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E. A. Krasavin, G. N. Timoshenko

RESEARCH PROGRAMME OF THE LABORATORY OF RADIATION BIOLOGY: ITS PERFORMANCE IN 2006 AND THE PROGRAMME FOR 2007

Report to the 101st Session
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Dubna 2006

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1. Scientific research programme for 2006

The research programme of the Laboratory of Radiation Biology (LRB) determined by the 1st priority theme was concentrated in 2006 on the following main directions: fundamental radiobiological and radiation genetic research with heavy charged particle beams, investigation of molecular photo- and radiobiological processes in eye structures, research in the field of molecular dynamics, radiation research and radiation protection at the basic nuclear facilities of the JINR and its environment.

2. Execution of the 2006 programme

2.1. Radiobiological and radiation genetic research

The study of molecular damages in peripheral human blood lymphocytes after irradiation by γ-rays and accelerated heavy ions was continued. The regularities of induction and reparation of double strand-breaks (DSB) in cells irradiated with ^{60}Co γ-rays and lithium ions (^{7}Li, linear energy transfer 20 keV/μm) by using comet assay analysis were studied. The histograms of cell distribution on the level of their DNA violation after γ-ray and heavy ion irradiation were obtained. It was shown that in control samples the value of "tail moment" is negligible but its distributions are revealed with growing of the irradiation dose. The distribution is shifted to the more values of the "tail moment". The relationships for DSB yield on the dose of used types of radiation were built on the basis of obtained results. The linear dose-effect dependences were revealed for γ-rays and heavy ions. The analysis of the results demonstrates that heavy ions are more effective on the induction of DSB in comparison with γ-rays. The coefficient of relative biological effectiveness of accelerated lithium ions is 1.6±0.1.

The regularities of induction and reparation of DSB under influence of inhibitors of DNA synthesis arabinofuranosyl cytosine (Ara C) and hydroxyurea (HU) in γ-irradiated cells were studied. The combination of these agents blocks not only the replicative but also the reparative synthesis of DNA. As was shown the short gaps

in DNA transform to the enzymatic DSB, as result of the S_1-endonuclease impacts of the opposite strand of DNA. It was established that in comparison with the normal conditions the number of DSB increases up to ~3 times after 2h in irradiated cells under Ara C+ HU influence. The differences in DSB number in irradiated cells under normal conditions and under Ara C+ HU after 5h rich up to 7. This explains the effective DSB repair in cells under normal conditions and transformations of single strand gaps into enzymatic DSB under inhibitors of DNA repair synthesis. At the following experiments with heavy ions when mainly direct DSB will be formed in DNA are planed to study the influence of inhibitors DNA synthesis on the yield of DSB and kinetics of their repair.

The investigations in the range of low doses of ionizing radiation were extended. The additional data confirming nonlinearity of dependence of chromosome aberration frequencies on the dose in human peripheral blood lymphocytes were obtained. Namely irradiated cells displayed hyper radiosensitivity (HRS) at very low doses with a pick around 5-7 cGy determined by chromatid type of aberrations. With the subsequent increase of the dose to 10-15 cGy, the aberration yield decreased significantly and demonstrated an inverse dependence on the dose. At $\geq$ 50 cGy the dose-effect curve became linear with a less steep slope as compared to the initial one increased radioresistance (IRR). Analysis of literary data concerning the mechanisms of low dose action allowed us to hypothesize about possible cellular processes underlying HRS and IRR. The most probable cause of highest aberration yield in the region of extremely low doses is radiation-induced drastic elevation of generated endogenous reactive oxygen species (ROS). At the same time, the ensuing decrease of aberration frequency may be caused by activation of cytoprotective signaling pathways (mostly Erk protein kinase) that is aimed to reduction of oxidative stress. In order to verify this hypothesis we started the work with human mammary carcinoma cells cal51 allowing us to apply variety of inhibitors and activators of cellular processes that suppose to be involved in realization of atypical phenomena of low dose radiation.

For the investigations of high LET radiation-induced chromosomal aberrations DNA-proportional deviations distribution in individual variability were continued in collaboration with biophysical group of Institute of Biology (Keltce, Polska).

In all published studies concerning with individual radiosensitivity the lymphocytes were exposed only by low LET radiation. Our studies showed that the inter-donor variability acts as a potential source of mistakes at absorbed dose quantification. Choosing chromosome 2 for analysis could be minimize this error. Moreover, present results support the point of view that the ratio of centric/dicentric rings (F-ratio) could be a signpost to estimate to high LET exposure.

