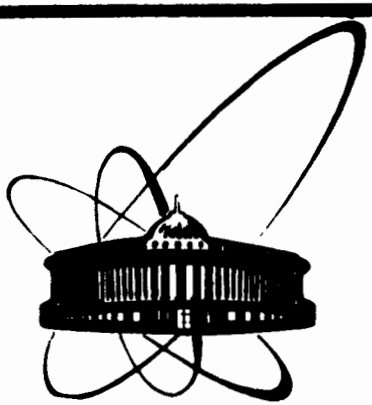

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TWO PATHWAYS OF DNA DOUBLE-STRAND BREAK REPAIR IN G1 CELLS OF SACCHAROMYCES CEREVISIAE

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INTRODUCTION

G1 cells of the diploid yeast Saccharomyces cerevisiae are known to be capable of the liquid holding recovery (LHR), i.e., a significant increase in γ-or X-irradiated cell survival during keeping the cells in a non-nutrient medium /1,2/ This process is completed within 24-72 h, depending on the irradiation dose. Later experiments demonstrated that LHR is due to the repair of DNA DSB induced in the cells by radiation/3,4/. The lack of repair in haploid yeast cells irradiated in G1 phase of the cell cycle is suggestive of a recombinational mechanism. Recently, it has been found that in the G1 phase the diploid cells of S.cerevisiae are capable of an additional type of LHR which, in contrast to the previously established 'slow' type, has got the name of 'fast' recovery /5/ Fast recovery is observed after plating the irradiated cells on a solid nutrient medium containing 1.5 M NaCl or KCl/5,6/. In this case, the survival of irradiated cells rapidly increases, reaching a plateau during 30-40 min of holding the cells in water at 28°C. Thus, the rate of the fast recovery exceeds by one or two orders of magnitude the corresponding value for the slow recovery. The fast recovery, unlike the slow one, cannot be revealed by plating the cells on the standard solid medium (i.e., containing no KCl), for in this case this process is completed prior to the first post-irradiation cell division. Irradiated G1 haploid cells are incapable of fast recovery, in contrast to those irradiated in the logarithmic phase of culture growth (in which up to 70% cells are in the S or G2 phases of the cell cycle /7/), when this process is rather effective/8/).

The aim of this paper was to demonstrate that the fast post-irradiation recovery is a special pathway of DNA DSB repair which differs from that previously established in G1 diploid cells. The genetic control of fast repair of DNA DSB has been studied. Mutations

rad51, rad52, rad54 and rad55 have been shown to block the fast repair, whereas in diploid mutants homozygous for rad50, rad53 and rad57 the efficiency of this process is close to that for wild type cells.

MATERIALS AND METHODS

The isogenic haploid and diploid strains of S.cerevisiae, i.e., 2873-2A (a HTH_1-HTH_2-HTH_3) and 2873-1B (a HTH_1-HTH_2-HTH_3 /α HTH_1-HTH_2-HTH_3) were used. The diploid strain was heterozygous only for the mating type locus/9/.

Besides, we used a series of diploid radiosensitive mutants kindly supplied by Dr. T. Saeki, i.e., XS800 (RAD/RAD); XS1806 (rad50-1/rad50-1); XS806 (rad51-1/rad51-1); XS1898 (rad52-1/rad52-1); XS1889 (rad53-1/rad53-1); XS1935 (rad55-3/rad55-3); XS1988 (rad57-1/rad57-1). For a more detailed description of the strains see the work of Saeki et al./10/.

Diploid strain T3 (rad54/rad54) kindly supplied by Prof. I. A. Zakharov/11/ was also used in this study.

Yeast cells were grown on a solid nutrient medium YEPD (3 days, 28°C); for DNA sedimentation analysis the YEPD contained 150 μM [8-^{14}C]-adenine (44 mCi/mM). The cells were then resuspended in sterile distilled water; the suspension was layered on top of a linear sucrose gradient (40-60%) and cenrifuged in a bucket rotor at 1000 x g at room temperature for 15 min, after which the top fraction containing non-budding cells was withdrawn. The cells were washed twice with water. The resulting suspension consisted of cells of an approximately equal size. The proportion of budding cells was less than 1%.

