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**Գ.00.04 կենսաքիմիա մասնագիտությամբ
կենսաբանական գիտությունների դոկտորի
գիտական աստիճանի հայցման ատենախոսության**

ՍԵՂՄԱԳԻՐ

ԵՐԵՎԱՆ 2022

MINISTRY OF EDUCATION, SCIENCE, CULTURE, AND SPORTS OF RA
YEREVAN STATE UNIVERSITY

MARUTYAN SEDA VIKTOR

THE METABOLIC CHANGES AND DNA STRUCTURAL DAMAGES IN X-IRRADIATED YEASTS *C. guilliermondii NP-4*

SYNOPSIS

**of dissertation for conferring of science degree of
Doctor of Biological Sciences
in the speciality of 03.00.04 - Biochemistry**

YEREVAN -2022

Ատենախոսության թեման հաստատվել է Երևանի պետական համալսարանում

Գիտական խորհրդատու՝	կենս.գիտ.դոկտոր, պրոֆեսոր Լ.Հ.Նավասարդյան
Պաշտոնական ընդդիմախոսներ՝	կենս. գիտ.դոկտոր, պրոֆեսոր Մ.Ա.Սիմոնյան
	կենս. գիտ.դոկտոր, պրոֆեսոր Պ.Ա.Ղազարյան
	կենս. գիտ. դոկտոր, դոցենտ Գ.Յու.Մարմարյան
Առաջատար կազմակերպություն՝	ՀՀ ԳԱԱ Հր.Բունիաթյանի անվան կենսաքիմիայի ինստիտուտ

Ատենախոսության պաշտպանությունը տեղի կունենա 2022թ. մայիսի 6-ին, ժ.14:00-ին, Երևանի պետական համալսարանում գործող ՀՀ ԲՈԿ-ի Կենսաֆիզիկայի 051 մասնագիտական խորհրդի նիստում (0025, Երևան, Ա.Մանուկյան փ. 1, ԵՊՀ, կենսաբանության ֆակուլտետ):

Ատենախոսությանը կարելի է ծանոթանալ Երևանի պետական համալսարանի գրադարանում:

Ատենախոսության սեղմագիրն առաքված է 2022թ. մարտի 24-ին:

051 մասնագիտական խորհրդի գիտական քարտուղար,

կենս.գիտ.թեկնածու, դոցենտ Մ.Ա.Փարսադանյան

The theme of the dissertation has been approved at Yerevan State University

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	Dr. of Biological Sciences, Professor P.A.Ghazaryan
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Leading organization:	H.Buniatyan Institute of Biochemistry NAS RA

The defense of the dissertation will be held on 6th May 2022, at 14:00, at the session of 051 Scientific Specialized Council on Biophysics of SCC of RA at Yerevan State University (0025, Yerevan, Alex Manoogian str. 1, YSU, Faculty of Biology).

The dissertation is available at the library of Yerevan State University.

The synopsis has been sent on the 24th March, 2022.

Scientific Secretary of 051 Specialized Council,
PhD, Associate Professor M.A.Parsadanyan

INTRODUCTION

Topic's significance: Currently, the risk of extreme conditions (ionizing or non-ionizing radiation, technogenic pollution of the environment, heat shock, starvation or malnutrition, etc.) on living organisms is increasing day by day. Living organisms are endowed with great abilities to adapt to the ever-changing conditions of the environment, which enable them to survive in extreme conditions. During this time, the organisms develop certain defence mechanisms, which are the basis for the development of means of protection of organisms from extreme impacts. From this point of view, the study of yeasts, which are more resistant to external factors than mammals, is of great importance. Nowadays, ionizing radiation has a significant place among the unfavourable factors of the environment, the harmful effect of which is realized on the organism first at the level of genetic material, and then directly at the level of proteins that are the result of its expression. The ionizing radiation-induced DNA damages lead to structural damages of chromosomes and the reproductive death of cells. These damages can be repaired by the normal functioning repair system of DNA, ensuring the post-irradiation survival of the organisms. However, radiation-induced damages of the components of the repair system may result in loss or misrepair of DNA damages in the cell, but the data obtained in this area are still insufficient to develop anti-irradiation means. The cell's repair system has radioprotective potential, so its study could provide a basis for developing means to enhance organisms' ability to survive in extreme conditions.

Research of radiation-induced DNA structural damages in the cells, metabolic abnormalities, and repair possibilities of those structural damages during the post-irradiation repair period can be performed in lower eukaryotic cells, yeast. The latter is similar to mature eukaryotic cells in their cellular structure and subcellular organoids [Zhang *et al.* 2018], with structural-functional features of genetic material [Muhammadi *et al.* 2015], with the identity of the important cellular metabolic pathway [Poswal et al, 2017]. They are unicellular eukaryotes, which have a typical cellular structure; their nucleus is surrounded by a nuclear membrane; the genetic material is divided into several chromosomes; they multiply by simple mitosis or germination [Zhang et al., 2018]. Yeasts have a short life cycle, they multiply rapidly and can be grown under strictly controlled biochemical laboratory conditions without the high cost of complex equipment. For this reason, yeasts are widely used in biochemistry, microbiology, radiation biochemistry as a convenient model for the study of eukaryotic cells [Salazar *et al.* 2016].

At the Research Laboratory of Biochemistry of YSU Department of Biochemistry, Microbiology, and Biotechnology of YSU the changes in the metabolic processes of yeasts *Candida* under the influence of various extreme external factors (ionizing and non-ionizing radiation, heat shock and nitrogen starvation, etc.) are studied for many years. In this work, the changes of some metabolic processes in yeasts *Candida guilliermondii* NP-4 are presented under influence of X-irradiation and after post-irradiation repair.

The purpose and objectives of the research: This work aimed to study the viability of *C. guilliermondii* NP-4 yeasts, changes in some metabolic processes and the morphology of yeasts, as well as changes in the physical-chemical properties of DNA under the influence of X-irradiation and after post-irradiation 24 hours repair process.

The following tasks have been set for the work:

1. study the growth dynamics of irradiated yeasts *C. guilliermondii* NP-4 within 24 hours of post-irradiation growth, until the establishment of the stationary phase of growth, their colony-forming ability and vitality;
2. study the morphological-functional changes in yeast cells and subcellular organoids after X-irradiation and post-radiation incubation
3. study the changes of fluorescence, electrophoretic, and melting parameters of the DNA of yeasts under influence of X-irradiation and after post-irradiation repair period,
4. study the activities of deamination enzymes of nucleotides in yeasts and their changes under influence of X-irradiation and after post-irradiation repair,
5. study the hydrolysis intensity of polyphosphate nucleotides ATP, ADP, GTP, and GDP in yeasts, their changes under influence of X-irradiation and after post-irradiation repair,
6. study the ATP-hydrolysis intensity in yeast mitochondria and its change under influence of X-irradiation and after post-irradiation repair,
7. study the changes in volutin granules (polyphosphates) in yeasts under influence of X-irradiation and after post-irradiation repair by electron microscopy analysis.

Scientific novelty and practical value of the study

1. After X-irradiation of yeasts *C. guilliermondii* NP-4, a 2-hour delay in all stages of the life cycle is observed in comparison with non-irradiated yeasts, which may be due to G_2-block.
2. The colony-forming ability of X-irradiated yeasts *C. guilliermondii* NP-4 is 2.8 times small than in the case of non-irradiated yeasts, although there are no significant differences between the two variants in terms of biomass accumulated during the stationary growth phase. This phenomenon can be explained by the possibility of the formation of giant cells.
3. X-irradiated yeasts *C. guilliermondii* NP-4 do not exhibit vitality. Vital activity is restored during post-irradiation repair, but it remains significantly lower than the value of non-irradiated yeasts.
4. Under influence of X-irradiation in the DNA of yeasts *C. guilliermondii* NP-4, the structural damages occur which are deepened in the post-irradiation repair period. The number of damages increases when the cells are irradiated at 0°C, as the activity of repair enzymes is suppressed at low temperatures.
5. Yeasts *C. guilliermondii* NP-4 undergoing post-irradiation repair reveals additional fractions of DNA of lower molecular weight, which may be explained by enzymatic re-damaging due to "mis-repair" of DNA damages, as well as the emergence of new molecular forms of DNA due to recombination repair of DNA damages.

6. The melting temperature of DNA of X-irradiated yeasts *C. guilliermondii* NP-4, contrary to expectations, increases, and the melting interval decreases, which may be due to the formation of DNA-DNA and DNA-protein bindings.
7. The activities of deaminases of purine and pyrimidine nitrogenous bases, nucleosides, and nucleotides in *C. guilliermondii* NP-4 yeasts are changed under influence of X-irradiation and post-irradiation repair ensuring the participation of these compounds in nucleotides salvage pathways.
8. Under influence of X-irradiation, the giant cells and filamentous forms of cells appear in the population of yeasts *C.guilliermondii* NP-4. The volutin granules appear in the cytoplasm of X-irradiated yeasts, which are contained in vacuoles of non-irradiated yeast cells. During post-irradiation growth, these granules become smaller and spread throughout the cytoplasm.
9. Contrary to expectations, during the post-irradiation repair of yeast, the deepening of radiation-induced damages of DNA takes place. The formation of additional DNA single strands breaks, double strands break, DNA-DNA, and especially DNA-protein bindings are formed.

The obtained data can be used to prevent the effects of ionizing radiation on living organisms, to increase the efficiency of post-irradiation repair processes, to develop biochemical protective means, especially by developing agents that have a stimulating effect on the cell repair system. Data on changes of deamination intensity of purine and pyrimidine compounds can be used for medical purposes to diagnose genetic disorders in humans at the embryonic level in the clinic. These data may also be useful in assessing the increase in the number of SH-containing compounds in the environment, which may be manifested, in particular, during radiotherapy of cancer. The distribution of volutin particles in the cytoplasm and vacuoles can be considered as a suitable target for the evaluation of ionizing radiation-induced cell damages, their repair, and compensatory processes in cells.

Main points to present at the defence

1. Under influence of X-irradiation, a two-hour delay in the growth phases of yeasts occurs compared to non-irradiated yeast, which is the overall response of these cells to any external stress.
2. Under influence of X-irradiation, the volutin granules appear in the cytoplasm of X-irradiated yeasts, which become smaller during post-irradiation growth, and spread throughout the cytoplasm. These granules are an additional source of energy for yeasts *C. guilliermondii* NP-4 and are used to repair radiation-induced damages of DNA.
3. Under influence of X-irradiation, structural damages occur in DNA, which deepens during the further repair period.
4. During the post-irradiation repair period of yeasts, the metabolic changes are partially restored, but the DNA damages do not repair completely. A yeast cell polymorphism is revealed in repaired yeasts population, which can be due to mis-repair of DNA damages.

As a result, repaired yeast cells are formed, which differ significantly in the structure of their DNA, so they are different from each other by their morphology.

Work approbation: The main results of the dissertation were discussed at scientific seminars of Department of Biochemistry, Microbiology, and Biotechnology of YSU, of "Biology" Institute of YSU, and scientific conferences; 2nd International Symposium "Problems of Biochemistry, Radiation and Space Biology", Moscow-Dubna, Russia, 2001; 3rd International Symposium "Problems of Biochemistry, Radiation and Space Biology", Moscow-Dubna, Russia, 2007; International Science and Technology Center, Seminar "Gut microbiota composition in Crohn's disease patients", Project A-1227, Agavnadzor, Armenia, 2009; 15th European Microscopy Congress (EMC -2012), Manchester, UK, 2012; The 59th Biophysical Society Annual Meeting. Baltimore, USA, 2015; The 3rd International Scientific Conference of Young Researchers; Dialogues on Science, Yerevan, Armenia, 2015; 8th International Congress «Weak and super weak fields and radiations in biology and medicine», Saint-Petersburg, Russia, 2018; International Conference "Modern Trends in Biochemistry, Radiacion and Space Biology; The Great Sissakian and the significance of his Research", Yerevan, Armenia, 2019.

Publications: The main results of the dissertation were published in 26 scientific articles. The dissertation was discussed at the session of the Department of Biochemistry, Microbiology, and Biotechnology of YSU (No. 12, 25.06.2021).

Volume and structure of dissertation: The dissertation contains the following chapters: Introduction, Literature Review (1st chapter), Methods of Investigation (2nd chapter), Results and Discussion (3rd chapter), Summary, Conclusions, List of Literature (total 291 papers and books), List of Abbreviations. The dissertation consists of 271 pages, 51 pictures, 12 tables.

MATERIAL AND METHODS

The strain used in the study was yeast *C. guilliermondii* NP-4 (wild-type) [Navasardyan, 2003]. Before each experiment, the yeast museum culture was reseeded on Petri dishes containing 2% wort agar. The yeasts were grown in the thermostat at 30°C, for 24 h [Davtyan et al, 2001].

