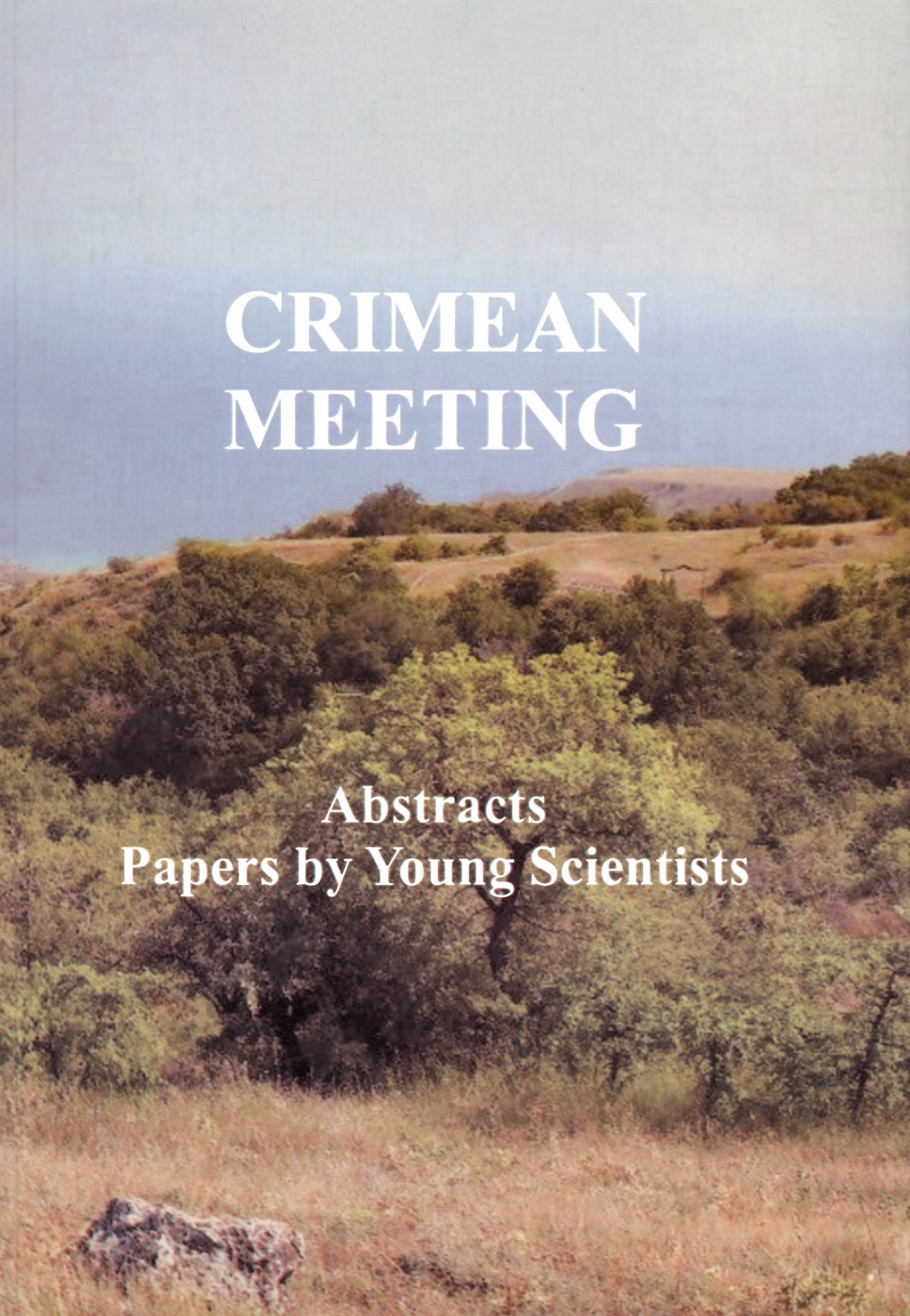
CRIMEAN MEETING



Abstracts
Papers by Young Scientists

Joint Institute for Nuclear Research

CRIMEAN MEETING

Third International Conference, Dedicated to
N. W. Timofeeff-Ressovsky
«MODERN PROBLEMS OF GENETICS, RADIOBIOLOGY, RADIOECOLOGY AND EVOLUTION»

Third Readings after V. I. Korogodin & V. A. Shevchenko

NATO Advanced Research Workshop
«RADIOBIOLOGICAL ISSUES
PERTAINING TO ENVIRONMENTAL SECURITY AND ECOTERRORISM»

Alushta, 9–14 October 2010

ABSTRACTS
PAPERS BY YOUNG SCIENTISTS

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Genetics

Genome mechanisms
Hypermutagenesis
Mechanisms of adaptive mutagenesis
Epigenetics
Mobile elements
Medical genetics
Discussion: System features of cells

Radiobiology

Genetic instability, bystander effect and radioadaptation
Radiation sensitivity
Signaling and DNA repair
Late effects of ionizing radiation
Non targeted effects
Epidemiology of ionizing radiation
Bystander effects between organisms in populations
Biological dosimetry
Discussion: Effects and mechanisms induced by low dose irradiation at cell, tissue and population levels.

Radioecology

Radionuclide migration in environment
Effects of ionizing radiation on populations and ecosystems (including low dose effects)
Ecological dosimetry;
Problems of radiation protection of environment;
Problems of rehabilitation of radiation polluted areas
Sea radioecology
Discussion: System response of biogeocenoses on radiation effects including low dose radiation.

Evolution

Hereditary changes as a process;
Inheritance of acquired characters;
Heredity and infection, horizontal transferring and symbiosis;
Viruses and information change in biosphere;
Speciation
Discussion: Evolution of biosphere

ABSTRACTS

of Presentations

GENETICS

THE HIGH LEVEL OF CHROMATID EXCHANGE ABERRATIONS INDUCED BY IRRADIATION DURING G2 STAGE IN HUMAN PLURIPOTENT CELLS

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The erroneous repair of DNA double-stranded breaks (dsb) is ultimate reason of chromosome aberration formation. DNA damage response includes rapid chromatin remodelling and relaxation. Embryonic stem cells (ESC) are rich in less compact euchromatin and, as differentiation progresses, cells accumulate highly condensed, transcriptionally inactive heterochromatin regions. ESC and their differentiated derivatives can serve as an isogenic model to study the influence of higher-order chromatin structure on DNA damage repair.

Using G2-chromosome radiosensitivity assay we found significantly increase (up to 2 - 10 fold) of chromatid exchange rate 2 hours after 1 Gy irradiation in two lines of human ESC compared with their differentiated fibroblast-like derivatives and primary fibroblast line HS27. The frequency of chromatid exchanges in ESC and differentiated cells exhibited clear quadratic dose response resulting from two-hit events of irradiation energy deposition. Two-hit nature of chromatid exchanges suggests non-homologous end joining (NHEJ) implicated in their formation.

The chemical inhibition of key NHEJ component DNA-dependent protein kinase (DNA-PK) by NU7026 caused significant decrease of radiation-induced chromatid exchanges in hESC. Chromatid exchanges were insensitive to DNA-PK inhibition in differentiated cells although NU7026 led to 4-6 fold increase of chromatid breaks detected in metaphases 2 hour after 1Gy irradiation in both cells types used (pluripotent and differentiated).

Therefore we can conclude: (1) NHEJ is active during late G2 cell cycle stage in ESC and differentiated cells; (2) DNA-PK suppresses chromatid break formation after irradiation during G2; (3) most but not all radiation-induced chromatid exchanges observed in hESC are products of DNA-PK–related dsb misrejoining.

MICRO - ALGAE AS MODEL SYSTEM FOR STUDYING OF GENOTYPE AND INDUCED RESISTANCE TO OXIDATIVE STRESS

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Evidences are available that different stress conditions could activate similar defense mechanisms in various biological systems.

For better understanding of mechanisms underlying genotype and induced resistance to oxidative stress both mutant strains of unicellular green alga *C. reinhardtii* and Antarctic algae have been used as a model system.

Our hypothesis was that Antarctic algae from habitats with extreme environmental conditions have evolutionary developed effective cellular defense mechanisms.

The aim of our work was to identify reliable molecular and biochemical markers of resistance to oxidative stress and to elucidate the relationship between genotype and induced resistance (adaptive response). Micro - algae stress response has been investigated at different levels- cellular, molecular, biochemical.

The contribution of repair capacity of DSB DNA, antioxidant and chaperons systems has been analyzed.

The development of plant-based biomarker test systems could be considered as a very important because it corresponds to strategies for protection of the gene pool of cultivars, biodiversity preservation and genome stability of plant populations -natural and agricultural.

Acknowledgement: This work was funded under the projects "Antarctic algae – model system for oxidative stress resistance", Bulgarian Ministry of Education, Young and Science and "Molecular mechanisms of induced resistance in plants to oxidative stress", agreement between RAS and BAS.

HOW *LILIUM* EXTRACT CAN MODIFY GENOTOXIC EFFECT OF ZEOCIN IN LOWER AND HIGHER PLANTS?

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At present the availability of genotoxic chemicals in the environment is considered as a huge problem because their potential to induce cell damages at different levels, mutations, genome degradation and changes in population structure, serious diseases, including cancer. Prophylaxis against induced environmental mutagenesis aims at reduction of the genetic load by introduction of exogenous compounds with antimutagenic properties towards specific chemical or physical agents. The current knowledge in environmental mutagenesis clearly illustrates that there do not exist any universal antimutagen(s). The specific action of the tested potential antimutagens depends mainly on several factors: the type of the compounds; the sensitivity of the test-system used, experimental design and endpoints applied. Extract *of L. candidum* or the so called "white Madonna lily" was chosen, because in folk medicine this plant has been used for a treatment of different diseases. The aim of this work was to evaluate bioactivity of *Lilium* (LE) on various plant test-systems (lower and higher plants) and to clarify how LE can modulate genotoxic (DNA damaging) potential of zeocin. Various experimental assays and endpoints were exerted. Constant field gel electrophoresis (CFGE) and the alkaline comet assay were used in green alga *Chlamydomonas reinhardtii* and *Pisum sativum*. Chromatid aberrations (CA) and micronuclei (MN) were used to detect genotoxic effects in *Hordeum vulgare*. In systems tested by us it was revealed that zeocin-induced genotoxic effect could be modified by LE treatment. Effects obtained are strongly depended on the experimental designs.

Acknowledgement: This work was funded under the projects BG-SK/206/2009 and SK-BG-0017-08

A TEST OF KIMURA'S MUTATION-RATE CONJECTURE

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In the 1960s, Motoo Kimura suggested that genomic mutation rates would evolve to an equilibrium value that balanced downward pressure from the deleterious impact of most mutations against the ever-increasing "physiological" cost of further reducing rates. By the 1990s it was clear that most organisms displayed only one of two mutation rates. RNA viruses displayed rates of 0.1–1 per genome replication, whereas microbes with DNA genomes displayed rates of 0.0025–0.005 per genome replication. Four animals with rather diverse rates per sexual generation appeared to display rates only slightly higher than those of DNA microbes when converted to rates per germline cell division, provided that those rates were normalized to that fraction of the whole genome in which mutations produce a fitness impact. These generalities implied that mutation rates were finely evolved values but generated two mysteries: why were the DNA rates so similar despite the extreme diversity of organismal life histories and replication strategies, and what was so special about "0.003"? As rates were collected from ever more diverse microbes, we sought examples that might deviate informatively from the general rule. The first success was the thermophilic crenarchaeon *Sulfolobus acidocaldarius*, which exhibited both a low rate and a paucity of base substitutions relative to indels. A survey of (thermophiles versus mesophiles) × (archaea versus bacteria) revealed dN/dS values that were indifferent to archaea versus bacteria but were substantially reduced in thermophiles compared to mesophiles, suggesting that purifying selection was stronger in thermophiles, a result expected from increased constraints on protein structure at high temperatures. The second success was the thermophilic bacterium *Thermus thermophilus*, where markedly low base-substitution rates were also observed. Thus, as Kimura would have predicted, environmentally increasing the deleterious impact of base substitutions (through their resulting missense mutations and perhaps their impact on RNA secondary structures) provided sufficient downwards pressure to generate the (still unknown) ways in which thermophiles maintain lower mutation rates.

MODIFICATION OF SOME ORGANISMS CHARACTERISTICS BY NONIONIZING ELECTROMAGNETIC RADIATIONS

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The biological efficiency of the red coherent light, the infrared low-energy laser radiation (IR LLR) and the extremely-high-frequency (EHF) electromagnetic radiation (EMR) is investigated on some biological objects. The EHF radiation in 40-50 GHz frequency range of output signal considerably influences upon reproductive functions of some cultural cereals. For barley the most efficiency is revealed for 40 and 50 GHz frequencies. The statistically reliable excess of indexes of crop capacity (c/ha) and weight of 1000 grains (g) at the average on 20 % over the EMR-untreated control is defined in both cases. Also similar EHF EMR effects are observed in these groups (40 and 50 GHz) in F_1 generation. In this case excess over the control data is more considerable.

Considerable inhibiting effects of noise mm radiation (53.57-78.33 GHz) are revealed in Drosophila melanogaster (Drosophilidae; Diptera) at the level of fecundity, body weight and other characteristics. Influence of single components of photodynamic therapy as well as their complex influence on investigated signs of Drosophila melanogaster shows well-expressed supressing character. The most inhibiting effects are revealed in following cases: a) while photosensitiser (PS) is treated individually - for 5% mass share of PS in nutritious medium; b) while complex of photodynamic therapy components is treated - for 0.50% mass share of PS and 200 J/cm^2 irradiation dose.

Then the influence of IR LLR (λ=890 nm) and EHF EMR on some features of an individual development rate of Aphidoletes aphidimyza (Cecidomyiidae; Diptera) is researched. The IR LLR treatment is not effective at the level of reproduction characteristics. The increase of Aphidoletes aphidimyza fecundity due to EHF EMR treating is revealed for 50 GHz frequency. In this case the fecundity increasing over the control data is reached to 3.8 times. The obtained results may be used in applied entomology in purposes of quantity suppression of the agricultural vermin insects.

INVESTIGATIONS OF EPIGENETIC CHANGES DURING THE AGING OF PLANTS

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PROTEIN INHERITANCE AND EPIGENETICS

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Several prion diseases (infectious amyloidoses) controlled by a single gene *PRNP* are known in mammals. Prion is a conformationaly modified protein encoded by *PRNP*. About ten prions discovered in lower eukaryotes, predominantly in *Saccharomyces* yeast, are cytoplasmic inherent determinants (unlike in mammals): several transcription and translation factors are among them. These are the examples of so called "protein inheritance".

Prionization inactivates protein in some instances and does not in the others. Possible adaptive value of prionization mechanism is under discussion now. The mechanism is involved in cytoplasmic incompatibility in *Podospora anserina*, in development of cytoplasmic mammalian stress granules, in long term potentiation in mollusk *Applisia* and in several other processes. Yeast prions are the most convenient model for the study of prionization process and the mechanism of protein inheritance. System *SUP35* gene - [*PSI*] prion had been studied in more detailes. Several cis- and trans- acting factors regulating prionization process and prionization network were described. These facts should be taken into consideration in seeking antiprion agents.

The protein inheritance as an epigenetic phenomenon modifies the central dogma of molecular biology, which describes the biological template principle. Conformational templates or the second order templates for proteins are proposed in addition to sequence templates or the first order templates for nucleic acids. Regulation of translation termination process trough eRF-3 (Sup35p) prionization in *Sacch. cerevisiae* is being discussed as a model system for the study of interaction between the two types of template processes.

FLAVONOID BIOSYNTHESIS GENES IN WHEAT AND WHEAT-ALIEN HYBRIDS: STUDIES INTO GENE REGULATION IN PLANTS WITH COMPLEX GENOMES

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Pigmentation by flavonoid pigments is the oldest trait employed for studies in genetics. Flavonoid biosynthesis (FB) genes contributed to understanding and discovery of many genetic and epigenetic phenomena, such as basic underlying *principles of heredity,* gene polymery, transposable elements, epigenetic gene regulation, posttranscriptional gene silencing. In the current study, FB genes were used as a model to investigate how genes function in a foreign background in wide hybrids of cereals and to study regulatory-target gene relationships in allopolyploid wheat genome (*Triticum aestivum*, AABBDD, $2n=6x=42$). We performed genetic and/or physical mapping of 26 FB regulatory and 11 target gene loci in wheat and its relatives. From these genes, *Rc* and *F3h* represented the most convenient regulatory-target gene model. *Rc* (red coleoptile) controls pigmentation of coleoptile. In wheat, 3 homoeologous *Rc* genes (*Rc-A1*, *Rc-B1*, *Rc-D1*) were mapped to chromosomes 7AS, 7BS and 7DS, respectively. In diploid relatives of wheat, the *Rc* genes were localized to chromosome 4R of rye, 7H of barley, 7S of *Aegilops speltoides* and 7D of *Ae. tauschii*. The presence of the dominant *Rc* allele induces expression of *F3h* (flavanone-3-hydroxylase) in coleoptiles. Quantitative examination of *F3h* expression using real-time PCR on cDNA from coleoptiles of the genotypes (wheat cultivars and wheat-alien hybrids) carrying different combinations of the *Rc* and *F3h* genes led us to the following conclusions: (1) FB gene regulation cuts across genomes of allopolyploid wheat; (2) regulatory FB genes contribute more to the functional divergence between the diploid genomes of allopolyploid wheat than do the structural genes; (3) phenomenon of transcriptional dominance takes place in the wheat-rye chromosome substitution line; (4) regulatory *Rc* genes of different Triticeae species are able to activate wheat target genes *F3h*, demonstrating good cooperation of the wheat and alien FB gene systems within the hybrid genomes; however (5) the bigger genetic distance between wheat and a donor species, the lower transcriptional level of wheat *F3h*, suggesting that successful insertion of alien gene into recipient genome still does not guarantee the desirable level of its transcription, because of the donor-recipient gene expression networks divergence.

CYCLIN-DEPENDENT KINASE CDC28: INVOLVEMENT IN CELL CYCLE REGULATION, GENOME STABILIZATION, CHECKPOINTS AND REPAIR

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Cell cycle dependent kinases (CDKs) play a central role in cell cycle regulation. There are 7 CDK and 8 cyclins in the human and only CDK1/CDC28 and 7 cyclins in budding yeast. CDKs are highly homologous (40-75% similarity among hCDK1 through hCDK7 and 62% for hCDK2 and yCDC28). Yeast CDC28 is an excellent model for studying CDK regulation and their role in cell division and genetic tolerance. CDC28 is active and required during all phases of the cell cycle. Lesions of CDC28 functioning have pleiotropic manifestations: for example the *cdc28-srm* mutation decreases the mitotic stability of the native chromosomes and recombinant circular mini-chromosomes, increases cell sensitivity to damaging agents, and fails the cell cycle arrest in G_0/S, G_1/S, S/G_2, G_2/M. It seems likely that in budding yeast CDC28 mediates cellular response induced by DNA damage including checkpoint control and repair. The well known checkpoint genes *RAD9, RAD17, RAD24,* and *RAD53* have been found to belong to one epistasis group called the *RAD9*-group as regards cell sensitivity to γ-radiation. An analysis of the radiosensitivity of double mutants has reveled that the mutation *cdc28-srm* is hypostatic to each of the checkpoint mutations *rad9Δ* and *rad24Δ,* and additive to *rad17Δ* and *rad53.* Thus epistatic interactions have demonstrated *CDC28* belongs to this epistasis group and a branching *RAD9*-dependent pathway. *CDC28* can also participate in a minor mechanism involved in determining cell radiation sensitivity independently of the mentioned *RAD9*-dependent pathway. In the human, CDK have proto-oncogenic properties. Failure to arrest the cell cycle in CDK mutant contributes to cancer development and may cause resistance to standard treatments. Major advances in the understanding of cell cycle regulation mechanisms provided a better knowledge of the molecular interactions involved in human cancer. This progress has led to the promotion of new therapeutic agents. Moreover, the components of the cell cycle are probably involved in other non-cancerous diseases (for example, mitochondrial diseases) and their role must be defined.

In yeast, CDC28 influences the mitochondrial genome stability. Molecular mechanisms of CDC28 participation in cell tolerance are considered – in particular the hypothesis that links genetic stability in yeast to changes in the chromatin and mitochondrial genome organization dependent on the gene *CDC28.*

CHROMATIN AND DNA DAMAGE REPAIR

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In eukaryotic cells the inheritance both accurate DNA sequence and her chromatin organization is key position for the genome stability. Different DNA lesions induced by endo- and exogenous factors creation the problem for this stability. For all comprehension as cell can accomplish this task it is integrated the knownledge about nature these lesions, its detection and repair into chromatin surroundings. Numerous types of DNA lesions and repair pathways and complex chromatin organization complicate this comprehension. Resent progress in each from these spheres helps to clear on molecular and cellular levels significance of these processes interaction. In this report I shall view modern conceptions about regulation of repair processes on chromatin level. I shall view types of histone modifications and its influence repair regulation and the role of remodeling complexes in different branches of repair processes.

A HIGHLY MUTABLE X^{Z3314}-CHROMOSOME OF *DROSOPHILA MELANOGASTER* ISOLATED FROM THE WILD POPULATION OF ZVENIGORODKA (UKRAINE)

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Analysis of variability of X-linked genes in *Drosophila melanogaster* strain Z3314 over the period of 2003-2010 has revealed the following mutations (*yellow-1*; *white*; *lozenge*; *singed*; *cut*) generated over the studied period, as well as the phenotypic expression of "dark eyes" mutation, the genetic background of which remains non-identified. Some of the above mutations occurred in the course of experimental crosses to X-linked females, while the others resulted from inbred crosses during the strain maintenance. The most unstable of those mutations was *singed-3314* allele (mutation frequency from $4{,}6Ч10^{-4}$ to $1{,}4Ч10^{-3}$). Over the period from 2006 to 2010 we detected 15 mutation events in the strain carryind that allele. All derivatives of *singed-3314* turned to be unstable, as well. The directions of occurring mutations are as follows: *sn* to *sn+* and *sn+* to *sn*. Another allele, *losenge-3314 (lz)*, also turned to be unstable, as we detected the mutations from *lz-3314* to *lz+*, as well as a single mutation from *lz-3314* to *lz-3314sl.* In the course of analysis of X-chromosome from Z^{3314}strain on the occurrence of recessive lethal and visible mutations (Muller-5 method), we detected a high level of lethal mutation generation (the mutation frequency of $7{,}2Ч10^{-3}$), which is comparable to the mutation frequency of Canton-S X-chromosome irradiated by 300 Roentgens at the radiosensitive stage of spermatogenesis. We observed the occurrence of a recessive mutation with "dark eyes" phenotype localized in chromosome 2, as well as a mutation modifying the pattern of *dorsccentralis* and *scutellaris* macrochaetae localized in chromosome 3, which we identified as *rase* mutation. The latter mutation is not annotated in Genome brouser, as well as the correspondence of that mutation to a particular nucleotide sequence of *Drosophila melanogaster* was not found. We detected a full-size *hobo* element harbored in the genome of Z^{3314} strain, however, the *in situ* hybridization approach failed to detect *hobo* in *yellow* or *white* chromosomal sites. The strain carries a truncated version of P element, as well. We carried out structural analysis of the regulatory and coding sequences of yellow gene, however, we failed to detect any significant deletion or insertion localized to that site. We, thus, assume that some other mutability factors may be present in that strain, which are expressed under inbred maintenance or crosses to the laboratory stocks.

APPEARANCE AND PROPAGATION OF PRION-LIKE POLYMERS IN YEAST CELLS AND THEIR INTERACTION WITH PRIONS

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Long polyglutamine stretches form amyloid polymers in yeast cells, but these polymers do not propagate, presumably due to poor recognition by the prion-replicating chaperone Hsp104. We proposed that Hsp104 recognition requires the presence of hydrophobic residues within the polymerizing domain. To check this, we constructed several series of hybrid proteins with polyglutamine stretches fused to the nonprion domains of Sup35. The polyglutamine stretches could contain 20% of tyrosine (Y), tryptophan (W), leucine (L), alanine (A), valine (V), serine (S) and other amino acids. The presence of Y, W, A, V and S dramatically reduced the size of SDS-resistant polymers of respective proteins compared to pure polyQ. Thus, the hypothesis that hydrophobic residues are the main determinants of Hsp104 recognition was not confirmed, since several amino acids which are not considered to be hydrophobic still enhanced polymer fragmentation. Alternative hypotheses are currently being examined. In contrast to natural yeast prions (e.g. [*PSI*$^+$]), which appear *de novo* very rarely, polyQX (where X is any residue) polymers, except for polyQL, appeared with high frequency, being present in the majority of cells. Thus we can conclude that the amino acid content of an amyloidogenic protein can influence both the appearance of polymers and their fragmentation.

Polymers formed by proteins with extended polyglutamine domains can also induce the formation of SDS- or sarcosyl-insoluble polymers of three tested chromosomally-encoded Q/N-rich proteins, Sup35, Rnq1 and Pub1. These polymers were non-heritable, since they could not propagate in the absence of polyglutamine polymers. Sup35 prion polymers caused the appearance of non-heritable sarcosyl-resistant polymers of Pub1. Since eukaryotic genomes encode hundreds of proteins with long Q/N-rich regions, polymer interdependence suggests that conversion of a single protein into polymer form may significantly affect cell physiology by causing partial transfer of other Q/N-rich proteins into a non-functional polymer state.

EVOLUTION OF COMPLEX STRUCTURAL GENE *ADE7,4 (PUR2,5)* METHILOTROPHIC YEAST *PICHIA METHANOLICA*

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In the methylotrophic yeast *Pichia methanolica* MH4 sequenced a complex structural gene *ADE7, 4 (PUR2, 5),* controlling the second and the fifth stages of *de novo* purine biosynthesis. Polypeptide size of 478 amino acid residues consists of two functional domains GARS-113 aa and AIRS -365 aa, thus a marked shortening domain purD. Similar *PUR2,5* genes are found in all studied members of taxon " fungi". The protein is homologous to other proteins of genes orthology fungus, but closer to the *Candida albicans*. A comparative phylogenetic analysis of the two sequenced genes of *Pichia methanolica* MH4 *ADE7, 4 (PUR2, 5)* and *ADE1 (PUR6)* on a sample of 20 unicellular eukaryote taxa of "plants", "fungi", "animals", and 3 species of primitive multicellular organisms- Nematodes and Trichoplax. Comparison of gene organization *PUR2, 5* and *PUR6* with prokaryotic showed that these genes correspond to genes of bacterial monofunctional *purD (PUR2)*, *purM (PUR5)* and *purK, purE (PUR6).* The first complex gene *PUR6* was formed through the merger of genes *purK* and *purE* in taxon "plants", followed by joining the domain *purC (PUR7)* only in the first multicellular animals-*PUR7, 6 (purC, E).* The second complex gene was *PUR2, 5 (purD, M)* of fungi, has received additional domain *purN (PUR3)* in unicellular animals *PUR2, 5,3 (purD,M,N).* Myxomycetes, oomycete and diatoms formed a complex genes *PUR7, 6* and *PUR2, 3,5* previous animals, but they are not related phylogenetically. Of the 6 monofunctional genes of prokaryotes were formed 2 gene complex animals. Revealed highly homologous α-proteobacteria - domains purM fungi and animals and gene purD plants. Thus, fungi is origin of typical animal gene *PUR2, 5,3,* and algae-gene *PUR7, 6.*

DE NOVO MUTATIONS IN Y-CHROMOSOME STR LOCI REVEALED IN PATERNAL LINEAGES OF SIBERIAN TUNDRA NENTSI POPULATION

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Y-chromosome short tandem repeats (STR) markers are widely used in human population genetic studies and forensic applications. Estimation of Y-STR mutation rate has a key role for dating the origin of Y chromosome lineages and for paternity tests. Previous studies demonstrated significant interlocus difference in mutation rate and the positive correlation to STR repeat length. The different ethnic groups and various Y-chromosome haplogroups defined by single-nucleotide polymorphisms were characterized by different Y-STR mutation rates. To date no Y-STR pedigree mutation studies were reported in native Siberian populations. Siberian Tundra Nentsi population possesses many unique genetic features and represents a good model for genetic, ecological as well as genealogical studies because of large family sizes, available ancestry information and relatively isolated life style in the extreme north environment. We thoroughly selected 50 paternal lineages with deep genealogical depth from 3 to 6 generations with the total number of 330 males. The number of descendants varied from 2 to 25 per paternal lineage, in average 7 males per lineage. We also included in our analysis six Komi, three Russian and one Khant families who lived in the same villages. Totally 35 STR loci from non-recombining part of Y-chromosome were studied: DYS19, DYS385A, DYS385B, DYS388, DYS389-1, DYS389-2, DYS390, DYS391, DYS392, DYS393, DYS426, DYS438, DYS439, DYS442, DYS447, DYS448, DYS449, DYS454, DYS455, DYS456, DYS457, DYS458, DYS459, DYS460, DYS464-1, DYS464-2, DYS570, DYS576, DYS607, CDY-1, CDY-2, YCA-1, YCA-2, H4, and C4. Eleven males (4 %) had different alleles in 6 or 7 STR loci compared to their paternal ancestors. We believe that those men were born out-of-wedlock or were adopted. Traditionally native Siberian people adopt and raise all children who lost their parents. De novo mutations were observed in 21 out of 35 Y-STR loci. We found relatively higher number of mutation gains (60%) versus losses. Our study has thus shown that the chosen Y-STR loci represent a powerful tool to estimate mutation rates for forensic and population genetic purposes.

HETEROGENEITY OF THE CELL POPULATIONS AND GENOME INSTABILITY DURING COLONY ADAPTIVE REGROWTH IN YEAST *SACCHAROMYCES CEREVISIAE*

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The colony adaptive regrowth manifests itself as the development of papillae on the surface of the aging yeast colonies. This phenomenon reflects appearance of better-adapted cell subpopulation. Although widely accepted that adaptive regrowth is important for adaptation of cell population to stress conditions, there is short of information about state of the "secondary" cell populations and genomic changes associated with adaptive regrowth.

Using contrast light and luminescence microscopy we have examined morphology and state of nuclear and mitochondrial genomes of the adaptive regrowth cell population. The morphological analyses by contrast light microscopy have demonstrated heterogeneity of the adaptive regrowth cell populations. Yeast cells from papillae exhibited great diversity with respect to cell size, shape and cell cycle stage. The most of the cells had typical traits of the stationary phase but dividing cells with various sized buds were observed. We have detected cells of irregular shapes, with shrinkage and various degree of cell deformation. The cell size varied over a wide range, the diameter of some cells was significantly bigger that the mean value.

The state of nuclear and mitochondrial genomes of the cells from papillae has been estimated by luminescence microscopy of DAPI vital stained cells. The structural nuclear anomalies, end-points of cell-cycle failure and loss of mitochondrial DNA were chosen as parameters of genome instability. The most observed nucleus anomalies were spindle-shaped and "tailed" nuclei. The abnormalities of cell division were manifested as appearance of cells with several nuclei and cells with big unnucleated buds. In some cells process of the nucleus fragmentation was registered. The fragmented nuclear material was spread in cell volume or concentrated as crescent shape near cell wall. In the cell population of adaptive regrowth we found *rho*^{0} cells (cells without mitochondrial DNA).

Thus our data suggest that cells from yeast adaptive regrowth colonies are characterized by high morphological heterogeneity and destabilization of genome.

POST-TRANSCRIPTIONAL GENE REGULATION BY SMALL RNAs AND RNA BINDING PROTEINS

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In recent years it has become apparent that a large fraction of all genes in animals is regulated post-transcriptionally by small RNAs.

Furthermore, animal genomes contain hundreds of genes with RNA binding domains. It is clear that many of these RNA binding proteins have important and specific functions in mRNA localization and stability as well as in regulating protein production. However, only recently technologies have become available to probe post-transcriptional regulatory networks on a genome-wide scale.

I will briefly review previous efforts to understand more about the function of microRNAs in post transcriptional gene regulation. I will then present ongoing work where we use high throughput quantitative proteomics, next generation sequencing and computational approaches to unravel the biological function of small RNAs and RNA binding proteins in well defined in-vivo systems such as *C.elegans*.

ENDLESS PURSUIT OF DOUBLE-STRAND BREAK ENDS

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In the pursuit of radiation-induced cellular damage that manifests in cell killing and genome instability, but is reversible by the phenomenon of liquid holding, DNA double-strand breaks (DSBs) have been shown to be the major culprit. It is now widely accepted that DSBs provide both beneficial and deleterious effects and that elaborate systems were evolved to address their detection, processing and repair. Understanding the mechanisms of DSB induction, processing and eventual biological consequences as well as just the detection of DSBs remain a challenge--especially for randomly induced breaks. We have recently developed systems in budding yeast to address i) induction of random primary and secondary DSBs; ii) processing of DSB ends; iii) genetic control of DSB induction and repair; iv) the transition from DSB to chromosome break; and v) genome instabilities associated with DSBs. Using a circular chromosome, we find that resection of random, dirty-end DSBs induced by ionizing radiation or derived from MMS single-strand damage is rapid and is primarily due to the MRX complex. Along this line, the circular chromosome system is now being developed to address DSBs in human cells. Interestingly, the transition from DSB to chromosome break at a unique DSB in yeast is largely prevented by the nuclease function of Exo 1 as determined by separation of florescent markers that flank a DSB. Based on a tetraploid gene-dosage model, the role of cohesin is not only to enhance DSB repair, but it also directs recombinational repair events to sister chromatids thereby preventing loss of heterozygosity. However, the presence of repeated sequences open the genome to gross rearrangements. These findings along with recent work on synergy between DSBs and mutagenesis demonstrate the genomic vulnerability to DSBs and the genetic investment in their orderly processing.

DAMAGE-INDUCED LOCALIZED HYPERMUTABILITY

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DNA damage is repaired by a variety of systems. Defects in repair can result in genome-wide increases in damage-induced mutability. Alternatively, hypermutability could be confined to small sections of the genome that experience high levels of damage and/or reduced repair. This could be the case for damage in long (thousands of bases) stretches of single-strand (ss) DNA formed during abnormal DNA transactions, such as replication fork uncoupling, delay in repair of a double-strand break (DSB) or at uncapped telomeres. We confirmed this hypothesis using specially designed yeast systems in which long stretches of damaged ssDNA formed near DSBs or at uncapped telomeres were restored to the double-strand (ds)-state (Yang et al., *PLoS Genetics*, 4(11): e1000264). We further explored the size and continuity of hypermutable regions via large-scale sequencing including re-sequencing of the entire genomes. We conclude the following: i) the likelihood of mutations in damaged ssDNA can be over 10,000-fold greater than in dsDNA; ii) mutations occur primarily via trans-lesion synthesis (TLS) at the sites of damage rather than by error prone synthesis on the undamaged template; iii) several long stretches of damaged ssDNA can be efficiently restored to ds-state within the same cell thereby generating regions of damage-induced hypermutability in multiple genomic locations; iv) hypermutability can occur without artificial creation of long ssDNA, since yeast cells grown in the presence of a weak mutagen can contain up to 30 strand-biased mutations in a region covering as much as 100 kb, while the remaining 12 Mb of genome contain only as few as 10 mutations; v) based on specificity and strand bias of mutations, hypermutability can arise from ssDNA associated with DSB resection or uncoupled replication forks. Our findings suggest that long ssDNA can be formed within chromosomes in response to DNA damage. Multiple mutations arising in damaged long ssDNA may provide rapid diversity and selective advantage in adaptive evolution and could be an important source of carcinogenesis.

GENOME INSTABILITY IN DIFFERENT POPULATION GROUPS

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Induced genome instability is caused by environmental contamination and characterized by higher susceptibility to genotoxic stress and delayed effects such as increased frequencies of genetic endpoints in distant terms after mutagenic exposure. Genome destabilization can lead to cell malignancy, be transmitted through the progeny to many generations and affect human health. Therefore estimation of genomic integrity to reveal genome destabilization is of great importance for prognosis of cancer and general morbidity risks in different population groups. As a novel approach to detect genome instability, we proposed to measure the level of endogenous DNA damage, cell sensitivity to oxidative stress and DNA repair kinetics for 3 h cell incubation after their treatment with H_2O_2 (100 μM, 1 min, 4°C) *in vitro*. For this purpose, the alkaline comet assay was used. The heparinized samples of peripheral blood were obtained from following groups of subjects: healthy donors as a control; patients with Nejmegen breakage syndrome (NBS) and Williams syndrome (WS); workers of motor-car industry. Informed consent was obtained from each subject (or from their parents in the cases of genetic disorders) for inclusion in the study and before the collection of blood samples. The proposed approach allowed us to detect increased basal levels of endogenous DNA damage and decreased DNA repair capacity in both NBS and WS patients. The latter fact was unexpected for WS, since this disease is associated with the microdeletion of the specific region at chromosome 7, which was not accompanied by significantly increased frequencies of spontaneous chromosome aberrations. In contrast to WS, results of the comet assay in NBS patients well agreed with the data of cytogenetic analysis. In the group of motor-car industry workers, the average parameters of genome integrity were close to control ones. Interestingly, no correlation was established between age and individual levels of endogenous and H_2O_2-induced DNA damage. However, a few individuals with the features of genome instability were found among 150 subjects examined. The possible reasons of genome destabilization in the cases associated or not with occupational pollution will be discussed.