Cell suspensions ($1 \cdot 10^6$ cells/ml) were irradiated with γ-rays of ^{137}Cs at a dose rate 33 Gy/min at 0°C. To determine cell survival, the suspensions were diluted with water at 0°C and plated on YEPD or YEPD + 12%KCl.

For DNA sedimentation analysis, the cells were harvested on Millipore HAWP filters ($2 \cdot 10^7$ cells per gradient) at 0°C and resuspended in a solution containing 2% 2-mercaptoethanol, 0.15 M NaCl and 0.04 M EDTA, pH 8.3 with subsequent incubation at 30 C for 15 min. The cells were then harvested on filters, washed with water, resuspended in a solution containing 1 mg/ml of Zymolyase 5.000 (Kirin Brewery, Japan), 1 M sorbitol and 1 mM EDTA, pH 7.5 and incubated at 35°C for 20 min.

To elucidate fast DNA DSB repair, we added KCl to a mercaptoethanol-containing solution (final concentration of 10%). After incubation in this solution, the cells were washed with 10% KCl. The zymolyase solution contained 10% KCl instead of sorbitol.

Suspensions of protoplasts (0.1 ml) were layered onto 4.6 ml gradients (15-30% sucrose, 1 M NaCl, 0.02 M EDTA, pH 7.2) with a 0.1 ml top layer of the lysing solution (2% sarcosyl, 3% sodium deoxycholate, 5% sodium dodecylsulfate, 0.02 M EDTA, 0.01 M Tris-HCl, pH 8.0/12/). The thus formed gradients were run in a Beckman SW 50.1 rotor at 10000 rpm at 20°C for 25 h.

The gradients were fractionated from top to bottom into 0.1 ml fractions. Each fraction was placed on a Whatman 3MM filter. RNA hydrolysis was performed in 0.5 N NaOH (37°C, 18 h). The filters were then placed into 10% trichloroacetic acid (TCA) (4°C, 15-20 min), washed twice with 5% TCA and twice with 96% ethanol and then acetone, dried and counted in a toluene-based scintillation fluid, using a Nuclear Chicago Mark II scintillation counter.

The number average molecular weight M_n of yeast DNA and the average number of DSB per genome were calculated by the method of Resnick and Martin/13/.

RESULTS AND DISCUSSION

Previously, it was shown that 10% KCl inhibits the fast post-irradiation recovery of diploid yeast. In our experiments, diploid cells of yeast strain 2873-1B γ-irradiated with 460 Gy were either

Table 1. Fast post-irradiation recovery of γ-irradiated (460 Gy) diploid yeast cells (strain 2873-1B), held in various media.

Survival, % [a]				
Immediate plating	Plating after holding the cells (28°C, 1 h) in			
	water	10% KCl	1 M sorbitol	0.04 M EDTA, pH 8.3
3.5±0.3	14±2	1.8±0.2	16±2	14±2

[a] Survival was determined by plating the cells on YEPD + 12% KCl.

immediately plated on a solid medium YEPD + 12% KCl or held in water or 10% KCl at 28°C for 1 h before being plated on YEPD + 12% KCl. As can be seen from Table 1, the survival of cells held in water increased from 3.5 to 14%, whereas that of cells held in 10% KCl decreased to 1.8%. Holding of γ-irradiated cells in 1 M sorbitol or 0.04 M EDTA (pH 8.3) did not affect the efficiency of the fast recovery compared with holding in water (Table 1). This indicates that under standard conditions of yeast cell lysis with an intermediate step of protoplast formation, the fast recovery is of the same efficiency as that after holding the cells in water. Therefore, the whole procedure of protoplast formation, i.e., incubation with mercaptoethanol and zymolyase, was performed in the presence of 10% KCl.

