





Z.M. Biyasheva^{1,2} , M.Zh. Tleubergenova^{1,2*} ,
Y.A. Zaripova^{1,2} , D.Zh. Nurhan^{1,2} , A.M. Shaizadinova^{1,2}

¹Al-Farabi Kazakh National University, Kazakhstan, Almaty

²Scientific Research Institute of Problems of Biology and Biotechnology, Kazakhstan, Almaty

*e-mail: tleu.madina96@gmail.com

INDUCTION OF REPORTER GENES EXPRESSION BY IONIZING RADIATION AND TOXINS INFLUENCE ON THE *DROSOPHILA MELANOGASTER* GENOME

In the human environment, there are many factors that can cause genotoxic and mutagenic effects and one of them is ionizing radiation. Radioactive substances like radon, thorium, radium and their decay products during decomposition are able to emit not only highly penetrating gamma radiation, but also form large heavy alpha-particles. Alpha-particles have a low penetrating power, but high ionization energy, about 10 times greater than gamma-rays. Consequently, we researched genotoxic effects of alpha-radiation using a test-system with reporter genes on the *Drosophila melanogaster*. In nature, the main source of alpha-particles is colorless radioactive gas radon, its isotopes and daughter decay products. It is technically difficult to create sufficiently intense and directed beams of α -radiation on the long-lived radon isotope – ²²²Rn. Therefore, for experiments was chosen the ²³⁸Pu (Plutonium) isotope, which has the necessary α -line at decay equal to 5.5 MeV. The genotoxic activity of alpha-particles was tested on *Drosophila melanogaster* with genotype containing genes of luminous proteins. Working of the genetic construction based on the GAL4-UAS system, often used in research to determine gene expression. Genotype also included GADD45 protein gene and GFP gene (green fluorescent protein). This genes combination allows to observe glow of *Drosophila* organs and tissues after stress factor impact. The sensitivity of construction was checked by chemical mutagen cisplatin, which led to the larvae death at high concentrations, and caused glow of organs at low concentrations. The larvae were exposed to alpha-radiation for 20 hours. Preparations were analyzed on a light microscope with a blue filter and glow of the larval organs was observed. The glow intensity was depending from radiation source. In other words, higher radiation dose caused brighter glow, which correlates with synthesis of luminous protein level. This proves that alpha-radiation from radon and its decay daughter products has a genetic effect.

Key words: alpha radiation, radon, *Drosophila*, GFP, genotoxicity.

З.М. Бияшева^{1,2}, М.Ж. Тлеубергенова^{1,2*},
Ю.А. Зарипова^{1,2}, Д.Ж. Нурхан^{1,2}, А.М. Шайзадинова^{1,2}

¹Әл-Фараби атындағы Қазақ ұлттық университеті, Қазақстан, Алматы қ.

²Биология және биотехнология проблемалары ғылыми-зерттеу институты, Қазақстан, Алматы қ.,

*e-mail: tleu.madina96@gmail.com

Drosophila melanogaster геномына иондаушы сәулелену мен токсиндерге ұшыраған кездегі репортерлік гендерінің экспрессиясын индукциялау

Адамның қоршаған ортасында иондаушы сәулелену сияқты генотоксикалық және мутагендік әсерлерді тудыруы мүмкін көптеген факторлар бар. Радон, торий, радий және олардың ыдырау өнімдері сияқты радиоактивті заттар ыдырау процесінде тек жоғары өтетін гамма-сәулеленуді бөліп қана қоймай, ірі ауыр альфа-бөлшектер түзе алады. Альфа-бөлшектер, кіші өткізгіш қабілеті бар болса да, гамма-сәуледен 10 есе үлкен иондаудың жоғары энергиясына ие. Осыған байланысты біз *Drosophila melanogaster* жеміс мушкасында репортер гендері бар тест-жүйені пайдалана отырып, α -сәулеленудің генотоксикалық әсерлеріне зерттеулер жүргіздік. Табиғатта альфа-бөлшектердің негізгі көзі түссіз радиоактивті газ радон, оның изотоптары және ыдыраудың еншілес өнімдері болып табылады. Радон – ²²²Rn ұзақ өмір сүретін изотопында жеткілікті қарқынды және бағытталған альфа-сәулелену шоғырын жасау техникалық қиын болғандықтан, модельдеуші эксперименттер үшін 5,5МэВ тең ыдырау кезінде қажетті альфа-желісі бар ²³⁸Pu (Плутоний) изотоп таңдалды. Альфа-бөлшектердің генотоксикалық белсенділігін

тестілеу *Drosophila melanogaster*-де, құрамында жарқырайтын ақуыз гендері бар генотипті алдын ала анықтаумен жүргізілді. Генетикалық конструкцияның жұмысы гендердің экспрессиясын анықтау үшін зерттеулерде жиі қолданылатын GAL4-UAS жүйесіне негізделген. Генотипте ДНК реттілігі бұзылғанда синтезделген GADD45 ақуыз гені және GFP (жасыл флуоресцентті ақуыз) гені болды. Гендердің бұл комбинациясы стрестік фактордың әсерінен кейін дрозофиланың органдары мен тіндерінің жылтырауын байқауға мүмкіндік береді. Алынған конструкцияның сезімталдығын алдын ала цисплатиннің химиялық мутагенінің көмегімен тексерілді, ол жоғары концентрациясы кезінде дрозофиланың дернәсілдерінің өлуіне алып келді, ал төмен концентрациясы кезінде органдардың сәулеленуін тудырды. Дернәсілдер 20 сағат ішінде альфа-сәулелену әсеріне ұшыраған. Препараттарды талдау көк сүзгішпен жарық микроскопта жүргізілді және дернәсілдер органдарының жарқырауын бақыланды. Сәулеленудің қарқындылығы сәулеленудің көзіне байланысты, яғни сәулеленудің дозасы көп болған сайын жарқыраған ақуыз синтезінің деңгейімен корреляцияланатын шам соғұрлым жарық болады. Бұл радон және оның еншілес ыдырау өнімдері дрозофила дернәсілдеріне әсер еткенде генетикалық әсері бар екенін дәлелдейді.

