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# RBE OF $\alpha$ PARTICLES AND POSTRADIATION RECOVERY IN A TEMPERATURE CONDITIONAL MUTANT OF DIPLOID YEAST FOR VARIOUS CRITERIA OF CELL DEATH

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The influence of the linear energy transfer (LET) on the relative biological effectiveness (RBE) and the liquid-holding recovery (LHR) ability was studied for a temperature conditional mutant of diploid yeast. Both the RBE and the rate of the LHR were much lower for the reproductive killing at 36 °C and cell killing without division at both 23 and 36 °C. However, the probability of recovery was independent of culture temperature being identical for both the low- and high-LET radiations. It was concluded that the LHR process itself is not damaged after densely ionizing radiation while the enhanced RBE value and the reduced rate and volume of recovery after high-LET radiation may be entirely caused by the increased yield of the irreversible radiation damages.

Изучено влияние линейной передачи энергии (ЛПЭ) на относительную биологическую эффективность (ОБЭ) и способность к восстановлению при выдерживании в непитательной среде условного температурного мутанта диплоидных дрожжей. Как ОБЭ, так и скорость восстановления в непитательной среде были намного ниже для репродуктивной гибели при 36 °C и для клеточной гибели без деления как при 23, так и 36 °C. Однако вероятность восстановления не зависела от культивируемой температуры и была одинаковой для излучений с низкими и высокими ЛПЭ. Делается вывод, что процесс восстановления в непитательной среде сам по себе не повреждается при действии излучений с высокими ЛПЭ, а повышение значений ОБЭ и пониженная скорость и объем восстановления после действия излучений с высокими ЛПЭ полностью обусловлены формированием необратимых радиационных повреждений.

## INTRODUCTION

The unexpected role of the specific repair pathways in the RBE values of high-LET radiation was demonstrated for bacterial [1–3], yeast [4–6] and mammalian [7–9] cells. The data presented in these communications demonstrate that (i) the RBE values of densely ionizing radiations were higher for more radioresistant wild-type cells in comparison with those for radiosensitive mutants defective in some reparation systems; (ii) the rate of recovery was reduced for high-LET radiation. However, it is still unknown whether high-LET radiation affects the recovery process itself or it only produces a higher number of irreversible damages that cannot be repaired at all. Both these possibilities may be realized simultaneously. This

question remains to be determined. It is known that the diploid temperature conditional radiosensitive mutant of *Saccharomyces cerevisiae* (strain g580L, *rad54-3*) is capable of repairing radiation-induced DNA double strand breaks (DSB) if they are cultured at the permissive temperature of 23 °C during postirradiation period [10]. The RBE of $\alpha$ particle was higher for this postirradiation temperature while it was smaller at the restrictive temperature of 36 °C due to a considerable depression of DNA double strand breaks repair [5]. The RBE values of $\alpha$ particle was higher for reproductive yeast cell killing than for cells killing without any postirradiation division and incapable of recovery [11]. Thus, it would be of interest to get an additional evidence of cell recovery participation in the RBE of densely ionizing radiation by comparing the RBE value of $\alpha$ particles for the reproductive cell killing and for the interphase cell killing without postirradiation division for the temperature conditional radiosensitive mutant cultured after irradiation at 23 and 36 °C. The other goals of this study were: (i) to elucidate to what extent the reduced recovery after high-LET radiation would be due to damage to recovery mechanisms themselves and/or to the formation of irreversible unrepairable damage; (ii) to estimate whether the reduced cell ability to recover at 36 °C is related with the increased yield of irreversible damage or due to the decrease in the probability of recovery. In this study, the liquid-holding recovery (LHR) will serve as an indicator of the cellular repair activity.

