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RESEARCH PROGRAMME OF THE LABORATORY OF RADIATION BIOLOGY: ITS PERFORMANCE IN 2005 AND THE PROGRAMME FOR 2006

Report to the 99th Session
of the JINR Scientific Council
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Dubna 2005

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1. Scientific research programme for 2005

The Laboratory of Radiation Biology was established by order of JINR Director academician of RAS V.G. Kadyshevsky signed in June 2005. This decision is an proper result of long formation of one part of fundamental biology and radiation investigation in the Institute. The research programme of LRB is determined by the 1-st priority theme, that was concentrated in 2005 on the following main directions: fundamental radiogenetics research at heavy charged particle beams, investigation of radiobiological processes in eye structures, research in the field of molecular dynamics, radiation investigations and radiation protection at the basic nuclear facilities of the JINR and in environment.

2. Execution of the 2005 programme

2.1. Radiation research

The physics support of the biological experiments *was carried out. Several experiments with carbon ions (480 MeV/nucleon) and protons (1 GeV) were performed at the nuclotron.*

The radiation protection conception for the SAD project consists in constructing a low-power integral system on the basis of 660 MeV proton accelerator and a sub-critical core fuelled with uranium-plutonium mixed oxide was completed in the frame of first design stage. For investigation of the neutron induced radioactivity of the soil around the phasotron some holes were bored at the phasotron border. The of soil samples from different places and depth were measured by gamma-spectrometers. It was found the negligible quantity of the induced radioactivity in these samples as compared with natural soil.

The radiation shields for portable neutron generators amounting to mobile and stationary castoms posts for identification hidden explosive and narcotic substances were constructed.

The radiation detector responses study. The work on X-ray spectrometer application for radiation investigations was started. The calculations of the X-rays detection efficiency with thin NaI(Tl) and Si-detectors were carried out by the "Titan" code.

In the frame of the participation in the planet surface research programme the first calculation of the collimated neutron detector efficiency for the Moon spacecraft was done.

Area and occupational personnel radiation monitoring in the field of the JINR nuclear installation was continued.

2.2. Radiation genetic research

The study of induction of mutations different nature by ionizing radiation in yeast *Saccharomyces cerevisiae* was continued. 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 reversion to Lys^+ and Hom^+ is due to deletion of a single nucleotide predominantly. Induction of mutations by γ-ray was studied earlier. The induction of mutations by UV-light and heavy ions is investigated at present. Induction of mutations in haploid yeast cells by ^{7}Li-ions with LET=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 from UV-light and γ-ray 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 the several *SRM* genes have been identified and some of them have been localized (*SRM5/CDC28, SRM8/NET1,*

SRM12/HFI1). Now the attempt to clone and identify *SRM2* gene is carried out. It is planned to study influence of *srm* mutations on stability of recombinant plasmids. We showed that *srm2* mutation decrease stability of centromeric (YCp50) and non-centromeric (YRp12) ARS-containing plasmids.

Together with Institute of Max-Plank (Berlin) the *srm5/cdc28-srm* mutation was investigated. Sequencing analysis of *cdc28-srm* revealed a single nucleotide substitution of glycine with serine 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 (Dr. Kholmurodov Kh.T) the investigation of influence of *cdc28-srm* mutation on the structure of kinase and interactions with substrates and regulatory proteins was carried out. 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 G16S (G20S in CDC28) in these complexes shows 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 of distance between ATP and substrate. Apparently this is the reason of kinase activity inhibition.

2.3. Radiobiological research

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 6 donors. At the doses 1-5 cGy the cells shown the highest radiosensitivity

(hypersensitivity - HS), mainly due to chromatide-type aberration, which are typical to those spontaneously generated in the cell and believed not to be induced by irraidation 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 have become linear. Individual variability in amplitude and position of HS pick was observed. In spite of this, statistically significance of non-linearity of dose-effect curves and availability of HS region were evidenced by regression analyses applied to all donor's data. The similar response to low doses of ionizing radiation with LET 0,3-16 keV/μm was revealed.

