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PROPHAGE λ INDUCTION BY IONIZING RADIATION OF DIFFERENT LETs

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INTRODUCTION

The SOS regulatory system controls the response of Escherichia coli to a variety of treatments that damage DNA[1]. In a lysogen RecA protein acts as a protease and cleaves both the LexA and λ repressor, and turns on SOS functions and lytic pathway growth. The induction of the λ prophage as an indirect assay gives information for the SOS system state. The experiments dealing with the λ bacteriophage induction began in the 50s[2-4]. As inducing agents, they used mainly ultraviolet (UV) irradiation, X-rays and later γ-irradiation[5]. Until recently, one has not performed any work with accelerated heavy ions. In order to clarify the mechanism of lethal and mutagenic action of ionizing radiation of different linear-energy transfer (LET) it is important to have information on E.coli SOS response to the same radiations. It is known that the increasing LET is connected with increasing of DNA lesions which are repairable only in the slow repair processes[6]. In this connection, the degree of SOS response expression would show if the above indicated lesions take part in a signal formation that switches on SOS functions. The present studies have examined the dependence of the λ prophage induction frequency on the dose of radiations with different LET, and similar dependences under variety of pre-irradiation cultivation conditions, and variety of cell genotypes.

MATERIALS AND METHODS

Bacterial Strains

The strains used in our work are presented in table 1. Strain Cr^{r}_{str} was used as an indicator of plaque forming units in the induction experiments. Strain Ay(λ) was used as a donor for bacteriophage λ wild type.

Media, Growth of Bacteria and Irradiation

The cells were grown overnight in liquid nutrient broth (meat-pepton broth (MPB) made at the Institute of Epidemiology and Microbiology "N.F.Gamalei", Moscow). In some experiments we

Table 1. Bacterial strains

Strains	Genotype	Source
HfrH	thi	Institute of Genetics Academ.
HfrH(λ)	as HfrH, but λ	Sci., USSR
30S0	thi, lac, recA37	-"-
30S0(λ)	as 30S0, but λ	-"-
W3110	B1, thy36, deoC	Institute of Biophysics Ministry of Health
W3110(λ)	as W3110, but λ	-"-
JC5491	Hfr, Thr, ile, val, B1, recBC	-"-
JC5491(λ)	as JC5491, but λ	-"-
P3478	polA1, B1, thy	-"-
P3478(λ)	as P3478, but λ	-"-
C	str^{r}	-"-
GC244	lexA4, his	-"-
GC244(λ)	as GC244, but λ	-"-

used poor (M9) and rich (AMP) nutrient media. The M9 media consist of M9-buffer and glucose and thiamin, 0.08% and 0.0001%, respectively. M9-buffer was 19.7 mM NH_4Cl; 43.7 mM Na_2HPO_4; 23.2 mM KH_2PO_4; 9 mM NaCl; 1 mM $CaCl_2$; 1 M $MgSo_4$. AMP media (made at patent medicine's factory of Leningrad's meatworks) are prepared on the basis of aminopeptide and diluted threefold in 0.15 M NaCl.

The cells were diluted 1:20 and incubated with shaking at 37°C to approximately $2x10^8$ cell/ml. Cultures were centrifuged at 2000 g for 15 min and resuspended in 10^{-2} M $MgSO_4$. 1 ml of the culure was filtrated through Dacron nuclear filters (diameter of filter pores is 0.5 m). This method of separation of a lysogenic bacterial culture from the free phage (FP) permits one to reduce the background of FP by about two orders of magnitude[7].

Gamma-irradiation was performed with a $^{137}C_s$ gamma-source. 0.01 ml of the samples disposed on the nuclear filter surface were maintained at approximately 0°C during irradiation. Filters were put up on the nutrient meat-pepton agar (MPA). The dose-rate was approximately 25 Gy/min.

We used beams of heavy ions produced by the heavy many-charged ion accelerator (Laboratory of Nuclear Reactions, Joint Institute for Nuclear Research, Dubna). The samples were irra-

diated in the same manner as the gamma-irradiated samples.

All dilutions after irradiation were done in 10^{-2} M $MgSO_4$. After convenient dilution the cells were plated on glass petri dishes containing approximately 20 ml of MPA for colony forming units determination. To determine the production of infective centers 0.1 ml of the aliquot was added to melted soft-agar together with 0.1 ml of overnight indicator bacteria and poured on the MPA plates.

