LABORATORY OF RADIATION BIOLOGY

In June 2005 the Division of Radiation and Radiobiological Research was reorganized into the Laboratory of Radiation Biology (LRB). The scientific programme of LRB was determined by the first-priority theme of the Topical Plan for JINR Research and International Cooperation that focused efforts on the following main directions:

- radiation genetics and radiobiology;
- photoradiobiological research;
- molecular modeling of biophysical systems;
- physics of radiation protection;
- radiation monitoring at the JINR nuclear facilities and personnel radiation control.

RADIATION GENETICS AND RADIOBIOLOGY

The study on induction of mutations of different nature by ionizing radiation in yeast *Saccharomyces cerevisiae* was continued [1]. Mutagenic property of ionizing radiation was characterized by using four different mutator assays. They were a forward mutation rate assay that detects mutations inactivating the arginine permease gene (Can^r mutations) and reversion assays detecting mutations that revert a 4-base insertion in the *LYS2* gene or that revert a +1T insertion in a stretch of 6 T's in the *HOM3* gene. The reversions to Lys^+ and Hom^+ are due to deletion of a single nucleotide predominantly. Induction of mutations by γ rays was studied earlier. The induction of mutations by UV light and heavy ions is presently investigated. Induction of mutations in haploid yeast cells by ^{7}Li ions with LET of 17 keV/μm was tested. The curves had nonlinear character.

A plasmid system is used for quantitative analysis of extended deletion (about several kbp) formation by ionizing radiation. Dose dependences of induced deletions on UV light and γ rays had nonlinear character. The heavy ions (^{7}Li, 17 keV/μm) also induce this type of mutation. The curves had nonlinear character as well.

The study of genetic control of genetic stability and DNA damage-induced arrest of cell cycle progression (checkpoint) was continued. Earlier several *SRM* genes were identified and some of them were localized (*SRM5/CDC28, SRM8/NET1, SRM12/HFI1*). Now an attempt to clone and identify *SRM2* gene is made. It is planned to study influence of *srm* mutations on the stability of recombinant plasmids. It was shown that *srm2* mutation decreases the stability of centromeric (YCp50) and noncentromeric (YRp12) ARS-containing plasmids.

Together with the Max Planck Institute (Berlin), the *srm5/cdc28-srm* mutation was investigated. Sequencing analysis of *cdc28-srm* revealed a single nucleotide substitution of serine for glycine in position 20 (G20S) in the conservative G-rich loop of protein kinase CDC28. The central role that cyclin-dependent kinases play in the timing of cell division and the high incidence of genetic alteration of CDKs or deregulation of CDK inhibitors in a number of cancers makes CDC28 of yeast *Saccharomyces cerevisiae* very attractive as a model for studies of mechanisms of CDK regulation. Together with group of MD simulations, the investigation of influence of *cdc28-srm* mutation on the structure of kinase (Fig. 1) and interactions with substrates and regulatory proteins was carried out [2]. The crystal structure of human CDK2 that has served as a model for the catalytic core of other CDKs, including CDC28, was used. Nanoseconds long molecular dynamics (MD) trajectories of human CDK2 (fully active complex pT160-CDK2/cyclin A/ATP/substrate) were compared. The MD simulations of substitution of G16S (G20S in CDC28) in these complexes show conformational changes of CDK2 structure leading to the moving of the G-loop away from ATP and opening of the CDK2 substrate binding box. Deformation of G-loop has consequences such as increase in the distance between ATP and substrate. Apparently this is the cause of kinase activity inhibition.