Түйін сөздер: альфа-сәулелену, радон, дрозофила, GFP, геноуыттылық.

З.М. Бияшева^{1,2}, М.Ж. Тлеубергенова^{1,2*},
Ю.А.Зарипова^{1,2}, Д.Ж. Нурхан^{1,2}, А.М. Шайзадинова^{1,2}

¹Казахский национальный университет имени аль-Фараби, Казахстан, г. Алматы

²Научно-исследовательский институт проблем биологии и биотехнологии, Казахстан, г. Алматы

*e-mail: tleu.madina96@gmail.com

Индукция экспрессии репортерных генов при воздействии ионизирующей радиации и токсинов на геном *Drosophila melanogaster*

В окружающей среде человека существует множество факторов, способных вызывать генотоксические и мутагенные эффекты, такие как ионизирующее излучение. Так радиоактивные вещества, как радон, торий, радий и их продукты распада, в процессе разложения способны выделять не только высоко проникающее гамма-излучение, но и образовывать крупные тяжелые альфа-частицы. Альфа-частицы, обладая малой проникающей способностью, несут высокую энергию ионизации, примерно в 10 раз большую, чем гамма-лучи. В связи с этим нами были проведены исследования генотоксических эффектов α -излучения с использованием тест-системы с репортерными генами на плодовой мушке *Drosophila melanogaster*. В природе основным источником альфа-частиц являются бесцветные радиоактивный газ радон, его изотопы и дочерние продукты распада. Поскольку создавать достаточно интенсивные и направленные пучки α -излучения на долгоживущем изотопе радона – ²²²Rn технически затруднительно, то для моделирующих экспериментов был выбран изотоп ²³⁸Pu (Плутоний), имеющий необходимую α -линию при распаде равную 5,5 МэВ. Тестирование генотоксической активности альфа-частиц проводили на *Drosophila melanogaster* с предварительным конструированием генотипа, содержащего гены светящихся белков. Работа генетической конструкции основана на системе GAL4-UAS, часто используемой в исследованиях для определения экспрессии генов. В генотипе также присутствовали ген белка GADD45, синтезируемый при нарушении последовательности ДНК, и ген GFP (зеленый флуоресцентный белок). Эта комбинация генов позволяет наблюдать свечение органов и тканей дрозофилы после воздействия стрессового фактора. Чувствительность полученной конструкции заранее проверили с помощью химического мутагена цисплатина, который при высокой концентрации приводил к гибели личинок дрозофилы, а при низкой концентрации вызывали свечение органов. Личинок подвергали воздействию альфа-излучения в течение 20 часов. Анализ препаратов проводили на световом микроскопе с синим фильтром и наблюдали свечение органов личинок. Интенсивность свечения зависела от источника облучения, то есть, чем больше доза излучения, тем ярче было свечение, что коррелирует с уровнем синтеза светящегося белка. Это доказывает, что при воздействии на личинок дрозофил α -излучения от радона и его дочерних продуктов распада наблюдается генетический эффект.

Ключевые слова: альфа-излучение, радон, дрозофила, GFP, генотоксичность.

Introduction

Environmental protection takes one of the major places in the sustainable development of human so-

ciety. Genetic testing is aimed at detecting and evaluating the genetic hazard of environmental factors present in the human environment. Radioactive contamination engages a central place in the processes

of atmospheric air, water, soil pollution. Radioactive contamination is characterized by the presence of radioactive substances on the surface, in the air, in the human body or elsewhere in quantities exceeding specified levels and can cause mutagenic and carcinogenic effects. [1]. Thus, isotopes of uranium, radon and its daughter products of decay (DDP) are involved in the creation of natural radiation, repeatedly and for a long time affect a person. They determine the complexity of the environment and are participants in the synergistic effects of pollutants. In this regard, to determine the possible genetic effects of physical or chemical agents, not individual tests are used, but a battery (set) of tests that meet the requirements of sensitivity and specificity [2]. Currently, systems with linked sex chromosomes have been used to account for visible mutations and recombinations, test systems with reporter genes of a luminous protein on *Drosophila melanogaster*, the “comet” method on small rodents, etc. [3].

Radon and its decay daughter products are the radioactive substances creating radioactive contamination. Radon and its DDP are sources of alpha-particles or positively charged helium nuclei that are easily retained by the human skin without penetrating the body, and have a short run, so it seems that they do not pose a danger. In nature, there are different sources of these pollutant-radioactive isotopes of uranium and radon. Radon is a gas, and therefore easily enters the human lungs along with the inhaled air and inside the body when it decomposes, it releases α -particles. Under the influence of this ionizing radiation, malignancies and various mutations may appear in the body [4]. Given this, it is undoubtedly necessary to assess the genotoxicity of the most common sources of α -radiation — radon and its daughter decay products. According to the USA Department of health, radon is the second most common (after smoking) factor that causes lung cancer of the predominantly bronchogenic type. Lung cancer caused by radon radiation is the sixth most common cause of cancer death [5, 6]. Radon radionuclides account for more than half of the total radiation dose that the human body receives on average from natural and man-made radionuclides in the environment [7].

Radon is a radioactive gas and its decay products can enter the body together with the inhaled air and this leads a significant danger to human health. Radon, also, can accumulate in residential and industrial buildings from gas and running water. Radon is released from the earth's crust everywhere; its concentration varies significantly for different points of the globe. For instance, Almaty region can

be classified as radon-dangerous territories, because of the large number of tectonic faults presence, which increase emanation of radon. For solving this problem, it is necessary to monitor state of environment, to create a system, which can determine the sources and factors of man-made impact, to identify biosphere elements which are most sensitive to impact and to evaluate the degree of this impact [8].

