

SURVIVAL OF YEAST UNDER THE ACTION OF THE PHOTON IONIZING RADIATION

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Received January, 18, 2007; accepted October 30, 2007; published online November 10, 2007

Bacteria and yeast are classical object for researchers studies. Influence of different type of irradiation on the yeast have been analyzed. The data show that radioresistance depends on the growth phase. Different types of survival curve in relation to the type of irradiation were observed. It should be emphasized that after UV irradiation the survival curve contains a specific "shoulder" fragment, which are not observed in X-ray and gamma-Co⁶⁰ irradiation.

INTRODUCTION

Bacteria and yeast are classical object for genetic, biochemical and radio-biological research since their biological properties allow us to find out the nature of separate elementary processes with a relevant degree of accuracy. These processes indicate a sufficiently smaller accumulation of secondary alterations in comparison to those observed in the cells organized in a more complex way.

Radioresistance of different types of bacteria in to the action of ultraviolet [Saeki et al (1988)], X-ray irradiation [Galeotti et al. (1981)] or gamma-irradiation [Petin (1979), Durand et al. (2002)] varies very wide range. The data are relative as radioresistance substantially depends on the state of culture at the moment of irradiation, therefore different researchers obtained different quantitative characteristics of bacteria radioresistance.

The character of dose survival dependences for different types of bacteria differs greatly: radioresistant species show a large "shoulder" in the survival curve while radiosensitive show simple exponents. For instance *Pseudomonas fluorescens* are much more radiosensitive than *Micrococcus radiourance*. The latter species is characterized by an exceptionally high radioresistance. Radioresistance of cells of this type depends in general on the irradiation conditions.

The *Micrococcus radiourans* bacterium characterized by an extraordinarily low radiosensitivity is used as a model to study the nature of the processes which have a great influence on a uniquely high radioresistance. This bacterium has sufficiently large cells combined in groups of two-four.

In a state of spore bacterial cells are able to withstand different factors, in particular ionizing radiation. For

example, for *Bacillus subtilis* spores the lethal dose is two times higher than that for normal cells. The peculiarities of reproduction of mushrooms and organization of the nuclear apparatus of their cells predetermine a sufficiently high radioresistance of these organisms.

Radiosensitivity of the *Saccharomyces cerevisiae* is investigated in a number of experiments since cultures of this species were used to study various radio-biological phenomena [Petin (1983), Durand et al. (2002)]. However, radiobiological properties of some other types of fungi for which one can get mainly multishock survival curves are also determined. It is quite natural taking into account the heterokaryon nature of fungi [Korogodin et al. (1996)].

The increase of linear energy transfer magnitude is accompanied by relative biological efficiency of radiation [Petin and Kim (2005)]. The effect of dose rate in the case of irradiation of fungi with ultraviolet rays, X-ray radiation or gamma-radiation does not take place. Hitting of survival curves (value of extrapolation number) for heterokaryon organisms correlates with the number of nuclei in cells. In *Saccharomyces* radiosensitivity grows in the polyploids strains from haploid cells to triploid. The subsequent growth of poliploidicity is followed by the reduction of radioresistance of cells. It testifies to the fact that radiation inactivation of cells is substantiated by the appearance of recessive and dominant lethal mutations. In fact, dominant lethals are mostly connected with chromosomal alterations, their probability of appearance rises with the increase of number of chromosomes in a cell.

The presence of fungi mutations which are characterized either by higher radioresistance or very

high radiosensitivity enabled us to investigate genetic control of radioresistance of a cell. Radiosensitive mutants are selected for *Basidiomycetes*, *Ustilago maydis*, yeasts of *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, *Neurospora crassa*, *Pullularia pullulans*. Some of these mutations have blocked critical elements of the DNA reparation system, others are characterized by deficient processes of post-radiation repair which can be the reason for the delay of cellular division.

EXPERIMENTAL RESULTS

Yeasts were exposed to the action of radiation at a room temperature. Three types of irradiation were used:

1. Ultraviolet radiation (ultraviolet – B) with wave-length of $\lambda = 300$ nm, density of power was $0,01$ W/mm².
2. X-ray radiation (anode is hard tungsten with white radiation), density of power was $0,001$ W/mm².
3. Gamma-radiation, the source was Co⁶⁰.

In the re-calculation photon energies are accordingly: 4 eV, $5 \cdot 10^4$ eV and $1.3 \cdot 10^6$ eV. It is more appropriate to present dose dependences of the effect on radiation in the coordinates of “survival vs surface density of energy, J/mm²”. In case of the impenetrating gamma-radiation we find more convenient to introduce the “survival vs mass density of energy, J/kg (Gy)” coordinates.

