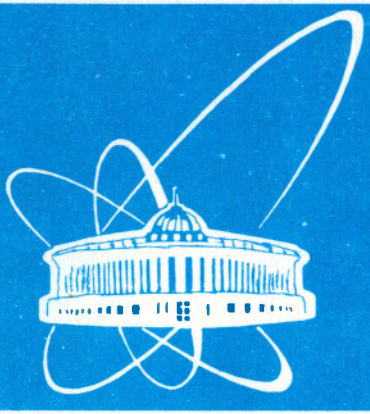

СООБЩЕНИЯ
ОБЪЕДИНЕННОГО
ИНСТИТУТА
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Дубна

E19-97-371

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λ-PROPHAGE INDUCTION IN *E.coli* CELLS BY RADIATION WITH DIFFERENT LET

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1997

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Зависимость индукции профага λ в клетках бактерий *E.coli* от радиации с различной ЛПЭ

Исследована индукция профага λ в клетках *E.coli HfrH*(λ) после облучения ускоренными ядрами гелия, ионами бора, углерода, дейтронами и α-частицами. Установлена дозовая зависимость фракции индуцированных клеток и определена эффективность индукции (λ *i p*). Показано, что зависимость λ от ЛПЭ описывается кривой с максимумом.

Работа выполнена в Отделении радиационных и радиобиологических исследований ОИЯИ.

Сообщение Объединенного института ядерных исследований. Дубна, 1997

Bonev M.N., Collev S.D.
E19-97-371

λ-prophage Induction in *E.coli* Cells by Radiation with Different LET

λ-prophage induction in *E.coli HfrH*(λ) strain after irradiation with α-particles, accelerated helium ions, boron and carbon ions, as well as deuterons is investigated. The dose dependence of the fraction of induced cells is measured and its initial slope (λ-induction potency — λ *i p*) is determined. It is shown that the dependence of λ *i p* on LET is a curve with a maximum.

The investigation has been performed at the Division of Radiation and Radiobiological Research, JINR.

Communication of the Joint Institute for Nuclear Research. Dubna, 1997

Introduction

The response of *Escherichia coli* to a variety of treatment that damage DNA is controlled by the SOS regulatory system [1]. SOS induction leads to increased mutagenesis [2], SOS mutagenesis [3], increased repair of DNA [4], W-reactivation and W-mutagenesis [5,6], inhibition of cell division [7] and also to the induction of prophage lambda [8,9]. The biochemical steps of SOS induction are initiated by the activation of RecA protein after blocking of DNA synthesis has occurred [10]. The activated RecA protein cleaves both the LexA repressor which leads to the SOS response, and the λ-repressor which initiates the lytic pathway of cell growth [1]. Therefore the induction of λ-prophage reflects the repair processes in the cells after action of DNA damaging agents.

Early experiments on λ-prophage induction used ultraviolet and X-rays as inducing agents [11,12]. Similar investigations have been made using γ-rays [13]. Recently we published [14] the results of λ-prophage induction in repair-deficient and wild type *E.coli* strain by γ-rays and heavy ions. The data shown that λ-prophage induction increases with increasing LET up to 70 keV/μm. In this paper we present the effect of α-particles, boron ions and carbon ions, which possess LET bigger than 100 keV/μm, on the λ-prophage induction. The relative effectiveness of heavy ions in λ-prophage induction can provide an information on the nature of those DNA injuries triggering the both lytic development of bacteriophage λ and SOS response. Such information would help, particularly, in increasing our understanding of SOS *(UmuC-mediated)* mutagenesis in *E.coli.*

Materials and Methods

Bacterial strains. The *E.coli* strains used in our experiments were : *HfrH(λ)* and *C(str^r).*

Lysogenic induction and survival assay. Bacteria were growth overnight in liquid nutrient broth (Gamaleya Institute of Microbiology, Moskow), diluted 1:20, and incubated at 37^{0}C to approximately 2.10^8 cells/ml. The suspension was centrifuged at 8000 g for 15 min and resuspended twice in 0.01 mol/l $MgSO_4$. Lysogenic bacterial cells were separated from the free phage particles by means of filtration through Dacron filters with pore diameter 0.5 μm (Joint Institute for Nuclear Research, Dubna). This method enable us to reduce the background of the free phage particles by two order of magnitude.