All the plates were incubated at 37°C for at least 18 h. The radiations used in the present work are shown in table 2.

Table 2. Physical features of the used radiations

Radiation	Energy MeV/nucl.	LET KeV/μm	Dose rate Gy/min	Source
γ-rays	-	0.3	25	$^{137}C_s$
D_2^+	8.8	5	720	A-200
D_2^+	1.65	18	720	A-200
He_4^{++}	8.0	22	600	A-200
He_4^{++}	1.7	72	600	A-200

Survival and Lysogenic Induction

The survival (S) was presented as a ratio $S = N_s/N_o$, where N_s is the number of colony forming units after radiation and N_o is the number of non-irradiated bacteria. The prophage λ induction frequency (I) was determined as a ratio of the amount of the observed plaque forming units (N_i) to the amount of the colony forming units N_o: $I = N_i/N_o$. The dependence of I on the dose (D) was fitted by the function: $I(D) = \alpha D \exp(-\beta D) \times (1 - \exp(-D_0^{-1}D))$. Here D_0^{-1} designated the bacterial strain radiosensitivity $1/D_{37}$ and α, β are parameters.

Statistical Methods of Treatment

The parameters A,B of the survival curves presented in the semilog scale by the equation $Y = Ax + B$, were calculated by the least squares method. The parameters α and β of the function I(D) were determined by Rozenbrock's method /8/.

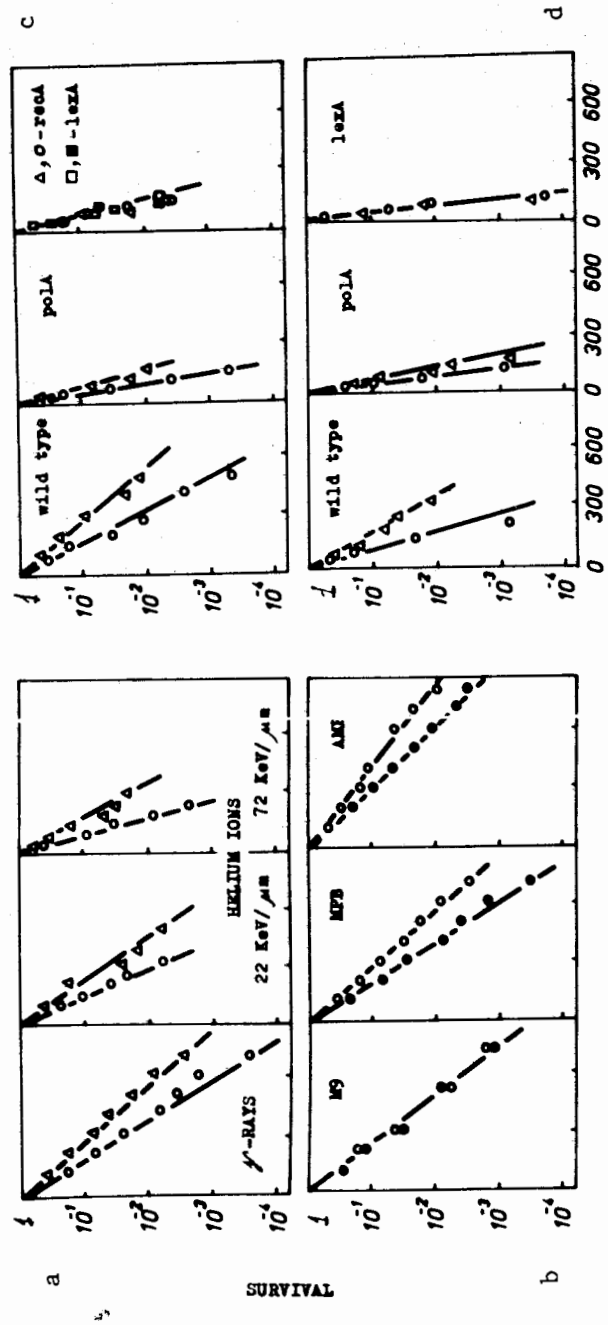

Fig.1. The survival curves: a) Irradiated cells HfrH; (Δ) - nonlysogen, (o) - lysogen, b) HfrH cells cultivated up to the γ-irradiation in different media; (o) - nonlysogen, (●) - lysogen, c) γ-irradiated cells; (Δ) and (□) - nonlysogen, (o) and (■) - lysogen, d) exposure of cells to accelerated helium ions with LET = = 22 KeV/μm; (Δ) - nonlysogen, (o) - lysogen.

