PHAGE-INDUCED RADIORESISTANCE OF LYSOGENIC BACTERIA

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SUMMARY Lysogen which contains heat-inducible prophage was subjected to an appropriate heat treatment to allow only early events following phage induction. Using this system, it can be shown that early steps of phage development are responsible for increased radioresistance of the bacterial host in terms of survival and postirradiation DNA breakdown.

INTRODUCTION From the radiobiological standpoint, one of the interesting features of many phages (virulent and temperate) is that the radiation-induced damage to their genomes is susceptible to host repair enzymes. That is why Escherichia coli B, for example, can reactivate ultraviolet-irradiated phages T1, T3, and T7 (1). Certainly, the utilisation of host repair enzymes can help the viruses in their struggle for survival; moreover, phage genomes, in general, do not specify effective mechanisms for the repair of their own radiation damage (2). Therefore, the similar advantage to the host which might accrue from its relation with the phage would hardly be expected. However, when bacteria lysogenic for λcI857, a heat-inducible phage mutant, are subjected to an appropriate heat treatment prior to gamma-irradiation, they become apparently less sensitive. This increase in radioresistance is associated with the presence of the phage and is obviously due to the commencement of the phage development.

Direct evidence for such a special case of phage-induced radioresistance has not yet been demonstrated. We have found this phenomenon and it will be described in the present communication.

MATERIALS AND METHODS λcI857 (hereafter called t857) was obtained through the courtesy of Dr. A. Levine (Paris). Escherichia coli C600 (t857) was prepared in our laboratory. This lysogenic strain and its non-lysogenic counterpart, E. coli C600, were cultivated at 30°C in tryptone broth, containing 5 g Bacto-tryptone and 13 g Oxoid nutrient broth per liter of water. Labelling of DNA, when required, was accomplished by growing overnight a small culture (2 ml.) in tryptone broth, supplemented with 0.2 mg/ml. deoxyadenosine and 4 μg/ml. ^{14}C-thymidine (specific activity: 56.2 mCi/mmole; Radiochemical Center, Amersham).

Cells grown to the stationary phase (about 6 x 10^9 bacteria/ml.) were treated as follows. They were first diluted into prewarmed tryptone broth and incubated either at 30°C or 42°C for various lengths of time. Thereafter they were cooled down to 0°C by dilution in ice-cold tryptone broth. This suspension was then divided into two equal portions, one being subsequently irradiated and the other serving as control. Radiation was from a ^{60}Co source at a dose rate of approximately 94,000 R/hour. Viable cell number was determined on nutrient agar plates, containing 5 g Bacto-tryptone, 13 g Oxoid nutrient broth, 3 g Oxoid bacteriological peptone and 12 g agar per liter of water. Colonies were counted after 24 hours of incubation at 30°C.

To determine postirradiation DNA breakdown, ^{14}C-thymidine labelled cells were incubated at 30°C in the presence of chloramphenicol (20 μg/ml.). The drug was added, before the beginning of postirradiation incubation, in order to prevent the reincorporation of breakdown products (3). Aliquots (0.5 ml.) were withdrawn at suitable time intervals and mixed with an equal volume of ice-cold trichloracetic acid. The DNA containing precipitates were collected on "Millipore" filters and counted on a gas-flow counter.

RESULTS AND DISCUSSION t857 is a phage carrying a double mutation in the cI region and is characterised by heat induction and by the failure of ultraviolet induction (4). Also, t857 cannot be induced by gamma-irradiation, as shown

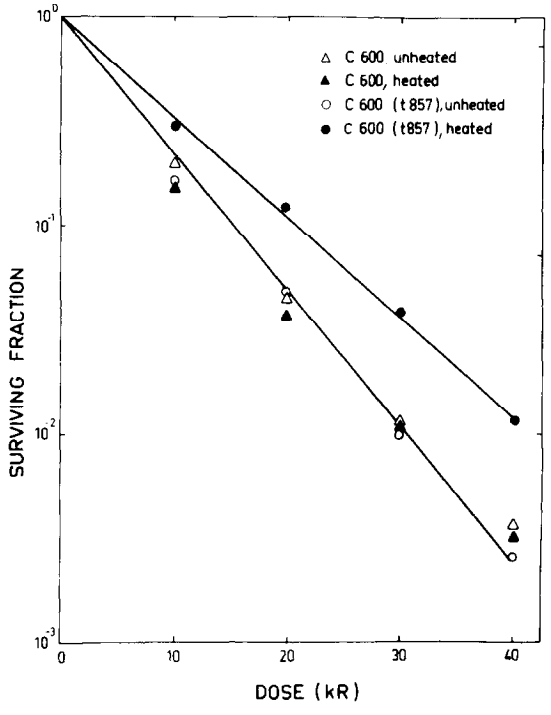

Fig. 1. Shows survival curves of heated (5 minutes at 42°C prior to irradiation) and unheated cultures of E. coli C600 and E. coli C600 (t857).

by the following two pieces of evidence. The first is that E. coli C600 (t857) bacteria, unlike lysogens with wild-type prophage, do not produce phages after gamma-irradiation. Secondly, when bacteria are incubated at 30°C, that is, at the non-inducible temperature, the survival curves of E. coli C600 and E. coli C600 (t857) coincide (lower curve in Fig. 1). In contrast to this, the presence of wild-type prophage is associated with a striking increase in radiosensitivity of the lysogenic cells (5) probably due to two different killing effects: phage production and gamma-irradiation. There are indications, however, that the presence of defective prophage is responsible for higher radioresistance of E. coli 15T⁻ in comparison to its cured derivative, JG151 (6). Unfortunately, the nature of mutation of this prophage is unknown. Moreover, it could even not clearly be demonstrated that the induction process itself is responsible for an increase in radioresistance. In order to investigate the possible

role of prophage induction in the development of host radioresistance, the following conditions must be provided: (a) the inducing agent has to be harmless to bacteria, (b) following induction, phage development must be arrested (either by using phage mutants or by an appropriate experimental procedure) prior to the stage which leads to the cell death, and (c) gamma-irradiation must be excluded as inducible agent.