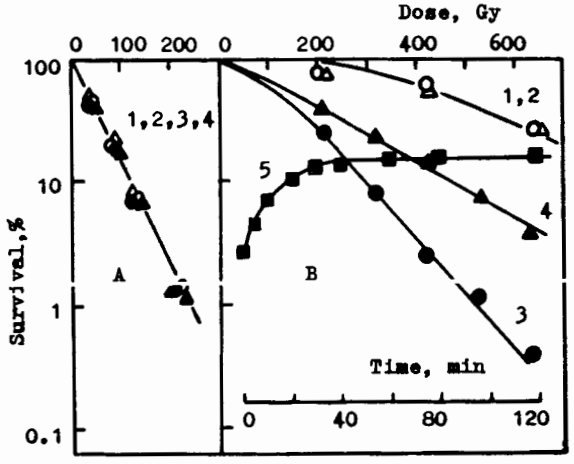

Fig. 1. The dose-survival curves of haploid 2873-2A (A) and diploid 2873-1B (B) cells after irradiation with γ-rays. 1, 3 - plating immediately after irradiation; 2, 4 - plating after keeping the cells in water - (28°C, 1 h); 5 - cell survival after irradiation with 460 Gy versus time of keeping in water (28°C). Open symbols - plating on YEPD; dark symbols - plating on YEPD + 12%KCl.

Fig. 1 shows the survival curves of haploid 2873-2A (A) and diploid 2873-1B (B) S.cerevisiae cells after their irradiation with γ-rays. The irradiated cells were plated on YEPD or YEPD + 12% KCl immediately after irradiation or after holding in water (28°C, 1 h). As can be seen from the Figure, the survival curves of haploid cells do not practically differ in all experimental variants. In the case of diploid cells the situation is quite different. The survival of irradiated cells is practically the same in both experimental variants, when the cells were plated on YEPD (Fig. 1B, curves 1 and 2).

However, the survival of γ-irradiated cells held in water (curve 4) markedly exceeds that for cells immediately plated on YEPD + 12% KCl (curve 3).

Fig. 1 also shows the dependence of the survival of γ-irradiated (460 Gy) diploid cells on the time of their keeping in water before plating on YEPD + 12% KCl (curve 5). The fast recovery is completed within 30 min; cell survival does not change as a result of subsequent holding for 1.5 h. Since in this case the survival does not reach the corresponding value for cells plated on YEPD, it follows that the sensitisation effect of KCl is not only due to the inhibition of this type of cell repair, but also depends on some other factors[5].

Fig. 2 shows the DNA sedimentation profiles in a neutral sucrose gradient for diploid cells of the 2873-1B strain irradiated with 570 Gy. Earlier it was demonstrated that the position of mitochondrial DNA from irradiated and non-irradiated cells in the gradient does not change because of the small size of the mitochondrial genome which

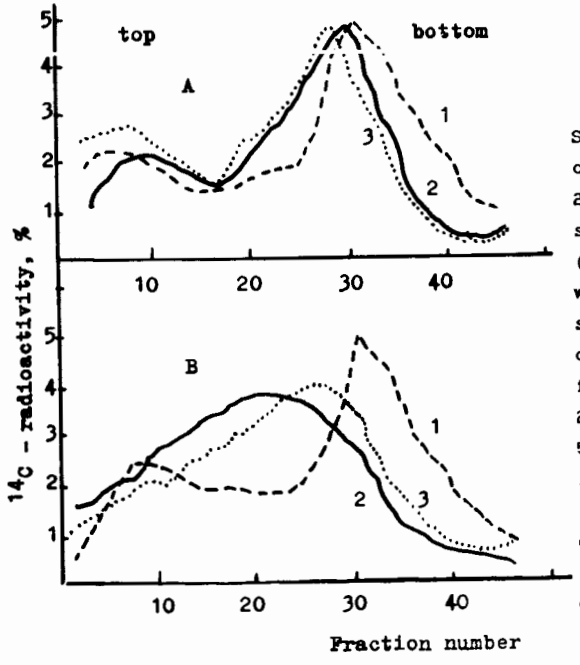

Fig. 2. Sedimentation profiles of DNA from diploid 2873-1B cells in neutral sucrose gradients (15-30%). Protoplasts were prepared by a standard procedure (A) or in the presence of 10% KCl (B). 1- control; 2 - γ-irradiation with 570 Gy, lysis immediately after irradiation; 3 - γ-irradiation with 570 Gy, lysis after keeping the cells in water (28°C, 1 h).

practically excludes DSB induction at the irradiation dose used. Thus, the mitochondrial DNA does not interfere with the results of chromosomal DNA sedimentation analysis data /3/. Protoplasts of irradiated cells were prepared by a standard procedure (Fig. 2A) or in the presence of 10% KCl (Fig. 2B). In the same experiment, cell survival was estimated by plating the cells on YEPD and YEPD + 12% KCl.