The liquid medium for yeast growth – Yeasts were grown in a liquid medium with the following composition: 3.8 mM $(NH_4)_2HPO_4$, 17.5 mM $NH_4H_2PO_4$, 1 mM K_2SO_4, 1.6 mM $MgSO_4x7H_2O$, 100mM D-glucose, (pH=5,5) [Navasardyan et al, 2017]. The medium was sterilized in an autoclave (TR250N, Korea) for 20 min, at the pressure of 1 atm, by temperature 100°C. $3x10^{-6}$ g / l of sterilized biotin was added to the medium as a microbial growth factor.

Yeast biomass production - Yeasts were reseeded in 1000 ml Erlenmeyer flasks containing 200 ml of liquid medium and were grown on a shaker (200 ppm) at 30°C, for 24 h [Navasardyan et al, 2017]. The yeast biomass was separated from the culture medium

by centrifugation at 3500 g, for 10 min. Biomass amount was determined by nephelometric and weighting methods.

X-irradiation of yeasts - The yeasts were exposed to X-irradiation at the Department of Physics of YSU, at International Laboratory of Physical Ecology, by X-machine Dron-3 (Russia) with X-tube BSW-29Mo (λ=0.71x10^{-8}cm) or BSW-25Cu (λ=1.54x10^{-8}cm), (U=35 kV, I=15 mA, duration of irradiation 30 min.) [Marutyan et al, 2016]. The exposure power of radiation was 1080 Gy / h, the total dose of radiation was 540 Gy.

Survival of X-irradiated yeasts was recorded after 24 h incubation at 30°C in the liquid cultural medium by hourly measurements of optical density (OD) of the culture medium with KFK-2MP (yellow filter). The exponential curve of survival was constructed considering the dependence of ratio $\frac{N}{No}$ from irradiation dose D, where N_0 – the initial amount of yeast biomass exposed to X-irradiation by dose D; and N – the biomass of survived yeasts [Philippov et al., 2016].

Growth dynamics of yeasts were recorded during the first 24 hours of post-radiation growth of cells by measuring the OD of the culture medium at each hour of growth. The specific growth rate of yeasts was calculated according to the equation $\mu = \frac{1}{x}\frac{dx}{dt}$, where x is the amount of accumulated biomass (mg/ml) [Tashiro et al, 2019]. The cell duplication time t_d was recorded by the equation $\mu = \ln 2/t_d$. The latent phase duration was recorded at the point of intersection of the abscissa axis with the tangent in the log phase of growth [Olivares-Marin et al, 2018].

The colony-forming ability of yeasts was calculated based on the number of colonies formed on Petri dishes containing 2% wort agar. The yeasts were grown in a thermostat at a temperature of 30°C for 48 hours until the appearance of visible macro colonies [Davtyan et al., 2001].

The vitality of yeasts was recorded based on the monitoring of fluorescence intensity of intracellular NADP(H) of yeasts (1 mg dry biomass/mL) during the forced transition from aerobic to anaerobic conditions (AA transition) [Kuřeca et al., 2009]. The intracellular NAD(P)H fluorescence intensity was recorded by the FluoroMax™ spectrometer, on a time basis, in a 10 mm quartz cuvette, the excitation wavelength was 340 nm and the emission wavelength was 440 nm. The AA transition in yeasts was provoked by creating alternatively aerobic and anaerobic conditions in the cell suspension. This was ensured by sparging either air or nitrogen (0.1L/min) into the cuvette with suspended yeast protoplasts. The $FI_{340/440}$ signal was first recorded online every 0.5 s during aerobic conditions (air sparging) until a constant output signal was reached (FI_{AE}). Then the airflow was switched to nitrogen which led to a step-wise increase of the $FI_{340/440}$ signal due to oxygen depletion in cells. The signal was recorded until a steady-state value was obtained (FI_{AN}). The yeast vitality was

expressed as a relative increase of NAD(P)H fluorescence (FI_{rel}, %) between aerobic and anaerobic value by this equation:

$$FI_{rel}=100(FI_{AN}-FI_{AE})/\ FI_{AE}$$

Scanning electron microscopy of yeasts - For electron microscopic investigations of non-irradiated, irradiated, and repaired yeast suspensions were fixed in 2.5% glutaric aldehyde solution in 0.1 M cacodylate buffer [Hovnanyan et al, 2015]. Post-fixation was realized by 1% solution of OsO_2 in the same buffer at room temperature for 1 h. Studies of preparations were realized by the transmission electron microscope Tesla BS-500 and scanning electron microscope Tesla BS-301 (Tescan, Czech Republic). Analysis of images was done using software "VideoTest Structure 5. Nanotechnology" [Hovnanyan et al. 2010]. The micro photos were scanned at dilution of 900 pixels/inch with software Draw X5 11 Photoshop CS5. The work was carried out at the Institute of Fine Organic Chemistry of NAS RA.

DNA isolation from yeasts and purification was realized by modification [Davtyan et al, 2010] of Marmur's method [Marmur et al, 1962] developed by us. The presence of residual peptides in DNA solutions was tested by the Lowry method [Lowry et al., 1951]. The results showed that in the purified DNA samples proteins were practically absent.

Spectral properties of yeast DNA were recorded by spectrophotometer GENESIS 10S UV-VIS (Thermo Fisher Scientific Inc., USA) in the UV range of electromagnetic radiation (λ=230-320 nm). The following values were obtained for yeast DNA solutions: A_{260}/A_{230}=2 և A_{260}/A_{280}=1.9. Thus, the obtained DNA samples fully meet the literature standards of purity and nativity [Britten et al, 1974].

The electrophoretic properties of yeast DNA were recorded in 0.8% agarose gel (Serva, Germany) [Lee et al, 2012], in tris-acetate buffer (400 mM tris-OH + 0.5 M EDTA, pH=8). The electrode voltage was 60 V. The gel was stained with ethidium bromide solution (2 mg/mL, 30 min) and viewed under UV light on a Chemiscope. The gel was photographed on film Micrat-300 through a red filter. The tape negative was developed with the "Ultraskan"-LKB densitometer in integration mode.

Yeast DNA fluorescence parameters were recorded using the fluorescence spectrometer FluoroMax™. Data processing and graph construction were carried by software DM3000F. 10 mm quartz cuvettes with polished Teflon plugs were used. The fluorescence parameters were recorded by titration of the DNA with ethidium bromide [Margaryan և et al. 2017]. The value of λ=510 nm was chosen as the excitation wavelength for the DNA-ethidium bromide complex. The eukaryotic (from chicken erythrocytes) (Reanal) and prokaryotic (*E.coli*) DNA fluorescence parameters were also recorded. The biomass of *E.coli* bacteria was obtained from the Laboratory of Molecular Biology and Biotechnology Laboratory of ANAU.

Yeast DNA melting parameters were recorded with the Pye Unicam 8-100 spectrophotometer. The heating was carried out by temperature programming with a linear growth rate of 0.7°C/s. Absorption was measured with a programmable HP97S counter. 10 mm quartz cuvettes with polished Teflon plugs were used [Marutyan, 1999]. Measurements were done in the temperature range of 50-100°C. The measurement error was 0.025°C for temperature recording and $5x10^{-4}$ for OD recording. The melting temperature was determined according to the data of the integral melting curve at a value of 0.5 of 1-θ. The mean GC-content of DNA was determined by the empirical formula T_m = 176- (2.6-X) (36-7.04lg [Na +]) [Frank-Kamenetsky, 2017]. The melting range was determined by the points of intersections of straight lines corresponding to the values 0 and 1 of 1-θ with the tangent of the curve in the point of the melting temperature. The differential DNA melting curve is obtained by deriving the OD data by temperature [Grigoryan, 2013].

Obtaining of water-soluble protein extract from yeasts - Yeast cells are pre-frozen to -10°C and then pressed with a pre-frozen press (French press, Russia). The homogenate was mixed on a magnetic mixer for 20 min in distilled water to obtain a water-soluble protein extract. The resulting mixture was centrifuged at 15.000 g for 20 min [Навасардян, 2003].

Study of deamination of purine-pyrimidine compounds in yeasts – Intensity of deamination of purine (adenine, adenosine, AMP, ADP, ATP, guanine, guanosine, GMP, GDP, GTP) and pyrimidine (cytosine, cytidine, CMP, CDP, CTP) compounds was recorded by the amount of released nitrogen by Bertlow method [Martinek, 1963]. The OD of the samples was measured with a photoelectron colorimeter KFK-2M (Russia)at λ=640 nm. The intensity of deamination was assessed according to a pre-obtained calibrating curve, according to which 1 unit of deamination activity corresponds to 1 μg o released nitrogen per 1 mL of solution.

The mitochondrial fraction of yeasts was separated by differentiated centrifugation [Egorova et al., 2013]. The yeast homogenate was centrifuged in 0.25 M sucrose at 0 °C for the following steps:

1. Homogenate centrifugation at 3000 g, for 10 min
2. Supernatant centrifugation at 15000 g, for 15 min
3. Supernatant centrifugation at 30,000 g, for 20 min (Beckman L2)

The resulting precipitate, the mitochondrial fraction of yeasts, was homogenized in a glass homogenizer with 0.05 M Tris-HCl buffer (pH=8.5) to break down the mitochondrial membranes.

The ATP-ase activity of yeasts was determined by an amount of released inorganic phosphorus [Борисова и др., 2017]. The amount of inorganic phosphorus was determined according to the previously obtained calibrating curve. The specific ATP-ase activity was calculated according to the amount of protein.

Data processing was performed by software Statistika 10.0 (StatSoft). Each experiment was repeated at least 7-10 times. The errors were given in tables and error bars were presented on figures. Standard errors such as standard deviation were calculated using the appropriate function of Microsoft Excel 2013. The changes were validated by calculation of Student's validity criteria (P); the differences between experiments (irradiated and non-irradiated cells) were valid if $P<0.05$.

RESULTS AND DISCUSSION

Survival of X-irradiated yeasts *C.guilliermondii* NP-4

One of the main quantitative characteristics of radiation damage is the dependence of the viability of biological objects on the radiation dose, the graphic representation of which is the "dose-impact" curve. If the viability of cells is taken as a measure of impact, then the corresponding "dose-effect" curve is called the survival or viability curve [Borshchegovskaya et al., 2019]. The preliminary data of our studies [Navasardyan, 2003] showed that yeasts *C. guilliermondii* NP-4 have been characterized by the sigmoid curve of survival, their LD_{50} was 720Gy [Navasardyan et al, 2017], and extrapolation number was 2, i.e. yeasts *C. guilliermondii* NP-4 are inactivated by a double-hit mechanism. Since the sigmoid curve of viability is typical mainly for higher eukaryotes [Joiner, et al., 2009; Filippov et al., 2013, Sutherland, 2014], then the sigmoid appearance of the viability curve obtained for *C. guilliermondii* NP-4 yeasts indicates that these cells resemble higher eukaryotes in terms of viability and can be considered as a suitable model for their study.

During the first 24 hours of post-irradiation growth of yeasts, which includes the latent, logarithmic, and stationary phases of the life cycle, the growth kinetic parameters of irradiated and non-irradiated yeasts were determined. For non-irradiated yeasts, the lag phase duration was 6 hours, and for X-irradiated yeasts, it was 2 hours longer than for non-irradiated yeasts and ended at 8 h of growth [Navasardyan et al, 2017]. At the end of the lag phase, adapting to environmental conditions, the cells of the two cases began to rapidly multiply, and undergo an exponential or log phase of growth. The logarithmic phase of growth in both cases was approximately 12 hours. However, for irradiated yeasts, it begins at the 8^{th} hour of growth, that is, 2 hours later than for irradiated cells, due to a two-hour delay in the lag phase. This delay can be explained by the fact that X-irradiation causes structural changes in yeast DNA and proteins, including enzymes, which disrupt the cell life cycle, in particular, delay the mitosis phase, resulting in the decline in mitotic activity, G_2-block [Vakifahmetoglu et al., 2008]. The stationary phase of yeast growth for irradiated cells is also confirmed by a two-hour delay, but differences in the amount of biomass accumulated during the stationary phase between irradiated and non-irradiated yeasts almost disappear (Table 1). The specific growth rate of non-irradiated yeast is higher in the early logarithmic (8th hour of growth) and late logarithmic (16^{th} hour of growth) phases than in irradiated yeasts, and the difference was $\Delta\mu = 0.20\ h^{-1}$ u. $0.22\ h^{-1}$, respectively. Duplication time for irradiated yeast cells exceeded that of non-irradiated cells. Moreover, the increase in the kinetic parameters of irradiated yeasts in the late logarithmic phase is more pronounced than in the case of non-irradiated cells

[Navasardyan et al, 2017], which indicates that at this phase of growth irradiated yeasts grow more intensively than non-irradiated cells.

