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Interaction of Bacterial and Lambda Phage Recombination Systems in the X-Ray Sensitivity of *Escherichia coli* K-12

(DNA/repair/λ bacteriophage *red* and *gam* genes/*E. coli recB* gene)

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ABSTRACT ***E. coli* cells lysogenic for the thermoinducible prophage λcI857 can be transiently induced by a brief heat treatment. Although this treatment does not kill the cells, some λ products normally formed during vegetative phage development are made that can alter the response of host cells to x-irradiation by causing an increase in radioresistance. This increased resistance is particularly striking in the recombination-deficient *recB*$^-$ strain, which is normally much more radiosensitive than its *recB*$^+$ parent. After pulse-heating at 42°, the survival curve of *E. coli recB*$^-$ lysogenized with λcI857 does not differ from that of the wild-type strain. Since λ *red* mutants do not increase the radioresistance of *recB*$^-$ strains, both λ *red* gene products, λ exonuclease and β-protein, are required to compensate for the missing *recB* product. Furthermore, phage-induced radioresistance also occurs in *recB*$^+$ lysogens even when they carry λ *red*$^-$, but not when the λ prophage is *gam*$^-$. Thus, in wild-type cells, phage-induced radioresistance requires some interaction between the bacterial *recB* gene product (exonuclease V) and the phage γ-protein.**

X- or gamma-ray-induced damage of bacterial cells is subject to repair by several enzymatic systems that are specified by bacterial genes, a number of which have been located on the genetic map of *Escherichia coli* (1).

One interesting example of repair is seen in the case of phage-induced radioresistance of lysogenic bacteria (2). It differs from other types of repair operating on bacterial DNA in that it appears to depend on products specified by the bacteriophage. This type of repair can easily be demonstrated in *E. coli* cells lysogenized with λcI857, a thermally inducible phage mutant that is not induced by either ultraviolet (3) or gamma rays (2), and which was originally derived from λ *ind*. Lysogens containing λcI857 can survive a short heat treatment in spite of the synthesis of some proteins normally formed during vegetative phage development. Some of these products, which are apparently harmless to bacteria, can bring about a considerable increase in the survival of host cells exposed to ionizing radiation (2).

Our present study was undertaken to identify the λ gene(s) involved in phage-induced radioresistance of lysogens of wild type *E. coli* and also of the radiosensitive mutants carrying *recB*. Since the bacterial recombination system appears to contribute to the repair of radiation damage (4–6), we have tested the contribution of the λ recombination system in phage-induced radioresistance of these strains.

The data presented in this paper show that products of the genes of the recombination region of the phage λ can be utilized in the repair of bacterial DNA. In particular, proteins specified by λ *red* genes can effectively compensate for the lack of host-cell *recB* product in the repair of x-irradiated DNA.

Abbreviation: YET, yeast extract–tryptone growth medium.

MATERIALS AND METHODS

Bacterial Strains. The *E. coli* strains used in this study and their relevant genetic characteristics are as follows:

AB1157 (7), a standard recombination-proficient strain, obtained from Dr. E. A. Adelberg, and carried in our collections for many years.

AB2470 (4), *recB21* derivative of AB1157.

DM456 (8), Su$^-$ recombination-proficient strain, obtained from Dr. K. B. Low.

KL168 (9), Su$^-$ *recB21*, obtained from Dr. K. B. Low.

Bacteriophage Strains. The bacterial strains were made lysogenic by the following λ mutants:

λcI857 (3): all the λ strains used in this paper carry the marker cI857, which makes them thermoinducible.

λcI857 *red3* (10): obtained from Dr. C. Radding. *red3* is defective in the production of both λ exonuclease and β protein. This phage was tested and found to be recombination-defective by the spot test of Signer and Weil (12).

λcI857 *red329* (11): obtained from Dr. C. Radding. *red329* is defective in the production of λ exonuclease.