## 1. MATERIAL AND METHODS

The radiosensitive diploid yeast mutant of *Saccharomyces cerevisiae* (strain d580L, *rad54-3*) which is temperature conditional with respect to DSB rejoining cells was used in this study. This strain was obtained by us from Dr. D. Frankenberg (Germany). The cells were grown before irradiation up to the stationary phase of growth on a solid complete nutrient medium (1% yeast extract, 2% peptone, 2% glucose, 1.5% agar) for 7 d at 30 °C. To separate clumped cells, they were sonicated during 30 s at 30 W. Aliquots from the same cell suspension were irradiated with $^{60}$Co $\gamma$ rays (40 Gy/min) and with $^{239}$Pu $\alpha$ particles (25 Gy/min). The LET of a particle reaching the cell monolayer was estimated to be 134 keV/$\mu$m. Exactly at about this LET value the maximum RBE–LET relationship was registered for inactivation of many eukaryotic cell systems. Immediately after irradiation, a part of irradiated cells was plated onto nutrient agar (immediate plating) for the assay of the cell survival at 23 and 36 °C. Another part of the irradiated cell suspension was placed for the LHR at 23 and 36 °C and the cell survival was determined as a function of the incubation time (delayed plating). Plated cells were grown for 36 h on nutrient agar. It is known that dying cells can be inactivated either without any division (interphase killing) or after one or several reproduction cycles (reproductive killing). The cell survival was estimated microscopically using two criteria. First, cells that were capable of producing a microcolony consisting of more than 40 cells were considered to be viable (reproductive death). It is worth noting that the cell survivals estimated with macrocolony and microcolony counting coincided well. Second, the survival cells have to accomplish at least one division (cell killing without any division).

During the LHR process a number of the primary radiation damages is eliminated, resulting in an increased cell survival. This can be considered as a reduction of the initial dose $D_1$ to a certain effective dose $D_{\mathrm{eff}}(t)$ which is proportional to the mean number of the residual

damages, both reparable and irreversible, after a recovery for $t$ hours. It has been demonstrated for yeast cells [12, 13] that the decrease in the effective dose $D_{\rm eff}(t)$ with the recovery time $t$ could be fitted by an equation of the form

$$D_{\rm eff}(t) = D_1[K + (1 - K)\,{\rm e}^{-\beta t}], \tag{1}$$

where $K$ is the irreversible component of radiation damage, i.e., the fraction of radiation damage resistant to recovery, and $\beta$ is the recovery constant that characterizes the probability of the recovery per unit time. The ratio $K(t) = D_{\rm eff}(t)/D_1$ reflects the relative part of the primary radiation damage that has not been repaired during $t$ hours of recovery. If $t$ is sufficiently large (for yeast cells it is about 2–3 d), the recovery curves reach a plateau when the ability of the cells to recover is saturated or exhausted. For this case, we can write

$$K = K({\rm plateau}) = {\rm D_{eff}(plateau)/D_1}. \tag{2}$$

In this expression, $D_{\rm eff}$(plateau) is the effective dose corresponding to the plateau of the recovery curve, which is proportional to the mean number of the irreversible damages. Knowing the survival and recovery curves after cell exposure to low- and high-LET radiations, one can calculate the corresponding values of $D_{\rm eff}(t)$, $D_{\rm eff}$(plateau), $K(t)$, $K$, and $\beta$.

## 2. RESULTS AND DISCUSSION

Figure 1 shows the dependence of cell survival on radiation dose (*a*) and the duration of postirradiation recovery (*b*) for the reproductive killing of diploid yeast cells (*Saccharomyces cerevisiae*, strain g580L). The cells were exposed to graded doses of $\gamma$ rays (curves *1, 2*) and $\alpha$ particles (curves *3, 4*) and plated on nutrient medium immediately after irradiation (curves *1, 3*) and after 3 d of storage in conditions promoting the LHR (curves *2, 4*). The control and exposed cells were grown at 23 °C during postirradiation period. Cells incubated at the temperature permissive for DSB repair (23 °C) [5] yielded shoulder survival curves with a small extrapolation number. The RBE of $\alpha$ particles, defined as the ratio of doses producing 10% survival after low- and high-LET radiations, was found to be 4.6 (see table). This value is close to that observed for wild-type yeast cells [4, 14]. It means that these radiosensitive mutant cells being incubated at 23 °C show radiobiological responses similar to those of wild-type cells capable of recovery.