TEMPORAL REGULATION AND SPATIAL RESTRICTION OF MUTATION IN THE *ESCHERICHIA COLI* CHROMOSOME

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Mutation programs controlled by stress responses can limit mutation to times of stress, when cells are maladapted to their environment[1], and possibly to local genomic regions, changing basic ideas about the randomness of mutation that drives evolution[2]. The most well documented stress-induced-mutation mechanism occurs in starving *Escherichia coli* and requires the RpoS general/starvation- and SOS DNA-damage-stress responses, DinB error-prone DNA polymerase, and double-strand breaks (DSBs) and their repair[3], but was thought to be peculiar to an F' conjugative plasmid not a mechanism affecting evolution generally[4]. We show that this mechanism occurs in chromosomes of starving F⁻ *E. coli*. Endonuclease-induced DSBs increase mutation 50-100-fold, SOS-, RpoS-, DinB- and DSB-repair-protein-dependently. When added back, the F' enhanced this only 2-fold via an extra *dinB* gene. We demonstrate that the mutations are localized near DSBs regardless of DSB position in the chromosome and decrease exponentially up to 30-60kb away. Restriction of mutation in genomic space could allow rare cells in a population with an adaptive mutation to survive, and promote local concerted evolution within genes. Furthermore, fully half of spontaneous mutation during starvation requires SOS, RpoS, DinB and DSB-repair proteins indicating that this is a mutation program that stressed cells usually run. Mutation programs that temporally regulate and spatially restrict mutation could enhance the ability of cells to evolve.

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STRESS AND PSEUDOINHERITANCE OF ACQUIRED CHARACTERS

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Inheritance of acquired characters implies that modifications occurring in some species look exactly like inherited adaptations in other (not necessarily related) species. To be explainable by a flash of increased mutability or transposition events [1], this phenomenon should be accompanied by substitution of multiple mutations, which is somewhat difficult for a small population living peripherally close to tolerance limits. Furthermore, full-genome projects reveal an extremely high plasticity of genetic material. Abundance of polymorphisms in the genomes requires that the organisms address on a non-trivial evolutionary problem: reduce the cost of natural selection by letting only those mutations go that hold most promise for fitness. As a non-specific adaptation-related syndrome (after H. Selye), stress provides resistance to adverse factors (cross-resistance) in a short term; however, it depletes and kills in a long term. Thus, it appears as though a small population will be better off if it adjusts organism's *internal* environment so that they, faced with having to produce a prolonged stress response (by rapid entry into the cross-resistance phase or by dampening down the deleterious effects of stress in the anxiety and depletion phases) can function as normal as possible by selection among the mutations in a limited number of genes rather than try to adapt to each of the many adverse effects of the *external* environment separately by trying all available mutations. Because non-specific adaptation to the external environment will be provided by gene regulatory networks responsible for cross-resistance (and also by associated modifications, namely acquired characters), which have many times been validated in their evolutionary history, phenotypically this process will look (a) as though it were one of inheritance of acquired characters (which it is not), (b) as a case of parallelism in the rates and amplitude of variation that ensues from these modifications with a limited number of adaptively evolving genes. Examples of this evolutionary scenario have been found in bacteria, plants and animals.

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INHERITANCE OF LITTER SIZE AT BIRTH IN FARMED POPULATION OF SABLES (*MARTES ZIBELLINA L.*)

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Sable is the most valuable species for a fur breeding. Analysis of inheritance of litter size in the sable may give insight into evolution and diversification of the reproductive strategy of this species. The reproductive strategy of sables differs drastically from that of the majority of predators. The litter size (LS) of a farm sable is normally 1 to 12 puppies. Sable females are the most prolific at the age of 8-9 years and whelp stably till 12-14 years. But sables are notable for their late sexually mature state. Unlike minks and foxes the first dung by sables is often empty. It can be explained both by the species' genetic peculiarities and by the fact that young females haven't passed a selection for production capacity. We have carried out statistical analysis of the average LS in the farm pedigree of the sables. The study is based on the breeding data maintained at the Pushkinsky fur farm (Moscow region, Russia). Animals aggregated into a single large pedigree within whelping period 1994-2006 have been analyzed. The pedigree contained multiple crossings and loops. Statistical analysis has shown that the sample is statistically uniform and a trait distribution doesn't differ significantly from normal for the females which had crossed more than 3 times. To reveal a male's and female's influence on a trait display we have conducted a single-factor dispersive analysis that had shown an importance of mother's effect only. Segregation analysis performed has allowed to reveal a genetic determinacy of trait and ascertain a major gene inheritance model.

CREATION OF TESTS - SYSTEMS FOR EVALUATION GENETIC EFFECTS OF IONIZING RADIATION AND MUTAGENIC FACTORS OF ENVIRONMENT

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On a basis of half-dominant chlorophyll mutation type *chlorine*, with recessive lethal effect was created unique high-sensitive complex test – system for evaluation of frequency of various mutation by the minimal expenses of work and time, caused by the physical or anthropogenic agents of environment. The complex test - system allows to take into account frequency of mutation induction: somatic mutation, which was registered on a leaf surface of plants, concerning to various genotypic classes (+ / +, chl / +, chl / chl); recessive embryonic and chlorophyll lethal mutation, revealed by the embryonic test in seedpods of plants of the first generation; lethal mutation, arising in fourth chromosomal group of coupling.

Depending on the purposes and tasks of the researches the test - system allows to estimate quantitatively frequency of mutation and crossingover separately by the listed parameters or various combinations.

On a basis of marker line of *Arabidopsis*: *glabra (gl)* – lack of fuzzy, III coupling group; *angustifolia (an)* – narrow extended leaf, I coupling group; *viridicaulis (vc)* – light-green stem, IV coupling group; *erecta (er)* – erectoid type of plant, II coupling group; *triplex (tr)* - threefold seedpods, V coupling group; *xanthoseminalis (xas)* – yellow seeds in seedpods, IV coupling group. For association `of the listed genes in one genotype carried out numerous crossings, as a result of which 64 lines with various combinations of genes gl, an, vc, er, tr and xas are allocated. One, from this lines, that combine in one genotype five recessive genes, tr` vc` er`gl` an, is used by us for simplification genetic analyses, and also is effectively applied as test - system (similarly to test on specific loci, developed for laboratory mice) to study genetic effects on ionizing radiation. This multiple marks line was tested in radiobiological experiments and is recommended as unique test - system for evaluation genetic effects of ionizing radiation and mutagenic factors of environment.

ASSOCIATION OF GENETIC INSTABILITY IN THE NATURAL *DROSOPHILA MELANOGASTER* STRAIN 3314 WITH TRANSPOSABLE ELEMENTS ACTIVITY

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The distributions of hybridization sites of transposons *P* and *hobo* and retrotransposons *mdg1* and *mdg2* on polytene chromosomes of larvae of highly unstable *Drosophila melanogaster* stock 3314 and its mutant progeny were examined. The stock had been sampled in Zvenigorodka, Ukraine. Spontaneous mutations of the genes *yellow* (1A5), *white* (3B6), and *lozenge* (8D5-8D6) were detected in lines derived from stock 3314. According to our results, only the appearance of the *lz* mutation can be related to the activity of the transposable elements under study, because a *P* element hybridization site was found in site 8D of the corresponding line. The *lz* mutation showed instability characteristic of insertion mutagenesis. Its reversion to the normal phenotype was accompanied by disappearance of the *P* element from 8D. The distribution of TEs over X chromosomes of the strain was greatly variable. This observation points to high rates of TE transposition. Generally, elevated activity in a particular strain is characteristic for few TE families. In our study, all TEs examined were active, which is typical for a mutational burst. The mutant derivatives were maintained in the laboratory by crossing mutant males to females with coupled X chromosomes. It is likely that the instability of the strain 3314 genome is induced or, at least, increased by cross to a balancer stock, because crosses of geographically or genetically distant Drosophila stocks often induce hybrid dysgenesis, which causes various genetic aberrations. The instability in the 3314 genome may also have been caused by dysgenic crosses owing to the genetic heterogeneity of the natural population or repair system disturbances.

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STRESS-INDUCED MUTAGENESIS MAY BE MEDIATED BY ALTERED ACTIVITY OF P450 CYTOCHROME IN MICE

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Zoosocial stressors (among others) present a serious threat because they have considerable negative effects on organisms. It is well known, that stress influences the immune and neuro-, endocrine systems. Stress has been implicated differently in either the course or cause of many diseases, for example: depression, cancer, and different infectious diseases. It is not enough to know of stress effects and its causes, but besides it is necessary to understand all of its negative consequences. It is also important to take into account an increasing frequency of stresses in human life. Model organisms may be very helpful for research of mutagenic effects of different stressors. Some of the data implies that the stress condition has a mutagenic effect. It was shown that several olfactory stressors increase level of mitotic disturbances including chromosome aberrations in mouse bone marrow cells. The mechanism of this effect in mouse bone marrow cells after pheromonal treatment is unknown. We suppose that stressors may cause changes in liver P450 activity resulting in more effective metabolic activation of promutagens or reactive oxygen species production. To check this hypothesis we compared the ability of liver homogenates from stressed by pheromone and intact mice which are not subjected pheromonal treatment to activate 2-aminofluorene using the Ames test. The results indicate that the conversion of 2-aminofluorene to mutagenic compounds in the Ames test was higher in the S9 fraction from the livers of stressed mice than in control group of mice. Stressors influence on the level of mitotic disturbances including chromosome aberrations in bone marrow cells was estimated by anaphase-telophase method. The results show that the frequency of mitotic disturbances in mouse bone marrow cells was significantly increased after pheromonal treatment. We have shown here that a mitotic disturbances in mouse bone marrow cells induced by zoosocial stressors are accompanied by increased activity of liver enzymes. It is not clear yet whether the change P450 cytochromes activity is a cause of induction of mitotic disturbances. Further investigations are required.

MEDICAL GENETICS

GENETICALLY ALTERED ANIMAL MODELS AS TOOLS IN MOLECULAR MEDICINE

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Worldwide, the highest morbidity and mortality results from such cardiovascular diseases as, hypertension, myocardial infarction, cardiac and renal failure as well as stroke. Since the cardiovascular system and its regulation is quite complex, study of these disorders has been grossly limited to whole organism models. As a result, in recent years, transgenic technology has played a significant role in the discovery of specific gene products for cardiovascular regulation and disease etiology. Genetic manipulation in rats and mice has generated animal models with altered expression of numerous genes. In the presentation, classical and novel technologies available for the alteration of the rodent genome will be described and some animal models with modifications in genes important for cardiovascular regulation will be mentioned.

MOLECULAR SYSTEMS BIOLOGY OF CANCER: AN URGENT REPLY TO THE ACTUAL CHALLENGE OF NON-CONVENTIONAL CHEMOTHERAPY

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Following the revolution in cancer radiotherapy in the past decade (transition from non-conformal to highly conformal exposure methods), the chemotherapy of malignant tumors is undergoing currently a shift from non-specific cytostatics to non-conventional target drugs: monoclonal antibodies (mabs), kinase inhibitors (nibs) etc. The drugs of a new generation influence selectively the complex sub-cellular molecular machinery that makes a malignant neoplasm proliferate infinitely. Since the signaling mitogentic pathways imply dozens of proteins and genes, at the present time dozens of potential target drugs are being tested, either pre-clinically or clinically, and about ten drugs are allowed now for patient care, at least in Russia.

At the stage of drug design and pre-clinical tests, pure experimentation may not provide the exhaustive information on the alterations that the drug brings up to the cell signaling process, due to enormously large number of protein and gene states that need to be explored and checked. However, the *in silico* approach of systems biology can help us much in understanding the overall structure and crucial details of mitogenic signaling.

Having quantified and experimentally tested the large-scale models of mitogenic and survival signaling, which are initiated by EGF, heregulin and insulin stimulation, as well as non-linear synergistic interaction of these signaling networks, we are seeking now for the effective ways (like evolutionary and genetic algorithms) for fast end effective multi-criterion optimization (fitting) of a multi-dimensional vector of free parameters of the model. To perform the process of parameter fitting, we need also to develop the methods for sensitivity, uncertainty and errors analysis in complex models of biochemical networks. The methods and computer codes that we are currently developing, we would apply for *in silico* studies of combined mitogenic/survival and mitogenic/cytokine molecule signaling pathways.

CHEMICAL-INDUCED PLURIPOTENCY AND CHROMOSOMAL DISTURBANCES DURING REPROGRAMMING PROCEDURE

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The goal of this study was to define the role of chromatin-modificating factors in forming pluripotency phenotype of differentiated nuclei in human somatic cells.

Human differentiated embryonic fibroblasts were used in this study. Cells were treated with 5-aza-2-deoxycitidine, sodium butyrate and cycloheximide remodeling chromatin structure and affecting genes expression. Treatment effects were estimated on genomic and chromosomal levels. Modern complex molecular-cytogenetic methods were used(Real-Time PCR, FISH, methyl-specific PCR).

It was established that 5-aza-2-deoxycitidine or sodium butyrate led to increased expression of typical for ESC *OCT4* and *NANOG* genes. A full revivification of *OCT4* expression was shown only in one of 15 cultures after treatment with 5-aza-2-deoxycitidine and cycloheximide taken together. Synchronous revivification of both genes was detected only in one culture after short treatment with cycloheximide. Also, it was found that revivification of *OCT4* and *NANOG* expression in embryonic fibroblasts after influence of chromatin-remodeling compounds was accompanied by growth of frequency of monosomy on chromosomes 12, 17 and induction of micronuclei on chromosomes 12, 16 and 17. Diverse sensitivity of imprinting centers and imprinted genes with 5-aza-2-deoxycitidine treatment were revealed . Processing all cultures of fibroblasts with it did not cause any disturbance in the status of methylation locus *SNURF-SNRPN, IGF2/H19, CDKN1C*. At the same time, in all cultures there was demethylation of *KCNQ1OT1* imprinting center on mother's homolog that led to the loss of this locus imprinting. Probably, this imprinting center is a "hot point" of variable epigenetic modifications. On the other hand, we have established that ESCs treatment with 5-aza-2-deoxycitidine led to decrease of *oct-4* expression. The morphology of ESC colonies was changing and spontaneous ESC differentiation occurred in culture. These data show that epigenetic status of pluripotent genes is important for reprogramming.

Thus, monitoring of chromosomal instability and analysis of methylation status must be an obligatory step during somatic cell reprogramming experiments.

STUDY OF FREE-RADICAL PROCESSES DURING ROTENONE MODELING OF PARKINSON DISEASE

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The study of biological organisms, their tissues and chemiluminescence (ChL) in biochemical complexes has made it possible to discover the role of some physical- chemical processes in biological samples. Free radical reactions in the object which are connected with lipid peroxidation processes belong to these biophysical experiments. The study of such reactions is of vital importance both for physiologically normal organisms and for the description of different pathological processes. Recent investigations have revealed that free radical damages can lead to grave pathologies. Here included Parkinson Disease (PD) which is a neurodegenerative disease.

We isolated striatum (left and right segments), brainstem, neocortex (left and right segments), cerebellum, spinal cord, thymus, heart and liver of white male rats after rotenone injection in the right striatum (12 μg/ml). The tissue homogenates (20 mg/ml in Tris-HCl buffer, pH=7.4) was taken on 5th, 10th and 15th days after injection for ChL analysis on a quantometric device with FEU-139 photomultiplier. Lipid peroxidation was studied in marked nervous tissues using the method of accumulation of a malonic dialdehide (MDA). Determination of SOD activity was done using the reaction of autooxidation of adrenaline in pH=10,2.

It was shown with ChL and above mentioned biochemical methods that processes of lipid peroxidation after rotenone injection in the right striatum dramatically changed in a time-dependent manner. The studies have shown that the loss of grey matter cells is mainly due to apoptosis of substantia nigra cells, oxidative stress and mitochondrial damages. It is known that a number of pesticides and herbicides cause these pathologies and it is essential to find out the mechanisms of the PD and the role of modern factors in the development of the disease.

MOLECULAR-GENETIC STUDY ON MYOCARDIAL INFARCTION RISK FACTORS IN COMPARISON WITH THIS DISEASE RATE

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One of the most important causes of arterial thrombosis is a high fibrinogen level. Thr312Ala polymorphisms of fibrinogen α-chain gene and Val34Leu ones of Factor XIII gene are responsible for structural changes of fibrin. These polymorphisms as well as 4G/5G insertion/deletion in PAI-1 gene (inhibitor of plasminogen activator) influence clot formation at one of the last stages of the coagulation cascade. That's why they were chosen for myocardial infarction (MI) risk factor study. FVL mutation is a well-known risk factor of venous thrombosis and its role in predisposition to arterial thrombosis was known to be rather controversial, so we studied this mutation too.

The methods of polymerase chain reaction (PCR) with specially designed allele specific primers were used. DNA extracted from dried bloodspots was used as a matrix for PCR. Blood samples of 175 patients with MI and 270 people of control group were examined.

The frequency of FVL mutation in the group with MI was shown to be 2.3 times as higher as in the control group (OR= 2.3). It can be concluded that this mutation is a significant risk factor not only for venous thrombosis but also for arterial one (for MI in particular). Genotype distribution in the group of patients with MI and in the control group was shown to be different - the frequencies of TA genotypes for Thr312Ala and LL homozygotes for Val34Leu in patients were revealed to be higher as compared to the control group (OR=1.4 and 2.0 respectively). As for PAI-1, it was shown that 4G/4G genotype of this gene can predispose to MI (OR=1.4) and 5G/5G one can be protective (OR=0.9).The obtained results were compared with the life style of patients. No positive correlation with excess weight, smoking, high blood pressure and pancreatic diabetes was revealed. This can mean that MI in these people was caused to a greater extent by genetic factors than by environmental ones. The frequencies of MI in different European countries were compared to the population frequencies of genetic risk factors predisposing to cardiovascular diseases.

ON ELIMINATION OF RADIONUCLIDES FROM THE ORGANISM USING THE PREPARATION FOLIUM PINI

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Natural vegetable extract Folium Pini is rich with such active biological components, wich have extremely important functions in mans vitality and viability. Theser components are vitamins: A retinol, E the coferol, K carotene and C, photosterones of biologically active lipidsw (saturated and unsaturated fatty acids), proteins, free and essential amino acids and other biologically active compounds. These enumerated components have particular significance in treatment of cancer diseases.

It has been established thet fir extract "tkis nobati" accelerates the elimination of cesium (Cs) from the organism. Further the tests were continued in relation to ather radionuclides. For this first of all there was checked the preparation "folium Pini" in wich only radioactive potassium (40K) 190 Bk/kg was found out. After this the urine of 20 patients was checked before taking the extraction in vich besides the radioactive potassium one of the isotopes of radionuclide uranium(235U) was detected which amount was fluctuated within 3-6 Bk/kg. It is interesting to note that before taking the preparation the analyses were conducted for 10 days or 10 days urine was checked in each patient daily, or with 24 h accumulation which excluded the randomness.

Such approval was given by the head of the Farmaceutic Department, professor G.Abuladze of Nuclear Physics of the Tbilisi State University professor L.Kurdaze.Radioprotective properties of the preparation have been studied in different departments of the Georgien National Oncologikal Center under the leadership of professor R.Vepkhvadze.

RADIOPROTECTIVE DRUGS OF PLANT ORIGIN

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Nowadays the results of Chernobyl accident are felt in Georgia. Within the increase of radiation background and also augmentation of radiation of alimental products caused the fact that number of oncological diseases is increased. It needs to be mentioned that one of treatment ways for this type of patients is medical irradiation.

Therefore, it has been necessary to eliminate the radio active isotopes from human organism, consumers of such alimental products, also to decrease the negative results of medical irradiation.

In Georgia for the moments two radioactive drugs of plant origin are used widely "Tkis Nobati" and "Mendjuni". They represent sum of plant origin extracts containing carotinoids, flavonoids, tanids, chlorofils and other compounds. For convenience of patients drugs are developed in the forms of sugar syrops, gelatine capsules, rectal suppositories.

Drugs are received by patients with tumor of different organs such as lungs, uterus, stomach etc after medical irradiation.

The analyze of urine of the patients has demonstrated that if health organism eliminates the quantity of ^{238}U 3-4 Bek/L after drug's administration, the products quantity ^{238}U-^{234}Th is sharply increased 200-300 Bek/L meanwhile the quantity of ^{40}K is not changed.

Meanwhile the elimination of ^{137}Cs has been investigated from animal's organism which was administrated in different doses. It has been shown, that in control group half period of eluation of ^{135}Cs has been $14 \pm 1,5$ day. And in the group of animals, which received the "Tkis Nobati", this time was decreased till $8,4 \pm 0,75$ day. The letal result was not shown in this group.

The mentioned above gives the possibility to make the conclusion about high effect of used drugs.

REDUCED RB1 EXPRESSION CAUSES IMPAIRED GENOME STABILITY IN BONE-CELLS AND PREDISPOSE FOR RADIATION-INDUCED OSTEOSARCOMA

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Osteosarcoma is the most frequent bone-tumor in children, with some rare genetic defects causing a strong inherited predisposition. This bone malignancy can also arise as a late complication following medical or occupational radiation-exposure, with evidence of a gene-environment interaction. The purpose of this study was the identification of the susceptibility genes, and the analysis of the cellular pathways that are involved in a genetic predisposition for radiation bone-tumorigenesis.
Using a model of alpha-radiation induced osteosarcoma in mouse strains of different susceptibility, we recently mapped a mayor susceptibility locus to chromosome 14. A combination of QTL analysis and mapping of allelic imballances suggested that the *Rb1* tumor suppressor gene is the most likely candidate gene. The BALB/c allele of the *Rb1* promoter was found to carry a hexanucleotide duplication, which is predicted to generate two additional binding sites for WT1 and SP1. Reporter assay and direct *Rb1* mRNA quantification confirm that this insertion causes an approximately 1.8fold change in expression level. A mouse mutant carrying a bone-specific Cre-Lox deletion for Rb1 confirmed, that the monoallelic loss of this tumor-suppressor gene is sufficient to increase osteosarcoma frequency from 11% in wt to 46% in Rb1+/- animals following alpha-irradiation of the bone.
In osteoblast cell-lines established from the Cre-Lox mice we found evidence that - in contrast to the canonical *Rb1* tumor-suppressor function - a 50% reduced gene expression is already sufficient to cause a cellular effect. In addition to the expected effect on cell-cycle regulation we could also show an impaired genome stability. This non-canonical function was suggested before, but our studies provide the first experimental evidence and imply that an individual cancer predisposition might be caused by low-penetrance gene polymorphisms.
This study was supported by grants from the EU Euratom FP6 program and by the Parents Initiative of Pediatric Cancer Patients.

VITAMIN D RECEPTOR GENE POLYMORPHISM IN BELARUS POPULATION AND PATIENTS WITH AUTOIMMUNE PATHOLOGY

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Vitamin D participates in various metabolic paths and modulates also the autoimmune processes in organism through the intracellularly located vitamin D receptor (*VDR*). This study was conducted to evaluate association between four *VDR* SNPs: FokI (rs2228570), BsmI (rs1544410), ApaI (rs11168271) and TaqI (rs731236) and genetic susceptibility to autoimmune diseases in Belarus. 695 healthy native Belarusians (population control) and 82 patients with autoimmune thyroid diseases (AITD) or type 1 diabetes were genotyped using PCR-RFLP analysis. The genotype and allele frequencies are presented in tables. Among all the polymorphic sites studied only for *VDR*/FokI SNP genotype distribution was significantly different in patients comparing to controls (X^2=8,49; P<0,05). Though the allele frequencies did not differ significantly between patients and population cohort, the *VDR* haplotypes distribution was quite different in patients comparing to controls (X^2=45,02; P<0,01). Almost the quarter of patients (24,7%) were heterozygotes for all four *VDR* positions (TtAaBbFf), while such genotype had only 9% of all population control. Just one patient had TtAaBbff gaplotype (1,2%), but it was much more common for the control group - 37 (5,3%). The role of definite *VDR* polymorphic sites and their combinations (haplotypes) in predisposition to autoimmune diseases for Belarus patients is hard to define yet, so we need to enlarge the patients cohort. genotypes requires further studies using more lager patient cohorts.

Table 1-*VDR* genotypes in patients with autoimmune diseases and in population control, %

	VDR/Tag			*VDR*/Apa			*VDR*/Bsm			*VDR*/Fok*		
	TT	Tt	tt	AA	Aa	aa	BB	Bb	bb	FF	Ff	ff
Patients	36,1	54,2	9,6	28,0	53,7	18,3	20,5	48,2	31,3	20,7	67,1	12,2
Population	46,3	41,1	12,6	34,8	41,5	23,8	17,9	50,7	31,4	29,1	50,3	20,7

Table 2-*VDR* allele frequencies in patients with autoimmune diseases and in population, %

	VDR/Tag		*VDR*/Apa		*VDR*/Bsm		*VDR*/Fok	
	T	t	A	a	B	b	F	f
Patients	63,3	36,7	54,9	45,1	44,6	55,4	54,3	45,7
Population	66,9	33,1	55,5	44,5	43,3	56,7	54,2	45,8

SNPs IN THE HIV-1 TATA BOX AND THE AIDS PANDEMIC

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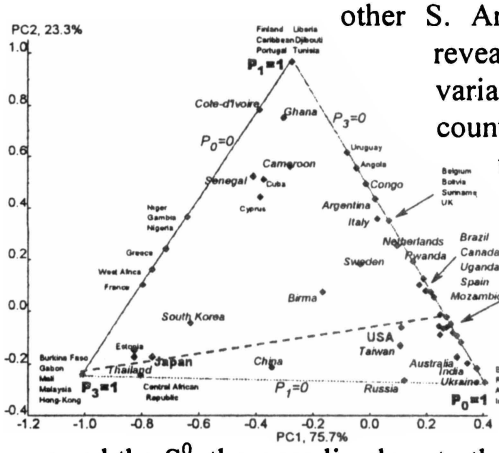
In 2662 TATA boxes (2311 isolates) 146 alleles (S) were found. We named as norm S^0 the *agatgctgCATATAAgcagctgcttt* (59%). The 2nd-most frequent (11%) S took the core *CATAAAA* prevailing in SO-Asia. The K_D of the TATA-binding protein (TBP) estimated by: $-\ln[K_{D,TATA}(S)]=10.9-0.23\ln[K_{D,TBP/dsDNA}(S)]+0.15PWM_{TATA,Bucher}(S)-0.2\ln[K_{D,TBP/ssDNA}(S)]$;
10.9 is the nonspecific affinity; $-\ln[K_{D,TBP/dsDNA}]$ is the contribution of TBP sliding along DNA; $PWM_{TATA,Bucher}$ is the one of TBP/TATA recognition; $-\ln[K_{D,TBP/ssDNA}]$ is the one of the stabilization of the TBP/TATA complex. In each of 70 countries we found the frequency of occurrence weighted by the number, n, of the associated S: P_0 is the S^0; P_1 is the non-S^0 alleles with S^0 affinity; P_2 is the high-affinity S; P_3, is the low-affinity $S^{CATAAAA}$; P_4 is the other S. Analysis of prevalence of the 146 S revealed principal components PC1 (75.7% variance) and PC2 (23.3% variance). The countries with $n<10$ are typed in plain; those with $10\leq n<99$, in italics; those with $n\geq100$, in bold. All the countries fall within a triangle with the vertices $\mathbf{P_0=1}$, $\mathbf{P_1=1}$, $\mathbf{P_3=1}$ and the sides $P_0=0$, $P_1=0$, $P_3=0$. The countries lie close to the P_3 was interpreted as in neutral drift around the S^0, the ones lie close to the P_0 and P_1 - as under selection to low-expressing forms HIV-1 or neutral drift around the $S^{CATAAAA}$, the ones lie close vertex $\mathbf{P_3=1}$ to the side P_3 (under broken line) - as under selection against S^0-like and high-affinity S. So, each of countries is associated with one of three trends in HIV-1 evolution: neutral drifts around the S^0 or the slowly replicating $S^{CATAAAA}$, adaptive growth of the $S^{CATAAAA}$ frequency.



Acknowledgements: RAS programs 21, 22, 23, "Biosphere origin and evolution of geo-biological system"; IP 119, State Contract № P721, RFBR 09-04-01641-a

PROGNOSIS AND VERIFICATION OF THE *TATA* SNP EFFECTS

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SNPs represent the most general form of genetic variability of the human. Computer study of SNP in the regulatory regions of genes consist of the SNP annotation and prediction of the influence of SNP on the phenotype. The first task is successfully solved *in silico*, the second one is the challenge of the medical genetics and bioinformatics. For creation of the test systems that would enable *in silico* prognosis of the SNP of TATA-box effect on phenotype we have (1) *in silico* analyzed the effects of the 53 TATA box polymorphisms in 34 human genes and 38 TATA box polymorphisms in 27 genes of commercial and laboratory animals and plants on the TATA box/TATA-binding protein (TBP) affinity and compared polymorphism's impacts on the value of affinity with known phenotipical effects of polymorphisms and (2) experimental tested hypothetical *in silico* predictions of the TBP-affinity for SNP associated with human diseases. The TBP-affinity was *in silico* estimated by the original equilibrium equation for the four subsequent steps of TBP/TATA box binding:

$$-ln[K_{D,TATA}(S)] = 10.9 - 0.23 ln[K_{D,TBP/dsDNA}(S)] + 0.15 PWM_{TATA,Bucher}(S) - 0.2 ln[K_{D,TBP/ssDNA}(S)],$$

It was hypothesized that biochemical manifestation of the SNP (sequence S^0 mutated to $S^{\#}$), in case that $\delta(S^0 \rightarrow S^{\#}) > 0$, would be the excess of the protein, relative to the normal state, $\delta = 0$, and in case that $\delta(S^0 \rightarrow S^{\#}) < 0$, it would be the protein deficiency. The *in silico* prognosis were found to be statistically significant for 32 out of 53 human polymorphisms and 13 out of 38 another polymorphisms upon the expected frequency of $p_0 = 0.05$, established by Student's *t* test. Results of experimental verification of change TBP/TATA dissociation constants as a result SNPs well agreed at qualitative level with the prognosis.

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MARKER CHROMOSOMES IN INFERTILITY PATIENTS

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Supernumerary marker chromosomes (sSMC) are the most complicated cases of cytogenetic investigation because of their structure and heritage. Genetic counseling and prognosis of healthy offspring in families with sSMC include recommendation of preimplantation genetic diagnosis (PGD). In this study we present the results of investigation of two sSMC cases: identification, gamete distribution and interchromosomal effect in patients with reproductive failure.

A patient (aged 31), his wife and his parents were karyotyped in one family and only a patient (aged 40) – in another one. Both patients had infertility. We made spectral karyotyping (SKY) and metaphase chromosome microdissection followed by PCR with degenerate oligonucleotide primer (DOP). Fluorescent hybridization *in situ* (FISH) was performed for chromosomes (4 or 15, 13, 18, 21, X and Y) in sperm and somatic cells.

Using metaphase chromosome microdissection we identified the sSMC as the chromosome 4 derivate (47,XY,+mar.ish der(4)) in the 1st case. It was present in 83% lymphocytes and in 32% spermatozoa. The sSMC of the 2nd patient was morphologically bigger and was identified as chromosome 15 inverted duplication (47,XY,+inv dup(15)(q11)) by SKY technique. Using FISH sSMC was found in 89.5% lymphocytes and in 40% spermatozoa.

There were no interchromosomal effects of sSMC in the 1st patient. Aneuploidy level of the chromosome 4 was 3.2% in sperm and 1.2% in lymphocytes. We revealed an interchromosomal effect of sSMC in the 2nd patient on disjunction of sex chromosomes: 4.4% somatic and germ cells had X/Y aneuploidy. Aneuploidy level of chromosome 15 was 0.8% in spermatozoa and 3.2% in lymphocytes.

Distribution of sSMC in gametes appeared to have been approximately equally probable except the frequency of sSMC loss. The latter was found based on somatic cells investigation. Increased aneuploidy level was revealed for chromosomes 15, X, Y in inv dup(15) case and for chromosome 4 in der(4) case.

RADIOBIOLOGY

BIODOSIMETRY 1.0: FREE SOFTWARE FOR RADIATION BIOLOGICAL DOSIMETRY

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Purpose: To develop a free computer program for radiation biological dosimetry with support of translocation analysis and different platforms.

Materials and methods: Lazarus/FPC (*1*) has been used for development of the program. Improved sequence-based data on the values of relative DNA contents of chromosomes contents were chosen (*2*) for calculating of genomic translocation frequencies from raw experimental data using approach proposed by Lucas et al (*3*).

Results: The user-friendly program, BioDosimetry 1.0, for radiation biological dosimetry by translocation analysis has been developed at the Joint Institute for Nuclear Research. The BioDosimetry 1.0 is a novel, intuitive and free program for Microsoft Windows and UNIX-like systems with the Graphical User Interface. The software provides the introduction of raw results of translocation analysis of human lymphocytes, the choice of male or female human genome and chromosomes "painted" by FISH. The program automatically calculates the share of genome "painted" by FISH, coefficient for transforming of experimental data on the whole human genome and estimates the dose of radiation.

Conclusion: The free computer program with support of translocations analysis has been developed for use in radiation biological dosimetry.

Availability: The computer program BioDosimetry 1.0 is freely available at http://www.jinr.ru/programs/jinrlib/biodosimetry/.

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MOLECULAR RADIOBIOLOGY OF THE ANIMALS GENES: FROM N.W. TIMOFEEFF-RESSOVSKY TO THE PRESENT DAY

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The most fundamental problems of present-day molecular radiobiology of the higher animals genes, such as nature of radiation-induced heritable gene/point mutations and efficiency of densely ionizing radiation, notably neutrons, in gene/point mutation induction are known to be primarily posed and resolved, to a first approximation in accord with level of genetics of the day, by a classical N. W. Timofeeff-Ressovsky's works performed on *Drosophila* germ cells. At present, in spite of a rapid progress in molecular biology of animals genome and unique genes as well, molecular aspects of mutation induction and processing in germ cells still remain to be solved. As a further evolution of the principal N.W. Timofeeff-Ressovsky's studies which have given the first-priority for Russian school of radiation genetics, a large-scale experiments on induction and molecular (PCR) analysis of γ-ray- and neutron-induced gene/point mutations at the complex *vestigial* (*vg*) gene of *D. melanogaster* have been carried out. According to our results the *vg* gene/point mutants induced by both γ-rays and neutrons may result from the four different types of DNA alterations: (i) micromolecular changes non-detected by PCR; (ii) a "single-site" deletion; (iii) partial deletions of 2-3 contiguous gene regions, and (iv) "complex" lesions as a combination of several independent small partial deletions. However, although the mutational spectra are close for both radiation studied, the relationship among the mutational types is quite different for γ-rays and neutrons. In particular, γ-rays are more efficient in induction of first and third mutation type whereas neutrons induce fourth type more frequently. As a whole, γ-rays and neutrons induced intragenic DNA alterations are small. This picture differs from that in animal somatic cells where the massive and total deletions of gene are predominant.

BIOLOGICAL EFFECTS OF 7.5 MEV ELECTRONS

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The charge composition of cosmic space radiation fields consists of different particles from electron and positron to Uranium nucleus, which has wide energy diapason from some tens up to 10^{18} -10^{19} eV. Great electron flows can be observed; because of negligible permeability of electrons, they do not induce significant radiobiological effect due to protection of modern cosmic apparatus. The doses can increase sharply up to some Gy/day due to electron contribution, when thickness of shielding would be decreased up to 0.1-0.01g/cm^2 (V.M. Petrov*)

The property of complete radiation fields needs to investigate the biological effects of electrons especially. We use microtron MT- 7.5 (YerPhI) in our experiments.

In this paper, effects of electron (7.5 Mev) on bacteria E.coli AB1157 (wild type), AB 2463 (sensitive mutant, rec A13$^-$), and BL 1114 (superresistant mutant, Gам^r 444) will be investigated.

Cytogenetic investigations of blood of persons were provided / Cytogenetic analysis for radiation dose assessment; A manal. (Vienna,IAEA,2001,Techn.Reports.Se, N 405,126p.); registration of active radicals by method of chemoluminescent analyses (facility Photon Counting Head H9319 Hamamatsu).

*Third Readings after N.M. Sissakian, 2004, Yerevan, p.283-302

THE IMPACT OF BYSTANDER EFFECTS AND ADAPTIVE RESPONSES IN THE BIOLOGICAL RESPONSES TO LOW DOSE/LOW FLUENCE IONIZING RADIATION: THE MODULATING EFFECT OF LINEAR ENERGY TRANSFER

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The health risks of low level radiation remain ambiguous and have been the subject of intense debate. To reduce the uncertainty in evaluating these risks, research advances in cellular and molecular biology are being used to characterize the biological effects of low dose radiation exposures and their underlying mechanisms. Radiation type, dose rate, genetic susceptibility, cellular metabolic state, growth stage, levels of biological organization and environmental parameters are among the factors that modulate interactions among signaling processes that determine the outcome of low dose exposures.

Using mice, and normal human or rodent cells maintained in culture, and a variety of biological endpoints, we have shown that exposure to low dose/low dose-rate ($\leq$10cGy over days) from ^{137}Cs or ^{60}Co γ-rays triggers signaling events that protect cells from endogenous oxidative damage or damage due to a subsequent challenge dose of ionizing radiation. DNA repair, oxidative metabolism and cell cycle checkpoints are implicated in the biological responses observed and involve differential regulation of signaling processes. With importance to the assessment of health risks, exposure to low dose/low dose rate γ-rays significantly reduced the frequency of neoplastic transformation to below the spontaneous level. Moreover, adaptive effects induced by low dose/low linear energy transfer (LET) radiation were communicated to neighboring non-targeted cells and protected the latter against stress from a subsequent exposure to low or high LET radiation.

In contrast to adaptive responses in cells/tissues targeted with low dose/low LET radiations, persistent stressful effects were observed in cells/tissues targeted with low fluences of high LET radiations, including α-particles and high charge/high energy (HZE) particles. The stressful effects were not only confined to the target but were also propagated to neighboring cells/tissues and persisted in progeny cells.

