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Lambda Bacteriophage Gene Products and X-Ray Sensitivity of *Escherichia coli*: Comparison of *red*-Dependent and *gam*-Dependent Radioresistance

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When gene products of lambda bacteriophage are introduced into a cell by transient induction of a lysogen, increased resistance of the cells to X rays results. This phenomenon has been called phage-induced radioresistance. Genetic studies show at least two classes of induced radioresistance. The first type depends on the products of the λ *red* genes and is observed in bacteria that are mutated in the *recB* gene. It is thought that the λ *red* products compensate for the missing RecBC nuclease in the repair of X-ray damage. An optimal effect is obtained even when the λ *red* products are supplied 1 h after irradiation. The lesions that are affected by the *red*-dependent process are probably not deoxyribonucleic acid strand breaks because the extent of deoxyribonucleic acid strand rejoining is not altered by the *red* products. The second type of phage-induced radioresistance requires the *gam* product of λ and is observed in wild-type and *polA* strains. The λ *gam*$^+$ gene product must be present immediately after irradiation to exert its full effect. In its presence, DNA breakdown is decreased, and a greater fraction of DNA is converted back to high molecular weight. Strains carrying *lex*, *recA*, or certain other combinations of mutations do not show any detectable phage-induced radioresistance.

Several lines of evidence suggest a relation between recombination and the repair of deoxyribonucleic acid (DNA) in X-irradiated cells. Strains that are recombination deficient because of a mutation in *recA*, *recB*, *recC*, or *recF* genes are also more sensitive to the lethal effects of X rays than are Rec$^+$ strains (3, 8). Other studies have shown that the survival of X-irradiated *Escherichia coli* is enhanced by the presence of certain gene products introduced into the cell by the transient induction of bacteriophage λ (27, 28). In wild-type bacteria, the γ protein of λ is required for phage-mediated radioresistance (28), whereas in *recB* bacterial strains, the two products of the λ *red* genes (λ exonuclease and β protein) are essential (28). Lambda exonuclease (11) and β protein (21) are known to participate in λ recombination (20, 25), whereas γ protein inhibits the RecBC nuclease in whole cells (29, 30) and in extracellular purified systems (24). It therefore seems likely that there may be at least two separate mechanisms for phage-mediated resistance, both of which are related to proteins involved in recombination.

In this paper, we extend the earlier genetic studies to other bacterial strains carrying genetic defects in DNA metabolism, and also present biochemical results that show further differences between *gam*-dependent and *red*-dependent radioresistance.

MATERIALS AND METHODS

Bacterial and bacteriophage strains. The properties and sources of the bacterial strains used are listed in Table 1.

The bacterial strains were lysogenized with the following λ mutants: λ*cI857* (26) (all the λ strains used in this paper carry the marker C1857 which makes them thermoinducible); λ*cI857 red3* (25) (*red3* is defective in the production of λ exonuclease and β protein); λ*cI857 gam 210* (31) (*gam 210* is an amber mutation defective in the production of γ protein).

X-ray survival. Lysogenic bacteria were grown at 30 C in YET broth containing 5 g of yeast extract, 10 g of tryptone, 10 g of NaCl, and 120 mg of NaOH per 1,000 ml of water. In the case of *polA* cells, 5 μg of thymidine per ml was added. Unless otherwise stated, all experiments were done with stationary-phase cells. For transient induction by pulse heating, the cells were treated as follows. An aliquot (0.1 to 0.5 ml) of bacteria was first diluted 10- to 50-fold with prewarmed YET broth and incubated at 42 C with aeration. At suitable time intervals, 0.1-ml samples were withdrawn and chilled by dilution with 0.9 ml of ice-cold YET broth. This suspension was divided into

TABLE 1. *Bacterial strains*

Bacterial strain no.	Relevant genotype	X-ray sensitivity[a]	Recombination proficiency	Source	Reference
DM456	Wild type	R	+	K. B. Low	17
AB2470	*recB21*	S	−	Our collection	9
JC5183	*recB21 recC22 sbcA5*	R	+	C. M. Radding	1
JC7623	*recB21 recC22 sbcB15*	R	+	C. M. Radding	14
JC8218	*recB21 recC22 xonA1*	R	−	A. J. Clark	12
JC3916	*recF143*	S	+	A. J. Clark	
JC3881	*recB21 recC22 recF143*	S	−	A. J. Clark	
JC8111	*recB21 recC22 sbcB15 recF143*			A. J. Clark	7
DM803	*lex-1*	S	+	K. B. Low	18
P3478	*polA1*	S	+	K. B. Low	4, 6

[a] R, Resistant; S, sensitive.

two portions, one being subsequently irradiated and the other serving as control. The viable cell counts in the figures are presented on the basis of the original culture before dilution and treatment.

