LABORATORY OF RADIATION BIOLOGY

In 2011, the Laboratory of Radiation Biology (LRB) continued research in the framework of Topic 04-9-1077-2009/2011 «Research on the Biological Effect of Heavy Charged Particles with Different Energies» in the following fields: fundamental radiobiological and radiation genetics research with heavy charged particles; research on the effect of accelerated charged particles on eye structures (the lens and retina); molecular dynamics research; mathematical modeling of induced mutagenesis in bacterial cells; radiation research and radiation protection of JINR's basic facilities and the environment. The Topic has been prolonged to 2014 (04-9-1077-2009/2014).

RADIATION GENETICS AND RADIOBIOLOGY

Research has been continued on the regularities in the formation of DNA damage of different types under ionizing radiation with the enzymatic DNA comet analysis method. The application of endonuclease III (EndoIII) and formamidopyrimidine glycosylase (Fpg) repair enzymes allows modified pyrimidine and purine bases to be transformed into DNA single-strand breaks (SSB). With the use of modifying enzymes in alkaline and neutral DNA comet analysis, comparative dose dependences were obtained of the formation of DNA SSB and modified purines and pyrimidines, as well as DNA double-strand breaks (DSB) and clustered DNA DSB under irradiation with ^{60}Co γ-rays (Figs. 1 and 2) [1, 2].

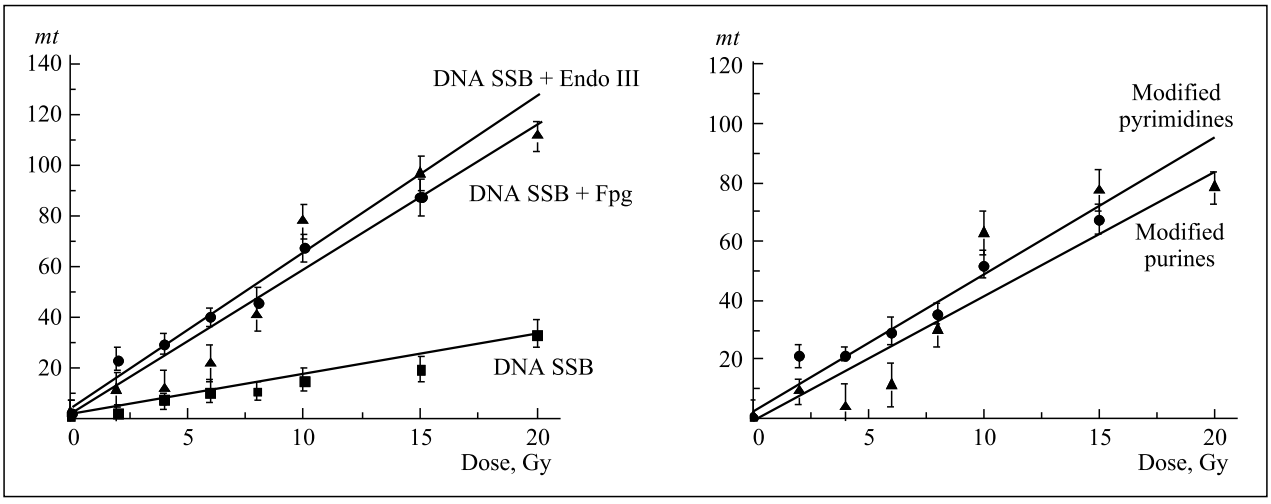

Fig. 1. The dose dependences of the DNA SSB and modified base yield in the presence of enzymes under irradiation with ^{60}Co γ-rays

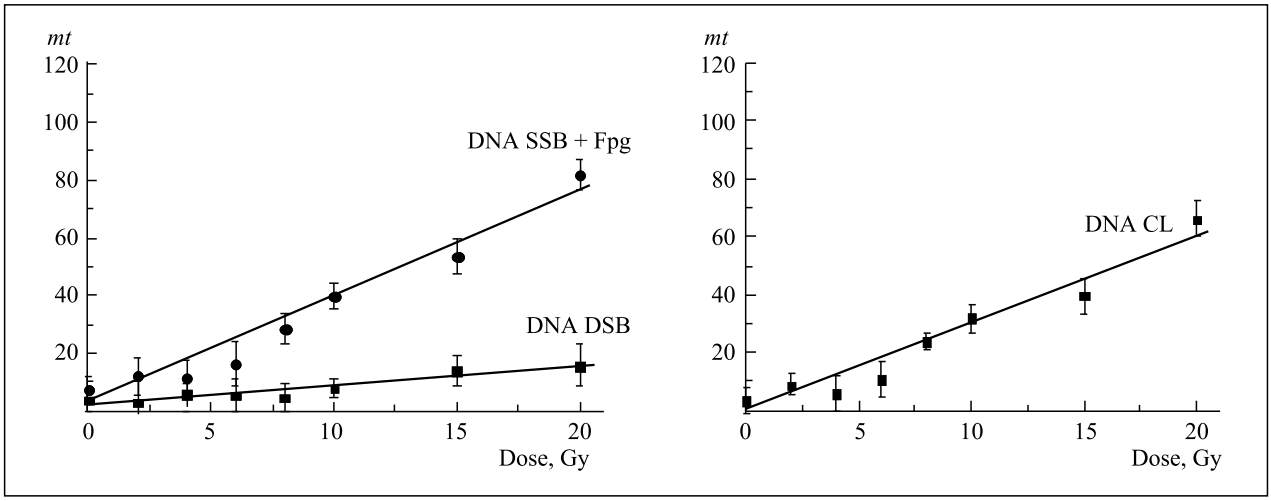

Fig. 2. The dose dependences of the DNA DSB yield in the presence of the Fpg enzyme and clustered lesion (DNA CL) yield under irradiation with ^{60}Co γ-rays