λcI857 *red113* (10): obtained from Dr. C. Radding. *red113* is defective in the production of β protein.

λcI857 *gam210* (13): obtained from Dr. C. Radding. *gam210* is an *amber* mutation. Neither *gam210* nor *red3* is able to grow on *E. coli* strains carrying *polA* (13). We showed that *gam210* and *red3* can complement each other during growth on a *polA* strain, and used this test to confirm the presence of *gam* in this phage strain.

λcI857 *bio69* (14, 15): obtained from Dr. K. B. Low. The *del* and the *red exo* genes are deleted from this strain.

λcI857 *red270 gam210 del8* (Spi$^-$; see ref. 14): obtained from Dr. J. Zissler. *del8* is a pleiotropic mutation affecting the expression of *amber red* and *gam* mutations on an Su$^+$ host.

Experimental Procedure. Lysogenic cells were grown overnight for 18 hr at 30° with aeration in YET broth containing 5 g of yeast extract, 10 g of tryptone, 10 g of NaCl, and 120 mg of NaOH per 1000 ml of water. All results in this paper are for stationary phase cells grown in this way.*

* In experiments with logarithmic phase cells not included here, the results for *recB*$^-$ cells were qualitatively and quantitatively similar to those in this paper; however, wild-type cells in the logarithmic phase of growth showed little or no phage-induced radioresistance.

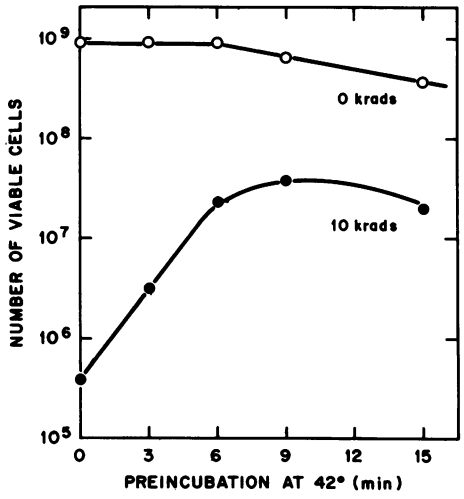

FIG. 1. Effect of pulse-heating on survival of control and x-irradiated *E. coli* AB2470 *recB21* lysogenic for λcI857 *red*$^+$ *gam*$^+$. Symbols: unirradiated control (○); 10 krads (●).

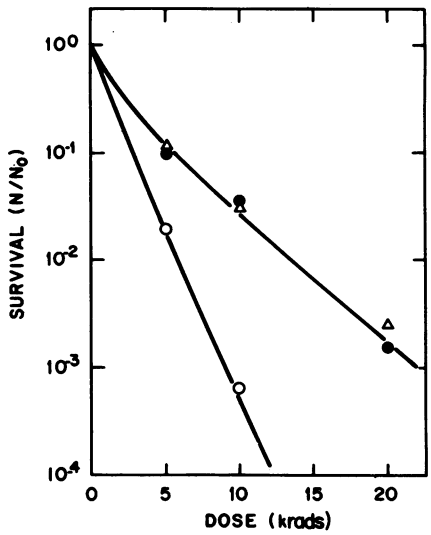

FIG. 2. Surviving fraction of heated and unheated lysogens as a function of x-ray dose. Symbols: AB2470 *recB21* lysogenic for λcI857, unheated (○), or pulse-heated at 42° for 6 min (●); unheated AB1157 *recB*$^+$ lysogenic for λcI857 (△). N is number of viable cells; N_0 is numbers of cells before irradiation.

An 0.1-ml aliquot of the overnight culture was then diluted 50-fold with prewarmed YET medium in order to achieve an immediate shift in temperature from 30° to 42°. The bacteria were thereafter incubated at 42° with aeration. At suitable time intervals, 0.1-ml samples were withdrawn, chilled by dilution with 0.9 ml of ice-cold YET broth, x-irradiated, and kept at 0° until plating.