X irradiation was carried out with a Siemens Stabilipan 250 operated at 250 kV and 15 mA without added filtration. The dose rate was about 4,000 rads per min. Viable cell number of both unirradiated and irradiated cultures was determined by spreading bacteria (after appropriate dilutions) on YET agar plates (YET broth supplemented with 2% agar). Colonies were counted after 1 to 2 days of incubation at 30 C.

Sedimentation of bacterial DNA in alkaline sucrose gradients. The lysogens were labeled in their DNA by growing in YET broth supplemented with 200 μg of deoxyadenosine per ml and 1.2 μg of [^{3}H]thymidine (specific activity 2 Ci/mmol) per ml. When the cells had grown to about 2×10^8 cells/ml, they were centrifuged, washed, and resuspended in fresh YET broth supplemented with 5 μg of unlabeled thymidine per ml. After an additional 45 min of growth at 30 C, the cells were diluted 10-fold and incubated for 4 to 6 min either at 30 or 42 C. The cells were then transferred to an ice bath, and the bacterial suspension was divided into two portions, with one being irradiated while the other was kept as the control. These cells (0.25 ml) were mixed with equal volumes of 1% Sarkosyl in 2×10^{-2} M EDTA. Immediately, a sample (0.15 ml) of this mixture was layered onto the top of a 5-ml, 5 to 20% linear alkaline sucrose gradient (0.3 N NaOH, 0.7 N NaCl, 10^{-3} M EDTA). Gradients were centrifuged at 30,000 rpm in a Spinco SW50.1 rotor for 90 min at 20 C. After centrifugation, fractions of 0.2 ml were pumped out from the bottom of the tubes by inserting capillary tubes through the gradient.

For the determination of acid-precipitable radioactivity, fractions were collected directly on Whatman 3MM filter disks (23 mm in diameter) and treated with 5% trichloroacetic acid, ethanol, and acetone as previously described (22). In those experiments in which total radioactivity was measured, fractions were collected directly into vials and counted after addition of 0.05 ml of 1 N HCl and 8 ml of scintillation fluid composed of 6.6 g of *p*-bis(2,5-phenyloxazoyl)-benzene, 82 mg of 2,5-diphenyloxazole, 600 ml of Triton X-100 detergent, and 167 ml of water per 1 liter of toluene.

Coefficient of repair. In certain experiments we have observed that after irradiation, the DNA can be affected in at least two different ways. It can either be converted to DNA of higher molecular weight, or it can be broken down to smaller pieces as a result of nucleolytic degradation. As a consequence, a heterogeneous collection of DNA sizes results. Therefore, we think that it is inappropriate to characterize the sedimentation distributions in terms of a molecular weight average, particularly if the acid-soluble fraction is excluded from consideration. For this reason, we have introduced a method which compares the extent of overlap between different distributions of radioactivity in alkaline sucrose gradients. When two gradients have the same number of fractions, as in our experiments, the overlap can be calculated directly from the following expression:

$$\text{overlap} = \sum_{i=1}^{n} Z_i$$

where $Z_i = C_i$ if $C_i \leq X_i$, or $Z_i = X_i$ if $X_i < C_i$, where C_i is the fraction of the total recovered radioactivity in the ith fraction of an alkaline sucrose gradient from control cells, X_i is the fraction of total recovered radioactivity in the ith fraction of an alkaline sucrose gradient from the irradiated cells, and n is the total number of fractions in each gradient. The rationale behind this approach is that in the hypothetical case of complete repair, the sedimentation distribution of radioactivity in a gradient will be identical with that of an unirradiated control (i.e., the overlap = 1), whereas deviations from this distribution are a consequence of incomplete repair (i.e., overlap < 1). Since there is some overlap of the control curve with that of irradiated cells at zero time, we define a parameter, which is called the "coefficient of repair," to exclude this background, as follows:

$$\text{coefficient of repair} = \frac{\text{overlap}_t - \text{overlap}_{t_0}}{1 - \text{overlap}_{t_0}}$$

where overlap_t and overlap_{t_0} refer to overlap calculated at time t and t_0, respectively. From this, it is seen that the coefficient of repair has values ranging

from 0 for the distribution obtained immediately after irradiation to 1 for a distribution resulting from complete repair and identical to that of the unirradiated control. An example of the experimental determination of the coefficient of repair is demonstrated in Fig. 1.

We would like to emphasize that the coefficient of repair is only a convenient indicator of repair, and cannot be used to obtain a quantitative estimate of molecular weight or the number of strand joining events. In this paper, the coefficient of repair is defined on the basis of the overlap at t_0, which is convenient for use with ionizing radiation because the strand breaks are found immediately at t_0. Under experimental conditions used in our studies, the coefficient of repair has values that fall between 0 and 1, the theoretical maximum. However, under other circumstances, where the overlap is less at time t than at t_0, a negative value will result for the coefficient of repair. For example, in the case of excision repair of UV damage in wild-type *E. coli*, the overlap would decrease to a minimum value during the early part of the postirradiation period, and then at a later time would increase to a higher level. In a case such as this, it would be preferable to define the coefficient of repair in terms of the minimum overlap rather than the overlap at t_0.

