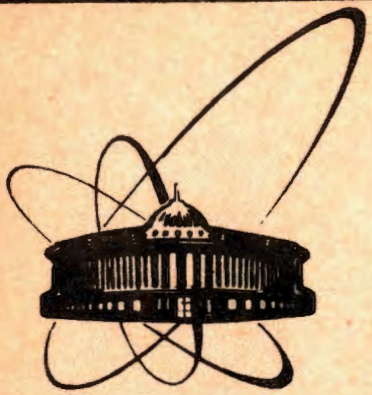

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N.V.Levkovich, A.I,Chepurnoy

A STUDY OF GAMMA-RAY MUTAGENESIS IN SACCHAROMYCES CEREVISIAE. ANALYSIS OF REVERSION PRODUCTION KINETICS IN A WILD-TYPE HAPLOID STRAIN

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Introduction

A new method of quantitative estimation of mutants (Chepurnoy *et al.*, 1988, 1989) based on the use of nuclear filters (Fleroff, 1984) and the knowledge of mutant revealing kinetics in selective media allowed showing, with *leu2* reversions as an example, that formation of γ-induced mutants is a protracted process during postradiation growth of irradiated cells. The time for formation of induced mutants depends on the cell cycle phase during irradiation. In the exponential phase it can be up to 15 hours; in G_1, up to 6 hours, and in G_2 most of the induced mutants are formed in the period between 12 and 15 hours of postradiation growth of irradiated cells. If there is no DNA replication, γ-induced mutants are not formed. Reparative processes responsible for higher radioresistance of cells in the G_2-phase of the cell cycle, as compared with the G_1-phase, do not affect the formation frequency of γ-induced reversions, which was the same in both cases at all doses in the range from 24 to 200 Gy. In this range the mutation frequency depended on the dose linearly. In experiments with a synchronized cell culture it was shown that γ-induced reversions are formed during postradiation replications. There is no reversion in phases G_1 and G_2 of cell cycle. In the present paper we continue studying mechanisms of formation of γ-induced mutants by analysing formation kinetics of induced mutants during postradiation culturing of the irradiated cell culture.

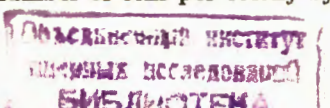
Materials and Methods

Strains

Haploid strains NA3-24 *(a leu2-1 lys1-1 can1-23 RAD)* of yeast *Saccharomyces cerevisiae* were used in this study (Chepurnoy and Mikhova-Tsenova, 1988). The mutants observed were *leu2* reversions.

Experimental procedures

Using the method of ordered plating (Von Borstel, 1978), we suspended the yeasts in water, exposed them to 24 Gy/min γ-radiation Cs^{137}, and plated them with a 220-pin inoculator on a lavsan nuclear filter (Fleroff, 1984) covering a solid medium in Petri dishes. Thus, about 220 macrocolonies emerged in every Petri dish. After growing the cells at 30°C for a given time interval, we resuspended a few tens of colonies in water and determined the number of cells per colony by microscopy. Buds and



parental cells were counted as separate cells. Other colonies were transferred together with the filters to selective media for selection of Leu$^+$ revertants, which are clearly identifiable in these media as colonies of secondary growth.

Media

The growth medium for the NA3-24 strain cells was a minimal medium M3 (Zakharov *et al.*, 1984) containing lysine (30 mg/l) and leucine (30 mg/l). The selective media included M3 with lysine for detection of Leu$^+$ revertants. To determine the number of cells that survived the irradiation, we used the full medium P (Zakharov *et al.*, 1984).

Quantitative estimation of mutants

The dynamics of appearance of revertants on selective media is usually characterised by a curve with two peaks: the first population corresponds to the revertants formed before the transfer of the cultures on the selective medium, whereas the second one corresponds to those formed after the transfer, during residual growth (Chepurnoy and Mikhova-Tsenova, 1989). Quantitative estimation of mutants in the culture at the moment of transferring the cells to the selective medium was based on their number in the first wave of the 'appearance curve'. The mutation frequency was calculated from the proportion of colonies without revertants:

$$R = \frac{1}{n} ln(N/N_o), \qquad (1)$$

where n is the number of cells survived in a colony, N is the total number of colonies, N_o = (N–M) is the number of colonies without mutants, M is the number of colonies with mutants. The number of the survived cells was determined by the ability of cells to form visible colonies when plated on a full medium.

Synchronisation of cells

Cells were synchronised by Williamson and Scopes method (1962). For the NA3-24 strain this method gives a very high degree of synchronism. Only 3% of cells had buds after three cycles of synchronisation.