RESULTS

The survival curves are found in fig.1. We find that the radiosensitivity (D_o^{-1}) of strains with $recA^+/lexA^+$ genotype increases as well as LET. Furthermore, the difference $\Delta D_o = D_o(\lambda^-) - D_o(\lambda^+)$ approximately remains a constant. In contrast to it, ΔD_o of the cells cultivated up to the γ-irradiation in various nutrient broths decreases in the direction rich ⟶ poor media. λ prophage induction in strains which possess $recA^+/lexA^+$ genotype had a common peculiarity. The induction frequency dependence on the dose shaped a curve with a maximum (fig.2-4). The initial linear slope of I(D) is defined by the parameter α which represents quantitative increment of the induced cell number per unit dose. We named α the inductivity. The parameter α increases as well as LET and an alteration poor ⟶ rich media does it. The I(D) function's parameters are shown in table 3. The parameter β defines I(D) decay. Unlike I(D) for $recA^+/lexA^+$, the dependence I(D) for recA, lexA and recBC strains was a constant (fig.5).

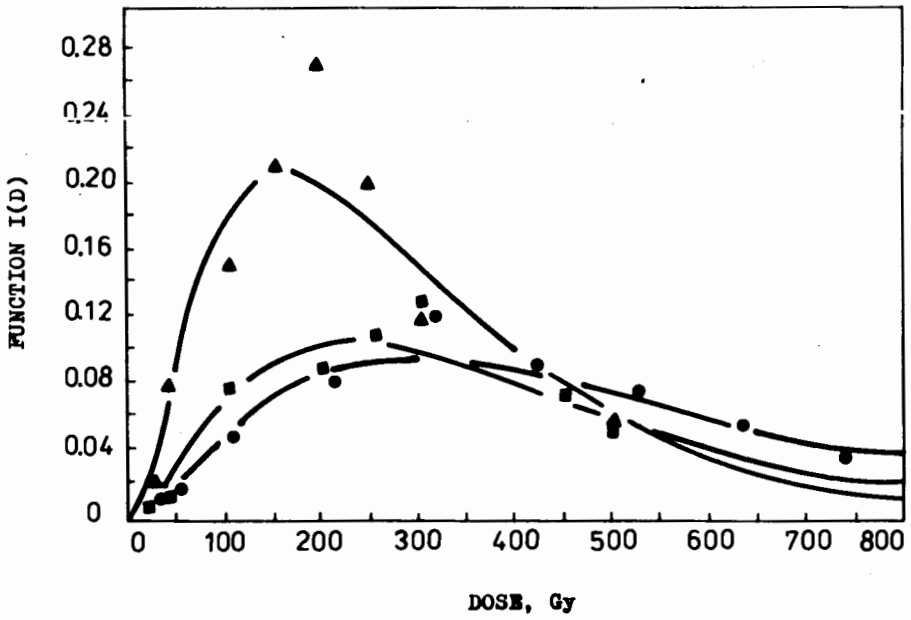

Fig.2. The function I(D) for E.coli HfrH(λ) after; (•) - γ-irradiation, (■) - accelerated helium ions with LET = = 22 KeV/μm and (▲) - accelerated helium ions with LET = = 72 KeV/μm.