RESULTS

Radioresistance dependent upon gam or red gene products of λ bacteriophage. Previous studies (27, 28) have shown that the transient induction of λ bacteriophage in an *E. coli* lysogen increases the survival of the host bacteria after exposure to X rays. An example of this effect is shown in Fig. 2. It is seen that the survival of unirradiated cells is only slightly affected by a brief incubation at 42 C, whereas the same heat treatment before X irradiation greatly increases the number of survivors. The survival is defined as the number of viable cells found after X irradiation divided by the number of viable cells in an unirradiated control culture exposed to 42 C for the same length of time. In most subsequent figures, only the survival is plotted, whereas the actual viable counts are omitted.

Since the earlier experiments demonstrated that λ gam^+ was required for phage-induced radioresistance in wild-type *E. coli* and that λ red^+ was necessary in a *recB* strain, we have investigated other bacterial strains in an effort to correlate phage-induced radioresistance with phage and bacterial genotypes. The results in Fig. 3, obtained with strains carrying *recB* combined with various other genes, show that in each case a mutation in the λ *red* gene abolishes the phage-induced radioresistance.

For wild-type bacteria, the phage-induced radioresistance observed with λ gam^+ does not occur when the λ phage carries a *gam* mutation (Fig. 4). It is also seen from Fig. 4 that transient induction of a *polA* lysogen leads to a dramatic increase in survival after exposure to X rays. When the prophage carries an amber gam^- mutation, the effect is greatly diminished. In connection with the residual response observed with the gam^- mutation, it may be significant that Lehman and Chien (15) observed a low level of normal polymerase I in this *polA* strain which is probably due to a low level of suppression of amber mutations. In similar experiments with wild-type and *polA* bacteria lysogenic for λ *red*, the same level of phage-dependent radi-

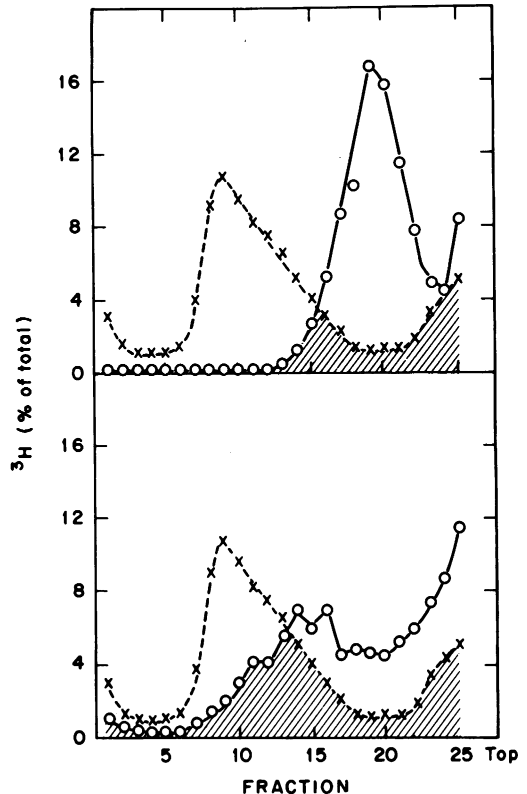

FIG. 1. *Example of the determination of the coefficient of repair from the overlap of DNA sedimentation distribution in alkaline sucrose gradients. Symbols:* ×, *both panels, unirradiated control;* O, *top panel, immediately after irradiation with 10 krads;* O, *bottom panel, incubated for 60 min at 30 C after irradiation with 10 krads. The shaded portions represent the overlap at* t_0 *(top) and* t_{60} *(bottom) as described. These values are 0.31 and 0.60, respectively, from which the coefficient of repair is calculated to be 0.42. (These data are for polA1 strain lysogenic for λcI857 and heated at 42 C for 4 min just before irradiation.)*

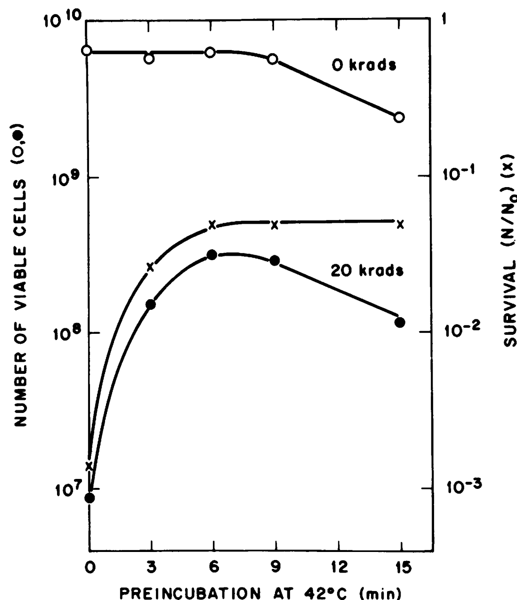

FIG. 2. *Effect of pulse heating on survival of control and X-irradiated E. coli recB21 recC22 sbcA lysogenic for λcI857. Viable cell counts are presented on the basis of the original culture before dilution and treatment. Symbols:* O, *viable cells/ml in unirradiated control;* ●, *viable cells/ml after irradiation with 20 krads;* ×, *ratio of the number of viable cells in the control to the number of viable cells in the irradiated culture.*

