Inactivation of Prophage in Ultraviolet-Irradiated *Escherichia coli*: Dependence on *recA* Gene Activity

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The fate of the prophage part of the lysogenic chromosome was followed in the course of post-ultraviolet incubation. For this purpose, λ cI857 ind prophage, which can be induced by heat but not by ultraviolet light, was used. The prophage, initially more resistant than its repair-proficient host cell, was rapidly inactivated. This inactivation was not caused by the impaired capacity of irradiated cells to support growth of the phage. Over the entire dose range tested, little, if any, sensitivity difference between the host and the prophage was found at the end of cell division delay. Rapid inactivation of the prophage was also observed in *uvr* cells after small doses of ultraviolet light. The same small doses did not cause inactivation in lysogens carrying a mutation in the gene *recA*. This suggests that the functional gene *recA* is required for inactivation of the prophage part of the lysogenic chromosome.

UV-induced damage of bacterial cells is subject to repair by several enzymatic systems specified by bacterial genes. These enzymatic systems can reverse the lethal effects of photoproducts in the bacterial DNA (13, 15). In the course of post-UV incubation, an increase in the biological activity of the irradiated DNA is therefore expected. However, our recent experiments with λ cI857 ind lysogens show that, instead of an increase, a drop in the biological activity of the prophage DNA takes place during a certain period of post-UV growth (8). This drop was suggested to be a consequence of the impaired chromosomal function in dying cells (8). The study of prophage inactivation in UV-irradiated *Escherichia coli* may thus aid us in the understanding of the specific causes of cell death under adverse circumstances.

In this paper we extend our earlier study on prophage inactivation in repair-proficient bacteria. We show that, over a wide range of UV doses tested, the process which inactivates the prophage is completed (or almost completed) before the end of cell division delay. Also included in this paper are the results with other bacterial strains carrying genetic defects for DNA repair that point to the key role of the *recA* gene in this inactivation process.

**MATERIALS AND METHODS**

**Strains.** The phage mutant used in our study was λ cI857 ind (12). This double mutant is characterized by thermoinducibility (12) and by the failure of UV- or gamma-ray induction (10, 12, 14). The following strains of *E. coli* K-12 were used: AB1157 (2, 5), a standard recombination- and repair-proficient strain; AB1886 *uvrA6* (4) and AB1885 *uvrB5* (4), excisionless derivatives of AB1157; and AB2453 *recA13* (6), a recombination- and repair-deficient derivative of AB1157. Strain AB1157 was obtained from W. D. Rupp (Yale University, New Haven, Conn.), while other bacterial strains were supplied by B. J. Bachmann (Yale University, New Haven, Conn.). Lysogenic derivatives of these strains carrying λ cI857 ind were prepared in our laboratory.

**Growth and irradiation of cells.** If not otherwise stated, the experiments were done with the stationary phase cells which had been grown at 30°C in TP medium (8). For UV irradiation, bacteria were centrifuged, washed, and suspended in 67 mM phosphate buffer (pH 7.0) to a density of 1 x 10⁷ to 5 x 10⁹ cells/ml. During irradiation the cell suspension was stirred continuously. The source of 254-nm light was a 30-W low-pressure mercury germicidal lamp (Philips). When repair-proficient AB1157 bacteria were irradiated, the dose rate was 1.5 W/m². For UV-sensitive mutants, the dose rate was lowered to 0.05 W/m². This was achieved by covering the lamp with nontransparent black paper except for a 30-mm segment in the center. For post-irradiation growth, the cells were collected by centrifugation and resuspended in the same volume of fresh TP medium.

**Post-irradiation platings.** At intervals during post-irradiation incubation, samples were taken for the determination of (i) the viable cell number, (ii) thermoinducibility of the λ cI857 ind lysogens, and (iii) capacity of nonlysogenic cells to promote growth of the unirradiated phage.

To determine viable cell count, appropriate dilu-
tions of the bacterial culture were spread on the surfaces of nutrient agar plates (TP medium supplemented with 3 g of bacteriological peptone [Oxoid] and 12 g of agar [Oxoid]). The colonies were counted after 1 to 2 days of incubation at 30°C.