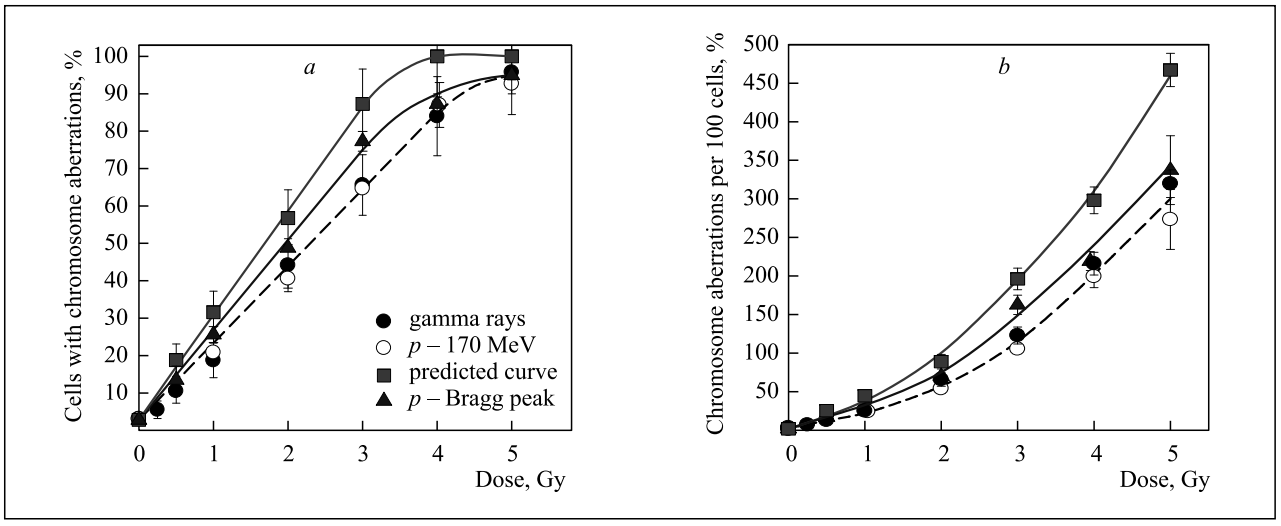

Fig. 3. The dependence of the frequency of the formation of lymphocytes with chromosome aberrations (*a*) and the total amount of chromosome aberrations (*b*) on the dose of irradiation with therapeutic beam protons: ■ — a curve calculated taking into account the contribution of the radiosensitive fraction of G_2 lymphocytes irradiated in the Bragg peak region; ▲ — nondividing lymphocytes irradiated in the Bragg peak region; ○ — nondividing lymphocytes irradiated with 170 MeV protons and ● — gamma rays

A series of studies were completed at the synchrocyclotron medical beam to evaluate the radiosensitivity of cells in different cell cycle phases. It was found that the radiation effectiveness factor taking into account the biological aspect (the radiosensitive fraction of lymphocytes) increased to 1.45 (Fig. 3).

Research has been continued on the mechanisms of radiation-induced apoptosis in human lymphocytes. The kinetics was studied of the change in the level of the expression of genes controlling the synthesis of the P53 protein, caspase 8, and caspase 9. The maximal level of P53 protein expression was observed after approximately two-hour post-irradiation incubation of cells (Fig. 4), which is in agreement with the data obtained by other authors using different cell cultures. It has been shown that caspase 9 is actively expressed in human lymphocytes after irradiation, and the level of caspase 9 expression is observed to increase for up to 24 hours during incubation. The level of caspase 8 expression begins to increase approximately 6 hours after gamma-irradiation and continues to grow for up to 24 hours. The data obtained correlate well with the earlier results on apoptotic cell induction, the maximal level of the formation of which is also observed after approximately 24-hour post-irradiation incubation [3–5].

Regularities were studied in adaptive response induced in three donors' blood lymphocytes by different doses of gamma radiation (2–15 cGy). A high degree of variability was found in the manifestation of adaptive response — both between different donors and in repeated tests of one donor (Fig. 5).

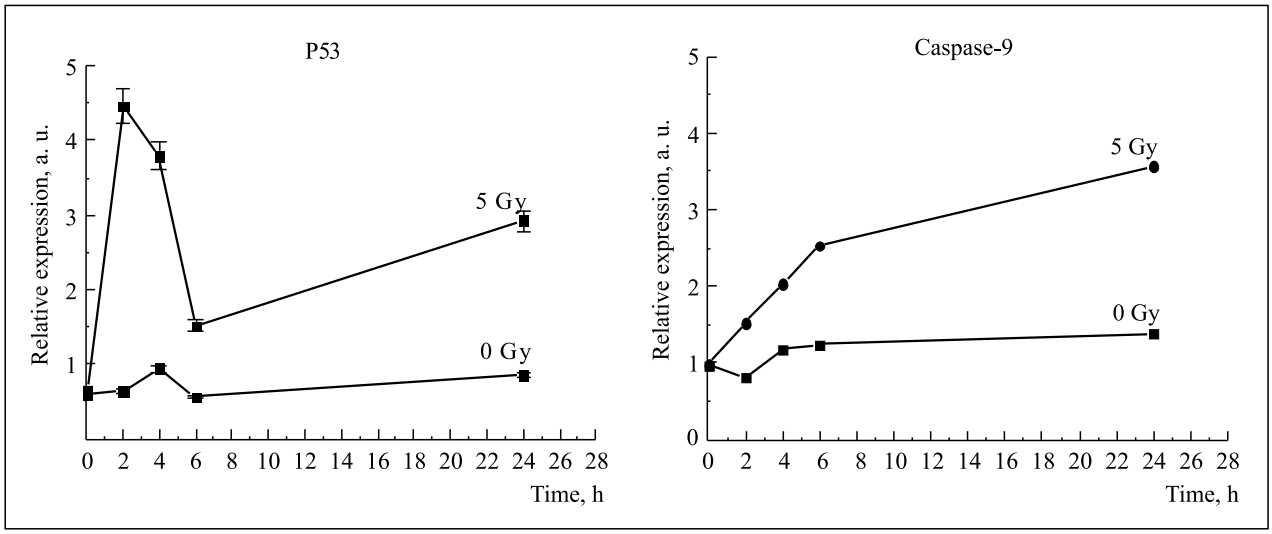

Fig. 4. The kinetics of the expression of genes controlling the synthesis of the P53 protein and caspase 9 after irradiation with ^{60}Co γ-rays