In collaboration with biophysical group of GSI (Darmstadt, Germany) the series of experiments have 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/nuclon C ions; 11 MeV/nuclon Ni ions). It was found that irradiation of confluent cultures of fibroblasts both with sparsely and densely ionizing radiation cause 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 post-irradiation shows the increase of aberration yield with time up to twofold 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 biophysical group of Institute of Biology (Keltce, Polska) the series of experiments have 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 grow of exposure to heavy ions in such fields as aircraft and space travel and due to their increasing 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 inter-donor variability 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, present results support the view that the ratio of centric rings/dicentrics (F-ratio) could be a signpost to estimate to high LET exposure. Thus, this is especially interesting for the radiobiology of heavy ions. Present results cause next questions in the field of radiosensitivity to high LET radiation and the further investigating are need.

The estimation of influence of therapeutic proton beam (phasotron JINR) action on the human cells after cytogenetical damages in peripheral blood lymphocytes. The peripheral blood lymphocytes were used as a model for the study of human cell damages. The purpose investigation was the study of quantity and quality particularities of chromosome aberrations arising and estimation of proton beam efficiency for initial energy of 170 MeV and the Bragg peak by cytogenetical tests. The effects of initial beam protons and γ-rays at all dose diapason did not differ practically. The Bragg peak protons are higher efficiency also as in compared to protons of initial beam and γ-rays. So the fraction of cells with multitude aberrations (3 and more) exceeded in 2,5-3 times their number after irradiation by protons 170 MeV (27 % and 10 % accordingly). After the dose of 5 Gy multitude aberrations arise in more number of cells and this destruction decreased (89 % and 81 % accordingly).

In real practice an irradiation of tumors is realized dose diapason with a several directions (up to 7). This method decreases a damage of normal tissue. Analysis of obtained data has shown that effects shall differ the most essentially at summery dose of 3-4 Gy on the tumor. So at the 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 more if it take into consideration that, firstly, irradiation causes a delay of cell division start, as many in vertigator have shown. It is pronounced for heavy damaged cells by high radiation doses and part of them does not arrive 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 cell was not registrated during microscoping and secondly, tumors cell population is asynchronous and the most part of cells is more radiosensitive then the cells of normal tissue and the irradiated lymphocytes, that are in G_0-phase of cell cycle.

The study of individual radiosensitivity of chromosomes of human peripheral blood lymphocytes to heavy ions was started. The aim is to verify the heterogeneity of lymphocytes sensitivity to high LET radiation. There was checked whether the distributions after high LET radiation-induced chromosome aberrations among the whole-chromosome dependent on the DNA content of the painted chromosomes. The first results indicate inter-donors variability of radiation-induced aberrations in the painted chromosomes 2, 8 and 14. It was found that the chromosome 2 is less sensitive than expected and the extent of inter-donor variability is minimal. The chromosome 8 is more sensitive that it was expected. Moreover, significant inter-donor variability of aberration frequency was indicated. These observations are consistent with the findings of others investigators. On average, in chromosome 14 similarly like chromosome 8 were observed the relative The comparison of complex exchange frequency with our results after γ-rays irradiation shows the absence of significantly increasing of aberrations frequency. However, present dates show the inter-chromosomes as

well as inter-donors differences. Similarly as in the even of simply exchange in the chromosome 2 were found the lowest level of complex exchanges while the highest frequency of complexity was observed in the chromosome 8. However, present dates show the inter-chromosomes as well as inter-donors differences. Similarly as in the even of simply exchange in chromosome 2 were found the lowest level of complex exchanges while the highest frequency of complexity was observed in chromosome 8. The results suggested that the inter-donor variability 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.

2.4. Photo-radiobiological 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 on 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 on 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 of 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 of 100 % H_2O at 20 °C before and after heating to 95 °C.

As a result of the experiments for the first time were obtained the structural data about α-crystalline by the small angle scattering method in wide range of high temperature (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 mechanism of shaperon similar α-crystalline activity depending on temperature prehistory of this protein.