We conclude that coupled with epidemiology, the knowledge of cellular and molecular processes underlying low dose radiation-induced biological effects should further refine our estimates of radiation risks at low doses.

NEUROSPECIFIC PROTEINS AND CYSTEINE PROTEASES IN THE BRAIN STRUCTURES AND SERUM BLOOD OF THE RATS UNDER INFLUENCE OF LOW DOSES RADIATION

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The low doses of ionizing radiation influence are at the special position among factors damaging central nervous system. One of the reasons of radiated-induced brain damages is disturbance of blood brain barrier (BBB). According to that, the attention was attracted to glial fibrillar acid protein (GFAP) – astrocytic-specific protein of brain intermediate filaments. The aim of the work was both the researching of GFAP content dynamics in different brain structures, blood serum and the investigating of lysosomal cysteine cathepsin B activity after x-ray irradiation of rats in dose 25 cGr. The dynamics of soluble GFAP form concentration in neocortex, hippocampus, corpus striatum, cerebellum, middle brain and pons varolii tissues was determined under influence of x-ray radiation in dose of 25 cGr. The researches were held in 1, 12, 24, 120 and 168 hours after irradiation affect. The quantitative estimation of neurospecific protein' content was made by ELISA. Concentration of GFAP exponentially increased in blood serum in 24 hours after irradiating and since 168 hours it became maximal. It was established the increasing of tissue lysosomal cysteine cathepsin B activity in blood serum under influence of ionizing radiation. The changes of neurospecific protein' content and cathepsin B activity found in blood serum of irradiated rats could be informative test of irradiation damaging action. The increased level of GFAP content in blood testifies not only to modifications in BBB functioning and increasing of its penetration, but also to possibilities of CNS damages, caused by astroglial cells which are able to modulate neuronal differentiation. The determination of these neuroantigens in blood serum could give precise information about pathologic processes of brain caused by ionizing radiation in low doses. Thus, irradiative damage of brain is associated with metabolism disturbances and membrane-damaged processes, which lead to BBB penetration changes, and, as a result, to brain neurospecific protein elimination in blood.

PHEROMONES AND ADAPTIVE BYSTANDER-MUTAGENESIS IN MICE

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Discussing the causes of mutagenesis and its connection with the genome integrity we usually focus on chemical and physical mutagenic factors. But in different species of higher organisms there is at least one more indirect mechanism to control stability of genetic machinery in dividing cells and therefore connected with mutational changes. The mechanism deals with the exchange of information between conspecifics about their state and its spreading among communicating animals.

The genomic instability has been studied by cytogenetic analysis of chromosome aberrations and other mitotic disturbances (MD) in dividing bone marrow cells of CBA mouse strain. It was shown in the laboratory mice that volatile substances excreted by small unisex groups of animals into the environment induce genomic instability in conspecifics of same sex. At the same time there is no such effect in animals of opposite sex.

It is well known that overcrowded mouse females begin to produce pheromone 2,5-dimethylpyrazine (DMP) which induce genomic instability and other negative effects in recipient animals of both sexes (Даев, 2007; Jemiolo, Novotny, 1994). Thus, by means of DMP females spread the information about their unhealthy state induced by high density.

We have shown that DMP sniffing induce different types of MD in bone marrow cells of CBA recipient males and females. In mouse males DMP sniffing induces sperm head abnormalities, dominant lethals and inhibits immune system.

It has shown also that excreted volatiles (EV) of animals after their whole body irradiation or physical stressor action induce similar (but not so strong) cytogenetic disturbances in EV-sniffing recipient animals (Daev et al., 2007).

Therefore, house mouse and, evidently, other animal species could play a role of r promutagenic factor for their conspecifics which acts through chemical communicational mechanism and central neuronal network.

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CYTOGENETIC BYSTANDER-EFFECT IN MOUSE MALES AFTER IRRADIATION

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Deleterious action of irradiations in living organisms is well known. But aside from direct damaging action of radiation there are other negative consequences. In mammals similar damaging effects of radiation can be expanded among animal groups by mechanism named "bystander"-effect.

We have studied an action of the exposure of intact CBA mouse males (for 24 hrs) to volatile excretions of whole body irradiated males (4Gy, 0.7cGy/sec, ^{60}Co, "Gammacell 220"). Soiled bedding had been collected 24 hrs after irradiation of donor male groups and placed under the wire bottom of standard cage with recipient animals. Direct contact with soiled bedding was prevented. Similarly, control animals were exposed to soiled bedding of intact males. One more group of recipients was exposed to soiled bedding of stressed males (swimming) [Daev et al., 2007].

It is shown by ana-telophase analysis in bone marrow cells that the level of mitotic disturbances is higher in groups exposed to volatile excretions of irradiated (9.8%) or stressed animals (6.9%) than in control group (4.3%). Cytogenetic analysis of spermatocytes II shows similar results in spite of absence of the effect in spermatocytes I.

Thus, irradiated as well as stressed animals produced volatile substances which destabilize mitotic and meiotic divisions in recipient mouse males. It is possible to consider analyzed disturbances of different types as premutational or mutational events. In case of germ cells some of the aberrations could influence progeny of recipient males.

Both mechanisms of direct and indirect action of irradiation should be considered when performing radiation effects studies. The olfactory interaction in mice is an example of one possible way of negative effect expansion among animals after irradiation only few of them. Because "bystander"-induced genetic instability in somatic and especially germ cells can play an important role in animal's fate after stressor action or irradiation, clearly more work is required. Apparently, "indirect" effects of irradiation in humans should be also studied more closely. The research is supported by RFBR grant N 09-04-00693.

LIKE FATHER LIKE SON: TRANSGENERATIONAL GENOMIC INSTABILITY IN MAMMALS

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Mutation induction in the directly exposed cells is currently regarded as the main component of the genetic risk of ionizing radiation and chemical mutagens. However, recent data on the delayed effects of exposure to ionizing radiation represent a new challenge to the existing paradigm. The results of numerous *in vitro* studies show that ionizing radiation can not only induce mutations in the directly exposed cells, but can also lead to delayed effects, with new mutations arising many cell divisions after the initial irradiation damage.

Apart from the studies on mutation rates in somatic cells, considerable progress has been made in the analysis of radiation-induced instability in the mammalian germline, where the effects of radiation exposure were investigated among the offspring of irradiated parents. Our results show that mutation rates at tandem repeat DNA loci and protein-coding genes are substantially elevated in the germline and somatic tissues of non-exposed offspring of irradiated male mice. According to our data, this remarkable transgenerational destabilization can be attributed to the presence of a subset of endogenous DNA lesions. We have recently shown that paternal treatment by the alkylating agent ethylnitrosourea also results in the transgenerational effects, thus implying that this phenomenon is not initiated by a specific sub-set of DNA lesions and is most probably triggered by a stress-like response to a generalized DNA damage.

Our data imply that instability detected in the non-exposed offspring is caused by some DNA-dependent signal transmitted from the irradiated father and implicate an epigenetic mechanism for the transgenerational instability. The potential implication of these results for the estimates of genetic risks for humans will be discussed.

BIOPHYSICS OF HEAVY IONS

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The study of biological effects of heavy ions is attracting increasing interest in the scientific community for two main reasons. First, heavy charged particles can be used to treat solid cancers replacing conventional X-rays. Exciting clinical results are flowing from Japan and Germany, and several new hadrontherapy centers are indeed under construction in Asia and Europe. Second, heavy nuclei at high energy represent a major risk for safe human exploration of the Solar system, because they provide substantial fraction of the equivalent dose absorbed by astronauts in space. Therefore, basic studies on the biological effects of heavy iosn are urgently needed to tackle these two topics. In Europe, a large research activity is carried out at the GSI in Darsmstadtm, where the SIS accelerator can produce heavy ions up to energies of 2 GeV/n, and the future FAIR facility now under construction will push the limit to 45 GeV/n. GSI has pioneered heavy ion therapy in Europe, and is now the main center for space radiation research, supported by the European Space Agency (ESA). Special technological achievements, such as raster scanning and the microbeam (see figure above) are only available at GSI. The current research programs at GSI in the field of clinical radiobiology and psace radiation protection will be presented.

STUDY OF THE INFRARED LIGHT (850 NM), MODULATED BY 101 HZ, ACTION ON THE CROSS ADAPTIVE RESPONSE INDUCTION AND THE REACTIVE OXYGEN SPECIES PRODUCTION IN MICE *IN VIVO*

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In recent decades, great interest among researchers is the phenomenon of radiation adaptive response (AR). Its essence lies in the fact that prior exposure to low doses leads to increased stability of the object to the late effects of radiation in large doses of damaging. It is known that reactive oxygen species (ROS) are involved in the induction of AR. The phenomenon, when adapting and challenging exposures are factors of different nature, is called the cross AR. The cross AR is seen as a form of defense the organism against the mutagenic effect caused not only by ionizing radiation, but chemical agents. At the present time in medical practice there are many different devices, whose action is based on the use of infrared light (IRL) for the treatment of inflammatory diseases. In view of this, the aim of this work was to study the magnitude and dynamics of induction of the cross AR under the action of IRL in bone marrow and blood of mice in vivo.

White mongrel SHK male mice were used. IRL irradiation at a wavelength of 850 nm, modulated by a frequency 101 Hz, was performed using a light therapy device (22 mW/cm^2). Irradiation of mice with X-rays was performed on RUM device (200 kV, 1 Gy/min) as a positive control. To induce the AR used the scheme of radiation adaptive response. The level of cytogenetic damage was assessed using a micronucleus test. The production of ROS was estimated by luminol-dependent chemiluminescence (ChL).

The experiments performed showed that the irradiation of mice induces a cross AR, which can be identified as a cytogenetic and ChL methods. The cross AR did not differ in magnitude and dynamics from the radiation-induced AR and stored up to 2 months. The results also demonstrate that the level of ROS production plays a major role in the early stages of AR on mice. As a cross and radiation AR revealed only 5 h after adapting doses, when the level of ROS production was reduced to control value. The data obtained may indicate that the induction of the cross AR by IRL and radiation AR by X-rays in mice *in vivo* occurs by a similar mechanism.

ALTERATION OF mtDNA COPY NUMBER, MITOCHONDRIAL GENE EXPRESSION AND EXTRACELLULAR DNA CONTENT IN MICE AFTER IRRADIATION AT LETHAL DOSE

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High level of transcription and copy number of mtDNA is a guarantee of mitochondrial normal functioning. We have analyzed the number mtDNA copies and the steady-state levels of the mitochondrial and nuclear transcripts encoding several subunits of the oxidative phosphorylation system in blood cells of mice after irradiation. Animals were irradiated using X-Ray unit RUT-250-14-1 at the dose rate of 2.0 Gy/min, at 15 mA current and 200 kV voltage. Cu-Al filter (1 mm+1 mm) was used in all experiments. Mice were sacrificed 1, 5, 24 or 72 hrs after irradiation followed by decapitation and collection of whole blood samples. The control group (untreated) included 10 mice while each experimental time point is represented by 5 mice. Real-time PCR analysis of the expression and copy number of four mitochondrial genes – *cytb*, *atp6*, *nd4*, *nd2* – and d-loop region in mice blood was performed after irradiation of mice at the X-ray at a doses of 1 and 10 Gy. Real-time PCR was performed using the Applied Biosystems 7500 Real-Time PCR System per the manufacturer's recommendations. Analysis of mice blood cells population after 1 Gy irradiation revealed that at this dose the massive apoptotic cell death did not occur. Nevertheless, the content of mtDNA in blood cells was reduced twofold, whereas the ratio mtDNA/nDNA in serum increased. When mice were irradiation at the lethal dose of 10 Gy, a fivefold reduction in the number of lymphocytes was observed. Irradiation at 10 Gy led to stimulation of mtDNA transcription and replication within the first five hours. Later (24-72h) the content of mtDNA in survived cells decreased to the level observed in blood cells irradiated at 1 Gy dose. This decline in mtDNA/nDNA ratio was not a consequence of reduction in the number of mitochondrial fragments, but due to increase in nDNA content. Apparently, the alteration of mtDNA/nDNA ratio after irradiation can be used as a marker of the exposure to radiation, for instance during radiotherapy of oncology patients.

PHYSIOLOGICAL AND BIOCHEMICAL REACTIONS OF AMARANTH SORT "AMBER" ON MOMENTARY γ – IRRADIATION AND THEIR MODIFICATION OF EXTRACT RHODODENDRON AUREUM

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Amaranth - an annual herbaceous plant with a large number of biologically active substances (BAS): amino acids, trace elements, vitamins, etc. Amaranth's oil sort "amber" has a high content of squalene in the seeds.

Use of Rhododendron aureum extract due to the fact that (BAS) of this plant have higher structural diversity, such as kolhifolin - highly active compound cytostatic action.

The effect of γ-irradiation (0.5 Gy, 50.0 Gy and 500.0 Gy) and treatment of Rhododendron aureum extract in the generation of P on the physiological-biochemical reaction of the first generation (F_1) of amaranth sort "Amber" was investigated.

Laboratory germination of seeds of amaranth F_1 generation derived from irradiated parents was 54.0 – 64.0%. With additional processing of irradiated seed extract of Rhododendron aureum germination was 79.0 – 92.0%. The control germination in the variant without irradiation and extract treatment – 30.5%.

Established that under irradiation seeds generations P, in the F_1 generation the intensity of incorporation of ^{3}H-thymidine and ^{14}C-leucine in 3.4 - 4,7 and 2.1 – 2.4 times respectively, higher than the control. Additional processing of the Rhododendron aureum extract of seeds reduces the intensity of DNA and protein synthesized systems caused by exposure to doses of 0.5 and 50.0 Gy. In the variant with 500 Gy irradiation and additional extract treatment had an increase of intensity of these processes 5.4 and 2.9 times respectively, compared with the control.

To study the antioxidant (AO) systems in amaranth seedlings were determined: the content of low molecular AO, the activity of superoxide dismutase and peroxidase. In all variants decreased content and activity of AO in cells amaranth's seedlings was observed. With the exception of variant with the irradiation dose of 500 Gy, where the peroxidase activity exceeded the control by 50%.

EPIGENETICS OF RADIATION-INDUCED GENOME INSTABILITY

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Ionizing radiation (IR) is an important diagnostic and treatment modality, yet it is also a potent genotoxic agent that causes genome instability and carcinogenesis. The mechanisms of IR-induced genome instability still remain enigmatic. Epigenetic alterations which comprise mitotically and meiotically heritable changes in gene expression that are not caused by changes in the primary DNA sequence, are increasingly being recognized for their roles in health and disease. Three major areas of epigenetics—DNA methylation, histone modifications and small RNA-mediated silencing, are known to have profound effects on controlling gene expression. Yet, the exact nature of the epigenetic changes and their precise roles in IR responses and IR-induced genome instability still need to be delineated. We hypothesized that changes in global and regional DNA methylation and regulatory microRNAs play pivotal roles in IR responses and IR-induced genome instability. To test this hypothesis we utilized an established in vivo mouse model to study epigenetic alterations in the IR-target organs thymus and spleen after exposure to low (0.01, 0.05 and 0.1 Gy) and high (1 and 5 Gy) doses of X rays. First we determined the precise global and locus-specific DNA methylation patterns in the thymus and spleen tissues of IR-exposed of mice using the methylated-DNA-immunoprecipitation approach. We alsoanalyzed global gene expression patterns. We noted that IR exposure led to significant changes in DNA methylation over a variety of loci involved in MAPK signaling, transcription regulation, cell cycle control, apoptosis and DNA repair. DNA methylation changes were correlated with altered expression of selected genes. In parallel we determined microRNA expression profiles in thymus and spleen tissues of control and exposed animals. We noted that IR exposure caused very profound alterations in the splenic and thymic microRNAome. MicroRNA changes were paralleled by significant changes in the expression of miRNA processing machinery. Furthermore, microRNA changes led to significant alterations in the expression of microRNA target proteins. Molecular changes were paralleled by IR-induced alterations in cellular proliferation and apoptosis.The model of hierarchy and cross talk between different constituents of epigenetic information (DNA methylation and microRNAome), the maintenance and regulation of radiation responses and genome stability will be presented.

PHYSIOLOGICALLY ACTIVE HUMIC SUBSTANCES AS A FACTOR FOR DECREASING MUTAGENICITY OF DNA-DAMAGING PHYSICAL AND CHEMICAL AGENTS

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Many industrial pollutants are capable of causing mutations in cells of various organisms, leading to genetic pathologies in living organisms. Thus it is imperative not only to control the mutagen effects, but also to develop rehabilitation measures for preventing an increase in background levels. The most important measure is considered to be the use of wide-spread active humic substances which can degrade toxico-mutagenic properties of soils and increase non-specific resistance of organisms to the action of dangerous ecological factors. Multiple research on plants, microorganisms and laboratory animals has shown evidence that, in case of 50-60% of growth-depression of the mitotic cycle caused by specific inhibitors (such as pesticides and ionizing radiation), humic substances can promote full restoration of biological processes. This is proved by normalizing the relevant metabolisms by increasing the DNA reparation system, and improving the functional state of subcellular structures. The radio-modifying effect of humic substances is also proved in a wide variety of research programmes. Results of specific research on a Chinese hamster fibroblast cell culture and experiments with fibroblastic-like human cells, shows that humic substances reduce the level of spontaneous and radiation damage. Also, the humic preparations available in the cell-growing medium before, during and after exposure to radiation, promote the repair of the DNA single fiber ruptures and the normalising of their functioning. Thus, high physiologic activity of humic substances and their anti-toxic and anti-mutagen properties, are due to favorable influences on the protein, the synthesizing cell system, biological membranes, sub-cell structures and the cell division processes making the base for ontogenesis. Their unique structure and sorptive properties enable the limiting of ecological toxicant migration effects within the soil-to-plant system especially within industrial areas. They also increase the resistance rates of the plants and other organisms with regard to a variety of unfavorable environmental factors. This determines, for example, their protective and ecological functions at the level of the biogeocenosis. The observed decrease of genetic damage to the biota and human cells makes humic substances a promising agent for protecting the genetic fund and preventing genetic consequences of the technogenesis.

INFLUENCE OF VARIOUS REGIMENS OF GAMMA-NEUTRON IRRADIATION ON RADIATION SENSITIVITY OF MELANOMA B-16 CELLS

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One of the most important questions of clinical radiology is overcoming primary and secondary radioresistance of malignant tumours. It can be reached by influence on tumours of ionizing radiation with high linear energy transfer, in particular, neutrons. Application of neutron therapy both alone and in various schemes gamma-neutron therapy with differing contribution of a neutron component to the total dose is known. At the same time is opened a question on optimization of irradiation schemes.

The aim of our studies was comparison the some regimens of gamma-neutron irradiation of murine melanoma B-16 cells *in vitro*. This test-system is a cellular line the resistant to photon radiation. The cells were irradiated at the pulse neutron generator ING-031 (VNIIA, Moscow, En = 14.8 MeV) and the gamma facility "Luch" (^{60}Co). Biological efficiency of radiations by criterion of cells clonogenic growth was estimated. We carried out the comparison of several regimens of gamma-neutron radiation with various sequence (gamma→neutron; neutron→gamma; gamma+neutron simultaneously) and the contribution of neutron component to the total dose (10 and 30 %). The obtained data on cells clonogenic growth after mixed gamma-neutron effect with 10 and 30 % the contribution of neutron were below expected values. It allows to assume about presence of superadditive effect of radiation with high and low LET. Significant effect for irradiation with various sequences of gamma and neutron at 10 % the neutron contribution to the total dose was not obtained. Higher cells death at 30 % the neutron contribution for gamma→neutron in comparison with inverse sequence was reached.

Thus though the data of studies have preliminary character, it is possible to draw a conclusion about increase opportunity of cells radiation sensitivity, which was resistant to photon radiation by applying mixed neutron-photon irradiation.

THE DUCKWEED AS A BIOMODEL FOR THE RESEARCH ABOUT THE POPULATION LEVEL OF POSTRADIATION RESTORATION IN PLANTS

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It is known that each organization level of a live organism is characterized by certain mechanisms of postradiating restoration at the formation of various radiobiological reactions. For example, the basic processes at cellular, tissue and organism levels are reparation and regeneration whereas at cenosis level the leading processes are often the forms of population restoration. Besides, in spite of the fact that the population restoration at cenosis level is continuously linked with restoration at the lower organization levels, at this level the specific forms of restoration characterized for only this level are seen. It is natural that studying of the mechanisms of response to the influence of damaging factors needs new methodological approaches on various forms of population restoration with the use of adequate test systems. For this purpose the species of duckweed was used by us. It was seen that this test-system is characterized by the two levels of response to radiation influence. The first one - at a rather low level of radiation influence (up to 50Gy) when decrease in intensity of leaf growth as well as in colony formation was observed and the second one - at a high level of radiation influence (up to 200Gy) when a crushing of colonies took place and an increase in quantity of undeveloped plant leaves was seen. Thus, thanks to the step character of response of culture duckweed it becomes possible to definite quantity indicators for the investigated populations, not only at the influence of concrete physical and chemical factors but also at multifactorial influences that is often difficult to be calculated. It can be concluded that at the first level of damage an increase of plant resistance to unfavorable factors takes place that is due to the inhibition of growth processes.

COMPUTER MOLECULAR DYNAMICS STUDY OF NANOSYSTEM AND PROTEINS USING HIGH-PERFORMANCE SIMULATION TECHNIQUES

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In the paper we investigate the dynamical and structural properties of nanosystems and biological (protein) molecules based on the advanced computer molecular simulation methods and techniques.

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THE SERVICE EXPERIENCE IN EMERGENCY BIOLOGICAL DOSIMETRY USING CYTOGENETIC ASSAY

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The accidental radiation exposure requires individual dose assessment to help provide early medical aid and following health care. Our laboratory investigates radiation exposure using cytogenetic biodosimetry assay. The examined subjects comprised *low, intermediate* and *high* dose groups. The *low dose* group includes residents of contaminated areas of central Russia and Semipalatinsk nuclear test site. 2250 subjects were sampled repeatedly in 1989-2003. The enhanced levels of dicentrics were found but it could not confirm that they had been protractedly exposed to doses in excess of 1.0 Gy. The *intermediate dose* group includes 925 Chernobyl clean-up workers occupationally exposed to cumulative doses 0.1-0.3 Gy. The *high dose* group includes survivors of Chernobyl accident, emergency cases with industrial radiation sources, victims of radiation accidents of nuclear submarines and others. The doses range 0.5 – 9 Gy, so for the most of them admission diagnosis was ARS. A dicentric assay and FISH technique were synchronously applied over long post exposure period up to 20 years. The subjects showed fast decrease of dicentrics with time with a half-life of 4 months. The translocation technique works well as a retrospective dosimeter up to about 3 Gy. Higher acute doses seem not to be so well measured probably because many stem-cells are unable to produce progeny. The extra group comprised of engineers who entered highly radioactive areas of Chernobyl sarcophagus. Their accumulated doses ranged 1 – 17 Gy over period of 5 years. All of them are professionals with clear understanding of the risk of their health. The translocation yields remained at a high level showing no tendency to decrease and thus proved reliable for retrospective biodosimetry. We investigated how to improve accuracy of cytogenetic biodosimetry: how well *in vitro* dose response calibration for FISH translocations corresponds to translocations yield in lymphocytes taken from people a long time after acute exposure and how suitable are the late translocations and ESR of tooth enamel. It was shown that both persisting stable translocations and ESR spectroscopy signals are suitable with similar efficiency for retrospective biodosimentry after acute whole-body exposure. A residue of dicentrics may be seen decades after high dose irradiation.

8-OXOGUANINE IN CELLS OF DIFFERENT ORGANS OF CBA MICE AS A MARKER OF RADIATION EXPOSURE *IN VIVO*

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Radiation-induced modification of DNA bases is considered to play a significant role in carcinogenesis. 8-oxoguanine is one of the well-known DNA damages; however its induction in mice under irradiation in vivo studied poorly.

The aim of this investigation was to determine the possibility of estimating 8-oxoguanine in cells of the bone marrow, liver and lung after exposure to ionizing radiation in vivo. CBA mice were irradiated with dose of 2 Gy (dose rate 2.47 Gy / min) using ^{60}Co source. Age of animals was 3 months. Fixation of cells by freshly prepared 3.7% formaldehyde performed 30 min after irradiation. Then cells were dehydrated in methanol. After that samples were stained with OxyDNA Assay Kit (Calbiochem-Novabiochem GmbH, Germany), containing FITC-conjugate, which specifically binds to 8-oxoguanine. In addition DAPI was used for DNA staining. The evaluation of average of FITC fluorescence intensity in area of the cell nuclei was performed on microscope Leica DMI4000 B.

In unexposed mice the mean fluorescence intensity (M ± SE) in nuclei of bone marrow cells was 31,8 ± 1,4, hepatocytes - 11,9 ± 0,18, lung epithelial cells - 6,1 ± 0,31 relative units. After irradiation the mean fluorescence intensity of FITC significantly increased ($p < 0,05$) in the hepatocytes and lung epithelial cells up to 12,6 ± 0,17 and 7,5 ± 0,16 relative units, respectively.

Thus, it was shown the possibility of using this method in conditions in vivo. Basic levels of fluorescence of bone marrow cells, hepatocytes and lung epithelial cells were significantly different. We have plan on a further study of radiation-induced DNA oxygenated damages in short- and long-term periods after exposure at different doses.

STUDY OF X-RAY AND LASER IRRADIATION INFLUENCE ON THE POLLEN OF THE MUTANT ZEA MAYS BZ-2M

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In the study of environmental contamination by various physicochemical mutagens one of the leading places is given to the Zea mays. We studied mutant Zea mays bz-2m, homozygotic for a locus adh $^{-}$ obtained from the USA (University of California, Berkeley). The pollen analysis for sensitivity to the different types and doses of irradiations (X-rays and laser) were carried out in a range of the taken doses (40, 60, 80, 100, 120 Gy) and exposition (30 and 60 min). The results demonstrated that pollen fertility rate both calculated right after irradiation, and after incubating period was higher than in control, thus no dose dependence were established. Applied doses and expositions in general do not suppress pollen viability rate, except for doses 100-120 Gy, and do not influence at all on fertility, that allow to conclude about pollen radioresistance. Mutant pollen bz-2m possesses considerable radioresistance to X-rays and stability to the laser irradiation. Morphobiological features and reproduction of the mutant Zea mays bz-2m cultivated in Ararat plain are considerably lower comparing to the initial samples which results of its ecological inadaptability and in consequence of reaction norm of its genotype in new conditions. Zea mayscobs M_1 received from pollination of the mutant plants by X-rays irradiated pollen conserve characteristic for it four types of grain phenotypes, as well as appearance dependence of expected phenotypes of seeded grains that can be explained, obviously, by genotypic resistance of the mutant.

NEUTRON RBE CALCULATION IN TERMS OF RECOIL PROTONS LET SPECTRA

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One of the most interesting problems of the modern radiobiology is the relative biological efficiency (RBE) determination for radiation with high linear energy transfer (LET). For neutron sources, due to huge spectra variation, this problem becomes almost unsolvable, except direct experiment. We have been trying to use recoil proton LET spectra for RBE calculation for arbitrary neutron spectra. It can be possible if protons yield the major part of the neutron dose. Based on our calculations it's indeed so: at least 75% of neutron dose (excluding dose from secondary γ-quarks) are dose from recoil protons for different neutron spectra. The second problem is protons LET spectra. These spectra we have calculated in terms of ICRU data [1].

We have modified survival-LET dependency formula from [2] for *V79 Chinese hamster* cells for LET spectrum:

$$S_F(D,L) = \exp[-\frac{S}{0.16}\int_L \{1-\exp(-(L/L_1)^2)\}\frac{f_D(L)}{L}dL * D] \qquad (1)$$

L – LET, D – dose, S – cell nucleus area, L_1 - independent constant.

For *V79 Chinese hamster* cells the best fit was estimated with S=50 μm^2 and L_1=152 keV/μm.

After integration (1) for recoil protons of 0.2-0.4 MeV neutron group, we had estimated $S_F(D) = \exp[-0.84 * D]$. It agrees well with experimental data from [3], where survival was $S_F(D) = \exp[-0.86 * D]$ for mono-energetic 0.32 MeV neutrons.

Thus, recoil protons LET spectra are suitable for neutrons RBE estimation and the main limitation factor is a deficient knowledge in area of theoretical radiobiology of high-LET radiation.

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GENETIC EFFECTS OF IONISING RADIATION – SOME QUESTIONS WITHOUT ANSWERS

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Nowadays there are no proves of radiation induced mutation existence in human. Four categories of irradiated people were historically investigated: the survivors after Hiroshima and Nagasaki bombing, Chernobyl accident liquidators, patients receiving radiotherapy, people who were exposed to radiation in the course of their work (occupational exposure). No mutation increase was revealed. What are the causes of this? Methods of induced mutation analysis in human are not satisfactory and don't allow to reveal induced recessive mutations, but why wasn't dominant mutation growth registered? What is the difference between human and animals which easily demonstrate radiation induced dominant mutations?

There is no direct evidence of negative influence of low radiation doses too. All health human investigations in populations from regions with high radiation background (in Guangdong province in China, Kerala state of India and states of Iowa and Illinois of USA) had revealed no genetic effects and no harmful consequences for health and lifespan. Indeed there are considerable statistical problems involved in estimating the risks of low radiation doses. The confidence intervals are very large – up to many times greater than the ICRP risk factor at the upper limit, and consistent with a negative factor at the lower limit.

Nevertheless the "hormesis" phenomenon as well as radioadaptive response prove positive effects of low radiation dose – low priming (conditioning) doses protect organism against high second (damaged) doses.

There are a lot of questions concerning radiation induced bystander effect – is this effect positive or negative? Why did evolution preserve this phenomenon if it is negative? What happens after irradiation - adaptive response or bystander effect or both these phenomena simultaneously? May be they arise consequentially - at first bystander effect increases a number of damaged cells in order to switch on protect reactions in organism and induce adaptive response which appears about four hours later?

RADIATION-INDUCED NON-TARGETED EFFECTS OF LOW DOSES OF RADIATION: WHAT? WHY? AND HOW?

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Recent advances in our understanding of effects of radiation on living cells suggests that fundamentally different mechanisms are operating at low doses compared with high doses. Also, acute low doses appear to involve different response mechanisms compared with chronic low doses. Both genomic instability and so called "bystander effects" show many similarities with well known cellular responses to oxidative stress. These predominate following low dose exposures and are maximally expressed at doses as low as 5mGy. At the biological level this is not surprising. Chemical toxicity has been known for many years to show these patterns of dose response. Cell signaling and coordinated stress mechanisms appear to dominate acute low dose exposure to chemicals. Adaptation to chemical exposures is also well documented although mechanisms of adaptive responses are less clear. In the radiation field adaptive responses also become important when low doses are protracted or fractionated. Recent data from our group concerning bystander effects following multiple low dose exposures suggest that adaptive responses can be induced in cells which only receive signals from irradiated neighbours. We have determined using genetically distinct mice, with different radiosensitivities, that bystander effects occur in vivo and vary according to genetic background. We have data showing delayed and bystander effects in fish and in prawns following in vitro irradiation of haematopoietic tissue. These data have implications for environmental radiation protection of human and non human species alike. Simple extrapolations from high to low dose exposure may need to be re evaluated. This presentation discusses our knowledge about these low dose radiobiological effects in non-human biota and draws attention to the problem of defining the important questions relevant for the level of biological organisation being studied.

UNUSUALLY THE SAME γ-RAY- AND NEUTRON-INDUCED MOLECULAR CHANGES REVEALED BY PCR AT THE *DROSOPHILA BLACK* GENE

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The priority N.W. Timofeeff-Ressovsky's works on *Drosophila* germ cells aimed to study the nature of heritable gene/point mutations induced by low-LET radiation , on the one hand, and to assess the efficiency of neutrons as high-LET radiation in gene/point mutation induction , on the other hand, were primarily responsible for the development of the most fundamental and, at the same time, applied fields not only in a classical radiation genetics of animal genes, but in the present-day molecular radiobiology of eukaryotic genes as well. As the advancement of N.W. Timofeeff-Ressovsky's works on *Drosophila* germ cells within the framework of these fields, PCR-assay of γ-ray- and neutron-induced gene/point mutations at *Drosophila black* (*b*) gene, as a mini-target, was carried out. To locate precisely mutation changes on the gene map, the *b* sequence was divided into 3 overlapping fragments. Us PCR results shown, five out of 17(29,4%) γ-ray- and only one out of 13 neutron-induced *b* mutations had lost either of gene fragment studied. All other *b* mutants had wild-type fragment pattern. A predominance of micromolecular DNA alterations not detected by PCR in a full spectra of mutational changes at the *b* gene after action of both γ-rays and neutrons is quite unexpected finding of our studies particularly when taken into account a wide spread notion that neutrons as a high-LET radiation are more effective in induction of massive and total gene deletions than low-LET radiation. The equal efficacy of γ-rays and neutrons in induction largely of a micromolecular DNA changes within a small gene-target in germ cells might be explained within the framework of the theory of track structure (action of δ-electrons) and/ or of the highly specific structural organization of the gene and genome as a whole in animal germ cells in comparison with that in animal somatic cells.

ROLE OF GENES IN DNA DAMAGE RECOGNITION AND REPAIR IN *DROSOPHILA MELANOGASTER* LIFE SPAN REGULATION IN RESPONSE TO LOW DOSE IRRADIATIONS

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During lifetime organisms primarily deal with the action of chronic low dose stress factors, in particular, with ionizing radiation. As a rule, the effects caused by the influence of such environmental factors have nonlinear character, resulting from the interaction of damage genesis and accumulation processes, and resisting mechanisms of stress response. To assess the mechanisms of the complex influence of environmental factors on living organisms it is necessary to investigate the dynamics of such an integrated parameter as life span. It depends on many factors, including behavior characteristics of cellular and physiological processes. In particular, the system of DNA damage recognition and repair is of great importance in this context. The comparison of life spans of objects with fixed set of genotypic differences can be an efficient experimental approach in the study of cellular mechanisms on the basis of the life span parameter.

This research work is aimed at investigating the life span changes at *Drosophila melanogaster* individuals with defects or overexpression of genes of DNA damage recognition and repair.

It has been shown that *Drosophila melanogaster* individuals with defects of genes of DNA damage recognition and repair (in particular *mei-9, mei-41* and *p53*) have the less life span and the hypersensitivity to chronic influence of low dose ionizing radiation in comparison to *Canton-S* wild type strain. *D-GADD45* overexpression leads to life extension of *Drosophila melanogaster* individuals compared to individuals of the parental lines, which is preserved under conditions of chronic irradiation and is an evidence of their better adaptation to the influence of spontaneous and induced environmental factors. Thus, the obtained experimental data demonstrate the role of mechanisms of DNA damage recognition and repair in the life span regulation and determination of the response to the influence of chronic low dose gamma irradiation.

INVESTIGATION OF ADAPTIVE ABILITY OF *DUSCHEKIA FRUTICOSA* GROW ON TERRITORY WITH INCREASED NATURAL RADIATION BACKGROUND

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Influence of increased natural radiation background (INRB) on variability physiological and biochemical characteristics (quotients of general antioxidant protection, reparations and the general relative stability of cells` genome) and radiostability of seed brooding of *D. fruticosa* is investigated.

Shown positive correlation between activity superoxide dismutase and survival of seed brooding *D. fruticosa* depending to INRB vegetation place of mothers plants, that can rate how one of the adaptive way of this species to chronic radiation stress.

It is established, that with increase of expositions doses variability of cells general antioxidant protection is increase. It is shown, that vegetation of plants at doses range from 18,2 to 25,0 pC/(kg·s) led to increase of variability of biochemical characteristics of seed brooding, that in combination with increased containment of summary low molecular antioxidants in cells were the congenial factor for formation of their high radiostability to an acute exposition.

PHARMACOLOGICAL PROTECTION OF ORGANISM AGAINST CHRONIC RADIATION EFFECTS

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The struggle against pathological influence of radiation on the organism with the help of protective substances has been started as long as in the laboratory of B run by N.V. Timofeev-Ressovsky, true in smaller scales than the works on removing incorporated radionuclides (Semyonov, Tregubenko, Moskalyov, Streltsova, Lutchnik, Porjadkova, Timofeeva-Ressovskaya, Buldakov, Kulikova, Budko, Katch, Born, 1946-1955). The drop of numbers of the cells of critical tissues are the basis for the effects of acute irradiation but under the long low-intensive radiation influence – the chain of regulatory-metabolic changes. Such peculiarities create different approach to the pharmacological protection, which basic components are shown in this work. 1) Chemical compounds the molecules of which include radioprotective component are obtained in the result of directed synthesis. Low toxicity allows to inject the preparation multiply, predominantly perorally, in pharmacological doses (Rasina, Chupakhin, 2005). 2) Small mammals of natural populations – small common field mice (Apodemus S. uralensis) from the Eastern-Ural Radioactive Trace were used as a model object in order to study the effectiveness of the compounds (Rasina, Orekhova, 2007). Their samplings have been grouped taking into account the factors of population-ecology. 3) Testing of correcting activity of newly synthesized compounds has been carried out with the help of formed for these purposes complex of biochemical indices which characterizes functional-metabolic changes in the organism as the level of radioresistance and physiological adaptation (Rasina, Orekhova, 2009). The correcting effect of separate compounds has manifested itself in decrease of the intensity of peroxide oxidation of lipids of liver, spleen, erythrocytes and blood plasma, in activation of antioxidant system which is accompanied the increase of ceruloplasmin fraction as the index of neutralization of stress-factors components (Frieden, 1981) and the increase of DNA synthesis encouraging cell-tissue regeneration, conservation of structural-functional integrity of biomembranes and the rise of radioresistance of organism (Sharigin, Pulatova and others, 2005). The work has been done with the partial support of the programme of the development of leading scientific schools (S.S. – 1022. 2008. 4) and scientific-educational centers (contract 02. 740. 11. 0279).