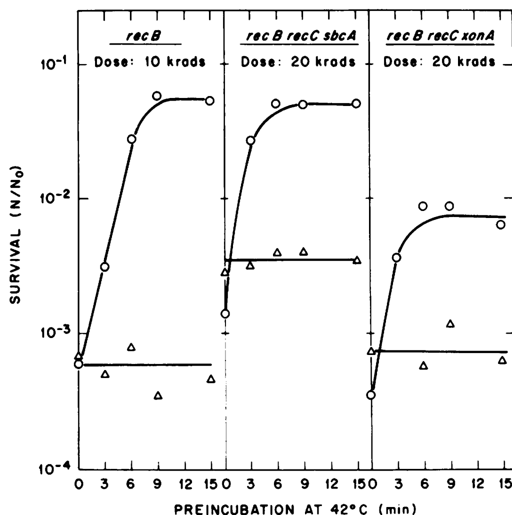

FIG. 3. *Effect of λ red 3 mutation on X-ray survival after pulse heating of several E. coli strains carrying λcI857 prophage. Symbols:* O, *strains lysogenic for λcI857 red⁺;* Δ, *strains lysogenic for λcI857 red 3. The lysogenic strains were derived from: AB2470 recB21, JC5183 recB21 recC22 sbcA5, and JC8218 recB21 recC22 xonA1.*

oresistance was obtained as when the bacteriophage was *red*$^+$. Thus, in these strains, bacteriophage-induced radioresistance requires the λ *gam* gene product, but occurs normally in the absence of λ *red* products. This contrasts with the results obtained with the *recB* strains (Fig. 2) where the λ *red* products were necessary to observe phage-induced radioresistance.

Although wild-type and many other strains show bacteriophage-induced radioresistance, the presence of certain bacterial mutations prevents this effect. The results with one such strain carrying *lex* are shown in Fig. 5. Other strains which show no effect include *recA* and *recB recC sbcB* mutants. The results with these and other strains in experiments similar to those shown in Fig. 2 to 5 are summarized in Table 2. From this table certain generalizations can be made. In strains that show λ-induced radioresistance, those which are mutated in the *recB* locus require λ *red*$^+$, whereas *recB*$^+$ strains show a normal effect with λ *red*$^-$ but require λ *gam*$^+$. In the cases examined, phage-induced radioresistance requires either λ *red*$^+$ or λ *gam*$^+$, but does not require λ *red*$^+$ and λ *gam*$^+$ simulta-

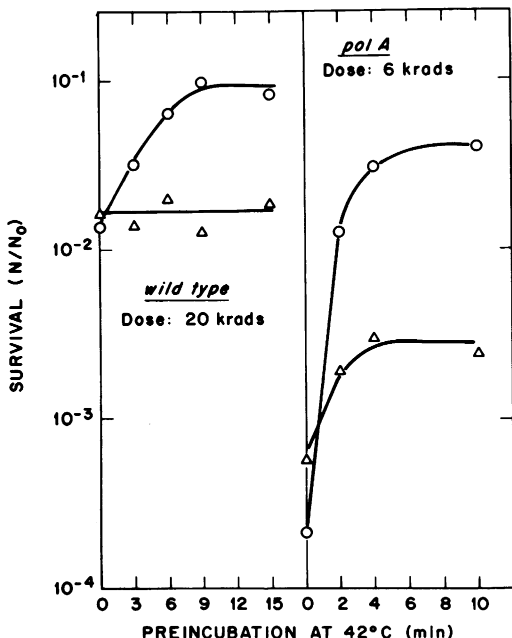

FIG. 4. *Effect of λgam210 mutation on X-ray survival after pulse heating of wild-type and polA strains of E. coli carrying λcI857 prophage. The polA cells were in the logarithmic phase of growth. Symbols:* O, *strains lysogenic for λcI857 gam⁺;* Δ, *strains lysogenic for λcI857 gam210.*

neously. (Because the *gam*210 mutation is an amber, its effect could be checked only in Su⁻ strains.) Thus, from a genetic point of view, bacteriophage-induced radioresistance can be classified as either *red*-dependent or *gam*-dependent.