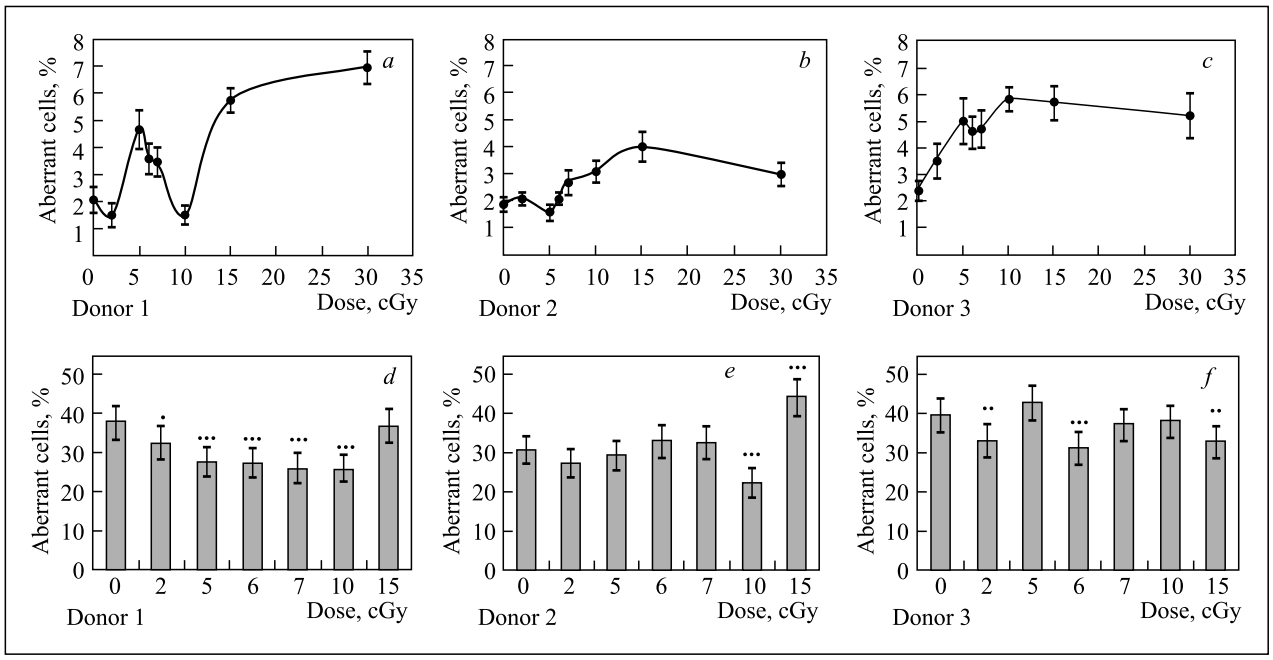

Fig. 5. *a–c*) The frequency of aberrant cells induced by γ-rays of three donors' G_0 lymphocytes. *d–f*) Adaptive response induced by different priming doses in the same donors' lymphocytes (parallel blood samples) shown as the aberrant cell frequency

It has been shown that it is impossible to establish an optimal dose for each individual, which would have a radioprotective effect if used for priming in each of the performed experiments. The same dose could induce opposite effects in all three donors' lymphocytes at different times of the study. Therefore, the extreme instability in the manifestations of this phenomenon does not allow it to be considered as a universal phenomenon that could be used in clinical practice or taken into account in evaluating radiation risks.

Experiments have been continued to study the effect of reactive oxygen species (ROS) and different modifiers on the formation of chromosome aberrations in cal51 cells of human mammary carcinoma under low doses of ionizing radiation (up to 5 cGy). It has been shown that the use of different modifiers, which have a high mutagenic potential and affect the endogenously-generated ROS, makes a significant contribution to the induction of chromosome damage in the low-dose region (Fig. 6). This fact allows one to suggest that radiation-induced oxidative stress and, as a consequence, general disorder in cell homeostasis can significantly affect the fate of the irradiated cell.

The analysis has been continued of the induction of DNA double-strand breaks (DSB) and formation of DNA deletions in lower eukaryote cells after exposure to ionizing radiation. The large size of the plasmid and its nucleosome structure allow the obtained data to

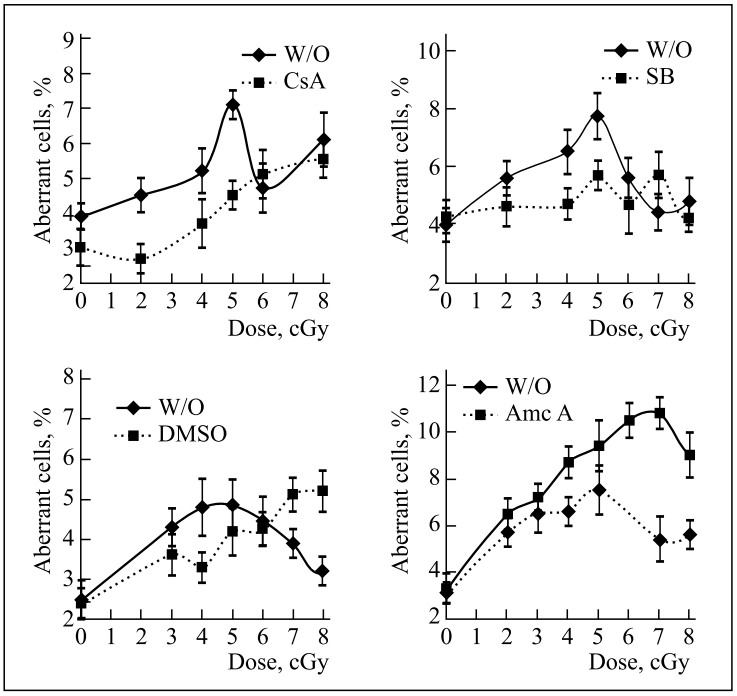

Fig. 6. The effect of modifiers on the yield of aberrant cells: W/O — without modification, DMSO — dimethylsulfoxide (2%), CsA — cyclosporineA (1μM), SB — SB203580 (10μM), Amc A — antimycin A (2μg/ml)

