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RADIOBIOLOGICAL MECHANISM IN CELLS.

DSB and Inactivation Mechanism

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1. INTRODUCTION

A model analysis of dsb formation in yeast cells and of their repair in non-growth conditions has been presented in the preceding paper [1]. Now an attempt will be undertaken to perform a similar analysis with the help of survival curves, if cells are plated in different periods of time after irradiation. In comparison with modelling dose dependences of dsb numbers this approach has certain advantages as well as disadvantages.

One of disadvantages lies in the fact that one stage more has appeared between irradiation and the final effect. To perform the mentioned analysis we must introduce an additional hypothesis concerning a causal relation between a dsb number in a cell (or their distribution in a cell) and an inactivation probability. On the other side, such an approach could help to test different hypothesis and thus contribute to the solution of this important question. Another complication may follow from possible differences of repair rates in growth and non-growth conditions.

The fact that some relations concerning dsb formation and inactivation probability will be more simple than formulas for determining actual numbers of reparable and irreparable dsb might be regarded as advantageous. Moreover, the survival curves (or a ratio of inactivated cells) can be determined with a greater precision than dsb numbers, which are estimated with the help of other formulae from a distribution of molecular lengths. There is, of course, a significant limitation of this approach, as it can be applied successfully only to cell types for which haploid and diploid forms with the same set of chromosomes are available.

2. EXPERIMENTAL DATA

Two isogenic strains of Saccharomyces cerevisiae of wild type have been used for survival studies: the diploid strain 28-73-1B and the haploid one 28-73-2A. Cells were incubated in YEPD for 5 days and centrifugated in sucrose-density gradient to obtain a single-cell suspension. Irradiation was performed in water suspension in glass tubes.

Two types of radiation were used. In the first case cells were irradiated by different doses of ^{137}Cs gamma rays (dose rate 0.62 Gy/s). In the other case fission neutrons from IBR-30 reactor were applied to; reactor worked in pulse regime with the frequency 5 s^{-1} and the pulse length 100 μs; irradiation was performed with dose rate 0.03-0.12 Gy/s.

After irradiation the cells were kept in non-growth conditions (destillated water or phosphate buffer at 30 °C) for different time intervals before plating.

For survival studies the cells were plated on nutrient agar YEPD; survival was determined by macrocolony method. The obtained results for both the yeast strains and both the radiation types are given in Tables 1 and 2.

Table 1

Survival ratios for haploid and diploid cells of Saccharomyces cerevisiae at different times (in hours) after irradiation by gamma-rays

Haploid cells		Diploid cells			
D (kGy)	S (%)	D (kGy)	S (%)		
			t = 0	t = 6.5	t=28.5
0.053	46.4	0.5	60.8	100.0	-
0.106	23.0	1.0	22.4	70.3	90.0
0.151	9.1	1.6	4.86	9.0	-
0.249	2.4	2.3	0.84	2.88	29.4
0.4	0.25	3.0	0.13	0.46	9.08
		4.0	0.023	0.035	0.93

3. CAUSAL RELATION BETWEEN A dsb NUMBER AND CELL INACTIVATION

It follows from ref. [2] that practically all reparable dsb are repaired after 3 days in non-growth conditions. The question arises what happens in growth conditions. It follows from older results [3,4] obtained with a similar type of diploid cells that primary lesions repair in a similar way, but the time for such a repair is limited. All cell damages seem to be fixed at the

Table 2

Survival ratios for haploid and diploid cells of Saccharomyces cerevisiae at different times t (in hours) after irradiation by neutrons

Haploid cells		Diploid cells				
D (kGy)	S (%)	D(kGy)	(%)			
			t = 0	t = 21	t = 44	t = 70
0.01	23.0	0.101	29.2	38.6	56.2	-
0.01	45.0	0.13	17.6	28.9	34.1	51.8
0.02	10.0	0.19	5.02	6.21	-	17.5
0.02	13.8	0.271	0.81	3.35	6.47	10.2
0.03	5.8	0.406	0.023	0.53	0.55	1.05
0.04	2.4					
0.04	2.7					

moment when a given cell passes to mitosis. And one is forced to ask what is the relation between cell inactivation and a number of dsb which have remained unrepaired.

