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THE REGULARITIES OF FORMATION OF GENE AND STRUCTURAL MUTATIONS IN *ESCHERICHIA COLI* CELLS AFTER HEAVY ION IRRADIATION

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The results of induction of point and deletion mutations by radiation with a broad range of linear energy transfer (LET) in *Escherichia coli* cells are presented. The linear-quadratic function for point mutation induction was shown in comparison with linear dependence for deletion mutations. The relative biological efficiency (RBE) as a function of LET is described by dependence with a local maximum. The greatest RBE coefficients for the lethal effects, gene and deletion mutation induction were obtained under different LET of heavy charged particles.

Представлены результаты индукции точковых и делеционных мутаций у бактерий *Escherichia coli* излучениями широкого диапазона линейных передач энергии (ЛПЭ). Показано, что в отличие от линейно-квадратичной зависимости выхода генных мутаций от дозы излучений дозовая зависимость выхода структурных мутаций описывается линейной функцией. Относительная биологическая эффективность (ОБЭ) излучений как функция их ЛПЭ в обоих случаях имеет локальный максимум. Наибольшие значения ОБЭ для летальных эффектов, индукции генных и делеционных мутаций реализуются при различных величинах ЛПЭ тяжелых заряженных частиц.

INTRODUCTION

The study of the regularities and mechanisms of mutagenesis induced by heavy charged particles in the living cells is one of the important problems in radiation genetics. The importance of the investigations is connected with regulation of personnel irradiation in mixed fields of radiation and protection of crews from Galactic heavy ions for long space flights, with the other important practical tasks. In the course of solving the problem it is important to study not only the total number of induced mutations in different cells. Of major interest are the data on the frequency of gene and structural mutations. The comparative research of the frequency of point and structural mutation induction in mammalian cells after irradiation with heavy charged particles over a wide range of linear energy transfer (LET) is a complicated problem. Such information is easier to obtain in experiments with bacterial cells.

There are different approaches to study of chromosomal mutations in prokaryotes [1, 2]. The first one is based on the determination of deletion directly in chromosome. The second one uses the artificial test systems (plasmids and episomes). The structural mutations in bacterial chromosome can be determined either by the detection of inability of mutant cells to the wild type reversion or by the detection of mutations in both flanking genes.

The investigation was aimed to study the regularities of induction of structural mutations in *Escherichia coli* cells after irradiation with γ rays and accelerated heavy ions in a wide range of LET. The test system based on the detection of deletion mutations in *ton B* and *trp* flanking genes was used [3].

MATERIALS AND METHODS

The following strains of *E. coli* cells were used in experiments: *B*, *W3110*, *X7026*. *E. coli T20 (col B-K260), M32-T19 (col M-K260), C600 (col D)* strains were used as a colycin producents. To obtain the colycins, the optimal method was developed. The lysate of $\varphi 80$ phage with $1 \cdot 10^{10}$ titter of phage particles/ml was used. Overnight culture for detection of deletions in *ton B* gene was grown in full LB medium. After dilution the culture was grown up to $3{-}4 \cdot 10^8$ cells/ml and then was cooled and irradiated. The irradiated cells were inoculated in fresh LB medium ($1{-}3 \cdot 10^8$ cells per 100 ml) and incubated for 6–8 h at 37 °C. The cells were plated on selective medium (LA medium with colicyn — 30% of volume) for detection of mutations. Before cell plating the samples were treated with phage $\varphi 80v$ lysate to identify *col* $B^{\mathrm{res}}\varphi 80^{\mathrm{res}}$ cells. The deletion mutations in the samples were evaluated after plating the *col* $B^{\mathrm{res}}\varphi 80^{\mathrm{res}}$ colonies on the selective medium with LA, M56 + citrat-Na, M56 + citrat-Na + Trp, M56 + Trp + Cr. The *ton B trp*$^-$ deletions were measured after 48 h. The reversions to *ton* B^+ phenotype were evaluated on M56 + Trp + Cr medium. The incubation of cells was for 36–40 h at 37 °C. The absence of back mutations was estimated as deletion mutation.

The mutation rate was measured as the ratio of mutant colonies (N_m) to the number of surviving cells (N). Dose dependence $N_m/N(D)$ was fitted to the following function:

$$N_m/N = kD^{\chi},$$

where D is the radiation dose. The parameters k and χ were determined for each $N_m/N(D)$ dependence by an optimizing procedure.

A ^{137}Cs γ-ray source giving a dose rate of about 20 Gy/min was used. Irradiation with heavy ions was performed at the heavy ion accelerator U200 (Joint Institute for Nuclear Research). The dose rate of heavy ions was 12 Gy/min. In experiments with γ rays the cell suspension was irradiated in glass tubes or in special glass plates. Because of the low penetration of low-energy heavy ions, bacteria were irradiated only in glass plates 300 μm deep and 10 mm in diameter. In this case 0.01 ml of the cell suspension was placed on sterile glass plates and covered with a Mylar film 15 μm thick.