Table 1

Kinetic parameters of growth of yeasts *C. guilliermondii* NP-4 (n=7)

Growth phases	Non-irradiated yeasts			X-irradiated yeasts		
	Number of colonies, x10^7 cell/mL	OD, rel. unit	μ^*, h^{-1}	Number of colonies, x10^7 cell/mL	OD, rel. unit	μ^*, h^{-1}
Lag (latent phase)	0.070±0.003	0.45±0.02	- **	0.07±0.009 p<0.05	0.45±0.03 p<0.05	-
Early logarithmic (t=8 h)	15.5 ± 5.1	1.45±0.12	0.47±0.03	0.48±0.02 p<0.002	0.94±0.01 p<0.05	0.27±0.02 p<0.01
Late logarithmic (t=16 h)	32.3 ± 2.2	15.32±1.31	0.66±0.04	12.0 ± 1.1 p<0.01	10.92±0.84 p>0.05	0.44±0.03 p<0.05
Stationary (t=21 h)	33.6 ± 3.1	20.41±1.84	-**	12.0 ± 1.1 p<0.01	18.75±0.75 p>0.05	-

Note: * μ – the specific rate of growth of yeasts,
p- was calculated as the difference between non-irradiated" and X-irradiated cells
** - has not been decided "

This apparent discrepancy can be explained by a 2-hour delay in the logarithmic phase of growth of irradiated yeasts, which results in a relatively high growth rate for irradiated yeast cells approaching the stationary phase as the non-irradiated yeast begins to fall. This can be explained by the fact that by the time the non-irradiated yeasts reach the stationary phase of growth when their growth rate is relatively lower than in the logarithmic phase, the irradiated yeasts are still in the logarithmic phase of growth, where their growth intensity is still quite high.

The colony-forming ability of *C. guilliermondii* NP-4

X-irradiated yeasts lag behind non-irradiated cells in terms of colony-forming ability at all phases of growth (Table 1). In the early logarithmic phase of growth, for non-irradiated yeasts, the number of colonies is increased by 200 times compared to the initial moment [Davtyan et al., 2001; Navasardyan et al., 2017]. At the end of the logarithmic phase, the colony-forming ability of non-irradiated yeasts is doubled compared to the early logarithmic phase, and in the stationary phase, i.e. from the 21st hour of growth, a constant number of colonies formed by non-irradiated yeast is established. For X-irradiated yeasts, the number of colonies increases only 7 times in the early logarithmic phase compared to the initial moment of growth, 25 times at the end of the logarithmic phase, and the number of colonies formed by these yeasts does not change during the transition to stationary

phase. Thus, in terms of colony formation, in the stationary phase of growth X-irradiated yeasts lag 2.8 times behind the non-irradiated cells [Davtyan et al., 2001], although there are no significant differences in the amount of accumulated biomass.

The vitality of *C. guilliermondii* NP-4

The term "vitality" describes the quality of microbial catalysts, such as the ability of yeast of fermentation, which can be used to judge the metabolic activity of microbes [Sigler et al. 2006]. Several quantitative methods have been proposed to evaluate the vitality of cells, but none of them is widespread for determining the vitality of yeast cells [Kuřek et al. 2009]. Now some methods are developed which are based on the use of intracellular fluorophores [Podrazky et al., 2005]. We used an approach, based on the registration of changes of intracellular NAD(P)H fluorescence intensity for non-irradiated and X-irradiated yeasts, during the aerobic-anaerobic forced transition (AA transition) period [Kuřek et al. 2009]). This transition is triggered by the sparging of liquid nitrogen into the yeast-containing medium, which expels oxygen from the medium. The anaerobic conditions in the cells environment were created, that stops the oxidation of NADP(H) in cells, increase the amount of NADP(H) in yeast cells, and observe the step-wise increase of NADP(H) fluorescence intensity. The change of NAD(P)H fluorescence intensity (FI340/440), measured as the response to AA transition can be considered as an indicator of yeast vitality [Navasardyan et al, 2011]. The data obtained by this method show that non-irradiated yeasts are characterized by approximately 53% vital activity (Table 2).

In X-irradiated yeasts *C. guilliermondii* NP-4, no change in the intensity of NAD(P)H was observed during the aerobic-anaerobic transition (Fig.1), i.e. no vital activity was observed in the irradiated yeasts. After the post-irradiation repair period, the vital activity of yeasts was partially restored to 9%, i.e. even after 24 hours of repair, the yeasts lag significantly behind the non-irradiated cells in terms of vital activity. It can be concluded that the metabolism of X-irradiated yeasts undergoes significant changes, including impaired ability to fermentation. During the post-irradiation repair period, different metabolic processes are restored, due to which the vitality of yeasts is partially restored, but it remains still approximately 6 times lower than the initial value typical of non-irradiated yeasts.

Morphological properties of yeasts *C. guilliermondii* NP-4

Non-irradiated yeasts *C. guilliermondii* NP-4 have a round, oval or oblong shape, a smooth surface (Fig. 2a), and the intercellular contacts are visible, as observed in the process of germination [Hovnanyan et al, 2015].

Measurement of *C. guilliermondii* NP-4 cell sizes using the program "Morphology" has shown the following: diameter was 1.15 - 2.71 μm, length - 3.22 μm and buds - 0.318 μm (Fig. 3a). In addition, adhesion of yeast on the plate surface with porous zirconia and multiform division of cells and a multitude of buds were established. SEM analysis of yeasts colonies showed that the clarification of the structured particularities of the different forms

of intercellular contacts zones is with the cell wall and in fastening to the plate substrate of zirconia (Fig. 3b) [Hovnanyan et al, 2015].

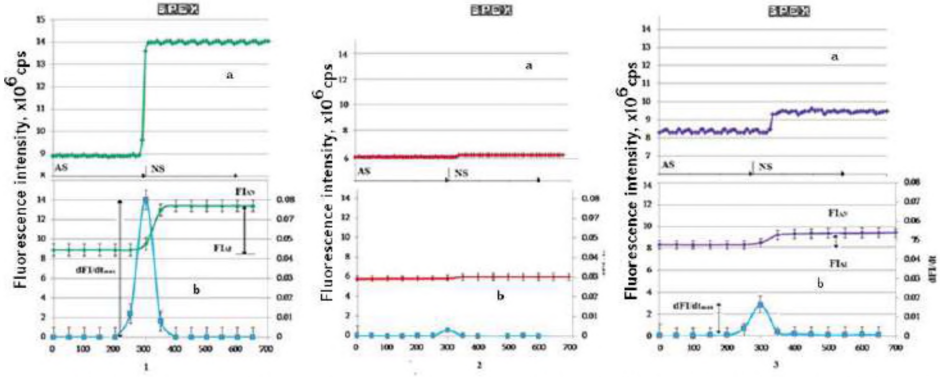


Fig.1. Changes of fluorescence intensity of intracellular NAD(P)H of yeasts *C. guilliermondii* NP-4 during the aerobic-anaerobic forced transition. 1. Non-irradiated yeasts, 2. X-irradiated yeasts, 3. Repaired yeasts, a) FI signal recorded by a fluorescence spectrometer (green, red and purple lines), b) smoothed FI signal and its first-order derivative: dFI/dt) according to [Kuřec et al., 2009].

Table 2

Fluorescence intensity parameters of intracellular NAD(P)H of yeasts *C. guilliermondii NP-4* under forced AA-transition (n=5, p<0.05)

Object	FI_{AE}, x10^6 cps	FI_{AN}, x10^6 cps	dFI/dt_{max}	FI_{rel}, %
Non-irradiated yeasts	9.0±0.4	13.8±0.9	0.07	53.3
X-irradiated yeasts	7.05±0.3	7.1±0.4	0.016	0
Repaired yeasts	8.15±0.4	8.89±0.5	0.03	9.0

Note:

FI_{AE} – Fluorescence intensity of intracellular NAD(P)H in aerobic conditions,

FI_{AN} - Fluorescence intensity of intracellular NAD(P)H in anaerobic conditions

FI_{rel} – a relative increase of fluorescence intensity during forced AA-transition

TEM analysis of yeasts showed (Fig. 2b) that the yeast cells had an amorphous cell wall (CW) adjacent to the plasma membrane; a dense granular cell plasma where the nucleus, mitochondria, and cell vacuoles (CV) of the same density are present. In cell vacuoles, the volutin granules (V) are seen [Hovnanyan et al., 2020]. They are surrounded by a membrane (Fig. 2c), and their structure most likely corresponds to fluctuating or so-called "dancing vacuoles" containing volute grains [Hovnanyan et al, 2020].

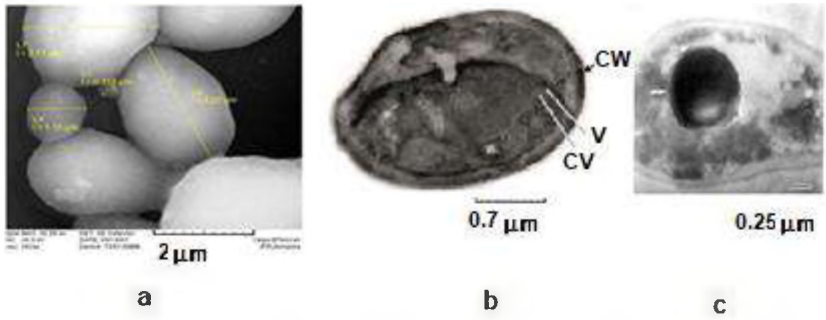


Fig. 2. Electron microscopic images of yeasts *C. guilliermondii* NP-4 (explanations in the text).

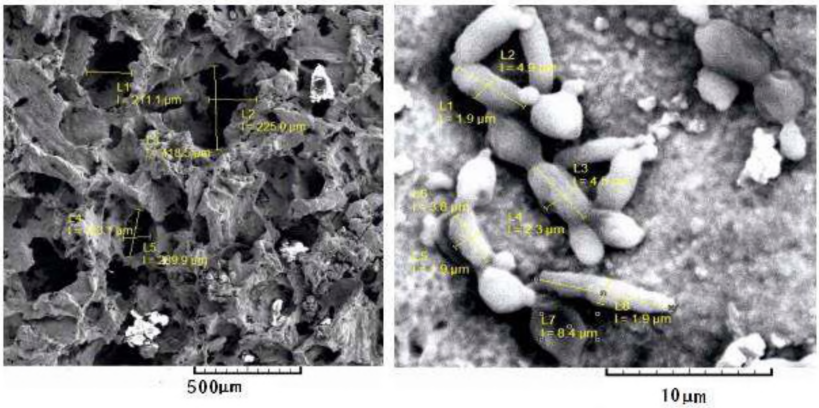


Fig.3. The ceramic porous of zirconia plate (a) and biofilms of *C. guilliermondii* NP-4 cells; (b) adhesion.

In the population of irradiated yeasts appear filamentous (Fig. 4a) and giant (Fig. 4b) forms of cells [Marutyan et al., 2019]. Examination of their ultra-thin sections with a transmission electron microscope (Fig. 4c, d) showed that dense polyphosphate granules, volutin granules, approximately 400 nm in diameter were released from the vacuoles into the cytoplasm, which is more pronounced in filamentous cells (Fig. 4d). At the same time, in the irradiated yeasts (Fig. 4 c) there is a cell wall deviation, loss of clarity of the plasma membrane configuration, and nucleus destruction [Hovnanyan et al., 2020].

Most of the yeast cells after post-radiation repair are similar to non-irradiated yeasts in their morphological features (Fig. 5a), in which intracellular compartments were restored during post-irradiation growth, and by their ultrastructural image, these cells are similar to native yeast cells [Hovnanyan et al., 2020].

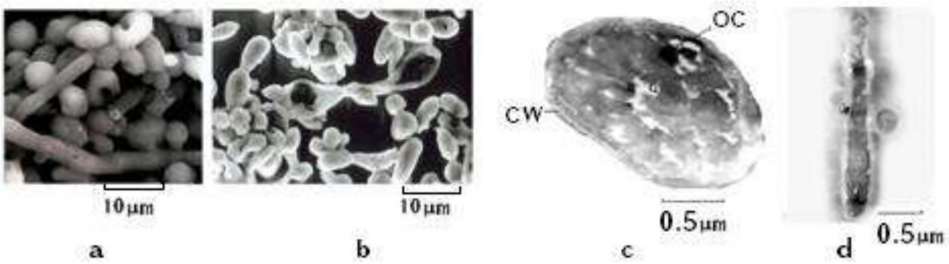


Fig.4. Electron microscopic image of X-irradiated yeasts *C. guilliermondii* NP-4 (explanations in text).