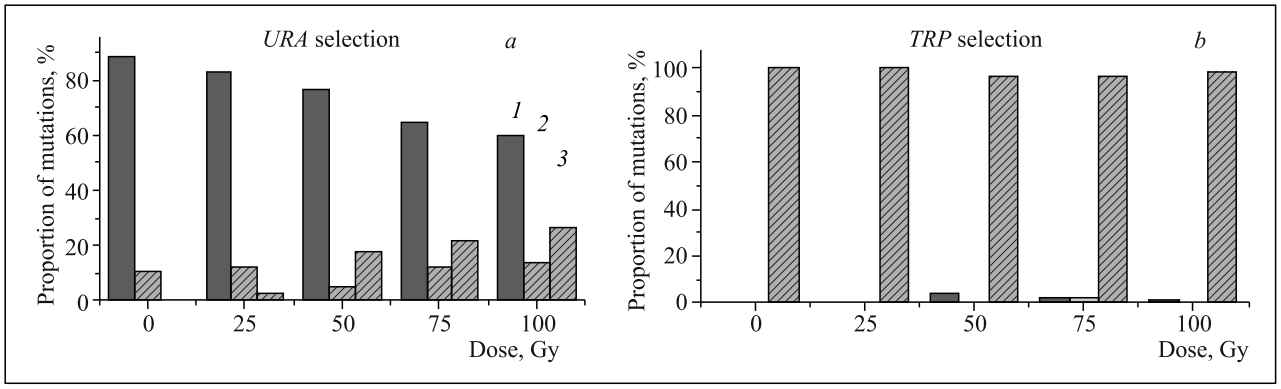

Fig. 7. Spectrum of mutations induced by γ-rays for two systems of selection (*URA3* (*a*) and *TRP1* (*b*)): *1* — deletion includes two genes, *2* — deletion includes three genes, *3* — deletion includes four genes

be extrapolated to chromosome-type DNA. Five genes were inserted into the shuttle vector that had regulatory elements of supporting plasmid in bacterial and yeast cells. With the use of one of them (the URA3 gene) as a selective marker, the loss of the other four genes was studied. Mutants with different spectra of lost genes were isolated (Fig. 7) [6–9].

DNA was isolated from 20 mutants; the precise localization of the deletions is being determined. Work has been started to isolate deletion mutants using other selective markers (TRP1 and LEU2), which will probably allow expanding the deletion size and localization spectrum.

PHOTORADIOBIOLOGICAL RESEARCH

Research has been continued on the effect of accelerated protons, gamma radiation, and the methylating agent methylnitrosourea (MNU) on the retina. Based on data analysis, a concept has been proposed of the genotoxic threshold of retina cell death initiation. The detection of changes in the electroretinograms (ERG) of mice after total gamma-irradiation and MNU introduction in cytotoxic and nontoxic concentrations shows that the ERG is an early and highly sensitive parameter of degenerative changes in the retina under the effect

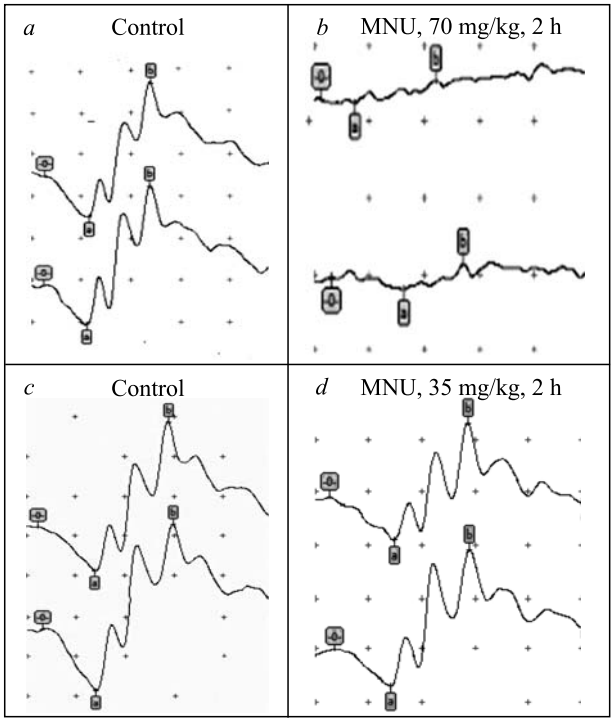

Fig. 8. Electroretinogram profiles of intact mice (*a, b*) and 2 hours after the intraperitoneal introduction of MNU in a cytotoxic dose of 70 mg/kg (*b*) and 35 mg/kg (*d*). The markers indicate (left to right) the ERG start and the ERG a- and b-wave extremums

of the supermutagen MNU (Fig. 8). A decrease in the ERG amplitude was observed 1–3 hours after exposure, while morphological changes in photoreceptors and their death — after at least 24 hours. The ERG amplitude continuously decreases with time, and, finally, the ERG profile completely flattens. Nontoxic concentrations of MNU ($<$ 70 mg/kg) caused significantly weaker and reversible decrease in the mice's ERG amplitude. The total gamma-irradiation of mice with a dose of 14 Gy did not cause any changes in the ERG during 10 hours after exposure. These data support the concept of the genotoxic threshold of induced retina degeneration in mice. It was also shown that not only does the early and irreversible loss of the ERG profile point to the loss of the retina's functional activity, but it also is an early marker of retina degeneration [10, 11].

The research has been supported by the «Fundamental Sciences for Medicine» grant from the Presidium of the Russian Academy of Sciences.

Research has been continued on the cataractogenic effect of ionizing and ultraviolet (UV) radiation in experimental animals. A single mechanism of the development of the cataracts of radiation and UV genesis and the senile cataract was revealed. It has been shown that the ionizing radiation-induced damage of the lens protein plays no significant role in cataract formation. The UV irradiation of the β_L crystallin leads to a dose-dependent lesion in the secondary structure of the protein, decomposition of aromatic amino acids, and molecule photolysis accompanied by the formation of covalent-bonded dimers and complexes of photolysis products formed by noncovalent bonds. At the same time, the contribution of radiation damage to the diffusion lens opacification is greater than that in the case of UV irradiation (Fig. 9).

