

GENETIC EFFECTS AFTER HEAVY ION IRRADIATION OF HAPLOID AND DIPLOID YEAST CELLS*

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Abstract. We have investigated the biological effects induced by different accelerated ions (^4He , ^{11}B , ^{12}C , ^{15}N , and ^{20}Ne) with different energies and linear energy transfers (LETs) and determined their relative biological effectiveness (RBE) for lethal damage and gene mutations. In particular, base pair substitution induction by ionizing radiation in haploid and diploid yeast *Saccharomyces cerevisiae* has been studied. We have detected the GC-AT transition in the haploid strain and the AT-TA transversion in the diploid strain. The RBE dependence on LET for lethal mutations is described by a curve with a local maximum at LET of about 100 keV/ μm . It is shown that the mutation frequency increases with increasing the dose up to 1000 Gy for diploid cells irradiated by different ions. A decrease in RBE with increasing LET has been observed for diploid cells. However, for haploid cells irradiated at doses of up to 100 Gy, the curves seem to have a plateau. The RBE dependence on LET for haploid cells is different and also has a plateau. But for substitution induction in haploid cells, an ion beam with a high LET (177 keV/ μm) is less mutagenic than the one with a low LET (44–127 keV/ μm). Therefore, we have obtained different biological effects of accelerated ions for haploid and diploid cells.

Key words: Accelerated heavy ions, base pair substitutions, yeast *Saccharomyces cerevisiae*

1. INTRODUCTION

Interest in studying the biological effects of heavy ions has grown in connection with active space exploration and considering future Mars missions [1]. Cosmic radiation is primarily composed of protons (~95 %) and heavy nuclei (Li, B, C, N, O, etc.) with energy of >10 MeV/u. Heavy ions have a more pronounced lethal effect on cells. The biological effects of high-LET radiation are generally greater than those of low-LET radiation, such as γ -rays, which is due to the high ionizing density in the tracks of charged particles [2]. However, the differences in RBE for different types of mutation were not studied well. It is possible to model the action of cosmic radiation by using heavy ion accelerator beams at JINR, Dubna. Unicellular yeast cells were chosen as a model eukaryote. The use of different yeast genetic systems is convenient for studying the dose dependence of mutagenic effects. Additional interest is aroused by the use of yeast to detect, measure, and correlate the impact of space radiation on living organisms over long durations beyond Low Earth Orbit and heliocentric orbit in Bion-M and NASA's BioSentinel mission. It will be interesting to compare laboratory and outer space experiments.

2. MATERIALS AND METHODS

2.1. Media

We used a standard rich medium YEPD (2 % peptone, 1 % yeast extract, 2 % glucose), a medium with glycerol YEPG (2 % peptone, 1 % yeast extract, 3 % glycerol, 3 % ethanol), and YPGD (1 % yeast extract, 2 % peptone, 3 % glycerol, 0.1 % glucose). Minimal MM₃₀₀ medium and synthetic complete media (SM) with bases and amino acids (Sigma) were described in [3]. SM contained adenine, arginine, histidine, methionine, tryptophan, uracil at 20 mg/l; tyrosine, leucine, lysine at 30 mg/l and threonine at 200 mg/l supplemented to medium MM₃₀₀.

2.2. Trp5-Assay

Various *trp5* mutant yeast strains (*MAT α his3- Δ 200 ura3-52 leu2- Δ 1 trp5-x*) were obtained from Dr. G. F. Crouse (Emory University, Atlanta, Georgia, USA). They differ from each other only by single base substitutions within codon-50 of the *trp5* gene [4]. Each strain reverts only *via* a true reversion event. In the present work, we used strain 1663 of this set. Omission media was SM-trp.