The recovery patterns are presented in Fig. 1, *b*. Here the recovery kinetics after low- and high-LET radiations are compared at approximately equal levels of survival (i.e., at equal amounts of lethal lesions). The arrows indicate the examples of the effective dose, $D_{\rm eff}(t)$, determination following 30 h of recovery. It can be seen from Fig. 1, *b* that the survival of irradiated cells held before plating in conditions favouring LHR was greatly enhanced in comparison with that obtained after immediate plating for both the low- and high-LET radiations. The number of viable cells increased as a function of time, reaching a plateau after about 2–3 d. Differences in repair kinetics are also evident, with slower recovery for $\alpha$-particle irradiation. The probability of recovery per unit time ($\beta$) was estimated using the survival and recovery curves after cell exposure to low- and high-LET radiations and Eq. (1). The fraction of irreversible damage the ($K$) was calculated as the ratio of doses producing 10% survival or the mean lethal doses after immediate and delayed plating. Both

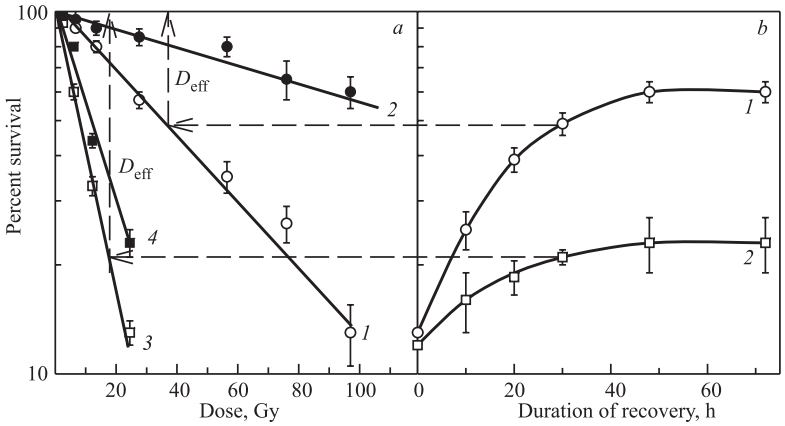


Fig. 1. *a*) The dependence of cell survival on radiation dose for the reproductive killing of diploid yeast cells (*S. cerevisiae*, strain g580L). The cells were exposed to graded doses of $\gamma$ rays (*1, 2*) and $\alpha$ particles (*3, 4*) and plated on nutrient medium immediately after irradiation (*1, 3*) and after a 23 °C holding period for 72 h at the permissive temperature for DSB repair (*2, 4*). *b*) The dependence of cell survival on the duration of postirradiation recovery after exposure to $\gamma$ rays (*1*) and $\alpha$ particles (*2*) and plated on nutrient medium after various recovery times at 23 °C. The control and exposed cells were grown at 23 °C during postirradiation period. The arrows indicate the examples of the effective dose, $D_{\text{eff}}(t)$, determination following 30 h of recovery. Data are the mean values calculated from at least three independent experiments. Error bars indicate the standard error of the mean. Curves were fitted to the data points by eye

these parameters are also included in the table after $\gamma$ and $\alpha$ irradiation. It is evident that the fraction of irreversible damage was markedly greater, i.e., the magnitude of postirradiation recovery was smaller, after $\alpha$ irradiation than that observed for $\gamma$ rays. In contrast, the probability of recovery was identical for both the low- and- high-LET radiations. Moreover, the probability of recovery of these mutant cells was identical with that observed earlier for the wild-type strain (XS800) [15]. Thus, the data presented suggest that recovery decreases with increasing LET of radiation. The decrease becomes apparent in both the rate and the volume of recovery. The independence of the probability of recovery from radiation quality means that the process of recovery itself stayed unchanged and a slower recovery may reflect a smaller yield of reversible damages produced by high-LET radiation. It can be concluded on this basis that the LHR process itself is not damaged after densely ionizing radiation and the enhanced RBE of the high-LET radiation may be related to the increased yield of the irreversible damage. Strong evidence for a causal relationship between rejoining of DSB, LHR and RBE of $\alpha$ particles has been reported [5]. We can suppose on this basis that the identical probability of the LHR observed in this paper may signify the identical probability of DSB rejoining after low- and high-LET radiations while slowing down and smaller volume of DSB rejoining may reflect the reduction in a number of DSB which are capable of rejoining.