At the same time, in the repaired yeast population, there are some giant cells and filamentous forms of cells, so some of the cells did not have time to repair from radiation damages during post-radiation growth. An increase in the amount of volutin is observed in the repaired yeast cell plasma (Fig. 5b, c) [Hovnanyan et al, 2015]. During post-radiation repair, large volutin granules (400nm) are broken down into small particles up to 25-150 nm in diameter, removed from vacuoles, and dispersed throughout the cell plasma. [Hovnanyan et al, 2020].

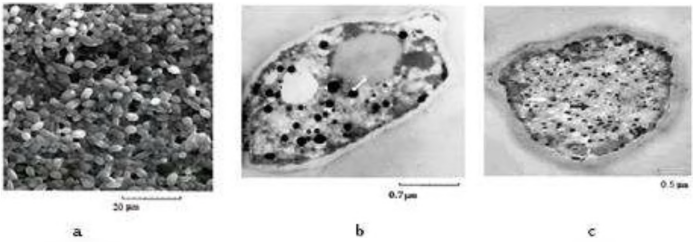


Fig. 5. Electron microscopic images of yeasts *C. guilliermondii* NP-4 after post-irradiation repair period.

It can be assumed that in yeast cells due to X-irradiation, volutin is used as an alternative source of energy to suppress the effects of stress and in order to restore cell vitality in a post-stress situation [Kulakovskaya et al., 2005].

Fluorescence properties of DNA of yeasts *C.guilliemondii* NP-4

X-irradiation brings to various structural damages of DNA, such as single-stranded (SSB) and double-stranded (DSB) breaks, intramolecular DNA-DNA and DNA-proteins bindings, etc. [Hall et al., 2012], which can inhibit the introduction of ethidium bromide into the double-stranded molecule of DNA. From this point of view, of great interest is the study of the mechanism of induction of structural damages to the genetic material of cells under the influence of X-radiation, the knowledge of the biochemical consequences of the resulting injuries, and the ways of their repair. Among the current issues in modern

radiation biochemistry is the issue of DNA stability, as the main link in the genetic system associated with the lethal effects of ionizing radiation. For this purpose, studies of yeast DNA fluorescent, thermodynamic and electrophoretic parameters were performed.

In the first phase of this part of the study, fluorescent parameters of complexes of ethidium bromide and DNA isolated from non-irradiated, X-irradiated, and repaired yeasts were studied.

When increasing the concentration of ethidium bromide in the DNA solution, in the cases of non-irradiated (Fig. 6a), as well as X-irradiated (Fig. 6b) and repaired (Fig. 6c) yeasts, an increase in fluorescence intensity is observed, which, starting from a certain concentration of ethidium bromide, remains unchanged. Thus, DNA is saturated with ethidium bromide [Marutyan 2013]. For non-irradiated and irradiated yeasts, the fluorescence intensities of DNA-ethidium bromide complexes at the saturation state (Fig. 6) do not differ significantly.

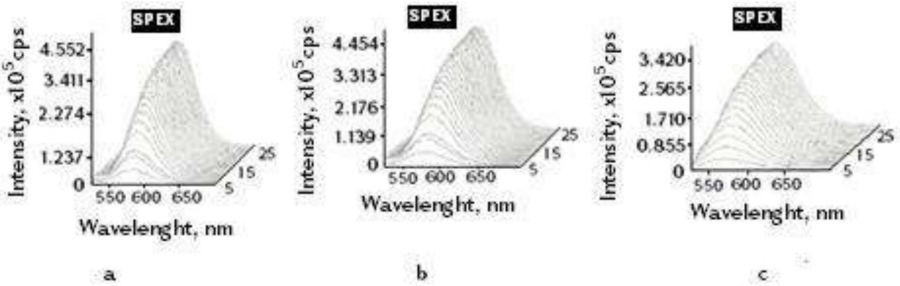


Fig.6. The titration of DNA of yeasts *C. guilliermondii* NP-4 with ethidium bromide (for explanations see the text)

However, in the case of repaired yeasts, the fluorescence intensity of the DNA-ethidium bromide complex in the saturated state is approximately 25% lower than that of the non-irradiated and irradiated yeasts. This fact indicates that fewer dye molecules have joined the repaired DNA, as the repaired DNA is likely to be more damaged than the irradiated DNA.[Марутян и др., 2015]. For non-irradiated yeasts, the DNA semisaturation with ethidium bromide is observed at the ratio C_{EB} : C_{DNA} = 1: 18 (Table 3), where 18 base pairs of DNA corresponds to 1 molecule of ethidium bromide in DNA solution. [Marutyan et al, 2015]. A similar state picture is obtained when studying the DNA fluorescent parameters from high eukaryotic organisms. [Davtyan et al, 2010; Marutyan et al, 2018]. As a control, we performed titration with ethidium bromide of DNA isolated from chicken erythrocytes (Reanal). It has been shown that the ratio C_{EB} : C_{DNA} in the semi saturated state of DNA with ethidium bromide is 1:12. Thus, the saturation rate is lower in the case of high eukaryotic DNA than in the case of low eukaryotic yeast DNA. [Marutyan et al., 2018]. This fact can be explained by the fact that highly evolved eukaryotes, such as birds, which are more evolutionarily advanced, have higher levels of DNA organization than yeasts. For comparison, a study of bacterial DNA fluorescence parameters was performed, too [Davtyan et al, 2010]. It has been shown that bacterial DNA is saturated with dyestuff

faster than DNA isolated from eukaryotic organisms [Marutyan, 2015]. Such differences in the rate of saturation with ethidium bromide may be due to the structural features of prokaryotic DNA. The DNA of eukaryotes differs from bacterial DNA by an excess of genetic material, i.e., there are a large number of repeating sequences in the eukaryotic genome [Alberts et al, 2014; Ugarkovich 2005], which probably leads to the fact that DNA in both high- and low- eukaryotes is saturated with dye at higher concentrations of dyestuff than prokaryotic DNA.

Table 3

The concentrations of DNA and ethidium bromide and their interaction in the state of semi saturation of DNA isolated from different objects with EB (n=5, P<0.05)

Object	C_{EB}, $x10^{-6}$ M	C_{DNA}, $x10^{-5}$M	C_{EB}:C_{DNA}
Chicken erythrocytes	6.32±0.5	7.49±0.6	1:12
Non-irradiated yeasts	4.23±0.2	7.61±0.5	1:18
X-irradiated yeasts	3.56±0.3	7.63±0.6	1:21
Repaired yeasts	3.28±0.3	7.65±0.4	1:23
E.coli bacteria (wild type)	1.83±0.1	7.72±0.6	1:42

Note: C_{EB} – concentration of ethidium bromide in DNA solution,
C_{DNA} – DNA concentration,
C_{EB} : C_{DNA} – the ratio of concentrations of ethidium bromide and DNA

The semi saturation of DNA of irradiated yeasts with ethidium bromide is observed at the ratio C_{EB}: C_{DNA} = 1:21, and in the case of repaired yeasts at C_{EB}: C_{DNA} = 1:23 [Marutyan, 2013]. The X-irradiation of yeasts at 0°C, when the activity of enzymes involved in DNA repair process during irradiation is suppressed (Table 4), the semi-saturated state of DNA with ethidium bromide is confirmed at the ratio C_{EB} : C_{DNA} = 1:29, and after post-irradiation repair – at C_{EB} : C_{DNA} = 1:31 [Marutyan et al., 2015]. Thus, the DNA of yeast irradiated at 0°C is more damaged than the DNA of yeasts irradiated at room temperature. When the yeasts are irradiated at room temperature and then kept at 0°C for 1 hour, when, according to the literature, the degree of DNA damages is significantly reduced [Drašol V., 1972], at semi -saturation the state the ratio C_{EB} : C_{DNA} = 1: 39 is observed (Table 4).), i.e. it is significantly higher than when yeasts are exposed to X-irradiation at room temperature or 0°C [Marutyan et al., 2015].

Thus, X-irradiation of yeast at 0°C results in more damages to the double-stranded structure of DNA than when irradiated at room temperature, as at low temperatures the activity of repair enzymes is probably suppressed during irradiation. And when irradiated at room temperature, these enzymes are already active during irradiation, they manage to repair some of the DNA damages. At the same time, there is

an increase in the degree of damages of the repaired DNA compared to the X-irradiated DNA.

It is possible, that the repair enzymes may be damaged or inactivated by X-irradiation, leading to incorrect DNA repair or enzymatic re-damages [Haber, 2021], and consequently, to DNA double-stranded breaks, and other structural damages.

Table 4

Semi-saturation of DNA of yeasts *C. guilliermondii* NP-4 with ethidium bromide under different conditions of irradiation (n=5, P<0.05)

object	Irradiation temperature			
	0° C		20°C, 1h at 0°C after irradiation	
	$C_{ЭБ}$, x10 $^{-6}$M	$C_{ЭБ}$:$C_{ДНК}$	$C_{ЭБ}$, x10 $^{-6}$M	$C_{ЭБ}$: $C_{ДНК}$
Non-irradiated yeasts	4.23±0.4	1:18	4.23±0.4	1:18
X-irradiated yeasts	2.83±0.2	1:29	3.56±0.3	1:21
Repaired yeasts	2.65±0.1	1:31	2.07±0.2	1:39

Note: C_{EB} – concentration of ethidium bromide in DNA solution,

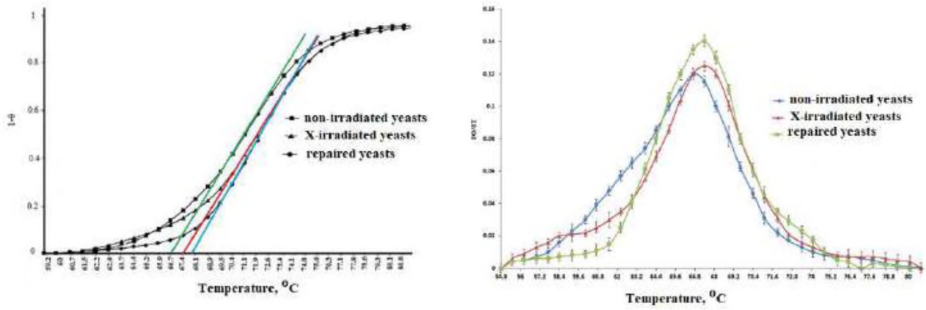
C_{DNA} – DNA concentration,

C_{EB} : C_{DNA} – the ratio of concentrations of ethidium bromide and DNA

The thermodynamic properties of DNA of yeasts *C. guilliermondii* NP-4

DNA melting parameters can provide important information about changes in DNA secondary structure [Tankovskaia et al, 2018]. Thus, the comparative evaluation of melting parameters - the melting temperature and melting interval of DNA of yeasts *C.guilliermondii* NP-4 was realized. The integral curves of DNA (Fig.7a) show that the DNA melting temperature of irradiated yeasts is increased by 0.67°C compared to the DNA of non-irradiated cells (Table 5); in repaired cells, it increases by 0.23°C compared to the DNA of irradiated cells, and the difference with non-irradiated yeasts is 0.9°C [Marutyan et al, 2016]. The melting temperature of DNA after X-irradiation is decreased by 0.74°C compared to non-irradiated DNA, and by 0.46°C for repaired yeast DNA. The melting interval of DNA after X-irradiation is decreased by 0.74°C compared to non-irradiated DNA, and by 0.46°C in repaired cells. Thus, compared to non-irradiated cells, the melting interval of DNA repaired cells is reduced by 1.2°C [Marutyan et al., 2016].

In fact, under the influence of X-radiation, and during the subsequent repair process, the changes in the DNA of yeasts lead to an increase in the internal stiffness of the molecule, which in turn makes the molecule of DNA more "hard to melt" [Gaziev, 1999; Gulyaeva et al., 2006]. The differential curve of melting of DNA of irradiated *C. guilliermondii* NP-4 yeast (Fig. 7b) in the low-temperature range (57-59°C) there is an additional peak, the melting deceleration of the DNA AT-rich sites of irradiated and repaired yeast DNA is visible [Marutyan et al., 2016].



ig.7. The melting integral (a) and differential (b) curves of DNA of yeasts *C. guilliermondii NP-4* [Marutyan et al, 2016].

Table 5.

The melting parameters of DNA of yeasts *C. guilliermondii* NP-4 (n=5, P<0.05)

Yeasts	Melting temperature, T_m, °C	Melting interval, ΔT, °C	Average GC-content, x 1000 base pair
Non-irradiated	66.75±0.15	9.6±0.4	0.32±0.02
X-irradiated	67.42±0.13	8.86±0.20	0.33±0.03
Repaired	67.65±0.14	8.4±0.3	0.34±0.03

X-irradiation may cause some damages to DNA AT-rich sites that do not repair during the subsequent incubation of yeasts, but they increase more. At the same time, the peak of the satellite DNA (75-77°C) typical for non-irradiated yeasts is shifted to the right in the differential melting DNAs of irradiated yeasts, and in the case of repaired yeast, it is less pronounced [Marutyan et al., 2016].