The series of experiments on whole blood samples irradiated with heavy ions ^{11}B, ^{17}Li and ^{20}Ne have been done. The frequency of dicentric + centric rings in the first post-irradiation metaphases and PCC excess fragments of human peripheral blood lymphocytes of several donors has been studied. The obtained results are in agreement with previous studies data.

Together with the Institute of Biochemical Physics RAS the investigation for item "New experimental and theoretical approaches for study of biophysical and molecule-cytogenetical chromosome instability mechanisms induced by radiation with different LET to prognosis of radiation cancergenic risks" has been performed. Obtained data allow us to estimate the possible mechanisms of chromosome instability for the human and mammalian cells and show the following direction for such problems investigation.

The new method was developed to detect of hidden defects in membranes of human blood erythrocytes. The method consists in membrane electroporation application for detection of hidden disorders of membrane electrodynamics characteristics of erythrocytes. We had shown the possibility to use the method for detection of damaging action of radiation on the membranes. Research of laws of gamma ray action in wide range of radiation dozes on membranes of human blood erythrocytes has been continued.

Several directions of our genetic research were developed in this year. One of it is an induction of different types of mutations from ionizing radiation with yeast *Saccharomyces cerevisiae* as model system of eukaryotic cells. We continued to use tester strain systems for detecting various types of mutations:

- large deletions on plasmid model. UV-light and gamma-irradiation efficiently induced deletions. Mutation *rad53* decreased a frequency of induced mutations.
- intergenic mutations - a forward mutation rate assay that detects all mutations inactivating the *CAN1* gene. It's showed a linear dependence of induced mutations upon $3x10^{-6}$ (survival 0.2%) after UV-irradiation.
- base-single deletions – frame shift assays detecting mutations that revert 4-base insertion in the *LYS2* gene or +1T insertion in a stretch of 6T's in *HOM3* gene. The rate of spontaneous *lys2* -revertion was $4x10^{-8}$ and of *hom3* -revertion – $3x10^{-8}$. The UV-light induced frame shift mutations more effectively. For dose 134.4 J/m^2 frequency of frame shift mutations for revertion to Lys^+ is $2x10^{-5}$ and for revertion to Hom^+ is $7x10^{-6}$. Dose dependence curves of frame shift mutation induction were linear for survival upon to 0.2%.
- tester system for base substitution is based on critical requirements for cycteine at position 22 of iso-1-cytochrome encoded by *CYC1* gene. In order to restore codon 22 and revert to wild type the defined substitution is necessary. All possible base-pair substitution – 2 transitions and 4 transvertions – can be monitored. It is obtained the curve of survival for all haploid and diploid strains after UV-light exposures. They are linear and sigmoidal, correspondently. The base pair substitution – transversion AT – TA induced by UV-light and neon ions with LET values 120 keV/μm was characterized. The shape of curves for diploids is similar and may be fitted by a linear-quadratic function in the case of UV-light exposure.

The studies of structure and functional elements of human and yeast kinases were continued. 3D structure of yeast kinase was built

on the base of crystal structure of human kinase. Phenotypes of different *cdc28* mutations were compared with structural rearrangements. It was showed the correlation between phenotypes (radiosensitivity, generation time and mitochondrial mutability) and rearrangements.

DNA mismatch repair (MMR) plays a major role in the recognition and correction of the mispaired base, increasing radioresistance, replication fidelity and maintaining genome integrity. Defects in MMR are the underlying cause for cancer susceptibility syndrome called HNPCC and account for 20% of sporadic cancers. High mutability and likelihood of cancer can be caused by mutations that reduce MMR or by external factors that directly inhibit MMR. Identifying such factors has important implications for understanding the role of the environment in genome stability. Cadmium (Cd^{2+}) is a known human carcinogen – ubiquitous metal with unknown biological function that can came into human's organisms mainly through environmental contamination and cigarette smoking. It showed Cd^{2+} inactivates the DNA mismatch repair (MMR) pathway.