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2.3. *Cyc1*-Assay

Diploid hemizygous yeast strains YMH51-YMH57 (*MAT α /MAT α cyc1-x/cyc1-1 cyc7-67/cyc7-67 ura3-52/ura3-52 leu2-3,112/+ cyh2/+ +/his1-1 +/can1-100*) of *cyc1*-tester set were kindly donated by Dr. M. Hempsey (Louisiana State University Medical Center, Shreveport, Louisiana, USA). These strains differ from each other only by single base substitutions within codon-22 of the *cyc1* gene [5]. The *cyc1*-tester system set for base substitution is based on critical requirements for cycteine at position 22 of iso-1-cytochrome *c* encoded by *CYC1* gene. In order to restore codon 22 and revert to wild type the defined substitution is necessary. Each strain reverts only via a true reversion event. In this assay all possible base-pair substitution – 2 transitions and 4 transversions – can

be monitored. In these strains the *cyc1-1* and *cyc7-67* alleles are complete deletions of their respective genes, therefore, revertants can not arise either by mutations that result in overexpression of iso-2-cytochrome *c* or by recombination between the *cyc1-x* and *cyc1-1* or *cyc7-67* genes. In the present work, we used strain YMH53 of this set. Revertants were selected on YEPG medium containing 0.1 % glucose; it was important to obtain efficient and reproducible reversion frequencies.

2.4. Irradiation

The source of γ -rays was ^{60}Co (the dose rate of 0.7 Gy/min and LET of 0.25 keV/ μm) from the therapeutic equipment “Rokus” (JINR, Dubna). Cells were irradiated in Eppendorf tubes and kept in ice for the prevention of DSB repair.

Table 1. Properties of ions used in this study

Ion beams	Energy (MeV/u)	LET (keV/ μm)	Rate (Gy/s)	Source
$^4\text{He}^{2+}$	32	21	2-31	U-400
$^4\text{He}^{2+}$	-	81	15-38	U-400
$^{12}\text{C}^{6+}$	7.5	203	14-18	U-400
$^{11}\text{B}^{5+}$	33.8	42	5-10	U-400M
$^{11}\text{B}^{5+}$	22.1	61	0.1-0.2	U-400M
$^{15}\text{N}^{7+}$	46	67	0.4-0.7	U-400M
$^{15}\text{N}^{7+}$	46	177	0.4-0.7	U-400M
$^{20}\text{Ne}^{10+}$	49.2	127	0.8-1.25	U-400M
$^{20}\text{Ne}^{10+}$	49.1	126	0.01-0.4	U-400M

Heavy ion irradiations (Table 1) were carried out at the Heavy-Ion Accelerators U-400 and U-400M in Dubna (JINR). Night cultures ($\sim 2 \times 10^8$ cells/ml) were grown in YEPD. The percentage of budding cells was less than 3 %. The cells were centrifuged, washed, and resuspended in water to a concentration of 10^9 cells/ml. For irradiation, dry 4 % agar in special containers was prepared and covered by a mylar film with a diameter of 20 mm. 100- μl samples were spread onto the films. Containers were kept on ice before and after irradiation. Immediately before irradiation containers were fixed on a cassette disk. Beam monitoring and automatic change of cell patterns nested on the disk were provided by the special electronic equipment. After irradiation, the cells were resuspended by transferring each film to a tube containing 2 ml of sterile water. After serial dilutions, the irradiated suspension was plated on SM-trp or on YEPD to assess mutagenesis and on the YPD cell to assess survival.

Statistical analysis was carried out using program OriginPro 2015. For fitting curves coefficient R^2 ranges from 0.97 to 0.99 with few exceptions ($0.92 < R^2 < 0.96$).

3. RESULTS

Yeast cells were irradiated using γ -rays, α -particles and different heavy ions with various LETs (Table 1), then cell survival was determined (Fig. 1a, 2a). Haploid and diploid strains were irradiated at a dose of up to 500 and 1200 Gy, respectively. Survival curves were characterized by exponential dose dependences. For all LETs examined, cell survival decreased with increasing dose. RBE had maximum values for the ion beam with LET of 127 keV/ μm (Fig. 3a).