The shapes of dose dependence curves of radio-biological effects can be rather various, in particular with several critical point. It shows that numerous systems influenced by radiation either directly or indirectly take part in forming the radio-biological effect. However, the shape of curves is usually simple. The linear (directly proportional) dependence between dose and quantitative value of degree of radio-biological effect is the simplest. The graphs of such dependences are straight lines. The curve of sigmoid or logistic shape turns out to be a very frequent case of the system sensitivity display. If a curve can be described by a relevant analytical expression, in case of need it is used as a mathematical model of radio-biological effect. Of special value are mathematical models whose parameters correspond to quite real characteristics of the irradiated object.

In Fig.1 the dependence of yeast survival on the dose of UV irradiation is presented. The curves are shown in a semilogarithmic scale.

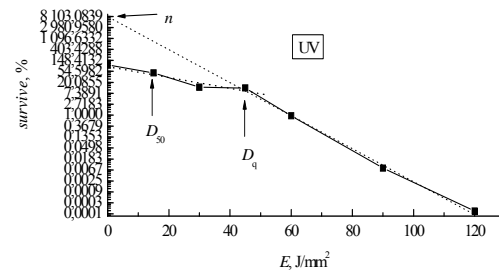


Fig.1. Dose dependences of the survival of yeast exposed to the ultraviolet rays.

Here are two distinct areas in the radiation dependence of the effect. The exponential form of “dose vs effect” curves enables us to define quite easily how many times larger the irradiation dose should be to reduce cell survival to a specific level.

The notion of semilethal dose concerns the population, that is a sufficiently large quantity of homogeneous cells that are subject to irradiation. In relation to a separate cell this quantity is transformed into its inactivation probability characteristics. Therefore, the dose D_{50} is related to the probability of 0.5, that is the cell is likely either to die or remain alive and was equal to 15 J/mm².

At small values of doses survival curves quite often deviate from the exponent and consist of two parts: initial, in which the growth of dose is not accompanied by a substantial reduction of survival and proper exponential. The first part of curve is called the “shoulder”. To interpret the curve with the “shoulder” such parameters are introduced: extrapolation number n and shoulder size D_q . The extrapolation number n is the coordinate of intersection of rectilinear extrapolation of the exponential part with the y -axis.

It is obvious that if the survival curve has a “shoulder”, the value of extrapolation number is still larger with the rise of radiosensitivity of the irradiated object. In the case of ultraviolet radiation for the given type of yeast it is equal to 8000 . The size of shoulder D_q is characterized by the value of dose which corresponds to intersection of the line extrapolating the exponential part of curve with the line parallel to the x -axis (the dose for cell renewing). It makes in this case of dose density $D_q = 45$ J/mm².

More powerful X-ray irradiation (Fig. 2) gives the dose dependence of effect without a drastic decline what is typical for the saturation start.

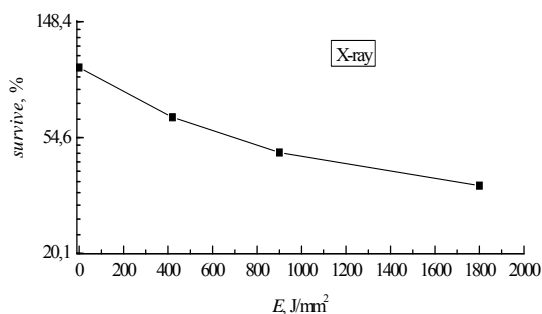


Fig.2. Dose dependences of the survival of yeast exposed to the X-ray irradiation

A similar behavior is peculiar to the dose dependence of yeast survival under the action of gamma-photons Co^{60} (Fig. 3). A characteristic shoulder in the dose dependence is not observed. The semilethal density of energy makes for the X-ray radiation 800 J/mm^2 and for gamma-radiation the dose is 400 Gy .

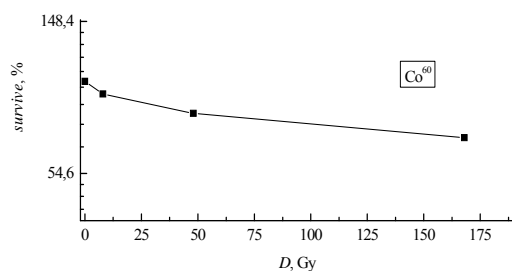


Fig.3. Dose dependences of the survival of yeast exposed to the gamma- Co^{60} irradiation.

