Korean J. Environ. Biol. 20(1) : 35~39(2002)

Cell Ploidy and Repair Ability Determine the Radioprotective Effects of Cysteamine in Yeast Cells of Various Species and Genotypes

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Abstract - The significance of cell ploidy and repair ability for the radioprotective efficiency of cysteamine was studied in DNA repair-proficient and repair-deficient yeast cells irradiated with ^{60}Co γ-rays. Results have been obtained for the cell survival of two groups of yeasts-diplont and haplont cells, both in haploid and diploid states. For diploid *Saccharomyces cerevisiae* yeast cells, the correlation between the radioprotective action of cysteamine and the cell repair capacity was demonstrated. Such a correlation was not clearly expressed for haploid yeast cells. In addition, evidence was obtained indicating that the degree of the radioprotective action was independent of the number of chromosome sets in haplont yeast *Pichia guilliermondii* cells and in some radiosensitive mutants defective in the diploid-specific recovery. It is concluded on this basis that the radioprotective action may involve the cellular recovery process, which may be mediated by a recombination-like mechanism, for which the diploid state is required. The results obtained clearly show that the radioprotective effect was dependent on DNA repair status and indicate that the mechanism of the radioprotective action may be realized on the level of primary radiation damage production as well as on the level of postradiation recovery from potentially lethal radiation damage.

Key words : radioprotective effect, ploidy, yeast cells, repair ability, evolution

Introduction

Cell repair ability is considered to be one of the most important biological properties that provides the reliable existence of biota among the increasing number of damaging factors. Cell ploidy is a crucial point determining this ability. The well-known diplont yeast cells exist as diploid cells in natural conditions, while their haploid cells were constructed genetically and are significantly more sensitive to ionizing radiation or ultraviolet light than diploid cells due to lack of the diploid-specific recovery (Mortimer 1958; Saeki *et al.* 1980). On the contrary, more rarely known haplont cells exist in nature as haploids and their diploid cells have been constructed in laboratory conditions. In such a case, the doubling of the cell genome was shown not to result in notable changes in the radioresistance because of a deficiency in the diploid-specific recovery (Korogodin 1993; Korogodin *et al.* 1996). Some results show the dependence of the radioprotective efficiency on the ability of various cells to recover from radiation damage (Bresler *et al.* 1978; Petin and Matrenina 1981; van Buul *et al.* 1997). Therefore, it would be of interest to compare the radioprotective action of any well-known chemical to haploid and diploid yeast cells of both di-

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plont and haplont species. Some radiosensitive mutants will also be analysed. In conclusion, a possible mechanism of the recovery protection and the role of the diploid-specific recovery in the evolutionary process will be discussed.

Materials and methods

Four diplont yeast strains have been used in this study: a haploid (RAD, strain S288C) and a diploid (RAD/RAD, strain XS800) wild-type yeast of *Saccharomyces cerevisiae* and their radiosensitive haploid (*rad*52, strain g160/2b) and diploid (*rad*52/*rad*52, strain XS 1898) mutants. These mutant diploid cells are defective in recombination repair (Saeki *et al.* 1980) and were initially obtained from Dr. S. Nakai (Japan). As haplont yeast, we used haploid (strain NRRLY-2076) and diploid (strain NRRLY 2075 × 2076) yeast cells of *Pichia guilliermondii*. They were initially obtained from Dr. Korogodin (Dubna, Russia). It was shown that *P. guilliermondii* diploid cells were defective in the diploid specific recovery (Korogodin 1993; Korogodin *et al.* 1996). Before irradiation (^{60}Co, 10 Gy min^{-1}), cells were incubated for 3~5 days at 30°C to the stationary phase. Cysteamine (0.01 M) was added to the cell suspension (10^6 cells ml^{-1}) 30 min before irradiation. The irradiation was carried out at room temperature. Immediately after irradiation, the samples were suitably diluted and plated on a nutrient agar to assay the cell survival by the colony forming ability. The survival response was determined by colony counts at the end of 5~7 days of incubation at 30°C. Other details have been described previously (Petin and Matrenina 1981).

Results

Fig. 1 shows gamma-radiation survival response curves of haploid and diploid wild-type strain of *Saccharomyces cerevisiae* irradiated without (open circles) and with 0.01 M cysteamine (closed circles). Similar curves of haploid and diploid *rad*52 mutant cells of *Saccharomyces cerevisiae* as well as haploid and diploid *Pichia guilliermondii* cells are presented in Figs. 2 and