On the basis of a qualitative analysis of different possibilities [5] the following hypothesis seems to be the most probable: A diploid cell is inactivated when in the moment of mitosis at least one pair of homologous chromosomes has remained damaged, i.e., when there exists at least one dsb in either chromosome of this pair.

As to haploid cells we will assume that such a cell is inactivated when at least one dsb has been formed in it [6].

These two assumptions will be a part of the model which will be tested with the help of the experimental data given in Sec.2.

4. MATHEMATICAL MODEL OF SURVIVAL CURVES FOR HAPLOID CELLS

Let us assume, similarly as in ref. [1] that an average number of dsb corresponding to one critical site is given by the relation

$$r_d = \rho_d D; \qquad (1)$$

the probability that at least one dsb has been formed in a given

critical site equals

$$p_d = 1 - \exp(-r_d). \tag{2}$$

The average number per one haploid cell is then equal to

$$n_g = p_d N, \tag{3}$$

where N is the number of all critical sites in the corresponding haploid cell. The inactivation probability (Poisson distribution assumed) will be given by

$$p_g = 1 - \exp(-n_g), \tag{4}$$

as one dsb in a cell is assumed to be sufficient for its inactivation.

It is, of course, necessary to admit that some deviations from Poisson law in dsb distributions over the whole population will exist and that introducing a correction given by an unhit part of the population will be reasonable. If x_g is an average number of dsb per one haploid cell hit by one beam particle (see /1/) the ratio of surviving cells will be given by

$$S_g = 1 - p_g p_t^{(g)}, \tag{5}$$

where one can put in the first approximation /1/

$$p_t^{(g)} = 1 - \exp(-n_g/x_g); \tag{6}$$

n_g in Eq.(4) should be again replaced by (see /1/)

$$n'_g = n_g / p_t^{(g)}.$$

5. SURVIVAL CURVES OF DIPLOID CELLS

The survival curve of diploid cells depends on the time after irradiation at which it is measured. The more time has elapsed since irradiation the greater the ratio of surviving cells (at least if a radiation of lower LET is applied to). However, there always remains a certain part of inactivated cells which corresponds to the existence of irreparable dsb. We will derive, therefore, first an expression for inactivation probability caused by these irreparable dsb.

One can start from the formulae (1) and (2) identical with those for the haploid cells. The dsb number corresponding to one section (segment), responsible for creation of irreparable

dsb, equals then (see /1/)

$$r_h = p_d N_h \tag{7}$$

and the probability that at least one dsb appears in a given segment is equal to

$$p_h = 1 - \exp(-r_h). \tag{8}$$

For an average number of pair-damaged homologous segments per one cell one obtains

$$r_2 = p_h^2 N/N_h. \tag{9}$$

The ratio of cells inactivated practically immediately after irradiation is then given by

$$p_2 = 1 - \exp(-r_2), \tag{10}$$

i.e., by the probability that at least one pair of homologous segments is damaged in a given cell.

Let us attempt now to determine the number of all inactivated cells. If our above-mentioned hypothesis about cell inactivation were applicable at once after irradiation the ratio of all inactivated cells would be given by

$$p_{c2} = 1 - \exp(-r_{c2}), \tag{11}$$

where

$$r_{c2} = p_c^2 N_c$$

is the average number of pair-damaged homologous chromosomes in a cell; the probability p_c is determined by

$$p_c = 1 - \exp(-r_c), \tag{12}$$

where

$$r_c = r_d N/N_c$$

is the average dsb number per one chromosome.