THE GENETIC DETERMINATION STUDY OF INDIVIDUAL RADIOSENSITIVITY CONSIDERING THE PREDICTION OF RADIOTHERAPY EFFECTS

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High frequencies of inherited DNA sequence variations (polymorphisms) are found in the human population. The involvement of polymorphic genes in the individual radiosensitivity is under intensive investigation. Here we present data on the frequencies of chromosome aberrations in lymphocytes of peripheral blood of 97 volunteers depending on genotypes by candidate genes before and after γ-irradiation with dose of 1 Gy *in vitro*. The frequencies of aberrations were estimated by analyzing not less than 1000 metaphases per person. The data of cytogenetic analysis were compared with the results of PCR–genotyping of 18 loci (23 sites) of xenobiotics detoxication *CYP1A1, CYP1B1, CYP2D6, GSTM1, GSTT1, GSTP1, NAT2*, oxidative stress *SOD2, CAT, GCLC,* repair *XPC, APEX-1, RAD23B, XRCC1*, *ERCC1*, *XPD*, apoptosis *Tp53* and *MTHFR* gene, which catalyses DNA methylation and synthesis. The significant differences by the frequencies of aberrations between "single-locus" genotypes were not found except for *GSTM1* locus, for which the enhanced frequency of spontaneous aberrations of chromosome type in "positive" genotypes compared to "zero" ones, i.e., homozygotes by deletion (OR=3,1, p =0.0095) was observed. The minimum frequency of spontaneous aberrations of chromosome type was recorded for carriers of double homozygotes by deletion of *GSTM1-GSTT1*: 0.0006 ± 0.0003 against 0.0027 ± 0.0003 for the rest of genotypes (p = 0.018 by the Mann–Witney test). The frequency of γ-induced chromosome aberrations was significantly higher for carriers of the TT *CAT* (T21A) genotype (p=0.027 by the Mann–Witney test) and for T allele *CYP1A1* (T606G) carriers (p=0.004 by the Mann–Witney test). The frequency of γ-induced chromosome aberrations correlated with total amount of minor alleles in loci *GSTP1, NAT2*, and *MTHFR* (r = 0.25 at p =0.0065). These results are in agreement with literature data that radiosensitivity is associated with functional polymorphisms in susceptible genes and correlates with the number of risk alleles.

MICROEVOLUTIONARY PROCESSES IN CHRONICALLY IRRADIATED POPULATIONS OF SMALL RODENTS

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In long-term monitoring work, natural populations of a model mammalian (mouse-like) species, the bank vole (*Clethrionomys glareolus, Schreber*), living over 22 generations under chronic radiation exposure in areas contaminated due to the Chernobyl accident, have been analysed in terms of ***the concept of microevolution***, developed by N.V.Timofeeff-Ressovsky. According to this concept, the studied populations, being under pressure of enhanced radiation exposure, are representing *elementary subjects of microevolution.* We used the standard chromosome aberration test in combination with various methodological approaches. By correlation of the cytogenetic results with the time course and intensity of the chronic exposure in five different sampling regions, multiple processes have been indentified in these populations, namely (1) the direct cytogenetic response of each investigated animal to its individual irradiation, (2) the transgenerational transmission and accumulation of damage and (3) the development of adaptive effects. The latter have been identified by *lower* frequencies of radiation-induced chromosome aberrations after whole-body acute gamma-irradiation with 100-1000 mGy of animals taken from a chronically irradiated population, compared to whole-body exposed animals from a previously unexposed reference population. The direct cytogenetic response and the transgenerational transmission of cytogenetic damage may be regarded as indicating mutations which act as *the raw material for microevolution*, and the observed adaptive effects appear as the *signs of a microevolutionary process* occurring in the chronically irradiated populations. Moreover in the chronically exposed populations, we have observed a long-term increased frequency of embryonic lethality, appearing as the sign of an effective *mechanism of selection* as one of *the factors of microevolution* and, at the same time, the payment of the population for adaptation. However, according to literature data, the development of radioadaptation is a complex and long-term process. Further investigations are necessary to clarify the genetic and possibly adaptive processes in chronically irradiated populations of mammals.

HORMETIC EFFECTS AND LEGAL DIFFICULTIES

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The hormetic response is a complex systems response that involves not only the responding cell but also surrounding (and not immediately affected) cells. We have demonstrated this in our laboratory through a positive bystander effect. However, the same cell type can also show a negative bystander effect; the response then is dictated by the surrounding cells (the system). This introduces an element of unpredictability into the response, as the response is determined by the status of the surrounding cells and not necessarily the type of insult.

This non-linear response makes legislation difficult as the response is determined (at least at low doses) by the state of the surrounding cells and not the damage to the targeted cell. The same dose could therefore be beneficial or lethal depending on the system biology. The other legal issue to be addressed is that of causation – was the response caused by the insult or the system? The difficulties that hormesis poses for regulators will be discussed.

NON-IONIZING AND NON-THERMAL RADIATION IMPACT ON WATER AND BIOLIQUIDS

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Increasing low electromagnetic field (EMF) pollutions disturb ecologists in the last years. However the mechanism of non-ionizing and non-thermal impact of EMF on bio-systems still is not clear. It is well known, that EMF changes physical and chemical properties of water *i.e.* the redox potential, acidity pH, conductivity, *etc.* Degassing of water produces the same effects. According to [1], a biological effect of weak electromagnetic fields can be caused by degassing of water and/or bioliquids occurring under EMF treatment. Degassing is a result of merge and emersion (coalescence) of synphase polarized nanobubbles. It is shown [2], that degassing by thermo-cycling increases pH and surface tension of water. These changes of the hydrophobic interaction modify the process of micelle structuring [3]. Dynamics of pH changes in water at degassing by centrifuge, as well as the erythrocyte sedimentation rate tests [4] show non-monotonic aftereffects. The research of influence of air dissolved in blood on dynamics of the blood clotting *in vitro* [5] leads to a conclusion that degassing can increase Ca^{++} activity. Many known experimental facts also may be explained in framework of the degassing model. Our theory can answer the main questions: 1) the primary targets of EMF is a micro- and nano-bubbles as discontinuities of the liquid density; 2) the way of accumulation of EMF impact is degassing of the treated liquid; 3) the biological effect is caused by changes of physical properties of the liquid after degassing.

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SOME ASPECTS OF RADIOECOLOGICAL MONITORING OF THE COMMON REED FROM WATER-BODIES WITHIN THE CHERNOBYL EXCLUSION ZONE

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The impact analysis of low doses of ionizing radiation on the breathers in natural populations is an important part of radiobiological studies of non-human biota. The main aim of our studies was to investigate some cytogenetic, morphological and reproductive rates of the common reed (*Phragmites australis* (Cav.) Trin. ex. Steud.) from different water bodies within the Chernobyl accident exclusion zone. The absorbed dose rate for littoral emergent plants in sampling water bodies was varied from 2,2E-02 to 1,2E-01 Gy·year^{-1}. The rate and main types of chromosome aberrations in roots meristems, morphological damages in seed germs, as well as rates of germinating ability and power were analyzed. There were registered rather low rate of germinating ability (14-48 %) and germinating power (<1) of seeds from all sampling water bodies with high levels of radioactive contamination in comparison to control ones. Against the general suppressed background the effect of relative stimulation of more affected seeds was observed. With increase of absorbed dose in range from 2,2E-02 to 1,2E-01 Gy·year^{-1} the number of germinated seeds was increased. At the same time the number of morphological damages of seeds was increased as well. The highest rate of the damages (up to 25 % of the total number of germinated seeds) was registered in plants from the most radioactive contaminated water bodies. There was determined the negative correlation between absorbed dose rate and chromosome aberration rate in roots of the common reed from sampling water bodies. The highest rate of chromosome aberrations (up to 17 %) were registered in plants with high level of morphological deviations in seeds germs. The data obtained from the complex analysis of natural populations of the common reed from the radioactive contaminated water bodies testify about rather high level of genetic efficiency of low doses of long-term exposure. There is observed a realization of radiobiological reactions on morphological and reproductive levels of plants from contaminated water bodies on the background of genetic instability induced by low doses.

INFLUENCE OF LOW DOSES OF DIFFERENT PHYSICAL AND CHEMICAL AGENTS ON THE INDUCTION OF CYTOGENETIC ADAPTIVE RESPONSE AND THE GROWTH OF SOLID TUMOR IN MICE *IN VIVO*

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In the last few years, the phenomenon of radiation-induced AR has aroused considerable interest among specialists concerned with the problems of radiobiology, cancer radiotherapy and ecology. It is considered as the form of the biological defense of the cells against the mutagenic action of oxidative stress induced not only by ionizing radiation and chemical agents but also by somatic diseases.

The goal of our investigation is a study of influence of low doses of physical and chemical agents on the cytogenetic damage in bone marrow cells and the oncogenesis, using the "adaptive response" test in mice in vivo.

Two-month-old SHK male mice were used. For the induction of AR, the animals were subjected to adaptive treatments with doses of 0.1 Gy of X-radiation (1 Gy/min), 0.16 Gy of chronic high-LET radiation (0.01 Gy/day), infrared light (IRL) at 850 nm modulated by a frequency of 101 Hz, and 100 μM hydrogen peroxide (i/v), and then were additionally irradiated with a challenging dose of 1.5 Gy of X-rays (1 Gy/min). Cytogenetic damage to bone marrow cells was detected by calculating micronucleated polychromatic erythrocytes. The influence of the adaptive treatments on the growth of solid tumor of Ehrlich ascite carcinoma was estimated by standard procedure.

It was found that: (1) the treatment of mice with low-dose of X-radiation, IRL and hydrogen peroxide induced cytogenetic ARs, which were equal in magnitude and dynamics of induction, and decreased the growth of solid tumor; (2) by contrast, the irradiation of mice with low dose of high-LET radiation induced no AR and increased the growth of solid tumor.

We suggest that the used adapting treatments bring the organism into a novel state characterized by increased resistance to radiation-induced cytogenetic damage and oncogenesis. The study may be of potential importance for clinical application of low-dose exposures in the therapy of certain kinds of tumors.

COMPARATIVE STUDY OF THE SOS DNA-REPAIR MECHANISM INDUCED BY UVC IN AEROBIC AND HYPOXIC CONDITIONS

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The hypothesis about inducible mutagenic SOS DNA-repair in bacteria was described for the first time in 1974. The SOS system is induced in response to the rare field of DNA-damaging agents which can lead to the forming of single-strand breaks in DNA. The SOS-repair is error-prone mechanism since it allows replication machinery to bypass lesions in DNA and to synthesize damaged DNA in the absence of matrix. Thereby, the SOS system is an original genetic program that can results in cell's adaptation to the changeable environment conditions and in accumulation of its evolutionary potential. The peculiarities of SOS-response was fully studied with UV-light as an inductor: genetic control, biochemical basis and structure of SOS-signal were clarified. However up to date there isn't any data about SOS-induction in hypoxic conditions, though hypoxia is known as one of the major factor of genetic instability and affects strongly on gene expression in both bacterial or mammalian cell.

The aim of our work was the comparative studying of the SOS-response induction and possibilities of its modulation in hypoxic and aerobic conditions.

The formation of SOS-response, as a result of genotoxic agents affecting, was monitored by the sfiA-gene expression (SOS-regulon) in E.coli strain with [sfiA::lacZ] structure (Quillardet Ph., Hofnung M., 1985). The isogenic strains of E.coli PQ37 uvrA [sfiA::lacZ] and E.coli PQ65 uvr+[sfiA::lacZ] were studied. Difference of strains – is in activity of the excision system UvrABC that repair UV-damages. We used short-wave UVC-irradiation (λ=254 nm) provoking direct DNA-damages and the formation of pyrimidine dimers mainly. The data were also compared with SOS-induction by 4-nitroquinoline 1-oxide (positive control) and nitric oxide donors.

We have experimentally proved for the first time that SOS-response develops in hypoxia conditions. Here we report that hypoxia specifically up-regulates the sfiA-expression induced by UVC, but comparison of SOS-induction by different SOS-agents exposes that presence of oxygen has differently directed effects.

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DIRECT AND REVERSE BYSTANDER EFFECT BETWEEN IRRADIATED AND UNIRRADIATED ORGANISMS: THE MODULATING ROLE OF CHEMOSIGNALLING IN ECOLOGY

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It was shown that mice or rats exposed to sublethal dose of ionizing radiation are able to decrease the immune reactivity of intact animals when them kept together in the same cage. Even one individual can cause such a disturbances of immunity in a group of intact animals. The data indicate that at early stage after exposure to sublethal doses of ionizing radiation mice secrete volatile substances (VSs) in urine which decreased thymus dependent humoral immune respose in intact animals (to 60-70% relative control). The stable effect observed as a result of one-day exposure allowed us to study the time course of secretion of immunosuppressive VSs by irradiated animals. These VSs possess attractive properties for the intact individuals. The irradiated mice showed an increased attractiveness to intact individuals. The biological significance of a combination of the immunosuppressive and attractive effects of VSs is unclear. It is supposed, that mammals possess of the distant immunomodulating chemosiqnal system, directed for immunoreactivity of individuals with immunodeficiency state.

It was established that VSs of intact mice restored the humoral immune response and other parameters of immunity in irradiated with a dose of 1 Gy animals. In this case, the irradiated recipients demonstrated an increase of humoral immune response to 140-170%. These VSs of intact mice activated at irradiation mice phagocytic activity peritoneal macrophages. Exposure of rats for the third day after irradiation (1 Gy) to the VSs of intact animals significantly increased the number of red blood cells, lymphocytes and granulocytes in the bloodstream.

Thus, direct and reverse bystander chemosignaling between the irradiated and intact, irradiated and irradiated animals mediate the modification of immunity and behavioral reactions of recipients. Apparently these untargeted effects of radiation spreading from one individual to another can have a significant impact on the viability of the entire population of animals.

THE CARRYING OUT OF NEUTRON BEAMS WITH ENERGY 10-16 MEV RADIOBIOLOGICAL STUDIES

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Radiotherapy is one of the main cancer treatment methods. Unfortunately the conventional radiation therapy methods are inefficient approximately for 30% patients. Therefore the complex approach to treatment of patients with radioresistant tumors is developing actively recently, neutron, proton and ion therapy methods are introduced to clinical practice. The new medical installations ensuring high therapeutic efficacy and necessary quality assurance of the radiotherapy are developed. The possibility of the therapeutic gain factor (TGF) increasing by means of low- and high-LET radiations interaction attracts a great attention.

This study was carried out on KG-2.5 accelerator (IPPE, Obninsk) neutrons with energy 10-16 MeV, dose rate ~ 0.08 Gy/min, derived by the reaction $^{7}Li(d,n)2^{4}He$. The "Luch" installation (^{60}Co) was gamma radiation source. Murine melanoma B-16 cells (*in vitro* study) and sarcoma M-1 transplantable to rats (*in vivo* study) were used as biological objects. The linear accelerator KG-2.5 neutron beam corresponds to analogous neutron sources in this energy range according to melanoma B-16 cell survival test (the relative biological efficacy (RBE) is equal to 1.8). RBE for sarcoma M-1 varies from 2.4 to 4.0 depending on scheme and dose of gamma-neutron irradiation (these results correlate with BR-10 reactor clinical data). The efficacy of gamma-neutron radiation with low neutron contribution (10-30%) is slightly higher that theoretically calculated efficacy for these irradiation parameters.

The clinical data showed that the sequence of gamma radiation and neutrons influence on antitumor effectiveness and TGF: preliminary gamma-rays exposure is preferable for a daily fractionating. The extending of interval between the fractions levels this advantage of gamma-neutron therapy.

X-RAY AND UVC-INDUCED BYSTANDER EFFECTS IN PLANTS

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Plants are capable of rapidly reprogramming patterns of gene expression, allowing fast acclimation and adaptation in response to specific environmental conditions. This ability depends on various signalling molecules operating within a plant and even between plants. Our previous experiments (Kovalchuk et al., Nature, 2003; Boyko et al., NAR, 2007; Boyko et al., 2010, PLoS ONE) showed that local exposure to stress results in systemic increase in homologous recombination frequency (HRF). We named this signal 'systemic recombination signal' or SRS. Although the nature of the signal was known, we hypothesized that plants are able to communicate this signal through phloem and through air.

For the experiment, we used transgenic Arabidopsis thaliana plants carrying in the genome luciferase gene serving as a substrate for homologous recombination. Cells in which recombination events took place are visualized in CCD luciferase camera after application of luciferine. Recombination events then are scored and recombination frequency calculated. In the experimental set-up we planted two groups of plants in Petri dish and covered one group with either aluminium or lead cover and irradiated the second group with either UVC (7,000 ergs) or X-ray (5 Gy). To test whether signal is communicated through media or through air, we used Petri dish with ¾ height dividers that separate the media but not the air exchange. In another set of experiments we placed two pots, one with irradiated and one with non-irradiated plants in sealed plastic bag for 4 days and scored recombination frequency in 7 days.

We found that both groups of plants, irradiated and non-irradiated grown in Petri dish had higher recombination frequency. Moreover, we found that both groups of plants grown in divided Petri dish also had higher recombination frequency. This suggested that the signal leading to increase in HRF is indeed airborne. The experiment with plastic bags also showed the increase in HRF in both groups of plants.

We conclude that irradiated plants exchange warning signals that could promote additional rearrangements in plant genome. This bystander effect could be one of the mechanisms of induced evolution, since increase frequency of rearrangements could potentially lead to diversification of genome composition in the progeny.

DETECTION OF A DISTANCE EFFECT IN PEA SEEDLING USING THE ANALYSIS OF GENES EXPRESSION

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Distance effect is phenomenon of transmitting biological signal from irradiated tissues to nonirradiated known to be characteristic for vertebrates. Possible induction of a distance effect in pea seedlings by ionizing radiation of doses 2 and 10 Gy was investigated. Totally irradiated plants (TIP) and root tips of plants with irradiated upper parts (IUP) were collected on the 1st and 4th days and their chromosomal aberrations (CA), mitotic index (MI) and relative growth rate (RGR) as well as transcription levels of *SAMS2*, *PCNA*, *P34*, *DHN3, Tub A1* genes were analysed. Comparison of CA, MI and RGR levels was not efficient enough to show any distance effect. Gene expression levels were evaluated via extraction of total RNA, synthesis of cDNA , specific PCR amplification followed by electrophoresis in agarose gel and quantification of gel band intensities with ImageJ processing program. Analysis of *SAMS2* gene expression in the IUP showed the increased expression level on the 4th day in comparison with expression level on the 1st day after the irradiation, while the TIP's expression level on the 4th day decreased. *PCNA* gene expression in the IUP showed similar results. Meantime expression level of *DHN3* in the IUP decreased on the 4th day and increased in the TIP. A variation of *P34* gene expression level is currently inexplicable. Analysis of expression level of *Tub A1,* used as a control, resulted in a relative stability at the current conditions. All in all the distance effect in pea plantlets was shown using the analysis of gene expression technique.

ROLE OF THE BOTTOM SEDIMENTS IN FORMING THE DOSE OF IRRADIATION OF DIFFERENT FISH SPECIES IN KIEV RESERVOIR

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In 2009 the formation of absorbed dose from incorporated radionuclides, water and bottom sediments on different species of fish from Kiev reservoir was studied. The aim of the work is the estimation of influence of fish behaviour from Kiev reservoir on forming the absorbed dose with taking into account the levels of radionuclide pollution of the ecosystem components. In our study the data about contents of radionuclides in water, bottom sediments and fish are used. Such species of fish as: *Rutilus rutilus* L.; *Tinca tinca* L.; *Blicca bjoerkna* L.; *Abramis brama* L.; *Carassius auratus* gibelio (Bloch)., *Hypophthalmichthys molitrix* Valenciennes; *Silurus glanis* L.; *Esox lucius* L.; *Stizostedion lucioperca* L.; *Perca fluviatilis fluviatilis* L. was studied. The migratory behaviour of fish and the spatial distribution of radionuclides by bottom area was considered. Absorbed dose from sum of incorporated ^{90}Sr and ^{137}Cs for fish was from 10 (*Hypophthalmichthys molitri*) to 112 (*Esox lucius*) $\mu Gy \cdot year^{-1}$. At the average, dose of irradiation from incorporated radionuclides for fish predators was in 3-4 times above, than for fish non-predators. Absorbed dose of water irradiation did not exceed 0,3 $\mu Gy \cdot year^{-1}$. Power of absorbed dose from bottom was depended on level of ^{137}Cs content in bottom soils, particularities of fish vertical distribution in water masses and from time of fish staying in zone of bottom sediment influence. The largest annual absorbed dose of irradiation from bottom was typical for *Tinca tinca* and *Carassius auratus* (7700 $\mu Gy \cdot year^{-1}$) from upper part of Kiev reservoir, the least - for *Stizostedion lucioperca* and *Hypophthalmichthys molitrix* (400 μGy/year) from lower part.

So, the 90 % of total annual absorbed dose of irradiation for different fish species from Kiev reservoir was determined by bottom sediments radionuclide pollution.

EPIDEMIOLOGY OF IONIZING RADIATION

RISK OF CHROMOSOMAL INSTABILITY STUDIED BY STATISTICAL MODELING

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It is known that irradiation stress induces late intracellular processes and adaptation. In this paper, we formulate the adaptation hypothesis. On its basis, we present statistical modeling. The modeling shows that multiple appearances of chromosomal abnormalities can be explained as a result of primary or late processes caused by DNA damage, with or without cell selection, which can be described with geometrical or Poisson distributions, respectively. The connections between the late intercellular processes (the "bystander effects") and late intracellular regulatory processes are considered. It is shown that combinations of low-dose-rate irradiation, high temperatures, and aging lead to synergistically increasing instabilities and decreasing seed survivals. Approaches to risk estimation of chromosomal instability are investigated in plant cells and in human blood lymphocytes. Risks of instability were estimated as values of distribution parameters dependent on radiation intensity in ecology and in generations of persons who experienced radioactive fallouts from nuclear tests. We conclude that statistical model of adaptation describes intra-and intercellular processes of genetic instability coupled with selection, and that the risks of these processes can be calculated as model parameters.

INTEGRAL ECOLOGICAL HYGIENIC ASSESSMENT OF ENVIRONMENTAL CONDITIONS AS HEALTH HAZARD

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Second half of 20-th century, as a result of creation of multiplane atomic engineering, has brought in an inhabitancy continuously growing quantity of the artificial technogenic radioactive substances new, as on population (ecosystems) character and by for the first time generated variants the combined influences with not less aggressive tehnogenno-toxic factors of environment.

At the same time, despite popularity of geography of distribution of radiating pollution of Ukraine, Belarus and Russia as a result of failure on the Chernobyl atomic power station, research of consequences of Chernobyl accident is still considered without taking into account tehnogenno-toxic influences, their intensity and inevitable distinctions in such situations of adaptable reactions of the population on change of structure of environment.

By us it is spent integral ecological hygienic assessment of environmental conditions in all areas of Bryansk region over a decade (from 1998 till 2007) is given as far as radiation (as the result of the Chernobyl catastrophe), toxic-chemical (as the result of industrial emissions accumulation) and combined radioactive-toxic components are concerned. Correlations of environmental changes with primary morbidity of infantile, adolescent and adult population are analyzed. Radioactive pollution density of the territories by ^{137}Cs is from 18,5 kBc/m^2 to 2149,7 kBc/m^2. The total technogenic pollution density (kg/person/year) by fluid and gas toxicants is from 1,1 to 149,9 kg/person/year, from 0,1 to 6,6 out of them are carcinogen 3,4 benzapyrene, from 0,1 to 60,5 – nitrogen oxides, from 0,1 to 28,0 – sulfur dioxide, from 0,7 to 57,8 – carbon oxide.

Data show sharp diversity of the infantile primary morbidity level of the areas under study, infantile morbidity growth in radiation areas in comparison with All-Russian morbidity, especially when background technogenic toxic metabolites (3,4 benzapyrene, nitrogen oxides, sulfur dioxide) are entered into the environment and also rigid correlation with toxic metabolites (r=0,85). There exist correlation with radioactive pollution density by ^{137}Cs only when there are combined radioactive-toxic environmental effects (r=0,56).

INDIVIDUAL PECULIARITIES OF GENOMIC INSTABILITY EXPRESSION IN CHILDREN BORN FROM IRRADIATED PARENTS AND GENETIC POLYMORPHISM

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High individual variability of genomic destabilization was observed in children born after the Chernobyl accident in 1987 - 2004 from irradiated parents (150 subj.) and manifested themselves in the diverse spectrum of transgenerational mutational effects (chromosome aberrations, TCR - mutations) and in different levels of their expression. The results of experimental studies confirming the reality of the induction of genomic instability in children living under conditions of prolonged exposure to the radiation factor (born in 1986-1998), also indicate the individual character of this phenomenon. In children with Elers-Danlos syndrome and Down syndrome whose cells have an increased radiosensitivity and used as a model for studies, various features of polymorphism in GST - genes, compared to the children of the control group were revealed. Thus, the possible contribution of the GST - family in the formation of radioresistance of cells and the organism as a whole was shown. Polymorphism of 5 detoxification genes: GSTM1 and GSTT1 (deletion polymorphism), GSTP1 (A330G), CYP1A1 (A4889G), NAT2 (T341C and G590A) and MTHFR (C677T) gene has been determined in two groups of children (45 subj., the children of the liquidators of the Chernobyl accident and the children born from the parents who lived for a long time on the radionuclide contaminated territories) and their parents (61 subj.). Elevated frequency of aberrant cells (AC) in irradiated parents - carriers minor T allele of MTHFR gene (TT and CT genotypes) compared to the individuals with the MTHFR (CC) genotype ($p = 0,007$) was revealed. The frequency of rare homozygotes GSTP1 (GG) and / or heterozygotes CYP1A1 (AG) in children with high frequencies of certain cytogenetic disorders (AC level of $\geq 2\%$) was 44 %, which significantly exceeded ($p = 0,032$) such rate in children with lower levels of chromosome aberrations (14,8 %). It has been shown that the rate of carriers of mutant alleles in two or three studied genes (genotypes GSTP1 (GG), CYP1A1 (AG), NAT2 (TC or CC)) is higher in children with elevated levels of chromosome aberrations (38,9%) as compared to patients with lower frequency of cytogenetic disorders (3.7%) ($p = 0,004$). The data obtained suggest a role of revealed molecular-genetic features in the expressivity of genomic instability in the children of irradiated parents and the necessity of further investigations.

EFFECTS OF BYSTANDER FACTORS *IN VIVO* FROM AFFECTED CHERNOBYL POPULATIONS

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In the investigation, the phenomenon of bystander effects (transfer of damaging factors from affected cells to intact cells) induced *in vivo* has been studied. This phenomenon is intensively studied all over the world, but its mechanism and the nature of inducing factors are still not known.

The purpose of this work was to study the bystander effect, induced in in vivo conditions – in blood of populations, irradiated by the Chernobyl accident (Chernobyl liquidators 1986-1987, workers of Polessky State Radiation Environmental Reserve (30 km area of Belarus around Chernobyl) and people living on contaminated by radionuclides territories of Gomel region).

Human keratinocyte cell culture was used as a test-system to evaluate the effects of bystander factors from blood serums of affected populations using micronuclei and viability assays.

It was revealed that bystander factors from the blood serum samples of affected populations were able to significantly increase the level of micronuclei and decreased viability of treated cells compared to intact and control cells. There were no statistically significant difference observed between intact cells (without serum added) and control cells (serums from healthy populations added). The highest level of damage was induced by the factors from serums of Chernobyl liquidators 1986-1987 yy., the least – factors from blood of people, living in contaminated territories of Gomel region (in some cases, individual data of these patients was not statistically different from control data).

The data clearly shows that blood serum samples of irradiated populations contain some kind of bystander factors, induced in vivo and able to cause cell damage when transferred to in vitro conditions. These factors are able to circulate in blood stream for more than 20 years (in case of 1986-1987 Chernobyl liquidators), possibly, creating self-maintaining vicious circle, could not be neutralized by antioxidants and do not lose their effect after freezing.

ANALYSIS OF P53, NPM, N-RAS GENES IN PERIPHERAL BLOOD OF PATIENTS IN LATE PERIOD AFTER RADIATION ACCIDENTS

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Protein p53 plays a significant role in maintaining genome stability. Both the transcription activity of P53 gene and forming of the complex p53 with Hdm2, which leads to destruction of the protein p53, determine the content of this protein . NPM and ARF regulates the content of p53, affecting both the transcription of the gene and interaction of this protein with Hdm2. Genes N-RAS, NPM, c-Myc stimulate proliferation that may have a role in tumor formation. In this study, we consider the transcription activity and mutations in genes NPM, p53, n-RAS, which were examined in peripheral blood of the healthy donors and the patients in late period after radiation accidents.

We designed an RT-PCR strategy to show among the patients the decrease of P53 expression and the constant level of NPM compared with the control group. Among the patients was found that the forming de novo mutations in "hot points" in areas of codons 246–250 exon 7 of P53 gene and codon 12 of N-Ras gene, which are damaged by intracellular oxidative stress, was met more often than in the control group. Compared to patients without such mutations, patients with mutations have been shown to have reliably lower expression of NPM. The forming of these mutations depended on level of p53 regulated by expression of NPM.

Our results provide evidence suggesting that the higher transcription activity of NPM supports stabilization of p53 in the cells, increase its level and intensification of genome stability control.

ADAPTIVE RESPONSE IN BLOOD LYMPHOCYTES OF PATIENTS SUFFERING FROM THE ALLERGIC SYNDROME OF THE SECONDARY IMMUNE DEFICIENCY

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The phenomenon of radiation-induced adaptive response (AR) consists essentially in the fact that preliminary irradiation with low doses leads to increasing of resistance of an object to the subsequent high-dose exposure. AR as a whole may be an important general biological mechanism for cell protection against the mutagenic and lethal effects of genotoxicants. The goal of this work was to study the capacity for AR in peripheral blood lymphocytes of patients with the allergic syndrome of the secondary immune deficiency.

The study included 12 women suffering from the allergic syndrome of the secondary immune deficiency and 17 healthy women. The induction of AR was studied *in vitro* in a culture of peripheral blood lymphocytes. Samples of whole blood were irradiated on a RUP X-ray device according to the following scheme of AR: preliminary irradiation with a dose of 10 cGy followed 5 h later by irradiation with a challenging dose of 2 Gy. The frequency of micronuclei (MN) in cytochalasin-blocked binuclear lymphocytes served as a criterion of damage.

It was found that (1) the mean frequencies of spontaneous MN in lymphocytes from immune deficiency patients is significant higher than in lymphocytes from healthy donors; (2) there is a clear correlation between the spontaneous and radiation-induced level of micronuclei in our groups of both sick and healthy donors; (3) AR was induced almost in all women examined, with the mean magnitude of AR in both groups being the same, but the interindividual variability of the magnitude of AR in the group of secondary immune deficiency patients was significantly greater. We suggest that increased chromosomal sensitivity of lymphocytes from sick women may be related to immune deficiency state. In contrast, AR induction did not depend on immune system, therefore we thing that adaptive response test may be not a marker for prediction of immune diseases.

SOMATIC GENE MUTAGENESIS IN PERSONS EXPOSED TO IONIZING RADIATION AT LOW DOSES

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The aim of this work was to study frequency of peripheral blood lymphocytes bearing mutations at T-cell receptor (TCR) locus in various human contingents exposed to ionizing radiation at doses up to 200 mSv. Flow cytometry was used to detect variant $CD4^+$ T-lymphocytes lacking CD3 as a result of mutations at TCR locus. The TCR-mutant cell frequency was assessed in 450 unexposed control donors and exposed persons, including 343 employees of Nuclear Power Engineering (NPE, mean cumulative dose±SE 100,0±6,6 мSv), 371 Chernobyl cleanup workers (100,0±9,1 мGy) and 525 persons living in radiation contaminated areas (5.1-13.8 mSv). Indicators of redox system (activity of antioxidant enzymes, content of malonic dialdehyde in blood plasma and intracellular level of nitric oxide in lymphocytes) were determined in NPE workers and compared with the TCR-mutant cell frequency.

Results of group analysis demonstrated an increase in frequencies of the TCR-mutant cells in all groups as compared to those in age-matched control groups ($p<0.05$ by Mann-Whitney test). In the exposed groups there is an appreciable proportion (12-18%) of individuals with elevated mutant cell frequencies exceeding the 95% confidence interval in age-matched control groups. If such persons are excluded from the analysis, mutant cell frequencies in the remaining persons do not differ from those in the control group. These findings imply that statistically significant increase in mutant cell frequencies in the irradiated groups is attributable namely to the subset of individuals with *high levels of mutant cells. The proportion of persons with elevated mutant* cell frequencies did not depend on dose in groups of the Chernobyl cleanup workers and the NPP workers. The response of irradiated residents displayed some extent a correlation with radiation dose. The TCR-mutant frequency was found to significantly correlate with content of malonic dialdehyde and intracellular level of nitric oxide.

GENETIC EFFECTS IN THE OFFSPRING OF PEOPLE EXPOSED TO IONIZING RADIATION – ARE THEY POSSIBLE?

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Ionizing radiation (IR) is a known mutagen; however, no distinct radiation-induced hereditary diseases have so far been demonstrated in human populations. High resistance is peculiar for mammalian immature germ cells (spermatogonia and dictyate oocytes) coinciding with the epidemiological evidence of Hiroshima and Nagasaki cohort. Mature and maturing gametes (i.e. close to conception period) are substantially more vulnerable; however, desire minor attention simply because it is highly unlikely to expose many people at these stages. Nevertheless, in case of extensive radionuclide contamination many people could be chronically exposed to low-dose IR which effects are poorly studied. Human data are even scarcer. In this respect, Chernobyl accident (April, 26 of 1986) provided unique opportunity to study the question.

Surveillance of congenital anomalies (CA) in Belarus is done within population-based registry. The prevalence of 9 CA, usually undoubtedly diagnosed among newborns, were traced since 1980. Within the post-accidental period an increasing trend was observed for majority of CA; however, the situation was similar for areas contrasting by radionuclide contamination assuming general cause of the trend, most probably not associated with IR. Significant positive association with weighted-average for the population annual exposure doses was marked within the very first years after the accident for polydactyly only (clinically trivial anomaly with considerable impact of hereditary cases). Moreover, significant pick of Down syndrome and polydactyly was marked at the beginning of 1987, i.e. among children conceived within the first months after the accident when exposure dose rates were considerably elevated due to decay of short-lived radionuclides.

Thus, despite high general resistance of mammalian germ cells to IR, exposure around conception period might be considered as a possible risk factor for CA passing by prenatal selection. Obtained results can not be considered as a strong evidence for cause-effect association because ecological nature of the study does not provide the opportunity to control for possible confounders and biases. The results need confirmation in the other exposed populations, preferably in properly designed analytical studies.

RADIOECOLOGY

ANALYSIS OF THE EFFECTIVENESS OF COUNTERMEASURES TO REDUCE ^{137}Cs ACCUMULATION IN PLANTS AFTER THE CHERNOBYL ACCIDENT

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Illustrated by the south-western districts of the Bryansk region, the dynamics of ^{137}Cs transfer factors to plants and countermeasures effects on the radionuclide accumulation in farm products have been estimated. A significant decline with time in TF ^{137}Cs to plants is shown. Over 20 years after the radioactive fallout this decline ranged for different plants from 7 to 85 times, being maximal in the first years. The accumulation of ^{137}Cs by plants is greatly influenced by the soil properties. Thus, the maximal transfer of the radionuclide to plants is reported on peaty soils followed by sandy soils and minimal - on sandy loam soils.

An important role of soil mineral fertilizing and liming has been demonstrated in reducing ^{137}Cs accumulation in plants. On agricultural lands which saw increased rates of fertilizing a significant (on average 3- to 7-fold) decrease in TF ^{137}Cs was reported compared to the sites which did not receive countermeasures. Particularly effective is the use of agroameliorants in the early years after radioactive fallout due to high ^{137}Cs mobility. Then the effectiveness of such measures declines because of the radionuclide fixation in the soil.

Three periods have been identified in the reduction of ^{137}Cs content in plants during 20 years after the Chernobyl accident estimated by change with time in the effective half-life of this radionuclide after radioactive fallout. In the first period after the ChNPP accident (1987-1991) this parameter varies within the 0.5-2.3 years range. In the second period (1992-1997) decrease in ^{137}Cs transfer slows down and amounts to 1.2-15.5 years. The third post-accidental period (1998-2006) is characterized by maximal values of the effective half-life (3.3-30.1 years). Currently, for some crops a decrease in ^{137}Cs content is defined by radioactive decay of this radionuclide and several crops show a trend for reaching this value.

The investigations have also demonstrated that in the same conditions ^{137}Cs is transferred to fodder products to a larger extent than to plant products, which needs to be taken into account while planning the crop rotation systems in radioactively contaminated areas.

RADIOECOLOGICAL STUDIES AT THE YENISEI RIVER

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The Yenisei is one of the largest rivers in the world. The mining-and-Chemical Combine (MCC) of Rosatom, situated on the bank of the Yenisei, 60 km downstream of the city of Krasnoyarsk, includes a reactor plant and a radiochemical plant. The MCC has been in operation for over 50 years and has contaminated the Yenisei floodplain with radionuclides. Annual expeditions of the Institute of Biophysics have revealed a number of facts suggesting that radionuclide concentrations in the Yenisei ecosystem are many times higher than their background levels. There are floodplain areas with abnormally high ^{137}Cs levels in the soil – up to 850 kBq/kg. Most of them are local spots, but there is also an extended anomalous riverside stretch in the town of Yeniseisk, 330 km downstream of the MCC. "Hot" particles with ^{137}Cs activity reaching 30 MBq/particle were detected in floodplain soil layers. Laboratory examination proved the reactor origin of those particles and made it possible to approximately date them. Sediment samples collected from the Yenisei River were found to contain layers with abnormally high levels of transuranics.