To determine the production of infective centers (plaque forming units - PFU), 0.1 ml of appropriately diluted suspension was added to 3 ml of top agar (7 g/l agar, 1 g/l yeast extract, 8 g/l NaCl and 3 nmol/l $MgSO_4$) together with 0.1 ml of suspension of indicator bacteria (4.10^8 cells/ml; C strain) and poured onto plates containing nutrient agar. The fraction of spontaneously induced cells that may occur on the plates during

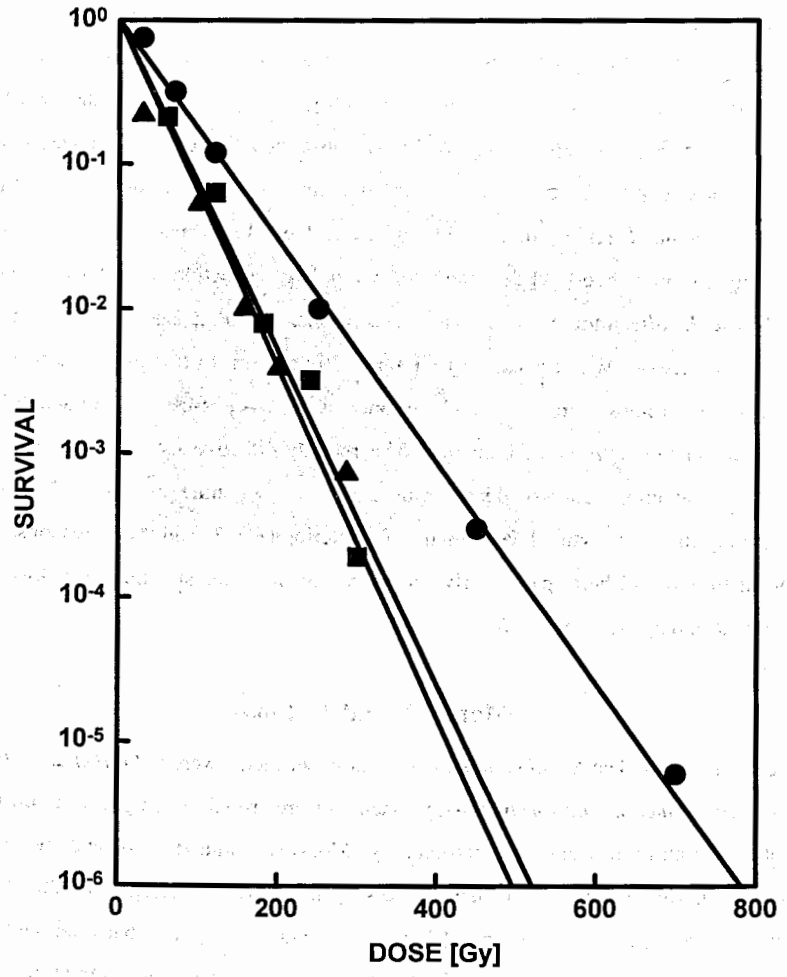

Fig. 1. Survival curves. HfrH(λ) cells treated with: ■, α-particles; ▲, boron ions; •, carbon ions.

overnight incubation was one or two order of magnitude lower than in the irradiated samples. The survival fraction (S) was calculated as the ratio $S=N_s/N_0$, where N_s is the number of surviving bacteria and N_0 is the number of the non-irradiated cells. The number of cells were determined by a standard methods, i.e. by counting the number of macrocolonies on the nutrient agar.