The molecular mechanism was explained of the interaction between exposures to ionizing and UV radiation in the process of damage development in crystallins. The accumulation of denatured crystallins in the lens results in their aggregation and, therefore, the enhancement of light scattering and lens opacity (a cataract).

Under the cataractogenic factors (aging, UV radiation, ionizing radiation, etc.), the following chain of pathology takes place:

- Nuclear apparatus is damaged; division is disordered; epithelial cells partly die.
- Defective fiber cells containing nuclei and mitochondria are formed from damaged epithelium.
- The violation of epithelium integrity caused by its cell death increases the penetration of oxygen into the lens.

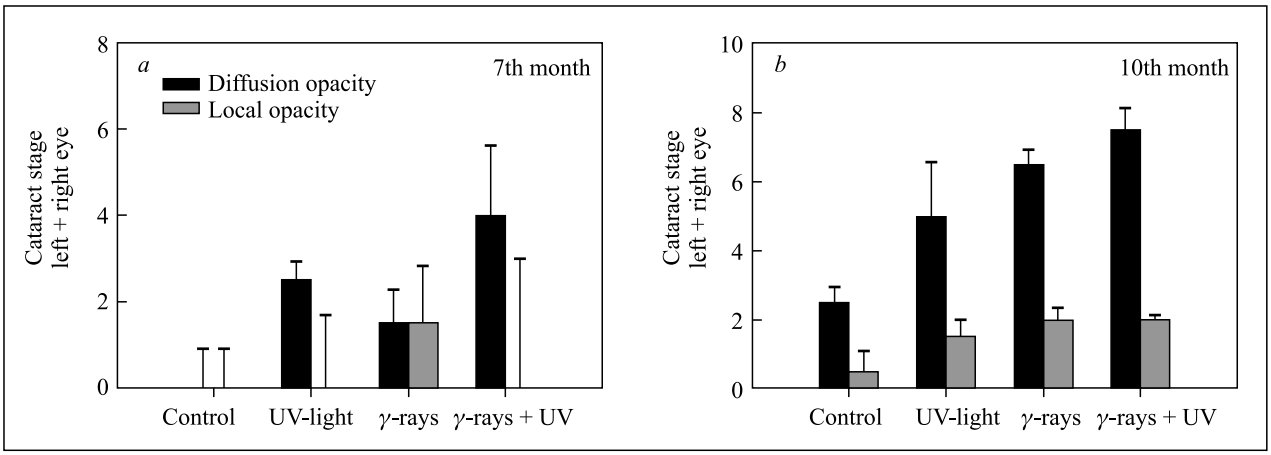

Fig. 9. γ-rays and UV radiation-induced cataract development long time after exposure

- The activity of mitochondria leads to a significant increase in the concentration of reactive oxygen species (ROS).
- ROS damage crystallin proteins.

Thus, the physical basis of the origin of lens opacity consists in the destabilization of the structure of crystallins — the main protein molecules of the lens — which develops under oxidative stress.

MATHEMATICAL MODELING OF RADIATION-INDUCED EFFECTS

Research has been continued on the mathematical modeling of DNA repair mechanisms in cells with different levels of genome organization and the development of model approaches to describing DNA damage induction by heavy charged particles.

New model approaches have been developed to describe the mechanisms of the induction of DNA lesions of different types on the atomic level by accelerated heavy ions. The radial distribution of volume energy and the absorbed dose in accelerated ^{4}He, ^{12}C, and ^{40}Ar ion tracks were calculated in the energy range of 3–20 MeV/nucleon. Comparison of the spatial location of the atoms of the adenine–thymine nucleotide pair and the calculated radial distribution of the dose and volume energy was performed [12]. The performed calculation will allow modeling the induction of primary DNA lesions of different types taking into account the precise location of specific atoms in the nucleotides (Figs. 10–12). This approach will allow taking into account the influence of bond breakage between the atoms of the molecule on the DNA damage yield. With the use of the obtained results, it seems to be possible to evaluate the probability of the induction of DNA lesions taking into account their clustering under irradiation by heavy charged particles.

The model describing the key processes in *Escherichia coli* bacterial cells during the excision repair of damaged bases was improved. On the basis of the stochastic approach to describing biochemical interactions, the mechanism of the removal of damaged bases with the participation of formamidopyrimidine glycosylase (the Fpg protein), which has several types of activity [13, 14], was described in more detail.

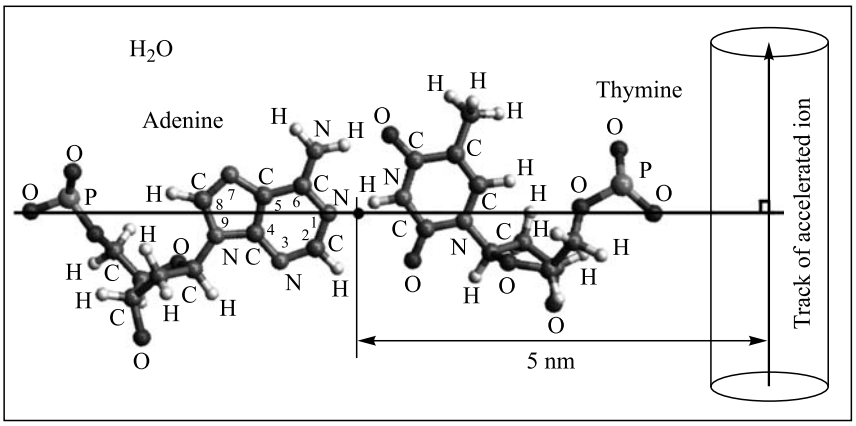

Fig. 10. A scheme of the spatial orientation of an adenine–thymine nucleotide pair relative to an accelerated ion track

