INDUCED RADIORESISTANCE IN FOUR STRAINS OF *ESCHERICHIA COLI*, TWO WITH LAMBDA LYSOGENS

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ABSTRACT Cells of *E. coli* that are *recA*$^+$ and *lex*$^+$ show a phenomenon of induced radioresistance. A preexposure to ultraviolet light, or ionizing radiation followed by incubation to allow protein synthesis, followed by treatment with rifampin to prevent further induction, renders the cells resistant to further doses of radiation. When this is attempted with lambda lysogens of the same strains, no radioresistance is seen, even though the preexposure is too small to induce lambda itself. If the lysogens are *ind*$^-$, namely λCI857, about the normal radioresistance can be developed by pretreatment. These findings suggest that the lambda repressors can bind to single-strand breaks caused by the inducing agent and can modify the course of induction.

INTRODUCTION

Cells of *E. coli* that are neither *recA*$^-$ or *lex*$^-$ show an induced radioresistance (IRR) when pretreated by ultraviolet light (UV), ionizing radiation, or nalidixic acid as inducing agents (1,2).

The experimental procedure is to give the inducing dose or fluence, incubate for 40 min, then block further transcription with rifampin, and irradiate with graded doses of X- or gamma rays. In the induced cells of suitable strains an increase is observed in the radioresistance that can be as high as three or four times that of induced cells. This increase is dependent upon the radiation genetics of the particular strain. Thus strains AB1886 (*uvrA*$^-$) and AB1157 show an increase between two- and fourfold, respectively, with this treatment.

It occurred to us that since the inducing dose for this phenomenon is considerably less than that needed to induce phage lambda (3–5) in lysogens of lambda, it might be possible to elicit the radioresistance without the induction of the prophage. However, it was found that radioresistance could not be induced in such lysogens. We then attempted to see whether this effect was concerned with the inducibility of the prophage by examining the inductionless prophage λCI857, in which we found that radioresistance could be about normally induced. The interaction between the lambda repressors and the induction system may be responsible for this behavior.

TABLE I

SOURCES OF STRAINS USED

Strain	Source
AB1157	Dr. R. Boyce, Biochemistry Dept., U. Florida
AB1157λ	Dr. R. Boyce, Biochemistry Dept., U. Florida
AB1157λCI857(*ind*$^-$)	Dr. Petersen, Bureau of Radiological Health
AB1186 (*uvrA*$^-$)	*E. coli* genetic stock center
AB1186λ	The Pennsylvania State University stocks (Dr. B. K. Lydersen)
AB1186λCI857(*ind*$^-$)	The Pennsylvania State University stocks (Dr. B. K. Lydersen)

MATERIALS AND METHODS

Table I shows the bacterial strains used. AB1157 is essentially wild type as to radiation sensitivity genes. Strain AB1886 is *uvrA*$^-$, deficient in the first step of the excision process. The lysogens were constructed by Dr. R. Boyce (University of Florida), Dr. Petersen (Bureau of Radiological Health), or previously by Dr. B. K. Lydersen at The Pennsylvania State University.

The technique of growing and plating the cells has been described by Pollard and Achey (1). The only unusual feature in this work is the addition of rifampin, to prevent further induction, 20 min before the gamma or X-ray irradiation is given.

For the irradiation with UV two sources were used. A B-H6 mercury arc (General Electric Co., Cleveland, Ohio) and double monochromator in the Zoology Department at Duke University provided 265-nm radiation, incident vertically downward on a photocell. Interposed between the photocell and the beam is a silica dish containing the sample to be irradiated. The amount of radiation is estimated first by reading with distilled water in the silica dish (I_0) and then with the sample in its culture medium in the dish (I_t). The average intensity can be calculated from these incident and transmitted radiation intensities, and is $(I_0 - I_t)/\ln(I_0/I_t)$. Other series of irradiations in the Penn State biophysics laboratory used a germicidal lamp, partly wrapped with tape to diminish the output, placed 30 cm above the dish. Since the available meter had insufficient sensitivity for observation of the transmitted light at this distance, average intensities were based on measurements within 10 cm of the light source. I_0 and I_t at 10 cm were read without the sample and then with it, permitting the measurement of I_0/I_t. The calculation of average intensity as described above was then made by measuring the incident intensity of the light at 30 cm with two meters, one of which was a Blak-Ray meter (J225, Ultra-Violet Products, Inc., San Gabriel, Calif.), and the other a Jagger meter (6) calibrated by Dr. R. A. Deering (Biophysics Laboratories, Penn State). The average intensity was then derived from the observed incident intensity and the knowledge of I_0/I_t.