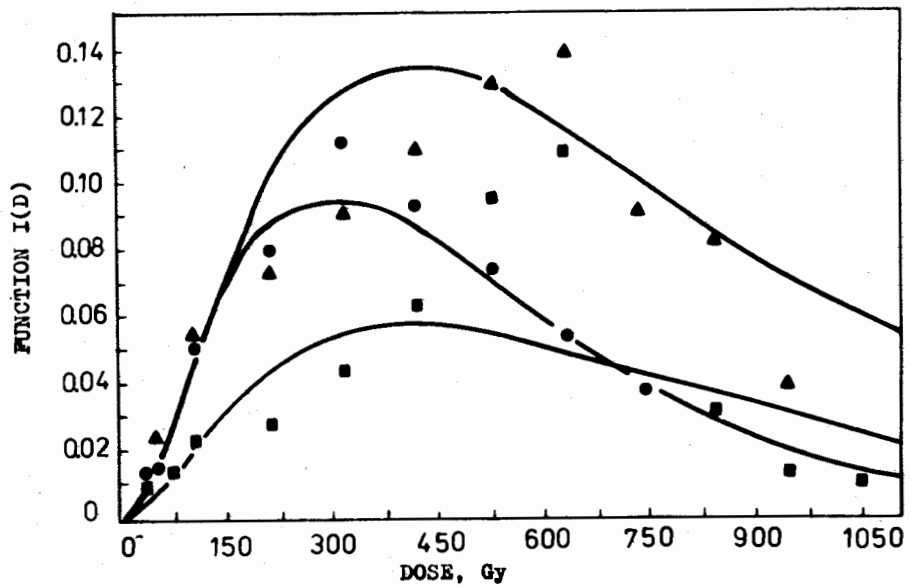

Fig.3. The function I(D) for γ-irradiated E-coli cells HfrH(λ) incubated up to the irradiation in: poor M9 media - (■), MPB media - (•) and AMP media - (▲).

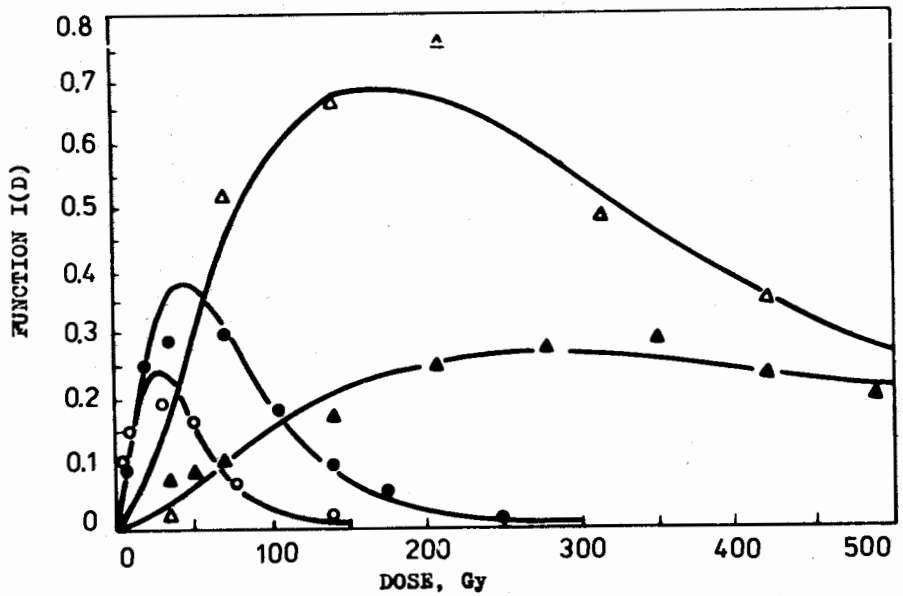

Fig.4. The function I(D) for: W3110(λ) - (Δ, ▲) and P3478 - (○, •). Open symbols - accelerated helium ions with LET = = 22 KeV/μm, closed symbols - γ-irradiation.

Table 3. Parameters of the dependence I(D) for indicated strains after γ-irradiation and accelerated heavy ions action.

Strain	Parameter ($\times 10^{-3}$)	Radiation (LET)				
		γ-rays 0.3 KeV/μm	Helium ions 22 KeV/μm	Helium ions 72 KeV/μm	Deuterons 5 KeV/μm	Deuterons 18 KeV/μm
HfrH(λ)	D_o^{-1}	17±0.5	24±2	34±3	15±2	25±2
	α	1.46±0.17	3.15±0.77	5.07±2.60	1.99±0.001	3.37±0.002
	β	5.43±0.17	8.40±0.79	10.77±2.37	2.00±0.002	4.56±0.002
W3110(λ)	D_o^{-1}	28.39±1.00	25.60±1.40			
	α	4.76±0.25	1.90±1.40			
	β	6.50±0.42	6.27±0.49			
P3478(λ)	D_o^{-1}	94.35±5.20	50.0 ± 2.3			
	α	56.80±6.50	54.2±12.5			
	β	48.00±2.72	54.9± 6.0			

A comparison between the induction levels of these mutants showed that the induction frequency increases in accordance with the sequence recA ⟶ lexA ⟶ recBC.