For the determination of thermoinducibility, portions of the lysogenic culture were diluted with fresh TP medium prewarmed to 42°C. After 20 min at 42°C, the free phage were inactivated by exposure for 7 min to the anti-λ serum, the K value (1) of which was 1.5 min⁻¹ in the final mixture. Thereafter, the irradiated samples were diluted, mixed with indicator bacteria in overlay agar, and plated on the nutrient surface. The plates were incubated at 37°C, and the plaques were counted the next day. To get the time course survival curves for thermoinducibility, the plaque count was corrected for the post-irradiation growth of surviving cells (11).

For the determination of capacity, samples of the host cells were centrifuged at 0°C, suspended in adsorption buffer (20 mM MgSO₄, 10 mM Tris; pH 7.2), and infected with the phage. Multiplicity of infection was usually 10 at a final cell density of about 10⁷/ml. After an adsorption period of 5 min at 42°C, the unadsorbed phage were inactivated by exposure for 7 min to the antiserum (K value was 1.5 min⁻¹ in the final mixture). The samples were then diluted and plated for plaques. The plates were first incubated for 1 h at 42°C and after that for 1 day at 37°C. The plaque count obtained in this way was also corrected for the growth of surviving host cells (11).

RESULTS AND DISCUSSION

During the early post-irradiation period, the survival of the plaque-forming ability of the heat-induced λ cI857 ind lysogens is higher than the survival of their colony-forming ability. The difference in UV sensitivity between the prophage and its host is lost, however, in the course of post-UV incubation. After a UV dose leaving 4% surviving cells, this sensitivity difference is completely lost just before the end of the post-irradiation division delay (8). We wanted to see whether the same result could be obtained with other UV fluences. Therefore, UV survival of the prophage was determined at points of post-UV incubation: immediately after exposure to UV light and at the end of division delay (Fig. 1A). It is seen that, immediately after irradiation, the dose response curve for plaques was well above that for colonies, whereas, at the end of division delay, the two curves coincided. The fall in the prophage survival could not be ascribed to a decrease in the capacity of irradiated cells to support growth of the phage: Fig. 1B

**FIG. 1.** Dose response curve for inactivation of heat inducibility (A) and capacity (B) immediately after irradiation and at the end of division delay. Due to the stationary phase of growth, cell division in the unirradiated cultures was blocked for about 100 min. UV irradiation set up a block which persisted longer and was directly related to the size of UV dose received: for each increase in dose of 40 J/m², cell division was delayed for 1 h. (A) To determine heat inducibility, we induced samples of E. coli AB1157 (λ cI857 ind) cells in fresh tryptone broth at 42°C either immediately after radiation (□) or at the end of division delay (■). The colony-forming ability of lysogens immediately after radiation (○) or at the end of division delay (●) was also determined. (B) To determine capacity to support growth of the phage, we infected samples of AB1157 cells with the λ cI857 ind and plated them for plaques either immediately after radiation (□) or at the end of division delay (■). The colony-forming ability of cells immediately after radiation (○) or at the end of division delay (●) was also determined.
shows that the capacity exhibited the same high resistance at the end of division delay as it did immediately after irradiation. Thus, some biochemical processes associated with the chromosomal DNA may be responsible for the death of the resident prophage in the repair-proficient host. Therefore, we next tested the fate of the prophage in some bacterial mutants carrying genetic defects for DNA repair. All these experiments were done at about 1% cell survival, and the viability time course curves for the prophage and capacity were followed (Fig. 2).

Figure 2A shows typical results obtained with the lysogens carrying uvrB mutation. The ability of uvrB cells to produce the viable phage on heat induction was retained for 1 to 2 h. After that heat inducibility was gradually lost. Kinetics of the inactivation was the same as in the wild-type parental strain until 4 h of post-UV incubation. By this time most of the cells had already lost their ability to be heat induced, whereas the capacity to support growth of exogenous phage was only slightly affected. At the later time, however, a decrease in the capacity was also observed. This decrease, i.e., cessation of cytoplasmic functions, might be responsible for the killing of the prophage at the times after 4 h of post-UV incubation. Note that only a small fraction (about 10%) of the initially viable prophage could be inactivated by this mechanism. Similar results to those presented in Fig. 2A were also obtained with the lysogens carrying uvrA mutation. The only difference was that, in this strain, a decrease in the capacity of irradiated cells to support growth of the phage was more pronounced at the later post-irradiation time.