The investigations of chromosome aberrations induction in human peripheral blood lymphocytes by low doses of ionizing radiation with different LET have been continued. The results obtained earlier were confirmed on blood samples of six donors (Fig. 2). At the doses 1–5 cGy the cells showed the highest radiosensi-

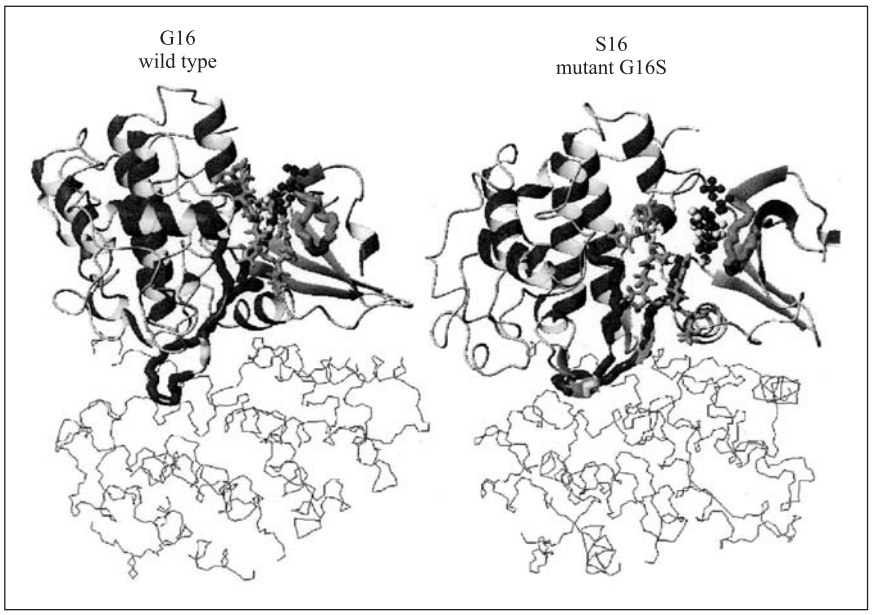

Fig. 1. The calculation of protein kinase structure by molecular dynamics method

tivity (hypersensitivity, HS), mainly due to chromatide-type aberrations, which are typical of those spontaneously generated in the cell and believed not to be induced by irradiation of unstimulated lymphocytes according to the classical theory of aberration formation. With increasing dose the frequency of aberrations decreased significantly, in some cases to the control level. At the doses above 50–70 cGy the dose–effect curve has become linear [3]. Individual variability in amplitude and position of HS peak was observed. In spite of this, significance of nonlinearity of dose–effect curves and availability of HS region were evidenced statistically by regression analyses applied to all donors' data. A similar response to low doses of ionizing radiation with LET of 0.3–16 keV/μm was revealed.

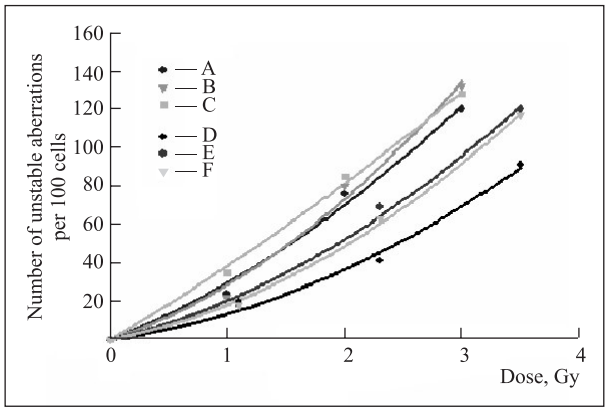

Fig. 2. Chromosomal aberrations in human lymphocytes of six donors after irradiation with 480-MeV/nucleon carbon ions

In collaboration with a biophysical group of GSI (Darmstadt, Germany), a series of experiments have been done on normal human fibroblasts to examine the relationship between cell proliferation and expression of the chromosomal damage after X-ray and particle irradiation (195 and 10 MeV/nucleon C ions; 11 MeV/nucleon Ni ions). It was found that irradiation of confluent cultures of fibroblasts with both sparsely and densely ionizing radiation causes drastic, probably permanent cell cycle arrest in initial G_0/G_1 phase in dose- and LET-dependent manner. As a result, only few irradiated cells can progress to first mitosis. Measurements of chromosomal damage in first cells cycle at multiple time points of postirradiation show the twofold increase of aberration yield with time for the lower doses of low-LET radiation. At higher doses and high-LET irradiation this effect is less pronounced or even disappears due to the rapid chronic cell cycle arrest of severely damaged cells and, as a result, their inability to reach mitosis. These observations are consistent with the other studies that indicate that this response is a specific strategy of fibroblasts to maintain genetic integrity of population and prevent the expansion of genetic alterations.