In this regard, environmental monitoring is a key system for ensuring the quality of the natural environment. A budding solution for environmental monitoring could be to find GFP markers which are induced in response to environmental pollution. For this reason, in this study was used the design of the two *Drosophila* lines Gadd45-Gal4 x UAS-GFP. If the Gadd45 is promoter, which strongly induced by irradiation with radioactive sources, while the Gal4 yeast protein inducing GFP expression. Accordingly, we conducted studies of the genotoxic effects of alpha radiation using a test system with reporter genes of a luminous protein on the fruit fly *Drosophila melanogaster*. Green fluorescent protein (GFP) has a number of desirable features for its use as a reporter in living cells and organisms [9] and is used to visualize gene expression in individual cells, tissues, and organs. Using the GFP reporter marker gene, it is possible to visualize the gene in body tissues without cell lysis, subsequent biochemical analysis, and distortions during tissue fixation and staining. Currently, the fluorescent protein is used as a marker in the study of genetic development programs and for solving applied problems, such as creating biosensors.

Nowadays, global environmental pollution caused by technogenic products, which increased mutagenic activity, carry the danger of exposure to genetic apparatus of living organisms. Living beings in industrial and natural conditions expose number of damaging environmental factors. Radiation and chemicals are the most dangerous and often occurring among them. Testing of chemical compounds is carried out in accordance with a number of international documents. This is the Good Laboratory Practice (GLP), a guide to preclinical (non-clinical) research by the world health organization. At the first stage of checking the genetic effects of environmental factors, relatively inexpensive short-term tests are used, which allow taking into account the frequency of occurrence of various types of genetic damage in plants, animals, and microorganisms. This is the Meller-5 *D. melanogaster* system for accounting for recessive sex-linked lethal mutations and a system for accounting for somatic recombination (mosaicism). However, new testing methods

are constantly being developed and implemented to determine the potential hazard of environmental factors. In this regard, we used *Drosophila melanogaster* genetic lines synthesized at the Institute of Molecular and Cellular Biology of SB RAS (Novosibirsk), which contained linked sex chromosomes. When using such genetic chromosomal structures, there is an effect of the position or change in the activity of the gene when changing its position in the genotype [10]. The system with linked sex chromosomes makes it possible to take into account visible newly emerging mutations in the X chromosome [11], and additional Y chromosomes in the genotype are the strongest modifiers of the position effect.

In addition to test systems using chromosomal rearrangements, reporter genes are now widely used, which can be used to register the activity of genes under the influence of a number of environmental pollutants [12]. This makes it possible to create a collection of lines that respond to a specific contaminant using a fluorescent tissue signal, which can be registered using a low-resolution fluorescent microscope. In this article, the environmental factor was taken as α -radiation, which is the basis of the radiation activity of radon gas, its isotopes, and DDP.

Materials and methods

Testing of the genotoxic activity of alpha particles was performed on *Drosophila melanogaster* with preliminary constitution of the genotype containing genes of luminous proteins. This method allows you to visually analyze the expression of the GFP gene in salivary glands, various tissues, and in the imaginal disks of drosophila under α -irradiation and determine the minimum dose of radiation.

The GAL4 / UAS system is a biochemical method based on the study of gene expression and their functions in various model organisms. This system was developed by Andre Brand and Norbert Perimon in 1993 and also is claimed to be a powerful tool for studying gene expression. There are many lines of model organisms that express GAL4 in certain tissues. Basically there are fruit fly lines, which are called the GAL4 line. Tissues of such *Drosophila* lines can be very specific. Besides, GAL4 has no significantly influence on the phenotype. The second part of the system is called reporter. These are lines of flies completely modified by reporter genes under the control of the UAS enhancer [13].

To create the genetic structure of the reporter genes of luminous proteins, the following lines were used: UAS-GFP (registration number in the

Bloomington collection-4776), GADD45-GAL4 (112149). Accordingly, males and females UAS-GFP lines contain in the autosomes the gene of green fluorescent protein (GFP) and the yeast promoter UAS; the GADD45-GAL4 genetic line has the transcription factor Gad45-Gal4 in the autosomes, where GADD45 is the driver, and the yeast protein GAL4 gene acts as a transcription activator [14].

The UAS-GFP line used contains the green fluorescent protein gene isolated from the *Aequorea victoria* jellyfish by Osama Shimomura, as well as the UAS (upstream activation sequence) obtained from the *Saccharomyces cerevisiae* yeast, which activates and enhances the transcription of the attached gene [13]. The Gadd45-Gal4 line contains a gene encoding a protein of the GADD45 family (growth arrest and DNA damage inducible 45) that participates in DNA repair processes, cell cycle arrest, apoptosis, and gene expression regulation [15, 16]. GADD45 family proteins are involved in transmitting stress signals in response to various physiological or environmental stress conditions [17], such as ultraviolet radiation, methyl methane sulfonate (MMS), or hydrogen peroxide, and various family members are involved in various reactions to cell and DNA damage [18, 19]. The GAL4 gene encodes a transcription activator protein that binds to the UAS sequence, which increases the promoter's affinity for polymerase and triggers the transcription process [13, 20].

The long-lived radon isotope ^{222}Rn was used as a source of alpha particles, which generates the main α -line with an energy of 5.5 MeV, but it is technically difficult to create sufficiently intense and directed beams of α -radon radiation on it, so for modeling experiments, three samples of the experimental spectrometric source of α -radiation were selected, containing: 1) the plutonium isotope 238 (^{238}Pu) with a radionuclide activity of $4.01 \cdot 10^4$ Bq; 2) the plutonium isotope 239 (^{239}Pu) with radionuclide activity of $3.80 \cdot 10^3$ Bq; 3) triplet (^{238}Pu , ^{239}Pu , ^{233}U) with radionuclide activity of $3.86 \cdot 10^4$ Bq. All work with flies was performed under anesthesia using sulfur ether (pro narcosi).