2.5. Computer molecular modeling of biophysical systems

Studies on the visual pigment rhodopsin basically 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, are 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 makes them one of the most intriguing targets for pharmacological innovations and drug interventions. Nowadays, over 50 % of the drugs currently used by humans are affect or involve the GPCR. Unfortunately, there yet remain a much of problems need to elucidate, 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 to interact with a G-protein, has to be of a fundamental question of these matter. It is known 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 the only to 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 a more well-defined tertiary structure. This was the only determined in 2000-year, thereby representing itself as an excellent candidate for the investigation of molecular details not only for visual processes but for the functional mechanisms of all receptor family as well. It is worth noting that until now we cannot answer yet many important questions so that to be able testify them through the direct experimental measurement. For example, the resolution of the recent diffraction data has not to be sufficient enough to determine the reliable 3-dimensional structure of the chromophore at physiological temperature scales, on which the high-quality excited-state calculations are generally fulfilled.

It is clear, that correct chromophore conformation is necessary to obtain a more precise model for the rhodopsin reaction center, viz. the protein binding pocket of rhodopsin. These questions can be addressed for computer simulations and model calculations. Thus, for today the only adequate computer simulation is providing the details of the function and complicated molecular motions, which are difficult, expensive, and time consuming, to be captured from the experiment or static structural data of an isolated condition. The dark-adapted state of rhodopsin with 11-cis retinal chromophore inside was investigated through the molecular dynamics simulation method. The main goal of the calculations was to understand the molecular interaction mechanisms of the 11-cis retinal with opsin, simulation the physiological regeneration of the visual pigment and to find the answer what kind of reorganization in rhodopsin structure makes this molecule as a super high sensitive receptor.

Computer molecular dynamics simulations of rhodopsin protein show that 11-cis retinal chromophore to be 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 for 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 as a ligand-antagonist in rhodopsin protein as a G-protein-coupled receptor. These intermolecular events may be considered as an 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 our simulation data, we conclude that the chromophore adaptation process in its binding site initiates the most important conformational rearrangement of the surrounding protein.

As results, 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.

3. Scientific research programme for 2006

3.1. Radiation research in 2006 will be concentrated on the following main lines:

- *Shielding calculations and design.* It is planned to continue the design of the radiation protection system for the SAD installation. The participation in the planet surface research programme and others JINR projects will be continued;
- *Physical support of radiobiological experiments.* The radiobiological experiments with heavy nuclei at nuclotron will be carried out for study of chromosomal aberration in human blood lymphocytes and for study of lens proteins aggregation under action of ionizing radiation with high LET;
- *Response Detectors Study.* The study of the responses of different type radiation detectors will be continued.

3.2. Radiobiological research in 2006 will be connected with:

- *Study of induction and reparation of double strand breaks of DNA in human lymphocytes after irradiation by accelerated heavy charged particles with broad region of LET;*
- *Study of regularities of unstable and stable chromosomal aberrations in human lymphocytes after irradiation by heavy ions with high energy;*
- *Investigation of mutagenic effects of heavy charged particles in low eukaryotic cells;*
- *Study of mechanism of cataract genesis induced by heavy charged particles, radiobiological effects of heavy ion irradiation on retina and visual pigmen, structure of visual pigment by small-angle scattering of neutrons method.*
- *MD simulations of DNA, RNA and proteins, studies of the mutation formation, computer molecular dynamics simulation analysis.*

4. 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 October 4 – 7. It was organized by the Scientific Council of RAS on radiobiology, SRC RF – Institute for Biomedical Problems, Institute for biochemistry physics of RAS, Institute of general genetics of RAS and 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 at 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, Belorussia, France, Germany, Poland, Bulgaria and JINR participated in the Conference and Workshop.

The education process at the chair "Biophysics" of the International University "Dubna" was continued. 65 students in sum are studying now on specialty "Radiation protection of people and environment". 22 new students were admitted in 2005 to the chair. The first graduation of the 7 students took place in 2005.

5. Administration activity

Personnel. The total personnel of the LRB were 106, including the Directorate staff 17.

Finance. Funding of research in the direction of radiation and radiobiological investigations in 2006 is shown in Table 1.

Table 1. Financing LRB in 2006.

Area	Financing plan (k$US)
08-9-1015-96/2008 (1-st priority)	331,0
Infrastructure	74,0
Total	405,0