In collaboration with a biophysical group of the Institute of Biology (Keltce, Poland), a series of experiments have been done. The aim of this study was to investigate if deviations from DNA-proportional distribution of high-LET radiation-induced chromosomal aberrations are individually variable. In all published studies dealing with individual radiosensitivity, lymphocytes were exposed only to low-LET radiation. The growth of exposure to heavy ions in such fields as aircraft, space travel and application in radiotherapy cause increasing interest in radiobiological sciences. This is the first study of individual radiosensitivity of chromosomes of human peripheral blood lymphocytes to heavy ions. Our results suggested that the interdonor variabil-

ity is a potential source of error in calculating the dose absorbed by one individual on the basis of a calibration curve generated with lymphocytes of a different individual and this error can be minimized by choosing chromosome 2 for analysis. Moreover, the present results support the view that the ratio of centric rings to dicentrics (F ratio) could be a signpost to estimate high-LET exposure. Thus, this is especially interesting for the radiobiology of heavy ions. The present results cause new questions in the field of radiosensitivity to high-LET radiation and show that further investigations are needed.

The estimation of influence of the therapeutic proton beam (JINR's Phasotron) on the human cells after cytogenetical damages in peripheral blood lymphocytes has been made. The peripheral blood lymphocytes were used as a model for the study of human cell damages. The purpose of the investigation was to study the quantity and quality particularities of arising chromosome aberrations and to estimate proton beam efficiency for an initial energy of 170 MeV and the Bragg peak by cytogenetical tests. The effects of initial beam protons and γ rays in the whole dose range did not differ practically. The Bragg peak protons are also of higher efficiency as compared to the protons of initial beam and γ rays. So the fraction of cells with multitude aberrations (3 and more) was 2.5–3 times their number after irradiation with 170-MeV protons (27 and 10% accordingly). After a dose of 5 Gy, multitude aberrations arose in a larger number of cells and this destruction decreased (89 and 81% accordingly) [4].

In real practice, irradiation of tumors is realized with several directions (up to 7). This method decreases a damage of normal tissue. Analysis of the obtained data has shown that effects shall differ most essentially at a total dose of 3–4 Gy on the tumor. So at a dose of 3 Gy, the fraction of cells with chromosomal aberrations is about 80%, but it does not exceed 10% in normal tissues. The difference may by even greater when it is considered that, firstly, irradiation causes a delay of cell division start, as many investigators have shown. It is pronounced for cells heavily damaged by high radiation doses and part of them does not reach mitosis at all. As we have noted, the fraction of these lymphocytes with great number of chromosome aberrations increased essentially. Therefore, it is very likely some part of such cells was not registered during microscoping and, secondly, tumors cell population is asynchronous and the most part of cells is more radiosensitive than the cells of normal tissue and the irradiated lymphocytes that are in G_0-phase of cellcycle.

PHOTORADIOBIOLOGICAL RESEARCH

The measurement of small-angle neutron scattering in the solution of α crystalline (concentration 8 mg/ml) with variation contrasts in four buffers has been carried out. The first buffer contained 100% H_2O, the second 77% H_2O – 23% D_2O, the third 32% H_2O – 68% D_2O, the fourth 10% H_2O – 90% D_2O. The preliminary analysis of observations was done. Radius of gyration and maximum size and volume of α crystalline in buffers with different D_2O concentration were obtained. The values of these structural parameters are practically independent of D_2O concentration in buffer and are similar to the parameters obtained by the method of small-angle X-ray scattering. The independence of gyration radius from D_2O concentration in buffer is evidence of homogeneous density of α-crystalline macromolecule. This is a new experimental fact.