The experiments were held at temperature about 20-25°C. Before intercrossing, virgin females were selected beforehand from one of the crossed lines. For this reason, all the flies were removed from the test tube before obtaining virgin females. So, every 6-8 hours' drosophila virgin females were collected. In this time (6-8 hours) fruit droplets, which hatched from eggs do not acquire sexual maturity, and males of this age are unable in female fertilization. The *Drosophila* fruit fly has elongated

light body and undirected wings about first 2-3 hours after the hatching from the pupa. These signs were also taken into attention in the process of virgin females selection. The female differs from the male by body sizes (female is larger than male) and the abdomen pointed tip. Males have pigmented end of the abdomen. The males were kept separate from the females before crossing. Females and males were from different lines. The 3-5 flies of virgin females were placed in the same tube with males. These flies were parent generation. Parental specimens remained in test tubes for 4-5 days. Then after this period flies were removed from the containers and left larvae which were developed from the laid eggs. The larval phase of development begins from the larva hatching moment and continues until pupation stage (120 hours). The larval phases are divided into three periods – the first – larva I age, second – larvae II age and third – larvae III age. The adult fly's organism is mainly formed from certain groups of cells called imaginal cells. Imaginal disks are not externally differentiated and located in the larva body separately. Imaginal disc is a larva's part of body, which further develop into organs of adult *Drosophila* during pupal transformation [21].

When constructing a genotype with a reporter gene of a fluorescent protein, virgin females of the UAS-GFP line were crossed with males of the Gad45-Gal4 line. We used III age larvae, which was received after crossing the *Drosophila melanogaster* lines UAS-GFP and Gadd45-Gal4. Then this construction was exposed by radioactive decay products of radon – ^{238}Pu and ^{239}Pu , and triplet (on 20 hours), which are the source of alpha radiation. Irradiation of the larvae was carried out in special wells in which the nutrient medium was placed. For this, a nutrient medium prepared according to the generally accepted technique with a thickness of 1 mm [22] was poured onto the bottom of glass cuvettes and put 50 imago of *drosophila* were placed. The source of α -particles was above the flies at a distance of no more than 20 mm. The analyzing method of the larvae include making preparation of imaginal disks on a slide. Imaginal disks were isolated from the larvae which were obtained from the crossing of two lines of fruit fly. Then, imaginal disks were placed on a slide in a physiological solution drop and covered by a cover slip. Two preparations were prepared on a slide in the same time. The first one is a larvae after exposure to alpha radiation and second is larvae, which were not mutagenized. Therefore, larvae were prepared, salivary glands and imaginal discs were isolated for fluorescence spectroscopy.

This combination of genes allows you to observe the glow of *drosophila* organs and tissues after exposure to radiation. At 25°C, stage III larvae can be obtained as early as the fourth day after the egg is laid. In our experiment, the larvae developed at 20°C, which significantly slowed their development, so the larvae of the end of stage II and the beginning of stage III were selected for 5-6 days after crossing the parents.

Results and discussions

The major problem of genetic toxicology is the risk assessment of mutations in somatic and germ cells under the influence of environmental pollutants. Identification of potential mutagens and carcinogens for humans is the main task of testing environmental pollution, including radiation. The purpose of this assessment is the scientific justification of measures aimed at reducing population genetic risk under exposure by genotoxic compounds. Experimental methods for studying the genotoxicity of chemical compounds are called test systems. Well-known genetics and widely used methods for studying the genetic effects of radiation and alkylating super-mutagen agents are used as test systems. These include methods for accounting for chromosomal aberrations and gene mutations in vitro and in vivo systems in mammalian cells, accounting for recessive mutations in *Drosophila* and dominant lethal mutations in rodents. Modern genotoxicity testing systems mainly include methods that have proven themselves in the study of hundreds and thousands of compounds with different structures. For them, such characteristics as sensitivity and specificity are defined. They have a well-developed testing protocol and optimal characteristics for the speed of the experiment and cost-effectiveness. Recently, new methods based on new principles for detecting mutagenic and DNA-damaging effects have been finding wider application. Nowadays, there are about 200 test systems and about 20 methods that are well developed and widely used. The following living organisms have been proposed as test organisms for recording mutagenic effects: cell culture or whole organisms, prokaryotes (*Salmonella typhitrium*, *Escherichia coli*, *Bacillus subtilis*), yeast (*Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*), fungi (*Aspergillus nidulans* *craispoispois*), *Vicia faba*, *Tradescantia*), insects (*Drosophila melanogaster*). Currently, strict requirements are set for testing, which are determined by the regulatory provisions of a number of international documents.

These include: Good Laboratory Practice-GLP, The Organization for Economic Co-operation and Development – OECD. One of the OECD function is the development and publication of officially agreed guidelines for testing genotoxic compounds. According to OECD rule No. 477 of April 4, 1984, one of the most used mutagenicity and genotoxicity test systems is the test system based on the *Drosophila melanogaster* fruit fly [23]. For this reason, testing of the genotoxic activity of alpha particles was performed on *Drosophila melanogaster* test system based on preliminary construction of the genotype containing genes of luminous proteins GFP.

To test the sensitivity to stress of the genetic construct with the GFP reporter gene, the larvae were exposed to the chemical mutagen cisplatin. Cisplatin is a cytotoxic drug, an inorganic substance, a complex chloride-ammonia of divalent platinum,

is a strong mutagen that is also used as an antitumor drug in medicine. It has pronounced cytotoxic, bactericidal and mutagenic properties. The basis of biological properties, according to the generally accepted opinion, lies the ability to form strong specific bonds with DNA. Research methods used in this field to study the development and prevention of mutagenesis.[24] The applied two concentration of cisplatin (125 mg / ml, 500 mg / ml), for this purpose, the larvae were placed in a test tube with nutrient containing cisplatin for 24, 8 and 4 hours. After a day and 8 hours, cisplatin showed 100% lethality, and the exposure for 4 hours was zero for this indicator.

