strain by the production of a large shoulder with little if any change in the final slope of the survival curve (Fig. 9). For the wild-type strain, maximum resistance against a second X irradiation was obtained with a priming dose of about 6 krad (data not shown).

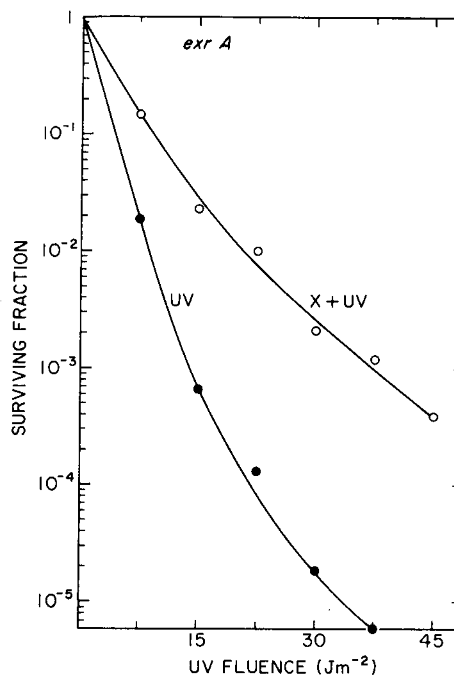

Figure 6. Survival curves for UV irradiated *E. coli* K-12 *exrA* (SR188) with and without prior X irradiation. Log phase cells were X irradiated (5 krad) in complete growth medium (survival = 2.3×10^{-2}), and then incubated at 37°C in complete growth medium for 90 min (no change in survival) before being diluted in buffer, and then exposed to various fluences of UV radiation. The symbols are as described in Fig. 2.

Table 2. Protection of different derivatives of *E. coli* K-12 against UV radiation by a prior exposure to X radiation

Strains	Average radiation protection factor*		Radiation doses	
		Corrected for synergism	1st (krad)	2nd ($J\ m^{-2}$)
wild-type				
(SR247)	301 (9)	4.9	12–15	50–120
(SR204)	151 (2)	37.5	15	80
uvrB5				
(SR22B)	18.1 (2)	3.2	15	10
uvrD3				
(DY184)	4.4 (2)	—	10	6
polA1				
(SR144)	29.0 (2)	2.8	5	10
recA13				
(SR63)	2.0	—	5	1.5
(SR70)	1.3 (2)	—	5	1.5
(SR261)†	1.2	—	5	1.5
recA56				
(SR47)	0.8	—	5	1.5
(SR176)	1.3 (2)	—	5	1.5
recB21				
(SR78)	1.5 (3)	—	5	50–56
(SR255)	1.1	—	5	50
recB21 exrA				
(SR239)	0.8	—	5	5
lex				
(SR203)	1.9 (2)	—	5	12
(SR205)	5.5 (2)	1.4	5	7.5–11.0
exrA				
(SR269)	1.2 (2)	—	5	10
(SR188)	14.4 (2)	—	5	12
(SR192)	15.7 (5)	—	5	12
exrA uvrB				
(SR283)	2.8	—	5	1.7
(SR14) (B_{S-1})	1.1	—	6	0.8
(SR15) (B_{S-1})	2.0	—	6	0.8
exrA polA				
(SR194)	16.0 (2)	—	5	6

*The protection factor was obtained by dividing the survival observed after both X and UV irradiation with the 60–120 min delay between the two irradiations (incubation at 37° in complete growth medium), by the survival observed with no incubation between the two irradiations. When more than one experiment was performed on a given strain, this is specified by the number in parenthesis. To correct for synergism, the survival after the split-dose irradiation was divided by the calculated survival (for no delay between the two irradiations) based upon the response of cells to the two individual irradiations. The dashes indicate that there was no synergism and therefore no correction was made for these strains.

†In preliminary studies (Smith and Martigoni, 1973), one *recA13* derivative (AB2487) showed X ray-induced resistance to UV irradiation (after UV fluences greater than $0.7\ J\ m^{-2}$) but no enhanced resistance to X irradiation. We lost this strain and a new isolate from the Yale Culture Collection (SR261) showed no enhanced resistance.