It can be seen that the γ-irradiation causes a shift of the DNA sedimentation profile towards fractions with lower molecular weights (as compared with non-irradiated control), i.e., the number average molecular weight of DNA, M_n, decreases as a result of irradiation (approximately 1.3 fold in the case of the standard protoplast preparation procedure and 1.6 fold in the case of the protoplast preparation procedure in the presence of 10% KCl). The survival of irradiated cells plated immediately on YEPD and YEPD + 12% KCl is 32 $\pm$ 3% and 0.60 $\pm$ 0.07%, respectively. Holding irradiated cells ($1 \cdot 10^6$cells/ml) in water (28°C, 1 h) prior to lysis does not practically change the DNA sedimentation profiles, when the protoplasts were prepared by a standard method (Fig. 2A, profiles 2 and 3) but causes a shift of the DNA sedimentation peak towards the fractions with higher molecular weights, when the protoplasts were prepared in the presence of 10% KCl (Fig. 2B, profiles 2 and 3). In the latter case, the M_n value increases approximately 1.2 fold. The survival of irradiated cells held in water is 36$\pm$ 3% (when plated on YEPD) and 4.7 $\pm$ 0.4% (when plated on YEPD + 12% KCl).

The experimental results can be interpreted in the following way. The increase in the molecular weight of DNA as a result of holding the irradiated cells in water results from a DNA DSB repair which cannot be detected by standard methods because of it being completed prior to protoplast lysis on the gradient top. The isolation of DNA from irradiated cells in the presence of 10% KCl, which inhibits the fast DNA DSB repair, permits to reveal the fast repair in experiment. On the other hand, it may be assumed that preparation of protoplasts from irradiated cells in the presence of 10% KCl results in the formation of additional DSB from DNA single-strand breaks (SSB) induced by irradiation. In this case, the increase in the molecular weight of DNA after holding the irradiated cells in water reflects the repair of DNA SSB, but not DNA DSB. This hypothesis can be refuted by the results of sedimentation analysis of DNA isolated from haploid S. cerevisiae cells irradiated in the G1 phase.

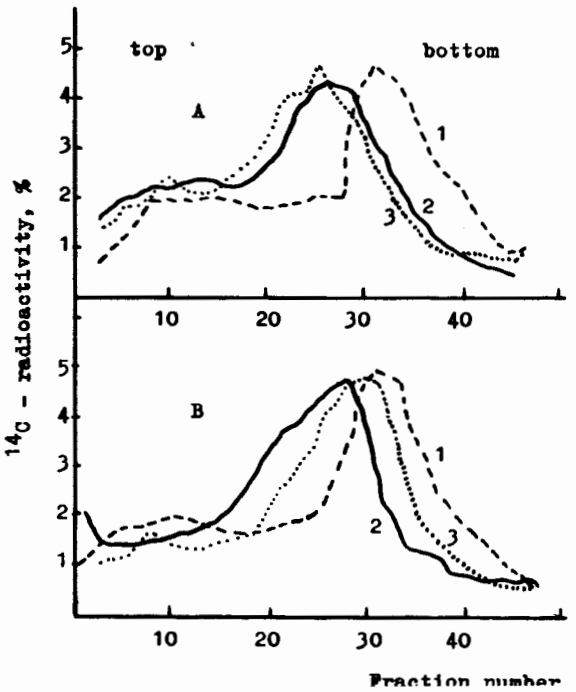

Fig. 3.
Sedimentation profiles of DNA from haploid 2873-2A (A) and diploid 2873-1B (B) cells in neutral sucrose gradients (15-30%). Protoplasts were prepared in the presence of 10% KCl. 1 - control; 2 - γ-irradiation with 460 Gy, lysis immediately after irradiation; 3 - γ-irradiation with 460 Gy, lysis after keeping the cells in water (28°C, 1 h).

Fig. 3 shows the sedimentation profiles of DNA from haploid (A) and diploid (B) cells γ-irradiated with 460 Gy as obtained in a neutral sucrose gradient. The protoplasts were prepared from irradiated cells in the presence of 10% KCl. It can be seen that holding irradiated cells in water (28°C, 1 h) does not change the DNA sedimentation profiles of haploid cells but causes a shift in the sedimentation profiles of diploid cells towards fractions with higher molecular weights (M_n is increased 1.2 fold). Therefore, if cell incubation with 10% KCl causes the transformation of some part of SSB to DSB , the observed increase in the molecular weight of DNA will not be diploid-specific.