The data described above prompted similar types of experiments using restrictive (36 °C) temperature for postirradiation cultivation. Figure 2 exhibits the results of these experiments. It is evident that at the restrictive temperature of 36 °C the *rad54-3* mutant yields exponen-

**RBE of $\alpha$ particles and parameters of LHR of conditional thermosensitive diploid yeast cell mutants of *Saccharomyces cerevisiae* (strain g580L, *rad54-3*) for various criteria of cell killing. The table includes the temperature at which LHR and postradiation cultivation was carried out**

| $T$, °C | The form of cell killing | RBE | $K(\gamma)$ | $K(\alpha)$ | $B(\gamma)$, $h^{-1}$ | $\beta(\alpha)$, $h^{-1}$ |
|---|---|---|---|---|---|---|
| 23 | Repro-ductive | 4.6 ± 0.4 | 0.28 ± 0.03 | 0.69 ± 0.05 | 0.05 ± 0.005 | 0.05 ± 0.005 |
| | Without division | 2.9 ± 0.2 | 1.00 | 1.00 | 0.00 | 0.00 |
| 36 | Repro-ductive | 3.0 ± 0.3 | 0.71 ± 0.06 | 0.68 ± 0.04 | 0.05 ± 0.005 | 0.05 ± 0.005 |
| | Without division | 2.5 ± 0.2 | 1.00 | 1.00 | 0.00 | 0.00 |

tial survival curves. One can see that radiobiological response is quite different from that observed at the temperature (23 °C) permissive for DSB rejoining. The mutant cells became more sensitive to low-LET radiation and their ability to undergo the LHR was also decreased. The decrease is apparent in both the rate and the volume of recovery. Here again, it was of interest to elucidate to what extent the reduced recovery was due to damage to the recovery mechanism itself and/or to the formation of irreversible unrepairable damage. We estimated the corresponding parameters describing the LHR process in these conditions. They are also listed in the table. It is clear that the probability of recovery ($\beta$) was identical for low- and high-LET radiation at both the permissive (23 °C) and restrictive (36 °C) for DSB rejoining temperatures. It means that an identical fraction of the reversible radiation damage is recovered per unit time independently of radiation quality at both temperatures investigated. The irreversible component was enhanced for restrictive temperature even for low-LET radiation and was similar to that observed for high-LET radiation. Therefore, the recovery process itself is not damaged after densely ionizing radiation and the enhanced RBE value of the high-LET radiation as well as the high radiosensitivity of mutant cells at the restrictive temperature may be related to the increased yield of the irreversible radiation damage.

The LHR processes are known to fail for interphase cell killing when they are inactivated immediately after irradiation without any postradiation division [11]. Figure 3 exhibits the survival curves for diploid yeast cells of *S. cerevisiae* (strain d580L) killing without division after $\gamma$- and $\alpha$-exposure. The irradiated cells were kept during the LHR procedure and then were incubated on nutrient media at 23 °C (Fig. 3, *a*) and 36 °C (Fig. 3, *b*). It can be seen that unusually tremendous radiation doses were used in these experiments after which a degradation of main cell compounds was observed [12]. Due to this fact no any recovery was recorded. Moreover, in all cases some decrease in cell survival was observed after LHR procedure (Fig. 3). This fact can be considered as an indication of the failure of the connection between interphase cell killing and cell ability to recover radiation damage and perhaps may be related with unrepairable radiation damages of vitally important cell structure. As the RBE value of densely ionizing radiation correlates with cell ability to recover from radiation damage, it can be expected that for cell killing without division the RBE of $\alpha$ particles would