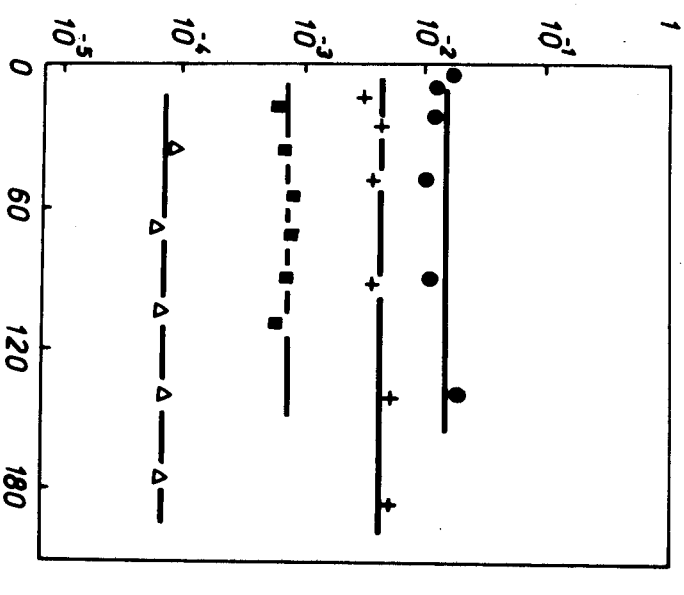

Fig. 5. λ prophage induction in: γ-irradiated cells; (Δ) - recA mutant and (■) - lexA mutant, exposed cells to accelerated helium ions with LET= = 22 KeV/μm; (+) - recBC mutant and (●) - lexA mutant.

DISCUSSION

The Lysogen Survial

The experimental data showed that the survival of the lysogen recA$^+$/lexA$^+$ strains was lower than that of the nonlysogen strains. After irradiation the lysogen dies because of both nonrepaired DNA injuries and a lysis due to the propage λ induction. In this connection a question emerges about the relationship of the two destruction types. One ordinary hypothesis was that the two lethal effects are independent. In order to check this hypothesis we constructed a mathematical model. We initiated the following symbols: P_r - the probability that a cell will die as a result of nonrepaired DNA injuries, P_i - the probability that a cell will die owing to the prophage induction. Using these symbols we can present the probability of the lysogen survival S_l in view:

$$S_l = 1 - P_r - P_i + P_r \cdot P_i. \qquad (1)$$

As a consequence of the hypothesis we can write: $P_r = 1 - S$, where S is the nonlysogenic survival. Taking in view that $I = P_i$, (1) becomes:

$$S_1 = S(1 - I). \qquad (2)$$

The value S_1 can be easily obtained from (2). In fig.6 the survival curves of the (λ^+), (λ^-) HfrH strains, the survival curve S_1 obtained from (2) and the λ prophage induction frequency after γ- and accelerated helium beam irradiation are given. Apparently, the degree of induction expression doesn't influence the exponential curve shape. On the basis of the fact that the exponential survival curves of the lysogenic strains had exponential behavior and not the one shown by the dotted line in fig.6 we concluded that the radiation destruction processes and prophage induction in lysogen are not independent. After radiation damage of DNA two RecA functions