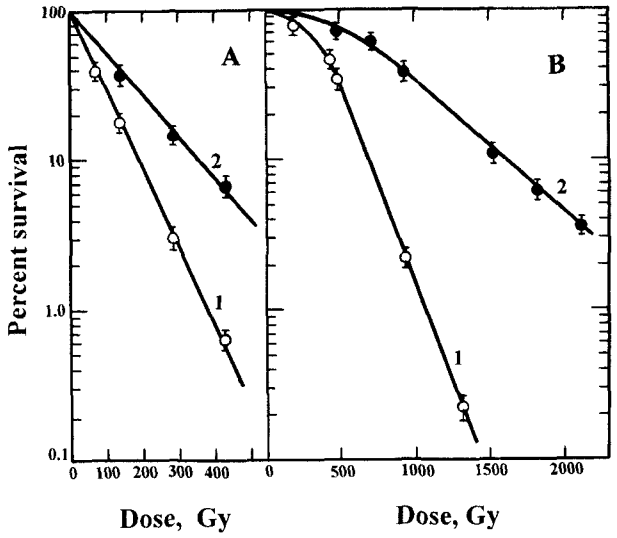

Fig. 1. The survival curves of haploid (strain S288C, A) and diploid (strain XS800, B) yeast cells of *Saccharomyces cerevisiae* exposed to γ-rays without (curves 1) and with (curves 2) 0.01 M cysteamine. The standard error of the mean of 5 replicate experiments is shown.

3, respectively. Each data point represents an average survival for 3~6 Petri dishes, each containing from 50 to 300 colonies. Each experiment was repeated a minimum of 3 times. Dose-effect curves have been drawn by visually fitting the experimental points. The efficiency of the radioprotective action was quantitatively estimated by the dose modifying factor (DMF), which was calculated in this paper by the ratio of the mean lethal doses of the survival curves obtained experimentally with and without cysteamine. The mean lethal dose (D_o) corresponds to the dose needed to reduce the survival S to $S \cdot e^{-1}$ or about 0.37 S in the exponential region of the survival curves. The DMF values calculated on the basis of experimental survival curves presented in Figs. 1-3 are listed in Table 1 for all yeasts analysed. As usual, homozygous diploid cells of the wild-type were much more radioresistant than their haploid cells (Fig. 1) due to diploid-specific recovery (Saeki *et al.* 1980) eliminating DNA double-strand breaks (Luchnik *et al.* 1977). From these data it is evident also that the efficiency of the cysteamine protection was more greatly expressed for the wild-type diploid (DMF = 2.8) than for the haploid (DMF = 1.8) yeast cells. This means that the diploid specific recovery can play a considerable role in

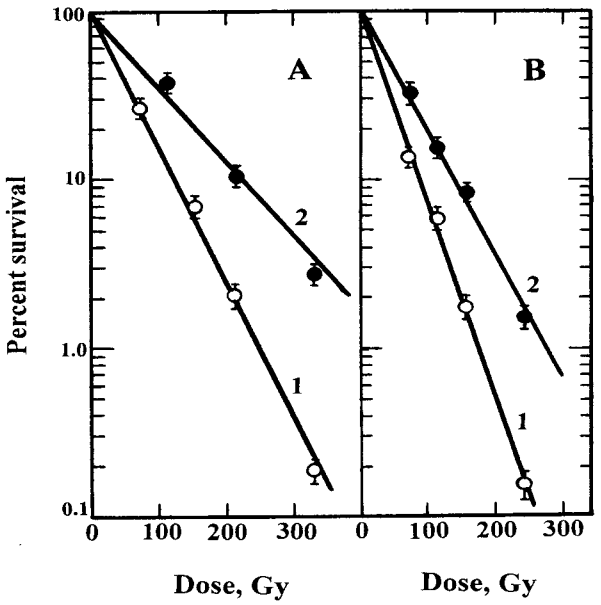

Fig. 2. The survival curves of haploid (strain g160/2b, A) and diploid (strain XS1898, B) yeast cells of *Saccharomyces cerevisiae* exposed to γ-rays without (curves 1) and with (curves 2) 0.01 M cysteamine. The standard error of the mean of 3 replicate experiments is shown.

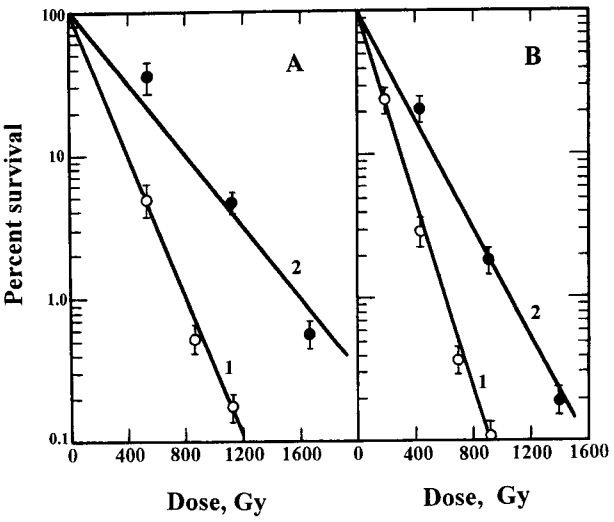

Fig. 3. The survival curves of haploid (strain NRRLY-2076, A) and diploid (strain NRRLY 2075×2076, B) yeast cells of *Pichia guilliermondii* exposed to γ-rays without (curves 1) and with (curves 2) 0.01 M cysteamine. The standard error of the mean of 3 replicate experiments is shown.