The meter used in some of the experiments, when the dose rate required that the distance from the light be 30 cm, read about six divisions. This measurement of the incident light could be made reliably to perhaps 7%, as the reading was quite steady. On occasion the transmitted light was fivefold reduced (about the extreme) and the reading of 1.4 scale divisions could be made with only about 25% accuracy. Since the absorption by the culture was not a function of the distance from the light source over the distance 30–10 cm, the method, as described, for measuring the I_0/I_t ratio was used. Knowing I_0 and I_0/I_t, one can then calculate I_t and apply the formula to derive the average dose.

The source of ionizing radiation was either a Picker 50-kV peak X-ray machine (Picker Corp.,

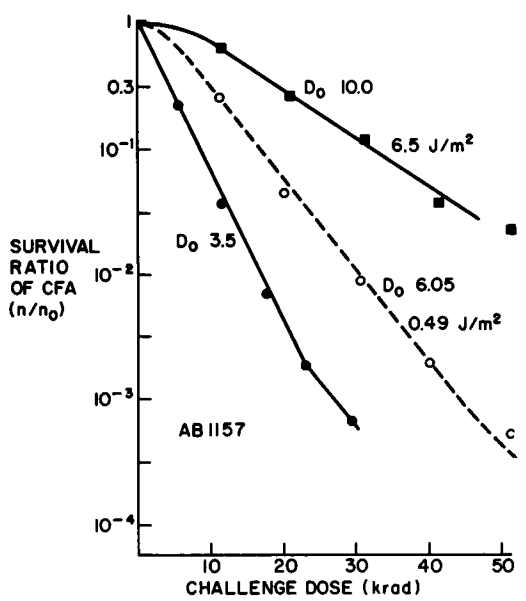

FIGURE 1

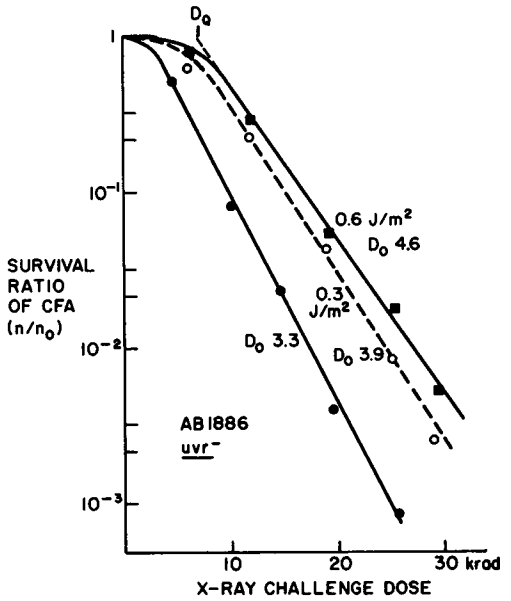

FIGURE 2

FIGURE 1 Induced radioresistance in strain AB1157 (wild type). Cells which have received 6.5 J/m^2 of 265 nm UV, incubated 45 min, given rifampin for 10 min, and then subjected to varying doses of ionizing radiation show a considerably larger ratio of colony-forming ability (CFA) remaining after each X-ray dose. A much smaller prefluence of 0.49 J/m^2 also produces a clearly observable radioresistance.

FIGURE 2 Data similar to those of Fig. 1, but for the *uvr*$^-$ strain AB1886. The radioresistance is less marked, but definite, and occurs for much smaller prefluences.

Cleveland, Ohio) or a Gammacell 200 (Atomic Energy of Canada Ltd., Ottawa, Ont.). The X-ray tube was a beryllium window OEG 50 (Machlett Laboratories, Inc., Stamford, Conn.) with 50 mg Al/cm^2 filtration.