Results

In Fig.1 there are the data on formation of reversions induced by 24 Gy γ-irradiation of synchronised NA3-24 cells in phase G_1. The data cover cell cycles of the first four reversions in periods of time.

As already mentioned, formation of induced reversions after irradiation

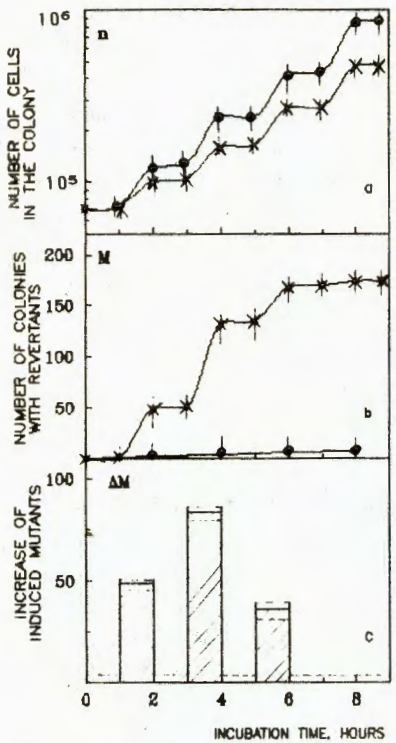


Fig.1. Formation of *leu2* reversions in NA3-24 cells in the four generations after irradiation with a dose of 24 Gy;
a — number of cells in one colony of a non-irradiated (•) and irradiated (×) culture,
b — number of colonies with *leu2* reversions in samples of N = 660,
c — increase in the number of colonies with induced postradiation generations

in phase G_1 is not confined to the first postradiation replication and is observed during the first 6 hours of postradiation incubation or in the first three generations of irradiated cells. As follows from the results obtained, formation of induced mutants is not monotonous (continuous, smooth) during the 6-hour incubation, which one could expect because of dispersion of division delay in damaged cells, but is connected to three successive phases of DNA replication.

In our opinion, induced reversions in the second and third phases of postradiation replication result from premutation damages which do not develop into mutations for several divisions.

Using the data on the increase in the number of reversions in generations 1, 2 and 3 one can calculate the probability of mutations (r) in a generation.

In Fig.2 there are the results of experiments on determination of frequencies of induced reversions in the 1st, 2nd and 3rd generations for different γ-radiation doses.

In the dose range 24–192 Gy the behaviour of r in generations 1–3 is

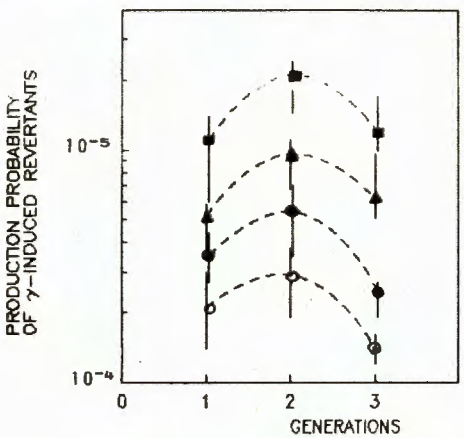


Fig.2. Probability of formation of γ-induced reversions in postradiation generations after irradiation of cells in phase G_1 with doses of 24 Gy (○), 48 Gy (●), 96 Gy (▲), 192 Gy (■)

the same, which, as we think, shows that there is the same mechanism for development of damages into mutations after irradiation of cells with different gamma doses, and allows averaging fractions of reversions for each generation.

Mutants formed in each generation as a fraction of the total number of mutants induced by different gamma doses are shown in the Table.

Table. Fraction of reversions formed during postradiation replications of γ-irradiated NA3-24 cells

		% of the total number of reversions		
Dose,Gy	Total	1st replication	2nd replication	3rd replication
24	100%	34	45	21
48	100%	32	50	18
96	100%	21	43	36
192	100%	22	47	31
Averages	100%	27±7	46±3	26±8

Averaging the data for each generation (on the assumption that there is the same mechanism of development of damages into mutations after irradiation of cells with different gamma doses) we find out that 27±7% of all γ-induced reversions are formed in the first postradiation replication of DNA, 46±3% in the second one and 26±8% in the third one.

In Fig.3 the general frequency of *leu2* gene reversion is plotted as a function of the gamma dose.