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 5000 rads/minute.

Both nonirradiated and irradiated cell suspensions were further diluted with buffered saline and spread on YET agar plates (YET medium plus 2% agar). Colonies were counted after 1 day (AB1157) or 2 days (DM456, AB2470) or 3 days (KL168) of incubation at 30°.

RESULTS

The Effect of Transient Induction of λ *on Radiosensitivity of the recB*$^-$ *Host.* As shown in the upper curve of Fig. 1, the AB2470 *recB21* strain lysogenic for the thermoinducible phage λcI857 can be heated for 6 min at 42° without any significant loss of colony forming ability. Since the cI857 repressor is inactivated within a few seconds (16), expression of normally repressed λ genes is expected to begin during this period. The presence of viral products is clearly evident in the change of the cells' response to x-rays. As a result of transient induction, i.e., of pulse-heating at 42°, a striking increase in radioresistance occurs (the lower curve of Fig. 1). Control experiments show that pulse-heating of nonlysogenic cells does not change the radiosensitivity (data not presented). The increased survival in the *recB* lysogen is more pronounced than that described earlier for wild-type lysogens (2). As seen in Fig. 2, the survival of the *recB*$^-$ lysogen heated for 6 min is very similar to that of wild-type lysogens. Heating for longer than 6 min can even increase the survival above that of the wild type, but such a treatment was not used in the experiment shown in Fig. 2, because it reduces the number of colony formers in the unirradiated control.

The Role of λ *Red Products.* In order to identify λ genes, whose products confer increased resistance on the *recB* bacterial host, we used lysogens carrying λ mutants *red* and *gam*. As illustrated in Fig. 3, the *recB*$^-$ lysogen carrying λcI857 *red3* does not show any detectable increase in radioresistance upon heating. Similar results were also obtained with *recB* strains carrying λcI857 *red329* and λcI857 *red113* (data not shown); moreover, the latter (defective in the production of β-protein) was slightly more sensitive after heat treatment.

Although not as striking as in *recB*$^-$ bacteria, phage-induced radioresistance also occurs in wild-type cells lysogenized with λcI857. It might be expected that, for this increased resistance, the products of λ *red* genes are needed too. The experiment presented in Fig. 4 shows, however, that this is not the case: a similar increase in resistance after pulse-heating occurs in wild-type lysogens carrying either λcI857 *red3* or λcI857 *red*$^+$.

The Role of γ*-Protein.* In addition to the *red* gene products, a protein coded by the λ gene *gam* (13) is also of interest in the study of phage-induced radioresistance because it may interact with certain bacterial recombination enzymes. Since *gam210*, used in our study, is an *amber* mutation, appropriate nonpermissive hosts were lysogenized for λcI857 *gam210*. Their radiosensitivities after pulse-heating were assayed. Fig. 5 shows that the *recB*$^-$ lysogen displays approximately the same increase in survival after transient induction of either *gam*$^+$ or *gam*$^-$ prophages. In marked contrast to this, the functional *gam*$^+$ phage gene is required to produce an increase in radioresistance of the wild-type strain (Fig. 6).

The requirement for the *gam* gene is also shown in additional experiments (data not shown). The wild-type cell lysogenized with λcI857 *bio69*, in which the genes for *del* and *red exo* are deleted (14), shows the same increase in resistance after pulse-induction as either the *red*$^+$ or *red*$^-$ lysogens (Fig. 4). However, the triple mutant, *del8 red270 gam210*, does not display the increased resistance upon pulse-heating. It is, therefore, obvious that the absence of the *gam* gene product abolishes the phage-induced resistance in the wild-type cell.