We analyzed point mutations induced by γ -rays and ions beams using two genetic assays (*trp5* and *cyc1*). Irradiation by γ -rays efficiently induced all types of base pair substitutions in *trp5*-assay, although GC-AT transitions were predominant [6]. In diploid *cyc1*-strains, GC-AT transition and GC-TA transversion were induced more efficiently after γ -irradiation [7]. In experiments with irradiation by heavy ions, we used only one haploid strain 1663 and one diploid strain YMH53 and tested GC-AT transition (strain 1663) and AT-TA transversion (strain YMH53).

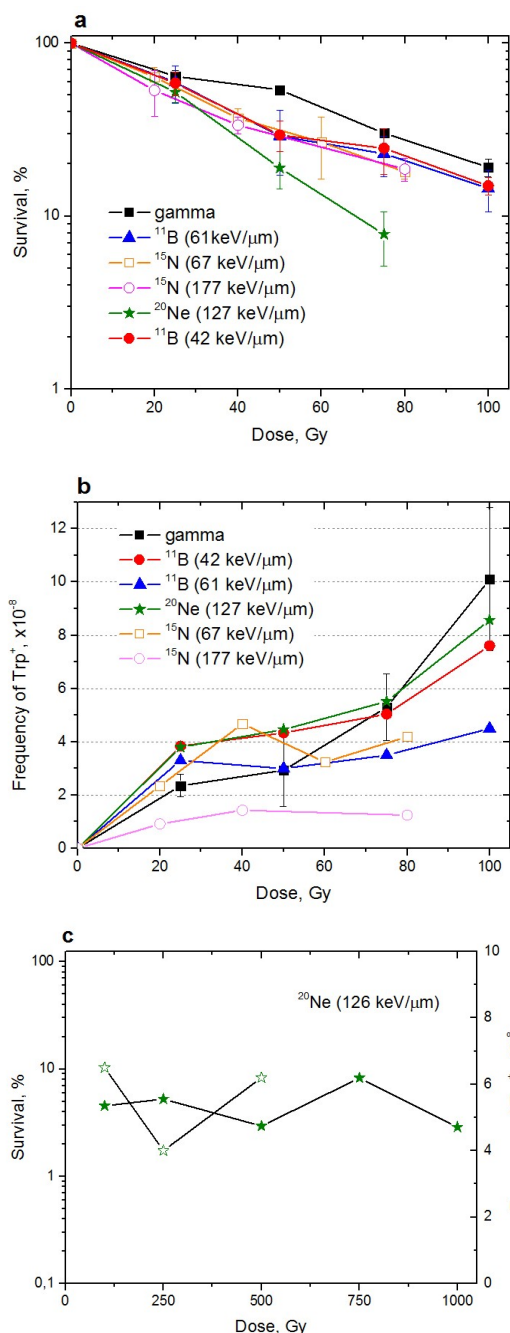


Figure 1. Survival (a,c) and frequency of GC-AT transition (b,c) after exposure of *trp5*-strain 1663 to ionizing radiation with different LET

Non-irradiated cultures had a small portion of revertant cells. The frequency of Trp⁺ revertants was $<4.8 \cdot 10^{-10}$ for *trp5*-strains 1663 and the frequency of Cyc1⁺ revertants was $5 \cdot 10^{-9}$ for *cyc1*-diploid strain YMH53. As we can see from Fig. 1b, in haploid cells, the frequency of mutation increased with increasing the dose of γ -radiation up to 100 Gy. We did not irradiate haploid cells with higher doses except ^{20}Ne

(127 keV/ μm) (Fig. 1c); we observed the plateau at high irradiation doses in the case of heavy ions, as we can see for ^{15}N (177 keV/ μm), ^{20}Ne (127 keV/ μm), ^{15}N (67 keV/ μm), ^{11}B (60 keV/ μm). In the other picture (Fig. 2b), we observed diploid strains. The frequencies of mutation induced by γ -rays and α -particles were increased up to 1000 Gy. The curves were fitted by linear-quadratic functions. However, mutations were practically not induced by carbon ions with LET of 203 keV/ μm . The curves were fitted by linear-quadratic (for 0.25 and 203 keV/ μm) and exponential (for 20 and 80 keV/ μm) functions.