Based on the data obtained, it can be concluded that in the DNA of irradiated yeasts, in addition to strand breaks, there are other damages, the effect of which replicates the effect of single-stranded and double-stranded DNA. In particular, this phenomenon may be caused by thymine dimers in the DNA molecule, DNA-DNA and DNA-protein bindings, which increase the stiffness of the structure of the DNA molecule.

The electrophoretic parameters of DNA of yeasts *C. guilliermondii* NP-4

In the next stage of the work, a comparative study of the electrophoretic mobility of yeast DNA was performed immediately after X-irradiation of cells and after 24-hour post-irradiation incubation [Marutyan et al., 2016], when the cell repair system is enabled to work. Immediately after X-irradiation, as in the case of non-irradiated yeasts, a single high molecular fraction is detected in the DNA electrophoregramm (Fig.8) (fraction 1),

which differs from non-irradiated DNA only slightly in terms of mobility [Marutyan et al., 2016].

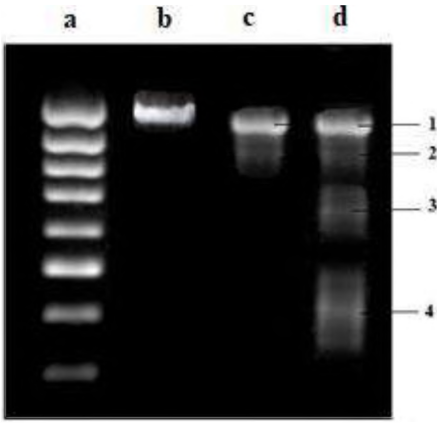


Fig.8. The electrophoregram of DNA of yeasts *C. guilliermondii* NP-4: a – marker DNA, b – DNA of non-irradiated yeasts, c – DNA of irradiated yeasts, d – DNA of repaired DNA [Marutyan et al., 2016].

Table 6

The molecular weights of DNA fractions of yeasts *C.guilliermondii* NP-4 (n=6, p<0.05)

	The molecular weight of DNA fraction, x 10^6 D		
Fraction	Non-irradiated yeasts	X-irradiated yeasts	Repaired yeasts
1	10.3 ± 0.4	9.2 ± 0.3	9.2± 0.3
2	-*	-*	4.2 ± 0.1
3	-*	-*	0.3 ± 0.02
4	-*	-*	0.08 ± 0.005

*- not found

In the electrophoregram of DNA after post-irradiation repair, in addition to the high-molecular fraction, there are also three additional fractions with relatively high mobility, fractions 2, 3 և 4 (Table 6), which indicates the double-strand breaks in the structure of DNA during X-irradiation and post-irradiation repair [Marutyan et al., 2016]. The additional fractions found in the repaired DNA electrophoregram are fragments of DNA, characterized by a molecular weight of 10^4 - 10^5 D.

Deamination of purine and pyrimidine compounds of yeasts *C. guilliermondii* NP-4

In higher eukaryotic organisms, the study of the metabolic exchange of structural monomers of yeast DNA, namely nucleotides, in particular deamination, may provide important information on metabolic disorders under extreme conditions, as the first step

of nucleotides, nucleosides, and nitrogenous bases degradation is its deamination. This process is catalyzed by specific enzymes, amino hydrolases, or deaminases.

Nitrogen bases and nucleoside deaminases are nowadays known as adenine deaminase or adenase (EC 3.5.4.2) [Pospišilova et al., 2006], adenosine deaminase (EC 3.5.4.4) [Zhou et al, 2014], guanine deaminase or guanase (EC 3.5). 4.3) [Fernandez et al, 2010], and guanosine deaminase (EC 3.5.4.15) [Dahncke et al, 2013]. The enzyme that deaminases AMP is also known as AMP-deaminase or adenylate deaminase (EC 3.5.4.6) [Bhagavan et al, 2015]. During cell metabolism, adenine is converted to hypoxanthine by deamination, guanine to xanthine, and AMP to IMP. The latter undergoes further hydrolysis by the enzyme nucleotidase to form inosine, which in turn is phosphorylated by the enzyme purine-nucleotide phosphorylase to hypoxanthine to form ribose-1-phosphate [Rolfes, 2006]. Cytosindeaminase (EC 3.5.4.1) Costanzi et al, 2007] and cytidine deaminase (EC3.5.4.5) [Ito, 2018] enzymes are known for the deamination of pyrimidine compounds. Cytosine, deaminated by the enzyme cytosine deaminase, is converted to uracil, which is not the normal nitrogen base for DNA, and a mutation occurs in the DNA sequence. The intermediate products of nucleotide deamination can be reinvolved into nucleotide resynthesis, or so-called salvage pathways, which is a unique energy-saving system for microbes, including yeasts [Kutmon et al., 2016].

Deficiency of deaminase enzymes in the organism leads to the development of various diseases, including Lash-Nihan syndrome [Dasgupta et al, 2014], mitochondrial DNA depletion syndrome, mitochondrial neurogastrointestinal encephalomyopathy, Ataxia telangiectasia [Fasullo et al, 2015], diseases of the organism's immune system, such as acute combined immunodeficiency [Bolognia et al, 2008], due to disorders of purine metabolism in the organism. Therefore, it is of great interest to study the changes in the metabolism of nucleotides, nucleosides, and nitrogenous bases under X-irradiation of yeasts, including the recording of changes in their deamination intensity.

The data we received showed that in yeast extract the activity of adenine deaminase, adenosine deaminase, guanine deaminase, and guanosine deaminase enzymes (Table 7) is very low.

In X-irradiated yeasts, an increase in the intensity of ADP and ATP deamination was observed, i.e. after X-irradiation, the metabolism of ATP is increased. From the point of view of deamination, the highest activity is observed for ADP [Marutyan et al, 2017]. There was a decrease in the intensity of deamination of GDP and GTP. Very low deamination intensity was found for GTP, and among guanine compounds, the highest deamination level was observed for GDP, which is, however, lower than that of ADP.

After post-radiation repair of yeasts, there is a decrease in the deamination intensity of both ATP and ADP, but in the case of both substrates, the deamination intensity remains higher than the value obtained for non-irradiated yeasts. At the same time, both the GTP and the GDP have been shown an increase in deamination intensity, but for both substrates, it remains lower than the values obtained for non-irradiated yeast. Surprisingly high intensity is shown in the case of GMP deamination, and the intensity of GMP deamination in repaired yeasts exceeds that of all guanine and adenine compounds, except ADP [Marutyan et al., 2019].

Table 7

The intensity of deamination of purine nucleotides in the water-soluble extract of yeasts *C. guilliermondii NP-4* (mg N_2/mg protein) ($p<0.05$, n=7)

	ADP, 30mM	ATP, 30mM	GDP, 30mM	GTP, 30mM
Non-irradiated yeasts	0.71±0.05	0.041±0.002	0.44±0.03	0.50±0.02
X-irradiated yeasts	1.14±0.08	0.39±0.03	0.28±0.001	0.060±0.003
Repaired yeasts	0.95±0.07	0.12±0.01	0.39±0.02	0.41±0.02

In non-irradiated yeasts, cytidine compounds are generally subjected to deamination by rather high intensity [Marutyan et al., 2014], which indicates that these compounds undergo stronger catabolism in yeasts than purine compounds (Table 8).

Table 8.

The intensity of deamination of cytidine compounds in water-soluble extract of yeasts *C. guilliermondii NP-4* (mg N_2/mg protein) ($p<0.05$, n=7)

	Cytosine, 30nM	Cytidine, 30mM	CMP, 30mM	CDP, 30mM	CTP, 30mM
Non-irradiated yeasts	0.88±0.07	1.17±0.11	0.23±0.02	1.22±0.1	1.095±0.03
X-irradiated yeasts	1.04±0.1	0.58±0.09	0.011±0.001	0.9±0.05	0.73±0.06
Repaired yeasts	1.47±0.12	0.78±0.04	1.05±0.1	1.36±0.1	1.22±0.8

Relatively high levels of deamination have been reported for CDP, CTP, and cytidine, which are probably metabolically quite active, and are highly catabolized in non-irradiated yeasts [Marutyan et al., 2014].

The X-irradiation generally suppresses the deamination of all cytidine compounds of yeasts except cytosine, and CMP exhibits a very low intensity of deamination.In repaired yeasts, the further increase in the deamination intensity takes place for all cytidine compounds. Moreover, the highest deamination intensity is observed in the case of cytosine, which exceeds the values typical for both irradiated and non-irradiated yeasts. There is also an increase in deamination intensity of CMP compared to both non-irradiated and irradiated cells.

During X-irradiation of yeasts and further repair, activation of GEP catabolism is almost not observed, i.e. it is not subjected to intensive catabolism, but is involved in various processes of plastic metabolism.

The hydrolysis of purine polyphosphate-nucleotides in *C.guilliermondii* NP-4 yeasts and mitochondria

In the next stage of the work, we carried out a comparative study of ATP-aze, ADP-ase, GTP-ase, and GDP-ase activities in non-irradiated, X-irradiated, and repaired yeasts *C. guilliermondii* NP-4 [Marutyan, 2019]. As the data have shown, in non-irradiated yeasts,

the hydrolysis intensity of ADP and GDP is twice as high as the hydrolysis activity of ATP and GTP (Table 9). In irradiated yeasts, no change in ATP-ase activity was observed, and the intensity of ADP hydrolysis increased by about 60%. In irradiated yeasts, the GTP-ase activity was not detected, and the intensity of GDP hydrolysis has not changed. Possibly, under the influence of X-radiation stress, yeasts use alternative energy sources, rather than ATP, which is a universal energy source. Particularly, in this case, ADP is used as an alternative source of energy. After post-radiation repair, there is a sharp increase in hydrolytic activity of ATP, ADP, and GTP compared with X-irradiated yeasts.

Table 9

The intensity of hydrolysis of purine polyphosphate-nucleotides in *C. guilliermondii* NP-4 yeasts after X-irradiation and post-irradiation repair (μg Pi/mg protein, n=5, p<0.05)

	ATP, 16μM	ADP, 16μM	GTP, 16μM	GDP, 16μM
Non-irradiated yeasts	8.1±0.8	16.1±1.4	8.03±0.68	16.2±1.4
X-irradiated yeasts	7.98±0.7	25.87±2.3	0	16.1±1.3
Repaired yeasts	40.2±3	40.1±3.9	39.9±3.7	16.3±1.2

Probably, during the post-irradiation repair, when the recovery high energetic processes take place in yeast cells, the energetic needs of cells are strongly increased, and as an alternative source of energy ADP and GTP are used. Particularly, the strong increase of GTP-ase activity may be caused by protein synthesis intensity, such as new proteins, like enzymes of SOS-repair, radioprotective proteins, etc. Under conditions of radiation-induced stress GDP does not play an important role in yeasts' energy metabolism.

In the mitochondria of yeasts, different values of ATP-ase activity were revealed at different phases of growth (Table 10) [Hovnanyan et al, 2020].

Thus, compared to the latent phase of growth, in the logarithmic phase, the ATP-ase activity increases by about 3 times, and approaching the stationary phase of growth, a 2.2-fold decrease in ATP-ase activity is observed compared to the logarithmic phase [Navasardyan et al, 2019].

Table 10.

The mitochondrial ATP-ase activity of yeasts *C. guilliermondii* NP-4 in different phases of growth (μg P_i/mg protein, n=5, p<0.05)

Growth phases	Non-irradiated yeasts	X-irradiated yeasts
Latent phase (t=0)	2.3 ± 0.2	1.7±0.1
Logarithmic phase (t=16 h)	6.8 ± 0.5	5.0±0.4
Stationary phase (t=22 h)	3.0 ± 0.29	1,5±0.08

The logarithmic phase of the yeast life cycle, which is accompanied by the strong growth of cells, is characterized by a high intensity of metabolism, for which the cells need a lot of energy, so the ATP-ase activity at this phase is higher than at the initial point. Approaching the stationary phase of the cell life cycle, the growth of yeasts is slowed down, so the metabolic rate and, consequently, the need for cells for energy decreases. Compared to the logarithmic phase, the ATP-ase activity also decreases, although, in comparison to the latent phase, it remains significantly higher.