MMR is a complex reaction that involves multiple proteins, that recognizes the mismatch, excises the DNA containing the error and resynthesizes the correct DNA sequence. In yeast, several genes have been identified, particularly MSH2, MSH3 and MSH6, that are homologues of MutS in *Escherichia coli*. Homologues of *E. coli* MutS have been found nearly in all organisms. Prokaryotic MutS proteins are encoded by a single gene and form of homodimers. Eukaryotic MutS proteins are heterodimeric. The initial recognition of mispair (a critical step in the pathway) is carried out by two protein complexes: the Msh2-Msh6 heterodimer, which recognizes base-base mismatches and frameshift (±1 bp) mispair, and the Msh2-Msh3 heterodimer, recognizes frameshifts and large insertion deletion mispairs (2-4 bp).

All members of the MutS family possess a conserved ATPase activity. Both mismatch recognition and the ATPase activities of MutS are essential for MMR even though each activity is

independently detectable. ATP binding and hydrolysis by the dimeric Msh protein complexes is a critical factor of MMR and can to modify the interactions of Msh2-Msh6 and Msh2-Msh3 with the mismatched DNA and other downstream factors. Cd^{2+} inhibits both Msh2-Msh6- and Msh2-Msh3-dependent human MMR activity *in vitro* and less inhibitory to its DNA mismatch binding activity and more mismatched duplexes. The inhibition of ATPase activity by Cd^{2+} is prevented by cysteine and histidine, suggesting that these residues are essential for the ATPase activity and are targeted by Cd^{2+}. Cysteine inhibits the ATP coupling and hydrolysis through the Msh2-Msh6 complex and inhibits the DNA coupling to some extent. The interactions of cadmium with Msh2 and/or Msh6 that are responsible for inhibition are unknown.

Two structures of MutS complexes have been reported already, the *Thermus aquaticus* (TAQ) and *E. coli* enzymes and its complex with heteroduplex DNA. A MutS subunit consists of five structural domains arranged in the shape of a comma. The globular domain I and domain IV are involved in DNA binding. Domain V contains the ATPase activity. Domain II, III and V retain similar structures in the presence or absence of DNA. MutS forms a stable dimer due to the extensive interactions between the ATPase domains.

The molecular modeling for the Msh2-Msh6 complex of yeast *Saccharomyces cerevisiae* was performed using MODELLER, based on the template structure of *E. coli* (the PDB file: 1E3M). The secondary-structure prediction algorithms and sequence alignment methods were implied. Since we are interested in studying the influence of ions Cd^{2+}, we modeled only the fifth domain-fragment (residues 543-765). MODELLER generates the three dimensional structure that relies on structure prediction and sequence alignment results followed by energy minimization using CHARMM force field. The ribbon structures were created with MOLMOL. Ribbon diagram of the Msh2-Msh6 nucleotide-binding sites and associated dimer interface.

In this case the basic structure of domain V of yeast Msh2-Msh6 complex was identified using homology modeling approach. Further from the MMR mechanism the Cd^{2+} -inhibition activity can be analyzed using the computationally generated structures.

2.2. *Photo-radiobiological research*

Based on computer simulation approach a molecular dynamics of dark-adapted state of the visual rhodopsin has been investigated. The analysis has been provided for the interactions of chromophore group, 11-cis retinal and surrounding amino acid residues in the Schiff base region. It was shown (Fig.1) that interaction of protonated Schiff base linkage with negative charged Glu113 is most likely not simple classical electrostatic one between two opposite charged groups. One can proposed that not only Glu113, but also Glu181 and Ser186 take part in the protonated Schiff base linkage stabilization. In accordance with our calculations Glu181 as a counterion interacts with Schiff base linkage through Ser186.

It was shown that UV irradiation causes a covalent modification of a crystallins. But an aggregation of molecules damaged is not occurred. But ab aggregation of molecules damaged is not occurred. It confirms a high stability of a crystallins. UV irradiation causes a covalent modification of b-crystallins, wich accompanied with protein aggregation and precipitation. UV irradiation of a- and b-crystallins mixture does not cause protein aggregation. We made a new method for separation of protein damage and aggregation. Using this method it was shown that molecular mechanism of a-crystallin chaperone0like function protection does not connect with stable complex formation.

It was shown that a-crystallin decreases the thermo stability of rabbit muscle GAPD, which connected to protein oligomeric structure.

