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The Role of Radiation Quality and Cell Ploidy in Genetic Instability

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Abstract

The survival and late appearance of colonies by isogenic haploid and diploid yeast cells of wild type *Saccharomyces cerevisiae* and their homozygous radiosensitive mutants surviving after irradiation with γ-rays and α-particles have been studied. It was obtained a great genetic instability (100%) for diploid cells of wild type with sigmoid survival curves and radiosensitive mutants characterizing by exponential survival curves. On the contrary, haploid cells exhibited a less pronounced genetic instability (20%) independent of the genotype. It is concluded that the genetic instability induced by ionizing radiation is mainly determined by the ploidy of the cells but not by the sigmoid form of the survival curve and their ability to recover from radiation damage as was traditionally assumed.

Keywords: radiation quality, cell ploidy, genetic instability, cell survival, γ-rays, α-particles

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1. Introduction

It is well known that the effect of the delayed formation of colonies on the solid nutrient media by cells surviving after irradiation is an example of the manifestation of genetic instability in progeny of irradiated cells. [1, 2]. This effect was not studied in the dependence cell ploidy. Moreover, genetic instability was observed for radiation with high linear energy transfer (LET) of radiation quality but the relative biological effectiveness (RBE) was not estimated qualitatively [3–5]. Therefore, the main purpose of this work was to investigate the delayed appearance of haploid and homozygous diploid *Saccharomyces cerevisiae* yeast cells of wild type and radiosensitive mutant surviving after exposure to γ-rays and α-particles. The further objective was to compare quantitatively the RBE of α-particles for lethal events and genetic instability using haploid and homozygous diploid yeast cells of various genotypes.

2. Materials and methods

A study was made using haploid and diploid strains of *Saccharomyces cerevisiae* of wild type (S288C, RAD; XS800, RAD/RAD) and radiosensitive homozygous mutants to following loci: strain XS774-4d (*rad*51), XS806 (*rad*51/*rad*51); g160/2d (*rad*52), XS1898 (*rad*52/*rad*52), g218/7c (*rad*54), XS1879 (*rad*54/*rad*54). We used sparsely (γ-rays of ^{60}Co, LET = 0.2 keV/μm, 20 Gy/min) and densely (α-particles of ^{239}Pu, 23 Gy/min) ionizing radiations. Cells from the same stock solution were exposed to γ-rays and α-particles. The LET of α-particles reaching a cell monolayer was estimated to be 130 keV/μm. Exactly at about this LET value the maximum of RBE-LET relationship was observed for eukaryotic unicellular organisms. Survival response was determined on the basis of the colony counts obtained at the end of 5–7 days of incubation at 30°C. Genetic instability was estimated by a percent of colonies produced later than in control. Each data point represents average survival from three to six Petri dishes. Points in all the figures are the means from at least three independent experiments. Error bars in all the figures indicate the standard errors of the mean.

3. Results

Figure 1 demonstrates the dependence of survival and genetic instability on dose of sparsely ionizing radiation for yeast cells: haploid strain S288C (curve 1) and isogenic diploid strain XS800 (curve 2). Similar data are shown in Figure 2 for the same strains after alpha exposure. A set of similar data for haploid and diploid cells of radiosensitive mutants characterized in all cases by exponential dose-effect curves has been obtained. The results for haploid strain XS774-4d (curve 1) and isogenic diploid strain XS806 (curve 2) after exposure to γ-rays (Figure 3) and α-particles (Figure 4) are presented. Several data with other radiosensitive mutants were also obtained. Radiobiological parameters of all these strains are summarized in Table 1. It is obvious that regardless of the quality of radiation, diploid cells characterizing by sigmoidal survival curves show a significant genetic instability (100%), while this effect is expressed in a smaller degree (20%) for all haploid cells with an exponential dose-response curve. These new data demonstrate evident genetic instability for all radiosensitive diploid mutants with exponential forms of survival curves but not for haploid mutants. It can be also seen that RBE of the α-particles determined by the ratio of the isoeffective

doses of γ-rays and α-particles was almost identical for cell survival and genetic instability. These findings are not new for cell survival, while they are fundamentally new for genetic instability.