The probability p_{c2} will diminish with the time available after irradiation and tend gradually to p_2. One could in principle make use of the repair function $r_2(t)$ derived in ref. /1/ for the dsb-number decrease and to estimate the quantity p_{c2} from this changing number in different periods of time after irradiation. However, it seems to be more useful to look for

a change of the quantity p_{c2} itself as the statistical distribution of dsb over the population changes in the course of time and then the assumption of Poisson distribution becomes untenable. We will determine this change with the help of equation

$$\bar{p}(t) = p_2 + (p_{c2} - p_2) r(t), \tag{13}$$

where

$$r(t) = \frac{\rho + 1}{\rho + \exp[(rt)^{\eta}]} \tag{14}$$

is parametrized in a similar way like the function $r_2(t)$ in /1/.

We have limited ourselves to the formula for a slow repair only, as it is possible to expect that the fast repair is practically finished before the mitosis starts. Its influence has been taken into account in another way (see Sec.6).

The cell survival is then given by

$$S_d = 1 - \bar{p}(t) p_t^{(d)}, \tag{15}$$

where

$$p_t^{(d)} = 1 - \exp(-n_d / x_d) \tag{16}$$

accounts for the influence of the unhit part of the population; x_d is an average dsb number formed by one beam particle per one hit diploid cell.

It is necessary to mention that a similar mechanism concerning the formation of irreparable dsb has been already considered in ref./7/; in that paper, however, the number of irreparable segment pairs has been correlated to the survival curve of V-79 cells obtained in immediate plating.

6. THE INFLUENCE OF A FAST REPAIR

The quantity p_{c2} defined by Eq.(11) is based on the number of all dsb created in the cell production by the radiation. We know, however, from ref./1/ that a dsb fraction repairs quite quickly and that this fraction can depend on the applied dose. Thus the determination of inactivation ratio in different periods of time after irradiation cannot be based on Eq.(13); it is necessary to use the equation

$$\bar{p}(t) = p_2 + (p_x - p_2) r(t), \tag{17}$$

where

$$p_x = p_2 + \xi (p_{c2} - p_2) \tag{18}$$

and $\xi \in (0,1)$. We will admit that ξ is a dose-dependent factor, this dependence being assumed in the form

$$\xi = \xi_1 + \xi_2 D + \xi_3 D^2 . \tag{19}$$

7. ANALYSIS OF EXPERIMENTAL DATA

The method of obtaining experimental data has been described in Sec.2. In our model analysis we have started from the values given in Tables 1 and 2. We have again taken

$$N_c = 17, \quad N = 1{,}5 \cdot 10^7 .$$

In the case of gamma radiation, we have obtained with the help of optimization procedure MINUIT the following values of the free parameters

$$\rho_d = 7.94 \cdot 10^{-6} \ \mathrm{kGy}^{-1}, \quad N_h = 301,$$

$$x_g = 8.1, \qquad x_d = 108,$$

$$T = 3.27 \ \mathrm{h},$$

$$\xi_1 = 0.77, \quad \xi_2 = 0.15, \quad \xi_3 = -0.022.$$

The corresponding repair functions are represented in Fig.1a. The resulting survival curves at different times after irradiation together with experimental points are shown in Fig.2.

Similarly, we have obtained for neutrons

$$\rho_d = 5.05 \cdot 10^{-5} \ \mathrm{kGy}^{-1}, \quad N_h = 535,$$

$$x_g = 7.8, \qquad x_d = 70.8,$$

$$T = 2.1 \ \mathrm{h},$$

$$\xi_1 = 0.94, \quad \xi_2 = 0.053, \quad \xi_3 = 0.24.$$

The corresponding repair functions are represented in Fig.1b; the resulting curves are shown in Fig.3.