In the mitochondria of irradiated yeasts, as expected, at the initial phase of growth, i.e. immediately after irradiation, there is a decrease in ATP-ase activity compared to non-irradiated yeasts, which can be explained by a general decrease in the vital activity and general metabolic rate of yeasts as a result of ionizing radiation. In the logarithmic phase of growth, when the irradiated cells begin to divide rapidly, the ATP-ase activity begins to increase and at 16 hours of growth, it is about 3 times higher than the value observed at the latent phase growth. In the stationary phase of growth, In the mitochondria of irradiated yeasts, the ATP-ase activity falls and has a value approximately as in the latent phase, which is significantly lower than the activity exhibited in the stationary phase of growth in non-irradiated yeasts. In comparison with the logarithmic phase of growth, the ATP-ase activity of yeasts decreases 3.3 times in the stationary phase of growth [Hovnanyan et al, 2020].

It is well known that the total ATP-ase activity of the cell is due to the ATP-dependent ion channels, for which the ATP-ase enzyme is responsible. In particular, the introduction of Na^+/K^+-ATP-ase, which acts on the plasma membrane of all cells, ensures the unbalanced distribution of Na և K ions in the cell and medium. Mitochondrial ATP-synthase, in turn, provides the rate of oxidative phosphorylation. Therefore, in extreme conditions, such as under the influence of ionizing radiation, when the metabolic activity of the cell undergoes significant changes, it also irreversibly changes both the total and the mitochondrial ATP-ase activity. The current concept of modern radiation biochemistry assumes that the main target of the harmful effects of radiation is the nucleus DNA of cells, the damage of which can be lethal to the cell. However, nuclear DNA is not the only target of cell inactivation or damage. From this point of view, the radiation damages of mitochondria are very important [Kam et al, 2013] both in terms of mitochondrial DNA structure and mitochondrial functional activity. In addition, radiation damages of mitochondria can be triggered by the direct effect of ionizing radiation, as well as by secondary effects, in particular by the emergence of free radicals and reactive oxygen species. It is clear that radiation damages of mitochondrial DNA can lead to errors in the structure and function of the proteins it encodes, particularly enzymes, which ultimately lead to a decrease in the degree of oxidative phosphorylation. As a result, both ATP synthesis and hydrolysis are reduced, so there is a lack of energy in the irradiated cells, although it was expected that the cell would have to expend much more energy to repair the radiation damage. The yeasts we have studied partially cover this deficiency of energy through alternative energy sources, such as volutin.

CONCLUDING REMARKS

Living organisms are characterized by a great ability to adapt to the ever-changing conditions of the environment, which allows them to survive even in extreme conditions. In such cases, cells activate certain biochemical defense mechanisms against stress, the study of which at the molecular level is still relevant today, as it continues to deepen day by day under extreme conditions (ionizing and non-ionizing radiation, increase in man-made pollution, high temperature, starvation, etc.) the risk of impact on living organisms. At present, a great deal of scientific material has been accumulated on the mechanisms and effects of ionizing radiation on living organisms [Horbay et al, 2011; Holley et al, 2012; Chvastunov et al., 2013], but many principle issues have not yet been clarified. In particular, the issues of radioactivity, survival, and vital activity of X-irradiated eukaryotes and prokaryotes, have not been sufficiently studied; the role of changes in their metabolism in the adaptive reactions of organisms in the conditions of irradiation and further recovery has not been clarified. The possible mechanisms of changes in the pathways of energy exchange during metabolism, the involvement of alternative energy sources in the regenerative processes in cells, etc. are insufficiently studied. It is clear that the main target of radiation exposure in living cells is DNA, in which structural damage is either lethal to the cells or causes a variety of mutations to be passed on to future generations. It can be noted that the damage to both nucleus and mitochondrial DNA is similarly dangerous for the cell. Highly sensitive targets of radiation exposure to cells are also proteins, which provide the basic structural-functional features of the organism, as well as participate in the realization of genetic information and transmission to future generations. It is obvious that the disruption of each of these links can lead to the distortion of genetic information, which results in the emergence of a mutated generation. It is logical to assume that under influence of X-irradiation, and especially in the post-irradiation repair period, when cells are working to eliminate the destructive effects of radiation on the DNA and the various enzymes involved in metabolism, several biochemical mechanisms of activation of stress-proteins and synthesis of those proteins start operating. The study of these mechanisms at the molecular level is one of the most important problems in modern radiation biochemistry, the solution of which will allow the development of effective mechanisms to protect living organisms from the destructive effects of radiation. Various microbes, including yeasts, are mostly used for these studies as suitable models of eukaryotic organisms.

In our work, a comprehensive study of the effects of X-irradiation on yeasts *C. guilliermondii* NP-4 was realized for the first time. Yeasts *C. guilliermondii* NP-4 are characterized by an S-shape or sigmoid survival curve, which corresponds to the appearance of a doze-effect curve known from the literature for other yeasts [Arlyapov et al., 2009]. Such curves are exhibited for high eukaryotes [Joiner, et al., 2009; Philippov et al, 2013; Sutherland, 2o14]. From the position of target theory [Gudkov et al,2015], such curves are referred to organisms that are inactivated by multi-hit mechanism, when there is one target in the cell, the damage of which requires more than one effective hit, or if the cells have several targets, which are deactivated by one effective hit, but for the death of the cell it is necessary to damage all targets [Kudryashev, 2004; Timoffeeff-

Ressoffskii et al., 2010]. It is also possible that the observed yeasts have an effective repair mechanism for the restoration of radiation-induced damages [Lind et al., 2003]. The extrapolation number for yeasts *C. guilliermondii* NP-4 is 2, and taking into account the fact that these yeasts are single-celled [Hovanyan et al., 2008], it can be concluded that yeasts *C. guilliermondii* NP-4 are inactivated by a double-hit mechanism.

LD_{50} for yeasts *C. guilliermondii* NP-4 is 720 Gy, which is up to 2.5 times more than the value known from the literature for yeasts *Saccharomyces* [Bala, 2012]. Such high radio stability of yeasts *C. guilliermondii* NP-4 can be explained, probably, by the presence of a strong cell wall consisting of a protein-carbohydrate complex typical of yeasts *Candida* [Meledina, 2015], by the radioprotective effect of histone proteins, which are part of chromatin structure. They act as radical scavengers by absorbing X-radiation-induced free radicals in the cell, or by the screening DNA protecting it from direct radiation [Gulyaeva et al., 2006]. High radio stability can also be facilitated by the presence of an effective mechanism for repair of DNA damages [Lind et al., 2003], which is attributed to both direct repair of DNA damages and activation of new repair protein genes and synthesis of radioprotective proteins.

In the growth dynamics of irradiated yeasts there is a two-hour delay in the latent phase, which can be explained by genetic abnormalities, which disrupt the life cycle of cells, delay the mitosis phase, resulting in a decrease in mitotic activity, G_2-block [Cuddihy et al, 2003]. The G_2 block is characterized by the fact that the irradiated cells pass to the prophase of mitosis at a normal rate and accumulate at that phase. The further cell division is delayed [Marutyan, 2016], which can be caused by the despiralization of chromosomes and the transformation into interphase chromosomes, as a result of which cells "return" from prophase to interphase. A possible mechanism is also the inhibition of the synthesis of proteins needed for mitosis, especially of proteins involved in the synthesis of centrioles [Navasardyan, 2003]. During the first hours of the growth of irradiated yeasts, part of the damages is repaired by the cell repair system, and the cell population enters the logarithmic phase of growth [Navasardyan et al, 2017]. Approaching the stationary phase of growth, the difference in the amount of accumulated yeast biomass between non-irradiated and X-irradiated yeasts almost disappears, while in terms of both growth kinetics and colony-forming ability, irradiated yeasts are inferior to non-irradiated yeasts. To explain this seemingly contradictory fact, the morphological changes of yeasts were observed by scanning and transmission electron microscopes. The data showed that in the population of irradiated yeasts appear giant and filamentous forms of yeast cells. Yeasts *C. guilliermondii* NP-4 produce filamentous and giant cells under the influence of other stressors, particularly nitrogen starvation conditions [Hovnanyan, et al, 2019], under influence of non-ionizing radiation [Marutyan, et al. 2021]. The relatively high OD of X-irradiated yeasts, which does not correspond to the number of colonies they formed, apparently due to the formation of giant cells, as these cells are significantly larger than non-irradiated cells, physiologically are stable, and have all cellular components, but do not multiply, so they do not form macro colonies, but due to their larger size, they have a higher intensity of light absorption, OD. In filamentous yeasts, the volutin polyphosphate

granules, an alternative source of energy, are enlarged and excreted from the vacuoles to the cytoplasm.

After post-irradiation incubation, in the *C. guilliermondii* NP-4 yeast population, the cells are dominated that are similar to non-irradiated cells, i.e. in most of the irradiated cells during post-irradiation growth, the cell compartments are being restored. However, they are also accompanied by elongated filamentous and giant cells, so some of the cells did not repair from radiation damages during post-irradiation incubation.

Also noteworthy is the change that occurs in the yeast vacuoles during X-irradiation, especially during the post-irradiation repair. In non-irradiated yeasts cytoplasm, there are vacuoles containing membrane-coated amorphous volutin granules, linear polymers of orthophosphoric acid (Fig.9). Volutin acts as a storage of certain ions, especially phosphorus, various enzymes, and enzymes involved in the regulation of cell metabolism. First of all, as a source of phosphate, it participates in the phosphorylation of ADP and thus participates in the maintenance of a stable amount of ATP in the cell. At the same time, volutin orthophosphate is also involved in the synthesis of nucleic acids, which is especially important in stressful situations when rapid DNA reparative synthesis happens. In irradiated yeast cells, along with other morphological changes, there is an increase in the size of volutin granules, their removal from the vacuoles to the cytoplasm. Volutin osmiophilic conglomerates enlarge in size, emerge from cell vacuoles and appear in the cytoplasm. During post-irradiation growth of yeasts, the vacuoles are completely emptied of volutin, which is broken down into small particles by the enzymes endopolyphosphatase and exopolyphosphatase in the cytoplasm and dispersed throughout the cytoplasm. These granules are composed of polymerized residues of orthophosphoric acid and on the periphery, they are covered with RNA, protein, and lipid complexes [Hovnanyan et al, 2015]. They are removed from the vacuoles during post-irradiation incubation, shredded, and dispersed throughout the cytoplasm.

Possibly, under influence of X-radiation, the activity of the enzyme exopolyphosphatase in yeast vacuoles is likely to increase, which breaks down large polyphosphate molecules, leading to an increase in the amount of volutin in the cytoplasm. The volutin further is used by the cell as an alternative source of energy to suppress the stress factor and to restore the cell viability in post-stress situations [Kulakovskaya et al., 2005].

We suggest that orthophosphate produced by these processes is used in the post-irradiation growth period when the cell undergoes complex regenerative difficult processes, which require energy. It is used in the energetic and synthetic processes of cells, on the one hand as an alternative energy source and on the other as an additional source of phosphate, for nucleic acid synthesis. Considering the data known from the literature on the use by yeasts of polyphosphates as a source of ions during yeast repair period [Овнанян et al., 2008], based on the experimental data obtained, we can conclude that, probably, the polyphosphates compensate for the deficiency of ATP in the irradiated yeast cells [Hovnanyan et al., 2008].

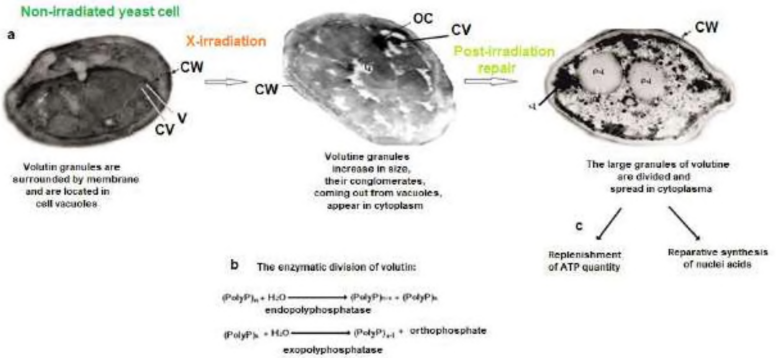


Fig.9. The changes in volutin of yeasts *C. guilliermondii* NP-4 induced by X-irradiation and post-irradiation repair: a – Localization of volutin in yeasts, b - enzymatic crushing scheme of volutin, c - possibilities of further use of volutin (CW - cell wall, CV - cellular vacuoles, OC - osmiophilic conglomerates of volutin, V-vacuoles).