The fraction of induced cells (I) was determined as ratio of the number of observed PFU (N_i) and the number N_0 of the non-irradiated bacteria; $I=N_i/N_0$. The dose-dependence I(D) of the fraction of induced cells was well described [14] by the following function:

$$I(D) = \alpha D \exp(-\beta D)(1- \exp(-D/D_0)), \qquad (1)$$

where $1/D_0$ is the radiosensitivity of the cells, α (or λip) and β are determined for each I(D) dependence by an optimizing procedure [15].

Irradiation. Low penetration (hundred μm) of α-particles and heavy ions required bacteria to be irradiated on the surface of Dacron nuclear filters. They were disposed on the surface of minimal agar and contained 0.01 ml of a bacterial suspension of 4.10^8 cells/ml for each dose. Up to 11 samples could be placed in a ring-shaped magazine. The samples were transported by remote control to the exit window of the ion beam. The homogeneity of the ion beam was checked using a colour change in a film in response to radiation. The LET values and dose determination were performed by semiconductor detectors and by an ionization chamber, respectively. The LET of the corpuscular radiation used were: α-particles - 110 keV/μm, boron ions - 105 keV/μm, and carbon ions - 205 keV/μm.

Results

The survival curves after a treatment of the cells by α-particles, boron and carbon ions are shown in Fig. 1. The sensitivity of the cells to α-particles as well as to boron ions are very close to each other. They are .027 Gy^{-1} and .028 Gy^{-1} respectively. The sensitivity after carbon ions treatment is smaller than that after irradiation by α-particles and boron ions,

it is 0.018 Gy^{-1}. Fig. 2 represents the fraction of induced cells per ml after α-particles, boron- and carbon-ions treatment. The behaviour of the induction curves for α-particles and boron ions are similar - its λIPs are 0.00221 Gy^{-1}, and 0.00227 Gy^{-1} respectively. The induction curve for carbon ions reaches a plateau at the high doses. The λip dependence on LET is shown in Fig. 3. The curve possesses a maximum at 50 keV/μm.

Discussion

The cell survival. As can be seen from Fig.1 the radiosensitivity of the cells in the region of LET values (100 - 200 keV/μm) falls with increasing LET. It is due to the fluctuation of the energy deposition which is significant at high LET of ionizing radiation [16].

The dose dependence of the fraction of induced cells. λ-Prophage induction can appear only when the λ-repressor level fallen to approximately 10 per cent of its initial level [17]. The cleavage of λ-repressor proceeds slowly and the derepression of λ-phage transcription is delayed as compared to the other SOS-controlled genes [18]. This fact supports the idea that the λ-prophage induction appears in those cells whose death is already inevitable.

The probability of λ-prophage induction per killed cell p(D) = I(D)/(1-S(D)) has been described as a mathematical function with maximum in the following simple form:

$$p(D) = \alpha D \exp(-\beta D) . \qquad (2)$$

Obviously the increasing part of this dependence characterizes the efficiency of ionizing radiation in inducing λ-prophage (the slope α, up to now named λ-induction potency - λip represents the number of induced cells per inactivated cell and per unit dose). The increase of p (D) with absorbed dose could possibly reflect the increase in DNA damage with dose of ionizing radiation. It is possible that some kind of DNA damage could lead to the activation of RecA protein, which could thereby lead to a derepression of λ-prophage.

At high doses, however, the probability p(D) decreases again. The results present in Fig.2 confirm such a behaviour of the λ-induction for the cells treated by α-particles