Figure 2B shows the results of experiments with the lysogens carrying recA mutation. The viability time course curve for the prophage was quite different from that obtained with uvrA or uvrB or wild-type strain: recA cells that had lost colony-forming ability retained the viable prophage until 5 h of post-UV incubation. Therefore, a slight decrease in the prophage survival was observed, but a corresponding fall in the capacity of irradiated cells could explain this decrease. We may thus conclude that rapid inactivation of the prophage, which is observed in both wild-type and uvr cells and which does not depend on the capacity, is controlled somehow by the functional recA gene product.

The above experiments were carried out with cells irradiated in the stationary phase of growth. During post-irradiation incubation, UV-induced processes in these bacteria proceed in a sequential and orderly way. This enabled us to dissect post-UV growth in several distinct phases and simplify our analysis of the complex cell response to UV light (submitted for publication).

![Fig. 2. Effect of uvrB mutation (A) and recA mutation (B) on heat inducibility and capacity during post-UV incubation. A percentage of the survival obtained after correction for the growth of surviving cells (11) is presented. The number of unirradiated colony formers at the time zero (ca. 2 x 10⁶ cells/ml) was taken as 100%. Data for the wild-type strain irradiated with 115 J/m² (1% cell survival) are included for comparison: (...) heat inducibility; (...) capacity. To get the survival level of 1%, we irradiated lysogenic and non-lysogenic AB1885 uvrB and AB2463 recA13 cells with 3 and 3.6 J/m², respectively. Symbols: O, heat inducibility; △, capacity to support λ infection.](http://jb.asm.org/)
Table 1. Colony-forming and plaque-forming abilities of log-phase wild-type and recA lysogens immediately after UV radiation and after 2 h of post-UV incubation

<table>
<thead>
<tr>
<th>Lysogen</th>
<th>h after UV</th>
<th>Colony forms (×10³)</th>
<th>Plaque forms (×10⁶)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB1157 (λ cI857 ind)</td>
<td>0</td>
<td>10</td>
<td>149</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>13</td>
<td>29</td>
</tr>
<tr>
<td>AB2463 (λ cI857 ind)</td>
<td>0</td>
<td>4</td>
<td>173</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>14</td>
<td>345</td>
</tr>
</tbody>
</table>

*Ca. 3 × 10⁶ cells/ml were exposed to 75 J/m² (3% cell survival).

In log-phase cells, on the other hand, the same UV-induced processes proceed along a shorter time line, and their sequence seems not to be so strictly controlled as in stationary phase cells. Therefore, one phase of post-UV growth merges into another. Apart from these differences (which might explain higher UV sensitivity of log-phase cells), essentially the same results were obtained with log- and stationary-phase bacteria. This is exemplified by the data recorded in Table 1. In accordance with our results with the stationary-phase bacteria, most repair-proficient lysogens lost their heat inducibility during a 2-h period, i.e., before the end of cell division delay. It should be noted, however, that the effect was not so fully expressed as in stationary-phase cells. Again, quite different results were obtained with recA cells. In contrast to wild-type bacteria, a significant increase in plaque count was observed after 2 h of post-UV growth. This increase could be due to the fact that UV-irradiated recA bacteria, including non-surviving but residually dividing cells (9), continue multiplying without any delay (7).

The data from Table 1 confirmed the results presented in Fig. 2: the functional gene recA is required for inactivation of the prophage incorporated in the lysogenic chromosome. However, the underlying molecular mechanism of this recA-dependent inactivation is not yet understood. The capacity data (Fig. 1B) indicate that the killing effect is not associated with the phase development in the bacterial cytoplasm. Also, recA-dependent UV reactivation (3, 15), although detected only under special set of irradiation conditions, suggests that the presence of the recA protein has the survival-promoting rather than the killing effect on A in the bacterial cytoplasm. Therefore, we are led to the conclusion that inactivation of the prophage is associated with recA-dependent processes acting on the bacterial chromosome during the time when cell division is blocked.

In our previous report we suggested that the fate of the prophage reflects the fate of its host chromosome (8). If this notion is correct, the recA gene product has a dual function: in addition to its well-established role in repair, it might somehow inactivate those chromosomes that have not been completely repaired. This would mean that cell death is programmed into the sequence of post-UV events. "Programmed" cell death seems quite unusual for a procaryotic organism and, therefore, further experiments are needed either to confirm or refute this concept.

LITERATURE CITED