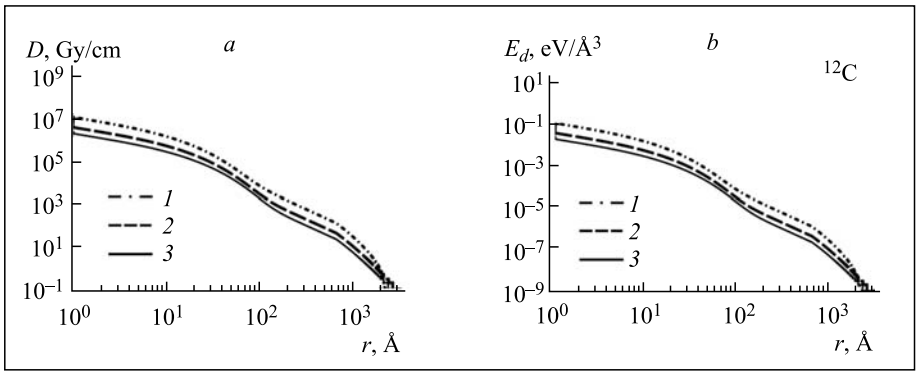

Fig. 11. The radial distribution of the absorbed dose (*a*) and volume energy (*b*) of 3, 10, and 20 MeV/nucleon carbon ion radiation (*1*, *2*, and *3*, respectively); *r* is the distance from the DNA geometric axis

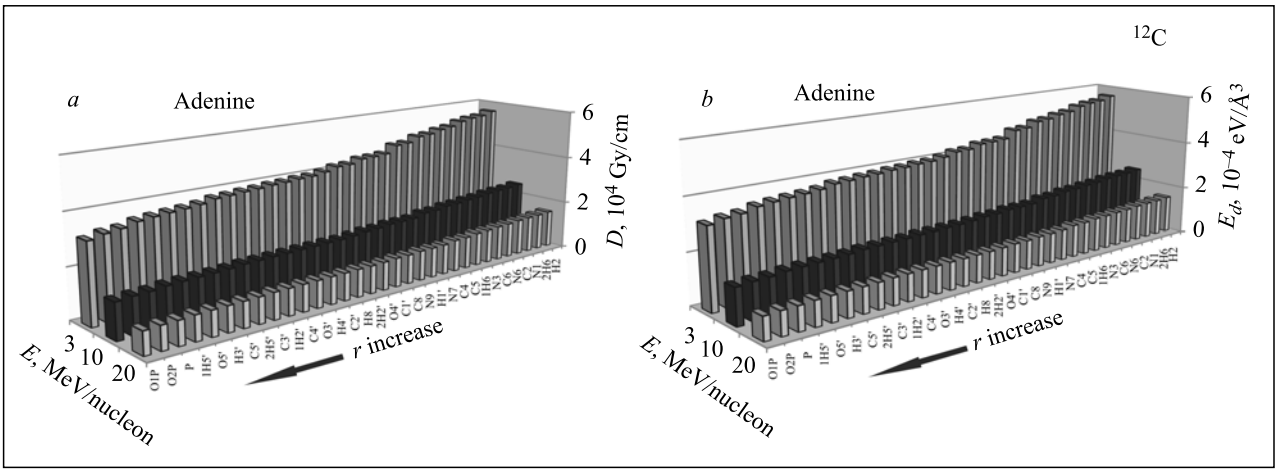

Fig. 12. The spatial distribution of the absorbed dose (*a*) and volume energy (*b*) for an adenine–thymine nucleotide pair in the case of accelerated carbon ions passing 5 nm from the DNA geometric axis

Work has been started to develop models of the genetic control of the molecular mechanisms of repair in higher eukaryote cells under ionizing radiations with different linear energy transfer. In prospect, it seems to be possible to apply the currently used mathematical methods to studying repair mechanisms in human cells and possible evaluation of their effect on different physiological functions of the organism. Theoretical studies of terahertz radiation on the DNA structure have been continued [15].

International cooperation in the group's two main research fields has been continued. In particular, scientists of the National University of Mongolia (Ulan-Bator, Mongolia), Cairo University and the National Cancer Institute (Cairo, Egypt), and the Radiation Dosimetry Department of the Institute of Nuclear Physics of the Czech Academy of Sciences (Prague, the Czech Republic) participate in research on modeling DNA damage and repair mechanisms.

COMPUTER MOLECULAR MODELING OF BIOPHYSICAL SYSTEMS

As part of the international scientific cooperation between JINR and the Arabic Republic of Egypt, an analysis was performed of the molecular dynamics of ethanol solvated by water on a platinum surface using the DL_POLY_2.19 code [16]. The structural and diffusion properties of the ethanol–water solution were studied in the temperature range of 250–600 K. A change in the self-diffusion coefficient of the 50 : 50% ethanol–water solution in the absence of a platinum surface showed an agreement between the calculated and experimental data: the error did not exceed 7.4%. The diffusion coefficients were observed to grow in the pres-

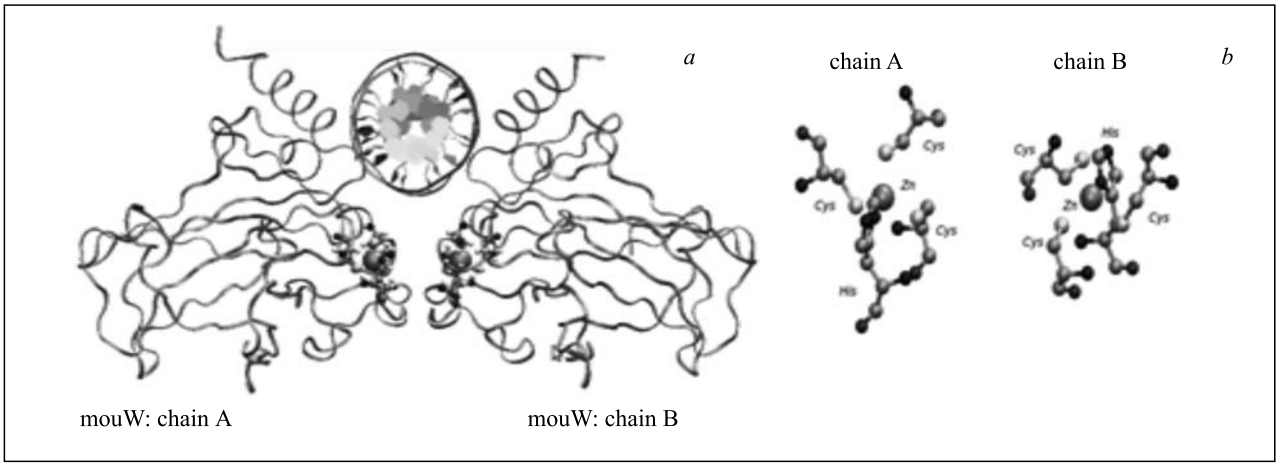

Fig. 13. *a*) The P53 oncoprotein structure. Two branches (A and B) of the protein are shown with a DNA fragment in the center. *b*) Two catalytic centers of the $[Zn(CYS)_3(HIS)_1]$ sites of zinc bonding

ence of a platinum surface, the estimated diffusion coefficients of both components of the solution (water and ethanol) being in agreement with the Arrhenius law. The structure functions of the radial distribution of atoms and density profiles were constructed, and correlations between them and self-diffusion coefficients of the system were illustrated.