A rather surprising result concerns the parameters x_g and x_d. According to a simple probabilistic interpretation we should

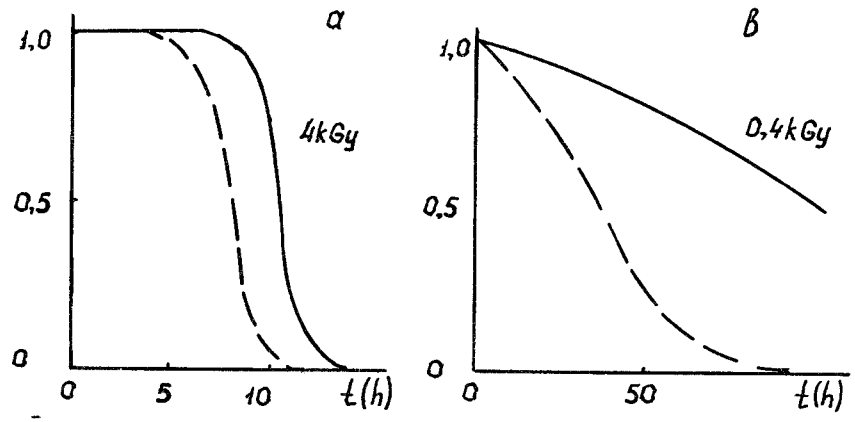

Fig.1. Functions describing the rate of dsb repair in diploid yeast cells Saccharomyces cerevisiae, strain 28-73-1B at different time after irradiation: a) by 661 keV gammas, b) by fission neutrons. The full lines correspond to the given doses; the repair is faster for lower doses, the dashed lines indicating the limits for non-irradiated cells.

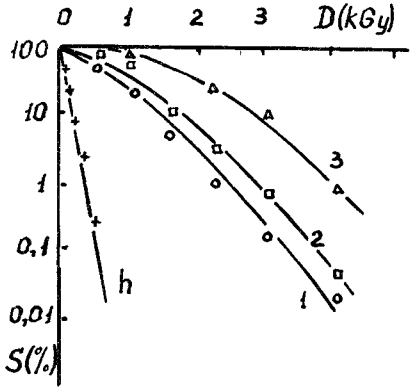

Fig.2. Theoretical survival curves for the parameter values given in the text together with experimental points from Tab.1 (gamma radiation); 1 - delay 0 h; 2 - 6.5 h; 3 - 28.5 h; h - haploids.

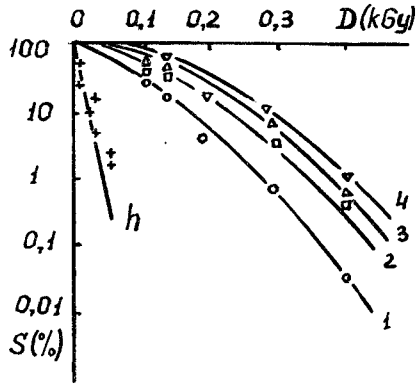

Fig.3. Theoretical survival curves for the parameter values given in the text together with experimental points from Tab.2 (neutrons); 1 - delay 0 h; 2 - 21 h; 3 - 44 h; 4 - 70 h; h - haploids.

expect that their ratio will be given by the numbers of critical sites in haploid and diploid cells only. The much greater difference must be related to the repair process in diploid cells, especially to the existence of fast and slow repair. While in the haploid cells x_g is an average from all dsb formed by radiation, x_d is an average over the cells exhibiting a slow repair only; the damages in cells with a small number of dsb repair so quickly that in our analysis these cells are regarded as practically unhit (see the quantities ξ and p_x).

On the other side, we should expect that the radiation with a higher LET would create a greater number of dsb per one cell. This would be surely so if the chromosomes were distributed uniformly over the whole cell nucleus. The obtained results can be easily brought into harmony with the chromosomes forming a narrow layer (~10 nm) under the nucleus membrane. In both the cases the energy is transferred to cells by secondary charged particles, i.e., by electrons or protons, and is transmitted in certain local deposites forming ion clusters. One can expect that the effective size of such clusters will not differ very much in both the particle types and will be comparable with the chromosome-layer thickness. Consequently, the number of dsb formed in individual cells cannot be very different.