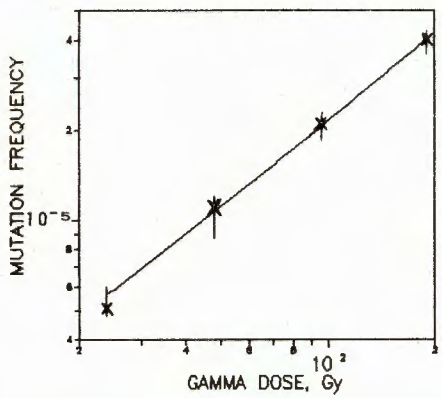


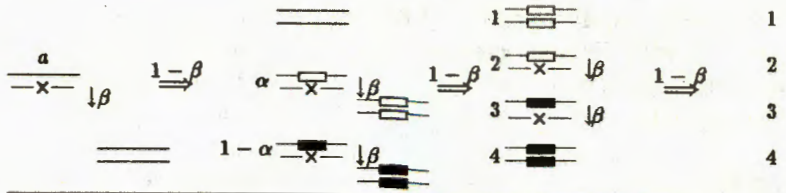
Fig.3. Formation frequency of induced *leu2* reversions as a function of the γ-radiation dose

In the dose range from 24 to 192 Gy there is a linear dependence of the mutation frequency on the irradiation dose. Thus, one can consider formation of γ-induced mutation to be a one-stroke process, i.e. one radiation damage is enough for a mutation to appear.

The one-stroke mechanism of reversion formation and an ascending-descending behaviour of the increase in induced reversions during postradiation incubation allow us to assume the following mechanism of formation of γ-induced reversions.

Let a be a number of cells with a radiation damage in the DNA strand opposite to the *leu2* gene (Fig.4). The damage can be repaired with a probability β. If the damage is not repaired to the moment of replication, then during replication a base causing reversion of the *leu2* gene will be found opposite to it with a probability α.

The number of reversions formed during the first postradiation replication will be $B_1^\gamma = a \cdot (1-\beta)\alpha$. During the second replication the number reversions formed on the solid medium will be $B_2^\gamma = a \cdot (1-\beta)^2(1-\alpha)\alpha$ and so on.



REPLICATIONS :	I	II	III
:	$a(1-\beta)\alpha$	$a(1-\beta)^2(1-\alpha)\alpha$	$a(1-\beta)^3(1-\alpha)^2\alpha$
:	$a(1-\beta)(1-\alpha)$	$a(1-\beta)^2(1-\alpha)^2$	$a(1-\beta)^3(1-\alpha)^3$

$$B_n^{\gamma} = a(1-\beta)^n(1-\alpha)^{n-1}\alpha; n \geq 1$$

FIG. 4. Hypothetical scheme of formation of γ-induced reversions with the premutation damage opposite to the transcribed DNA strand of the *leu2* gene (see explanation in the text). —×— — radiation damage, —▭— — reversion, —▬— — mutation, but not reversion

A general expression for the number of reversions formed during the n-th replication is

$$B_n^{\gamma} = a \cdot \alpha(1-\beta)^n(1-\alpha)^{n-1}, n \geq 1. \quad (2)$$

This relation is seen to be of the fall-down type.

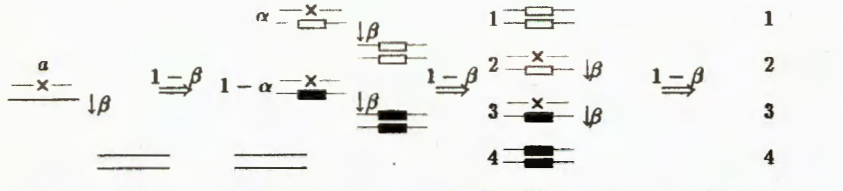
Now let's consider a case when the premutation damage is in the coding DNA strand of the *leu2* gene (Fig.5). If the damage is not repaired to the moment of replication, then during replication there can be any of four DNA bases opposite to it. In any case, no reversions are formed during the first replication. They can result from reparation between DNA replications:

$$B_{n\to n+1}^{\gamma} = a \cdot \alpha\beta(1-\beta)^n(1-\alpha)^{n-1}, n \geq 1;$$

and in further replications:

$$B_n^{\gamma} = a \cdot \alpha(1-b)^n(1-a)^{n-2}, n \geq 2. \quad (3)$$

As follows from the experiments, reversions are not formed between replications, i.e. $B_{n\to n+1}^{\gamma} = 0$. The dependence B_n^{γ} is a fall-down dependence, as in the previous case, but it begins from the second replication



