The analyses of remote descendants of yeast *Saccharomyces* cells irradiated by neutrons (0.85; 1.44; 14 MeV) or $^{60}$Co γ-rays

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**INTRODUCTION**

Interest in the biological effects of low doses of ionizing radiation, especially densely ionizing radiation, has been caused first of all by environmental contaminations resulted from technogenic nuclear facilities and power plant accidents. In such cases, living objects are exposed to radiation released by radioactive materials. On the other hand, the introduction of neutrons and high-energy sources into biology and medicine sets the task of studying the biological effects of radiation including the effects of small doses. In recent years, multiple experiments in which cells were exposed to low doses of ionizing radiation have yielded results which cannot be explained by target theory: genetic instability, bystander, hypersensitivity and induced radioresistance. It means that the processes involved in cellular responses to low-dose irradiation might differ from those when high doses of irradiation are delivered. Yeast has become popular in environmental radiation research. This is mostly because it is a convenient organism for studying the biological effects of ionizing radiation. The yeast *Saccharomyces* was used as the test eukaryotic organism which enables complete control over its chemical and physical environment.

**MATERIALS AND METHODS**

The diploid wild-type yeast *Saccharomyces* strain Megry 139-B was used. The cells were grown on solid nutrient medium (1% yeast extract, 2% peptone, 2% agar, 2% glucose) up to a stationary phase at 30° C. The fraction of budding cells did not exceed 1 – 2%. Cell suspension (1x10$^6$ cells/ml) was irradiated in a phosphate buffer (pH = 7.0).

**Irradiation**

The diploid cells were exposed to: neutrons at the channel B-3 of the BR-10 reactor (Ê – 0.85 MeV, dose rate – 1.0 Gy/min); fast neutrons with ultra-high dose rates (up to 6x10$^6$ Gy/s) or continuous neutron radiation (3x10$^3$ Gy/s) of the pulse reactor BARS-6 (Ê – 1.44 MeV); neutrons of the pulse neutron generator ING-03 (f = 50 Hz, Ê – 14.0 MeV, Russia); $^{60}$Co γ-rays (dose rate - 40 Gy/min, "Issledovateli").

After exposure to a dose range of neutrons (3.0 – 30.0 Gy) or to $^{60}$Co γ-rays (3.0 – 90.0 Gy), the cells were immediately plated on solid YEPD medium and incubated for 2 – 24 hours at 30° C for the light microscope analysis of cells budding and microcolony formation. The cells survival was obtained by measuring the colony-forming ability of irradiated cell populations. Cells were plated into Petri dishes post irradiation and incubated for additional 5 - 7 days at 30° C to enable the macrocolony formation. To determine the value of the relative biological effectiveness (RBE), cells were irradiated up to a dose of 700 – 800 Gy. RBE of radiation was estimated as a ratio of mean lethal doses (D$_0$) of γ-rays and neutrons. The RBE for lethality was calculated as follows: RBE = D$_0$γ/D$_0$n.
RESULTS AND CONCLUSIONS

In our work we studied and compared the biological effects of fission neutrons (\(\bar{E} \sim 1.44\) MeV) with ultra high dose rates (up to \(6 \times 10^6\) Gy/s). Diploid yeast cells of the wild type were irradiated in identical positions either with a single pulse (65 µs) or in continuous mode for one hour, the reactor power was approximately the same in both irradiation modes. The RBE value (1.74) of a single pulse of neutron irradiation was compared with the RBE value (1.79) of neutrons during continuous irradiation for the wild-type yeast strains (Tsyb et al., 2007). The similar survival resulted from the action of a single neutron pulse and the continuous action indicates that the time factor does not influence on the forming of elementary radiation damages in a cell. We have concluded that the biological effectiveness of neutrons of pulse reactor BARS-6 was independent on the dose-rate differing up to \(10^8\) fold. In our previous paper (Tsyb et al., 2001), the same RBE value (2.0) of neutrons (\(\bar{E} \sim 0.85\) MeV) of the B-3 channel of the BR-10 reactor at a continuous mode of the action was shown in the limits of experimental errors. The biological effectiveness of neutrons (4.1 – 3.1) was found to be the highest at small doses at 90 – 70% survival (Tsyb et al., 2007).