In cooperation with LRB's Japanese colleagues (Keyo University, RIKEN, and the Genomic Sciences Centre at RIKEN in Yokohama), research was performed on the molecular dynamics modeling of the structural properties of the P53 protein (Fig. 13). The influence of the Arg273His (R273H) amino acid substitution on the P53 → DNA binding region was studied [17–19]. Two dimer structures were modeled of the native and mutant Arg273His (R273H) amino acid of the P53 protein in the water environment at the same thermodynamic parameters.

It has been shown that the Arg273His mutation can strongly influence the interaction between the P53 protein and the DNA molecule — in particular, in the contact domain region, which can significantly change the picture of hydrogen bonds. Thus, the Arg273His amino acid substitution destroys the existing hydrogen bond in some cases, while in others it forms a strong P53 → DNA hydrogen bond, which is absent in the native protein. Conformational changes were studied in five key amino acid residues in the P53 → DNA binding region; their final relaxation states were compared with the mutant P53 protein. The results illustrate the molecular mechanisms of the P53 protein interaction and bonding with the DNA molecule, which can help understand the physiological aspects of the P53 oncoprotein functioning and its role in the development of cancer.

In 2011, the team of researchers, including Acad. M. A. Ostrovsky of the Russian Academy of Sciences, Kh. T. Kholmurodov, Dr. Phys. and Math., and T. B. Feldman, Dr. Biol., won JINR's Second Prize for Applied Research.

PROTECTION PHYSICS AND RADIATION RESEARCH

The upgrade was completed of the «Genome» facility for the fast automated irradiation of biological samples at nuclear beams of the MC400M cyclotron of the Laboratory of Nuclear Reactions.

Resolving the radiation safety issues for the NICA accelerator complex, which is being designed at JINR, has been continued. To calculate the shielding by engineering methods at the request of the contractor (the close corporation Kometa Complex Medical Technologies and Instruments), detailed simulations of double differential cross sections of nucleon production in the interaction of 4.5 GeV/nucleon gold nuclei with the material of the rings and fluence attenuation length and neutron dose dependences in usual concrete for neutron energies up to several GeV and for thick shielding were performed using the GEANT4 software. Two designs of the beam catcher localizing the nuclear beam halo losses were proposed. These catchers will be the main radiation sources of the collider, and their design will determine the collider's biological shielding. Double differential nucleon yields from the catchers; spatial distributions of the absorbed doses of secondary radiation in the elements of magnetic optics beyond the catcher; induced radiation accumulation dynamics in the catchers taking into account the planned collider operation schedule [20], etc., were calculated. On the basis of the decisions taken concerning the sources of nuclei losses and shielding configuration, a full-scale detailed calculation of the radiation conditions at the collider and in its environment are being performed using the GEANT4 software.

A portable autonomous version of the multisphere spectrometer of neutrons in a wide energy range with a LiI(Eu) slow neutron detector for work in field conditions was designed on the basis of a Lenovo netbook [21]. The spectrometer also includes a channel of monitoring the neutron field on the basis of a ^{3}He counter in a moderator. For spectrometer weight reduction, a compound moderator was fabricated, which consists of several polyethylene hemispheres nesting into each other. The spectrometer is designed to measure the fluence, effective dose, and spectra of neutrons in the radiation fields around nuclear physics facilities — in particular, the NICA complex.

Collaboration between JINR and the Institute of Space Research in the programme of planet research with nuclear physics methods has been continued. In particular, an experiment was conducted at the 600 MeV medical beam of the Phasotron at the Laboratory of Nuclear Problems to study the radiation resistance of quartz glasses of two types. The glasses were irradiated with 160–200 MeV protons in the absorbed dose region of 300–600 Gy. The study was aimed at choosing glasses for repacking the $LaBr_3$ crystal of the gamma-spectrometer of the Russian-made MGNS device on the board of the BepiColombo spacecraft for a mission to Mercury of the European Space Agency.

CONFERENCES AND EDUCATION

In 2011, LRB staff members took part in ten scientific conferences in Russia and four conferences in countries of Asia and Africa.

A round-table meeting «Topical Issues of the Radiation Safety of Long-Term Space Flights» timed to the 50th anniversary of the first manned space flight was held. During the meeting, strategic areas of research on the effect of ionizing radiations of different quality on biological objects and organisms were outlined. It was pointed out that experiments on the effect of high-energy heavy charged particles on the central nervous system and higher neural activity should be performed not only on small laboratory animals, but also on higher primates to model possible disorders in the cosmonauts' operating performance during long-term space flights. The necessity was proved of establishing a European-level vivarium with the participation of all parties concerned.

The Italian – Russian Round-Table Meeting «Astrobiology: New Ideas and Research Trends» was held, which was organized by the Embassy of the Italian Republic in the Russian Federation, JINR, and the Scientific Council on Astrobiology of the Russian Academy of Sciences. The subject area of the Round Table included a wide range of issues connected with the origin of life, evolution on the Earth and in the conditions of space, and the existence of life in the extreme conditions on the Earth and in space. A number of talks by Russian and Italian scientists were concerned with studying space dust and the search for organic molecules in space. Much attention was paid to problems of the search for exoplanets, carrying out long-term manned space missions beyond the Earth's magnetosphere, and application of nuclear physics methods for the analysis of terrestrial and extraterrestrial planets. During the Round Table, a number of important agreements on cooperation between Italian and JINR scientists were achieved. It was proposed that similar meetings with the participation of Italian and Russian scientists be held regularly.