Table 1: The comparison of the relative biological effectiveness of alpha-particles for cell survival and genetic instability for haploid and diploid yeast cells of various genotype.

Strain	Genotype	RBE for cell survival	RBE for genetic instability
S288C	RAD	2.1 ± 0.3	1.8 ± 0.2
XS800	RAD/RAD	4.8 ± 0.4	4.7 ± 0.3
XS774-4d	*rad51*	2.1 ± 0.2	1.8 ± 0.1
XS806	*rad51*/*rad51*	2.5 ± 0.1	2.5 ± 0.3
g160/2d	*rad52*	2.7 ± 0.3	2.0 ± 0.4
XS1898	*rad52*/*rad52*	2.7 ± 0.4	1.6 ± 0.2
g218/7c	*rad54*	2.4 ± 0.1	2.0 ± 0.3
XS1879	*rad54*/*rad54*	1.8 ± 0.1	2.6 ± 0.3

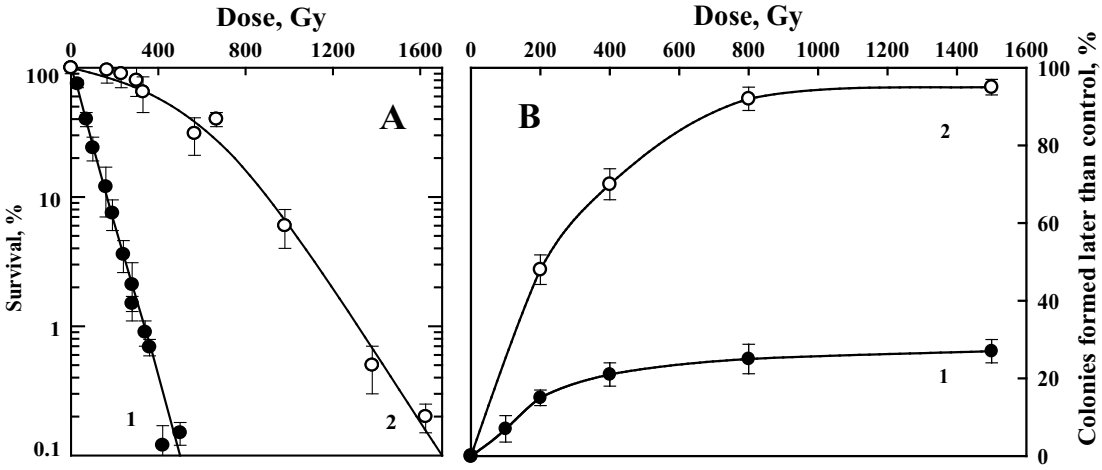

Figure 1: The dependence of survival (A) and genetic instability (B) on dose of sparsely ionizing radiation for yeast cells: haploid strain S288C (curve 1) and isogenic diploid strain XS800 (curve 2).

4. Conclusions

The delayed appearance of clones by cells surviving exposure to ionizing radiation, like other delayed effects such as chromosomal aberrations, reproductive cell death, genome rearrangement, malignant transformation, reduced cloning efficiency, micronucleus formation and heterogeneity among the progeny of irradiated cells,