The investigation of temperature realignment of α-crystalline macromolecule by the small-angle neutron scattering method has been carried out as well. The measurements have been done with the buffer containing 90% D_2O at temperatures 20, 50, 60, 65, 75, 85 and 95 °C during the heating and cooling stages and with the buffer containing 100% H_2O at 20 °C before and after heating to 95 °C. As a result of the experiments for the first time the structural data about α crystalline were obtained by the small-angle scattering method in a wide range of high temperatures (the measurements were carried out directly at high temperatures). These data will be used for analysis of α-crystalline realignment at high temperatures. This is important for understanding of the mechanism of shaperon-like α-crystalline activity depending on temperature prehistory of this protein.

COMPUTER MOLECULAR MODELING OF BIOPHYSICAL SYSTEMS

Studies on the visual pigment rhodopsin have been performed through the computer molecular dynamics simulations. The visual response of living cells and consequent cascade of biochemical events are mediated by a superfamily of membrane receptors known as G-protein-coupled receptors (GPCRs). They play a key role in all regulatory processes of living organisms. The signaling ways, being regulated by these receptor-proteins, determine many important biological processes, including the processes of sensor reception, endocrine regulation and synaptic transfer. The visual signals and broad spectrum of biochemical events sensed by these receptors make them one of the most intriguing targets for pharmacological innovations and

drug interventions. Nowadays, over 50% of the drugs currently used by humans affect or involve the GPCR. Unfortunately, there still remain many problems that need elucidation, say the molecular mechanisms of visual response, the mechanism of transmembrane signal transduction and so on. Understanding and precise description of the conformational changes, involving the transformation of an inactive GPCR into an activated form and being capable of interacting with a G-protein, has to be a fundamental question of this matter. It is known that all of the membrane GPCR-receptors possesses the same conformational entity, viz. the form of a seven transmembrane helical structure.

However, the detailed X-ray or NMR-structures of the GPCR, with the exception of the visual pigment rhodopsin, are still unknown. One may only suppose that the similarities in the sequences of these receptors have to imply that they have to share a common activation mechanism. Rhodopsin is the first GPCR with a less or more well-defined tertiary structure. It was determined in 2000, thereby representing itself an excellent candidate for the investigation of molecular details not only for visual processes but for the functional mechanisms of the whole receptor family as well.

Computer molecular dynamics simulations of rhodopsin protein [5, 6] show that 11-cis retinal chromophore is rearranged after its insertion in the chromophore pocket. Namely, the beta-ionone ring of the chromophore retinal is twisted in a time frame of 0.4 ns from the start of the simulation run. A clear correlation is observed between the beta-ionone ring twist and mobility of the cytoplasmic domain responsible for the G-protein binding and stabilization of alpha helix H-VI that is characteristic of the rhodopsin dark-adapted state. The changes in the behavior of nearest amino acid residues, surrounding the chromophore retinal, correlate with the beta-ionone ring twist. The computer simulation results are discussed with the actual role that 11-cis retinal chromophore plays being a ligand-antagonist in rhodopsin protein as a G-protein-coupled receptor. These intermolecular events may be considered as a transition process for the chromophore, to behave as a ligand and a powerful antagonist, so that to be switched to a state of an efficient agonist thereby activating the G-protein-coupled receptor. Thus, based on the simulation data, one can conclude that the chromophore adaptation process in its binding site initiates the most important conformational rearrangement of the surrounding protein.

As a result, the chromophore retinal has to lead the rhodopsin molecule not only to a state of a higher alert (viz. photoactivation), but it also stabilizes the inactive state of rhodopsin.