This is evidenced by a decrease in ATP-ase activity in X-irradiated yeast mitochondria at all phases of growth compared to non-irradiated yeasts (Table 12). It can be assumed that both in the logarithmic and in the stationary phase of growth, the irradiated cells need a large amount of energy because at these phases their multiplication (colony formation) intensity is quite high. However, in these phases of growth of X-irradiated yeasts, the ATP-ase activity is significantly lower than in non-irradiated yeasts. Thus, the amount of ATP in the cell, which is the main source of energy for any cellular activity, is clearly insufficient. A reason for a decrease of ATP-ase activity, which provides the cell with energy, can be radiation damages of mitochondria, which lead to a decrease in the level of oxidative phosphorylation in irradiated yeasts. Among these damages, the structural disorders of mitochondrial DNA is especially important. They triggered by both direct exposure to ionizing radiation and free radicals and reactive oxygen species, can lead to damages in the structure and functions of mitochondrial DNA-encoding proteins, including enzymes, which eventually leads to a decrease in the degree of oxidation phosphorylation. As a result, the ATP hydrolysis decreases, so there is a lack of energy in the irradiated cells, although it was expected that the cell would need much more energy to repair the radiation damages.

Therefore, to suffice the energy demand, an alternative energy source is used - volutin, which was stored in vacuoles before the cells irradiation and was not used in metabolic processes. In this way, the cells of microbes, particularly the yeasts, try to overcome the severe energy deficit to carry out the regenerative processes.

Among the current issues in modern radiobiology, the most important is the problem of DNA stability, as the main part of the genetic system, which is mainly associated with the deadly effects of ionizing radiation and is the main target of it [Tankovskaya et al, 2018]. The study of fluorescence, thermodynamic, and electrophoretic parameters of DNA of X-irradiated and repaired yeasts *C. guilliermondii* NP-4, showed that under

influence of X-irradiation the structural damages occur in DNA: single-strand and double-strand breaks, modifications of nitrogen bases, intramolecular and intermolecular bindings, which deepen during the further repair period. The study of yeast DNA fluorescence, thermodynamic, and electrophoretic parameters and their changes under influence of X-irradiation and after post-irradiation repair showed that X-irradiation causes structural damages to yeast DNA: single and double-strand breaks, nitrogen base modifications, intramolecular and intermolecular bindings which deepen during the further repair period. Radiation-induced single-strand breaks may turn into double-strand breaks during the repair period due to disruption of the repair enzymes, the so-called mis-repair, when, instead of repairing the damage at the single-strand break site of DNA, the DNA-polymerase causes a break in the opposite strand. As a result, double-strand breaks occur in the DNA, and the DNA molecule is fragmentized. This is evidenced by the appearance of low-molecular DNA fractions and DNA fragments in the electrophoregram of DNA of repaired yeast. It can be assumed that such a result is explained by the formation of two fractions of DNA with different electrophoretic mobility in the same cell or containing DNA molecules with different electrophoretic mobility in different cells. Such differences in DNA may be due to the DNA double-strand breaks repair by recombination in yeast cells [Piaza et al, 2017]. Probably this is the reason for the polymorphism of the repaired yeast population, which we showed as a result of electron microscopy studies. Therefore, it is logical that repaired yeasts, which are different from each other by their morphology, also differ significantly in the structure of their DNA (Fig.10).

Of great interest is the question of how yeasts with damaged DNA continue to multiply and leave a fertile generation. It can be assumed that in this case, non-histone proteins have an active effect on the functional activity of DNA, which are known to be of a high amount in yeast chromatin [Navasardyan, 2003].

Proteins probably form complexes with DNA in the form of DNA-protein bindings, which allows the DNA fragments to stay connected also in case when there are double-strand breaks in DNA, and successfully replicate or transcribe, ensuring a continuous process of transmission of genetic information. The facts of an increase in the melting temperature and a decrease in the melting interval of irradiated and repaired yeast DNA speak for themselves. It turns out that the structure of DNA is not destabilized by X-irradiation, but on the contrary, its stability is increasing. The most probable explanation for this phenomenon is the formation of bindings DNA-DNA and DNA-protein X-irradiation, which remain unrepaired during the post-irradiation repair period and make it harder for DNA to melt, leading to an increase in the melting temperature of DNA.

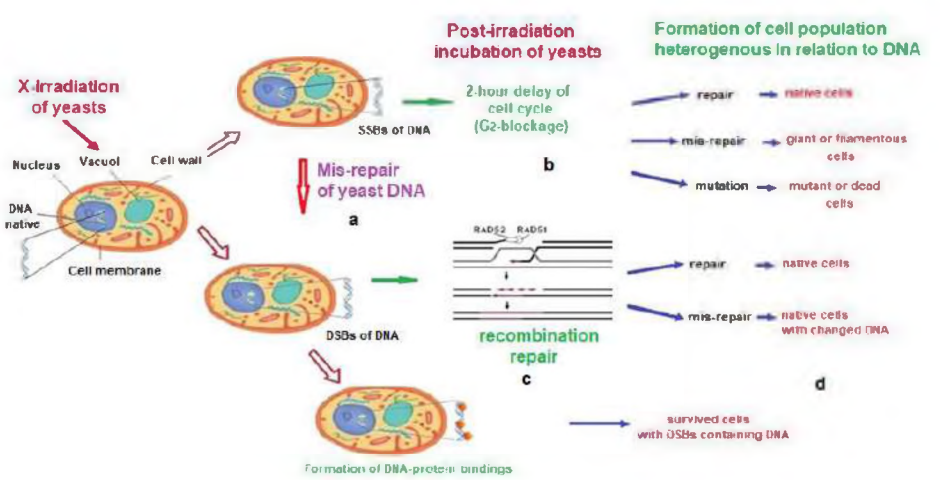


Fig.10. The possible survival scheme of *C. guilliermondii* NP-4 exposed to X-irradiation: a – Mis-repair of radiation-induced damages to DNA, b - G_2-blockage of the cell cycle, c - Recombination repair of double-stranded DNA fragments, d - Formation of cell population heterogeneous to DNA (during the post-irradiation repair the viable cells are formed DNA of which is significantly different from non-irradiated yeast DNA).

Under influence of X-irradiation, the abnormalities occur also in the metabolism of purine and pyrimidine nucleotides of yeasts. They are exchanged, and the first step of their metabolism is deamination, moreover, this process takes place both in the free state of the mentioned compounds and in the structure of nucleic acids, in particular, DNA. In the latter case, the deamination of nucleotides can lead to various mutations. The process of deamination is catalyzed by deaminases, the lack of which in the body leads to the development of various diseases, including Lesch-Nyhan syndrome [Dasgupta et al., 2014], mitochondrial DNA depletion syndrome [Bolognia et al, 2008], etc. Therefore, it was interesting to study the deamination changes of purine and pyrimidine compounds in yeasts under the influence of X-irradiation and after post-irradiation repair. It has been shown that ADP and GDP are deaminated with relatively high intensity in yeasts. The low intensity of ATP deamination is probably due to its energy role: ATP is used as a universal energy source in the cell and does not undergo intense catabolism. The same pattern was observed for GTP, which also has an important role in the cell: it involves intermediate phases of protein synthesis. The ADP and GDP are likely to serve as an alternative energy source for the studied yeasts. After X-irradiation, there is a decrease in the deamination intensity of guanine nucleotides, which may be due to the general decline in yeast metabolism under influence of irradiation which is manifested, for example, in the case of vitality: the vitality of the yeasts completely stops after X-irradiation. This conclusion is confirmed by a comparative study of the hydrolysis intensity of purine nucleotides - polyphosphates. Thus, the intensity of ATP hydrolysis in X-irradiated yeasts does not change significantly, moreover, it decreases compared to non-irradiated yeasts.

Therefore, it can be concluded that from a metabolic point of view, only the vital processes are preserved, which save only the cells from dying under influence of irradiation, while the other processes either stop or slow down a lot. ATP may be stored in the cells for further regeneration processes, and as an alternative source of energy during radiation, ADP is used, the hydrolysis intensity of which increases sharply compared to non-irradiated cells. This conclusion is supported by the fact that during irradiation, the hydrolysis of GTP is completely stopped, which indicates that during this time, the process of protein synthesis in the cells is likely to be interrupted. After post-irradiation incubation of cells, the vitality of yeasts significantly increases, but it remains very low compared to non-irradiated yeasts. At the same time, the intensity of hydrolysis of ADP, ATP, and GTP sharply increases, which proves that the processes of plastic exchange in yeasts are restored, the energy demand increases, therefore, in addition to ATP, adenine polyphosphate-nucleotide ADP is used as an energy source, as well as volutin. On the other hand, GDP does not play a significant role in the energy exchange of yeasts under radiation stress conditions. The study of the deamination intensity of purine compounds under influence of X-irradiation and after post-irradiation incubation of yeasts shows that purine nitrogen bases and nucleosides in the yeast do not undergo final catabolism, but are involved in nucleotide salvage pathways, which is a unique energy-saving system for microbes, including yeasts [Kutmon et al., 2016].

Thus, summarizing the discussion of the data obtained on the study of changes in the metabolism and some morphofunctional features of yeasts, we can say that X-radiation leads to profound changes in the morphology of yeasts, the structure of DNA, and various metabolic processes, which are partially repaired during post-irradiation incubation of cells, providing high radio stability of yeasts *C. guilliermon*dii NP-4 (LD_{50} = 720 Gy) and their survival after X-irradiation.

CONCLUSIONS

1. The latent phase of the life cycle of X-irradiated yeasts C. guilliermondii NP-4 is 2 hours longer than for non-irradiated yeasts, resulting in a 2-hour delay in subsequent phases.
2. The colony-forming ability of yeasts *C. guilliermondii* NP-4 decreases under the influence of X-irradiation, and according to the number of colonies, they are 2.8 times behind the non-irradiated yeasts.
3. In the population of yeasts *C. guilliermondii* NP-4 exposed to X-irradiation, giant and filamentous forms of cells appear, there is a loss of clarity of the cell wall and plasma membrane configuration, the amount of volutin in the cytoplasm increases. In repaired yeasts, partial repair of cellular compartments takes place, volutin granules remove from vacuoles, and spread throughout the cytoplasm.
4. In X-irradiated yeasts *C.guilliermondii* NP-4, the semi-saturation of DNA with ethidium bromide increases in comparison to non-irradiated yeast DNA. The semi-saturation of irradiated yeast DNA with ethidium bromide is observed at C_{EB} : C_{DNA} = 1:21, and for repaired yeast DNA at C_{EB} : C_{DNA} =1:23. For yeasts irradiated at 0°C, the DNA semi-saturation with ethidium bromide is at C_{EB} : C_{DNA}= 1:29, and after post-irradiation repair - at C_{EB} :C_{DNA} = 1:31.

5. Under influence of X-irradiation of yeasts *C. guilliermondii* NP-4 the melting temperature of DNA increases by 0.67°C, the melting interval decreases by 0.74°C. In the case of repaired yeasts, the melting temperature of DNA additionally increases by 0.23°C and the melting interval decreases by 0.46°C.
6. In the case of X-irradiated yeasts, the melting of AT-rich sites of DNA in differential melting curves are observed to slow down, the peak of the differential melting curve and the peak of melting of the satellite DNA (the range 75-77°C) shifts to the right.
7. After post-irradiation repair of yeasts *C. guilliermondii* NP-4, in the electrophoregramm of DNA, in contrast to X-irradiated yeast DNA, in addition to the high-molecular DNA fraction, three layers appear with relatively high mobility.
8. In yeasts *C. guilliermondii* NP-4, ADP is deaminated with relatively high intensity among purine nucleotides. For X-irradiated yeasts, the intensity of ATP deamination is increased by 9.5 times, and after post-irradiation repair, the intensity of deamination of both ADP and ATP is decreased.
9. Among the cytidine compounds, a relatively high degree of deamination is observed for CDP. In the case of X-irradiated yeasts, the deamination intensity of all cytidine compounds generally is suppressed, which is overcome during subsequent post-irradiation recovery.
10. In X-irradiated yeasts *C. guilliermondii* NP-4 the rate of hydrolysis of polyphosphate nucleotides, such as ATP, ADP, and GTP, increases.