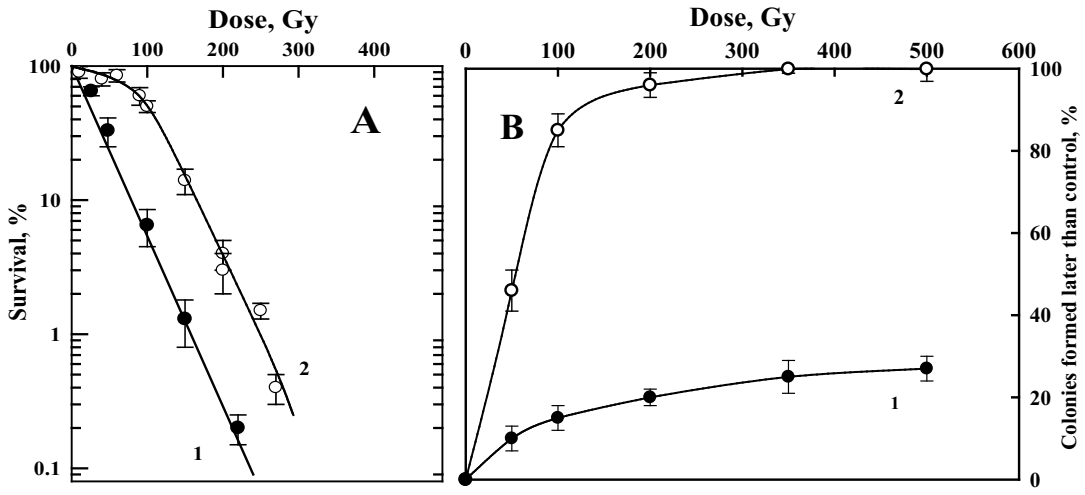

Figure 2: The dependence of survival (A) and genetic instability (B) on dose of densely ionizing radiation for yeast cells: haploid strain S288C (curve 1) and isogenic diploid strain XS800 (curve 2).

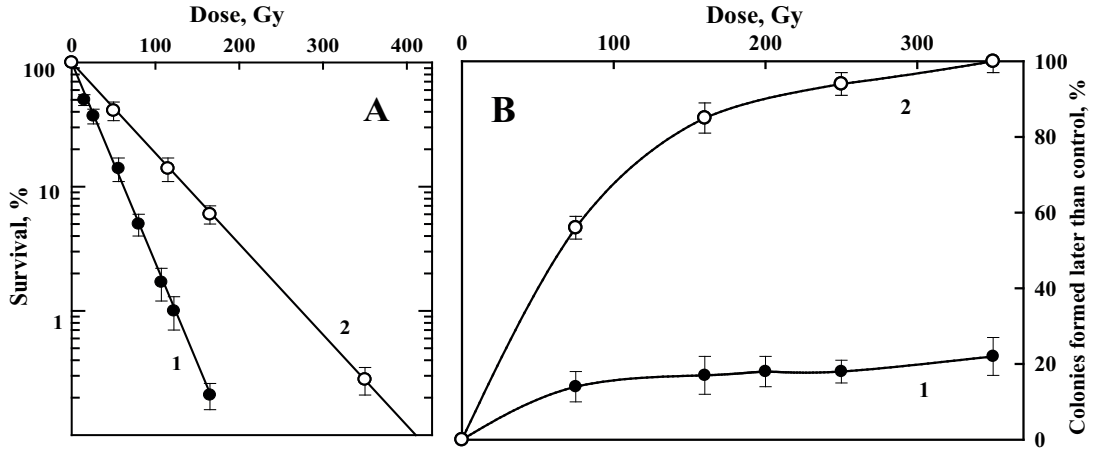

Figure 3: The dependence of survival (A) and genetic instability (B) on dose of sparsely ionizing radiation for radiosensitive mutants of yeast cells: haploid strain XS774-4d (curve 1) and isogenic diploid strain XS806 (curve 2).

exemplified genetic instability of irradiated cell. The common property of various delayed effects of radiation is the transmission of sublesioins to distant progeny. It was shown previously [6] that genetic instability is natural for diploid yeast cells with sigmoid shape of dose-response curves due to cell ability to recover from radiation damage, while this effect was negligible or even not observed for haploid strains with exponential survival curves. It might be the time to shift this paradigm. Indeed, the authors of the cited paper didn't use radiosensitive mutants with exponential survival curves both for haploid and diploid yeast cells exposed to low- and high-LET radiations. It is not excluded that genetic instability may be essentially determined by yeast cell ploidy and was not related with the shape of dose-response curve and cell ability to recover from radiation-induced damage. To test this suggestion,