RESULTS AND DISCUSSION

The mutation rate of *ton B* and *col B* genes in *E. coli X7026* cells as a function of the dose of γ rays, helium ions with different energies and carbon ions is shown in Figs. 1 and 2. A power dose-response relation was observed for all types of radiation. The highest efficiency in mutation induction was observed after irradiation with helium ions with LET = 20 keV/μm. At 78 keV/μm helium-ion irradiation the mutagenic effect was significantly reduced. As known, a power dose-response relation of mutagenesis is explained by the «two events» model [4]. These events are formed as a result of two independent «hits». The first hit forms the «premutational» lesion in observed gene and the second one is connected with induction of the SOS repair. This type repair transforms the «premutational» lesion to mutation. Therefore, a power dose-response character of mutagenesis reflects the important role of SOS repair in inducible mutagenic processes and the repair is essential condition in its realization.

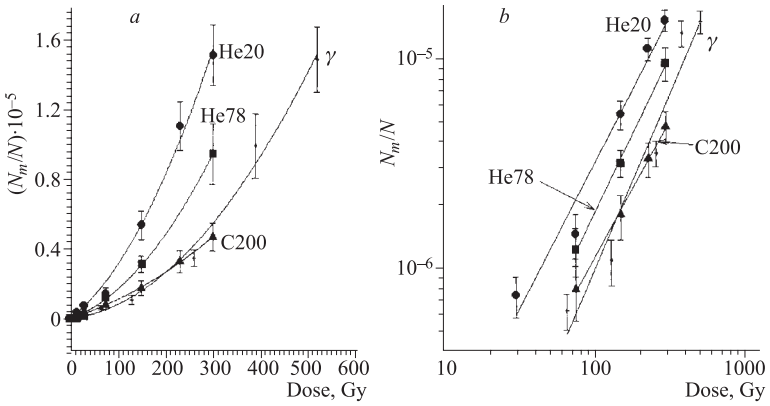

Fig. 1. The frequency of *ton* B^- mutation induction after irradiation of particles with different LET: *a*) the linear scale; *b*) the logarithmic scale

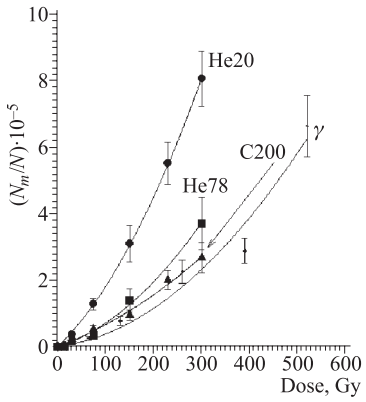

Fig. 2. The frequency of *col* B^- mutation induction after irradiation of particles with different LET (He20, He78, C200 indicate the LET of the particles)

The maintenance of power dose-response relation of mutagenesis after heavy-ion irradiation is determined by the following circumstances [4]. The microdosimetric analysis shows that irradiated cell population may be divided into three fractions. The first one is undamaged surviving cells, the second part is inactivated nondividing cells and the third one is «moderate» damaged cells, which can survive after reparation. With growing LET of the particles the fraction of undamaged surviving cells increases. On the contrary, the fraction of cells with lethal damages induced by the core of track particles decreases. As a result, the mutations form mainly in cell subpopulation, which is damaged by the δ electrons in track of the particles. This can explain the maintenance of power $N_m/N(D)$ relationship after the action of radiation with a wide range of LET, since the character of energy deposition by δ electrons in genetic structures with γ-ray and heavy-ion irradiation is the same.

The increase of mutagenic efficiency and RBE coefficients of charged particles with growing LET is determined by increasing yield of clustered single strand breaks (SSB) that are repaired by the mutagenic branch of the SOS repair system [4]. These damages are the molecular events that transform into mutations and induce the mutagenic DNA repair. The other character of $N_m/N(D)$ relationship was observed for deletion *ton B trp*$^-$ mutations. As can be seen from Fig. 3, the frequency of such mutations linearly increases with the doses of different types of radiation. The highest efficiency of mutagenic action was observed after irradiation with helium ions with LET $=$ 78 keV/μm. The mutagenic effect of carbon ions is lesser.

Taking into account the same character of $N_m/N(D)$ relationship for all types of radiation used in the experiments, it is easy to calculate the coefficients of relative biological efficiency (RBE) of heavy ions. The RBE can be calculated as the ratio D_γ/D_i, under the same level

of mutation frequency where the D_γ and D_i parameters correspond respectively to the doses of γ radiation and heavy ions for all levels of mutation frequency. The same way the linear type of dose-response deletion mutation induction allows calculating the RBE coefficients for these kinds of mutations.