While the enhanced resistance appears to remain constant over the 210 min of the experiment shown in Fig. 8 (X–X curve), full X ray survival curves run at various times after the initial irradiation demonstrated that the resistance of the cells had returned to near normal levels by about 240 min (data not shown). This contrasts with the results described above for cells in which the second exposure was to UV radiation.

The W3110 *exrA* strain also showed an enhanced resistance for the X–X split dose experiment that reached a maximum at about 60 min. At the point of maximum resistance, the full survival curve showed no shoulder, but about a 1.5-fold reduction in slope (Fig. 10).

Physiological requirements for radiation induced resistance. To avoid problems with synergism, the effects of amino acid starvation (Fig. 7) and treatment with 50 μg/ml chloramphenicol (Fig. 8) were studied using split dose X ray experiments. However, because the post-irradiation blockage of protein synthesis by either method is lethal to rec^+ cells (Fig. 7, 8; see

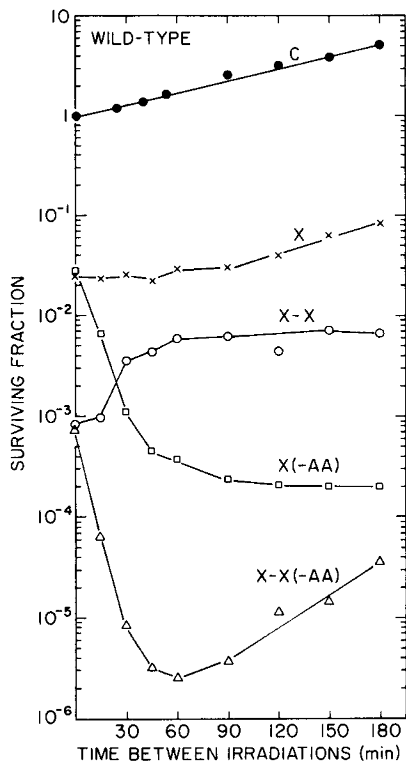

Figure 7. Effect of post irradiation amino acid starvation on X radiation-induction of resistance to X irradiation in *E. coli* K-12 wild-type (SR247). Log phase cells were X irradiated (15 krad) in minimal salts medium minus glucose (DTM), and then incubated for various times at 37°C in complete growth medium or in growth medium devoid of required amino acids (−AA). At various times, one sample was diluted into buffer and plated; a second sample was X irradiated (25 krad), diluted into buffer and plated. C, unirradiated control culture. X, X irradiated culture plated at the times indicated. X–X, X irradiated, incubated and X irradiated a second time at the times shown.

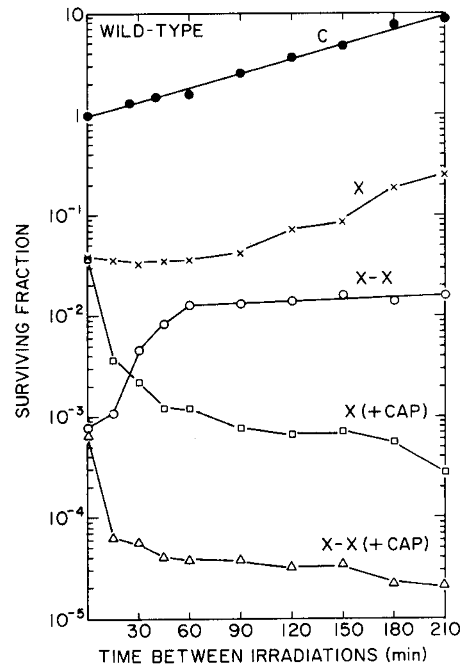

Figure 8. Effect of chloramphenicol on the X radiation-induced resistance to X irradiation in *E. coli* K-12 wild-type (SR247). The samples were irradiated in complete growth medium, otherwise the conditions and symbols are as described in Fig. 7. (+CAP), cells were incubated after irradiation in complete growth medium containing 50 μg/ml chloramphenicol (CAP) prior to the second X irradiation and then diluted and plated in the absence of CAP.

also Ganesan and Smith, 1972), such experiments are difficult to interpret. Nevertheless, the data suggest that chloramphenicol blocks the induction of resistance (Fig. 8). Similarly, amino acid stavation blocked the appearance of resistance for about 90 min, but subsequently resistance appeared slowly [curve X–X (−AA); Fig. 7].