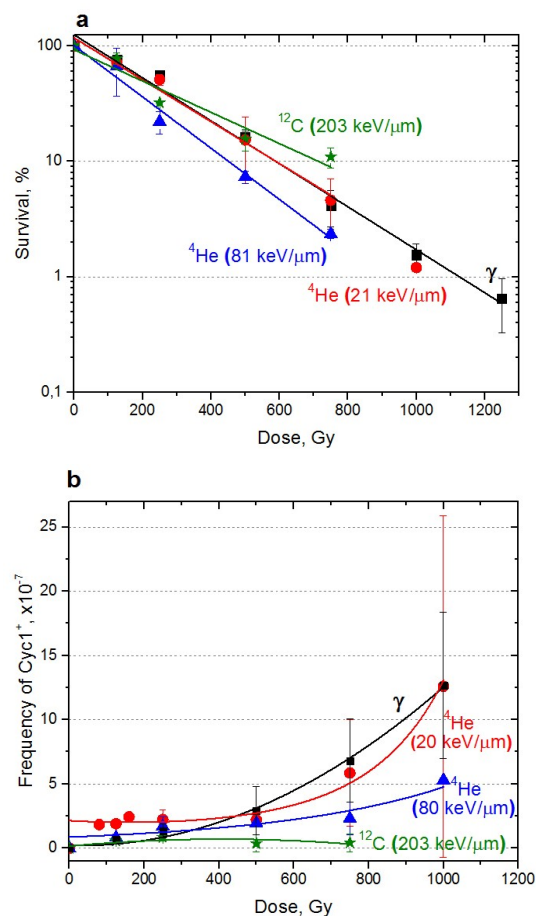


Figure 2. Survival (a) and AT-TA transversion (b) after the exposure of *cyc1*-strain YMH53 to ionizing radiation with different LET

For lethal effects, RBE was estimated as a ratio of curves slope for γ -rays to ion beams. The RBE dependence on LET for lethal mutations was described by a curve with a local maximum at LET of about 100 keV/ μm (Fig. 3a). However, the mutation frequency curves were not exponential as for lethal effects but had complex dependence. In this case, we got a few points reflecting the dependence of frequency at a different dose from LET. For haploid cells irradiated at doses of up to 100 Gy, the curves seem to have a plateau (Fig. 3b). For the substitution induction in haploid cells, an ion beam with a high LET (177 keV/ μm) was less mutagenic than the one with a low LET (44–

127 keV/ μm). However, a decrease in RBE with increasing LET has been observed for diploid cells (Fig. 3c). Therefore, we have obtained different biological effects of accelerated heavy ions for haploid and diploid cells.

4. DISCUSSION

Ionizing radiation induces various types of mutations, particularly base pair substitutions, most of which are an indirect effect of radiation and are caused by radicals induced by radiolysis of water, such as 8-oxo-dGTP. Base modifications may be mispairing and lead to base pair substitutions, for example 8-oxo-dGTP induces GC-TA and AT-CG transversions. Our experiments demonstrate that γ -rays generate base pair substitutions more efficiently than ion beams. Moreover, dose dependences of mutation increased up to 1000 Gy for the diploid hemizigotic strain and up to 100 Gy for the haploid strain in the case of low LET but had a plateau for haploid and diploid strains in the case of high LET. As reported by Matuo et al. (2017), it was a curve with a local maximum for γ -ray-irradiation at doses of up to 200 Gy and the maximum of gene mutations in *ura3* was localized at ~ 100 Gy but they did not operate with higher doses as in our experiments.