RESULTS

Fig. 1 shows the results obtained with the wild-type strain cell AB1157. It can be seen that a pretreatment of 6.5 J/m^2 markedly increases the resistance to the challenge dose of ionizing radiation. It also increases after a pretreatment of 0.49 J/m^2. There is an increase both in a shoulder seen at low doses and in resistance as a reciprocal of the limiting slope. For the wild-type strain the increase in the shoulder is not as marked as in other strains. The best indication of the change in radioresistance is in the value of the downward slope of the surviving fraction graph, usually expressed in reciprocal as D_0. It can be seen that a lower dose of radiation gives less protection and the value of D_0 is lower.

Similar data for strain AB1886 are shown in Fig. 2. It is interesting that in this *uvr* strain, the radioresistance is revealed in the shoulder more than in the slope. As an estimate of the change in the shoulder an intercept dose (D_Q) is used, as indicated.

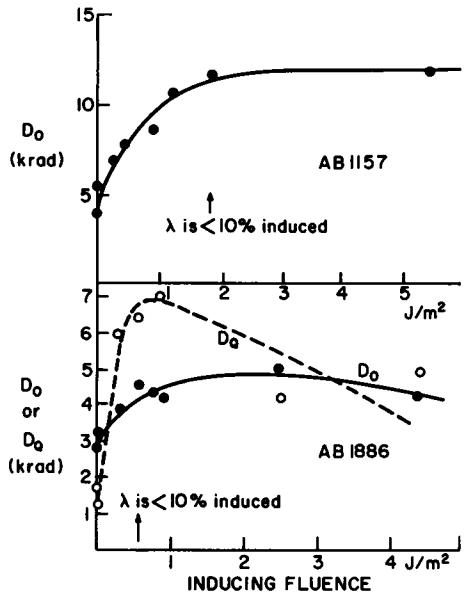

FIGURE 3

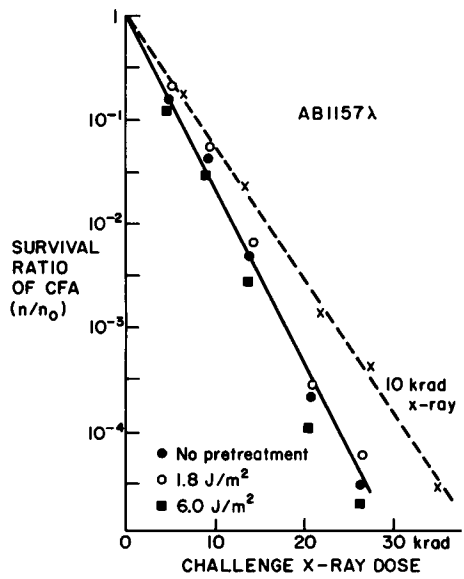

FIGURE 4

FIGURE 3 The variation of the slope of the survival curves, D_0, and, in the *uvr*⁻ case, of the intercept made by the line with the unity ratio axis, designated D_Q, with the inducing prefluence. Also indicated is the approximate dose at which lambda is induced in the corresponding lysogens. It is clear that there is considerable radioresistance at prefluences for which very little induction of lambda has occurred. Thus small prefluences should induce radioresistance in lambda lysogens.

FIGURE 4 The effect of three pretreatments on the sensitivity of AB1157λ. For the prefluence of UV there is no observable effect. A very slight effect seems to be present with a predose of 10 krads of X-rays.

Again, it can be seen that increasing the dose does have some effect both on the slope and on the value of D_Q, but also that for this strain of cells the doses necessary to elicit the response are smaller than for the wild type.

Results for a number of such dose-response curves are shown in terms of D_0 or D_Q in Fig. 3. The upper panel is for strain AB1157. As the inducing dose is increased to about 3 J/m², the value of D_0 changes, reaching 12 krads. For strain AB1886 a somewhat different pattern is seen. A relatively smaller change in D_0 occurs as the inducing dose increases and the change reaches a maximum somewhere between 1 and 2 J/m². A much more marked effect upon D_Q reaches maximum at around 0.8 J/m² in inducing dose. On both of these panels are shown our findings for the 10% induction of lambda, which suggested to us the possibility of inducing radioresistance substantially before induction of lambda. Two methods were used to observe the dose-response relation for the induction of lambda. The first was the observation of free phage released at 80 and 100 min of incubation at 37°C for various inducing doses. The second was the concomitant loss of colony-forming ability at the same doses. If cells form