It was interesting to analyze the region of low doses where the cell survival was 100%. What kind of descendants of irradiated cells was that? As a rule, at low doses of ionizing radiation with different LET the state of irradiated cell is usually studied and very seldom their descendants are analyzed. The cells which form large clones (macrocolonies) apparent to the naked eye on solid nutrient medium are believed to be able to propagate itself without bound or be reproducively survived. The cells which are not able to form macrocolonies are considered to be dead. It should be mentioned that it is not known yet whether the irradiated cells or their closest descendants died. The yeast cells survived after any exposure can also be determined by the formation of microcolonies on solid nutrient medium after incubation for 24 hours at 30°C (Korogodin, 1966). Within this time period, the cells form microcolonies consisting of thousands of budding cells which are compactly arranged. In our experiments, the cells exposed to various kinds of low-dose radiation formed typical macrocolonies in nutrient agar medium after 7 days incubation at 30°C, i.e. cell survival was 100%. We assumed that a cell population formed from an irradiated cell can be considered to be a carrier of some non-lethal damages which had been induced in an initial irradiated parental cell. Approximately at 10 – 12 hours of incubation, non-irradiated cells start forming small compact microcolonies. Irradiated cells form along with such microcolonies a lot of changed microcolonies as “chains”, “twigs” from 8 - 20 large cells (l = 40-50 µm, d = 20-30 µm versus l = 20-25 µm d = 10 µm for control cells) so called “spiders” resulted from slowing the reproduction rate of descendants of irradiated cells (Tsyb et al., 2006). It should be particularly emphasized that such forms of microcolonies are believed to be inactivation forms of the yeast cells exposed to lethal doses of ionizing radiation (Korogodin, 1966). In our experiments we used the doses which were by 5 – 10 times less than mean lethal doses.

At 18 – 20 hours of incubation, the non-irradiated cells formed typical microcolonies consisting of compactly arranged cells, and the irradiated cells formed along with typical microcolonies atypical looser regenerative microcolonies (~20%) with ragged edges (Fig. 1). We determined the dose-dependent biological effectiveness of neutrons for the formation of microcolonies with a changed shape which were absent in control (Fig. 2). The output of such forms of microcolonies is linear, and this test suggests that the effectiveness of two beams of reactor neutrons regardless of the dose rate (at a isoeffect level) is by 4 – 7 times
higher in comparison with gamma-radiation (Table 1). Neutrons with an energy of 14 MeV are by 2 times less effective than reactor neutrons (E – 0.85 MeV and 1.44 MeV). At 24 hours of incubation, almost all cells (98.0%) which had been exposed to γ-rays or neutrons formed typical microcolonies.

*Figure 1. Microcolonies formed by unirradiated control yeast cells (a); microcolonies formed by yeast cells irradiated with low dose neutrons (b): 1, 2 – nontypical microcolonies, 3. – typical microcolonies.

*Figure 2. Relationship between formation of nontypical microcolonies and dose of γ-rays (curv. 1) or 14 MeV neutrons (curv. 2), 1.44 MeV neutrons (curv. 3, ● – pulse, ○ – continuous), 0.85 MeV neutrons (curv. 4) for yeast cells. P<0.0001

*Table 1. Relative effectiveness of neutrons by the formation of changed microcolonies by yeast cells at equivalent effect levels

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<th>Nontypical microcolonies, %</th>
<th>Biological efficacy of neutrons*</th>
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<tr>
<td></td>
<td>n (0.85 MeV)</td>
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<tr>
<td>2.5</td>
<td>7.3</td>
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<tr>
<td>5.0</td>
<td>5.9</td>
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<tr>
<td>10.0</td>
<td>4.9</td>
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<td>15.0</td>
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<td>20.0</td>
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Thus, obtained data show that:
1. Ionizing radiation at non-activating doses induces non-lethal damages in yeast cells which lead to the disturbance of budding dynamics without loss of the colony-forming ability;
2. The biological effectiveness of neutrons within the range of non-lethal doses depends on the neutron energy;
3. The biological effectiveness of neutrons of the pulse reactor BARS-6 was independent on the dose rate differing up to $10^8$ fold for the survival and morphology of microcolonies.

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REFERENCES