These data suggest that holding irradiated G1 diploid cells in water (28°C, 1h) results in a DNA DSB repair. This repair is sensitive to 10% KCl. This indicates that the fast post-irradiation recovery of yeast cells is due to the DNA DSB repair which cannot be detected by the standard method, i.e., irradiated cell lysis in the absence of KCl.

The results of sedimentation analysis of DNA from haploid and diploid yeast in a neutral sucrose gradient as well as the

corresponding values for cell survival are given in Table 2. As can be seen from the Table, the increase in cell survival resulting from fast

Table 2. Number average molecular weight, M_n and survival of γ-irradiated cells under various post-irradiation conditions

Strain	Dose, Gy	Lysis conditions	M_n, rel. units		Survival, %[a]	
			Lysis immediately after irradiation	Lysis after holding in water (28°C, 1 h)	Plating immediately after irradiation	Plating after holding in water (28°C, 1 h)
2873-2A	0	KCl[b]	1.0	1.0	100	98±9
(haploid)	460	KCl[b]	0.73±0.02	0.76±0.02	<0.002	<0.002
2873-1B	0	KCl[b]	1.0	1.0	100	95±6
(diploid)	460	KCl[b]	0.72±0.02	0.88±0.03	3.1±0.7	16±4
	570	KCl[b]	0.63±0.02	0.77±0.03	0.5±0.2	5.1±1.2
	0	stan-	0.98±0.03	0.98±0.03	100[c]	105±10[c]
	570	dard	0.79±0.03	0.75±0.02	33±5[c]	35±6[c]

[a]Cell survival was determined by plating on YEPD + 12% KCl.

[b]Protoplasts of irradiated cells were prepared in the presence of 10% KCl.

[c]In this case the cell survival was determined by plating on YEPD.

post-irradiation recovery is correlated with the increase in M_n resulting from the DNA DSB repair. These data allow a rough estimation of the efficiency of DNA DSB repair. Thus, it was found that diploid cells of yeast strain 2873-1B irradiated with 460 and 570 Gy and then held in water (28°C, 1 h) repair of about 50% of the total number of the DNA DSB induced by irradiation.

Interestingly, the fast recovery efficiency of this strain described by the D_0'/D_0 ratio is equal to 1.9 ± 0.3, where D_0 and D_0' are the doses which decrease e-fold the survival on the exponential part of survival curves for the cells plated on YEPD+12%KCl immediately after irradiation and after holding in water, respectively (Fig. 1B, curves 3 and 4). Thus, the 2 fold decrease of the mean number of DNA DSB per cell as a result of repair is accompanied by a nearly two fold increase in the magnitude of D_0 for the survival curve due to fast recovery.

Now a question arises as to whether the DNA DSB repair observed herein is a separate pathway or it is part of a previously established slow repair[3] which takes place during a long-term (24-72 h) LHR. Indeed, one may suppose that the use of 10% KCl enables the detection of the initial stage of the DNA DSB repair, which under standard conditions of protoplast preparation is completed by the moment of protoplast layering onto the gradient. Nevertheless, the hypothesis according to which fast repair is a separate pathway of DNA DSB repair can be confirmed by the facts given below. Here it seems appropriate to emphasize that the fast post-irradiation recovery is due to the DNA DSB repair, whereas earlier observations[3] demonstrated that slow LHR is coupled with a slow repair of DNA DSB. Therefore one may infer from the survival data that yeast cells possess the ability to repair DNA DSB in G1 phase of the cell cycle.