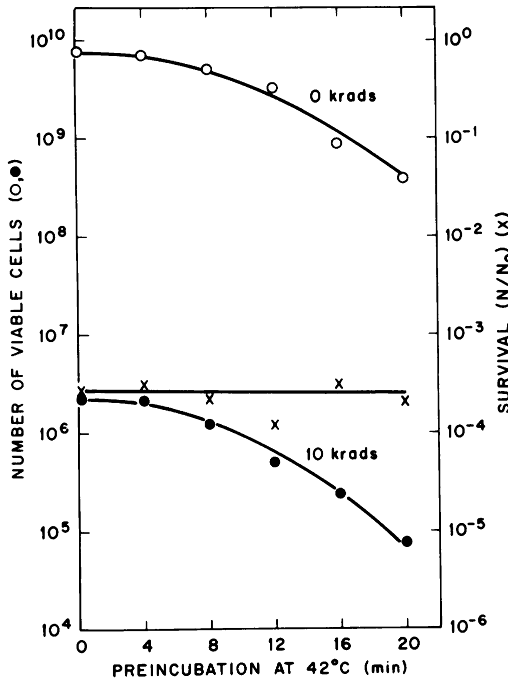

FIG. 5. *Effect of pulse heating on survival of control and X-irradiated E. coli lex-1 lysogenic for λcI857. Symbols: ○, viable cells/ml in unirradiated control; ●, viable cells/ml after irradiation with 10 krads; ×, the ratio of viable cells in the control to viable cells in the irradiated culture.*

Heating of lysogens after irradiation. In all experiments in earlier studies and in the preceding part of this paper, the λ gene products were provided by heating the lysogens just before exposure to X irradiation. Since it is of interest to know whether λ products are needed at early steps of postirradiation repair, we have done experiments in which the lysogens were irradiated and then heated 5 to 6 min at different times after irradiation. From the results in Fig. 6 with a *recB* strain, it is seen that the *red*-dependent radioresistance is fully expressed even when the heat induction of λ is delayed until 60 min after irradiation, whereas heating at later times results in a slight decrease in the effect.

Similar experiments to determine the time at which λ gene products are required in wild-type and *polA* strains are shown in Fig. 7. In these cases the gam^+ gene of λ is necessary for induced radioresistance, and, in marked contrast to the results in Fig. 6, a delay of only 10 min after irradiation nearly abolishes the observed effect. These results point out a further difference between *red*-dependent and *gam*-dependent radioresistance. In the *red*-dependent case, introduction of the λ gene products at times up to 1 h after irradiation suffices to produce the maximal effect, whereas in the *gam*-dependent situation, λ gene products are required during the first few minutes after irradiation to produce an optimal effect.

Effect of red products on rejoining of strand breaks in a recB⁻ strain. Since the

TABLE 2. *Effect of bacterial and bacteriophage genotype on lambda-induced radioresistance*

Bacterial genotype	X-ray survival without transient induction (%)	Effect of transient induction on X-ray resistance[a]		
		λ red^+ gam^+	λ red^- gam^+	λ red^+ gam^-
recB	0.04 (10 krads)	++	−	++
recB recC sbcA	0.1 (20 krads)	++	−	
recB recC xonA	0.03 (20 krads)	+	−	
recB recC sbcB recF	0.07 (8 krads)	±		
recB recC recF[b]	0.1 (8 krads)	−		
recB recC sbcB	0.1 (20 krads)	−		
recA[c]		−		
lex	0.04 (10 krads)	−	−	
polA	0.02 (6 krads)	++	++	±
recF	0.2 (10 krads)	+	+	
Wild type (DM 456)	1.4 (20 krads)	+	+	−

[a] −, No effect of transient induction on X-ray survival, dose reduction factor: 0.9 to 1.1; ±, slight effect, dose reduction factor: 1.1 to 1.3; +, moderate effect, dose reduction factor: 1.3 to 2.0; ++, strong effect, dose reduction factor: > 2.0. Dose reduction factor is log survival of uninduced cells/log survival of cells after transient induction.

[b] In some experiments, a slight increase in survival after transient induction was observed.

[c] Z. Trgovčević, D. Petranovic, and V. Zgaga (unpublished data).

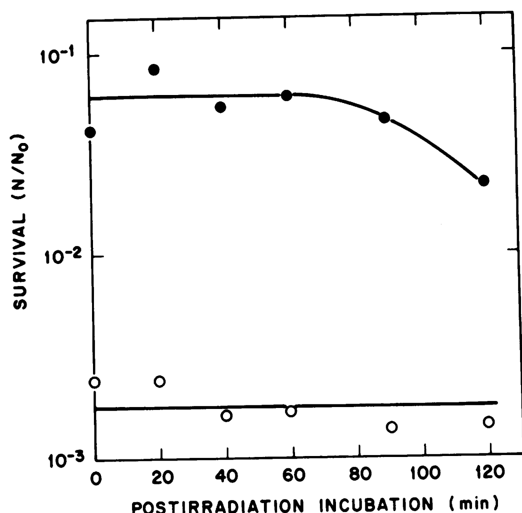

FIG. 6. *Effect of postirradiation incubation at 30 C on red-promoted resistance. At the times indicated, surviving cells of E. coli recB21 lysogenic for λcI857 and irradiated with 10 krads were either scored directly without any additional treatment* (O) *or were first pulse heated for 5 min at 42 C and then scored* (●).