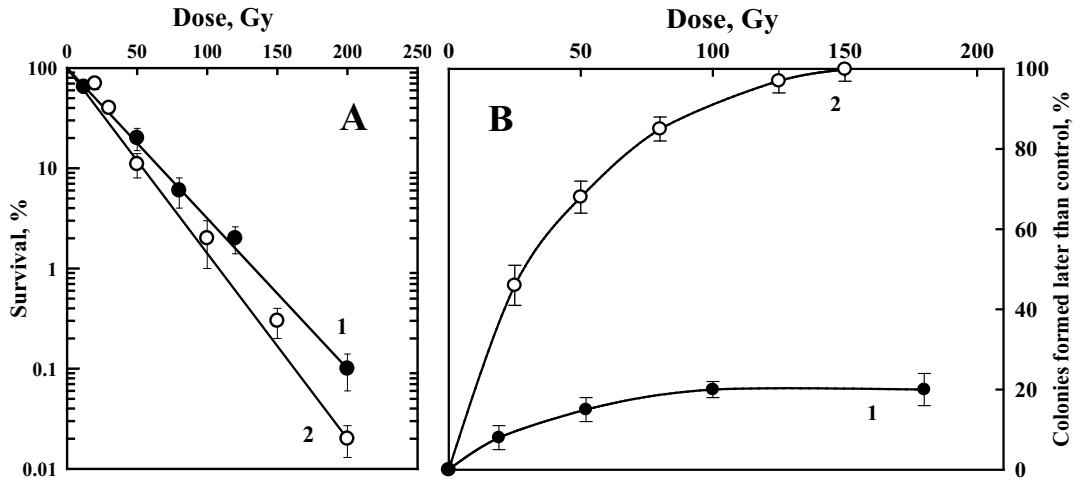

Figure 4: The dependence of survival (A) and genetic instability (B) on dose of densely ionizing radiation for radiosensitive mutants of yeast cells: haploid strain XS774-4d (curve 1) and isogenic diploid strain XS806 (curve 2).

we studied the delayed appearance of clones produced by haploid and homozygous diploid radiosensitive mutants surviving exposure to radiation with different LET.

The new original data presented in this paper may be summarized as follows. The degree of genetic instability is mainly determined by cell ploidy: both resistant and radiosensitive diploid strains in contrast to haploids demonstrate large extent of the delayed appearance of clones surviving after irradiation (100% vs. 20%). It is concluded that this effect is mainly determined by cell ploidy rather than the sigmoidal shape of survival curve and ability of cells to recover from radiation damage as it is traditionally assumed for *Saccharomyces cerevisiae* yeast cells.

The same increased efficiency of α-particles for cell inactivation and late formation of colonies by irradiated cells can be associated with greater efficiency of the densely ionizing radiation (Table 1) to form lethal radiation damage and the corresponding sub-lesions that persists in distant descendants of surviving cells after irradiation and thereby causes destabilization of the genome.

Although biological effects associated with radiation-induced genetic instability are widely discussed, the natures of events that initiate and retain instability in the progeny of irradiated cells as yet remain unclear. Careful discussion of molecular mechanisms underlying genetic instability described here are beyond the main scope of this work. However, some points may be mentioned. Many mechanisms have been proposed on how radiation exposure can give rise to genetic instability. There is evidence that repair of DNA double-strand breaks are important for the induction of delayed reproductive death [2]. While it was shown the nucleus is the target

for radiation-induced genetic instability, it is likely that single gene, gene family, a single radiation-induced mutation or DNA double-strand breaks are not sufficient for the induction genetic instability. Eukaryotic cells possess two DNA double-strand break pathways: homologous recombination and nonhomologous DNA end joining [7]. Unrepaired or misrepaired DNA double-strand break may result in both cell inactivation and genetic instability. It was supposed that the repair of radiation induced DNA double strand breaks may initiate the genomic instability and carcinogenesis [6]. Since in our paper the genetic instability was demonstrated for yeast strains defective in recombination (*rad*51/*rad*51, *rad*52/*rad*52), it may be reasonably assumed that homologous recombination cannot be considered as a reason for the delayed appearance of clones observed here. It may be concluded that the mechanism of different manifestation of the genetic instability by haploid and diploid yeast cells surviving radiation exposure is independent on cell ability to recover from radiation damage and could be conceivably related with some chromosomal damages, which are lethal for haploid but not for diploid cells. The nature of such kind of damage stays to be elucidated.

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