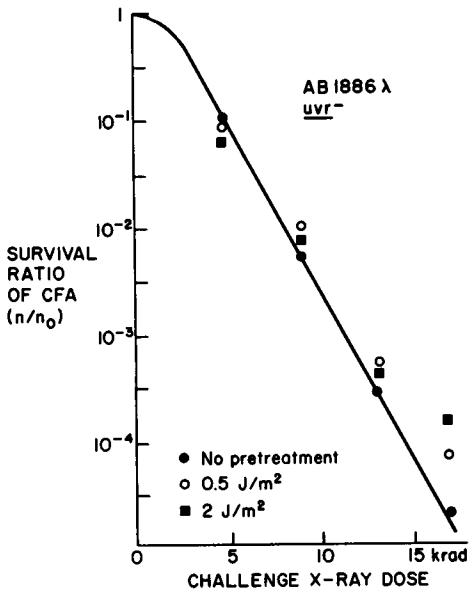

FIGURE 5

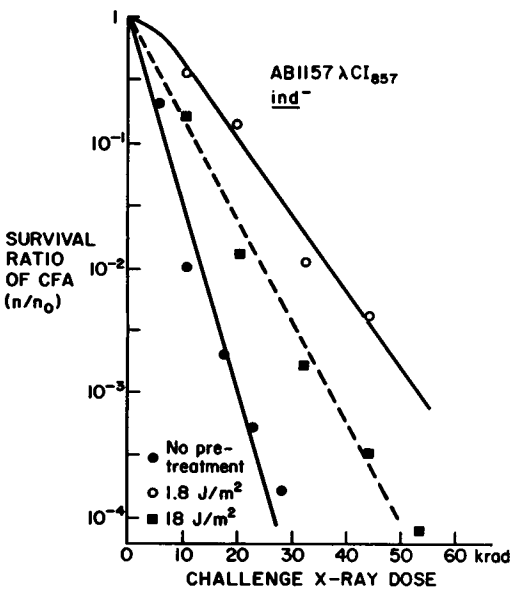

FIGURE 6

FIGURE 5 Similar data to those shown in Fig. 4, but for the *uvr*⁻ strain. No radioresistance is observed in the lysogen.

FIGURE 6 Observations on the noninducible temperature-sensitive strain λCI857. The *uvr*⁺ case here shows radioresistance clearly. The behavior is not quite identical to the nonlysogen, for the dose of 18 J/m² shows a reduced resistance to a greater extent than in the nonlysogenic cell.

colonies, they are certainly not induced for lambda. The less than 10% figures are based on these data. They are in substantial agreement with the findings of Monk et al. (5).

In Fig. 4 we show the effect of preliminary irradiations with X-rays or UV on the lambda lysogen of the strain AB1157. These cells are more sensitive to the X-ray challenge dose even without such pretreatment. It is also quite apparent that UV pretreatments with 1.8 J/m², which should not have induced any more than 10% lambda, and with 6.0 J/m², which would be more effective in induction, fail to increase radioresistance. There is a small amount of radioresistance for pretreatment with 10 krads of X-rays. Fig. 5 shows similar data taken with strain AB1886. Again, both a moderate and a larger dose of UV fail to develop radioresistance.

Results for the *ind*⁻ ts lysogen, AB1157λCI857, are shown in Fig. 6. With no pretreatment, the sensitivity is approximately the same as for the nonlysogen. A relatively small predose of 1.8 J/m² shows about the same amount of radioresistance as for the nonlysogen. If, however, the predose is increased to 10 times that amount, there is less protection in the lysogen. Thus, the phenomenon of induced radioresistance can be detected even in a cell lysogenic for lambda, with, however, some difference in the dose-respose relationship. The difference between the two responses seems to be in the