Obtaining a sensitive to stress, in our case, ionizing radiation, more precisely α -particles, genetic construction was made by crossing and using the principle of fluorescence induction, shown in figure 1.

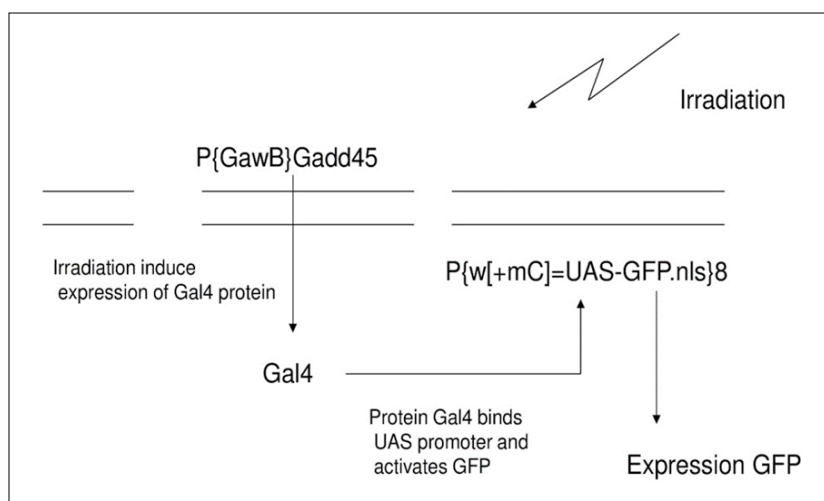


Figure 1 – The principle of operation of the genetic construction Gadd45-Gal4 + UAS-GFP exposed by α -radiation

Metal sources with the isotopes ^{238}Pu , ^{239}Pu and triplet (^{238}Pu , ^{239}Pu and ^{233}U) were used as sources of alpha particles. The absorbed dose, taking into account the weighted coefficient of α -radiation ($W\alpha \equiv 20$), was about 40-50 cGy, the time for the induction of GFP glow, which we found, was 20 hours, i.e. maximum gene expression does not occur immediately after irradiation, but one or more cell cycles are required in order to maximize the induction of repair gene activity.

After irradiation, the larvae were dissected. The analysis of the preparations was performed on a

light microscope with a blue filter ($\sim 375\text{nm}$). As a result, an image of the reporter's GFP induction in response to irradiation by different sources of alpha particles was obtained (Fig. 2).

The presented results show that the GFP reporter gene is more induced when irradiated with a ^{238}Pu source. A small glow was observed in the glands irradiated with the triplet and judging by the intensity of the glow, the ^{239}Pu isotope has a weak mutagenic activity close to that in the control, and the fluorescent protein is practically not synthesized (Fig. 3).

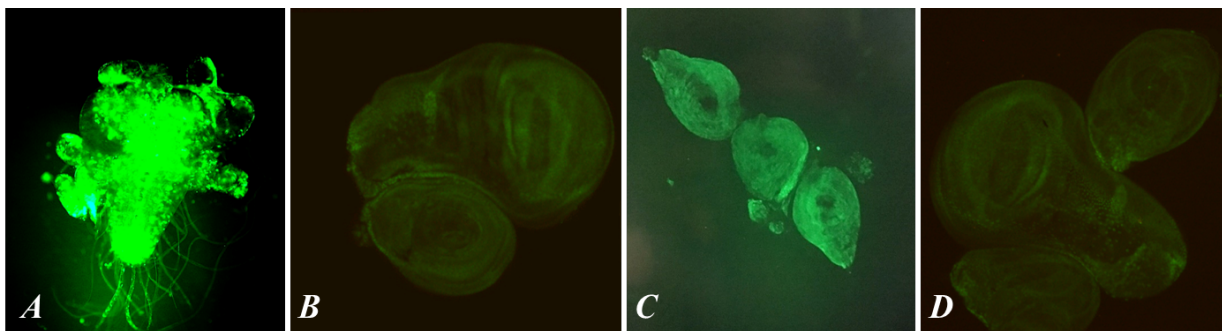


Figure 2 – Effect of α -radiation from different sources on imaginal disks of larvae: A – nervous ganglion (^{238}Pu), B – imaginal disks of a wing and legs (^{239}Pu), C – imaginal disks of legs (triplet), D – imaginal disks of a wing and legs (without irradiation)

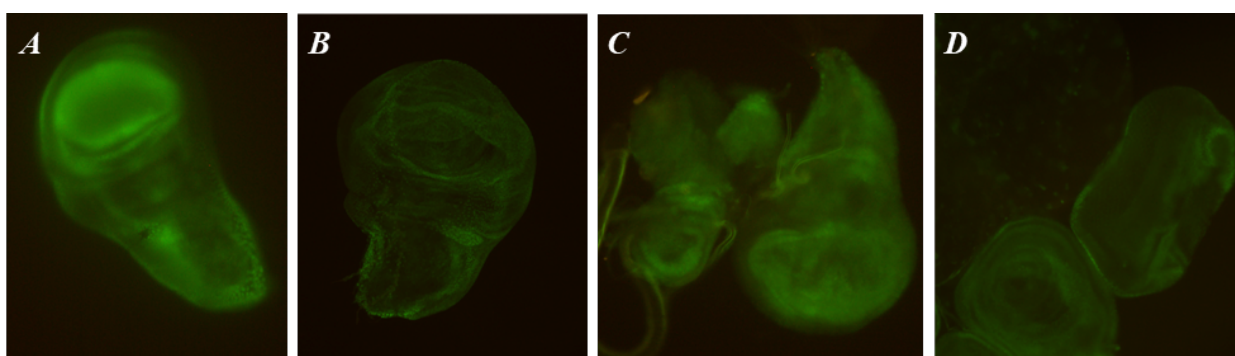


Figure 3 – Intensity of fluorescent induction depending on the source of α -radiation in the wing imaginal disks of *Drosophila melanogaster* larvae: A – ^{238}Pu , B – ^{239}Pu , C – triplet (^{238}Pu , ^{239}Pu and ^{233}U), D – control (without irradiation)