the mechanism of the radioprotective action. To test this hypothesis we used two couples of haploid and

Table 1. The efficiency (DMF) of radioprotective action of cysteamine (0.01 M) against γ-irradiation of haploid and diploid yeast cells of various species and genotypes

Species, ploidy, genotype	D_o, Gy with 0.01 M cysteamine	D_o, Gy without cysteamine	DMF
S. cerevisiae Haploid cells Wild type (RAD)	154±13	85±7	1.8±0.2
S. cerevisiae Diploid cells (RAD/RAD)	495±40	178±15	2.8±0.3
S. cerevisiae Haploid cells *rad*52 mutant	91±10	57±5	1.6±0.2
S. cerevisiae Diploid cells *rad*52/*rad*52	48±5	32±4	1.5±0.2
Pichia guilliermondii Haploid cells	329±24	165±15	2.0±0.2
Pichia guilliermondii Diploid cells	227±21	131±9	1.7±0.2

diploid yeasts, the diploid cells being defective in this kind of recovery (Figs. 2, 3). One can see that in these cases, the haploid radiosensitivity and the cysteamine efficiency were similar to those observed for the haploid cells of *S. cerevisiae* (Figs. 1-3, Table 1). It is apparent also that diploid yeast cells do not exhibit the enhanced resistance to ionizing radiation as do their haploid cells, but even they were slightly sensitive. At the same time, the cysteamine efficiency for diploid cells was a bit less than for haploid cells and much less than for diploid cells capable of the diploid-specific recovery (Figs. 1-3, Table 1).

Discussion

For many years it was widely accepted that the mechanism of the radioprotective action of chemical compounds against ionizing radiation was realized at a physicochemical stage of radiation damage production. The data obtained in this study and observed by others (Bresler *et al.* 1978; Petin and Matrenina 1981; van Buul *et al.* 1997) clearly show that the radioprotective effect was dependent on the DNA repair status. Kinetic schemes of the radiation damage formation include both

physicochemical and biochemical stages. It can be supposed that a physicochemical protection of the primary damage may be implemented by radical scavengers and its effectiveness would be independent of cell repair ability. According to the data obtained in this study, the DMF ≅ 1.6 can be considered as being realized at a physicochemical stage. The enzymatic repair occurs in the postradiation period at a biochemical stage of the radiation damage formation. The effectiveness of the repair ability protection, in accord with the results presented, is twice as high as the protection at the physicochemical stage.

It is known (Frankenberg-Schwager *et al.* 1984) that DNA double-strand breaks are responsible for yeast cell inactivation and their repair requires two homologous DNA duplexes (Luchnik *et al.* 1977). The experimental data obtained in this study show that diploid yeast cells capable of repairing this kind of radiation damage revealed the highest radioresistance and the greatest cysteamine efficiency. These results may be explained by the significance of the diploid-specific repair for both the radioresistance and the radioprotective effects of cysteamine. Radiosensitive diploid strains defective in the diploid specific recovery exhibited more radiosensitivity and significantly less radioprotective action. It is concluded on this basis that the radioprotective action may involve the cellular recovery process, which may be mediated by a recombination-like mechanism for which the diploid state is required.

The experimental data presented here may be explained by the key role of repair in the radioresistance of yeast cells of different ploidy. Apparently, haplont yeast cells are deficient in a repair system, the effect of which in diplont yeast cells are related to the presence of the double genome allowing a recombination process to occur. If radiation damages in haploid and diploid cells involve chromosome aberrations it can be expected that such lesions may arise at a higher frequency in diploid than in haploid cells because of differences in the target volume (number of chromosomes, DNA content, etc.). In such a case, the diploid cells must be more radiosensitive for cells incapable of recovery in comparison with haploid cells. If the dark recovery is not completely inhibited, then an intermediate situation may apply and haploid and diploid cells may show very similar radiosensitivity. Then the effectiveness of radioprotective action is expected to be nearly identical for haploid and diploid yeast cells as observed in this study (Table 1).

If the cell radioresistance to ionizing radiation may, to some extent, serve as a measure of cell sensitivity to other environmental factors inflicted genetic damage, one may suppose that only the yeasts capable of the diploid-specific recovery will have a selective advantage. On the contrary, for the yeasts incapable of the diploid-specific recovery, haploid cells may possess a selective advantage. It is not excluded that owing to this fact, the vegetative phase in natural conditions is presented by diploid cells in *Saccharomyces cerevisiae* and by haploid cells in *Pichia guilliermondii*. It follows that the development of repair systems, providing more full recovery of diploid cells from genetic damages in comparison with haploid cells may serve as a prerequisite for the transition, during evolutionary processes, from haplont to diplont cells.

Acknowledgements

This work was supported in part by the Russian Fund of Fundamental Research and Administration of Kaluga Region, Korea-Russia Scientist Exchange Program and the National R&D Program by the Ministry of Science and Technology of Korea.

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(Received 25 October 2001, accepted 4 January 2002)