studies of Kapp and Smith (10) indicate that the rejoining of X-ray-induced DNA strand breaks is less efficient in Rec⁻ strains than in Rec⁺ strains, one possible mechanism for λ bacteriophage-induced radioresistance could be to improve the repair of strand breaks. To answer this question, we examined the rejoining of X-ray-induced strand breaks in DNA under normal conditions and also under conditions where phage-induced radioresistance is expressed. The extent of strand rejoining is not altered by treatment that allows the expression of *red*-dependent radioresistance in a *recB* strain (Fig. 8). In more extensive kinetic studies with time points up to 60 min, we observed no effect of heat treatment on the rejoining of strand breaks in *recB*⁻ cells, nor was any difference between *rec*⁺ and *recB*⁻ cells found.

Effect of gam product on repair of strand breaks. Similar experiments were done with a *polA* strain to determine whether the presence of the *gam* product affected the repair of strand breaks. The results obtained with alkaline sucrose gradients in one experiment are presented in Fig. 9. The plots of acid-precipitable radioactivity after sedimentation in alkaline sucrose gradients show that the presence of the *gam* product after irradiation marginally, but significantly, increases the efficiency of repair (Fig. 9a). Thus, by comparing Fig. 9a with 9b, it is seen that the breakdown of irradiated DNA to acid-soluble material in the presence of the *gam* product is much lower than in its absence. Consequently, in the presence of the *gam* product, the actual fraction of DNA converted to high molecular weight is much greater.

A more complete set of results describing the effects of *gam* on the repair of DNA in the *polA* strain is presented in Fig. 10 in terms of the coefficient of repair described above. These experiments show that the coefficient of repair increases faster and to a higher level when the *gam* product is functioning. In the case of the *gam*⁻ mutation there is an intermediate response, which mimics the intermediate effect on cell survival (Fig. 6) and which may be due to a residual level of γ protein. Thus, in these three cases with the *polA* strain the repair of DNA, as

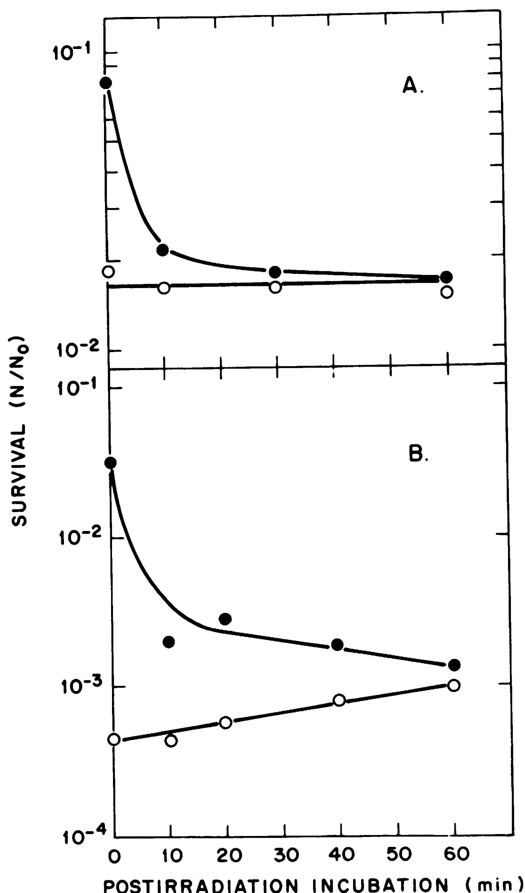

FIG. 7. *Effect of postirradiation incubation at 30 C on gam-promoted resistance in cells lysogenic for λcI857. Wild-type cells irradiated with 20 krads (panel A) and polA cells irradiated with 6 krads (panel B) were, at the times indicated, scored without any additional treatment* (O) *or were first pulse heated for 5 min at 42 C and then scored* (●).

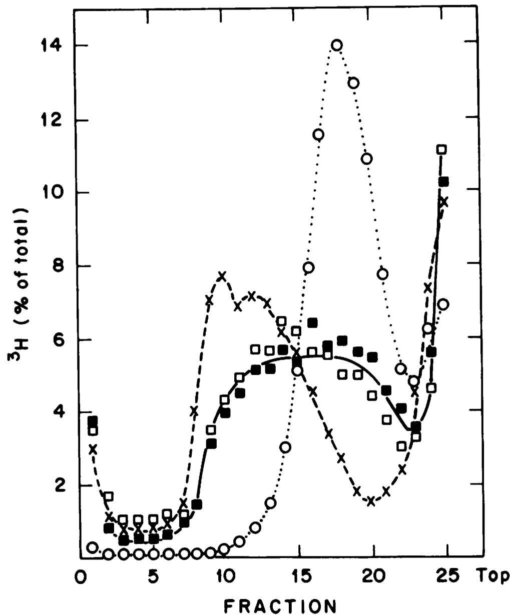

FIG. 8. *Sedimentation in alkaline sucrose of ^{3}H-labeled DNA from E. coli cells carrying recB21 and lysogenic for λcI857. Symbols: ×, unirradiated control; ○, unheated lysogens immediately after 10 krads; □, unheated lysogens after 10 krads and 45 min of incubation at 30 C; ■, lysogens heated at 42 C for 5 min, exposed to 10 krads of X rays, and incubated 45 min at 30 C.*

measured by the coefficient of repair, parallels the changes in survival measured under similar conditions in Fig. 4.