During irradiation, if DNA damage occurs, the expression of the GADD45 gene begins, which induces the synthesis of the GAL4 activator protein. The activator protein, in turn, attaches to the UAS sequence, increasing the promoter's affinity for polymerase, which leads to the beginning of transcription and synthesis of GFP in target tissues of drosophila larvae [25]. Consequently, the more GADD45 protein synthesis occurs, the more GFP is synthesized in cells. Enhanced expression of GADD45 proves that α -particles have an effect on the DNA (directly or indirectly) and cause changes which lead to activation of repair processes. A small amount of luminescence in the control related to the work of repair systems during the cell cycle. Also it connected with influence of other stressful factors, such as the temperature difference during transportation of the wells for exposure and background radiation. A high level of GFP in the organs of *Drosophila* larvae after

exposure proves that α -particles have genotoxic effects.

Therefore, the higher the radiation dose (depending on the source), the brighter the glow was, which correlates with the level of the fluorescent protein synthesis (Fig. 4). This showed that α -radiation affects the expression of the gene, i.e. it has genetic effects.

As a result of comparing the intensity of the salivary gland glow depending on the radiation source, it became clear that the isotope ^{238}Pu has the greatest mutagenic and carcinogenic activity. Also, a small glow was observed in the glands irradiated with the triplet, which also includes the isotope ^{238}Pu , but in a smaller amount. Judging by the intensity of the glow, the isotope ^{239}Pu has a weak mutagenic activity, as well as in the control, the fluorescent protein is almost not synthesized, so there is no glow. Based on this comparison, we can say that the isotope ^{238}Pu , which is the source of ionizing alpha radiation, has genotoxic properties.

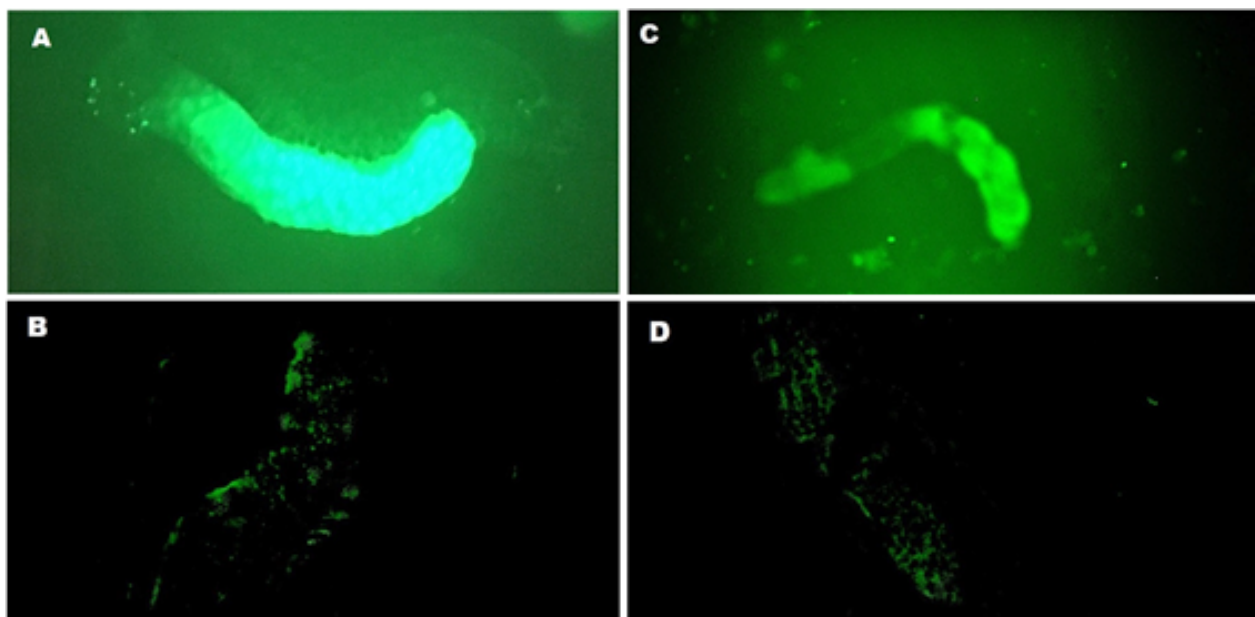


Figure 4 – Effect of α -radiation on the salivary glands of *Drosophila melanogaster*: A – ^{238}Pu , B – ^{239}Pu , C – triplet (^{238}Pu , ^{239}Pu and ^{233}U), D – control (without irradiation)

Although the radon isotope was not used in the experimental work, ^{238}Pu has a similar flux of alpha-particles (the decay energy of ^{222}Rn and ^{238}Pu is 5.5 MeV) and is substantially a pure alpha emitter. Besides, in nature there are isotopes of radon and its DDP with a higher decay energy. For this reason during the decay process of radon isotopes polonium isotopes can form, some of them are short-lived, but there are more long-lived isotopes (from several months to 100 years). DDP of radon, including polonium, can accumulate in the liver, kidneys, spleen and bone marrow. Unstable DDP of radon can ionize cells during alpha-decay, in which they accumulate. As a result of this process free radicals appear, the enzyme systems of cells are disrupted, which, possibly, leads to disruption of epigenetic processes in cells and in the progeny of irradiated cells. For example, it is known that polonium isotopes in a living organism behave like selenium, which, as a part of many enzymes, enters the active center of enzymes of the body's antioxidant-antiradical defense system, the metabolism of nucleic acids, lipids, hormones.