Monitoring of radionuclide levels in samples of aquatic organisms (submerged plants, zoobenthos, fish) from the Yenisei River in the vicinity of the MCC revealed a wide range of long- and short-lived artificial radionuclides. Certain species were found to contain up to 30 artificial radionuclides; ^{32}P concentrations were the highest. The first detailed analysis of radioactive contamination of forest ecosystem components in the Yenisei floodplain revealed abnormally high ^{137}Cs concentrations in the edible fungi, *Suillus granulatus* and *Suillus luteus*, which reached 10200 Bq/kg and were much higher than the safe limit accepted in Russia (2500 Bq/kg). Such berry shrubs as *Ribes nigrum* and *Rubus idaeus* were found to contain higher concentrations of radionuclides than other berry shrub species. Measurements of shrub samples collected in the Yenisei floodplain for many years detected such artifical radionuclides as ^{137}Cs, ^{60}Co, ^{90}Sr, transuranics (^{238}Pu, ^{239,240}Pu, ^{241}Am, ^{243,244}Cm), and ^{238}U.

CONTEMPORARY TRITIUM LEVELS IN MOISTURE-CONTAINING SYSTEMS IN THE REGION OF THE "MAYAK" ENTERPRISE

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Tritium, a radioactive isotope of hydrogen, enters the environment during operation of atomic reactors and nuclear fuel processing. The water medium in the basic place for tritium occurence in the nature. Tritium global background level in the water is 1 Bq/l, the technogenic background level is 5 Bq/l.

The inspection of water ecosystems in the region of the "Mayak" enterprise revealed tritium concentrations exceeding these levels: 6 to 113 Bq/l in non-technogenic reservoirs, 7 to 262 Bq/l in the snow cover, 13 to 80 Bq/l in the drinking water. Concentrations in open reservoirs and snow fallout decreased as the distance from the "Mayak" increased. We marked the tendency of tritium levels to decrease in lakes of non-technological usage: 8 and 6 –fold lower compared to 1982 and 1986. Concentrations depended on the predominating direction of winds formed by the mountain massi south-westwards of the enterprise.

Tritium concentrations also significantly depended on the depth of wells we suggest that underground water was the source for tritium entry. Tritium monitoring in rain precipitations in the region revealed an order higher concentrations near the enterprise than the technogenic background values in the control region. The results of the investigation snow that the "Mayak" is the source for anthropogenic tritium entry to the environment.

RADIOECOLOGY OF THE FUTURE: THE PROBLEM OF FINAL GEOLOGICAL DISPOSAL OF RADIOACTIVE WASTES

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The ethical principle according which the concept of "sustainable development" implies "*satisfying the needs of the present, without compromising the ability of future generations to meet their own needs*" is considered to be and absolute principle. Nevertheless also absolute principles have a their own range of application. Outside such a range they may lead to unwanted situations. With reference to radioactive wastes they should be managed in a way that secures an acceptable level of protection for human health and the environment, and affords to future generations at least the level of safety, which is acceptable today, But by referring to the rate of evolution of the human society with an extremely great increase in the last centuries, it is not possible to consider the far future generations as equivalent to the current one. In particular, the trend of evolution of the human society forecasts a strong acceleration never observed till now. Consequently the habits of the far future generations will be totally different from the today. Therefore also the criteria to be adopted for the safe containment of a geological repository must be take into account this fact. In fact it must be stressed that an excessive degree of protection implies, very often, only a waste of resources without any advantage and implying an absolute negative balance.
Keywords: sustainable development, radioactive waste management, protection, human health and the environment, evolution.

ENVIRONMENTAL-IMPACT STUDY OF RADIONUCLIDE-SPECIFIC REMOVAL FACTORS OF THE LHC SITE PA3

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CERN Large Hadron Collider (LHC) tunnel is almost entirely embedded in impermeable molasse except for a sector which is easily permeable for water and the water flow into the tunnel has been rather constant over the years: 22 litres per second. The water is pumped from the tunnel on the surface of the site PA3 where it is retained by an existing decantation basin and will be retained by another decantation basin in the future. The two basins will also decant particles and release water into a small stream from the site. The stream receives 22 l/s of the LHC infiltration water and supplies a lake in a commune in France. Despite of retention time, traces of activation products will remain in the released water and a study is needed to prove that the environmental and radiological impacts of the releases will be acceptable if not negligible. To complete the impact study, one needs to know the hydrological parameters of the stream (transit time, flow rate) and removal factors of decantation basins. Environmental impact studies require an estimation of activity of few radionuclides, namely ^{3}H, ^{7}Be, ^{11}C, ^{13}N, ^{14}O, ^{15}O and ^{24}Na. To estimate the stream's transit time and its flow rate a concentrated solution of NaCl as a tracer was used in output of the decantation basin. The study described radionuclide-specific removal factors for each radionuclide in the two decantation basins, which are defined as ratios of the activity densities in water when entering a basin and when leaving a basin. The study proved that hydrological parameters of the stream in the sector from the site PA3 until the lake are stable and with the second decantation basin, the activity densities of all radionuclides will be well below the usual detection limits.

LESSONS LEARNED FROM RADIOECOLOGICAL RESPONSE OF THE BLACK SEA TO THE CHERNOBYL NPP ACCIDENT

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Radioecological response of the Black Sea to the Chernobyl nuclear accident since 1986 up to present was studied as well as tendencies of interaction of its ecosystem with chernobyl radionuclides were determined. As the result four lessons were obtained.

Lesson 1. Primary pollution of the Black Sea with chernobyl radionuclides happened from athmospheric fallout in the beginning of May, 1986, followed discharges of the Dnieper, the Danube and other rivers as well as via the Dnieper irrigation system. *Lesson 2.* Radioactive contamination of biotic and abiotic components of the Black Sea ecosystem changed on the scale of time of sorption and metabolic interactions proportionally to concentration factors, values of which for ^{90}Sr and ^{137}Cs did not depend on time and concentrations of these radionuclides in aquatic environment. Mean values of concentration factors were coincided with values obtained in experiments with radioactive tracers. *Lesson 3.* Radiation-ecological situation in the Black Sea after the ChNPP accident did not exceed permissible levels for hydrobionts on dose and radiation-cytological criterians. Dose commitments on hydrobionts in the Kakhovka reservoir, the North-Crimean Canal, the Black and Aegean Seas were related to the "Radiation well-being zone", such in the Kyiv reservoir - to the "Physiological masking zone" and only in fishes of the CHNPP cooling water pond - to the "Ecosystems damage Zone". *Lesson 4.* Post-Chernobyl long-lived radionuclides were proved to be highly précised tracers of intencity of hydrological and biogeochemical processes in the Black Sea.

Implication 1. The main tendency of concentrations changes of the post-Chernobyl radionuclides in aquatic environment, bottom sediments and living components of the Black Sea ecosystems were in their exponential decrease on the time scale of 15-25 years. Life time of ^{137}Cs and ^{90}Sr in aquatic environment of the Black Sea was 25-40 % from the "life-time" of 97 % of their atoms as the result of complex influence of oceanographic and biogeochemical processes (under taking account of radioactive decay) . *Implication 2.* Potential-critical radiation-ecological zones in estuaries of rivers areas of the NW Black Sea are formed in connection with increasing of the Black Sea radionuclides amounts in the depth of bottom sediments. Discovered areas of such kind at the Dnieper-Bug estuary, in the near estuary zone of the Danube and to the less extent in estuaries of small rivers should be under the permanent rarioecological control which covers of determination of the "stock" and concentrations of radionuclides in them as well as dose rates of ionizing radiation for critical species of hydrobionts.

DISTRIBUTION OF RADIONUCLIDE PHYSICOCHEMICAL FORMS IN BOTTOM SEDIMENTS OF GLUBOKOYE LAKE IN THE CHERNOBYL EXCLUSION ZONE

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The bottom sediments, because of their chemical exchange with water, are the source of the second contamination of aquatic ecosystems by radionuclides. The study of radionuclide forms of being in the bottom sediments allows estimating migratory ability of long-living radionuclides in aquatic ecosystems. The aim of this work is study of ^{90}Sr and ^{137}Cs physicochemical forms distribution in different types of the bottom sediments in Glubokoye Lake within the Chernobyl accident exclusion zone. For this research we sampled cores of the bottom sediments as sand with warp and silt, which divided on 5 cm layers. The forms of being were determined by the method of fractionating. Radionuclides were found in exchange, carbonate, fixed with oxides and hydroxides of Fe and Mn, organic forms and mineral residue. The received data analysis showed predominance of ^{137}Cs in all layers of the bottom sediments. Some difference in accumulation by the ^{137}Cs studied types of the bottom sediments is registered. Thus the silty bottom sediments accumulate more of ^{137}Cs in an organic form and mineral residue on comparison with silty sands in all layers of the bottom sediments. In layers 10-15 and 15-20 cm of the silty bottom sediments is marked the greatest specific activity of ^{137}Cs in a mineral residue. The exchange form of ^{137}Cs prevails in sands with warp. In the silty bottom sediments an exchange form is shown only in a layer 0-5 cm. The part of exchange forms of ^{137}Cs relies on content of clay minerals and their organo-mineral forms which actively take in ^{137}Cs. Due to these properties ^{137}Cs has less migratory ability on comparison with ^{90}Sr. This radionuclide accumulates in an exchange form mainly. Also a high specific activity of ^{90}Sr in an organic form is determined. Accumulation of ^{90}Sr in a mineral residue in all layers of the bottom sediments is minimal.

DYNAMICS OF PRIPYAT URBAN LANDSCAPE SUPERFICIAL CONTAMINATION BY CAESIUM-137 : ESTIMATION BY THE RADIOMETRY EXPECTATION METHOD

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After evacuation of population because of catastrophe on Chornobyl NPP, demutation of urban landscapes there was in the settlements of Chornobyl disaster Zone. This process takes place on a background self-damage and biological degradation of urban landscapes such as building, pipelines, etc. (Tjutjunnik, Bednaja, 1998). Demutation strongly influences on migration of radionuclides in urban landscapes. The purpose of this work is an estimation of gamma field spatial distributing and superficial contamination dynamics of Pripyat urban landscapes by ^{137}Cs during 1986-2009. The results got by radiometric expectation method. The coefficient of radiometric decay expectation is calculated as: $K_{RA} = P_f/P_a$ (where P_f is a current dose-rate, P_a is the expected with ^{137}Cs decay dose-rate) (Ganzha, 2008). Value $K_{RA}<1$ shows geochemical loss of ^{137}Cs from the overhead layer of soil, $K_{RA}>1$ are places of accumulation of radionuclide. Application of method for the estimation of migration exactly ^{137}Cs is justified because this radionuclide in formation of dose-rate above the surface of urban landscape in 1988 was 72%, in 2008 - 99%. Conducted calculations of change of distributing of K_{RA} on Prypjat urban landscapes showed that a mean value of coefficient in 1990 had been 0,85 at the coefficient of variation 130% and 30% values >1 from inspected 145 pickets. As far as development of demutation processes of in 1999 a value K_{RA} was multiplied to 1,20 (coefficient of variation 220%) and 60% values >1. To 2009 the demutation process was some stabilized, that showed diminishing of coefficient of variation to 140% at an unchanging mean value K_{RA} – 1,20 and 50% values >1. The chart of the spatial distributing of anomalies K_{RA} in the different years changed depending on distributing of demutation process intensity on city territory.

BLOCKAGE OF RADIONUCLIDE UPTAKE BY LIVING ORGANISMS AS MAIN WAY OF RADIATION PROTECTION OF BIOTA AT CONTAMINATED BY RADIOACTIVE SUBSTANCES TERRITORIES

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In the contaminated territories all living organisms get up to 90% of the dose via internal irradiation. In plants it forms from radionuclides, which comes from the soil through the root system, together with elements of mineral nutrition, animals get it from feed and a man - with food of animal and vegetable origin. Therefore, the main route of protection from radioactive contamination of living organisms in this situation should not be considered as radioprotection - protection from the effects of ionizing radiation, but radioblocking - protection from uptake of radionuclides. Radioblocking should be performed at all stages of the food chain, started from soil through agricultural plants (fodder) to productive animals and human food. At the "soil-plant" stage of food chain content of ^{137}Cs and ^{90}Sr, the main dose-forming radionuclides can be reduced in 2-3 times by agro-chemical methods (liming, application of mineral phosphorus-potassium and organic fertilizers). At the "crops (fodder)-productive animals" stage by changing the diet feeding, the addition of mineral additional forage, enterosorbents, as well as the detention regime, introduction of a diet with an absolute predominance of clean feed before animal slaughtering, the content of radionuclides can be reduced in 3-4 times. At the "crop and animal breeding production – human food" stage due to the primary culinary and technological revisions it may be reduced in 1.5-5 times. Calculations based on the average diet of the inhabitants of the most polluted region of Ukrainian, Belarus and Russia Polissya, which did not significantly differ, as well as on the assumption that the main products, molding dose are milk, meat, potatoes, vegetables and mushrooms in some cases, evidence of that the dose of internal radiation can be successfully reduced in 2-4 times or even more.

THE ROLE OF PLANKTON IN THE MIGRATION OF RADIONUCLIDES AND HEAVY METALS IN AN ATOMIC POWER STATION COOLING RESERVOIR

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The work summarizes the results of many-year studies (1985-2006) of the state of planktonic communities and their ability to accumulate radionuclides and heavy metals in the Beloyarskaya Atomic Power Station (BAPS) cooling reservoir. The obtained experimental data prove that the liquid discharge from the BAPS affects the planktonic complex state, especially in the sanitary-protective zone where the discharge from the wastewater channel and spillway channel enters. A twofold reduction in the number of phyto- and zooplanktonic organisms was observed in the wastewater channel compared to the reservoir upper reaches. The water passing through the cooling sets twice or thrice reduced the phyto- and zooplanktonic number and biomass: the phytoplankton perished by 38 % (65 metric tons per day), and the zooplankton, by 55 % (6 t/day). ^{60}Co concentrations were significantly higher in the discharge zone compared to the control region. After the 2nd unit of the power station was out of operation concentrations of the radionuclides became lower. In the wastewater channel ^{90}Sr accumulation was about the same in the phyto- and zooplanktone, but ^{137}Cs accumulated in greater quantities in phyto- the in zooplanktone.

PECULIARITIES OF ^{137}Cs ACCUMULATION BY FISH *RUTILUS RUTILUS (L.), ABRAMIS BRAMA (L.), BLICCA BJOERKNA (L.)*

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In 1986 – 2009, there was studied ^{137}Cs accumulation by fish from Kaniv water-storage reservoir of Dnieper river and cooling pond of the Chornobyl NPP. It was found that benthophage fish *Rutilus rutilus* (L.), *Abramis brama* (L.), *Blicca bjoerkna* (L.) have more stable characteristics for ^{137}Cs accumulation compared to other fish species.

Distribution of ^{137}Cs by organs and tissues of *Rutilus rutilus* (L.), *Abramis brama* (L.), *Blicca bjoerkna* (L.) is similar to the one for other fish species. After ingress of ^{137}Cs to water ecosystem, relative equilibrium content of ^{137}Cs in this species and water is reached in 3 – 6 months. Relative content of ^{137}Cs for *Rutilus rutilus* (L.), *Abramis brama* (L.), *Blicca bjoerkna* (L.) from the same water reservoir does not differ significantlty. These species have similar smooth dynamics for content of ^{137}Cs. Accumulation coefficients for ^{137}Cs for these species do not depend on level of radioactive contamination of water reservoirs. There were not detected significant difference in content of ^{137}Cs for males and females. Compared to many other fish species, intraspecific difference in content of ^{137}Cs for *Rutilus rutilus* (L.), *Abramis brama* (L.), *Blicca bjoerkna* (L.) are not significant (not more than 2 – 3 times). It is essential that, for *Rutilus rutilus* (L.), *Abramis brama* (L.), *Blicca bjoerkna* (L.), there is no "size" effect on content of ^{137}Cs, i.e. specific content of ^{137}Cs does not depend on weight of fish.

These species are widespread in Europe. Above-mentioned peculiarities of ^{137}Cs accumulation by benthophage fish *Rutilus rutilus* (L.), *Abramis brama* (L.), *Blicca bjoerkna* (L.) allow to use them as convenient and relatively stable subjects for radiation control and monitoring, study of ^{137}Cs migration by food chains, modeling and forecasting of radioecological situations in water ecosystems.

APPLICATION OF THEORY AND MODELS RELIABILITY IN RADIATION ECOLOGY

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Developed our model and the theory radioapacity ecosystems, would introduce an appropriate option - radiocapacity factor to determine the status of the biota of the ecosystem. Radiocapacity - defined as the limit of radionuclide contamination of biota ecosystem is exceeded, which may experience depression and / or suppression of growth of the biota. Experimental and theoretical studies we have found that the higher the parameter radiocapacity biota in the ecosystem, the higher the level of prosperity and security of the biota in this ecosystem. In particular, in studies with plant ecosystems, it is shown that the ability of biota to accumulate and retain the radionuclide tracer - ^{137}Cs, analog elements of mineral nutrition of plants-K, shows the stability and reliability of the biota of the ecosystem. It is established that a reduction in the radiocapacity biota in plant ecosystem under the influence of chemical pollutants and gamma irradiation plant, clearly shows the reduction in welfare and safety of the biota.

Thus it can be argued that the parameters radiocapacity able to act as a measure of the reliability of each element of the ecosystem and the ecosystem as a whole. The higher radiocapacity factor and /or the probability of retention of tracer of each ecosystem element are less than the reliability integral part of the ecosystem. Using these parameters the reliability of the elements of the ecosystem, and knowing the structure of a particular ecosystem, we are able to adequately assess the reliability of the entire ecosystem, through its ability to provide distribution and redistribution of the tracer, which reflect sits stable state. On the basis of this new approach to assessing the reliability of ecosystems, we calculated the reliability of the examples of specific types of ecosystems (slope and mountain ecosystems, for example). It is shown that the slope and mountain ecosystems, by virtue of the sequential type of organization, have a low stability and reliability, in terms of ability to ensure the migration of pollutants according to the ecosystems. GIS technology permits to estimate of realiability real landscapes.

SPECTROMETRIC RESEARCHES OF CLETHRIONOMYS SP. FROM NEAREST 5 KM CHNPP ZONE

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The data of spectrometric researches of field voles radioactive contamination are presented in this work. The voles are a group of animals widely used in radiobiological and genetic researches.

Field voles were caught in the autumn of 2008 and 2009 year inside the 5-км area of ChNPP in «Red forest» (the research territory «Janov»).

Animals were caught used Sherman's traps. These traps were ranged from benchmark through each 4 m. 50 traps were placed in total. Catching was carried out during 3 days. Lines of traps were checked every day. In total 18 animals have been caught. The species of animals was determined by morphological criteria. All caught animals are immature, young of the current year. The weight of animals from 12,1 to 20,8 g.

A spatial distribution of radioactive fallout on the research territories is inhomogeneous and has «spotty» character even on the small areas.

Average values of ^{137}Cs contamination of research territory are ranged from 37 to 170 $MBq \cdot m^{-2}$. The presence of ^{60}Co, $^{154,155}Eu$, ^{241}Am were fixed by spectrometric methods in top layers of research territories soils. Whereas in investigated mice samples only presence of $^{134,137}Cs$ from above-mentioned radionuclides was detected. Values of radioactivity ^{137}Cs also is inhomogeneous and varies in a range from 22-2500 Bq/g at the different individuals caught on the same territories. Individual with anomalous high content of ^{137}Cs (16700 Bq/g) were found. The received data are summarized and discussed.

AN APPROACH TO ASSESS PREDICTED NO-EFFECT DOSES AND DOSE RATES FOR AGRARIAN BIOCENOSES

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Agrarian ecosystems are of special concern from the viewpoint of establishing safe levels of radiation impact on the environment since, on one hand, their contamination can affect human health via radionuclide uptake with food, and on other hand, agroecosystems are ones of the most sensitive to a number of environmental impacts including ionising radiation. While the existing system of radiation standards is effective in providing radiation protection of human and restricts radionuclides content in food chains, there are no guidelines on setting any limitations to directly protect agrarian ecosystems from negative effect of radiation. The aim of this work is to develop methods for an assessment of critical doses and dose rates that can result in significant radiation-induced effects in agrocenoses. This is realized on an example of cultivated plants which are one of the main components of agroecosystems.

Available information on dose dependences in such umbrella endpoints as reproductive potential, survival, morbidity, alterations in morphological and biochemical processes, genetic effects in crops, vegetables, fruit trees, etc are gathered from papers issued mainly in Russian scientific press during last 50 years. Data are maintained as database in MS Access that contains about 7000 entries; the work is ongoing. As critical, there are considered doses producing 50% changes of biological effect at acute impact, or dose rates resulting in 10% changes at chronic exposure of plants. There are three main exposure situations for plants: acute irradiation of seeds, acute and chronic exposure of vegetating plants. Critical doses and dose rates are assessed from dose-effect dependences constructed with data sets, referred to indexes of reproduction and survival. It is found that data on survival collected so far are rather insufficient to estimate critical dose (rates) for species of cultivated plants. From the available information, the predicted no-effect doses and dose rates for agrocenosis are estimated basing on reproduction endpoint. They range within 67÷80 and 15÷17 Gy at acute exposure of the most radiosensitive species in dormant and vegetation periods, correspondingly, and 3÷10 mGy/h at chronic exposure of vegetating plants. The estimates obtained are going to be improved with further development of the database and treatment approaches.

METHODS FOR PREDICTING ^{137}Cs CONTAMINATION LEVELS OF SOIL SUITABLE TO OBTAIN PLANT AND FODDER PRODUCTS IN COMPLIANCE WITH THE ADOPTED STANDARDS

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Now 25 years after the Chernobyl accident in some south-western districts in the Bryansk region levels of radioactive contamination of the agricultural land still remain rather high and part of the agricultural produce does not meet the adopted standards. This generates a need for developing methods of predicting the levels of ^{137}Cs contamination of arable and pasture-meadow land at which it is possible to obtain eco-friendly plant and fodder products.

Maximum permissible levels of ^{137}Cs contamination of agricultural lands have been predicted which allow plant production in accordance with the adopted standards using different scales of countermeasures in the affected areas. With the Chernobyl affected south-western districts of the Bryansk region as an example, time periods were evaluated after radioactive fallout when plant and fodder production meeting the radiological standards is feasible.

A comparative analysis has been made of estimated and measured levels of ^{137}Cs contamination of agricultural lands. The estimated contamination densities are always somewhat higher than real measurements. Although on haylands and pastures ^{137}Cs removal with fodder products is higher than with plant products obtained on arable land, reliability of the predictive calculations is not significantly influenced by the radionuclide removal with phytomass. With time after radioactive fallout, ^{137}Cs is not only removed with the agricultural products but does not migrate to the deeper soil layers either. The bulk of the radionuclide is deposited in the top 20 cm soil layer independent of its mechanical composition. Estimations based on radionuclide decay alone are fairly reliable, however for better accuracy these must take into account the scales of the applied agrotechnical countermeasures.

RADIONUCLIDES CONTAMINATION RISK ASSESSMENT AND PREVENTION OF THE TURF-PODZOLIC AND CHERNOZEM SOIL IN UKRAINE

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The prospects to increase the buffer capacity of soils by means of some amendments were estimated under the model conditions. Investigations were conducted in different areas of Ukraine. The first soil samples contaminated with radionuclides were taken in the Chernobyl, Polessky, Ivankovsky districts of Kiev Region. The second area was the territory along the south-eastern trace of radioactive contamination in the Dnipropetrovsky province. Contamination of soil and plant sample with radionuclides was estimated by radiometer. Agrochemical analyses of the soil samples were made by the standard methods. The objects under research were turf - podzolic sandy soils and typical medium - loamy black soil. The humic preparations from peat, brown coal and other local materials incorporated into contaminated soils were used as amendments and rehabilitation means for protection against radionuclides in the laboratory experiments. EDTA, phosphates and limestone were applied in addition to them at different doses. Twenty-day winter wheat seedlings were chosen as test plants. The field experiment to reduce radionuclide accumulation in winter wheat grain by humates, limestone, zeolite, and manure application was conducted in the Ivankovsky district of Kiev Region.

Decrease in radionuclide accumulation in winter wheat seedlings was 65, 42, 18 % due to the brown coal and peat humic preparation, zeolite, limestone incorporation in soil at doses of 1, 10, 2.5 g/100g respectively. Limestone amendment at dose of 7 ton/ha provided the total β-activity reduction in grain samples from $5.4 \cdot 10^{-9}$ *Ci/kg* to $3.7 \cdot 10^{-9}$ *Ci/kg*.

Several districts along the south-eastern trace of ^{137}Cs radioactive contamination within 1.2 Ci/km^2 were selected in the Dnipropetrovsk Region just after Chernobyl accident. At this time there are spots with ^{137}Cs density in soil samples within 0.45-0.5 Ci/km^2. Unfortunately, erosion processes within steppe landscapes reach 40-50%. Therefore, radionuclide migration should be taken into account to avoid erosion factor development in steppe landscapes and some wetlands.

DEVELOPMENT OF N.V. TIMOFEEFF-RESSOVSKY IDEAS BY URALS SCHOOLS OF RADIOECOLOGY

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Laboratory of biophysics and radiation biogeocenology was created by N.V. Timofeeff-Ressovsky into Institute of Biology, Ural Branch of Academy of Science in 1955. He is one of the founders of a new scientific discipline – the radiation biogeocenology (radioecology). N.V. Timofeeff-Ressovsky was wording the tasks of this scientific direction as in detail as on a large-scale. Realization and development of N.V. Timofeeff-Ressovsky ideas are widely represented in the scientific works his pupils and followers. They formed the Ural scientific of N.V. Timofeeff-Ressovsky School. Briefly estimating the whole passed way we can pick out two directions of researches. They are as follows. 1. Investigation of the mechanisms migration, accumulation and distribution of radionuclides in freshwater ecosystems. Within the framework of this direction there are carried a large-scale investigations of accumulation levels, distribution and migration of ^{90}Sr ^{137}Cs, $^{239,240}Pu$ in the Ob-Irtysh river ecosystem comprising territories in the Urals and Khanty-Mansisky national region. There were carried out of several years' radioecological investigations of the Beloyarsk nuclear power plant influence on the state of the cooling pond and other ecosystems. Detailed radioecological researches of the lakes contaminated as a result of the Kyshtym accident in 1957 upon nuclear enterprise "MAYK" in the Urals were carried out. It was fulfilled the monitoring investigation of the ^{3}H contents in the water ecosystems, situated in the influence zones of the atomic enterprises in the Urals. 2. Investigation of the influence of the nuclear fuel cycles enterprises on terrestrial ecosystems situated in the different regions of Russia (Yakutia, Urals) and Ukraine (Chernobyl). Such investigations include as determination of contamination levels in the different soil-cover components as estimate biological effects. The more scales radioecological investigations were carried out in the Eastern Urals Radioactive Trace (EURT). Radionuclides contents in soil and plant cover of the study area were determined. Total radionuclides store in EURT soils calculated; doze loads on some herbaceous plants evaluated. The aspects of flora diversity within EURT addressed and also the results enable to evaluate long-term effects of radiation and identify plant adaptations to the conditions of permanent ionizing radiation are given.

THE BEHAVIOUR OF CHORNOBYL RADIONUCLIDES IN SOILS AT NEAREST 5-KM CHNPP ZONE

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Researches of Chernobyl radionuclides vertical migration were carried out inside the 5-км area of ChNPP in «Red forest» territories in 1999-2002 *and 2009 years. A presence of* ^{60}Co, ^{90}Sr, $^{134,137}Cs$, $^{154,155}Eu$, ^{241}Am and $^{238-240}Pu$ isotopes was determined to the 30 cm depth. In areas with automorphed soils ^{137}Cs and ^{241}Am isotopes were detected to the 60 cm depth. Besides, in 2009 year ^{243}Am and ^{243}Cm isotopes were identified in samples of top layers of «Red forest» soils. Therefore a possibility of the presence of «fresh» radioactive fall-out in «Red forest» soils is discussed. Periods of semiclearing of upper 5-cm soil layer were obtained for all researched soils. The periods of semiclearing of upper 5-cm soil layer from transuraniums (^{241}Am and $^{238-240}Pu$ isotopes) are similar to the periods of semiclearing from ^{137}Cs, ^{90}Sr and are equal 30 years.

EFFECTS OF IONIZING RADIATION ON POPULATIONS AND ECOSYSTEMS

NATURAL COENOPOPULATIONS OF STELLARIA GRAMINEA FROM EAST-URAL RADIOACTIVE TRACE: EFFECTS OF RADIATION AND WEATHER CONDITIONS

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During the last four years we have been studying native coenopopulations of lesser starweed (*Stellaria graminea* L.) from East-Ural radioactive trace (EURT, Chelyabinsk and Sverdlovsk regions, Russia) and control localities. The radiation exposure at the investigated impact plants were on three orders of values more than at the non-radiation samples. High interannual variability of viability and radioresistance of seed progeny is established, and at the same time certain communication of indicators with level of the radioactive contamination has not been revealed. The most important source of variability of viability of seed progeny are weather conditions but significant influence of temperature and precipitation are revealed only for impact coenopopulations of lesser starweed. For example, direct dependence between the sum of effective temperatures for two summer months (June and August) and survival rate of sprouts on impact plots (analysis of covariance, F = 4.88–5.96, p=0.028–0.043) was shown. Also the absence of communication between the given indicators in background territory was registered. At the same time on the sum of precipitation for the same months inverse relationship on survival rate of seed posterity in EURT zone (analysis of covariance, F =5.20–9.23, p=0.0083–0.038) and again absence that on background sites has been revealed. It is established that the share of sprouts with anomalies in EURT samples is above an additional irradiation promoted revealing of the latent infringements. Negative correlation between survival rate of lesser sprouts and the quantity of low-molecular antioxidants on all sites is found.

ECOLOGICAL PROBLEMS OF CRIMEA

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The Crimea has big natural, socio-cultural and economic variety. Modern ecological condition of region is result economic and socio-cultural activity several last centuries. Great environmental changes took place in the second half twentieth century. In 50-80 years of twentieth century were constructed large chemical plants. Agricultural lands occupy 70 per cent of the territory of the Crimea, and near 400 thousands hectare be irrigated after construction of the North Crimean Canal.

The irrigation of the agricultural fields was reason of unfavourable processes in soils: reduction of soil fertility, compression of soils, secondary salinization. Breach of order at use of mineral fertilizers and pesticides was reason to soil pollution, coastal waters Black and Azov seas. The biological productivity of Azov Sea - most productivity sea of world - has fallen sharply. Alongside with marine pollution big role in reduction bioproductive was played by invasive species.

The condition of mountain crimean forests is worsened. Main reason: recreational load and infringement of interactions in biocenoses.

Only 25 per cent of area region occupies nature landscapes. It is insufficient for preservation of regional ecological balance.

In 90 years after disintegration of USSR and liquidation of collective farms standard of farming decreased, destruction of drainage systems took place. Took place significant reduction of industrial production and substances polluting reduced release. However decrease of population's welfare and growth of unemployment stimulated growth poaching, not normalized consumption nature biological production, cutting down of soil-protective forest belts.

The condition of regional nature environment can be improved with help of social and economic stimulation environmental activity.

CONDITION OF GENETIC MATERIAL IN A BONE MARROW CELLS OF VOLES IN SITES WITHIN 30 KM CHERNOBYL EXCLUSION ZONE IN REMOTE PERIOD AFTER ACCIDENT

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The estimation stability of biosystems to ionizing radiation *in situ* is a urgent (serious) problem at the present stage of development of nuclear technologies. Studying of genetic material of red marrow cells of voles - one of the accepted approaches to solution of this problem.

The purpose of studying - assess the condition of genetic material in a red marrow cells of voles, which were caught after 22 years of Chernobyl accident on the areas with different level of radionuclide_pollution, but close other ecological factors.

The condition of a genetic material was evaluated using the micronuclear (micronucleus) test.

Researches were conducted on 39 voles (*Clethrionomys sp.*) which were caught by autumn of 2008 on five testing areas in Chernobyl exclusion zone. Density of areas pollution has constituted from 0,074 - 0,185 10^3kBq/м^2 – ^{137}Cs and 0,074-0,111 10^3 kBq/м^2 – ^{90}Sr on conditionally control area, and reached(achieved) 37,0-37,37 10^3 kBq/м$^{2-}$ ^{137}Cs; 25,9-37,0 10^3 kBq/м^2 – ^{90}Sr at areas with a maximum level of pollution.

Frequencies of cells with micronuclei significantly and mean numbers of micronuclei in cells of animal groups from all testing areas increased compared to the control area after Chernobyl accident after 22 years approximately in 44 voles generation. A distinct tendency of increasing the degree of loading of cells with micronuclei with the density of radioactive contamination of territories of inhabitation of voles were revealed after Chernobyl accident after 22 years approximately in 44 voles generation.

The quantitative and qualitative analysis content of ^{137}Cs, ^{90}Sr in body of voles is carried out.

INVESTIGATION OF BARLEY PLANTS GENETIC POLYMORPHISM IN CONNECTION WITH LEAD TOLERANCE

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The lead is considered as one of the most dangerous heavy metal (HM). However mechanisms of action and genetic polymorphism of various plants and, in particular, different varieties of cultural plants to HM tolerance are investigated insufficiently. Thus, the purpose of the work was to study of lead tolerance of spring barley *(Hordeum vulgare M.)* from the VRIPI world collection and selection of samples with polar level of tolerance on germination, length of roots and shoots, vigour of seedlings. The further aim of the work was the study genetic polymorphism of the selected samples on isoenzyme spectra of some anti-oxidative enzymes and studies the correlation of barley tolerance level and features of spectra of these enzymes. Seeds (100 seeds in each sample) of 3 spring barley *(Hordeum vulgare M.)* samples were exposed to 9 Pb concentrations in range of 1 to 9 mg/ml taken to determination of lead critical dose. With use of the testing concentration (1.5 mg/ml) 80 samples from 100 were presently tested. It was found out high polymorphism of sample tolerance to lead. I.e., among 60 samples tested 16 had high tolerance and 11 had much lower. Reaction of seedling roots was strongly pronounced. Root length decreased sharply already at 1 mg/ml of lead. Shoot length shows light stimulation at the same conditions and was more tolerant to lead. In spite of the length suppressing numerous violations of root morphology: curvature of roots tips, branching, swelling formation, reduction of root quantity per seed, geotropism anomaly, color change etc. were observed. Mitotic index in root seedling roots increased up to concentration of lead 3 mg/ml, and then reduced up to the control level at concentration 5 mg/ml. The analysis of frequency of separate mitotic phases shows, that amount of divided cells is almost completely provided by prophases. To the share of other phases it is put a maximum about 7 %. It allows assuming the presence of powerful blocking influence of lead on process of cell division. Moreover, the detailed analysis of dependences a doze curves of metaphase and ana-telophase frequency shows, that it sharply reduces after concentration 2 mg/ml and to 5 mg/ml transition of cells in these mitotic phases practically stops. It probably connected with disruption of spin division apparatus. The preliminary analysis of chromosome aberration show sharp increase of heavy disturbances frequency at the minimal lead concentration (1-3 mg/ml): multipolar mitoses, chromosome sticking, chromatin destruction, etc.

EFFECTS OF CHRONIC IRRADIATION ON PLANT POPULATIONS

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An assessment of the state of plant and animal populations inhabiting polluted territories and the analysis of mechanisms of their adaptation to adverse environmental conditions undoubtedly has general biological importance. Consequently, studies that examine biological effects on non-human biota in natural settings provide a unique opportunity for obtaining information about the potential biological hazard associated with radioactive contamination. Nevertheless, up to now there is a distinct lack of quantitative data on the real long-term biological consequences of chronic radiation exposure lasting a long period of time. Actually, few studies exist that are directly relevant to understanding the responses of plant and animal populations to radioactive substances in their natural environments. The results of long-term field experiments in the 30-km Chernobyl NPP zone, in the vicinity of the radioactive wastes storage facility (Leningrad Region), at radium production industry storage cell territory (the Komi Republic), in the Bryansk Region affected by the Chernobyl accident, and in Semipalatinsk Test Site, Kazakhstan that have been carried out on different species of wild and agricultural plants are discussed. Although radionuclides cause primary damage at the molecular level, there are emergent effects at the level of populations, non-predictable solely from the knowledge of elementary mechanisms of the pollutants' influence. Plant populations growing in areas with relatively low levels of pollution are characterized by the increased level of both cytogenetic disturbances and genetic diversity. Radioactive contamination of the plants environment activates genetic mechanisms, changing a population's resistance to exposure. However, in different radioecological situations, genetic adaptation to extreme edaphic conditions in plant populations could be achieved with different rates.