The education process has been going on at the Department of Biophysics, Dubna University. Total student enrolment in the specialty «Human and Environmental Radiation Safety» is 62; five postgraduates attend the Radiobiology specialty programme. In 2011, six new students were accepted to the Department. 14 successfully completed their education and received Engineer-Physicist diplomas.

On the basis of the Departments of Chemistry, Geochemistry, and Cosmochemistry, a course of molecular dynamics is offered to graduate students of Dubna University.

REFERENCES

1. *Boreyko A. V. et al.* Effect of DNA Synthesis Inhibitors on the Induction and Repair of DNA Double-Strand Breaks in Human Lymphocytes under Radiations with Different LET // Part. Nucl., Lett. 2011. V. 8, No. 4 (167). P. 670–678 (in Russian).
2. *Kruglyak A. N. et al.* Regularities and Mechanisms of DNA Damage Formation in Human Lymphocytes under Radiations with Different LET // Stud. Sci. Conf. «Space Era: Society and Science». Korolev, 2011. In press (in Russian).
3. *Savelyeva M. G. et al.* A Study of Regularities and Mechanisms of Human Lymphocyte Apoptosis Induction by Gamma Quanta and 170 MeV Protons // Theses of the 10th Conference of young scientists, specialists, and students timed to the 50th anniversary of the first manned space flight. Institute of Medical and Biological Problems, Moscow, 2011. P. 61 (in Russian).
4. *Baranova E. V. et al.* Radiation-Induced Apoptosis Regularities and Mechanisms // Proc. of the 15th JINR Conference of Young Scientists and Specialists, February 14–19, 2011. Dubna, P. 298–301 (in Russian).
5. *Savelyeva M. G. et al.* Cytogenetic Effects of Cosmic Radiations // Stud. Sci. Conf. «Space Era: Society and Science». Korolev, 2011. In press (in Russian).
6. *Stepanova A. N., Koltovaya N. A.* Induction of DNA Fragment Loss by UV Light in the Yeast *Saccharomyces cerevisiae* // Dubna Univ. Bull. 2010. V. 23, No. 2. P. 8–11 (in Russian).
7. *Belokopytova K. V. et al.* Using the YCpL2 Shuttle Vector for the Analysis of Gamma Radiation-Induced Deletions in Haploid Strains of the Yeast *Saccharomyces cerevisiae* // Proc. of the 15th JINR Conference of Young Scientists and Specialists. Dubna, 14–19 February 2011 (in Russian).
8. *Belokopytova K. V., Koltovaya N. A.* Analysis of the Size of the Deletions in YCpL2 Plasmide DNA Induced by Gamma Rays in *Saccharomyces cerevisiae* // Theses of the 10th Conference of young scientists, specialists, and students timed to the 50th anniversary of the first manned space flight. IMBP, Moscow, 2011. P. 14–15 (in Russian).
9. *Belokopytova K. V. et al.* Dependence of Gamma-Induced Deletion Mutant Spectrum of *Saccharomyces cerevisiae* on the Selection Procedure // Thes. Rep. to the Rus. Conf. with Intern. Participation «Topical Issues of Toxicology and Radiobiology», St. Petersburg, 19–20 May 2011. P. 125 .
10. *Tronov V. A. et al.* Mechanisms of Radioresistance in Terminally Differentiated Cells of Mature Retina // Cell Tiss. Biol. 2012. V. 6(3) (in press).

11. *Tronov V. A. et al.* A Study of Apoptotic Signaling Underlying the Degenerative Changes in Mouse Retina Induced by Ionizing Radiation and Methylnitrosourea // Program of Fundamental Research of the Presidium of the Russian Academy of Sciences «Fundamental Sciences for Medicine», Thes. of Rep. 2011. P. 213 (in Russian).
12. *Aksenova S. V. et al.* Modeling the Spatial Distribution of the Volume Energy and Absorbed Dose of Radiation in the DNA Structure under Accelerated Heavy Ions. JINR Preprint P19-2011-45. Dubna, 2011 (in Russian).
13. *Belov O. V.* Modeling Base Excision Repair in *Escherichia coli* Bacterial Cells // Part. Nucl., Lett. 2011. V. 8. P. 141–148.
14. *Belov O. V.* Modeling Ultraviolet-Induced SOS Response in Translesion Synthesis-Deficient Cells of *Escherichia coli* Bacteria // Part. Nucl., Lett. 2011. V. 8. P. 61–66.
15. *Bugay A. N.* On Terahertz Solitons in DNA // Bulletin of the Rus. Acad. of Sci. Phys. 2011. V. 75, No. 12. P. 1683–1685 (in Russian).
16. *Kholmurodov Kh. et al.* Molecular Dynamics Simulation of the Interaction of Ethanol–Water Mixture with a Pt Surface // Natural Sci. 2011. V. 3, No.12.
17. *Kholmurodov Kh. et al.* MD Simulations of the P53 Oncoprotein Structure: the Effect of the Arg273 → His Mutation on the DNA Binding Domain // Advances in Bioscience and Biotechnol. 2011. V. 2, No. 5; Online: 2156-8502.
18. *Selwyne R., Kholmurodov Kh., Koltovaya N.* Homology Modelling and Molecular Dynamics of Cyclin-Dependent Protein Kinases // IT for Real World Problems, (Universities Press Series in Systems, Models, Informatics and Control) / Ed. Sree Hari Rao. 2011. P. 1–72.
19. *Kholmurodov Kh. et al.* MD Simulations on the Structure of Onco-Proteins P53: Wild-Type and Radioresistant Mutant Systems // Molecular Dynamics of Nanobiostructures. Nova Sci. Publ., 2011.
20. *Beskrovnaia L. et al.* Sumulation of Residual Activity in Steel and Copper Targets Induced by 950 MeV/n Uranium Ions // Part. Nucl., Lett. 2011. V. 8, No. 4. P. 364–367.
21. *Kotelnikov S. G. et al.* A Portable Autonomous Version of a Multisphere Neutron Spectrometer with a Monitoring Sensor for Measurements in Field Conditions // Instr. Exp. Tech. (in press) (in Russian).