In such a way, radon and its DDP are sources of ionizing radiation in nature and have genotoxic properties. In our work, we used a *Drosophila melanogaster* test based on the GAL4 / UAS biochemical system. This system allowed us to visually observe GADD45 expression with the benefit of GFP reporter gene and even compare expression levels after exposure under different sources of alpha-radiation.

Conflict of interest

All authors have read and are familiar with the contents of the article and have no conflict of interest.

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References

- 1 James R. DeVoe. Radioactive Contamination of Materials Used in Scientific Research. – Washington D.C.: National Academies, 1961 – 142 p.
- 2 Бондаренко Л. В., Дукельская А. В. Методы тестирования генетической активности факторов среды (практический курс) // Экологическая генетика. – 2007. – Т. 5, №1. – С. 42-44.

- 3 Шварцман П.Ю. Индуцированный соматический мозаицизм у дрозофил как тест-система для оценки генетической активности факторов окружающей среды // Генетика. – 1975. – Т. 11, № 8. – С. 171.
- 4 Karam P. A., Stein B. P. Radioactivity. – New York: Chelsea House, 2009.
- 5 Darby, S. Radon: A likely carcinogen at all exposures // Annals of Oncology. 2001. – Vol. 2, no 10. – P. 1341-1351.
- 6 Torres-Durán M., Ruano-Ravina A., Parente-Lamelas I. et al. Lung cancer in never-smokers: a case-control study in a radon-prone area (Galicia, Spain) // Eur Respir J. – 2014. – Vol. 44, no 4. – P. 850–852. doi: 10.1183/09031936.00017114.
- 7 Effects of Ionizing Radiation. UNSCEAR 2006 Report: Vol. II. Annex E: Sources-to-effects assessment for radon in homes and workplaces. – New York, 2008.
- 8 Грачев Н.Н. Средства и методы защиты от электромагнитных и ионизирующих излучений. – М.: изд-во МИЭМ, 2005 – 215 с.
- 9 Chalfie M. et al. Green fluorescent protein as a marker for gene expression // Science. – 1994. – Vol. 263. – P. 802-805.
- 10 Дубинин Н.П., Сидоров Б.Н. Зависимость действия гена от его положения в системе // Биол. журнал. – 1934. – Т. 3. – № 2. – С. 304–331..
- 11 Жимулев И.Ф., Беляева Е.С. Гетерохроматин и эффект положения гена и генетический сайленсинг // Генетика. – 2003. Т. 39. – № 2. – С. 187-201.
- 12 Moskalev A., Shapovnikov M., Snezhkina A. et al. Mining gene expression data for pollutants (dioxin, toluene, formaldehyde) and low dose of gamma-irradiation // PLoS One. – 2014. – Vol. 9, no 1. – e86051.doi.org/10.1371/journal.pone.0086051
- 13 Guarente, L. UASs and enhancers: common mechanism of transcriptional activation in yeast and mammals // Cell. – 1988. – Vol. 52. – P. 303-305.
- 14 Webster N., Jin J. R. Green S. Hollis M. Chambon P. The Yeast UASG is a transcriptional enhancer in human HeLa cells in the presence of the GAL4 trans-activator // Cell. – 1988. – Vol. 52, no 2. – P. 169–178. doi:10.1016/0092-8674(88)90505-3.
- 15 Hildesheim J., Bulavin D.V., Anver M.R., et al. Gadd45a protects against UV irradiation-induced skin tumors, and promotes apoptosis and stress signaling via MAPK and p53 // Cancer research. – 2002. – Vol. 62. – P. 7305–7315.
- 16 Liebermann D.A., Tront J.S., Sha X., et al. Gadd45 stress sensors in malignancy and leukemia // Critical reviews in oncogenesis. – 2011. – Vol. 16. – P. 129–140.
- 17 Moskalev A., Plyusnina E., Shaposhnikov M. et al. The role of D-GADD45 in oxidative, thermal and genotoxic stress resistance // Cell Cycle. – 2012. – Vol. 11, no 22. – P. 4222-4241. DOI: 10.4161/cc.22545
- 18 Liebermann D. A., Hoffman B. Gadd45 in stress signaling // J Mol Signal. – 2008. – Vol. 3. – P. 1–8.
- 19 Tamura R.E., de Vasconcellos J.F., Sarkard D., Liebermann T.A., Fisher P.B., Zerbina L.F. GADD45 proteins: central players in tumorigenesis // Curr Mol Med. – 2012. – Vol. 12. – P. 634–651.
- 20 Guarente, L., Hoar E. Upstream activation sites of the CYC1 gene of *Saccharomyces cerevisiae* are active when inverted but not when placed downstream of the “TATA box” // Proc Natl Acad Sci USA. – 1984. – Vol. 81, no 24. – P. 7860-7864.
- 21 Албертс А., Брей Д., Льюис Дж. и др. Молекулярная биология клетки. В 3 томах. – М.: Мир, 1994, – 77 с.
- 22 Kohler R. E. Lords of the fly: *Drosophila* genetics and the experimental life. – Chicago: University of Chicago Press, 1994, – 321 p.
- 23 Абилов С.К., Глазер В.М. Мутагенез с основами генотоксикологии: Учебное пособие. – М.; СПб.: НесторИстория, 2015.– 304с.
- 24 Jamieson E. R. Lippard S. J. Structure, Recognition, and Processing of Cisplatin–DNA Adducts // Chemical Reviews. – 1999. – Vol. 99, no 9. – P. 2467-2498.
- 25 Morin X., Daneman R., Zavortink M., Chia W. A protein trap strategy to detect GFP-tagged proteins expressed from their endogenous loci in *Drosophila* // Proc Natl Acad Sci USA. – 2001. – Vol. 98, no 26. – P. 15050–15055.