RADIATION STRESS AS A MODIFICATOR OF LIFE HISTORY IN RODENTS. IMPLICATIONS FOR RADIOLOGICAL PROTECTION OF POPULATION

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On the base of functional-ontogenic approach (Olenev, 2002,2004) the population structure of small rodents inhabiting the Eastern Urals Radioactive Trace zone (EURT) resulted from the Kyshtym accident 1957 and background areas were analysed. Objects of investigation - pigmy wood mice (*Apodemus (S.) uralensis*) and field mice (*Apodemus agrarius*) - are cyclomorphic mammals, which characterized by cyclic changes in the most biological parameters over a period of approximately one year, mass breeding and generation overlap in the presence of two alternative ontogenetic pathways. These pathways are supposed to divide natural population into groups of individuals with the same functional status, i. e. with the uniform patterns of growth and/or maturation rate as well as their participation in reproduction. It was shown under conditions irradiation (the EURT zone) that the dominant group in the population is individuals with the first type of ontogeny (the mature children of overwintered animals), which form the second generation. The high heterogeneity of overwintered group (it includes representatives of all generations born in the previous year) provides the possibility of genetic information transmission not only via successive change of generations, but also directly from the first generation born in given year to the first generation born in the next year of birth (transgeneration transmission). As a consequence the fecundity and abundance of mice in the impact zone are higher than in the background areas, which improves the adaptive structure of the population. The mechanism of switching to a certain type of ontogeny is mainly triggered by environmental factors of both natural and man-made nature. Thus, the ability to follow one of the two ontogenic pathways depending on existing environmental conditions is obviously of adaptive significance in the radiocontaminated zone. Evolutionary role of radiation stress in the modification of the life history of murine rodents is discussed. We emphasize the fact that the functional approach makes it possible to significantly reduce errors in assessing the radiological consequences and hence, can provide a reliable methodological basis for organization of biological monitoring.

AQUATIC BIOTA WITHIN THE CHERNOBYL ACCIDENT EXCLUSION ZONE: DOSE RATES, EFFECTS AND REHABILITATION

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Our studies were carried out during 1998-2009 in different water bodies of the Chernobyl accident exclusion zone. The absorbed dose rate registered in range from 1.3 mGy/year to 3.4 Gy/year. The highest rate was found in lakes within dammed flood land territory of the Pripyat River. The rate of chromosome aberration for snails' embryo from the control lakes was about 1.1-2.0%. About 3.3 and 5.7% of aberrant cells was registered in snail's embryo from the rivers of the exclusion zone. The highest rate of aberration was found in snails from lakes within the dammed territory on the left-bank flood lands of the Pripyat River - 21-23%. In the embryo of snails from some others closed water bodies registered in range 18-20%. The highest chromosome aberrations rate in root meristem of higher aquatic plants (17.8-10.8%) were registered in lakes within the left-bank flood lands of the Pripyat River, the lowest one (4.5-2.2%) - in plants from the river ecosystems. During 1998-2008 a tendency to decrease of chromosome aberration level in molluscs from all lakes of the exclusion zone was registered. The probabilistic prediction of the chromosome aberration rate for gastropod snails in lakes of the Chernobyl exclusion zone have shown that spontaneous mutagenesis level (2.0-2.5%) can be reach in Azbuchin Lake and Yanozsky Crawl in 2020s-2030s and in Dalekoye-1 Lake and Glubokoye Lake – in 2060s-2070s. In hemolymph of snails from Dalekoye-1 Lake, Azbuchin Lake and Glubokoye Lake the quantity of death cells averages 36.2%, 39.2% and 43.8% respectively, the part of phagocytic cells averages 44.3%, 41.2% and 45.0%, as well as decrease of the young amoebocytes quantity to 13.2%, 20.1% and 9.5% respectively. The insignificant quantity of abnormal cells and micronuclei has been observed as well. In the control lakes the part of death cells averages from 2.2 to 5.3% and the quantity of phagocytic was at level 3.0-4.2%. The quantity of young amoebocytes has increased here to 79.7-89.6%.

MULTIPLE-GENERATIONAL STUDIES OF LOW DOSE CHRONIC EXPOSURES TO A VERTEBRATE MODEL

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Relatively few experiments have been conducted on the effects to organisms following long-term exposures to low levels of radiation. Even fewer studies have examined the effects of radiological exposures to multiple generations of organisms. Speculations that damage will accumulate and be greater with each passing generation are plausible. Alternative, opposing views that adaptive response and repair mechanisms will counter the effects, such that damage does not increase with each generation, are equally plausible. Very few data sets exist to support one hypothesis over the other, particularly for chronic, low level exposures to vertebrate organisms. Our research explored multiple generational exposures of low dose irradiation to a model vertebrate organism, Japanese medaka (*Oryzias latipes*), one of the most widely used fish in comparative mutagenesis and carcinogenesis studies. A unique outdoor irradiation facility allowed us to examine effects to five generations of medaka that were continuous irradiated to different dose rates. The dose rates bracketed the IAEA guideline for acceptable chronic exposure levels to aquatic wildlife (10 mGy / d), and thus were a test to see if the guidelines were applicable for multiple generational exposures. The effects on reproductive endpoints that might influence population dynamics were examined. Our intention was to test the hypothesis that multiple generational exposures to low dose rate irradiation are no more damaging, as measured by reproductive characteristics that could potentially impact a population, than damage incurred from exposure to a single generation. Such knowledge is needed because chronic exposure to low levels of radiation is a more likely scenario for nuclear workers, and to wildlife exposed to routine releases from nuclear facilities.

ACCUMULATION PECULIARITIES OF THE MAIN DOSE-FORMED RADIONUCLIDES IN FISH OF THE CHERNOBYL NPP EXCLUSION ZONE

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^{137}Cs is one of the main dose-formed radionuclide in the organism of fish. Such regularity is registered for fish in the Kiev water reservoir, which is bound with Chernobyl Exclusion zone (relation of $^{90}Sr/^{137}Cs$ = 0.01-0.30). Other tendency is observed in the closed water bodies within the Exclusion zone such as lakes Glubokoye, Azbuchin, Dalekoye and Krasnensky former river bed of the Pripyat River. At comparatively stable specific activity of ^{137}Cs in tissue and organs of fish, from the end of 1990-s the specific activity of ^{90}Sr both in pray fish and predatory was increased. At that if in 1999 the correlation of ^{90}Sr to ^{137}Cs in benthophage fish was about 0.2, in 2000 - 0.6, in 2001 - 2.0 and in 2009 the average content of ^{90}Sr exceed content of ^{137}Cs in 2-5 times. For explored period at the increase of ^{90}Sr concentration in two times in water the concentration on radionuclide in fish tissue have grew in 5-6 times. The increase of ^{90}Sr in the organism of predatory fish passes slower and only occasionally insignificantly exceeds the content of ^{137}Cs. The highest specific activity of ^{137}Cs was observed in the fish muscles - 3549-55970 Bq kg^{-1}, and highest specific activity of ^{90}Sr registered in fish scale and range 56580- 83950 Bq kg^{-1} in Glubokoye Lake and 28600-42550 Bq kg^{-1} in Azbuchin Lake for the crucian carp as well as 42900-95810 Bq kg^{-1} for the redeye in Glubokoye Lake. The content of ^{90}Sr in predatory fish like perch from Glubokoye Lake was in range 55020-63260 Bq kg^{-1}, in the pike from Glubokoye Lake - 23600-35140 Bq kg^{-1}, and in the pike from Azbuchin Lake - 8210-11440 Bq kg^{-1}. Comparatively low specific activity of ^{90}Sr registered in fish muscles - 77-240 Bq kg^{-1} for the crucian carp from Azbuchin Lake, 273-310 Bq kg^{-1} for the tench from Glubokoye Lake, 252-310 Bq kg^{-1} for the perch from Glubokoye Lake, 17-66 Bq kg^{-1} for the perch from Dalekoye Lake, and 349-505 Bq kg^{-1} for the redeye from Glubokoye Lake. The highest content ^{137}Cs concentrated in fish muscles - 42-67%.

RADIATION FACTOR EFFECT ON VARIABILITY OF FLAVONOID CONTENT AND MORPHOMETRIC FACTOR IN TERMS OF *PENTAPHYLLOIDES FRUTICOSA*

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Variability of biochemical and morphometric parameters for *Pentaphylloides fruticosa* (L.) O. Schwartz (bush cinquefoil fam. *Rosaceae*) cultivated in of ^{90}Sr and ^{137}Cs radionuclide contamination gradient was studied. A fact of radiation effect on features with high variability was established using histograms of measurement results distribution and coefficient of variation (CV).

The experiment was carried out in Eastern Urals Radioactive Trace (EURT) zone. EURT formed as a result of an accident on "Mayak" Production Association in 1957. Method of computer analysis of magnified images was used for morphometric parameters survey. Identification and the quantitative content of flavonoids was performed by high-performance liquid chromatography (HPLC). It has been established, that the bush cinquefoil leaves contains 14 individual flavonoid components: quercetrin, kaempferol, hyperoside, isoquercitrin, quercitrin, astragalin and 8 compounds as derivatives of quercetrin or kaempferol .

It has been established that, as a rule, along side with radiation level increase, variability of morphological factors (length, width, area and perimeter of a leaf and terminal lobe a leaf, length of leaf stem and annual shoot, quantity of leaves on a shoot) and flavonoid content (total and according to groups) becomes lower. Variability of "individual" biochemical features, i.e. separate flavonoid components, on the contrary, tends towards CV increase as far as radiation load increments.

For the most part of the features studied, value increase for asymmetry ratio and excess coefficient is observed for radiation level increase. Double mode distribution in medium and maximum radionuclide-contaminated samples indicates coenopopulation heterogeneity according to morphometric factors and flavonoids content, and is an evidence of adverse effect of radiation on growth, development and metabolic processes of *Pentaphylloides fruticosa.*

METABOLIC MECHANISMS OF *APODEMUS (S.) URALENSIS* RADIORESISTANCE IN HABITAT POLLUTED WITH RADIONUCLIDES

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Peculiarities of metabolic homeostasis revealed in *Apodemus (S.) Uralensis* taken from the zone of the East Urals Radioactive Trace (EURT) have given a chance to determine energetic and structural components of radioresistance and their changes after the isolation from the radioactive habitat – keeping animals in the vivarium. Energetic component of animals resistance expressed in higher, with regard to the control, level of lipid and carbohydrate metabolism, oxidative power-forming processes in mitochondrions, characterizes stess-realizing strategy of adaptation caused by energy consumption increase on functional - metabolic activity of cells and tissues in unfavorable habitat conditions. Structural component of resistance expressed in terms of increase of RNA/DNA ratio and a part of cytoplasmic proteins, under decreasing DNA/total protein and a part of nuclear proteins, points to the change of functional activity of genom. Autocatalytic function underlying the base of proliferation and polyploidisation of cells decrease and geterocatalytic activity of genom causing RNA synthesis and processes of cells differentiation increase (Konyukhov, 1973; Morosov, Khavinson, 1985; Anatskaya, Vinogradov, 2004). Chronic activity of ionizing radiation of low power is dealt with as the inductor of accelerated differentiation and more rapid passing of cell cycle which corresponds to the processes of increased aging (Korogodin, Polycarpov, 1958; Wangenheim, 1976). Disconnection from radioactive habitat as a results of keeping animals in the vivarium brings researched indices of metabolic homeostasis to the initial level as the characteristic of phenotypic (physiological) component of the adaptation process in *Apodemus (S.) Uralensis* to the EURT conditions. The work has been done with the financial support of the programme of the development of leading scientific schools (NSh-1022.2008.4) and scientific-educational centers (contract 02.740.11.0279).

EFFECTS OF IONISING RADIATION ON NON-HUMAN ORGANISMS: A WORM'S EYE VIEW

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In recent years ecological impacts of ionizing radiation have emerged as an important research field. But there are still considerable knowledge-gaps regarding biological effects of chronic irradiation in wildlife, particularly for endpoints related to reproduction. Reproduction is considered to be one of the most sensitive radiation-associated endpoints, and determines not only the fate of the single organism, but may also influence the population dynamics and the balance of higher ecological units. Based on their radioecological properties and their important role in the soil ecosystem, earthworms have been identified by ICRP as one of the reference animals and plants (RAPs) to be used in environmental radiation protection. This paper will present results of a series of studies carried out on the effects of ionising radiation on earthworms, covering long term-effects on reproduction, as well as studies on biological processes such as recovery, acclimatization, adaptation and adaptive response, and a number of molecular biomarkers. The aim is to show how the studies can improve predictions of how individuals and populations will respond to chronic exposures of ionising radiation.

Reference: Hertel-Aas, T., Oughton, D.H. Jaworska, A., Bjerke, H., Salbu, B., Brunborg, G. (2007). Effects of chronic gamma irradiation on reproduction in the earthworm. *Eisenia fetida. Radiation Research.* **168**: 515-526. (ISSN: 0033-7587)

COMPARISON OF THE PLANT POPULATIONS UNDER CONDITION OF RADIOACTIVE AND CHEMICAL CONTAMINATION

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We carried out a comparative study of seed progeny taken from the dandelion coenopopulations exposed for a long time to radioactive (the East-Ural Radioactive Trace zone – EURT) or chemical (Nizhniy Tagil Metallurgical Combine impact zone – NTMC) contamination. Seed viability was found to decrease in a similar manner as the industrial stress of all types increased. Moreover, the radiation doses causing the first perceptible effects differ from the doses resulting in strong suppression (by 60 times) and when exposed to HM, this shift requires only a fourfold load increase. Adaptation potential of P-generation was assessed by its resistance to additional radiation and HM acute exposure. In provocation, the seeds from the background sample showed the least ability to adapt to additional HM exposure, but at the same time the seeds were rather resistant to radiation. Comparison of seed progeny from the EURT samples under conditions of provocation underlines non-specificity of the response. Coenopopulations from EURT, NTMC and background areas significantly differed from each other with respect to the quality and quantity composition of allozyme phenes. The analysis of clonal diversity showed the uniqueness of all coenopopulations in terms of phenogenetics. The data suggest that the genesis of each of coenopopulation took place in a peculiar manner: sets of the ancestor plants were different and their descendants altered genetically, bred and settled the area with some degree of success undergoing selection under pressure of natural environment factors and technology-related stress. Studies of F1-generation variability in radioresistance and metal resistance by family analysis showed that seed progeny from EURT impact zone possessed high viability but had latent injuries resulting in low resistance to additional man-caused impacts. In F1-generation from NTMC zone, high seed viability combined with increased resistance to provocative heavy metal and radiation exposure. No significant differences in responses to 'habitual' and 'new' factors, i.e. pre-adaptation effect, were found in samples from contaminated zones.

ECOLOGICAL RISK ASSESSMENT TO BENTHIC BIOCENOSES

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The most constructive and adequate approach to ecological risk assessment is the study of adaptive potential of natural populations. Genetic variability and adaptive possibility are connected with effective population size (i.e. the number of specimens which breed and determine genetic structure of next posterities). Effective population size depends on the dispersion of reproductive contribution of specimens. It is known that increasing of reproductive contribution dispersion leads to decreasing of effective population size and adaptive potential. For the assessment of specimens reproductive contribution a number (%) of posterity with spontaneous mutagenesis (up to 2% cells with chromosome aberrations) may be used as the criterion of full value posterity because one with higher number of cells with chromosome aberrations is less viable. Proceeding from data on the number (%) of full value posterity in population and the mean fecundity of females it is possible to calculate the number of full value posterity per a female (i.e. reproductive contribution). Obviously the increase of ecological risk may be expected if there is less than one full value embryo (or larva) per a female.

On the base of experimental data and cytogenetic studies of marine and freshwater invertebrates natural populations it was shown that different species had approximately equal part (%) of full value posterity at the same average level of chromosome mutagenesis for populations, independing on deleterious factors and females fecundity. This phenomenon made it possible to calculate specimens reproductive contribution for species with different females fecundity and to assess expected reduction of population adaptive potential and the increase of ecological risk at different levels of population damage.

Ranges of chromosome mutagenesis critical levels for different hydrobionts taxons were determined. An algorithm of ecological risk assessment to benthic biocenoses was discussed.

CHROMOSOME INSTABILITY IN *APODEMUS (S.) URALENSIS* AND *APODEMUS AGRARIUS* FROM RADIOCONTAMINATED ENVIRONMENT: INTERSPECIES COMPARISON

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The study of the genetic consequences of radiation incidents in rodent population is important from the point of view of long-term effects of chronic irradiation in biota.

The aim of the study was to compare intensity of mutation processes and accumulation of ^{90}Sr in bone tissue between two species of mice *Apodemus (Sylvaemus) uralensis* Pall., 1811 and *Apodemus agrarius* Pall., 1771 from the East Urals Radioactive Trace zone (EURT) resulted the Kyshtym incident 1957 (Russia).

Two plots from EURT zone with different soil pollution by ^{90}Sr - 2322 kBq/m^2 and 6740-16690 kBq/m^2 were investigated. Control sites were next: the adjacent area (44 kBq/m^2) and the geographically distant one (the global level of contamination). The accumulation of ^{90}Sr in bones of both species was increased with the level of soil pollution ($H=17,4-36,1$; $p<0,001$). The frequency of chromosome aberrations in bone marrow cells of mice from the impact zone was significantly higher than in control animals. For *A. (S.) uralensis* they were 3,14-4,20% (EURT zone) and 0,33-0,55% (control sites) ($\chi^2(df=3)=38,5$, $p<0,001$); for *A. agrarius* - 2,17-2,87% and 1,33-1,40% correspondently ($\chi^2(df=3)=8,6$, $p=0,035$). The levels of chromosome instability, which observed in mice from impact plots nowadays, were comparable with those ones were found in rodents from EURT zone in ten years after incident (Dubinin et al., 1972).

Significantly positive correlation of aberrant cell frequencies and accumulation of ^{90}Sr in individual level was shown in both species - *A. (S.) uralensis* ($Rs=0,51$; $p=0,007$) and *A. agrarius* ($Rs=0,25$; $p=0,042$). The frequencies of chromosome aberrations in *A. (S.) uralensis* were 1,5 times higher then in *A. agrarius* when accumulation of ^{90}Sr exceeded 50 Bq/g, whereas they were lower at background accumulation (0,5 Bq/g) (differences not significantly, $p>0,05$). So, mutagenic effect revealed in *A. agrarius* was less then in *A. (S.) uralensis* that is in agreement with the data concerning interspecies differences in radioresistance known for these species (Grigorkina, Pashnina, 2007). This study was supported by RFBR № 08-04-00638 and № 10-04-96101.

VIABILITY INDEXES OF THE COMMON REED'S SEEDS FROM WATER-BODIES WITHIN THE CHERNOBYL ACCIDENT EXCLUSION ZONE

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The most radiosensitive indicator of plants, as of all living organisms, is the process of generative reproduction. The seeds of plants are convenient object for investigation of chronic radiation influence on reproduction ability. For assessment of remote effects of chronic radiation influence on the air-aquatic plants there were sampled seeds of the common reed (*Phragmites communis*) of 2009 vegetation year from closed natural water bodies of the Prypiat River floodplain within the Chernobyl exclusion zone, man-made weakly flowing cooling pond of the Chernobyl NPP and from Verbne Lake, located within Kiev City, with background level of radioactive contamination. Absorbed dose for plants from these water bodies varies from 0.003-12.0 cGy $year^{-1}$. The vitality of seeds was assessed by the indexes of technical germinating ability, germinating power, period of appearance of the first and last germ and survivability. Our study shows the reducing of seeds vitality in conditions of small doses chronic radiation influence. There are registered low indexes of technical germinating ability, germinating energy, survivability and anomalously extended period of seeds germination. The dynamics of germination with the longest period and two maximums is registered for seeds of plants from water bodies with highest rate of absorbed dose – 12.0 cGy $year^{-1}$. The least rate of deviations in germination dynamics are observed in seeds of reed population from background radiation level lake. There is registered negative dose correlation for germinating power ($r = -0.95$) and survivability ($r = -0.64$). The index of technical germinating ability enough moderately correlates with absorbed dose ($r = -0.34$). Thus, vitality of seed progeny of the common reed, which grows in the gradient of radioactive contamination of water bodies of the Chernobyl accident exclusion zone, reduces with the increasing of dose rate, absorbed by plant. Obtained data about viability of seed progeny of dominant species of air-aquatic plants of littoral ecotones may be used in radioecological monitoring of radioactive contaminated water bodies.

EVOLUTION

ON EVOLUTION PROCESS OF MATHEMATICAL MODELLING VALUE METHOD

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The value method applying possibilities for evolution process prediction is discussed on a typical system branch chain reaction example.

The selection of the chain reaction kinetic model analysis is connected with high similarity of evolution process development task, where it is necessary to get results during short time, that in reality may process during long time. To get sensible solution via direct calculations is not always possible, as every small change, which is not always possible to calculate, in a chain process may be decisive. The necessity of a new approach for the problem solution is obvious and the success may also lead to new models of evolution development processes in other spheres.

On a typical sample of development process branch chain reactions the convenience of using value theory for predicting other development processes is shown.

FACULTATIVENESS PRINCIPLE, GENERALIZED GENOME CONCEPT AND HEREDITARY VARIABILITY

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Facultativeness in the genome structure and function reflects the general principle of the life organization and evolution: *the unity of the whole and the freedom of the parts.* The genome or cell hereditary system codes, maintans and transfers the hereditary information in both structurally and dynamically. Generalized Genome Concepts presents a genome as an ensemble of both obligatory and facultative elements, capable of functional facultativeness and dynamic (epigenetic) alterations. It is reasonable to discriminate between three kinds of heritable changes- Mutations, Variations and Epigenetic alterations. Structural facultativeness is expressed as a subdivision of cell DNA and RNA elements on two subsystems: Obligatory elements (OE) and Facultative elements (FE). FE include various kinds of repeats, mobile elements, amplicons, viral and foreign DNA, B-chromosomes, plasmids, cytobionts . The number and intracell topography of FE varies from cell to cell, in different tissues and individuals. Only the changes in OE were viewed as classical mutations. For diverse changes in the FE the term *variations* seems appropriate. Mutations and variations significantly differ on the character and mode of their occurrence. Variations may occur simultaneously in many cells/individuals and are induced by non-mutagenic factors. Spontaneous hereditary changes occur in the system ENVIRONMENT – FE – OE mainly via two-step mechanism. FE are the first to react to internal and external environmental challenges, then their activation induce gene and chromosomal mutations. Both variations and epigenetic alterations may have non-mendelian features, occur simultaneously in many individuals, sometimes reminding phenomenon of inheritance of acquire characteristics.

For humans we need to explore reality that every fertilized egg links both physicall and genetically three successive generations. If diverse environmental factors such as pollution, nutrition and lifestyle influence on the epigenetic dynamics of the oocyte in *F(n-2)* and *F(n-1),* they can cause genotype/phenotype changes in the *F(n)* cohorts. The trans-generational hereditary effects of chronic nutrional deficiency, global pandemies or of diverse environmental catastrophes such as the Chernobyl might be predicted.

MOLECULAR EVOLUTION OF THE *WOLBACHIA WRI, WMEL* AND *WPIP* GENOMES

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The *Wolbachia*-host associations have become advantageous tools for evolutionary studies of symbiont-host interactions. α-proteobacteria of the *Wolbachia* genus are widespread infectious agents in arthropods. The molecular mechanisms underlying their partnerships remain unclear.

Here, we perform a comparative analysis of the evolution mode of more than 650 orthologous protein-encoding genes from the *wMel*, *wRi* and *wPip Wolbachia* strains, which infected *Drosophila melanogaster*, *Drosophila simulans* and *Culex pipiens* respectively. The aim was to clarify *wMel* and *wRi* adaptation strategies to the host and diversify those strategies from *wPip* adaptation.

We identified genes under negative selection on the basis of analysis of nonsynonymous nucleotide substitutions and those under positive selection and neutrally evolving based on analysis of radical versus conservative amino acid replacement rates. The results allowed us to demonstrate features making similar and different the *wMel* and *wRi* bacteria in the course of their evolution. We distinguished three functional gene groups subject to the same evolution mode in both *wMel* and *wRi* strains: "translation, ribosomal structure and biogenesis" evolving more often under purifying selection; "carbohydrate transport and metabolism" evolve more often under positive selection; "replication, recombination and repair" evolving preferentially in neutral mode. Comparative analysis of the evolutionary changes in the amino acid composition of the *wMel* and *wRi* proteins and the number and functions of the positively selected *wMel* and *wRi* genes disclosed substantial differences in the *wMel* and *wRi* adaptation strategies. These differences were indicative of higher efficiency of positive selection for *wMel* than *wRi*. Our current results suggested that *wMel Wolbachia* strain became more cooperated with the host compared to *wRi* at the genome level. This suggestion is consistent with the discussed experimental data.

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COMPUTER SYSTEM FOR ANALYSIS OF MOLECULAR EVOLUTION MODES OF PROTEIN FAMILIES: RELATION OF MOLECULAR EVOLUTION WITH THE PHENOTYPICAL FEATURES OF ORGANISMS

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Recently, great doubts about validity of the nonsynonymous to synonymous nucleotide substitutions rate ratio as a robust criterion of positive selection were raised [1]. Alternative approaches to study the molecular evolution modes of proteins based on the radical to conservative amino acid replacement rate ratio (K_R/K_C) and the rate of change of various properties of amino acids (V_P) in the course of protein evolution. The essential drawback of these approaches is the need to know in advance positively selected change of certain amino acid properties.

Our novel SAMEM (http://pixie.bionet.nsc.ru/samem/) computer system is also based on the K_R/K_C and V_P, but has two crucial differences which allow us to fully overcome the drawback: we take into account all the known properties of amino acids, and we calculate the statistical relation of properties changes with certain user defined adaptive phenotypical features of organisms [2].

The opportunity to match evolutionary change of all amino acid properties with positively selected phenotypical features of organisms permits direct attribution of certain protein changes to positive selection events. The results of tests (Cyclins A, B, D, E; Notch; Delta; Serrate; TIR1) are consistent with experimental data. Thus, SAMEM system can be useful for comprehensive analysis of molecular evolution modes of various protein families.

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THE SYMBIOTIC ASSOCIATION OF *WOLBACHIA-DROSOPHILA MELANOGASTER*, BIOLOGY AND GENETICS

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Bacteria belonging to the genus *Wolbachia* are widespread among Arthropods and Nematods. An investigation into the relationship in a model symbiotic association of *Drosophila melanogaster* and *Wolbachia* can assist in getting a better understanding of those mechanisms. *Wolbachia* symbionts of *D. melanogaster* have been considered as a single common strain. Following the introduction of 5 genomic markers, *Wolbachia* were classified into 5 genotypes. We presents the results of prevalence analysis and the study of effects on the host biology of the three most common *Wolbachia* genotypes found in the wild populations of *D. melanogaster*, wMel, wMelCS, wMelCS2. More than 300 mutant host strains from the collection of the Lab of Genetics of Populations, IC&G were screened for *Wolbachia* infection and genotypic diversity as well as for haplotypic diversity of mtDNA.

Screening of wild populations of *D. melanogaster* of vast territory of North Eurasia over the quarter of a century for *Wolbachia* prevalence and genetic diversity yielded the levels of infection within 10-90% in all populations studied. We confirmed the previous reports indicating wMel to be the most prevalent genotype. No new genotypes of *Wolbachia* were found in the studied populations.

We described the diversity of mtDNA based on the sequence of mtDNA sequence of *D. melanogaster* and showed a strict association of those with infection by four *Wolbachia* genotypes found in the wild (wMel, wMel2, wMelCS, and wMelCS2).

Experiments on the effects of different *Wolbachia* genotypes on *D.melanogaster* fitness under optimal and thermal stress conditions demonstrated that, most often, *Wolbachia* infection does not influence the life span, but, in some cases, may increase or decrease this index in a sex-dependent manner.

Our experiments allowed to estimate the induction of cytoplasmic incompatibility by the three *Wolbachia* genotypes. wMel and wMelCS genotypes caused cytoplasmic incompatibility at the level of about 10%, while wMelCS2 did not.

DYNAMIC POLYMORPHISM OF FRUTICICOLA FRUTICUM (MÜLL.) AS ADAPTATION

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A hierarchic organization is characteristic of wildlife and its components. The rational hypothesis suggested by N. V. Timofeev-Ressovsky (1962) distinguishes four levels of life organization on the Earth having a single base of elementary structures and phenomena which underlies their specific features. According to darwinists, biogeocoenotic relations ("life struggle") are the motive power of the evolution. Life struggle is the evolution controlling mechanism. The control takes place in a biogeocoenosis, it depends on the relations between various specimens of a certain species and all non-organic and especially biotic factors facing them. Natural selection is only within a population and depends on its genetic structure and characteristics of individuals (Schmalhausen, 1973). The presence or absence on a shell of colour spiral bands (banding) is one of elementary colour systems of land snail shells. In genetically studied species this sign evidences of population polymorphism. For polymorphism, out of 25 thousand mollusks species only 14 species have been studied. It is important that in all the species studied the inheritance of banding was monogenous. Balanced polymorphism was stable in natural populations; colour type frequencies might exist for a long time; stabilizing selection was observed. Many-year studies of natural populations were made in 1968-2009 in the PreUrals (1-the preKama forest near Sarapul, 2- Bashkir steppe near Sterlitamak) and the TransUrals (the prePyshma forest, Sverdlovsk region, near Belsky settlement) in various biotopes. The effects of climate and fluctuating weather conditions in various years on the ratios of single-band (aa) and bandless (AA and Aa) morphs were found to be different in geographically different habitats. The effects were stronger in extreme habitats. Thus, the genetic variability of populations is realized by spatial and temporal variabilities, it is closely connected with the environment heterogeneity. A polymorphic population is less specialized than a monomorphic one, its greater genetic variability allowes to effectively use the environmental resources. The polymorphic structure significantly increases the population adaptive abilities. The investigations are partly supported by Federal program "Scientific and scientific-pedagogical personnel of innovative Russia (GK 02.740.11.0279)"

TIMOFEEV-RESSOVSKY'S PRINCIPLE OF CONVARIANT REDUPLICATION AND EVOLUTION OF LIFE

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N. V. Timofeev-Resovsky formulated the so called principle of "convariant reduplication" as the fundamental property of biological systems. Indeed, replication seems to be the one central, necessary, and in a sense, sufficient feature of all life. It is easy to show that natural selection is a simple epiphenomenon of error-prone replication. As pointed out by Eigen, for evolution to occur, a genome must replicate with an error rate below the error threshold. If the error threshold that is on the order of one error per genome per replication cycle is exceeded, the population of replicating genomes suffers the error catastrophe whereby a dominant sequence (wild type) cannot be transmitted through generations. However, later theoretical developments that I will survey show that the value and very existence of the error threshold critically depend on the structure of the fitness landscape. The replication principle is also intimately linked to the problem of the Tree of Life because of the inherent tree-like character of the replication process. I will discuss this connection and the current status of the Tree of Life concept in light of extensive horizontal gene transfer revealed by comparative genomics.

PARALLELISMS DURING MAJOR EVOLUTIONARY TRANSITIONS

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The ratio between randomness and directionality in evolution remains one of the most controversial questions in evolutionary theory. Parallel and convergent evolution is generally regarded as important evidence of directionality and partial predictability of macroevolutionary trends. Current paleontological data imply that multiple parallelisms represent a typical feature of many major evolutionary transitions (aromorphoses). Generally, the more we know about some particular transition, the more apparent it is that many important features of the derived taxon evolved independently in more than one clade within the ancestral taxon. This pattern reveals itself in numerous transitional fossils with mosaic distribution of ancestral and derived characters. The typical examples include: (i) 'ornithization' of theropods (Jurassic – Cretaceous), a process during which different avian characters (e.g., pennaceous feathers) evolved independently in several clades of theropod dinosaurs; (ii) 'arthropodization' (Ediacaran – Cambrian), a major evolutionary transition from wormlike ancestors to typical arthropods; (iii) 'hominization' (Pliocene – Pleistocene), or parallel evolution of derived 'human' characters in different lineages of australopithecines (as exemplified by several recently discovered fossils, e.g., *Kenyanthropus platyops* and *Australopithecus sediba*); (iv) 'mammalization' of theriodonts (Permian – Triassic), which involved parallel acquisition of various derived mammalian features in different lineages of theriodont reptiles; and many others. The same pattern of numerous parallelisms can be observed in smaller-scale evolutionary processes, e.g., during adaptive radiations of closely related founder populations on different islands or isolated lakes. Multiple parallelisms near the bases of many major and minor clades tend to obscure exact phylogenetic relationships between taxa and impair the applicability of classic cladistic procedures based on parsimony analysis. The reasons underlying this pattern are poorly understood, although some plausible hypotheses can be proposed. The fact that parallelisms are an ubiquitous feature of major evolutionary transitions and adaptive radiations implies that evolution in general is largely constrained and canalized by multiple ecological, morpho-physiological and genetic limitations.

THE DEVELOPMENTAL-GENETIC TOOLKIT AND THE PHENOGENETICS OF FORM

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All animal development makes use of a shared developmental-genetic toolkit, proteins and other molecules specified by genes that largely evolved to serve single-cell functions in the unicellular ancestors of the metazoans. How did these gene products come to serve entirely novel functions in embryogenesis? In establishing the field of *phenogenetics*, A. W. Timofeeff–Ressovsky, distinguished the fact of the phenotypic manifestation of a gene ("penetrance") from the form or degree of that manifestation ("expressivity"). This distinction opened the way to identifying the contextual associations that would permit an understanding of the action of genes in evolution and development. With respect to form in multicellular animals, this context prominently includes the physics of chemically and mechanically excitable "soft matter." Multicellular aggregates, but not individual cells, assume this material condition, which enables the generation of the characteristic morphological motifs of animal bodies and organs: interior cavities, multilayered tissues, segments, appendages, endoskeletons and so forth. This talk will discuss how a subset of the toolkit gene products, in the context of primitive multicellular aggregates of the Precambrian and early Cambrian periods, mobilized physical forces, processes and effects that operate on a larger scale than that of the individual cell. I propose that the resulting change in the genes' expressivity, rather than extensive change in gene sequence, led to the profusion of forms that constitute the basis of the animal phyla.

USE OF ANCIENT DNA GENOMIC SEQUENCING IN ASSESSEMENT OF EVOLUTIONARY CHANGES RESULTING FROM HUMAN ADAPTATION TO NORTH

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Recently, a paper has been published in Nature [1], describing a study based on nearly complete (79%) genomic sequencing of a man who lived in Greenland about 4000 years ago and belonged to the extinct Saqqaq culture. The authors compared the Saqqaq genome with 35 present-day populations, including those inhabiting Siberia (Nganasans, Dolgans, Evenks, Kets, Selkups, Chukchis, Koryaks and others), using Illumina 650,000 marker array. The obtained results provide evidence for a migration of the ancestors of Saqqaq Eskimos from Siberia into the New World some 5,500 years ago. Many of functional SNP assessment have been used to assign possible individual phenotypic characteristics of the Saqqaq man. It was established, that he had rhesus factor and second blood type A1 (II), present at high frequencies in populations of the eastern part of Siberia down to mid-China also at present. Furthermore, a combination of four SNPs at the HERC2-OCA2 locus was found, strongly associated with brown eyes among Asians. SNPs in chromosomes 2, 5, 15 and X suggest that the Saqqaq man probably possessed light brown skin colour, dark and thick hair and an increased risk of baldness. He also probably had shovel-graded front teeth - a characteristic trait of Asians and Native Americans, because associates with the same SNP that is characteristic of hair thickness. Found in chromosome 16 an AA genotype SNP is consistent with the Saqqaq individual having earwax of dry type that is typical for Asians and Native Americans, in contrast to wet type dominant in Europeans. In addition, the combined influence of 12 SNPs on metabolism and body mass index indicates that the Saqqaq individual was well adapted to a cold climate. This study, carried out by the European, US and Chinese scientists with Russian researchers being part of it, implies the great importance of international collaboration and demonstrates astonishing progress in human genetics.

[1] M. Rasmussen et al. (2010) Ancient Human Genome Sequence of an Extinct Palaeo-Eskimo. Nature, 463: 757-762.

SPECIES AGING AS MECHANISM OF THE GLOBAL CHANGES IN BIOSPHERE

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Global changes in biosphere are usually explained by the action of non-organic factors such as meteorites, volcanoes, transgressions, etc. However these factors cannot explain the selective character of replacements in fauna and flora: why some groups became extinct (e.g. dinosaurs), but the other ones persisted (e.g. crocodiles and turtles)? To find out the forces causing such events it is necessary to focus on the biological processes first of all.

The mechanism of the evolution, which causes global changes in biosphere could be characterized as follows: organisms inevitably tend to produce copies of themselves, but they are incapable to reproduce their exact copies; that is why species transform inevitably with the changes of generations, even if they are already well adapted to their environment; such a continuous transformation takes place in definite directions caused by various constraints, even if these directions are not rational or lead to the species extinction. Such a process results in the extinction of species and groups of species just as every individual dies because of aging. Such extinction creates a free space in biosphere, which stimulates a multiplication and correspondingly evolution of other species.

THE SPECULATION ON FORMATION OF EARLY BIOGENE ORGANIC ON THE EARTH

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Whatever the way (abiotic spontaneous self-organize, a panspermia, etc.) the first prokaryotes have appeared in an ***initially sterile*** environment planet the Earth. Their biomass has become the basis of all mass of various biotically synthesized organic matters which we observe today. Pioneers of a life on the Earth had the limited number of low-molecular weight abiotic synthesized inorganic growth sources of "biological atoms" - C, N, O, P, S. It is possible to argue on energy sources for realization of biosynthetic reactions in Pioneers cells. But abundantly clear that only after occurrence of the first generations of the free-living prokaryotes to growth and cell fission (more precisely, after their destruction and liberation of all cytoplasmic components habitual for us today), "the high-energy" biotically synthesized organic as much more attractive sources of carbon, nitrogen, phosphorus, began to collect on the Earth. Climatic and geological conditions of that time could promote non-uniform distribution on the planet of slowly accumulated the new "bio-organic" on separate prebiotically organized ecological niches. Most likely the water-soluble low-molecular weight compounds were distributed quite differently than high-molecular weight biopolymers. Thus an insoluble compounds could form the separate zones of the congestion which have become a petroliferous sites. On the whole, formation of the new "biotic-ecological" niches differing on a set growth sources for first prokaryotic inhabitants of the Earth, could form a basis for differentiation on more active inclusion in number of growth sources other new biogene compounds as well. In other words, the first mutations which have occurred in Pioneers cells on the Earth, could promote expansion of trophic possibilities of separate mutant variants of Pioneers in the conditions of formation and development of a variety of terrestrial biogene organic compounds. Thus, it is possible to assume, what exactly biosynthesis of terrestrial organic substrata has spur on the first events of evolution of prokaryotic organisms on the Earth about 3,7 billion years ago.