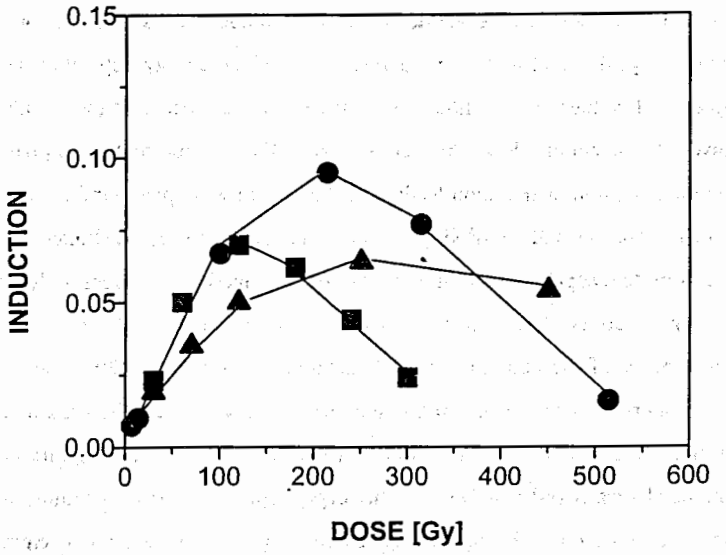

Fig. 2. Fraction of λ-induced HfrH(λ) cells by: ■, α-particles; ▲, carbon ions; •, boron ions.

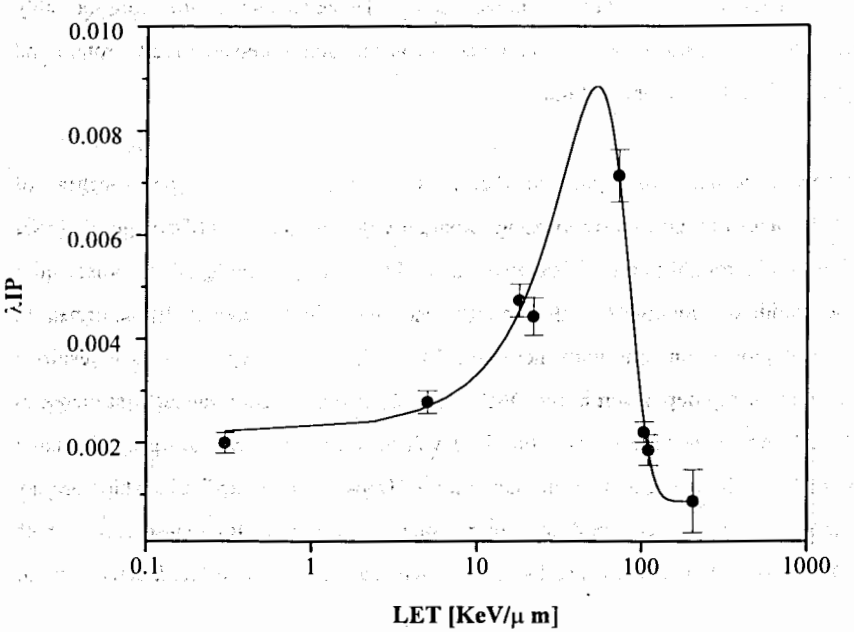

Fig. 3. Dependence of the λ-induction potency of HfrH(λ) cells on LET.

and boron ions. It does not observe the decrease of the induced cells after carbon ions treatment. Obviously, this fact is due to the fluctuation of the energy deposition taking place at higher LET values. The λ-induction of the cells may occur at the cellular or molecular level. At the cellular level the quantity exp(-βD) can represent the probability of λ-prophage inactivation or mutation leading to the loss of λ-prophage infectivity in a fraction of cells. The second possibility is that the content of the cellular inducer (activated RecA protein capable of maintaining λ-repressor and LexA-repressor cleavage) decreases at higher doses. The first hypothesis can be rejected on the basis of the negligible probability of the above-mentioned mutations occurring [19,20]. The second hypothesis is supported by the results of Craig and Roberts [21], who observed in an *in vitro* system that the proteolytic action of RecA protein depends on the concentration of the single-stranded breaks (ssb) of DNA. This dependence showed a maximum at an intermediate concentration of ssb and then decreased further when the ssb concentration increased [21,22]. It seems that the λ-repressor inactivation observed reflects recA proteolytic activity. However, a direct demonstration would be needed to show that recA protease activity *in vivo* falls at higher dose. The parameter β therefore possibly represents some characteristics of the biochemical interaction between recA protein and ssb arising in cells after irradiation.