REPLICATIONS :	I	II	III
:	0	$a(1-\beta)^2\alpha$	$a(1-\beta)^3(1-\alpha)\alpha$
:	$a(1-\beta)\alpha$	$a(1-\beta)^2(1-\alpha)\alpha$	$a(1-\beta)^3(1-\alpha)^2\alpha$
:	$a(1-\beta)(1-\alpha)$	$a(1-\beta)^2(1-\alpha)^2$	$a(1-\beta)^3(1-\alpha)^3$

$$B_n^{\gamma} = a(1-\beta)^n(1-\alpha)^{n-2}\alpha; n \geq 2$$
$$B_{n\to n+1}^{\gamma} = a(1-\beta)^n(1-\alpha)^{n-1}\alpha\beta; n \geq 1$$

FIG. 5. Hypothetical scheme of formation of γ-induced damage on the coding DNA strand of the *leu2* gene (see explanation in the text). —×— — radiation damage, —▭— — reversion, —▬— — mutation, but not reversion

and not from the first. The sum of two fall-down dependences (2) and (3), displaced with respect to each other, will have an ascending-descending behaviour, which we observed in the experiments.

Comparing the experimental data with the above formulas, we obtain $a = 0.3 \pm 0.2$, i.e. substitution of only one ($a = 0.25$) or at most two ($a = 0.5$) of four bases leads to a reversion.

Discussion

As pointed out above, the aim of this work was to study the mutagenic effect of γ-radiation on yeast *Saccharomyces cerevisiae* by analysing the kinetics of formation of induced mutants during postradiation incubation. The data obtained allow us to hope that the proposed approach may be helpful in obtaining new data on mutation mechanisms of *Saccharomyces cerevisiae*. If the study of mutagenesis by analysing the dependence of mutation frequencies on the radiation dose can be called an integral approach, the proposed analysis is a kind of differential one, taking into account more delicate aspects of induced mutagenesis. The observed fact that formation of induced mutants is a long process is itself interesting

and worth studying. It should be mentioned that this feature of mutagenesis was first described by Demerec (1946), but this aspect has remained in the shade up to now.

The proposed mechanisms of formation of induced reversions are not devoid of drawbacks, of course. Further investigations will probably reject these mechanisms, but they are quite acceptable as a tentative scheme, because they explain the ascending-descending behaviour of the increase in induced reversions. Unfortunately, to follow a further increase in induced mutants with this method is impossible because of the experimental error. Experiments with liquid media will probably yield additional information relevant to this. But the available data already allow some conclusions on the mutagenesis ways. In this respect, it is, of course, interesting and necessary to obtain and study similar kinetics of *RAD3, RAD6* and *RAD52* mutants using test systems both for back and direct mutations.

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Изучение γ-индуцированного мутагенеза
у Saccharomyces Cerevisiae.
Анализ кинетики образования
индуцированных реверсов у гаплоидных штаммов
дикого типа

Обнаружено, что после облучения синхронизированной культуры дрожжевых клеток γ-лучами в G_1-фазе клеточного цикла индуцированные излучением реверсы по гену leu2 образуются в трех первых пострадиационных фазах репликации ДНК, в промежутках между двумя фазами репликации ДНК образования индуцированных реверсов не наблюдается. Предлагается для изучения мутационных процессов использовать, помимо известных подходов, еще и анализ пострадиационной кинетики образования индуцированных мутантов. Предложены гипотетические схемы, объясняющие возрастающе-убывающий характер образования γ-индуцированных реверсов в период пострадиационной инкубации облученных клеток.

Работа выполнена в Лаборатории ядерных проблем ОИЯИ.

Препринт Объединенного института ядерных исследований. Дубна 1992

Levkovich N.V., Chepurnoy A. .

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A Study of Gamma-Ray Mutagenesis
in Saccharomyces Cerevisiae.
Analysis of Reversion Production Kinetics
in a Wild-Type Haploid Strain

After γ-irradiation of a synchronized yeast culture in the G_1-phase of the cell cycle, radiation-induced leu2 reversions are found to be formed in the first three postradiation phases of DNA replication. Without replication and in periods between two phases of DNA replication, formation of induced reversions was not observed. The analysis of postradiation formation kinetics of induced mutants is proposed to be used together with other know approaches for studying mutation processes. Hypothetical schemes are proposed to explain the ascending descending trend in formation of γ-induced reversions during postradiation growth of irradiated cells.

The investigation has been performed at the Laboratory of Nuclear Problems, JINR.

Preprint of the Joint Institute for Nuclear Research. Dubna 1992