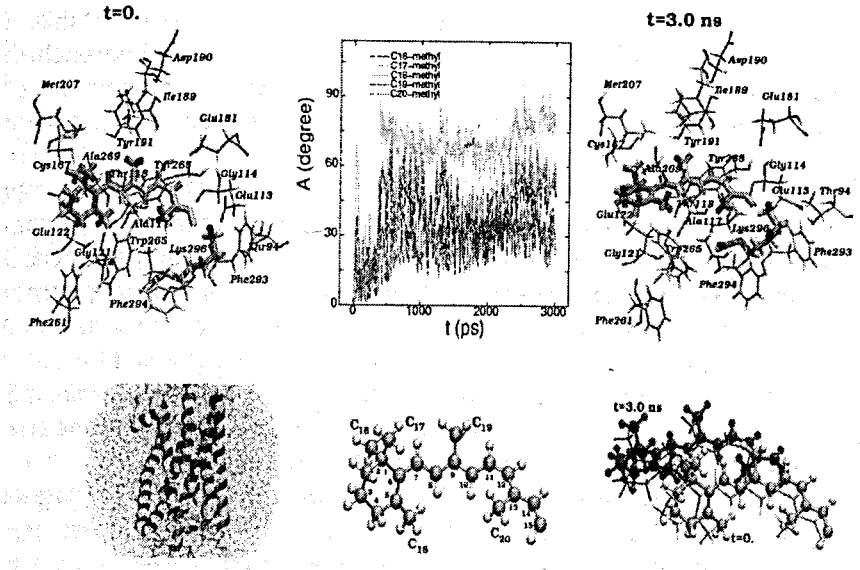

Fig. 1 Molecular dynamics of 11-cis-retinal in the rhodopsin chromophore center at the initial (t=0) and final (t=3 ns) simulation states are presented along with the torsion rotation angles of five methyl groups (C16-C20) (top). The positions of the11-cis-retinal atoms during the 3 ns dynamical changes are separately displayed (bottom). (View from the side of the rhodopsin molecule)

2.3. *Computer molecular modeling of biophysical systems*

In the above period of time the staff of CMM sector has performed the scientific research and educational activity within the following topics:

- Molecular dynamics of chromophore 11-*cis* retinal and surrounding amino acid residues in the chromophore binding pocket at physiological regeneration of visual pigment rhodopsin: computer simulation. Molecular dynamics calculations were performed for the time interval from t=0 to 3000 picoseconds, so that the configuration states of rhodopsin and free opsin were

analyzed and compared. It was demonstrated that the adaptation of the chromophore retinal in the opsin site causes a considerable influence on its protein binding pocket, as well as on conformations of the cytoplasmic part, but the extracellular part of the protein shows a comparably small changes. On the basis of the simulation results we discuss some molecular mechanisms for the rhodopsin protein function as a G-protein-coupled receptor in the dark state, i.e. for the chromophore retinal as a ligand-agonist stabilizing the inactive conformation of the rhodopsin (Fig.1).

- Analysis of the bonds between the ATP and catalytic subunit of kinases (wild and mutant) using md simulations of the active cdk2 crystal lattice
- Molecular-dynamics simulations of mechanism of SOS mutagenesis in escherichia coli, based on the studies of the conformation behavior of lexa and proteins involved in the formation of single- and double-stranded dna structure. In the present work nanoseconds long molecular dynamics (MD) simulations of the cyclin-dependent proteinkinases (CDK) with ATP complex were performed. The central role that CDK play in the timing of cell division and repair and the high incidence of genetic alteration or deregulation of CDK inhibitors in a number of cancers make CDC28 of yeast Saccharomyces cerevisiae very attractive model for studies of mechanisms of CDK regulation. The crystal structure of the human CDK2 has served as a model for the catalytic core of other CDKs, including CDC28. MD simulations of substitution CDK2-G16S in conserved G-loop shows an important of this amino acid and a conformational change of CDK2 structure resulting in the moving of the G-loop away from ATP and a new rearrangement of amino acids in the T-loop.
- application of the methods of quantum information theory to the visual information processing in retina
- application of genetic algorithms for simulation of proteins 3D structure

- application of wavelet transform for eliminating divergences in solution of stochastic differential equations and quantum field theory problems

It was proposed that information processing in brain, and in retina in particular, is most likely performed by means similar to hypothetic quantum computers, but at the presence of dissipation. Quantum mechanical tunneling effects are suggested to be responsible for the visual signal processing by bipolar cells in retina.