To determine the nutritional requirements for the expression of resistance to UV irradiation in the W3110 *exrA* strain, it was incubated under different conditions (i.e. buffer, medium, etc.) between irradiations. In all cases, an increase in resistance between irradiations was observed, however, the maximum amount of resistance observed in complete growth medium with or without chloramphenicol at 40 μg/mℓ was about 3 times that observed in buffer or in growth medium devoid of the required amino acid methionine. In spite of the reduced resistance in buffer or in the absence of required methionine, the fact that chloramphenicol had no effect suggests that protein synthesis is not absolutely required by the W3100 *exrA* cells in order to express enhanced

Table 3. Protection of different derivatives of *E. coli* K-12 against X radiation by a prior exposure to X radiation

Strains	Average radiation protection factor*	Radiation doses 1st (krad)	2nd (krad)
wild-type			
(SR247)	4.2 (3)	15	25
(SR204)	4.9	15	25
uvrB5			
(SR22B)	13.6 (2)	15	25
polA1			
(SR144)	9.8 (2)	5	5
exrA			
(SR269)	1.4	5	5
(SR192)	7.8 (2)	5	5
lex			
(SR205)	1.4	5	5
recA13			
(SR63)	1.9	5	5
(SR70)	1.1	5	5
(SR261)	1.9	5	5
recA56			
(SR47)	1.6	5	5
(SR176)	1.4	5	5
recB21			
(SR78)	1.5 (2)	5	5

*The survival observed after two doses of X rays separated by 60–90 min incubation at 37°C in complete growth medium, divided by the survival obtained with no incubation between the two irradiations. When more than one experiment was performed on a given strain, this is specified by the number in parenthesis.

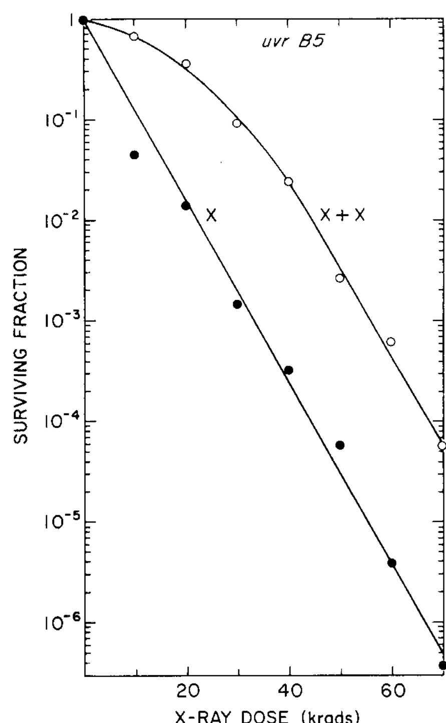

Figure 9. Survival curves for X irradiated *E. coli* K-12 *uvrB5* (SR22B) with and without prior X irradiation. Log phase cells were X irradiated (15 krad) in complete growth medium (survival = 1.8×10^{-2}) and then incubated at 37°C in complete growth medium for 90 min (no change in survival) before being further exposed to various doses of X radiation. ●, X irradiation only. ○, X irradiation plus incubation plus X irradiation normalized to the survival of the sample exposed to X radiation plus 90 min incubation as 1.0. The results are the average of two experiments.

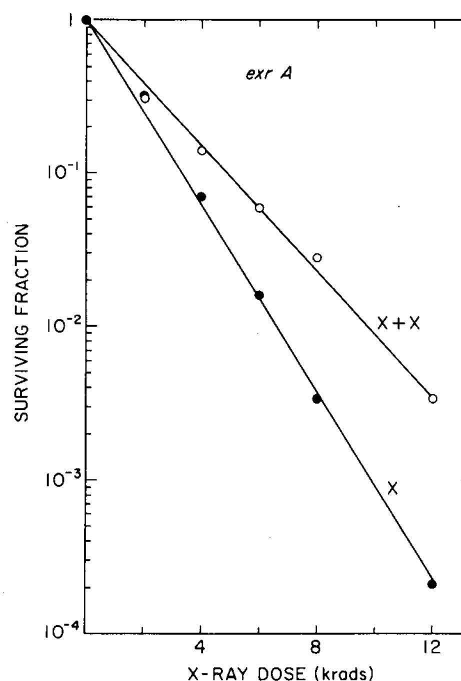

Figure 10. Survival curves for X irradiated *E. coli* K-12 *exrA* (SR192) with or without prior X irradiation. Log phase cells were X irradiated (5 krad) in complete growth medium (survival = 3.8×10^{-2}) and then incubated at 37°C in complete growth medium for 90 min (no change in survival) before being further exposed to various doses of X radiation. The symbols are as described in Fig. 9. The results are the average of two experiments.