DISCUSSION

Our results support the earlier suggestion that there are two separate types of radioresistance dependent on transient induction of λ bacteriophage (28). They can be differentiated by a requirement for either the gam^{+} or red^{+} genes of λ.

The genetic data show that *red*-dependent repair occurs in $recB^{-}$ strains. This suggests that λ *red* products are able in some way to compensate for the absence of the RecBC nuclease in the repair of DNA. The biochemically relevant reaction of these enzymes is uncertain, but the studies in this paper show that the strand rejoining of preexisting DNA, as measured by centrifugation in alkaline sucrose gradients, is not the critical step. Since the λ *red* products can be introduced 1 h after irradiation without any decrease in their effectiveness, it is evident that their presence is not needed at early stages of repair that occur immediately after irradiation. Perhaps in the $recB^{-}$ strain the damaged DNA survives longer or stays intact without degrading into a form which cannot be repaired. Alternatively, if these enzymes were involved in a post-replication mode of X-ray repair (23), the results obtained with the sucrose gradients would be expected, but there is no direct evidence for this hypothesis.

The effect of certain other mutations in the *recB* background on *red*-dependent radioresistance is observed in the *recB recC sbcA* strain, recombination pathways described by Clark (3). It is somewhat surprising that a very marked *red*-dependent increase in radioresistance is observed in the *recB recC SbcA* strain, since it is known that in this strain an adenosine 5′-triphosphate-independent nuclease (1, 3, 13) is made that compensates for the absence of RecBC enzyme. Perhaps the *red*-dependent radioresistance in this case can be explained by a "gene dosage" effect if the level of a new

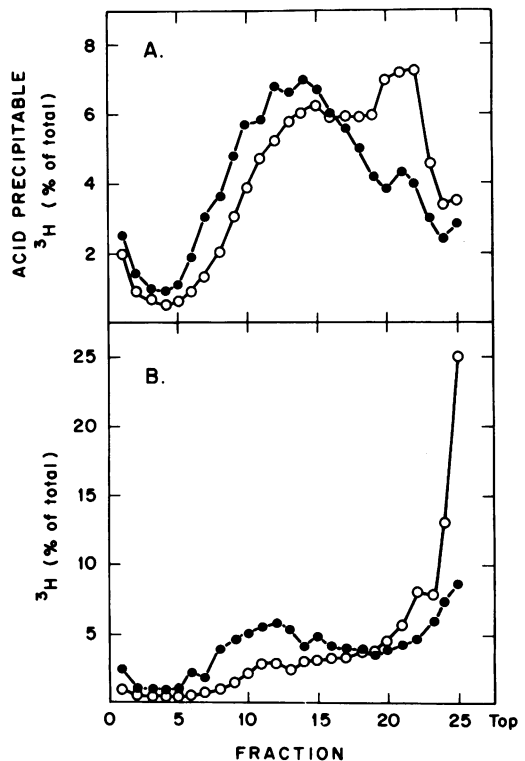

FIG. 9. *Sedimentation in alkaline sucrose of ^{3}H-labeled DNA isolated from polA cells lysogenic for λcI857. Lysogens were irradiated with 10 krads of X rays and then incubated at 30 C for 60 min. Panel A: acid-precipitable ^{3}H; panel B: total ^{3}H. Symbols: ○, no pretreatment; ●, pretreatment at 42 C for 4 min. The points in panels A and B are from a single experiment in which aliquots of the same cells were run in separate tubes in the same run.*