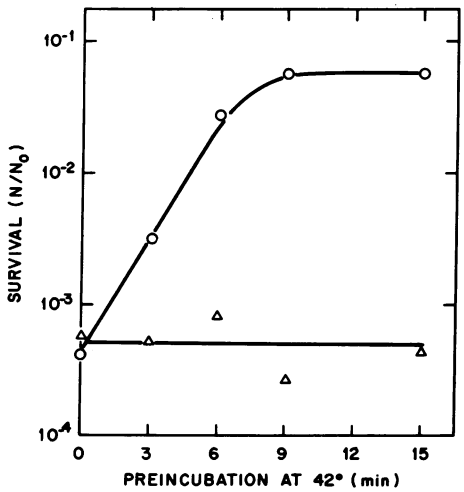

FIG. 3. Effect of *red* gene on the survival of pulse-heated *recB21 E. coli* irradiated with 10 krads. Symbols: AB2470 *recB21* lysogenic for λcI857 *red*$^+$ (○); AB2470 *recB21* lyosgenic for λcI857 *red3*(△).

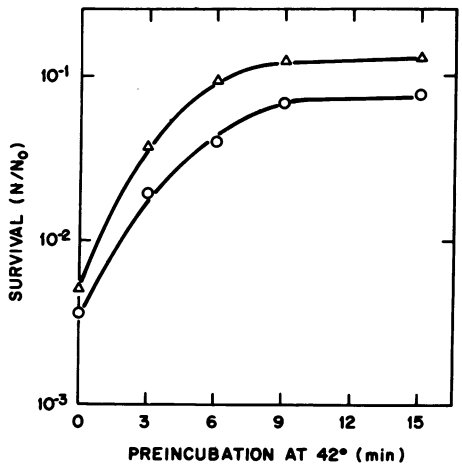

FIG. 5. Effect of λ *gam* gene on the survival of pulse-heated *recB21 E. coli* irradiated with 10 krads. Symbols: KL168 *recB21* lysogenic for λcI857 *red*$^+$ *gam*$^+$ (○); KL168 *recB21* lysogenic for λcI857 *red*$^+$ *gam210* (△).

DISCUSSION

The results can be summarized as follows. First, transiently induced λ can provide the *recB*$^-$ bacterial host with some products that increase the cell survival to at least wild-type level. Functional λ *red* genes for both λ exonuclease and β protein are needed to produce this effect.

Second, an increase in radioresistance of wild-type lysogens is observed upon transient heat induction, and this requires the *gam*$^+$ gene product of phage λ but not the products of the *red* genes.

An interpretation of the results obtained with the *recB*$^-$ host can be easily suggested. It is known that the phage λ can recombine by means of the host-cell recombination enzymes (12, 17). The host-cell recombination enzymes also can participate in the repair of ultraviolet lesions of the λ DNA (6, 18). Our results show that the λ *red* products can effectively substitute for the bacterial *recB* product in the repair of x-irradiated host DNA. The results of K. B. Low (personal communication), which demonstrate that the λ *red* gene proteins can contribute to bacterial recombinant formation in *recB*$^-$ or *recC*$^-$ cells, are in accord with our findings.

The experiments with the wild-type host that demonstrate an effect of the phage γ-protein are more difficult to interpret. Since the results of Figs. 3 and 5 show that γ-protein has no effect on the survival of x-irradiated *recB* strains, the changes in sensitivity observed in the wild-type strain require the simultaneous presence of γ-protein and the bacterial *recB*$^+$ gene product, and may be a consequence of an interaction between them. It has been previously suggested that γ-protein inhibits exonuclease V, the protein that is absent from *recB* and *recC* cells (19–21), and that γ-protein converts a *recB*$^+$ cell to a RecB$^-$ phenotype as a consequence of this inhibition (22, 23). From this model, it is predicted that the transient induction of the *red*$^-$*gam*$^+$ phage in the wild-type bacterium should inhibit exonuclease V and increase the radiosensitivity to that of the *recB* strain. However, in marked contrast to the response expected for the RecB$^-$ phenotype, the survival of the x-irradiated wild type strain increased after transient induction of the λ *red*$^-$*gam*$^+$ phage (Fig. 4). Thus, when survival