In our typical experiment the above-mentioned conditions were fulfilled. To induce only early steps of phage development E. coli C600 (t857) bacteria were heated for five minutes (7), and thereafter cooled and irradiated, as described in Materials and Methods. It can be pointed out that following this short heat treatment, the viable count of non-irradiated lysogens remains 100 per cent (cf. Fig. 2). As shown in Fig. 1, these bacteria exhibit higher resistance to gamma-rays when compared to unheated control. Radiosensitivity of non-lysogenic bacteria,

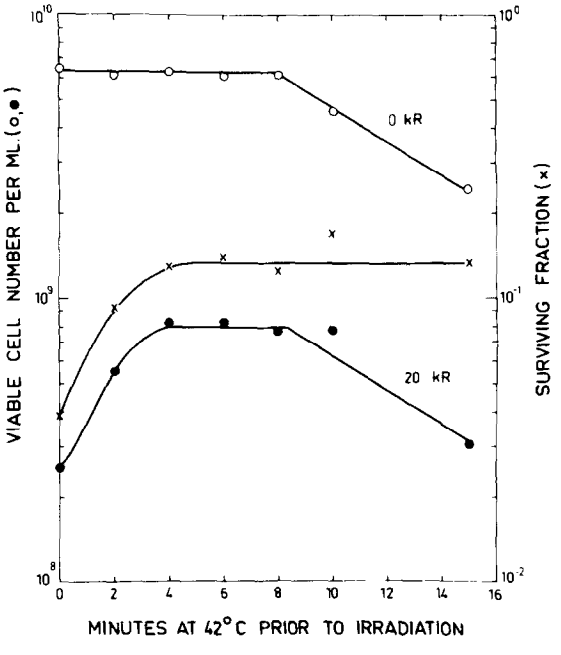

Fig. 2. Illustrating how the colony-forming ability of E. coli C600 (t857) changes with time at 42°C. Surviving fraction (radiosensitivity) shows the ratio of the number of heated and irradiated lysogens against heated but non-irradiated lysogens.

however, does not change due to heating (Fig. 1).

It can be seen from the uppermost curve in Fig. 2 that the number of colony-formers of E. coli C600 (t857) bacteria is not affected by heating during eight minutes. On the other hand, as the time at 42°C increases up to four minutes, an increasing proportion of lysogens becomes more resistant to the lethal effects of gamma-irradiation (Fig. 2, closed circles). After maximal radioresistance has been reached, it remains more or less constant throughout the experiment. This is evident from the curve showing the ratio of the number of heated lysogens which are subsequently irradiated, against the number of heated but non-irradiated lysogens (Fig. 2, curve traced by crosses). It turns out that even though lysogenic bacteria start to die after eight minutes at 42°C, the remaining survivors in control retain their increased radioresistance.

The described phage-induced radioresistance is obviously dependent on events occurring as a consequence of phage induction. In an effort to explain the effect of these events on the resistance of the bacterial host,

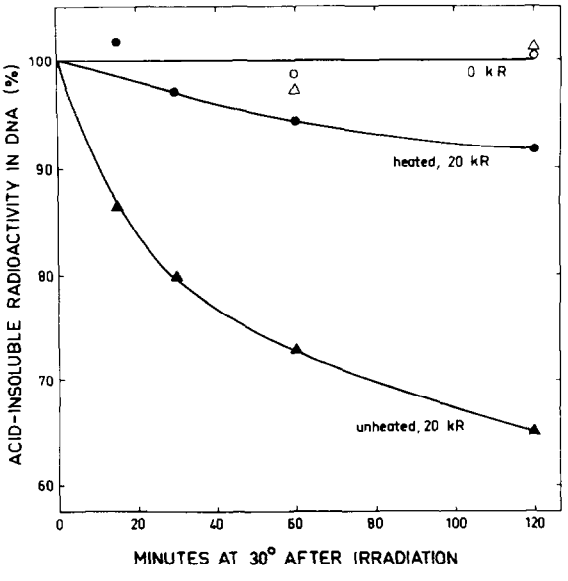

Fig. 3. Represents the kinetics of radiation-induced DNA breakdown in E. coli C600 (t857) cells. Before irradiation, bacteria were incubated for 5 minutes at 42°C (heated culture) and at 30°C (unheated culture), respectively.

radiation-induced DNA breakdown, which has been suggested as a major cause of cell death (3, 8), was studied in heated and unheated lysogens. The results are presented in Fig. 3. Indeed, as we expected, DNA breakdown is strongly inhibited in the heated culture. On the contrary, non-lysogenic bacteria degrade their DNA to the same extent, regardless of whether they are heated or not.

The data presented here show, beyond doubt, that some events following the phage induction can bring about a considerable increase in survival of the bacterial host after irradiation. Further investigation of the nature of these events is in progress in our laboratory.

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