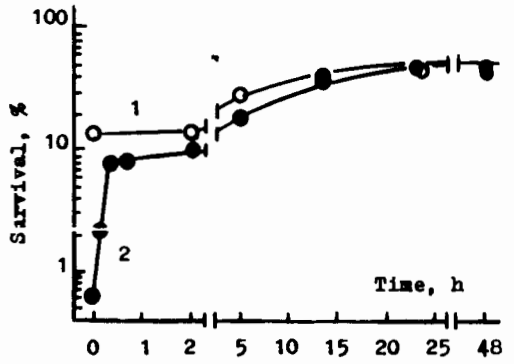

Fig 4. The survival of diploid XS800 cells γ-irradiated with 630 Gy versus time of keeping the cells in water (28°C). Open symbols - plating on YEPD; dark symbols -plating on YEPD + 12% KCl

The plots for the survival of γ-irradiated diploid cells (strain XS800) versus time of holding in water at 28°C before plating on YEPD (curve 1) or YEPD + 12% KCl (curve 2) are shown in Fig. 4. As can be seen from the Figure, the survival of cells plated on YEPD + 12% KCl increases during the first 30 min, reaching a plateau of 2-3 h duration (which coincides with the completion of fast DNA DSB repair); this is followed by a further increase in cell survival, corresponding to a slow LHR (slow DNA DSB repair). Plating the cells on YEPD enables the detection of only a slow recovery. In this way, the biphasity of kinetics of the post-irradiation recovery (DNA DSB repair) during holding the cells in a non-nutrient medium points to the existence of two separate pathways of DNA DSB repair in yeast G1 cells.

Additional evidence in favour of this hypothesis can be derived from the data on the genetic control of fast and slow DNA DSB repair.

The ability of yeast cells to cause fast or slow DNA DSB repair was estimated by the efficiency of fast and slow LHR.

Fig. 5 shows the survival curves of diploid yeast strains of a wild type (A) and radiosensitive mutants homozygous for rad50 (B), rad51 (C), rad52 (D), rad53 (E), rad54 (F), rad55 (G) and rad57 (H) after their irradiation with γ-rays. The irradiated cells were plated on YEPD or YEPD+12%KCl immediately after irradiation or after holding them in water (28°C, 1 h). As can be seen from the Figure, mutations rad51, rad52, rad54 and rad55 block fast post-irradiation recovery practically completely. Mutations rad51, rad52 and rad54 are also known to inhibit slow LHR/14, 15, 16/; however, in accordance with the DMF criterion (see the footnote "a" to table 3) for mutant homozygous for rad55 the slow LHR is nearly as efficient as in wild type strain cells (/15/ and our data). In contrast, in mutant homozygous for rad50 slow LHR is blocked completely (/14, 15, 16/ and our data), but the efficiency of fast recovery is approximately as high as in wild type cells (with regard to DMF) (fig. 5B).

The data on the genetic control of fast and slow LHR (fast and slow DNA DSB repair) are summarized in Table 3. In this way, the fast and slow repair of DNA DSB differ in terms of genetic control. The fact that mutations rad51, rad52 and rad54 block both fast and slow LHR testifies to the existence of common steps of fast and slow DNA DSB repair controlled by these genes.

Besides, it was shown that cycloheximide inhibits the slow LHR, but not the fast post-irradiation recovery/8/.

Table 3. The efficiency of fast and slow LHR[a] for radiosensitive diploid mutants of Saccharomyces cerevisiae

Mutation	LHR	
	fast	slow
RAD	1.8±0.2	2.0±0.3
rad50	1.8±0.2	1.0
rad51	1.0	1.0[b]
rad52	1.0	1.0[b]
rad53	1.7±0.2	1.5±0.2
rad54	1.0	1.0[b]
rad55	1.1±0.2	1.7±0.2
rad57	1.7±0.2	1.7±0.2

[a]The efficiency of fast (or slow) LHR was determined as a dose-modification factor (DMF). DMF = D'_{10}/D_{10}, where D_{10}, D'_{10} are the doses of irradiation reducing the survival to 10% for cells plated on YEPD + 12% KCl (or YEPD) immediately or after keeping in water at 28°C for 1 h (or 48 h), respectively.
[b]The data of Petin and Kabakova/15/.