DISCUSSION

Numerous investigations of bacteria finally proved the basic statement of radio-biology that the bacterial *DNA* is a target of the action of ionizing radiation [D.Averbeck and S.Averbeck (1994), Schiestl and Wintersberger (1992)]. At the same time it has been shown that radioresistance is controlled by genetic systems. For example, in cultures of *E. coli* there radio-stable mutants with frequency of about 10^{-5} further appear spontaneously and are selected as a result of action of ultraviolet radiation.

Episomes, genetic elements in bacteria that can replicate free in the cytoplasm or can be inserted into the bacterial *DNA* affect radioresistance of bacterial cells. Among the cells which survive after the irradiation there are such that cannot produce an unlimited quantity of divisions and must die. These cells form subclones called lethal sectors. Cellular death is obviously predetermined by not only induction of recessive and dominant lethal mutations but also lethal damages unconnected with the

structural changes of chromosomes. The especially radioresistant mutations are considered as a consequence of appearance of the cell reparation system.

Dose dependences of proliferation survival mostly acquire the form of exponential curves and in the system of semilogarithmic coordinates they are straight lines. Accordingly the analytical expression that describes the curve of survival dose dependence is an exponential function

$$N/N_0 = e^{-kD},$$

where N is the quantity of cells survived after the irradiation; N_0 is the quantity of the cells exposed to the rays of the D dose; k is a coefficient that characterizes the slope of exponent to the x -axis; e is a base of natural logarithm.

It is obvious that the steeper the curve, the smaller survival in case of dose growth what evidences to high radiosensitivity of cells. The molecular mechanisms of cell reparations, and repair of the radiation damage are dominant in revealing the nature of cell radioresistance (an illustration is a survival dose curves treatment made by means of these mechanisms, curves not being exponents).

It is confirmed by numerous experimental data that a critical event in a cell which causes its proliferation death is the formation of a double breaks of the *DNA*. Indeed, a one-strand *DNA* breaks recommence more effectively and thus plays a less important role in the cell death. In a theory double breaks of the *DNA* are generated when the two complementary stands of the *DNA* double helix are broken simultaneously at sites that are sufficiently close to one another that base-pairing and chromatin structure are insufficient to keep the two *DNA* ends juxtaposed [Jackson (2002)]

The number of two-strand breaks in the first case should be directly proportional to the value of the absorbed dose aD and in the second due to the statistics laws – to the dose squared bD^2 (a and b are factors that characterize the probability of forming two-strand break of the *DNA*). Though in this case survival curves differ from exponents, the theory of target is the basis which a two-strand *DNA* helix of the chromosomes.

The curves of survival cells with multihit targets differ from a simple exponential curve by the fact that in the region of small doses they are not exponential: in a semilogarithmic scale the survival curve consists of two parts, the proper exponential in the range of high values of doses and curvilinear “shoulder” in range of small doses. In the case of the *UV* irradiation a cell of yeast shows multihit of about 80. The biological sense of multihit can be seen for example in the amplification of genes whose activity is considered a

measure of the target inactivation. In the case of diploid cells which have the double set of chromosomes it is necessary to hit both alleles of a gene for inactivation and targets of these cells are two-shock.

Photons of higher energies (X-rays and γ -photons) as a result of a long track are characterized by a multistage process of the transmission of energy to a cell. The absence of a specific "shoulder" in the dose dependence in the range of small doses testifies to impossibility of the process of renewal of cells under the irradiation by high-energy photons. A long linear track is also formed by rapid photoelectrons which are generated in the process of interaction of a high-energy photon with the cell substance. Photoelectrons, in turn, predetermine a cell death related to the structural changes selected in the result of the ultraviolet radiation exposure [Karen *et al.* (1975), Nakai and Matsumoto (1967)]. Among the yeast cells survived after high-power irradiation there are radioresistant mutants able to divide. It shows a survive dose dependence which is less steep than the exponent.

CONCLUSIONS

The curves of survival of yeast cells at the ionizing radiation exposure differ from a simple exponential dependence: under the UV irradiation in the region of small doses in a semilogarithmic scale the survival curve consists of two parts, proper exponential in range of high values of doses and a curvilinear "shoulder" in the range of small doses. A cell shows a multihit of about 80. Photons of higher energies (X-rays and γ -photons) as a result of a long track are characterized by the multistage process of energy transfer to a cell. A specific shoulder in the dose dependence is not observed. For the radiations under consideration there is no partial repair of damage.

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