λ-Prophage induction as a function of LET. As is well known the broad spectrum of DNA damages are induced by ionizing radiation with different LET. Damage of DNA strands can be roughly divided into two classes [23]. The first (bulk of ssb) arises after the deposition of a relatively small amount of energy within the DNA molecule or near it; its rate of production falls with increasing LET. The second type arise as a result of substantial energy deposition in the DNA molecule; the production rate at first increases with LET and begins to fall only at hiht LET values (several hundred keV/μm). The latter class of DNA damage can arise in one strand of DNA as "complex" ssb, which are not repairable by a fast polA-dependent repair system in *E.coli* [24]. It can also arise in both DNA strands as double strand breaks (dsb), which is a lethal event in *E.coli* cells with one

chromosome per cell [25]. In addition to this, in the case of irradiation with high-LET particles, cell survival is related mainly to the fraction of non-hit cells. Therefore, the quantity p(D) in equation (2) presents the number of induced cells related to the killed subpopulation, i.e. to cells certainly hit by at least one particle. In this sense the λip can be regarded as a quantity properly reflecting some intrinsic cell response. On the whole the dependence of λip on the LET reflects the amount of the "complex" ssb, which as was mentioned above, have a maximum in the region of intermediate LET values (≤100 keV/μm).

References

1. Little J.W., Mount D.W. - Cell, 1982, v.20, p.11.
2. Witkin E.M. - PNAS, USA, 1974, v.71, p.1930.
3. Shinoura Y., Isee T., Glickman B.W. - Mutation Research, 1983, v.11, p.51.
4. Pollard E.C., Achey P.M. - Biophysical Journal, 1975, v.15, p.1141.
5. Weigle J.J. - PNAS, USA, 1953, v.39, p.628.
6. Defais M., Fauquet P., Radman M., Errara M. - Virology, 1971, v.43, p.495.
7. Witkin E.M. - PNAS, USA, 1967, v.57, p.1275.
8. Roberts J.W., Roberts C.W., Mount D.W. - PNAS, USA, 1977, v.74, p.2283.
9. Lu C., Echols H. - J. Mol. Biol., 1987, v.196, p.4.
10. Bertany G., - J. Bacteriol. 1951, v.62, p.301.
11. Marcovich H. - Nature, 1954, v.174, p.796.
12. Marcovich H., Latarget R. - Adv.Biol.Medic.Physics, 1958, v.6, p.75.
13. West S.C., Powell K.A., Emmerson P.T. - Mol.gen. Genet. 1975, v.141, p.1.
14. Bonev M.N, Kozubek S., Krasavin E.A., Amirtayev K.G. - Int.J.Radiat.Biol., 1990, v.57, p.993.
15. Velev G.V., Maniev V.M., Russakovich N.A. Communication of the JINR, Dubna, 1989, P11-89-329.
16. Красавин Е.А. - Проблемы ОБЭ и репарация ДНК, М.: Энергоатомиздат, 1989.

17. Jonson A.D., Poteete A.R., Lauer G., Sauer R.T., Ackers G.K., Ptashne M. Nature, 1981, v.294, p.217.
18. Walker G.C. Microb. Review, 1984, v.48, p.60.
19. Eisen H.A., Fuerst C.R., Siminovich L., Thomas R., Lambert L., Pereira da Silva L., Jacob F. Virology, 1966, v.30, p.224.
20. Mount D.W., Harris A.W., Fuerst C.R., Siminovich L. Virology 1968, v.35, p.134.
21. Craig N.L., Roberts J.W. Nature, 1980, v.283, p.26.
22. Cohen P.S., Resnik J., Sussman R. - J.Mol.Biol., 1983, v.167, p.901.
23. Munson R.J., Bridjest B.A. - Int.J.Radiat.Biol., 1973, v.24, p.257.
24. Bridgest B.A., Mottershead R.P. - Heredity, 1972, v.131, p.553.
25. Kosubek S., Krasavin E.A. - Neoplasma, 1984, v.31, p.684.

Received by Publishing Department
on December 10, 1997.