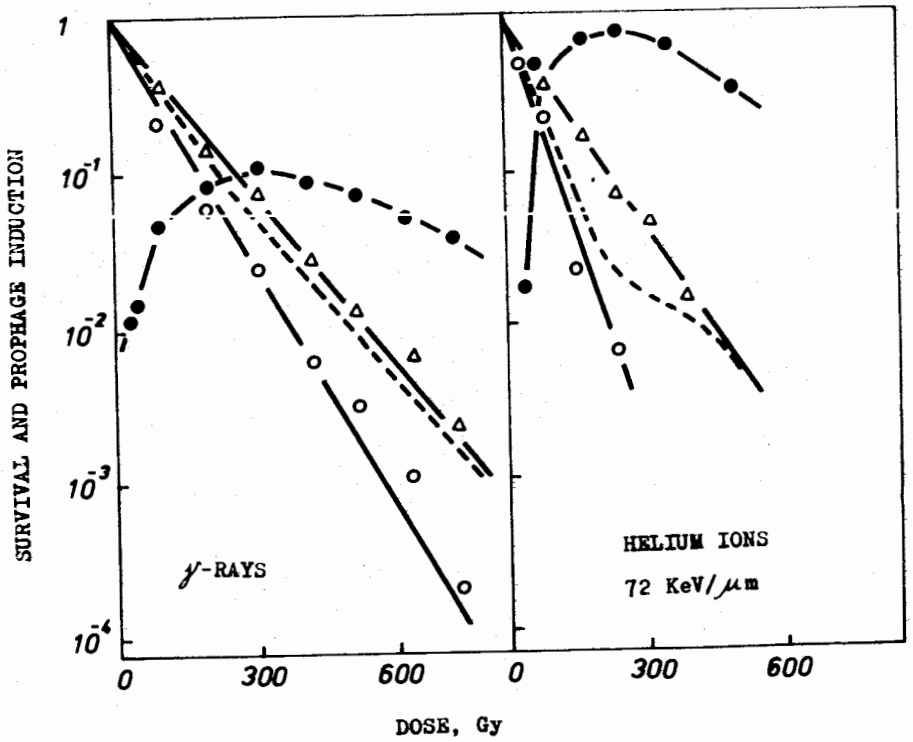

Fig.6. E.coli HfrH survival and λ prophage induction. Dotted line represents the theoretical curve obtained from equation (2). (Δ) - nonlysogen, (o) - lysogen, (•) - induction frequency.

attain paramount importance: proteolytic function leading to the starting of SOS response and the restrictive function in relation to DNA degradation processes following RecBC exonuclease operation. The presence of the λ repressor in a host-cell is "an additional weight" on $recA^+/lexA^+$-dependent repair, since the λ repressor competes with the LexA repressor in the process of their own cleavage from RecA protein and obstructs RecA protein to realize its restrictive function toward DNA degradation. In this connection we believe the derepression degree of SOS function and of RecA protein produced de novo in a lysogen is lower than the one in a nonlysogen cell. As a result, both DNA degradation and enzyme double-strand breaks (EDSB) increase. Thus the radiosensitivity of the lysogen enhances. This explanation is supported by the fact that cells cultivated in poor media up to the γ-irradiation indicated both slight λ prophage induction and a radiosensitivity equal to the isogenic (λ^-) strain (see fig.1b and 3).

The I(D) Dependence Behaviour

Since titration of the λ prophage induction had to be performed on a large cell population, the question arises: what is the I(D) dependence in an individual cell as compared to the I(D) dependence in an average cell population? One of two alternative situations might exist, depending on the action of the cellular inducer.

Hypothesis (1): A part of the cells are killed by cellular phages mutated in such a way that they lost their infective capacity. We rejected this hypothesis for the following reasons:

a) the frequency of the mutations mentioned above is quite smaller than the experimental values of I (ref./9/; see fig.2-4),

b) the dependence I(D) for lexA, recA and recBC mutants is a constant at definite radiation (see fig.5).

Hypothesis (2): the observed dependence I(D) reflects a cellular inducer activity. The following facts support this assumption. a) It is known that with the dose increasing, the common lenght of the single-stranded gaps (SSG) expands. So we can expect that dose increasing will cause a magnification of the connected RecA-DNA amount and, as a consequence of it, an intensification of the two RecA functions: proteolytic activity and DNA degradation restriction. b) In 1980 Craig and Roberts observed in vitro that the proteolytic activity of RecA protein depended on the single-stranded DNA (SSDNA) as a function with a maximum /19/.

On the basis of the above-mentioned consideration and taking into account the circumstance that the λ repressor inactivation reflects the RecA proteolytic activity and so switches on the lytic development of the bacteriophage λ, we believe that the dependence I(D) reflects the proteolytic RecA activity. Thus, we believe hypothesis (2) describes truthfully the experimental data.

λ Prophage Induction at Different LETs

After exposure of the cell to radiation a large spectrum of initial DNA damages are induced. Simultaneously with LET increasing, SSBs decay but DSBs and various "complex" DNA SSBs with end groups of the complicated structure augment[11]. These "complex" SSBs appear at a LET-dependent frequency as a curve with a maximum[12]. "Complex" SSBs are nonrepairable from polA$^+$-dependent repair, but are repairable only by recA$^+$/lexA$^+$-dependent one.

In this case, the yield of the initial breaks which are a substratum for various cellular exonucleases increases with LET. The exonuclease clearing causes SSG formations which may execute a signal role at the SOS system starting. Thus we may expect that the prophage inductivity will increase with LET. In reality, a similar increase in the inductivity takes place (see fig.2-4, table 3).