LIST OF PUBLICATIONS AS A PART OF DISSERTATION TOPIC

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4. Hovnanyan K., Marutyan S., Marutyan S.A., Hovnanyan M., Navasardyan L., Trchounian A. Ultrastructural investigation of acidocalcisomes and ATPase activity in yeast *Candida guilliermondii* NP-4 as "complementary" stress-targets"//Letters in Applied Microbiology, 2020, v.71, 413-419
5. Hovnanyan K.O., Gasparyan H.V., Marutyan S.V., Navasardyan L.H., Trchounian A.H. Comparative structural analysis of yeasts *Candida giulliermondii* NP-4 cultivated with and without nitrogen source//Proceedings of the Yerevan State University, Chemistry and Biology, 2019, v. 53(1), p.53-58.
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ՄԱՐՈՒԹՅԱՆ ՍԵԴԱ ՎԻԿՏՈՐԻ

ՆՅՈՒԹԱՓՈԽԱՆԱԿԱՅԻՆ ՓՈՓՈԽՈՒԹՅՈՒՆՆԵՐԸ ԵՎ ԴՆԹ-Ի ԿԱՌՈՒՑՎԱԾՔԱՅԻՆ ՎՆԱՍՎԱԾՔՆԵՐԸ ՌԵՆՏԳԵՆՅԱՆ ՃԱՌԱԳԱՅԹԱՀԱՐՄԱՆ ԵՆԹԱՐԿՎԱԾ ԽՄՈՐԱՍՆԿԵՐՈՒՄ

Ամփոփում

Հանգուցային բառեր՝ *C.guilliermondii NP-4* խմորասնկեր, ռենտգենյան ճառագայթում, ԴՆԹ-ի վնասվածքներ, վերապրում, կենսական ակտիվություն, պուրինային և պիրիմիդինային նուկլեոտիդների դեզամինացում, ԱԵՖ-ազային ակտիվություն, ձևաբանական փոփոխություններ, վոլյուտին

Առաջին անգամ իրականացվել է *C. guilliermondii* NP-4 խմորասնկերի վրա ռենտգենյան ճառագայթման ազդեցության բազմակողմանի ուսումնասիրություն: Ցույց է տրվել, որ ռենտգենյան ճառագայթահարումը հանգեցնում է *C.guilliermondii NP-4* խմորասնկերի նյութափոխանակության խախտումների, ԴՆԹ-ի վնասվածքների առաջացման, բջիջների մորֆոլոգիայի խորը փոփոխությունների, որոնք հետճառագայթային ռեպարացիայի ընթացքում մասնակիորեն վերականգնվում են, իսկ ԴՆԹ-ի վնասվածքները ամբողջությամբ չեն վերականգնվում, այլ ավելի են խորանում:

Ճառագայթահարված խմորասնկերի աճի դինամիկայում դիտվում է լատենտ փուլի երկժամյա ուշացում, ինչի հետևանքով խախտվում է բջիջների կենսական ցիկլը, դիտվում է միտոտիկ ակտիվության անկում՝ G_2-բլոկադա: Ճառագայթահարված խմորասնկերի աճի առաջին ժամերի ընթացքում ճառագայթային վնասվածքների մի մասը վերականգնվում է, և բջջային պոպուլյացիան անցնում է աճի լոգարիթմական փուլին, թեև աճի կինետիկական ցուցանիշներով և գաղութառաջացման ունակությամբ ճառագայթահարված խմորասնկերը զիջում են բնականոն խմորասնկերին: Ճառագայթահարված խմորասնկերի պոպուլյացիայում ի հայտ են գալիս խմորասնկերի թելանման և հսկա ձևեր, տեղի է ունենում վոլյուտինի հատիկների խոշորացում և արտազատում վակուոլներից դեպի բջջապլազմ: Հետճառագայթային աճեցման ընթացքում ճառագայթահարված բջիջների մեծ մասում տեղի է ունենում բջջային կոմպարտմենտների վերականգնում, թեև պահպանվում է նաև թելանման և հսկա բջիջների որոշ քանակություն: Վերականգնված խմորասնկերում վոլյուտինը դուրս է գալիս վակուոլներից, մանրանում և ցրվում ամբողջ բջջապլազմով մեկ և բջջի կողմից օգտագործվում է որպես էներգիայի այլընտրանքային աղբյուր՝ կոմպենսացնելով ճառագայթային վնասման հետևանքով բջջում առաջացած ԱԵՖ-ի դեֆիցիտը, որի առաջացման մասին վկայում է ԱԵՖ-ազային ակտիվության անկումը ռենտգենյան ճառագայթահարման ենթարկված խմորասնկերի միտոքոնդրիումներում աճի բոլոր փուլերում՝ չճառագայթահարված խմորասնկերի համեմատությամբ:

Ռենտգենյան ճառագայթահարման ազդեցությամբ խմորասնկային ԴՆԹ-ում առաջացած միաշղթա խզումները ռեպարացման ընթացքում վերածվում են երկշղթա խզումների՝ ռեպարացիոն համակարգի ֆերմենտների խաթարման և սխալ

ռեպարացման հետևանքով: Արդյունքում ԴՆԹ-ի մոլեկուլը կտրտվում է, ինչի ապացույցն է ռեպարացված խմորասնկերի ԴՆԹ-ի էլեկտրաֆորեգրամում ցածրամոլեկուլային ԴՆԹ-ի և ԴՆԹ-բեկորների ի հայտ գալը: Խմորասնկերի ԴՆԹ-ի երկշղթա խզման ռեկոմբինացիոն ռեպարացիայի արդյունքում միևնույն բջջում առաջանում են ԴՆԹ-ի տարբեր էլեկտրաֆորետիկ շարժունակությամբ օժտված ֆրակցիաներ, կամ տարբեր բջիջներում էլեկտրաֆորետիկ շարժունակության տեսակետից տարբերվող ԴՆԹ-մոլեկուլներ, ինչն արտացոլվում է վերականգնված խմորասնկերի պոպուլյացիայի պոլիմորֆիզմում: Հետևաբար, միմյանցից ձևաբանորեն տարբերվող խմորասնկերը էապես տարբերվում են նաև ԴՆԹ-ի կառուցվածքով: Վնասվածքներ կրող ԴՆԹ-ն, ամենայն հավանականությամբ, կարեր է առաջացնում ոչ հիստոնային սպիտակուցների հետ, ինչը հնարավորություն է տալիս ԴՆԹ-ի հատվածներին՝ մնալ միացած նաև այն դեպքում, երբ ԴՆԹ-ի մոլեկուլներում առկա են երկշղթա խզումներ, և հաջողությամբ ռեպլիկացվել կամ տրանսկրիպցվել: Սրա վկայությունն է ճառագայթահարված և վերականգնված խմորասնկային ԴՆԹ-ի հալման ջերմաստիճանի մեծացումը և հալման միջակայքի փոքրացումը:

Ռենտգենյան ճառագայթահարման ազդեցությամբ դիտվում է խմորասնկերի նյութափոխանակության ընդհանուր անկում, դրսևորվում է կենսական ակտիվության, պուրինային նուկլեոտիդների դեզամինացման ուժգնության անկում, ԱԵՖ-ի հիդրոլիզի ուժգնությունն իջնում է, իսկ ԳԵՖ-ի հիդրոլիզը՝ դադարում, այսինքն՝ պլաստիկ գործընթացները գրեթե դադարում են, բջիջներում տեղի է ունենում ԱԵՖ-ի խնայում հետագա վերականգնողական գործընթացների համար, իսկ որպես էներգիայի այլընտրանքային աղբյուր ճառագայթահարման ընթացքում ավելի շատ օգտագործվում է ԱԿՖ-ը: Հետճառագայթային վերականգնումից հետո զգալիորեն բարձրանում է խմորասնկերի կենսական ակտիվությունը, թեև այն մնում է խիստ ցածր չճառագայթահարված խմորասնկերի համեմատությամբ, կտրուկ բարձրանում է ԱԿՖ-ի, ԱԵՖ-ի և ԳԵՖ-ի հիդրոլիզի ուժգնությունը, այսինքն՝ խմորասնկերում վերականգնվում են պլաստիկ փոխանակության գործընթացները, մեծանում է էներգիայի պահանջը, ուստի որպես էներգիայի աղբյուր, բացի ԱԵՖ-ից, օգտագործվում են նաև ԱԿՖ-ը և վոլյուտինը: Պուրինային ազոտային հիմքերը և նուկլեոզիդները խմորասնկերում չեն ենթարկվում վերջնական կատաբոլիզմի, այլ ընդգրկվում են նուկլեոտիդների փրկության ուղիներում, ինչը էներգիայի և նյութերի խնայման յուրօրինակ համակարգ է մանրէների, այդ թվում՝ նաև խմորասնկերի համար:

МАРУТЯН СЕДА ВИКТОРОВНА

МЕТАБОЛИЧЕСКИЕ ИЗМЕНЕНИЯ И СТРУКТУРНЫЕ ПОВРЕЖДЕНИЯ ДНК У РЕНТГЕН-ОБЛУЧЕННЫХ ДРОЖЖЕЙ *C.guilliermondii NP-4*

РЕЗЮМЕ

Ключевые слова: *C. guilliermondii NP-4,* рентгеновское облучение, повреждения ДНК, выживание, жизнеспособность, дезаминирование пуриновых и пиримидиновых нуклеотидов, АТФ-азная активность, морфологические изменения, волютин

Впервые проведено многостороннее исследование влияния рентгеновского облучения на дрожжи *C. guilliermondii* NP-4. Показано, что облучение приводит к изменениям в метаболизме дрожжей, к образованию структурных повреждений ДНК, к глубоким изменениям в морфологии клеток. Метаболические изменения в процессе пострадиационной репарации частично восстанавливаются, а повреждения ДНК не восстанавливаются, а наоборот, углубляются.

В динамике роста облученных дрожжей наблюдается 2-часовая задержка латентной фазы роста, вследствие чего нарушается клеточный цикл, наблюдается падение митотической активности - G_2-блокада. В течение первых часов пострадиационной инкубации облученных дрожжей восстанавливается часть повреждений, и клеточная популяция переходит к логарифмической фазе роста, однако по кинетическим параметрам и способности колониеобразования облученные дрожжи уступают необлученным клеткам. В популяции облученных дрожжей выявляются нитчатые и гигантские формы клеток. В нитчатых клетках происходит увеличение волютиновых полифосфатных гранул – альтернативного источника энергии микроорганизмов, и его выделение из вакуолей в цитоплазму. При пострадиационной инкубации дрожжей в большинстве клеток происходит восстановление клеточных компартментов, однако сохраняется некоторое количество гигантских клеток и нитчатых форм. В восстановленных клетках волютин выделяется из вакуолей, уменьшается в размерах и распределяется по всей цитоплазме. Волютин используется дрожжевыми клетками как альтернативный источник энергии, компенсируя дефицит АТФ возникший в клетке вследствие радиационных повреждений. Об этом свидетельствует уменьшение АТФ-азной активности в митохондриях облученных дрожжей во всех фазах роста клеток по сравнению с необлученными клетками.

Часть однонитевых разрывов ДНК, образовавшихся под влиянием рентгеновского облучения, в процессе репарации превращаются в двунитевые, вследствие повреждения ферментов репарационной системы и так называемой неправильной репарации. Вследствие этого ДНК фрагментируется, о чем свидетельствует возникновение в электрофореграмме ДНК репарированных дрожжей низкомолекулярной фракции ДНК и ДНК-фрагментов. Репарация двунитевых разрывов ДНК дрожжей происходит в основном путем рекомбинантной репарации, вследствие чего возможно возникновение в одной клетке двух фракций

ДНК с разными электрофоретическими подвижностями, а также в разных клетках возникновение молекул ДНК с разными электрофоретическими свойствами. Такая возможность отражается в полиморфизме популяции восстановленных дрожжей. Следовательно, дрожжи, существенно отличающиеся морфологически, могут также отличаться по строению своей ДНК. Поврежденная ДНК, по всей вероятности, образует сшивки с негистоновыми белками, что позволяет фрагментам ДНК не расходиться даже в том случае, если в строении ДНК существуют двунитевые разрывы. О возможности образования таких сшивок свидетельствует, вопреки ожиданиям, повышение температуры плавления и уменьшения интервала плавления ДНК облученных и репарированных дрожжей.

Под влиянием рентгеновского облучения наблюдается уменьшение интенсивности дезаминирования пуриновых нуклеотидов, что обусловлено общим падением интенсивности метаболизма. Это отражается, например, в резком падении способности ферментации дрожжей, что наблюдается под воздействием рентгеновского облучения. Одновременно, рентгеновское облучение приводит к падению гидролиза АТФ по сравнению с необлученными дрожжами, а гидролиз ГТФ вовсе останавливается, т.е. пластические процессы в облученных клетках почти прекращаются. В качестве альтернативного источника энергии в процессе облучения чаще используется АДФ. После пострадиационного восстановления способность ферментации дрожжей значительно повышается, хотя остается довольно низким по сравнению с необлученными дрожжами. Резко повышается интенсивность гидролиза АТФ, АДФ и ГТФ, что свидетельствует о восстановлении в дрожжевых клетках процессов пластического обмена, увеличивается потребность энергии, поэтому в качестве источника энергии, кроме АТФ-а, используются также АДФ и волютин. Пуриновые азотистые основания и нуклеозиды в дрожжевых клетках не подвергаются конечному метаболизму, а включаются в пути спасения нуклеотидов, в ресинтезе нуклеотидов из промежуточных продуктов распада нуклеиновых кислот. Эти пути ресинтеза нуклеотидов представляют из себя своеобразную систему экономии ресурсов и энергии для микроорганизмов, особенно – для дрожжей, востанавливающихся от радиационных повреждений.