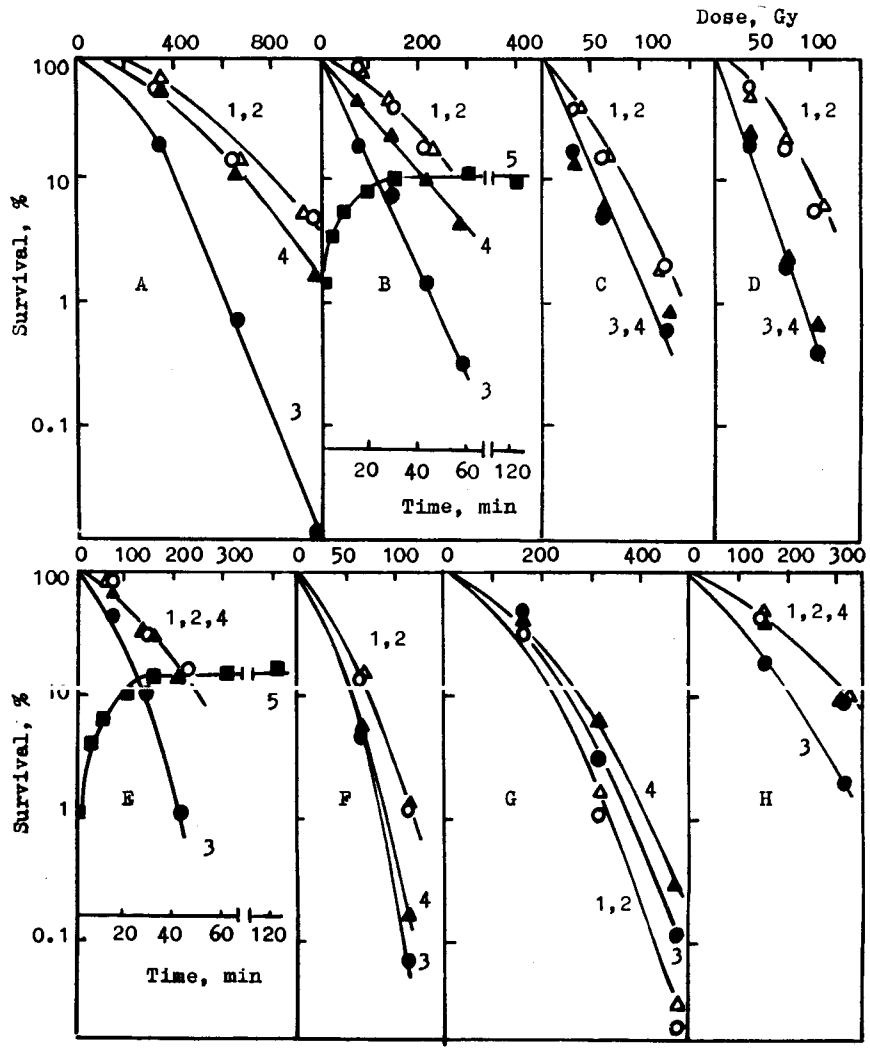

Fig. 5. The dose-survival curves of diploid wild type cells (A) and the mutants homozygous for rad50 (B), rad51 (C), rad52 (D), rad53 (E), rad54 (F), rad55 (G) and rad57 (H) after irradiation with γ-rays. 1, 3 - plating the cells immediately after irradiation; 2, 4 - plating the cells after keeping in water (28°C, 1 h); 5 - survival after irradiation with 210 Gy versus time of keeping the cells in water (28°C). Open symbols - plating on YEPD; dark symbols - plating on YEPD + 12% KCl.

These cumulative data suggest that fast DNA DSB repair is a separate pathway of repair in yeast G1 cells. This repair, being diploid-specific, occurs as a slow DNA DSB repair via a recombinational mechanism. The concrete mechanism of the fast repair is still obscure. It is also possible that the substrates for fast and slow repair are different types of DSB, e.g., "blunt" or"slant" DSB. It is also likely that repair promotes the elimination of DSB whose recovery does not require any significant repair synthesis of DNA. Nevertheless, it is obvious that the mechanisms of both fast and slow repair have several common steps, since both of them are controlled by RAD51, RAD52 and RAD54 genes.