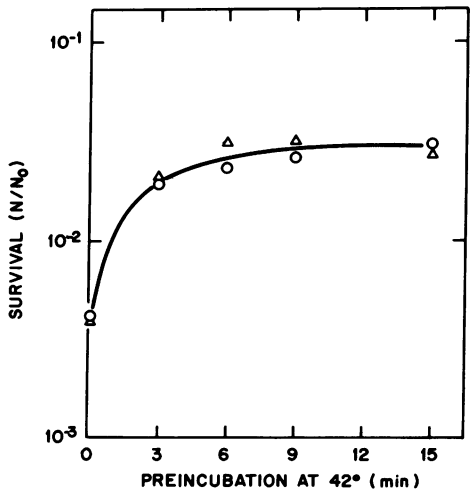

FIG. 4. Effect of λ *red* gene on the survival of pulse-heated *recB*$^+$ *E. coli* irradiated with 20 krads. Symbols: AB1157 *recB*$^+$ lysogenic for λcI857 *red*$^+$ (○); AB1157 *recB*$^+$ lysogenic for λcI857 *red3* (△).

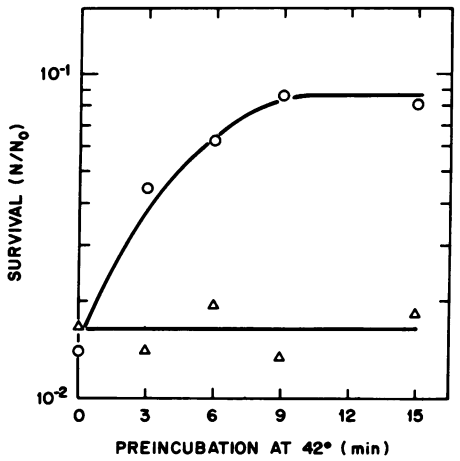

FIG. 6. Effect of λ *gam* gene on the survival of pulse-heated *recB*$^+$ *E. coli* irradiated with 20 krads. Symbols: DM456 *recB*$^+$ lysogenic for λcI857 *red*$^+$ *gam*$^+$ (○); DM456 *recB*$^+$ lysogenic for λcI857 *red*$^+$ *gam210* (△).

of x-irradiated cells is used as the criterion, γ-protein does not seem to convert *recB*$^+$cells to a RecB$^-$ phenotype.

Since *recB*$^+$ cells are more resistant than *recB* cells, the presence of exonuclease V benefits the x-irradiated bacteria. However, the simultaneous presence of γ-protein and exonuclease V is even better, since there is higher survival than in the absence of γ-protein. Although there are various interpretations of this effect, we shall consider three of them that involve interaction of the γ-protein and exonuclease V. The first possibility is a *differential inhibition* by γ-protein of the exonuclease V activities. The purified exonuclease V is a complex enzyme with at least four different known activities (24–26). Perhaps one (or more) of these activities increases the survival of x-irradiated cells while others decrease survival. In the absence of γ-protein, the balance of these activities would lie slightly toward the survival-promoting activity. If γ-protein preferentially inhibits the detrimental activity, the beneficial activity would then become more pronounced and result in the higher survival of the transiently induced x-irradiated cells.

Another possibility is that the presence of γ-protein results in only a *partial inhibition* of exonuclease V, and that an intermediate level of the exonuclease V activity is better for the x-irradiated cell than either a high level or its complete absence. For example, a limited nuclease attack might be required for successful DNA repair while extensive DNA degradation could prevent the successful completion of repair. This possibility is consistent with recent results of Sakaki *et al.* (27), who have purified the γ-protein and shown that it inhibits all known activities of the RecBC nuclease.

Finally, a third possibility is that γ-protein combines with exonuclease V to produce a *modified enzyme* with a new activity different from the original activities of exonuclease V. According to this notion, the altered enzyme is more efficient than the original exonuclease V in increasing the survival of x-irradiated cells.

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