resistance. In contrast to exr^+ strains (Ganesan and Smith, 1972), the W3110 *exrA* strain was not killed by post-irradiation amino acid starvation or treatment with chloramphenicol (data not shown).

DISCUSSION

We have observed that X-irradiated rec^+ lex^+ exr^+ strains become progressively more resistant to a second irradiation (X or UV) when incubated in complete growth medium for 60–90 min between irradiations (Fig. 1, 7). This enhanced resistance was characterized by the production of a shouldered survival curve with no change in the final slope for X irradiation (Fig. 9), whereas after UV irradiation both an enhancement of the shoulder and a reduction in the final slope were observed (Fig. 2, 4).

The *uvrD* strain showed some enhanced resistance when the X ray and UV irradiations were given in rapid succession (Fig. 2, 3). The protection of *E. coli* B_{S-1} (*uvrB exrA*) against X irradiation by UV irradiation at time zero has been reported previously (Smith and Ganesan, 1966).

In the *uvrD* strain, a maximum in resistance to UV radiation was achieved 45 min after X irradiation, and this resistance returned to the time zero sensitivity by about 150 min (Fig. 3). This enhanced resistance did not appear to decay with time in *uvrB* (Fig. 1) or wild-type cells (Fig. 7, 8). However, when full survival curves were performed on wild-type cells at various times after the inducing irradiation, we observed that the X ray-induced resistance to UV radiation was still maximal after 360 min of incubation, but the resistance to X radiation was almost back to normal by 240 min. Studies on the factors that affect the decay of resistance should prove as interesting as those that affect its induction.

The dose of X irradiation required to induce the maximum amount of resistance was about 6 krad when the second exposure was to X radiation but was ~15 krad when the second exposure was to UV radiation. We can offer no explanation for this difference in dose requirement.

The *recA*, *recB*, *exrA* (except in the W3110 background) and *lex* strains (in the W3110 or AB1157 backgrounds) did *not* show X radiation-induced resistance to killing by X or UV radiation (Table 2, 3). While the exceptions will be discussed below, the evidence is clear that the rec^+ lex^+ exr^+ genes are required for the X ray-induction of the type of resistance that is manifested by the *appearance* (after X irradiation) or the *enhancement* (after UV irradiation) of a shoulder on the survival curves.

While in the W3110 background (but not in the AB1157 background), the *exrA* strains (whether de-

rived from *E. coli* B_{S-1} or B_{S-2}) did show X ray-induced resistance, this was manifested *only* by a change in slope, whether the second exposure was to UV (Fig. 6) or to X radiation (Fig. 10). The resistance to UV radiation appeared to depend upon the presence of functional $uvrB^+$ and $recB^+$ genes (Table 2), but did not depend upon a functional $polA^+$ gene (Table 2), or upon protein synthesis, since the production of resistance was not blocked by treatment with chloramphenicol between irradiations.

In contrast to exr^+ strains (Ganesan and Smith, 1972), the *exrA* strains tested were not killed by post irradiation amino acid starvation or treatment with chloramphenicol. In this regard, the *exrA* strains are similar to the *recA* and *recB* strains (Ganesan and Smith, 1972).

While the *lex* and *exrA* mutants exhibit many similar properties (cf. Donch and Greenberg, 1974) they appear to be located at different sites on the *E. coli* chromosome (Chung *et al.*, 1975). This apparent difference in the *lex* and *exrA* mutations is consistent with our observation that in contrast to the *exrA* strains, the *lex* strains showed no induction of resistance whether in the AB1157 or the W3110 backgrounds (Table 2, 3).