The RBE on LET dependence for the lethal effect, induction of gene and deletion mutations is presented in Fig. 4. As can be seen, all the types of dependence are described by the curves with a local maximum. The position of the maximum is not invariant for the observed effects. The highest RBE coefficients were obtained at LET $\approx$ 100 keV/μm. The mechanisms that determined the character of RBE(LET) dependence are considered in detail in [5]. It was shown that the type of dependence is defined by the proportion of contribution in the lethal effect of irradiation direct and enzymatic double strand breaks (DSB) of DNA. The maximal values of RBE on the induction of point *ton B*$^-$ mutations were obtained after irradiation with helium ions with LET = 20 keV/μm. The same dependences were observed earlier [4] in experiments with gene mutation induction in *E. coli* cells and Salmonella tester strains (Ames test).

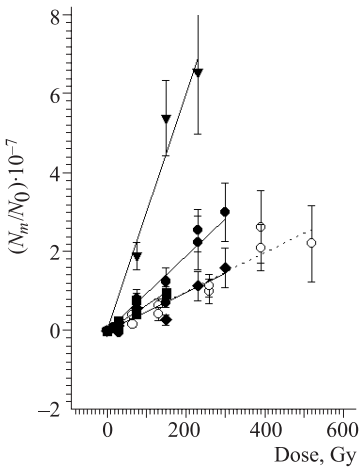

Fig. 3. The frequency of *ton B*$^-$ *trp*$^-$ mutation induction on the dose of radiation with different LET: ○ — γ rays; ● — He ions (20 keV/μm); ▼ — He ions (50 keV/μm); ■ — He ions (78 keV/μm); ◆ — ^{12}C ions (200 keV/μm)

Within the approaches developed in [4] one can explain the differences of RBE(LET) maximum position for the lethal and mutagenic effects of irradiation. These differences may be due to the different types of DNA damages involved in mutagenic and lethal effects of irradiation. The mutagenic lesions are connected with the base damages and the lethal effect is the consequence of DSB induction. The microdosimetric analysis of clustered SSB and DSB yields as a function of LET shows that the both dependences are described by the curves with a local maximum [6]. But the position of maximum for clustered SSB(LET) dependence is shifted almost by an order in the low region of LET in comparison with DSB(LET) dependence. This can explain the differences of RBE(LET) curves for the lethal effects of irradiation and for induction of gene mutations.

The dose-response relationships for the deletion mutations are described by linear functions (Fig. 3). This type of dependence is connected with induction of DSB that are involved in formation of deletion mutations. An influence was shown [7] of the repair of γ-induced DSB on the frequency of long deletions in artificial system with the use of episome that was integrated into bacterial DNA. The frequency of deletion mutation induction linearly increases with growth of doses from 25 to 200 Gy in a similar way as formation of DSB. On the other hand, the dose-response dependence for the substitutions and frame-shift mutations is described by a nonlinear function. On the basis of these results it was concluded that linear dose-response dependence for deletion mutations in γ-irradiated bacterial cells is determined by the following causes. The molecular damages that form the deletion mutations, unlike the clustered SSB that form the mutations from the point mutations, are the DSB of DNA. To fix such kind of mutations from the premutational lesions, there is no need to induce the mutagenic SOS DNA repair that plays a crucial role in formation of gene mutations.

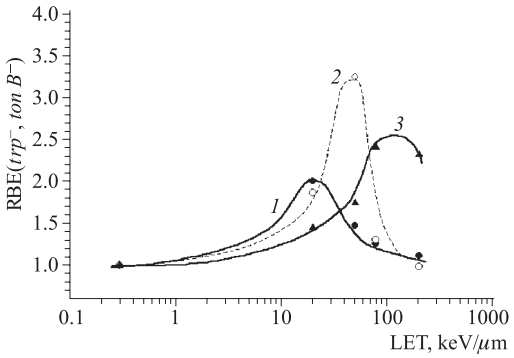

Fig. 4. The dependence of RBE on LET on the criterion of different type of mutation indication and on the lethal effect: ●, *1* — *ton* B^- gene mutations; ○, *2* — *ton* $B^- trp^-$ deletion mutations; ▲, *3* — lethal effect

On the basis of these data the position of the maximum of RBE(LET) dependence for deletion mutations may be explained. As can be seen from Fig. 4, the maximum of the dependence, in comparison with RBE(LET) relationship for the gene mutations, is shifted to the higher values of LET also for the lethal effects. This circumstance may indicate the uniformity of DNA lesions that are involved in formation of deletion mutations and the lethal effects of irradiation in *E. coli* cells. These damages are the DSB of DNA. The unlikeness of exact coincidence of these dependences probably reflects the different influence of enzymatic DSB in induction of the lethal and mutagenic effects of irradiation.

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