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Divisions of Survived Cells with Primary Radiation Damage

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1. Introduction

Single cells among both survived and inactivated parts of a homogeneous population respond differently to the same radiation dose. Survived cells produce on solid nutrient media colonies (different inactivation forms) of various sizes and morphology which appear within different time interval after irradiation [1]. Different manifestation of primary radiation damage can be quantitatively described by the probability model which is based on the supposition that clone formation is a probable process and the probability for the successful division of cell is determined by a number of damages. This means that the probability model is a peculiar synthesis of hit-and-target principle and biological stochastic. The model explains cell responses to radiation and quantitatively describes survival curves and the yield of inactivation forms of different yeast strains [2].

2. Probability Model

Irradiated single cells are damaged in a random fashion in accordance with the hit-principle, and the number of damages defines the probability P for the successful division. If the probability of damage expression (the probability of refusal) is α , the probability for the successful division of a cell with one damage is

$$P_1 = (1-\alpha)\,. \qquad (1)$$

For independent interaction of radiation damages, the probability for the successful division of a cell with i primary damage (sublethal lesion, hit) may be presented as

$$P_i = (1-\alpha)^i\,. \qquad (2)$$

The reduced probability for the successful division can remain unchanged through the successive division of cells [3]. Hence, the mechanism of clone formation is the well-known “birth-and-death” process. The process operates when an entity, in our case a single cell, either gives rise to progeny like itself (birth), or is removed in some way (death), and these two events occur in a random fashion. It would be of interest to obtain experimentally the distribution of survived cells in accordance with the number of primary lesions and after that, taking the population of cells with a known number of sublethal lesions, to determine various biological responses in the dependence of the primary radiation sublesions.

3. Materials and Methods

Two strains of diploid yeasts were used in the experiment; *Saccharomyces ellipsoideus (vini)*, strain Megri 139-B, and *S. cerevisiae*, strain 5*a*3b*α* that is heterozygous for the *ade2* mutation. Aliquots with 10^6 cells/ml from incubated cells for 3-5 days at 30°C were exposed to gamma radiation (^{60}Co, Gammacell 220, Atomic Energy Canada Ltd.). The survival was assessed by counting colonies in platings. Primary colonies, which appear after irradiation, were used to obtain subclones. Colonies, subclones of which grew simultaneously with a control and did not differ from the control phenotypically, were identified as stable (normal cell clones). Colonies, which formed slowly growing subclones and/or subclones that differed in morphology, were believed to be unstable clones.
To determine the content of mitotic recombinants in diploids that are heterozygous for the *ade1* and *ade2* mutations, colonies were replated on nutrient media YEPD and the number of white, red and sectored colonies was counted. The quantitative evaluation of respiratory mutants was performed as described in Ogur *et al*. [4], and the content of nonviable cells was determined by detecting, under a microscope, budding and nonbudding cells on the surface of the nutrient agar after growing for one day. Other details were described in the literatures [5,6].

4. Results and Discussion

The various macrocolonies produced from the irradiated diploid yeast cells with morphological changes may be attributed to the example of the expression of primary sublethal lesions. In case of the strain Megry 139-B irradiated with 600 Gy, the content of morphologically changed cells with two and three sublesions was significantly greater than that for clones without or with one primary sublesion (hit). Among the colonies produced after replating, saltant colonies were about 1% for clones without sublethal lesions, while they were higher than 80% for clones with two or three sublethal lesions.

The increased probability of unsuccessful division (refusal) should result in the existence of nonviable cells

in clones produced by the single cells survived after irradiation. Experimental data concerning the content of cells incapable to proliferation at optimal (30°C, standard nutrient media) and suboptimal (37°C, standard nutrient media + 7% NaCl) conditions for distant progenies of diploid yeast cells (strain Megry 139-B, 600 Gy) are presented in Table 1.

Table 1. The content of nonviable cells in clones produced from the diploid yeast cells survived after exposure to ionizing radiation

No. of primary sublethal lesions	No. of tested clones	The content of nonviable cells, %	
		Optimal condition	Suboptimal condition
0	28	17 ± 6	24 ± 7
1	50	20 ± 5	43 ± 11
2	22	27 ± 9	54 ± 15
3	28	36 ± 13	87 ± 13

The relative yield of nonviable cells in clones produced by survived diploid yeast cells increases with the number of inherited primary radiation sublethal lesions. Due to this fact, the growth effectiveness of cells under suboptimal conditions of culture was 76, 46 and 13% correspondingly to clones with 0, 2, and 3 primary sublethal lesions. Some unstable clones differ by a high segregation rate of respiratory mutants. Although respiratory mutants are virtually not encountered among primary colonies of irradiated diploid, these mutants can constitute more than 30% of all subclones in plating of some unstable clones. Diploid yeast cells (strain Megry 139-B) were irradiated in the stationary phase of growth (600 Gy). The data concerning the yield of respiratory mutants in clones with various numbers of primary sublethal lesions are presented in Table 2. With the tetrazolium overlag technique [4], cells with normal respiratory ability were colored in red color while clones consisting of respiratory mutants stayed white. It is obvious that irradiation resulted in an increased content of respiratory mutants in clones produced by irradiated cells. The effect was particular expressed for clones with a greater number of primary sublethal lesions (hits).

Table 2. The content of respiratory mutants in clones produced from the survived diploid yeast cells exposed to ionizing radiation

No. of primary sublethal lesions	Clones containing respiratory mutants (%)			
	0-2	>2-10	>10-50	>50-100
0	100	-	-	-
1	80	16	2	2
2	53	12	20	15
3	32	16	28	14

5. Conclusions

Unstable clones were formed among colonies grown from diploid yeast cells survived after irradiation. Cells from unstable clones exhibited an increased content of morphologically changed cells, nonviable cells and respiration mutants. Cells from unstable clones are characterized by the enhanced radiosensitivity. The degree of expression of the foregoing effects was the higher the greater the number of primary sublethal lesions was in the originally irradiated cell. The results were in accordance with the fact that the primary radiation lesion was not absolutely lethal for the diploid cell. With a certain probability depending on the total number of primary sublethal lesions and conditions of culture, disturbances were broke out in the successive divisions of the survived cells after irradiation.

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REFERENCES

[1] V.I. Korogodin, “Inactivation forms of yeast cells after exposure to ionizing radiation,” *Biophysica,* **3**, 206-214, 1958.
[2] Yu.G. Kapultsevich, V.G. Petin, “Probability model for cell responses to irradiation,” *Studia Biophysica*, **62,** 151-165,1977.
[3] Yu.G. Kapultsevich, “Quantitative Regularities of Cell Radiation Lesions,” Moscow: Atomizdat, 1978.
[4] H. Ogur, R. John, S. Nagai, “Tetrazolium overlag technique for population studies of respiration deficiency in yeast,” *Science*, **125**, 928-931, 1957.
[5] J.K. Kim, V.G. Petin, G.P. Zhurakovskaya, “Exposure rate as a determinant of synergistic interaction of heat combined with ionizing or ultraviolet radiations in cell killing,” *J. Radiat. Res.* **42**, 361-365, 2001.
[6] V.G. Petin, J.K. Kim, “Survival and recovery of yeast cells after combined treatments with ionizing radiation and heat,” *Radiat. Res*. **161,** 56-63, 2004.