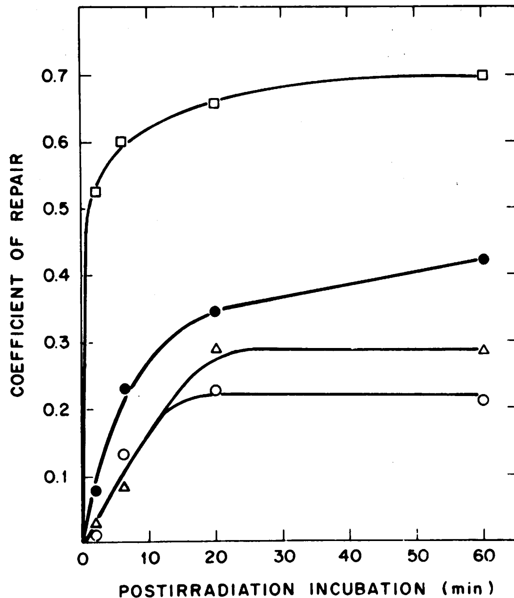

FIG. 10. *Effect of λ bacteriophage gene products on the kinetics of DNA repair as evaluated by changes in the coefficient of repair. All strains were irradiated with 10 krads of X rays at zero time and then incubated at 30 C. At the indicated times, samples were taken for centrifugation in alkaline sucrose gradients. From the distribution of total radioactivity in these gradients, the coefficient of repair was calculated as described in the text and in the legend to Fig. 1. Symbols: □, wild-type lysogenic for λcI857 gam⁺; ○, polA1 lysogenic for λcI857 gam⁺, no pretreatment; ●, polA1 lysogenic for λcI857 gam⁺, pretreated at 42 C for 4 min; Δ, polA1 lysogenic for λcI857 gam210, pretreated at 42 C for 4 min. The overlap at t_0 was 0.50 for the wild-type strain and 0.31 for the polA1 strains.*

endogenous nuclease is suboptimal.

Certain conclusions about the role of exonuclease I can also be drawn from the low level of *red*-dependent radioresistance in the strain carrying *recB recC xonA* and the absence of any λ-dependent resistance in the *recB recC sbcB* strain. It has been suggested that these strains may differ in their residual levels of exonuclease I, with more activity being present in a *xonA* than in an *sbcB* strain (12, 31). If this is correct, it may be that exonuclease I is required in conjunction with the λ *red* products for radioresistance to be expressed. Thus, the low level of radioresistance observed in the *xonA* strain may be due to some residual activity of exonuclease I, whereas in the *sbcB* strain there is too little exonuclease I to give any detectable effect.

From a similar set of experiments, it can also be argued that the *recF* product is needed for effective *red*-dependent repair, because little, if any, λ-dependent resistance is observed in the *recB recC recF* and *recB recC sbcB recF* strains.

When λ-dependent radioresistance occurs in cells that are $recB^+$ $recC^+$, the *gam* gene of λ is required. Our results show that γ protein must be present during the first few minutes after irradiation to exert its effect. Much of the radioresistance is lost when the heating to induce λ is delayed until only 10 min after X irradiation (Fig. 7).

The results with the alkaline sucrose gradients provide a partial explanation for the mechanism of *gam*-dependent radioresistance. These experiments show that γ protein decreases breakdown of DNA, whereas concomitantly a greater fraction of the DNA is converted back to a high molecular weight. It has already been demonstrated in other systems, in whole cells and in partially purified extracts, that γ protein inhibits the RecBC nuclease (24, 29, 30). This suggests that the RecBC nuclease may attack sensitive sites that are present shortly after irradiation and cause the DNA degradation. In the presence of γ protein, this attack is restrained or altered, thus decreasing DNA breakdown. It is evident that if both DNA strands are removed by DNA degradation (5, 19), there can be no subsequent repair. When damaged DNA is spared from breakdown by the presence of γ protein, it can then be a substrate for further reactions that may lead to successful repair. However, it should be emphasized that prevention of DNA breakdown by itself is not equivalent to successful repair; it only prolongs the lifetime of the damaged DNA molecule, allowing it to undergo subsequent repair processes. Unfortunately, the present state of our knowledge does not allow us to be more precise about the specific molecular alterations that are involved in this repair.

As we have pointed out earlier (28), there is an apparent paradox in the action of RecBC nuclease when related to the survival of X-irradiated cells. The presumed inhibition of the nuclease by the γ protein increases survival, whereas removing the active enzyme by introducing a *recB* or *recC* mutation decreases cell survival. A consistent explanation of these data is that the initial action of the enzymes is detrimental, perhaps by interfering with the repair of strand breaks, whereas its action at late stages may be necessary for the successful repair of lesions other than strand breaks. The possibility that the RecBC nuclease can act at late stages is suggested by the observation that the λ *red* products, which seem to compensate for the missing exonuclease V in $recB^-$ cells, exert their full effect when introduced as much

as 1 h after irradiation. Consistent with this idea is the finding of Birge (2) that the introduction of RecBC nuclease 2.5 h after the formation of zygotes can still stimulate recombinant formation.

It is unlikely that the RecBC nuclease is permanently inhibited by γ protein, since the transiently induced *polA1* lysogen shows normal viability. It has been demonstrated by Monk and Kinross (16) that the double mutant carrying the *polA12* allele, which leads to the synthesis of temperature-sensitive DNA polymerase I, and a *recB* mutation are inviable at restrictive temperature. This strongly indicates that the inhibition of RecBC nuclease by γ protein is incomplete or temporary or both. The continued synthesis of RecBC nuclease in transiently induced lysogens will eventually titrate out the γ protein, even if the initial inhibition has been complete. Consequently, the "late" repair process that requires RecBC nuclease can be successfully carried out.

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