Since the radiation-induced resistance that we have observed is of two types (i.e. one is characterized by the appearance or enhancement of a shoulder on the survival curve, while the second one is characterized only by a change in slope), they must be produced by at least two different mechanisms. Possible hypotheses to explain our results are:

(1) The production of a shoulder or the enhancement of a shoulder on a survival curve by a prior treatment of the cells with radiation is suggestive of the induction of repair enzymes. Because the post-irradiation blockage of protein synthesis by amino acid starvation or treatment with chloramphenicol is lethal to rec^+ cells (Ganesan and Smith, 1972), experiments to test for the enhancement of resistance while protein synthesis is inhibited are somewhat difficult to interpret. Nevertheless, the results suggest that chloramphenicol at 50 μg/mℓ blocks the appearance of resistance in an X–X split-dose experiment over the 3 h period studied (Fig. 8). When required amino acids were withheld, the appearance of resistance was blocked for about 90 min but then slowly increased over the next 90 min studied (Fig. 7). This delayed appearance of resistance could result from the utilization of amino acids obtained from protein catabolism. Thus, these data are consistent with, but do not prove the hypothesis that the synthesis of repair enzymes has been induced by the first irradiation.

A related hypothesis is that the radiation induces an inhibitor of enzymes that degrade DNA. The inhibition of non-specific DNA degradation should increase the efficiency of repair (e.g. Pollard *et al.*, 1974).

(2) The enhanced resistance may simply be due to the fact that the cells have progressed to a more resistant part of the cell cycle between irradiations. The work of Kelley and Rupert (1974) using synchronized cultures of *E. coli* are consistent with this hypothesis since wild-type and uvr^- cells showed a cell cycle dependent sensitivity to UV irradiation but rec^- cells did not.

A more specific statement of the cell cycle hypothesis is that the cells simply contain more DNA per cell (cell division is blocked by the first irradiation but metabolism continues) by the time of the second irradiation, and thus are more resistant. While this hypothesis might explain the resistance seen in the wild-type strain, and perhaps its absence in the *recA* strain, it cannot explain the absence of resistance in the *recB* and AB1157 *exrA* strains. While the *recA* strain is very sensitive to DNA synthesis inhibition after γ-irradiation, *recB*, *exrA* and even *recA recB* cells exhibit only a small inhibition of DNA synthesis that is not greatly different from that shown by wild-type cells (Gray, *et al.*, 1972).

(3) The repair enzymes may already exist in the wild-type strain, but they are somehow "activated" by the irradiation.

(4) Repair enzymes may be recruited to the vicinity of the DNA by the initial radiation damage, and, by virtue of being near the DNA during the second irradiation, they are able to accomplish a more efficient repair of the second damage.

(5) The damage produced by the first irradiation may simply be repaired before the second irradiation; the cells, therefore, having to cope with less damage per unit time. This should lead to a dose reduction factor (i.e. a change in slope) for the split-dose experiments, as we have observed for W3110 *exrA*.

Although this type of explanation may be satisfactory for the results for the W3110 *exrA* strains, it cannot explain the results for the *uvrD* strain where the resistance reached a maximum at 45 min and returned to the time zero value at $\sim$150 min. Even fewer lesions would be expected to be left unrepaired 150 min after the first irradiation, as compared with an incubation time of 45 min.

The repair of damage produced by the first irradiation cannot be the explanation for *recA* strains since they repair most of their X ray-induced DNA single-strand breaks (Youngs and Smith, unpublished data), and show no beneficial effect of split-dose irradiation. It could be argued, however, that there is some other type of lesion (e.g. base damage), that is not repaired in the *recA* strains, which is important in the enhanced resistance phenomenon.

In summary, the rec^+ lex^+ exr^+ genes are required for the X irradiation-induction of resistance to a subsequent exposure to X and UV radiation that is expressed by the production or enhancement of a shouldered survival curve.* The precise mechanism(s)

*A similar observation that *rec* and *lex* genes are required for the expression of the radiation-induced resistance to a subsequent irradiation has recently been reported by Pollard and Achey (1975).

(i.e. enzyme induction, enzyme activation, cell cycle dependence, etc.) by which this resistance is produced is far from clear. It would appear to be premature to select one hypothesis over all the others mentioned since it is quite likely that all are correct, but one may dominate for different strains and/or under different growth conditions.

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