PHYSICS OF RADIATION PROTECTION

The development of radiation protection conception for the SAD project (with participation of the LRB specialists) has been completed. A big volume of calculations was carried out for radiation environment in the dwellings of the subcritical assembly building taking into consideration different radiation sources — 660-MeV proton losses within the beam transport line and magnet optic elements, leakage neutrons from the shield of the assembly active zone, leakage neutrons from the solid Phasotron shield. On the grounds of the obtained data the allocation of the radiation impact areas inside the SAD building at different modes of the installation operation was proposed. The calculation of the neutron dose rate in environment around the LNP Phasotron, the estimation of the induced radioactivity of the equipment and in the magnet hall, as well as the estimation of the atmospheric injection activity, were done. The measurements of the leakage neutron dose spatial distribution at the SAD building site were carried out. The investigation of the neutron-induced radioactivity of the Phasotron border soil was done as well.

The calculation was performed and design of radiation shields against neutrons for mobile and stationary custom installations for identification of hidden explosive and narcotic substances was developed.

The radiation detector responses study was continued. The calculation of the multisphere neutron spectrometer response functions at neutron energy up to 20 MeV was finished [7]. The calculations of the X-ray detection efficiency for scintillation and Si detectors were carried out.

In the framework of the collaboration between the Space Research Institute (Moscow) and JINR on the planet surface research programme, the preliminary estimation of characteristics of the LEND device assigned for the Moon surface scanning was produced.

Three radiobiological experiments at the Nuclotron (with 480-MeV/nucleon carbon nuclei and 1-GeV protons), as well as at the U400M (boron-11 nuclei with energy 37 MeV/nucleon), were carried out in 2005. During the runs patterns of human blood lymphocytes and eye proteins were irradiated.

RADIATION MONITORING AT THE NUCLEAR FACILITIES AND PERSONNEL RADIATION CONTROL

The radiation monitoring for occupational exposure at JINR nuclear facilities is carried out by the automatic systems of radiation control (ASRC) and by portable radiometers and dosimeters.

In 2005 the individual dosimetry service maintained dose control for 1606 persons, including 54 visitors. The average individual yearly dose was 1.4 mSv at JINR. The maximum individual yearly dose was at FLNP (7.9 mSv).

The regular environmental monitoring of soil, plants and water from the river basins in the Dubna vicinity confirmed the conclusion that the environmental radiation pollution around the JINR has remained constant for a long time and is due to natural radioactivity and products of global fallout only. Any contribution to radioactivity pollution of the environment from JINR nuclear facilities was not found.

A large body of work on the radiation safety guarantee was executed at the IBR-30 dismantling in 2005. The exceeding of planned personal doses was not observed owing to the accepted arrangements. As a whole, the level of radiation protection and control at JINR corresponds to the federal rules and regularities in the field of nuclear energy.

SCIENTIFIC MEETINGS AND EDUCATIONAL ACTIVITY

The III international conference «The Genetic Consequences of Emergency Radiation Situations» and the workshop «The Actual Problems of Space Radiobiology during Long Orbital and Interplanetary Flights» coordinated with the conference took place in Dubna on 4–7 October. It was organized by the Scientific Council of RAS on radiobiology, SRC RF — Institute for Biomedical Problems, the Institute for Biochemistry Physics of RAS, the Institute of General Genetics of RAS and the Joint Institute for Nuclear Research. The main theme of the conference was examination of fundamental problems of radiation genetics with reference to people, animals and plants irradiated in crucial radiation situations. The conference and workshop scientific programmes included 52 plenary, sectional and poster reports. More than 85 physicists and radiobiologists from Russia, Ukraine, Belarus, France, Germany, Poland, Bulgaria and JINR participated in the conference and workshop.

The educational process at the chair «Biophysics» of the International University «Dubna» was continued. A total of 65 students are studying now on specialty «Radiation Protection of People and Environment». Tewnty-two new students were admitted in 2005 to the chair. The first graduation of seven students took place in 2005.

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