Various Pre-Irradiated Conditions

We specified that the increase in the inductivity takes place when cells are cultivated up to the irradiation in nutrient media altering from poor to rich one. In order to explain it, we assumed that α is directly proportional to the RecA protein amount. In agreement with this assumption there is a fact that in rich media the rybosom genes express themselves many-fold faster than they do it in poor media[13].

Genotype Role in the Alteration of λ Prophage Induction

Our experimental data testify that the expression of the λ prophage induction strongly depends on the genes participating in DNA repair (fig.4,5). We find in fig.4 that the dependence I(D) for polA mutant had the same shape as I(D) for the wild type strain. It is shown that the polA mutant SOS system works as efficiently as the wild type strain one does. We introduce

the following two symbols for the dose-modifying factor (DMF): $DMF_g = \alpha_m/\alpha_w$, where α_m and α_w represent the mutant's and wild type's inductivity; $DMF_s = D_{om}^{-1}/D_{ow}^{-1}$, where D_{om}^{-1} is the mutant's radiosensitivity and D_{ow}^{-1} is the wild type's one. Experimental data showed that DMF_g is three times more than DMF_s. This fact indicates that the participation of γ-sites in the formation of the lethal events differs essentially from the "prophage-virulent phage" switching over.

The DNA injuries, switching on the lytic cycle development of the bacteriophage λ are chiefly SS fragments or SSG_s[14]. DSB_s and $EDSB_s$ of DNA are lethal for E.coli cells. Many damaged DNA bases of polA mutant are repaired only by recA$^+$/lexA$^+$-dependent repair. As a result, a high level of EDSB, appears which determines the enchanced cell radiosensitivity[15]. On the other hand, the absence of polA-dependent repair increases the amount of DNA bind RecA protein and augments RecA protein molecules, which obtain an opportunity to acquire a protease conformation. Since a part of these injuries is recovered by recA$^+$/lexA$^+$-dependent repair, it is easy to understand that $DMF_g > DMF_s$.

After the action of the accelerated helium ions the isogenic strains W3110 and P3478 showed an inductivity that is larger than the one revealed after γ-irradiation. The reasons for this behaviour of the relative inductive efficiency (RIE) have been discussed. We only add to this that after irradiation the polA mutant gave a higher RIE value than the wild type did. This fact is understandable if one takes into account that various "complex" DNA injuries, otherwise repairable by polA$^+$-dependent recovery, are repaired in this case (polA mutant) by recA$^+$/lexA$^+$-dependent repair.

We believe the difference between the induction frequency of the recA, lexA, recBC mutants on the one hand and recA$^+$/lexA$^+$ genotype on the other hand is due to the little formation probability of the complex: DNA-RecA-λ repressor. The formation of this complex is obviously limited by the RecA protein amount for recA and lexA mutations, and by the SSG_s of DNA for recBC mutant. After the action of accelerated helium ions the lexA mutant showed an increase in the induction frequency with respect to the one observed after γ-irradiation. In our opinion, it is caused by the augement of the various "complex" DNA breaks, which appear more often after ion beam action, as a consequence of the energy dissociation features, than after γ-irradiation.

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Индукция профага λ ионизирующими излучениями с разной ЛПЭ

Изучена индукция профага λ γ-излучением и ускоренными заряженными ионами с разной ЛПЭ на разных штаммах E.coli. Зависимость частоты индукции от дозы I(D) для штаммов с $recA^+/lexA^+$-генотипом описывается кривой с максимумом. Индуцибельность этих штаммов возрастает как с ростом ЛПЭ, так и в направлении от бедной к богатой среде. В отличие от этого, зависимость I(D) для штаммов RecA, lexA, recBC является постоянной.

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Prophage λ Induction by Ionizing Radiation of Different LETs

The λ prophage induction caused by γ-irradiation and accelerated heavy ions with different LET was studied in variety Escherichia coli strains. The induction frequency on the dose I(D) shaped a curve with a maximum in the strains which possess $recA^+/lexA^+$ genotype. The inductivity of these strains increases as well as LET and an alteration poor ⟶ rich media does it. Unlike I(D) for $recA^+/lexA^+$, the dependence I(D) for recA, lexA and recBC strains was a constant.

The investigation has been performed at the Laboratory of Nuclear Problems, JINR.

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