EVOLUTIONARY RECENT FUNCTIONAL RESHAPING OF THE HUMAN GENOME BY TRANSPOSABLE ELEMENTS

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Transposable elements (TEs) are selfish fragments of DNA able to reproduce themselves into the host genomes. TEs typically occupy ~40-50% of mammalian DNA. Some TE inserts appeared in the DNA of human ancestry lineage after divergence with the chimpanzee ancestors, or less than ~6 million years ago. These human specific elements (hsTEs) represent only a minor fraction of the whole TE cargo of the human DNA. hsTEs are represented by the four families called HERV-K(HML-2), L1, Alu and SVA. The number of human specific copies for these families is approx. 150, 1200, 5500 and 860 copies per genome, respectively. hsTEs may be regarded as the perspective candidates for being molecular genetic agents of human speciation. Unlike most of random mutations and duplications, each new insert of hsTE has provided to the recipient genomic locus a set of functional transcription factor binding sites positively selected during the TE evolution. For example, clusters of novel inserts of Alu elements may serve as CpG islets, SVA elements provide functional splice sites and polyadenylation signals, whereas L1 and HERV-K(HML-2) elements donate enhancers, promoters, splice sites and polyadenylation signals. Significant proportion of the species-specific genomic deletions, duplications and translocations has been also generated due to ectopic recombinations between different individual TEs. Among the other, we report for the first time a detailed functional characteristics of the HERV-K(HML-2) hsTEs done at the genome-wide level. We identified 65 active *in vivo* human specific promoters contributed by these elements. We also show three cases of the HERV-K(HML-2) -mediated human specific regulation of functional protein coding genes taking part in brain development during embryogenesis. We found ~180 polyadenylation signals transferred by the hs SVA elements into the introns of known functional genes. Scaling of these data to the total number of the hsTEs predicts that hundreds of human genes are regulated "human-specifically" by these elements. Finally, we discovered a novel human specific family of TEs formed by a combination of the CpG islet of human gene *MAST2* and of the 3'-terminal part of SVA retrotransposon. This family is represented by ~80 members and is still highly active nowadays.

TRANSPOSABLE ELEMENTS AND GENOME EVOLUTION

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Our knowledge on the genome composition and diversity in various species has dramatically increased in recent years. We know now that an important proportion of most genomes are composed of repeated sequences such as transposable elements (TEs). For example, the human genome harbors more than 50% of such sequences, and the maize genome more than 80%. One of the main challenges of modern genetics and genomics is therefore to understand how TEs interact with genes and genomes, and how they are involved in phenotypic plasticity and thus in species adaptation to new environments and in evolution. The answers to these questions necessitate a fine understanding of the dynamics of TEs within and between genomes and populations in a large set of species that differ for their physiological and ecological traits.

SHORT PAPERS

by Young Scientists

GENETICS

FUNCTIONAL CHARACTERIZATION OF THE TRANSCRIPTIONAL REGULATORY ELEMENTS IN HUMAN RETROTRANSPOSONS HERV-K (HML-2) AND L1

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Retroelements comprise a considerable fraction of human genome. Human endogenous retroviruses are thought to be remnants of ancient retroviral infections of human ancestors' germ cells. HERVs can modify expression of host cell genes through their *cis*-regulatory elements concentrated in their long terminal repeats (LTRs). Although numerous HERV-related RNAs were identified in the human transcriptome, for most of them it remained unclear whether they are LTR-promoted or read-through products. For transcriptome-wide quantitative and qualitative detection of the HERV-K (HML-2) LTRs promoter activity, we developed a new technique called GREM (Genomic Repeat Expression Monitor), which may be applied to genome-wide isolation and quantitative analysis of any kind of transcriptionally active repetitive elements [2]. In our experiments we found that at least 50% of the human specific HERV-K (HML-2) LTRs are promoter active in human tissues, and identified 65 novel human specific promoters. We used a 5' RACE (rapid amplification of cDNA ends) technique to identify transcriptional start sites of LTR-promoted transcripts for human specific HERVs (HS HERVs) [1]. We have found that in addition to canonical promoter located within the U3 region of the LTR, HS HERVs also utilize an alternative transcription start site located on the border of the R and U5 LTR regions.

Human retroelements L1 are believed to be the only group of autonomous transposable elements currently active in humans. About 800 bp-long 5'-untranslated region (5'-UTR) of the human L1 harbors a unique internal transcriptional promoter. There are two opinions about which part of the 5'-UTR is the most important for its functional activity: either it is the first 5' 100-150 bp-long region [3] or it is the internal region of the 5'-UTR (+390...+662) [4]. We experimentally check these hypotheses using a reporter construct assay.

Materials and Methods

GREM technique includes hybridization of the PCR-amplified genomic sequences flanking human specific LTRs (HS LTRs) with cDNA, followed by selective amplification and cloning of hybrid DNA duplexes (Figure1).

The contribution of different 5'-UTR regions to L1 promoter activity was investigated using the constructs containing L1 5'-UTR with various deletions and reporter gene for firefly luciferase. We cotransfected cells with these constructs and with the normalization plasmid containing another reporter gene LacZ. We

measured both enzymatic activities (luciferase luminescence and beta-galactosidase activity) and concentration of the transcripts (using qRT-PCR) for both above reporter genes.

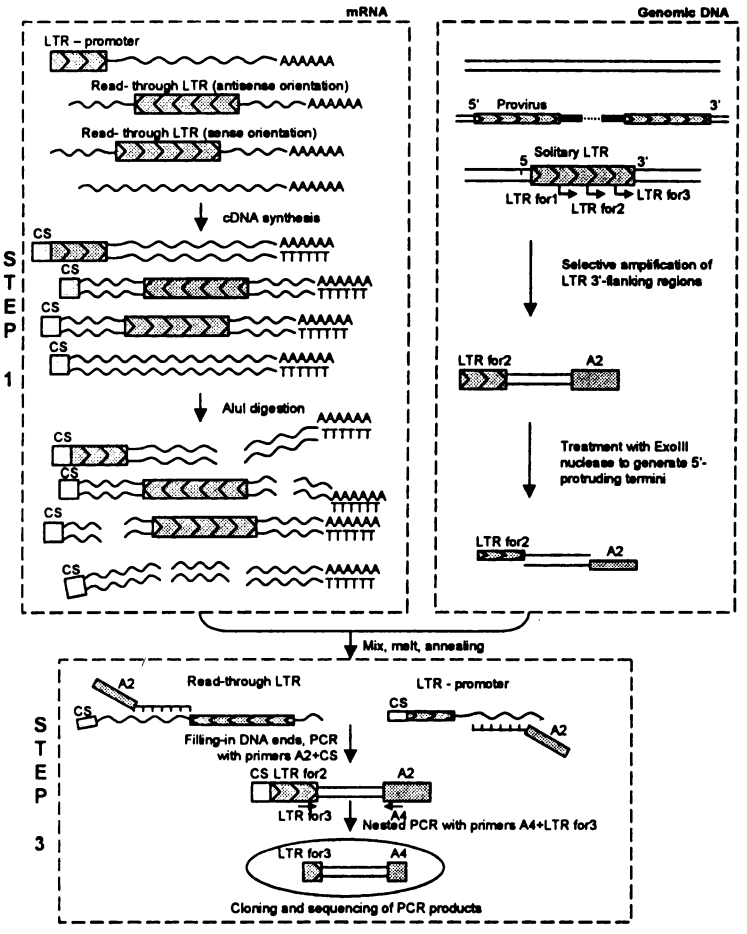

Figure1. Schematic representation of the GREM technique. The procedure includes three major steps: (Step1) genome-wide amplification of the genomic DNA flanking the 3' ends of target repetitive elements (here, HS LTRs). Treatment of resulting amplicon with ExoIII nuclease generates 5' protruding ends to be used at the third stage. (Step 2) At this stage cDNAs are tagged by a linker oligonucleotide (CS) at the RNA transcription start sites using the 'cap-switch' effect. cDNAs are then digested with AluI restriction endonuclease that has no recognition sites with HS LTRs. This step precludes amplification of LTR sequences read-through in the antisense orientation. (Step 3) Finally, the genomic DNA amplicon (Step1) is hybridized to the 5'-tagged cDNAs(Step2). The protruding DNA ends are filled in with DNA polymerase, and the hybrids obtained are nested PCR amplified with primers specific to the flanking genomic DNA adapter and cDNA 5' terminal tag sequence, respectively.

Results and Discussion

We have developed GREM, a genome-wide approach that makes it possible to focus on the repetitive elements' own promoter activity and to eliminate the background of read-through sequences. The resulting library of GREM clones can be used as a set of tags for individual transcriptionally active repetitive elements.

This approach combines the advantages of both 5' RACE and nucleic acid hybridization. We used GREM to study HERV-K (HML-2) LTRs promoter activity in normal and cancerous testicular tissues. We showed that the number of individual tags in the library was proportional to the content of mRNA driven by the corresponding promoter active repetitive element. We created the first genome-wide map of transcriptionally active HERV-K (HML-2) LTRs and found that at least 50% of all human specific HERV-K (HML-2) LTRs are promoter active. For five randomly chosen individual solitary LTRs, we precisely mapped transcription initiation sites using 5'RACE approach. In all cases, the transcription was driven from the same non-canonical promoter located on the border of the R and U5 regions within the HS LTR consensus sequence, which does not coincide with the canonical transcriptional start site located within the U3 region.

As for the 5'-UTR L1 investigation, our results demonstrate that the 5'-UTR region (+390...+526) is crucial for the L1 promoter activity, whereas the first 5' 100 bp-long region is significantly less important, which correlates with the data published by Olovnikov et al. [4] and argues with the generally accepted model published by Svergold [3].

Conclusion

We developed an experimental technique termed GREM for genome-wide isolation and quantitative analysis of any kind of promoter active repetitive elements. We created the first genome-wide map of promoter active human-specific endogenous retroviruses and individual solitary LTRs, and for the first time quantitatively characterized promoter activities of the particular elements. We argue with the generally accepted model by Svergold about regulation of human L1 transcriptional activity and provide evidence that the most important role there is played by an internal 5'-UTR region.

Acknowledgements

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ON EVOLUTION PROCESS OF MATHEMATICAL MODELLING VALUE METHOD

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Evolution is a movement process from low(simple) to high(complex), where main aim is to cancel the old and rise the new. This is adopted as a result of observations through many ages.

Is it possible to substantiate the development evolution theory via numerical experiments? Numerical calculations in the presence of the appropriate model are capable to prove that pending time some parameters quantity changes in the system sometime may lead to the same system quality changes. Proving the mentioned above it will be possible to prove the evolutional characteristic of the development, but mathematical modelling supposes initial conditions first of all which means even if proving the evolution development theory via numerical experiments, the creation of space and creation of life is still mysterious.

Following the value method applying possibilities for developing process prediction is discussed on a typical system branch chain reaction example.

Value analysis of the chain reactions of kinetic models

The selection of the chain reaction kinetic model analysis is connected with high similarity of evolution process development task, where it is necessary to get results during short time, that in reality may process during long time. To get sensible solution via direct calculations is not always possible, as every small change, which is not always possible to calculate, in a chain process may be decisive. The necessity of a new approach for the problem solution is obvious and the success may also lead to new models of evolution development processes in other spheres.

Let's discuss branch chain reaction mechanisms criticality phenomenon and try to give on transient period the parameters relation: criticality condition, using the parameters value in the functional.

If we choose as a objective functional the j-chemical element concentration arisen during reaction.

$$I(t) = c_j(t) = \int_0^t f_j(t)dt\,, \tag{1}$$

the i-th active center ψ_i value in the functional will be defined like this

$$\psi_i^j(t) = \frac{\partial f_j[f_1(t),\ldots,f_m(t)]}{\partial f_i}\Big/_{fi=fi(t_0)}. \tag{2}$$

This means that i-th active center value at every time point in j-chemical element creation contains the system evolutional development part. This is expressed so that as during value quantitative calculation it are counted not only the i-th active center direct influence is counted but also other components influence generated from it.

To calculate criticality state and ignition line, at first the reaction mechanism of appropriate kinetic equations system is written down

$$\frac{dc_i}{dt} = f_i(\mathbf{c}, \mathbf{k}), \qquad i = 1,2,..., m, \tag{3}$$

where c_i is the - i-th component concentration, k is primary reaction rate constant vector column. As a reaction system of critical state it's behavior in extremal state is suggested. In isotherm chemical reaction the extremum behavior of the system on kinetic parameter change is expressed $\delta N(t) = 0$, which is equivalent to the following:

$$I(t) = \int_0^t \frac{dN}{dt} dt \rightarrow extremum \tag{4}$$

where N is the system of summary concentration components.

In variations calculus it is known, that (3) and (4) conditions correspond to the Hamiltonian null value

$$H = \psi_0 f_0 + \sum_{i=1}^{n} \psi_i f_i = 0, \tag{5}$$

where $f_0 = \frac{dN}{dt}$, $\psi_0 = 1$, depending on minimum or maximum solved task. ψ_i – are concentration adjoint functions.

$$\frac{d\psi_i}{dt} = -\frac{dH}{dc_i}, \qquad i = 1,2,..., m \tag{6}$$

So the critical state of the system is described by (5) condition parallelly satisfying of (3) and (6) conditions. The Hamiltonian of the system during time is of invariant value. So H=0 extremum condition will be satisfied during all observation.

Let us discuss hydrogen-oxygen branch chain reaction (table 1) which is tested during various theoretical and experimental researches.

Table 1. Hydrogen-Oxygen system combustion kinetic model

N	Stages	Reaction rate constants
1	$H_2 + O_2 \rightarrow 2OH$	$1.5 \cdot 10^{-24}$
2	$OH + H_2 \rightarrow H_2O + H$	$5.53 \cdot 10^{-15}$
3	$H + O_2 \rightarrow OH + O$	$1.88 \cdot 10^{-15}$
4	$O + H_2 \rightarrow OH + H$	$3.87 \cdot 10^{-17}$
5	$H \longrightarrow 1/2H_2$	2.26
6	$H + O_2 + M \rightarrow HOO + M$	$1.19 \cdot 10^{-32}$

Reaction rate constants are represented by sm^3, sec. for $t=700^0C$ temperature. The criticality calculation is made starting from initial time in Δt intervals (in represented sample $\Delta t = 1.25 \cdot 10^{-4}$ sec); At $t=1.25 \cdot 10^{-4}$ sec. the initial value of the Hamiltonian for initial P_0 pressure is calculated. At the same time changing initial pressure value calculates the pressure of critical values, during which the Hamiltonian value is equal to 0. It is obvious that using this method the critical parameters may be calculated during the initial stage, not depending on reality for qualitative change for which we need seconds or million years and there is no need to make hard calculations which may lead to false results.

The initial $\psi_i(t_0)$, values for integrating equation (6) are calculated from (2) $\psi_H(0)=\psi_O(0)=\psi_{OH}(0)=\psi_{HO_2}(0)=1, \quad \psi_{O_2}(0)=\psi_{H_2}(0)=\psi_{H_2O}(0)=0.$

Initial substance concentration relations are as follows: $c_{O_2}(0):c_{H_2}(0)=1:2.$

Calculations of the first and second combustion limitations corresponding to mixtures of initial pressures have the following results` $P_1=0.14$ Torr, $P_2=18.01$ Torr.

In this case Hamiltonian will get 0 value for the mentioned two pressure values which are similar to experimental results. It is tested that the same sample for other more complex conditions are approved by experimental results.

CONCLUSION

Represented value method makes possible to calculate numerically the system combustion limitations and criticality parameters independent the reaction system complexity and there is no need to make direct calculations for the criticality state of the system: this is very important as the known problems are bypassed during mathematical modelling evolutional development processes which reach a deadlock.

MOLECULAR-GENETIC ANALYSIS OF FACTOR V LEIDEN MUTATION AND THR312ALA POLYMORPHISMS AS RISK FACTORS OF MYOCARDIAL INFARCTION

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Introduction

The problem of diagnostics of genetic predisposition to cardiovascular diseases such as infarcts, insults is very important because they are the leading causes of mortality nowadays. The involvement of factor V Leiden mutation in predisposition to arterial thrombosis and to myocardial infarction, in particular, is rather doubtful: some studies declare that factor V Leiden is linked with arterial thrombosis [1,2], while others show that it is not linked [3]. Due to this, we decided to investigate the effect of this mutation on predisposition to myocardial infarction.

One of the most important causes of arterial thrombosis is high level of fibrinogen, which is the one of the most important causes of arterial thrombosis due to its contribution to formation of fibrin clots consisting of thinner and compacted fibers with smaller pores between them. The fibrinogen level correlates with environmental risk factors, such as hypertension, smoking, diabetes, age and so on. [4]. Thr312ala polymorphism is responsible for structural changes in fibrinogen molecule. This polymorphism influences clot formation at one of last stages of coagulation cascade. That's why investigation of Thr312Ala polymorphism of α-chain of fibrinogen as risk factors of myocardial infarction is of current concern.

Subjects and methods

Blood samples of 100 patients with acute myocardial infarction were investigated for factor V Leiden mutation and for Thr312Ala polymorphism. These samples on a Guthrie cards were provided by National Center of Research and Applied Medicine "Cardiology". A control group consisted of 100 people of more than 50 years old without cardiovascular pathology in anamnesis. Blood samples of the control group were provided by Medical Centre of the National Academy of Sciences of Belarus.

For both Thr312Ala polymorphism and factor V Leiden mutation we used dried blood spots as a matrix for PCR, using method of enhanced direct amplification described by Makowski [5]. The presence of factor V Leiden mutation was detected by amplification refractory mutation system (ARMS) method, as described by Scobie [6] with some modifications. The Thr312Ala polymorphisms were detected using adopted method by Carter et al [7]. Odds rate (OR) was calculated by traditional method.

Results and discussion

Factor V Leiden. The presence of heterozygous (AG) genotypes were detected in 7% patients with myocardial infarction, none of the patients was homozygous for factor V Leiden (AA). In the control group 3% heterozygous genotypes and none homozygous ones were revealed. So, the rate of heterozygous genotypes in the group with myocardial infarction was 2.3 times as high as in control group. The frequencies of factor V Leiden alleles were 3.5% in the cases and 1,5% in the control (Table 1).

Table 1. Genotype and allele distribution of Factor V Leiden mutation

Genotypes	Cases	Controls	OR	95% CI
	N=100	N=100		
GG	93 (93,0%)	97 (97,0%)	0.41	0.10-1.64
AG	7 (7,0%)	3 (3,0%)	2.43	0.61-9.69
AA	0 (0%)	0 (0%)	-	-
Alleles	N=200	N=200		
G	193 (96,5%)	197 (98,5%)	1.64	0.44
A	7 (3,5%)	3 (1,5%)		

We found 3,0% of heterozygous carriers in the control group of Belarus people. This complies well with the population frequencies obtained by other authors in Russia and Belarus [8, 9].

Thr312Ala. We found the following genotype distribution in the group of patients with myocardial infarction: 59% TT, 39% TA, 2% AA. Genotype distribution in the group of healthy people was as follows: 64% TT, 29% TA and 7% AA (Table 2).

Table 2. Genotype and allele distribution of Thr312Ala polymorphism of fibrinogen α-chain

Genotypes	Cases	Controls	OR	95% CI
	N=100	N=100		
TT	59 (59,0%)	64 (64,0%)	0.81	0.46-1.43
TA	39 (39,0%)	29 (29,0%)	1.57	0.87-2.82
AA	2 (2,0%)	7 (7,0%)	0.27	0.05-1.34
Alleles	N=200	N=200	-	-
T	157 (80,0%)	157 (78,5%)	1.00	0.62-1.61
A	43 (20,0%)	43 (21,5%)		

Literature data about Thr312Ala polymorphism relation to genetic predisposition to cardio-vascular diseases are very limited and controversial. It was shown [7], that this polymorphism influences the poststroke mortality in subjects with atrial fibrillation. However these data were not corroborated in another paper [10]. So, the scientific literature data about Thr312Ala polymorphism are insufficient for clear conclusion about its relationship with cardiovascular diseases.

Conclusion

Assessing the frequency acquired in the group with myocardial infarction, we found 2.3 time increased rate in comparison with the control group. This indicates the role of factor V Leiden as a significant risk factor not only for venous thrombosis but also for arterial one (for myocardial infarction in particular).

According to our data the frequency of TA genotypes of Thr312Ala polymorphism in patients with myocardial infarction was 1,3 times as high as in the control group (39,0% and 29,0% respectively). This fact may be an evidence for the impact of this polymorphism on the genetic predisposition to myocardial infarction.

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MOLECULAR CHANGES IN IRRADIATED THYROID GLAND AND THYROID CARCINOMAS OF MICE

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Thyroid carcinomas arising from follicular epithelial cells are the most common endocrine malignancy in man. During studies performed on the population of the Marshall-Islands and after the accident at the Chernobyl nuclear power plant in 1986 a large increase in benign thyroid nodules and thyroid cancer, especially among children, was shown (1, 2). This study aimed to investigate molecular changes in thyroid follicular carcinoma (FTC) developed in highly susceptible mouse strain and intact thyroid tissue following irradiation that could be involved or predispose to thyroid cancer formation.

Material and Methods

Array Comparative Genomic Hybridization (array CGH) was performed to determine DNA copy number changes in 5 cases of FTC (Figure 1) and 1 case of thyroid hyperplasia. The tumours developed following low dose exposure of the thyroid by bone seeking alpha-emitter Th 277 injected in FVB/N mice (Table 1). Fluorescent labelled reference and tumour DNA were hybridized onto custom- made mouse BAC arrays covering the entire genome.

Table 1. Case, diagnosis and sex of mice

Case number	Tissue	Diagnosis	Sex
032	Thyroid gland	Follicular thyroid carcinoma	male
457	Thyroid gland	Follicular thyroid carcinoma	male
909	Thyroid gland	Follicular thyroid carcinoma	male
910	Thyroid gland	hyperplasia	female
1026	Thyroid gland	Follicular thyroid carcinoma	male
1375	Thyroid gland	Follicular thyroid carcinoma	male

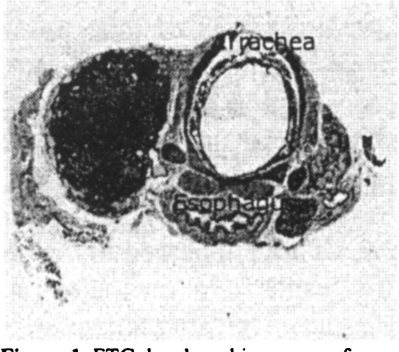

Figure 1. FTC developed in mouse of FVB/N mouse strain exposed to Th-227.

Detection of the phosphorylation of histone H2AX at the serine 139 (gH2AX) was performed to determine efficiency of follicular cells to repair DNA double strand

breaks (DSBs) following irradiation. Immunofluorescence was done on paraffin-sections of mouse thyroid gland of mice of JF1 and FVB/N mouse strains that were previously irradiated with 1 and 8Gy of whole body X-irradiation.

Results and Discussion

DNA from 5 follicular thyroid carcinomas and one case of thyroid hyperplasia were analysed using array CGH. In three out of six cases (032, 910 and 1026) a deletion of the entire chromosome 14 was observed. All array CGH profiles are shown in Figure 2.

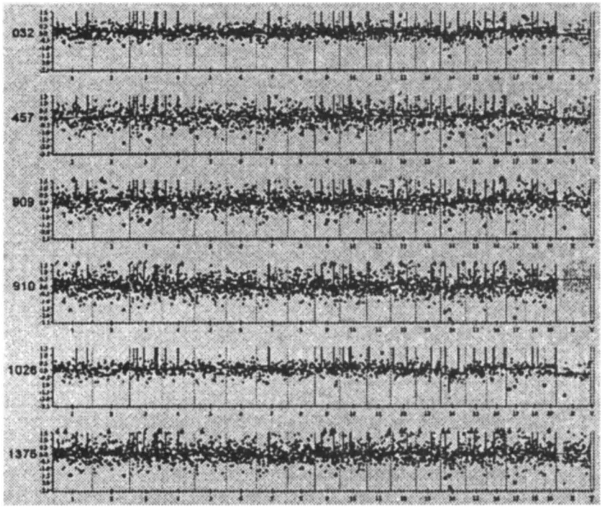

Figure 2. Array CGH profiles. Result of array CGH analysis of 6 thyroid tumours in FVB/N mice. Numbers along abscissa relate to chromosome, from left to right is the distance from p-arm telomere. Numbers on the ordinate axis give relative copy number changes in tumour DNA as a logarithm to base 2. From top to bottom case 032, 457, 909, 910, 1026 and 1375. Each single point represents hybridisation of tumour DNA relative to normal DNA on one distinct genomic BAC probe.

A similar pattern of chromosome 14 deletions was already reported in thyroid tumours of other mouse strains following high-dose exposure to I-131(3) and therefore suggests that this deletion is not associated with genetic predisposition in different mouse strains to thyroid tumourigenesis, but rather reflects a general molecular mechanism of thyroid cancer formation. Thyroid follicular cells did not show existence of gH2AX foci either in samples irradiated with 1Gy or in samples irradiated with 8Gy. On the contrary, gH2AX foci appeared in the cells of connective tissue and trachea (Figure 3). This result is in contradiction with previously published data (4), where a dose-dependent induction of gamma H2AX foci was observed in irradiated cell-cultures of primary human thyroid cells. One could assume, that the growth state of cells influence the expression of the gamma H2AX protein following DNA damage. *This might coincide with the termination of thyroid* growth, starting in mice at an age *of about 6 months (present data used* 5 – 8 month old animals). The thyroid gland in mice then begins to decline with increasing number of dilated follicles. These follicles contain abundant colloid that is functionally inactive and do not become iodinated after stimulation. On

the other hand, cells in culture force to divide and growth quickly and this might explain different observation of gH2AX foci formation in-situ and in-vivo. Recent observation could also explain age-dependent risk to thyroid cancer formation after radiation exposure.

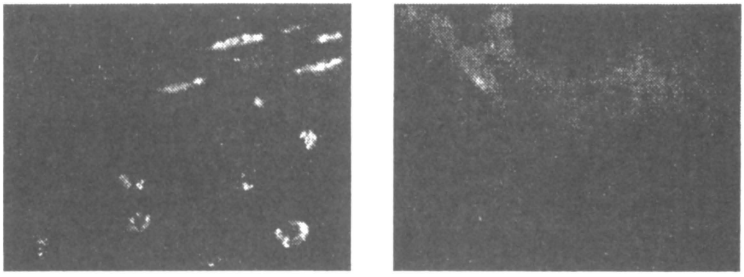

Figure 3. Images of fluorescence stained gamma H2AX foci. Shown sample of mouse thyroid tissue, irradiated with 8Gy. Left: part of the trachea with gH2AX foci inside of the nuclei. Right: part of thyroid gland, without gH2AX signal.

Conclusion

The observation suggests that congenital predisposition of FVB/N mice is caused by very early steps of the malignant transformation of thyrocytes, without any association to deletion of chromosome 14 in tumours itself. The absence of detectable gamma H2AX foci in irradiated thyroid gland with gamma H2AX foci in adjacent non- thyroid tissue suggests that this is a peculiarity of the thyroid follicular epithelial cells. It is hypothesised that resting thyroid epithelial cells of adult mice express reduced gamma H2AX, which might be associated with reduced DNA repair and low susceptibility to radiation carcinogenesis.

Acknowledgments

I would like to thank my supervisor Dr. Michael Rosemann, Head of the Institute of Radiation Biology Prof. Dr. Michael J. Atkinson, Prof. Dr. H. Zitzelsberger and to the Head of the European Master course in Radiation Biology Prof. Claus R. Trott for giving me possibility to submit this work as part of my Msc degree.

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DELETION OF THE *SACCHAROMYCES CEREVISIAE RAD30* GENE ENCODING DNA POLYMERASE η INCREASES THE FREQUENCY OF PRIMARY LESIONS IN GENETIC MATERIAL

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Introduction

Replicative DNA synthesis is a faithful process employing high fidelity DNA polymerases. The price for this high fidelity is the replicating polymerases incapacity to replicate over the damages occurring on the DNA template. Most DNA lesions block the progress of the replication fork, which can lead to the cell death. To overcome this block, the cell uses specialized low fidelity DNA polymerases, which synthesize DNA across the lesions (TransLesion Syntheses, TLS). DNA polymerase η (Polη) encoded by the *RAD30* gene is the primary TLS polymerase responsible for error-free bypass of cis-syn cyclobutane pyrimidine dimers (CPDs), one of the major lesions resulting from UV irradiation. Though, Polη plays a major role in accurately bypassing particular types of DNA lesion, such as CPDs and 7,8-dihydro-8-oxoguanine occurring during oxidative stress, Polη is able to bypass a broad range of other DNA lesions as well with less accuracy. Moreover, it exhibits the lowest fidelity among other DNA polymerases on undamaged DNA in vitro [1, 2, 7].

In the present work, we have studied a role of DNA polymerase η in genome stability maintenance. To test the influence of the *RAD30* gene deletion on occurrence of different changes in genetic material we have used an approach, named the α-test. The α-test was developed in our laboratory earlier for yeast *Saccharomyces cerevisiae* [3]. It is possible to distinguish between different types of mutational changes (point mutations, recombination, chromosome or chromosome arm loss) and temporary lesions in genetic material by using the α-test. All the events may be scored in the α-test by analyzing phenotypes of illegitimate hybrids if both arms of the chromosome III are mapped [5]. Such illegitimate hybrids arise with low frequency when hybridization of two heterothallic yeast strains of the same mating type α occurs. Frequency of illegitimate hybridization increases after DNA damaging treatment due to disturbance of the *MAT* locus expression.

Moreover, a modification of the α-test (selective system of illegitimate cytoduction) allows us to detect preliminary lesions in the genetic material both fixed and converted to mutation after completion of the repair. Cytoduction is uncompleted hybridization, when the cytogamy is not followed by the nuclear fusion and diploid cell formation. Using selective medium it is

possible to isolate cytoductants – haploid cells with mixed cytoplasm and nucleus of the recipient cell. Analysis of illegitimate cytoductants phenotype lets us to distinguish between primary lesions and inherited changes of genetic material both spontaneous and induced by genotoxic agents. Unfortunately, the system of cytoduction does not allow to register some genetic events which are lethal in haploids. Therefore, these two variants of the α-test are complementary and together let us to register all range of genetic events [3].

We have investigated the effect of the *RAD30* deletion both in selective systems of cytoduction and hybridization.

Material and Methods

Yeast strains. Following yeast strains where used in the present work: two isogenic strains in illegitimate hybridization test: K5-99-35B-D924 (wild type) and K5-99-35B-D924-*rad30Δ* (*rad30Δ* mutant); two isogenic strains in illegitimate cytoduction test: 26Б-D924 and 26Б-D924-*rad30Δ*. Yeast strain D926 was used as a partner for hybridization in both tests [6]. Yeast strains were grown in liquid YAPD media at 30°C. To check the strains viability the solid YAPD media was used as described before [4]. Media for selection of hybrids and cytoductans were prepared on the base of synthetic media as described earlier [6].

Selective systems of illegitimate hybridization and cytoduction. Measuring of illegitimate hybridization and cytoduction frequencies was performed as described earlier [6]. Phenotypes of illegitimate hybrids and cytoductants were checked on drop-out media series. Based on their phenotypes cytoductants and hybrids were divided into appropriate classes. The frequency of each class was assigned.

Results and Discussion

The absence of Polη was shown to have no influence both on spontaneous and on induced by UV-light frequency of illegitimate hybridization in yeast *S. cerevisiae*. Detailed analysis of events, which led to hybridization, suggests that deletion of the *RAD30* has no significant effect on chromosome loss, chromosome arm loss, recombination and transposition processes. Therefore, we propose that Polη does not participate in the maintenance of chromosome integrity as another TLS DNA polymerase (Polζ) does [8]. However, we obtained a significant increase in frequency of mutational and temporary changes in the *MAT* locus in *rad30Δ* strain (approximately, 2.5 times). Since, it is not possible to distinguish mutations and correctly repaired lesions in illegitimate hybridization test, we investigated effect of the *RAD30* deletion in selective system of cytoduction. In this study, we showed that deletion of the *RAD30* gene leads to increase in illegitimate cytoduction frequency (in 3.5 times) after exposure to UV-light. In contrast, no significant increase of cytoduction was observed without treatment. Moreover, we investigated what percent of temporary lesions was

repaired accurately and what percent was transformed to inherited changes (Fig. 1). We have shown increasing of noninherited lesions after exposure to UV-light. It obviously means that the temporary lesions are repaired more efficiency in the absence of Polη. Since Polη can accurate bypass only CPDs and it is inaccurate on other templates, we postulate that in the absence of Polη most lesions, aroused after UV-treatment, are repaired correctly.

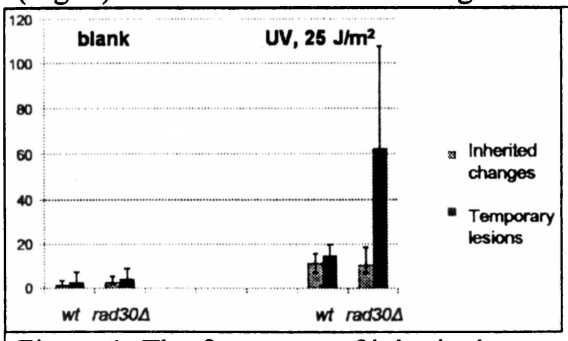

Figure 1. The frequency of inherited changes and temporary lesions without and after UV-treatment in wild type and *rad30Δ* strains.

Conclusions

The contribution of yeast DNA polymerase η in genome stability maintenance by using the α-test was examined. Polη does not influence both chromosome loss and rearrangements, suggesting that functions of Polη are restricted only to participation in the TLS as opposed to Polζ. We suppose that the increase of noninherited lesions in the absence of Polη reflects the efficiencies of photoreactivation, NER, and/or relative efficiency of lesions bypass by another TLS polymerase ζ.

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THE GENETIC BASIS OF ANTHOCYANIN BIOSYNTHESIS IN WHEAT, RYE AND WHEAT-RYE HYBRIDS UNDER NORMAL AND STRESS CONDITIONS

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Anthocyanins are pigmented compounds in plants, which are known to play a protective role under different stress conditions. For example, anthocyanin content tends to increase under UV-B irradiation, drought, cold, toxic metals in soils and pathogens attack [1]. In bread wheat (*Triticum aestivum* L.) and rye (*Secale cereale* L.), anthocyanin pigments are found in different organs such as culm, leaf, auricle, pericarp, coleoptiles and anther [2]. However, the genetic mechanisms underpinning formation of these traits as well as contribution of the pigmentation to stress tolerance have not been widely studied in wheat and rye. The aim of the current study was to investigate molecular-genetic mechanisms underlying anthocyanin pigmentation of the coleoptiles in wheat, rye and wheat-rye hybrids, and to estimate the role of the pigmentation in salt tolerance in wheat seedlings.

Materials and Methods

A variety of molecular-genetic methods such as gene cloning, mapping and expression analysis by RT-PCR (reverse transcription PCR) and qRT-PCR (quantitative RT-PCR) were used [3]. Primers specific to a variety of the wheat and rye anthocyanin biosynthesis (AB) genes were designed by OLIGO program [4] and used in PCR and RT-PCR on wheat–rye 'Chinese Spring'–'Imperial' addition lines [5], wheat-rye chromosome substitution line 'L 2R(2D)' [6] and wheat 'Chinese Spring' deletion lines [7]. Seedling growth parameters of wheat cv. 'Saratovskaya 29' ('S29') carrying *Rc* gene determining weak anthocyanin pigmentation and near-isogenic line 'i:S29Pp1Pp2' having additional *Rc* gene conferring strong coleoptile pigmentation, along with 2 complementary *Pp* genes for anthocyanin pigmentation of pericarp [8], was assessed under 0, 100, and 200 mM NaCl.

Results and Discussion

Cloning and mapping of the AB genes. Nucleotide sequences for a total of eight AB structural genes were cloned and mapped in wheat and rye for the first time (Table 1). The localization of the AB structural genes in wheat and rye genomes was in agreement with the rearrangements established earlier [9] between wheat and rye chromosomes. Besides the AB structural genes, the regulatory genes determining tissue-specific transcription of the AB structural genes are known. In coleoptiles, biosynthesis of anthocyanins is determined by the presence of the dominant allele in the *Rc* (red coleoptile) locus. Wheat has three homoeologous copies of the *Rc* gene (*Rc-A1*, *Rc-B1* and *Rc-D1*) mapped to the homoeologous group 7 chromosomes [10]. In the current study,

the rye *Rc* gene was localized in the syntenic region of rye genome (chromosome 4RL) and designated *Rc-R1* (Table 1). Assignment of wheat and rye AB genes to certain chromosomes enabled selection of suitable genetic models to study regulatory specificities of wheat/rye AB system and gene expression at the alien genetic background.

Table 1. The AB genes and their chromosome locations in wheat and rye. **Bold** – the genes cloned and mapped in the current study.