The electrons and slower protons coming from fission neutrons differ, however, very much in the space density of such ion clusters. While the damage of the chromosome layer caused by electrons will be accompanied by a very small damage of other parts of the given cell (or cell nucleus) the mentioned protons will always cause a great damage in the whole cell. This additional damage would not be probably fatal if not accompanied by dsb in chromosomes; it can, however, substantially influence the repair rate of potentially lethal dsb. The shape of the repair function of life-important dsb will depend on how many damages must be repaired in the given cell (or cell nucleus). In both the cases there exists a slight dose-dependence, which seems to be a little stronger for neutrons. This can bear upon the fact that the number of hit cells and the average dsb number in these cells will exhibit different dose dependences.

The value of effective time T, which is available in growth conditions before mitosis, can be regarded only as an approximate one as it can be rather strongly influenced by the parametrization chosen for the repair function, and partially also by the fact that the analysis is based on several survival curves only. The value obtained for the gamma radiation agrees, of course, with that reported earlier (see [4]).

It is quite natural that the probability ρ_d of dsb formation per a unit dose is several times greater for neutrons. From these values one can determine the number of dsb corresponding the energy absorbed by the DNA itself. If the DNA mass of the diploid yeast cell is taken as being $1.6 \cdot 10^{-14}$g (and the number of all critical sites $3 \cdot 10^7$) one obtains for the dsb yield

$G_\gamma = 0.24 \cdot 10^{-2}$ eV and $G_n = 1.44 \cdot 1/^{-2}\, eV^{-1}$; if the ρ_d value found for 30 MeV electrons in ref.[1] is used one gets $G_e = 0.066 \cdot 10^{-2}$ eV^{-1} All these values rising gradually with the correspinding LET seem to be mutually consistent. The dsb yield for the lowest LET agrees also with the values obtained by a direct measurement (comp., e.g., ref. [8]).

And finally one must mention the average length of the homologous segments. In the first case we have obtained that an average chromosome consists approximately of 3000 such segments while in the other case of 1700 only. This difference can follow from different space distributions of energy-transfer events. Both these values are, of course, much greater than ~200 segments obtained in ref.[1] for another yeast cell strain with the same genetical system. At this moment the only explanation of this rather great difference can be given by the very bad repair ability of the latter cell strain.

8. CONCLUSION

The presented model of the whole inactivation mechanism consists of two different approaches. While the formation of primary lesions (description of physical and chemical phases) has been pictured by an actual model representing main parts of the given mechanism, the biological phase (repair of primary damages) could be described with the help of a phenomenological mathematical formula only. All free parameters have been determined from experimental survival curves with the help of an optimization procedure.

The next stage must consist in interpreting the phenomenological repair functions as well as the values of individual parameters describing the destruction process on the grounds of other pieces of knowledge. E.g., the values of ρ_d, x_g, x_d (resp. ξ) can be related to microdosimetric or other microscopic characteristics of individual radiation types, the repair functions should be interpreted with the help of more detailed repair models, a.s.p. The presented results suggesting some new ideas for a further more detailed analysis could be very helpful.

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Амиртаев К.Г. и др. E19-82-792
Механизм воздействия излучения на клетки.
Двунитевые разрывы ДНК и механизм инактивации

Определялась выживаемость клеток дрожжей в различное время после облучения γ-квантами или нейтронами. Предложен механизм инактивации и проведен анализ полученных экспериментальных данных. Рассматриваются некоторые следствия, касающиеся различия механизма действия излучений с разной ЛПЭ.

Работа выполнена в Лаборатории ядерных проблем ОИЯИ.

Сообщение Объединенного института ядерных исследований. Дубна 1982

Amirtaev K.G. et al. E19-82-792
Radiobiological Mechanism in Cells.
DSB and Inactivation Mechanism

Survival for a kind of yeast cells in different periods of time after irradiation by gamma rays and neutrons has been measured. An inactivation mechanism has been proposed and a model analysis of the mentioned experimental data performed. Some consequences concerning the difference of radiobiological mechanism for different LET have been mentioned.

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

Communication of the Joint Institute for Nuclear Research. Dubna 1982