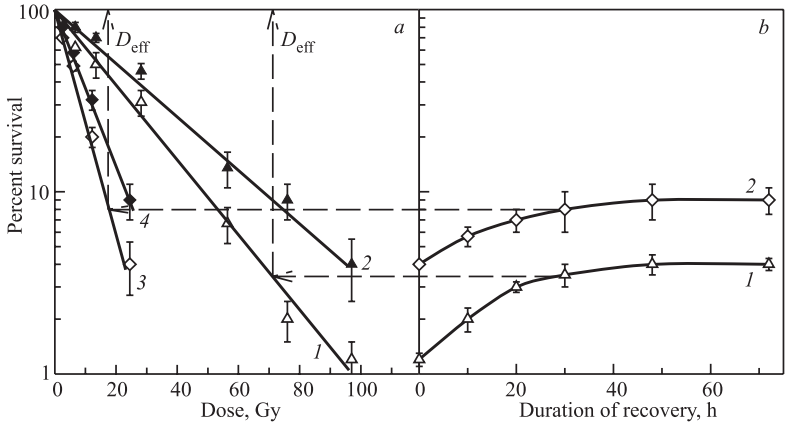


Fig. 2. The same as in Fig. 1, but the cells were recovered and grown during postirradiation period at the restrictive temperature of 36 °C

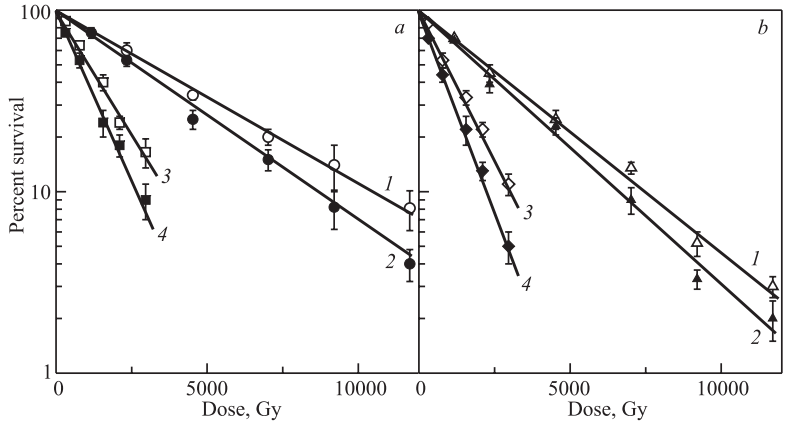


Fig. 3. The dependence of cell survival on radiation dose for diploid yeast cell (*S. cerevisiae*, strain g580L) killing without division. The cells were exposed to graded doses of $\gamma$ rays (*1, 2*) and $\alpha$ particles (*3, 4*) and plated on nutrient medium immediately after irradiation (*1, 3*) and after the LHR procedure for 72 h (*2, 4*). The cells underwent the LHR and were grown during postirradiation period at the permissive (23 °C (*a*)) and restrictive (36 °C (*b*)) temperature for DSB repair. Data are the mean values calculated from at least three independent experiments. Error bars indicate the standard error of the mean. Curves were fitted to the data points by eye

be smaller in comparison with that for the reproductive cell killing. This prediction is justified by the data presented in the table and calculated on the basis of Figs. 1–3. It is evident that the RBE of $\alpha$ particles for cell killing without division was smaller than that for reproductive cell killing, lowering from 4.6 to 2.9 for 23 °C and from 3.0 to 2.5 for 36 °C. These reduced RBE values are closed to those observed for radiosensitive yeast mutants incapable of recovery [4].

As was mentioned above, the action of ionizing radiation on living cells is determined by both physical properties of the ionizing radiation and biological ability of cells to recover from potentially lethal radiation damage. The data of this paper can be considered as an indication of the fact that the observed RBE value for nonrecoverable condition (RBE $= 2.5-3.0$) may be caused by the physical property of high-LET radiation, while further increase of this value (up to 4.6) may be due to the cell ability to recover from radiation damage inflicted by low-LET radiation.

In conclusion, the finding that the fraction of irreversible damage was greater for high-LET radiation in comparison with that for low-LET radiation corresponds to a number of experimental data observed at cellular and molecular levels [3–9]. The explanation of these results can be originated from the fact that high-LET radiations are known to produce clustering of ionizations and excitations in DNA [15], which produce more severe and complex damage than low-LET reference radiation. The independence of the probability of recovery from the radiation quality means that the slowing down of cell ability to undergo LHR after high-LET radiation is associated with a great number of irreversible unrepairable damages rather than the impairment or damage to recovery process itself.

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