REFERENCES

1. Korogodin, V.I. *Biofizika* (USSR), 1958, v.3, 703-710.
2. Patrick, M.H., Haynes, R.H. and Uretz R.B. *Radiat. Res.*, 1963, v.21, p. 144-163.
3. Luchnik, A.N., Glaser, V.M. and Shestakov S.V. *Molec Biol. Reps.*, 1977, v.3, p.437-442.
4. Frankenberg-Schwager, M., Frankenberg, D., Blocher, D. and Adamczyk, C. *Radiat. Res.*, 1980, v.82, p.498-510.
5. Glasunov, A.V. and Kapultsevich, Yu.G. *Radiobiologia* (USSR), 1982, v.22, p.62-69.
6. Boreiko, A.V., Nasonova, E.A. and Glasunov, A.V. *Radiobiologia* (USSR), 1984, v.24, p.543-546.
7. Williamson, D.H. *J. Cell. Biol.*, 1965, v.25, p.517-528.
8. Glasunov, A.V. and Kapultsevich, Yu.G. *Radiobiologia* (USSR.), 1983, v.23, p.344-348.
9. Naumov, G.I. and Tolstorukov, I.I. *Genetika* (USSR.), 1972, v.8, p.95-100.
10. Saeki, T., Mashida, I. and Nakai, S. *Mutat. Res.*, 1980, v.73, p.252-265.
11. Zakharov, I.A., Kovaltsova, S.V. and Marfin, S.V. *Genetika* (USSR), 1979, v.15, p.41-48.
12. Blamire, J., Cryer, D.R., Finkelstein, D.V. and Marmur, J. *J. Molec. Biol.*, 1972, v.67, p.11-24.
13. Resnick, M.A. and Martin, P. *Molec. Gen. Genet.*, 1976, v.143, p.119-129.
14. Rao, B.S., Reddy, N.M.S. and Madhvanath, V. *Int. J. Radiat. Res.*, 1980, v.37, p.701-705.
15. Petin, V.G. and Kabakova, N.M. *Mutat. Res.*, 1981, v.82, p.285-294.
16. Vishnevetskaya, O.Yu., Luchnik, A.N., Arutunova, L.S., and Shestakov, S.V. *Genetika* (USSR), 1983, v.19, p.26-32.

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Глазунов А.В., Глазер В.М., Капульцевич Ю.Г. E19-88-374

Два пути репарации двунитевых разрывов ДНК у диплоидных дрожжей Saccharomyces cerevisiae в G1-фазе клеточного цикла

Известно, что G1-клетки диплоидных дрожжей S. cerevisiae способны к медленной репарации двунитевых разрывов /ДНР/ ДНК при выдерживании в непитательной среде. Здесь показано, что диплоидные дрожжи S. cerevisiae, γ-облученные в G1-фазе клеточного цикла, способны к быстрой репарации ДНР ДНК, которая завершается за 30-40 минут выдерживания клеток в воде при 28°C. В результате этого кинетика репарации ДНР ДНК при выдерживании клеток в непитательной среде имеет двухфазный характер: первая фаза - "быстрая" - завершается за 30-40 минут, вторая - "медленная" - за 48 часов выдерживания клеток в воде при 28°C. Мутации rad51, rad52, rad54, rad55 подавляют быструю репарацию ДНР ДНК, в то время как rad50, rad53, rad57 практически не влияют на эффективность репарации этого типа. Показано, что быстрая и медленная репарация ДНР ДНК у диплоидных клеток S. cerevisiae в G1-фазе клеточного цикла являются отдельными путями репарации ДНР ДНК у дрожжей.

Работа выполнена в Лаборатории ядерных проблем ОИЯИ.

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Two Pathways of DNA Double-Strand Break Repair in G1 Cells of Saccharomyces cerevisiae

The G1 cells of the diploid yeast Saccharomyces cerevisiae are known to be capable of a slow repair of DNA double-strand breaks (DSB) during holding the cells in a non-nutrient medium [3,4]. In the present paper, it has been shown that S. cerevisiae cells γ-irradiated in the G1 phase of cell cycle are capable of fast repair of DNA DSB; this process is completed within 30-40 min of holding the cells in water at 28°C. For this reason, the kinetics of DNA DSB repair during holding the cells in a non-nutrient medium are biphasic, i.e., the first, "fast" phase is completed within 30-40 min; whereas the second, "slow" one, within 48 h. Mutations rad51, rad52, rad54 and rad55 inhibit the fast repair of DNA DSB, whereas mutations rad50, rad53 and rad57 do not practically influence this process. It has been shown that the observed fast and slow repair of DNA DSB in the G1 diploid cells of S. cerevisiae are separate pathways of DNA DSB repair in yeast.

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