The results of the research work by the staff of the CMM sector were presented in All-Russian and International Conferences, Symposiums and Seminars, as well as published in the domestic and foreign journals.

2.4. Radiation research

The main radiation component of the radiation fields at the working accelerators is neutrons with very wide energy range. The neutron spectra behind the JINR nuclear facilities are very differing depending on the accelerated particle energies, shielding materials, geometry. "source - shield" and so on. The following tasks were done for the accounting period:

- the systematization of the neutron spectra at the JINR accelerators and reactor was done for determination of the neutron ambient dose dependence on the shape of neutron spectrum. It needs for definition of the real range of the normalization coefficients at the area radiation monitoring.
- the calculations of different types of radiation shields for mobile and stationary installations for identification hidden explosive and narcotic substances were performed.
- the calculation of the local shields of two electron accelerator scrapers for the IREN project was done.
- in the frame of the participation in the planet surface research program the calculation and the experimental study of the collimated neutron detector characteristics for the Moon spacecraft was carried out.

- supported of the biological experiments with the blood lymphocytes, plant cells and laboratory mouse irradiation by the carbon ^{12}C ions with energies 200 and 500 MeV/amu was done at LHE Nuclotron. The series of radiobiological experiments was carried out with the neon, lithium and boron ion beams at the LNR U-400M.

3. Scientific research programme for 2007

Research and development will include:

- Investigation of peculiarities and mechanisms of stable and unstable chromosome aberrations induction in human cells, point and structural mutation induction in yeast cells by irradiation with heavy charged particles
- Investigation of effects of low doses radiation with different LET on the chromosomal apparatus of cells and problem of cell recovery;
- Study of damaging effects of heavy charged particles on human eye retina and rodopsin;
- The estimation of influence of carbon beams action on the human cells;
- Theoretical modeling of ionizing radiation interaction with matter (including interactions with biological structures), shielding and activation calculations;
- Prognostication of radiation situation at new nuclear facilities, large experimental installations and spacecraft boards;

As the results the following items will performed:

- New data on action of accelerated multi-charge ions on the genetic system of higher organisms sells.
- New data on damaging action of heavy charged particles on human eye structure (lens and retina)
- Developed methods for recognition and quantitative analysis of hidden damages in biological membranes, first of all, at

investigation of effects due to small dose of heavy charged particles.

- Mathematical modeling of radiation induced effects of charged particles by molecular dynamics methods.
- Improved calculation methods of radiation transport through matter and radiation induced damages formation.
- Calculations of radiation shields of new accelerators and large nuclear installations. It's planed to start the calculation of the new reconstructed Nuclotron shields as well the calculations of the absorbed and effective equivalent doses in biological objects irradiated by Galactic Space Radiation.

4. Scientific meetings and educational activity

On 5-9 June 2006 the 4th International Workshop on Space Radiation Research and 17th Annual NASA Space Radiation Health Investigators' Workshop was held in Moscow and St. Petersburg. The major roles in this meeting organization have been belonged to the JINR Laboratory of radiation biology and the Institute for Biomedical Problems of RAS. The modern problems of classical and space radiobiology, space radiation protection, dissymmetry, etc. were discussed at the Workshops. The special attention was given to Moon exploration program, Mars manned flight program and to study of possible application of obtained data to applied medicine, biology and radiation protection as well. More than 100 scientists from Russia, USA, Germany, Italy, Japan and JINR took part in it.

During 18-21 September at International Conference Hall the 2nd International Conference MSSMBS'06 "Molecular Simulation Studies in Material and Biological Sciences" has been held at JINR, Dubna. The main topics of the workshop were the molecular simulation studies of nano- and biostructures. For the purposes organizers invited the key experts from Japan, Russia, Armenian, Dutch, and Ukrainian universities and institutes.

The education process at the chair "Biophysics" of the International University "Dubna" was continued. 69 students in sum are studying now on specialty - "Radiation protection of people and environment" and 19 new students were admitted in 2006 to the chair. The 2nd graduation of the 8 students took place in 2006.

5. Administration activity

Personnel. The total personnel of the LRB were 79, including the directorate staff 12.

Finance. Funding of research in the direction of radiation and radiobiological investigations in 2007 is shown in Table 1.

Table 1. Financing LRB in 2007.

Area	Financing plan (k$ USA)
08-9-1015-96/2008 (1st priority)	393.9
Infrastructure	141.4
Total	535.3