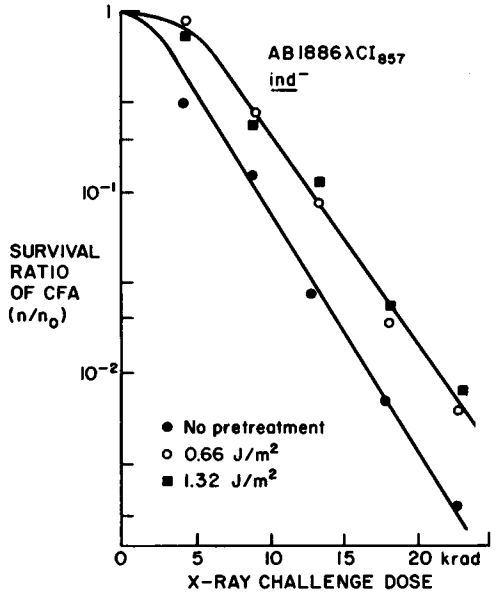

FIGURE 7 Data similar to those in Fig. 6, but taken with the *uvr*⁻ lysogen. Radioresistance is apparent, but slightly less than in the nonlysogenic strain.

lambda repressor, which cannot be inactivated by treatment either with ionizing radiation or UV, but can be inactivated by temperature shift. In these experiments the temperature was kept at the permissive temperature of 30°C throughout.

Fig. 7 shows the results for the *uvr*⁻ strain with the same phage λCI857 strain. Protection by pretreatment is somewhat less than observed in the nonlysogen, but it is quite definite.

These results lead us to the conclusion that the normal lambda lysogen does not show the phenomenon of radioresistance if UV is used as the inducing agent, but does show it, although in rather less than normal amount, if ionizing radiation is used. If, however, the lysogen is *ind*⁻, something close to the normal induction of radioresistance is observed.

DISCUSSION

A single underlying interpretation of induced radioresistance (IRR) has not yet been advanced. IRR does require the *recA*⁺, *lex*⁺ genotype and conforms in dose-response relationships and inducing agents to other manifestations of induced repair. In this paper we report results with two lysogens at inducing doses that will induce lambda in only 10% or less of the cells and which produce less than 10% loss of colony-forming ability. We do not see IRR under these conditions. On the other hand, lysogens of λCI857, which can be induced by temperature shift but not by radiation treatment, can be induced for radioresistance in a nearly normal way.

These findings suggest to us that where lambda repressors are present, they are in-

volved in some way with the induction process, not only for lambda induction, but also for the inducible radioresistance. The inducible radioresistance requires *recA*$^+$ and *lex*$^+$ genotypes and hence is expected to be correlated with the induction of the *recA* gene product, now shown to be the same as "protein X" by Little and Kleid (7). The induction of lambda is also correlated with the need for the *recA*$^+$ genotype. However, where lambda induction by radiation or nalidixic acid occurs, the time before free phage appears is 20–30 min longer than when λCI857 is induced by temperature shift. In our experiments, with the cells at the lower temperature, this last lysogen, which is not inducible by radiation, gives nearly normal IRR, while the radiation-inducible lambda does not. If it were supposed that the radiation induction of lambda involves first the derepression of *recA*, and then the transcription of an agent that inactivates the lambda repressors, the 20–30 min is not out of line. However, our work suggests that the presence of the lambda repressors alters the dose needed for IRR. It is as though even with an inadequate dose to induce lambda though (in the nonlysogen) quite adequate to derepress *recA*, the presence of inducible lambda prevents either the derepression of *recA* or of its expression in IRR.

In seeking for some factor that might account for this, we call attention to the work of Sussman and BenZeev (8), who found that the lambda repressors bind to single-strand breaks while those of λCI857 do not. If the action of radiation were to cause single-strand breaks necessary for the derepression of *recA*, then the binding of lambda repressors to these breaks might prevent the next step, the induction of the *recA* gene product. Careful dose-response observations on the development of protein X in lambda lysogens might shed light on this point. However, since it is quite possible that irradiation of bacteria carrying inducible lambda with doses too low to cause induction as measured by cell killing or phage production will cause expression of some lambda genes, our experiments do not exclude the possibility that lambda proteins other than the lambda repressor might be responsible for the observed phenomenon.

We are grateful to Dr. Kenneth Krell of the Bureau of Radiological Health for suggesting that we try the *ind*$^-$ strain of lambda, to Dr. R. Petersen for supplying a lysogen, and to Cynthia Bennett for summer technical assistance in our Penn State laboratory.

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