The sequence analysis of mutation in *ura3* gene showed γ -ray/carbon ion induced transversions (66.7/68.7%), transitions (20.0/13.7%) and frameshifts (13.3/17.6%) [8]. Transversions were mainly GC-TA and GC-CG and all of the transitions were GC-AT. We also obtained that the transversion frequency was higher than the frequency of transition [6]. However, for *trp5*- and *cyc1*-systems, γ -rays induced all types of transversions and transitions. It seems that the sample size of *ura3*-mutants was not sufficient. It is very interesting that the spectrum of substitutions induced by heavy ions was sequence dependent and that there were hotspots [8]. Yeast mutation sites were localized near linker regions of nucleosomes, whereas mutations induced by γ -rays were located uniformly throughout the gene.

Comparison of the base pair substitution spectrum of *trp5* haploid and *cyc1* diploid assays demonstrated the differences of spectra and frequencies [8]. The differences in the haploid *trp5* and diploid *cyc1* reversion assays could be due to their arising from fundamentally different mechanisms. It seems very likely that the reversion in the diploid cell occurs during the heteroallelic recombination with the homolog. The mitotic gene conversion of *leu1-1/leu1-12* in diploid cells was induced very efficiently by ionizing radiation [9]. The frequency of recombination increased at the dose of up to 800 Gy and was $\sim 8\%$ of living cells. Moreover, it was shown that double-strand breaks and their repair can be important sources of mutations which occur wherever breaks are located [10, 11, 12]. It is interesting to compare results obtained for definite point mutations with spectrum of nonspecific mutations located on large fragment of chromosome.

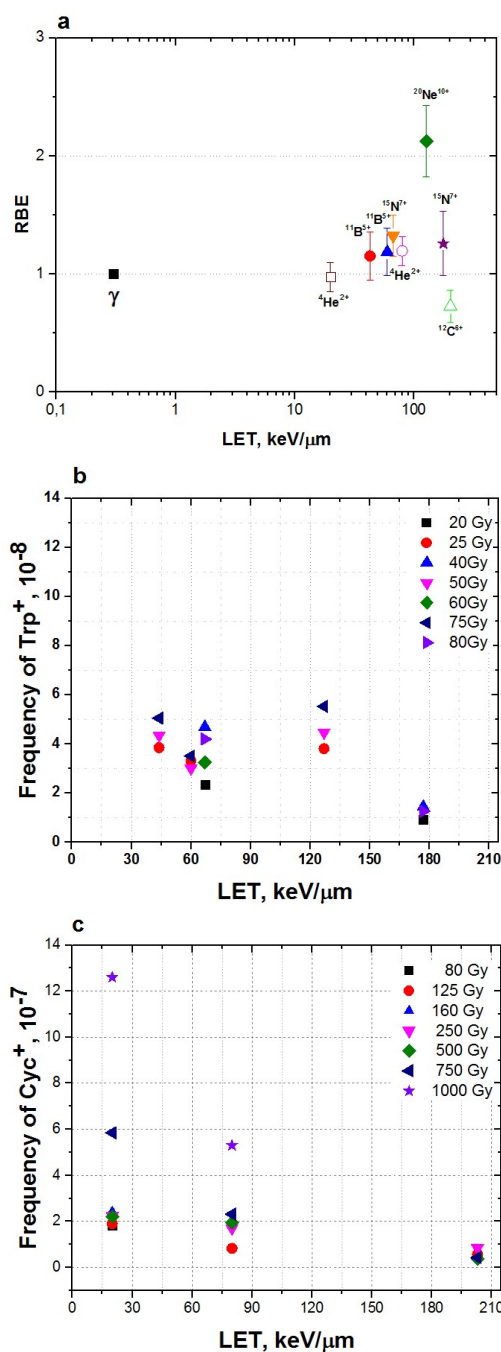


Figure 3. The RBE of lethality of haploid and diploid cells (a), the frequency of haploid *Trp*⁺ (b) and diploid *Cyc*⁺ (c) mutants for different irradiation doses as a function of LET. Designation at (a): open symbol – diploid, closed symbol – haploid

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