References

- 1 James R. DeVoe. Radioactive Contamination of Materials Used in Scientific Research. – Washington D.C.: National Academies, (1961) 142 pp.
- 2 Bondarenko L.V., Dukel'skaya A.V. “Metody testirovaniya geneticheskoy aktivnosti faktorov okruzhayushchey sredy.” [“Testing methods for the genetic activity of environmental factors”] *Ekologicheskaya genetika* 5, no 1 (2007): 42-44. (In Russian).
- 3 Shvartsman P.Y. “Indutsirovanny somaticheskij mozaitizm u drozofily kak test-sistema dlya otsenki geneticheskoy aktivnosti faktorov okruzhayushchey sredy.” [“Induced somatic mosaicism in *Drosophila* as a test system for assessing the genetic activity of environmental factors”] *Genetika* 11, no 8 (1975): 171. (In Russian).
- 4 Karam P. A., Stein B. P. Radioactivity. New York: Chelsea House, 2009.
- 5 Darby, S. “Radon: A likely carcinogen at all exposures.” *Annals of Oncology* 2, no 10 (2001): 1341-1351.
- 6 Torres-Durán M., Ruano-Ravina A., Parente-Lamelas I. et al. “Lung cancer in never-smokers: a case-control study in a radon-prone area (Galicia, Spain).” *Eur Respir J* 44, no 4 (2014): 850–852. doi: 10.1183/09031936.00017114.
- 7 Effects of Ionizing Radiation. UNSCEAR 2006 Report: Vol. II. Annex E: Sources-to-effects assessment for radon in homes and workplaces. New York, 2008.
- 8 Grachev N.N. “Sredstva i metody zashchity ot elektromagnitnykh i ioniziruyushchikh izluchenyi.” [“Means and methods of protection against electromagnetic and ionizing radiation”]. М.: изд-во МИЭМ (2005) 215 p. (In Russian).
- 9 Chalfie M. et al. “Green fluorescent protein as a marker for gene expression.” *Science* 263 (1994): 802-805.
- 10 Dubinin N.P., Sidorov B.N. “Zavisimost' deystviya gena ot yego polozheniya v sisteme.” [“Dependence of the action of a gene on its position in the system”] *Biol zhurnal* 3, no 2 (1934): 304–331. (In Russian).

- 11 Zhimulev I.F., Belyayeva Y.S. "Geterokhromatin i effekt polozheniya gena i geneticheskiy saylensing." ["Heterochromatin and gene position effect and genetic silencing"] *Genetika* 39, no 2 (2003): 187-201. (In Russian).
- 12 Moskalev A., Shapovnikov M., Snezhkina A. et al. "Mining gene expression data for pollutants (dioxin, toluene, formaldehyde) and low dose of gamma-irradiation." *PLoS One* 9, no 1 (2014): e86051.doi.org/10.1371/journal.pone.0086051
- 13 Guarente, L. "UASs and enhancers: common mechanism of transcriptional activation in yeast and mammals." *Cell* 52 (1988): 303-305.
- 14 Webster N., Jin J. R. Green S. Hollis M. Chambon P. "The Yeast UASG is a transcriptional enhancer in human HeLa cells in the presence of the GAL4 trans-activator." *Cell* 52, no 2 (1988): 169-178. [doi:10.1016/0092-8674\(88\)90505-3](https://doi.org/10.1016/0092-8674(88)90505-3).
- 15 Hildesheim J., Bulavin D.V., Anver M.R., et al. "Gadd45a protects against UV irradiation-induced skin tumors, and promotes apoptosis and stress signaling via MAPK and p53." *Cancer research* 62 (2002): 7305-7315.
- 16 Liebermann D.A., Tront J.S., Sha X., et al. "Gadd45 stress sensors in malignancy and leukemia." *Critical reviews in oncogenesis* 16 (2011): 129-140.
- 17 Moskalev A., Plyusnina E., Shaposhnikov M. et al. "The role of D-GADD45 in oxidative, thermal and genotoxic stress resistance." *Cell Cycle* 11, no 22 (2012): 4222-4241. DOI: 10.4161/cc.22545
- 18 Liebermann D. A., Hoffman B. "Gadd45 in stress signaling." *J Mol Signal* 3 (2008): 1-8.
- 19 Tamura R.E., de Vasconcellos J.F., Sarkard D., Liebermann T.A., Fisher P.B., Zerbina L.F. "GADD45 proteins: central players in tumorigenesis." *Curr Mol Med* 12 (2012): 634-651.
- 20 Guarente, L., Hoar E. "Upstream activation sites of the *CYC1* gene of *Saccharomyces cerevisiae* are active when inverted but not when placed downstream of the "TATA box." *Proc Natl Acad Sci USA* 81, no 24 (1984): 7860-7864.
- 21 Alberts B.A., Bray D.D., Lewis J.J. "Molecular cell biology: 3rd Edition." Garland Science (March 1994) 1408 p.
- 22 Kohler R. E. "Lords of the fly: *Drosophila* genetics and the experimental life." Chicago: University of Chicago Press (1994) 321 p.
- 23 Abilev S.K., Glazer V.M. "Mutagenез s osnovami genotoksikologii: Uchebnoye posobiye." ["Mutagenesis with the basics of genotoxicology: Textbook."] M.: SPb.: NestorIstoriya (2015) 304 p. (In Russian).
- 24 Jamieson E. R. Lippard S. J. "Structure, Recognition, and Processing of Cisplatin-DNA Adducts." *Chemical Reviews* 99, no 9 (1999): 2467-2498.
- 25 Morin X., Daneman R., Zavortink M., Chia W. "A protein trap strategy to detect GFP-tagged proteins expressed from their endogenous loci in *Drosophila*." *Proc Natl Acad Sci USA* 98, no 26 (2001): 15050-15055.