Enzyme (trait)	Rye genes	Chromosome locations in rye	Wheat genes	Chromosome locations in wheat
chalcone-flavanone isomerise	***Chi***	**5R**	***Chi-B1, Chi-D1***	**5B, 5D**
flavanone 3-hydroxylase	***F3h***	**2R**	*F3h-A1, F3h-B1, F3h-B2, F3h-D1*	2A, 2B, 2D [11]
anthocyanidin synthase	***Ans***	**6R**	*Ans-A1, Ans-A2, Ans-B1, Ans-B2, Ans-D1*	6A, 6B, 6D [12]
anthocyanidin-3-glucoside rhamnosyltransferase	***3Rt***	**5R**	***3Rt-B1, 3Rt-D1***	**5B, 5D**
anthocyanidin pigmentation of coleoptile	***Rc-R1***	**4R**	*Rc-A1, Rc-B1, Rc-D1*	7A, 7B, 7D [10]

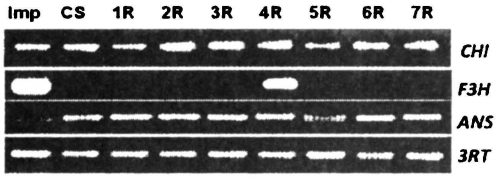

Fig.1. RT-PCR in 4-day-old coleoptiles of wheat–rye ('Chinese Spring'–'Imperial') addition lines. *Imp* 'Imperial', *CS* 'Chinese Spring', *1R, 2R...7R* 'Chinese Spring'–'Imperial' addition lines carrying the corresponding rye chromosome. 'Imperial' and 4R addition line have anthocyanin pigmentations in the coleoptile.

The regulatory specificities of wheat/rye AB system in comparison with other plant species. Using the addition lines it was shown that *Chi*, *Ans*, and *3Rt* were co-expressed in either pigmented or non-pigmented coleoptiles, whereas *F3h* was expressed only in the pigmented coleoptiles (Fig.1). Thus, in wheat/rye coleoptiles, biosynthesis of anthocyanins is specified by activation of *F3h* in the presence of the dominant *Rc* allele. In many plant species, *F3h* is expressed independently of the genes, determining tissue-specific pigmentation (for example, expression of *F3h* is observed in non-pigmented tissues in grape [13] and petunia [14]). This strong regulatory-target relationship between *Rc* and *F3h* in wheat and rye was further used to study the relationship between alien structural and regulatory genes in wheat-rye hybrids.

Conservation of wheat and rye AB regulatory networks. To study the effect of wheat *Rc* on rye *F3h*, we used wheat–rye chromosome substitution line 'L 2R(2D)' having anthocyanin pigmentation in the coleoptiles controlled

by the wheat *Rc* gene. In this line, instead of the wheat *F3h* homoeologue *F3h-D1* the rye *F3h* gene is present. Primers specific to wheat *F3h-A1*, *F3h-B1*, and the rye *F3h* gene were used in qRT-PCR performed on cDNA derived from 3-, 4-, 5-, and 6-day-old coleoptiles of 'L 2R(2D).' Although some transcriptional dominance of wheat homoeologues over the rye *F3h* gene was observed (at the third day the expression level of rye *F3h* gene was significantly lower than that of *F3h-A1*, *F3h-B1*, Fig.2), it can be concluded that wheat regulatory gene *Rc* can activate rye target gene *F3h*. *Vice versa*, in wheat-rye chromosome addition lines, rye *Rc* activated wheat *F3h* (Fig. 1).

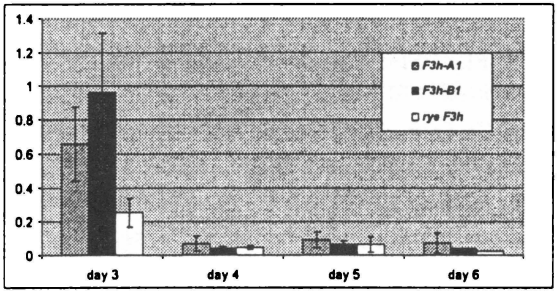

Fig.2. The expression of wheat *F3h-A1*, *F3h-B1*, and the rye *F3h* gene in the coleoptile of wheat–rye chromosome substitution line 'L 2R(2D)'.

Taken together, these results demonstrated good integration of the wheat–rye AB gene systems within the hybrid genomes.

The role of anthocyanin pigmentation in salinity stress resistance. While the growth of wheat seedlings under salinity stress was suppressed, the significant increase of anthocyanin content in both 'S29' and 'i:S29Pp1Pp2' was observed. The comparison of the seedling growth parameters between these near-isogenic lines showed higher values for the line with strong anthocyanin pigmentation ('i:S29Pp1Pp2') in comparison with the weakly colored line ('S29'), suggesting anthocyanin pigmentation of the coleoptile and grain pericarp may improve salinity tolerance of wheat seedlings.

Conclusion

There are species-specific peculiarities in anthocyanin biosynthesis regulatory networks of different plant species. However, wheat and rye show genetic relationship, which is enough close for coordinated work of their anthocyanin biosynthesis gene systems, when wheat and rye genomes are combined in a single nuclear. It is suggested that anthocyanin pigmentation of coleoptile and grain pericarp may improve salinity tolerance of wheat seedlings.

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STRESS-INDUCED MUTAGENESIS MAY BE MEDIATED BY ALTERED ACTIVITY OF P450 CYTOCHROME IN MICE

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Zoosocial stressors (among others) present a serious threat because they have considerable negative effects on organisms. It is well known, that stress influences the immune and neuro-, endocrine systems [1]. Stress has been implicated differently in either the course or cause of many diseases, for example: depression, cancer, and different infectious diseases [2]. It is not enough to know of stress effects and its causes, but besides it is necessary to understand all of its negative consequences. It is also important to take into account an increasing frequency of stresses in human life. Model organisms may be very helpful for research of mutagenic effects of different stressors. Some of the data implies that the stress condition has a mutagenic effect. It was shown that several olfactory stressors increase level of mitotic disturbances (MD) including chromosome aberrations in mouse bone marrow cells [3]. The mechanism of this effect in mouse bone marrow cells after pheromonal treatment is unknown. We suppose that stressors may cause changes in liver P450 activity resulting in more effective metabolic activation of promutagens or reactive oxygen species production. To check this hypothesis we compared the ability of liver homogenates from stressed by pheromone and intact mice which are not subjected pheromonal treatment to activate 2-aminofluorene (2-AF) using the Ames test [4]. The results indicate that the conversion of 2-AF to mutagenic compounds in the Ames test was higher in the S9 fraction from the livers of stressed mice than in control group of mice. Stressors influence on the level of MD including chromosome aberrations in bone marrow cells was estimated by anaphase-telophase method. The results show that the frequency of MD in mouse bone marrow cells was significantly increased after pheromonal treatment.

Materials and Methods

Three- to four-month-old highly inbred CBA males weighing 21± 1 g were obtained from the Rappolovo laboratory in animal care center of the Russian Academy of Medical Sciences. All mice were kept in groups of five in standard polypropylene cages in the vivarium of the Laboratory of Animal Genetics of the St. Petersburg State University. After a two-week adaptation, part of CBA males were subjected to pheromonal stressor 2,5-dimethylpyrazine (2,5-DMP) in a separate room. For this purpose, a perforated capsule containing an absorbent-cotton tampon was placed above the grating

of each cage, and 1 ml of a 0,01% aqueous solution of 2,5-DMP was applied onto the tampon. This concentration of the pheromone was approximately equal to its natural concentration measured in overcrowded cages with female house mice. Any direct contact with the tested substance other than the olfactory one was excluded. Control animals were subjected to the same procedure except that water was used instead of 2,5-DMP. The liver and bone marrow were fixed after 24 h pheromonal treatment. The ability of the S9 fraction obtained from the livers of stressed and intact mice to activate 2-AF was determined using the Ames test. MD in the dividing bone marrow cells for both groups of animals was measured using the anaphase-telophase assay.

Results and Discussion

Pheromonal treatment increases the ability of liver S9 fraction to convert 2-AF to mutagenic compounds in Ames test. Fig 1. shows the results of a standard experiment in which the conversion of 2-AF to mutagenic compounds by liver S9 fraction from stressed and intact mice was compared.

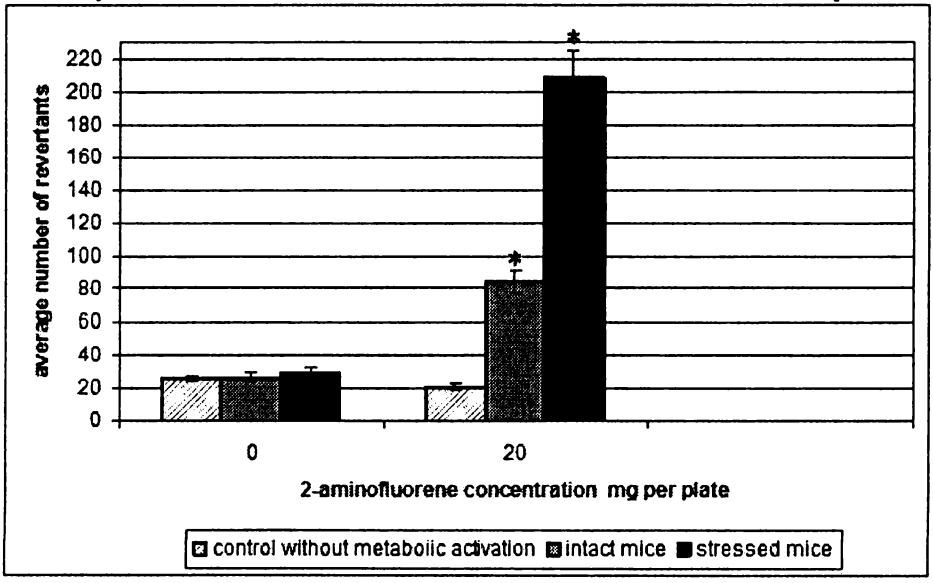

Fig 1. The number of revertants produced per plate in presence of various concentrations of 2-AF and 40 mg/ml of S9 protein for the stressed and intact mice. * - significance of differences between stressed and intact mice was estimated using Mann–Whitney–Wilcoxon test (p=3,187556947578e-005).

The Ames test is generally used as a rapid method to detect chemical carcinogens. In our experiments, we used the Ames test to compare the ability of liver homogenates obtained from stressed by pheromone and intact mice to metabolize 2-AF to mutagenic compounds. The results indicate that the Ames test can be used to compare quantitatively the ability of liver tissue to activate 2-AF. We have found that liver enzymes from intact mice were less active than liver enzymes from stressed mice based on the Ames test.

Anaphase-telophase assay indicates pheromonal treatment significantly increases MD level in mice bone marrow cells. Fig 2 shows stressors influence approximately doubled the frequency of MD in mice bone marrow cells.

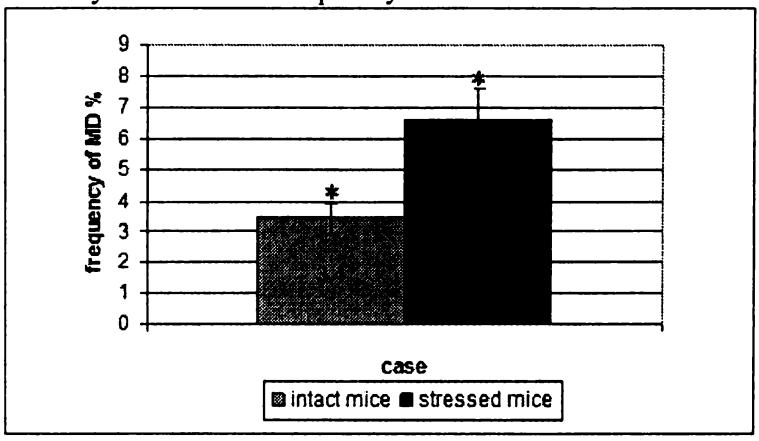

Fig 2. Frequency of MD in mice bone marrow cells after 24 h pheromonal treatment. * - significance of differences between stressed and intact mice was estimated using Fisher test (p=0,0001).

Conclusion

We have shown here that mitotic disturbances in mouse bone marrow cells induced by zoosocial stressors are accompanied by increased activity of liver enzymes. Hypothesis about direct connection between stress, changes in cytochrome P450 enzyme activity and mitotic disturbance to be checked in future.

Acknowledgements

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MARKER CHROMOSOMES IN INFERTILITY PATIENTS

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Supernumerary marker chromosomes (sSMC) are the most complicated cases of cytogenetic investigation because of their structure and heritage. Genetic counseling and prognosis of healthy offspring in families with sSMC include recommendation of preimplantation genetic diagnosis (PGD) [1, 3]. In this study we present the results of investigation of two sSMC cases: identification, gamete distribution and interchromosomal effect in patients with reproductive failure.

Materials and Methods

A patient (aged 31), his wife and his parents were karyotyped in one family and only a patient (aged 40) – in another one. There were two cases of spontaneous miscarriages in the history of the 1st family and primary infertility in the 2nd one. The 1st patient had normozoospermia but the 2nd patient - oligoteratozoospermia (according to the standard WHO criteria).

For chromosome examination, PHA-stimulated peripheral blood cells were used. Spectral karyotyping (SKY) was carried out using the SKY kit (Applied Spectral Imaging, USA). Metaphase chromosome microdissection followed by PCR with degenerate oligonucleotide primer (DOP) and labeling with TAMRA in additional cycles of PCR was performed. Whole chromosome painting (WCP) was made by standard protocol. Fluorescent hybridization *in situ* (FISH) was performed on slides with metaphase chromosomes and from cultivated leucocytes and on slides with fixed spermatozoa with probes Abbott Molecular Inc. We investigated distribution of sSMC in spermatozoa and aneuploidy frequency for chromosomes (4 or 15, 13, 18, 21, X and Y) in sperm and somatic cells using scoring criteria [2].

Results and Discussion

Both patients had sSMC, identified by standard karyotyping. CBG and NOR staining did not allow to define composition of sSMC.

The sSMC of the 1st patient was morphologically smaller than of the 2nd one. SKY and FISH failed to identify the sSMC [4].

Using metaphase chromosome microdissection, followed by FISH with labeled micridissection probe and then WCP identified the sSMC as the chromosome 4 derivate (47,XY,+mar.ish der(4)). It was present in 83% lymphocytes and in 32% spermatozoa. This sSMC had maternal origin (42.5%

mosaicism in his mother). The sSMC of the 2nd patient was morphologically bigger and was identified as chromosome 15 inverted duplication (47,XY,+inv dup(15)(q11)) by SKY technique. Using FISH sSMC was found in 89.5% lymphocytes and in 40% spermatozoa.

We analyzed an interchromosomal effect of both sSMC using 5-colour FISH (chromosomes 13, 18, 21, X and Y) for lymphocytes and spermatozoa. There were no interchromosomal effect of sSMC in the 1st patient. Aneuploidy level of the chromosome 4 was 3.2% in sperm and 1.2% in lymphocytes. We revealed an interchromosomal effect of sSMC in the 2nd patient on disjunction of sex chromosomes: 4.4% somatic and germ cells had X/Y aneuploidy. Aneuploidy level of chromosome 15 was 0.8% in spermatozoa and 3.2% in lymphocytes.

Table 1 Aneuploidy level (%) of chromosomes 4, 13, 15, 18 , 21, X and Y in cultivated lymphocytes and spermatozoa of two patients with sSMC

Chromosome abnormality	**Patient 1**	**Patient 2**	**Chromosome abnormality**	**Patient 1**	**Patient 2**
Monosomy 4	0.4	n/a	Nullisomy 4	0.4	n/a
Trisomy 4	0.8	n/a	Disomy 4	2.8	n/a
Monosomy 13	0.4	0	Disomy 13	0	0.4
Trisomy 13	0	1.2			
Monosomy 15	n/a	0.8	Disomy 15	n/a	0.8
Trisomy 15	n/a	2.4			
Monosomy 18	0.4	0.4	Disomy 18	0.4	0.4
Monosomy 21	0.4	0	Disomy 21	0.8	1.6
Trisomy 21	0.4	0.4			
Monosomy X	0.8	1.6	Disomy X	0.4	0.4
Disomy X	0.8	0.4	Disomy Y	0.8	1.6
Monosomy Y	0	1.2	Disomy XY	0.4	1.2
Disomy Y	0	1.6	Nullisomy XY	0.8	1.2
Total aneuploidy (13, 18, 21, X, Y)	3.6	6.8	Total aneuploidy (13, 18, 21, X, Y)	3.6	6.8
Total sex chromosome aneuploidy	2.4	4.4	Total sex chromosome aneuploidy	2.4	4.4

Conclusion

In both cases sSMC were identified with molecular cytogenetic methods: SKY was sufficient in one case, and microdissection and FISH with labeled

microdissection probe were necessary in another case. The individual interphase FISH strategies were developed for both cases.

We studied gamete distribution for both cases of sSMC that was very important for genetic counseling of the patients.

Distribution of sSMC in gametes appeared to have been approximately equally probable except the frequency of sSMC loss. The latter was found based on somatic cells investigation. Increased aneuploidy level was revealed for chromosomes 15, X, Y in inv dup(15) case and for chromosome 4 in der(4) case.

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RADIOBIOLOGY

MOLECULAR RADIOBIOLOGY OF THE ANIMALS GENES: FROM N.W. TIMOFEEFF-RESSOVSKY TO THE PRESENT DAY

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The most fundamental problems of present-day molecular radiobiology of the higher animals genes, such as nature of radiation-induced heritable gene/point mutations and efficiency of densely ionizing radiation, notably neutrons, in gene/point mutation induction are known to be primarily posed and resolved, to a first approximation in accord with level of genetics of the day, by a classical N. W. Timofeeff-Ressovsky's works performed on *Drosophila* germ cells [1, 2]. At present, in spite of a rapid progress in molecular biology of animals genome and unique genes as well, molecular aspects of mutation induction and processing in germ cells still remain to be solved. As a further evolution of the principal N.W. Timofeeff-Ressovsky's studies which have given the first-priority for Russian school of radiation genetics, a large-scale experiments on induction and molecular analysis of γ-ray- and neutron-induced gene/point mutations at the complex *vestigial* (*vg*) gene of *D. melanogaster* have been carried out. The first results obtained are described below.

Materials and Methods

Random samples of 31 γ-ray- and 11 neutron-induced gene/point (in a classical meaning) *vg* mutations were obtained over the large-scale experiments the physical and biological details as well as the genetics and cytology of these mutations, were described earlier [3]. Here, it should be noted that doses of γ-rays and neutrons used were iso-effective relative to survival of F_1 flies up to imagoes (5-60 Gy for γ-rays ^{60}Co and 2.5-20 Gy for fission neutrons 0.85 MeV, respectively). Genomic DNA were isolated from $vg^x/Df(2R)$ *vg88c28* (a multilocus deficiency uncovering the *vg* gene with adjacent lethal gene-markers) single-locus hemizygotes using Diatom™DNA Prep 100 Kit (Lab. "Isogene", Russia). For PCR-screening and precise location of the mutational DNA lesions over the entire map of the gene *vg* (2R: 49D-E; 15107 bp, 8 exons, 7 introns), its sequence was divided into 14 overlapping fragments. PCR primer pairs for the fragments were designed so that to obtain appropriate products-amplicons (380-2180 bp) in optimized PCR. The products were separated in 1% agarose gels stained with ethidium bromide (0.5 μg/ml) and photographed using a charge-coupled device camera

under UV transillumination .The absence of PCR product for a fragment indicated a partial gene deletion, and all such reactions were repeated.

Results and Discussion

According to the results of PCR- screening, 8 out of 31 (25.8%) γ-ray- and 1 out of 11 (9.1%) neutron-induced *vg* mutants showed no change in the fragment pattern suggesting that the mutational DNA lesions underlying these mutants are enough small to be detected by PCR. Further, 10 out of 23 (43.5%) γ-ray- and 5 out of 10 (50%) neutron-induced *vg* mutants had lost either of gene fragment studied (so-called a "single-site" deletion mutations) (Fig. 1).

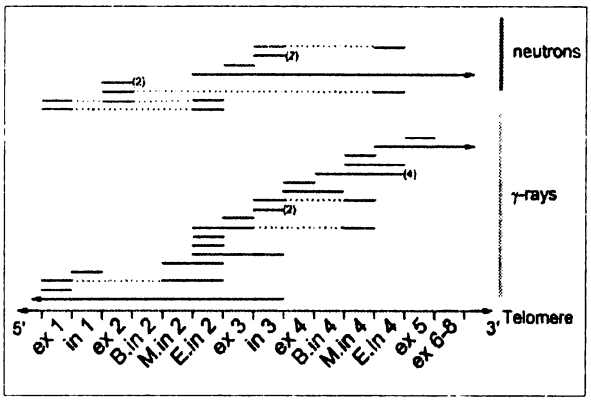

Figure 1. The size and location of the lost gene regions for γ-ray- and neutron-induced *vg* gene/point mutations (in sum for all doses studied). The number in parentheses shows the amount of mutations with the same pattern of PCR. The bottom scale depicts schematically the array of overlapping exonic (ex) and intronic (in) fragments of the *vg* gene studied; B; M; E – Beginning, Middle and End of large 2 and 4 introns.

The ten other γ-ray-induced *vg* mutants had partial deletions of 2-3 adjacent fragments and only one case, as in neutron series, revealed absence of a half of the gene. The rest three (13%) γ-ray- and 4 (40%) neutron-induced *vg* mutants contained two or three independent "single-site" deletions divided by a normal gene sequences (so-called "complex" mutants). Thus, our findings demonstrate that the *vg* gene/point mutants induced by both γ-rays and neutrons may result from the four different types of DNA alterations: (i) micromolecular changes non-detected by PCR; (ii) a "single-site" deletion; (iii) partial deletions of a contiguous gene regions, and (iv) "complex" lesions as a combination of 2-3 independent small partial deletions. Thus, although the mutational spectra are close for both radiation studied, the relationship among the mutational types is quite different for γ-rays and neutrons. In particular, γ-rays are more efficient in induction of point mutations with a "single-site" DNA lesion whereas neutrons induce more frequently the "complex" point mutations based on the clusters of independent DNA lesions. As a whole, the basic mutational alterations underlying both γ-ray- and neutron-induced gene/point *vg* mutations are represented by intragenic partial deletions the size of which vary in extent from single gene fragment to several adjacent fragments. This molecular picture of

radiomutability of the gene in *Drosophila* male germ cell is drastically distinct from that in mammalian or human somatic cells irradiated where a massive partial and total deletions of the gene-reporters are dominant [4].

Conclusion

Just as our quantitative assessment of the mutagenic efficiency of γ-rays and fission neutrons in induction of gene/point *vg* mutations in *Drosophila* sperms has shown a close mutation rates for these radiations (0.3 – 0.6 and 0.7 – 0.9 x 10^{-7} /locus /rad for γ-rays and neutrons, respectively, in range of the doses studied) [3], so described here our qualitative data show a close molecular nature of mutational changes induced by radiations under study among which a small partial deletions are the prevailing type of DNA alterations detected by PCR. These findings are somewhat unexpected in the light of current concept [4] that high-LET radiations, including neutrons, not only are more effective in mutation induction but also induce more large-scale deletions than low-LET radiation. In this connection, it is felt that the LET may not be the only factor determining the mutation spectrum but other conditions such as track structure or the cell type, genome state, the size of the gene-target and its position on the chromosome (in the interior of genome) may also play a role.

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STUDY OF THE INFRARED LIGHT (850 NM), MODULATED BY 101 HZ, ACTION ON THE CROSS ADAPTIVE RESPONSE INDUCTION AND THE REACTIVE OXYGEN SPECIES PRODUCTION IN MICE *IN VIVO*

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In recent decades, great interest among researchers is the phenomenon of radiation adaptive response (AR) [1]. Its essence lies in the fact that prior exposure to low doses leads to increased stability of the object to the late effects of radiation in large doses of damaging. It is known that reactive oxygen species (ROS) are involved in the induction of AR, they activate a signal transduction process and specific gene expression. The phenomenon, when adapting and challenging exposures are factors of different nature, is called the cross AR. The cross AR is seen as a form of defense the organism against the mutagenic effect caused not only by ionizing radiation, but chemical agents and is probably the most important biological reserves increase resistance to adverse environmental influences. Therefore, we think that the problem of finding adaptogens are able, as small doses of radiation to transfer the body to the adapted state, is actual. At the present time in medical practice there are many different devices, whose action is based on the use of infrared light (IRL) for the treatment of inflammatory diseases. The aim of this work was to study the magnitude and dynamics of induction of the cross AR under the action of IRL in bone marrow and blood of mice in vivo.

Materials and Methods

White mongrel SHK male mice 22-24 g (8 weeks of age) were used. IRL irradiation at a wavelength of 850 nm, modulated by a frequency 101 Hz, was performed using a light therapy device with mode of power of 22 mW/cm^2. Irradiation of mice with X-rays was performed on RUM device with a voltage of 200 kV at a dose rate 1 Gy/min. The production of ROS in blood cells was estimated by luminol-dependent chemiluminescence (ChL). For the ChL measurements we used a CHEMILUM-2001 device. ChL was measured at 37°C sequentially from 12 mini-dishes (V= 200 μl). The serial measuring time was 2.5 sec. Registration ChL from intact and treated cells was made in parallel probs.

To determine the level of polychromatophil erythrocytes (PCEs) with micronuclei (MN), animals were killed and prepared cytologic preparations of bone marrow. Each experimental point was obtained using no less than five mice and analyzing no less than 2000 PCEs. We have previously shown that irradiation of mice IRL did not affect on the level of spontaneous cytogenetic damage in bone marrow cells. Also, experiments were conducted to determine

the radiosensitivity of a dose of 1.5 Gy in depilated and undepilated mice. The value of AR in depilated and undepilated was the same and equal to the value of radiation AR, obtained by irradiation of mice by the standard scheme of AR (0.1 Gy + 1.5 Gy), so all further experiments were carried out on undepilated mice. The minimum time of exposure to IRL required for the induction of cross AR was equal a single irradiation for 10 min [2].

Results and Discussion

Fig. 1. presents data of the dependence of the yields of PCEs with MN on the interval between the adapting irradiation with IRL (a) and the X-rays (b) and challenging treatment with X-rays at a dose of 1.5 Gy. It was found that the decrease of cytogenetic damage as after the adapting exposure IRL, and after

adapting exposure of a dose of 0.1 Gy X-rays is observed not earlier than 5 h and remained at this level up to 2 months that is observed AR. In mice irradiated with IRL 0.5 h before the exposure to X-rays by the scheme used in studies of radiation protectors, no decrease in the number of PCEs with MN was found (1.5 Gy – 7.44±0.53; IRL + 0.5 h later 1.5 Gy – 6.7±0.45).

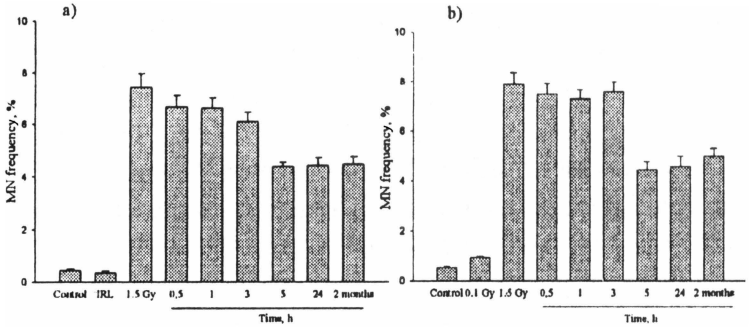

Fig. 1. Dependence of the yields of PCEs with MN in bone marrow on the interval between the adapting irradiation with IRL (a) and the X-rays (b) and challenging treatment with X-rays at a dose of 1.5 Gv

To compare the mechanisms of induction of AR under the action of IRL and X-rays in mice simultaneously with the measurement of cytogenetic damage in bone marrow was evaluated level of ROS production in blood. Fig. 2. presents data of the dependence of the level of ROS production in blood cells on time between the adapting irradiation with IRL (a) and the X-rays (b) and challenging treatment with X-rays at a dose of 1.5 Gy. It was found that the decrease in the level of ROS production to control values as after the adapting exposure of IRL, and after adapting exposure of X-rays irradiation is observed after 5 h, as well as the value of cytogenetic damage remains at this level up to 2 months. A similar dynamics of the induction of AR was observed on various objects with low-dose γ-radiation as an adaptive agent [3].

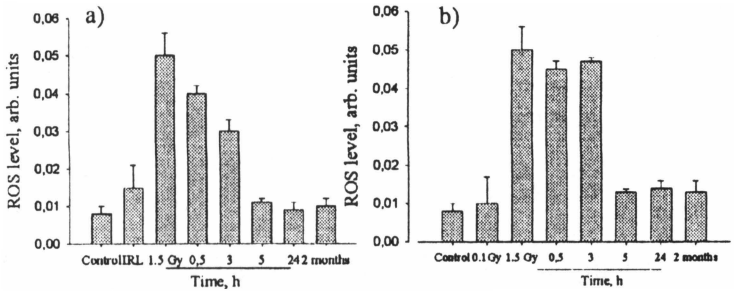

Fig. 2. Dependence of the level of ROS production in blood cells on time between the adapting irradiation with IRL (a) and the X-rays (b) and challenging treatment with X-rays at a dose of 1.5 Gy

Study of dependence of the level of ROS production in blood cells of mice with time after exposure IRL and 0.1 Gy X-rays showed that maximum level of spontaneous ROS production in both cases, 0.5 h after irradiation and gradually decreased to control values by 5 h, i.e. to date, which revealed the cross AR and the radiation AR on the cytogenetic test in bone marrow (Fig. 3).

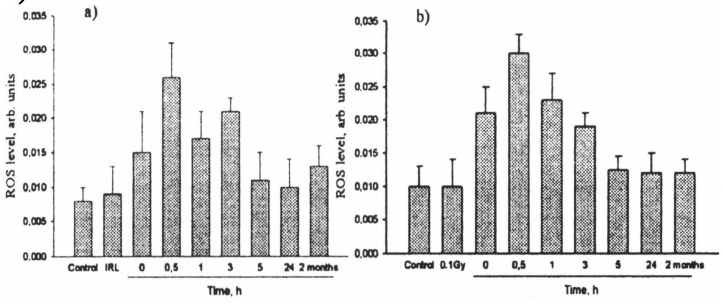

Fig. 3. Dependence of the level of ROS production in blood cells of mice on time after IRL treatment (a) and X-rays treatment (b) at a dose of 0.1 Gy

Conclusion

The experiments performed showed that the irradiation of mice with IRL (850 nm) modulated by a frequency of 101 Hz induces a cross AR, which can be identified as a cytogenetic and ChL methods. The cross AR did not differ in magnitude and dynamics from the radiation-induced AR and stored up to 2 months. The results also demonstrate that the level of ROS production plays a major role in the early stages of AR on mice. As a cross and radiation AR revealed only 5 h after adapting influences, when the level of ROS production was reduced to control value. The data obtained may indicate that the induction of the cross AR by IRL and radiation AR by X-rays in mice *in vivo* occurs by a similar mechanism.

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BYSTANDER EFFECT BETWEEN IRRADIATED AND NOT IRRADIATED WHEAT SEEDS

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The phenomenon of allelopathy among plants when some species can make depressing influence on the growth of others by escaped chemical substances is well known [1, 2]. It has been found that injured seeds within one species can have an allopathic activity [2].

Ionizing radiation as an injured factor has some advantages compared to thermal and mechanical influence because it is dosed precisely enough and disorders which it causes on plant objects are well explored.

Materials and Methods

Researches were performed on the air-dry soft winter-annual wheat seeds of Moscovscaya 39 sort. The next groups of seeds were used in the experiments: control (intact seeds, not exposed to any influences and stored separately) irradiated and contacted (intact, not irradiated but stored with irradiated seeds).

The group of irradiated seeds were exposed to gamma-irradiation of ^{60}Co on the irradiation installation "Researcher", using doses of 200, 400, 600 Gy, with the capacity of 50 Gy/min. All groups of seeds were in filter paper bags (100 seeds in each bag). The influence of irradiated seeds on intact and not irradiated seeds (the group of contact seeds) was simulated. The bag with intact seeds was put in immediate contact with the bag of irradiated seeds in to the cardboard box. Sprouting took place in Petri dishes with the optimal moisture and temperature of 19-21 °C, sand was used as substrate. These experiences were held four times.

The influence of irradiated seeds on not irradiated ones was determined by the sprouting energy, laboratorial germination and intensity of initial growth. The results were presented in arithmetic average and its average error. The reliability of differences were estimated using the Student test.

Results and Discussion

These researches showed that the influence of ionizing radiation on air-dry wheat Moscovscaya 39 seeds reduces their growth parameters. A month after irradiation the length of a plantlet and a root was reduced to a considerable extent and it depended on the dose of irradiation. Sprouting energy and germination were disturbed less. Further diminution of the length of plantlets and roots of the irradiated seeds, was observed when the period of storage

increased up to three months (table 1). At the same time the energy of sprouting and their germination didn't differ from the control.

Table 1. The initial growing processes of wheat seeds gamma-irradiated in different doses and their influence on not irradiated seeds in three months of the joint storage.

Variants of the experience	Sprouting energy, %	Germination, %	Plantlet length, cm	Root length, cm
Control	91.0±1.0	83.0±1.2	10.48±0.21	8.59±0.21
Irradiated dose 200 Gy				
Irradiated	75.0±7.6	69.0±8.9	4.70±0.26*	3.86±0.17*
Contacted	83.0±6.5	91.0±1.85*	9.75±0.29*	7.70±0.24*
Irradiated dose 400 Gy				
Irradiated	90.0±3.6	82.0±4.1	1.90±0.04*	1.96±0.06*
Contacted	82.0±7.9	86.0±4.9	8.28±0.30*	6.73±0.23*
Irradiated dose 600 Gy				
Irradiated	83.0±8.5	80.0±2.8	1.65±0.03*	1.35±0.05*
Contacted	79.0±9.7	88.0±5.6	8.13±0.16*	7.10±0.15*

Remark: * - reliable difference from the control of Student test

The main object of our research were the seeds contacted with irradiated ones. These seeds also had changes in the indices of growth but they had another character (table 1). So the length of roots after joint storage with irradiated seeds during a month didn't reduce in comparison with the seeds which were irradiated. It increased for a fact (in control – 7.43±0.17 cm and 8.85±0.28 cm and 8.69±0.23 cm – for seeds contacted with irradiated ones with the dose of 200 Gy and 400 Gy). The length of seed plantlets contacted with irradiated by the dose of 600 Gy was on the contrary reduced (contacted seeds – 9.01±0.26 cm, control – 10.76±0.22 cm). Such indices as sprouting energy and germination in the groups of contacted wheat seeds didn't differ from the control.

Increasing of length of the joint storage of contacted seeds together with irradiated up to three months led to the diminution of plantlets and roots length (table 1). Sprouting energy of seeds contacted with irradiated ones had been reducing by that term unlike the damages of plantlets and roots length.

Thus, the results of these investigations affirm that joint storage of irradiated wheat seeds with intact ones lead to essential changes of growth indices of the last ones. It is caused by the discharge of volatile substances (ethylene and the others) of seeds injured by ionizing irradiation. Besides, it depends on time of joint storage and irradiation doses of the seeds.

Conclusion

So it is supposed that the influence on the growth indices of contacted seeds depends on metabolism state of irradiated seeds because the stimulating effect decreases and it is replaced by the inhibiting one when the dose of irradiation increases. At the same time the inhibiting effect doesn't have precise dependence on an irradiated dose. The effect presented in this work corresponds to strengthening principle formulated by N.W. Timofeeff-Ressovsky [3].

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ECOLOGICALLY UNSUCCESSFUL COMPLEX FACTORS' INFLUENCE OF ENVIRONMENT ON HEALTH CONDITIONS AMONG INFANTILES ON THE TERRITORIES OF BRYANSK REGION

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Second half of 20-th century has brought continuously growing quantity of artificial technogenic radioactive substances into environment, they are new, because of its ecosystem character (Aleksahin R.M, Akimova T.A, Kuzmin A.P, Haskin V.V., etc., 2001) and because of its combined technogenic toxic environmental factors (Pivovarov J.P., Kirillov V.F, Korolik V.V., Mikhalev V.P, 2001). The number of territories where dose's power of radioactive substances' radiation in ten times outnumbers its backgrounds, that existed in preatomic epoch and variations that are inherent to modern environment with combined radioactive toxic influences, which are increasingly raising (Pivovarov J.P., Mikhalev V.P, 2004). At the same time, in spite of the fact that geography distribution of radiation pollution in Ukraine, Belarus and Russia as a result of the Chernobyl catastrophe, consequences' research of the Chernobyl catastrophe is still considered without tehnogenno-toxic influences, their intensity and inevitable distinctions in such situations of people's reactions, on the change of structure of environment (Pivovarov J.P., Mikhalev V.P, 2009).

More over, Bryansk region, as a result of the Chernobyl Catastrophe, has become the unique territory, as because it has new territories, that where unknown before with combined radioactive toxic and radioactive isolated components when the dozes were equal and also because this region has highly radioactive polluted Sothern-western territories (Mikhalev V.P, 2001). Studying of consequences of sharp environmental pollution diversity, the level of forming population's health, living in such conditions is very important and necessary for predicting the efficiency of technogenic toxic investments into people's reaction on radioactive pollution, as a result of the Chernobyl Catastrophe (Pivovarov J.P., Mikhalev V.P, Kirillov V.F, Korolik V.V., Jampolsky J.A., 2001, 2004, Bylatcheva M.B., 2005, Tsyganovsky A.M, 2009).

Materials and Methods

We've made an integral ecological hygienic assessment of environmental conditions in all areas of Bryansk region over a decade (from 1998 till 2007) by its radiation (as the result of the Chernobyl catastrophe), toxic-chemical (as the result of industrial emissions accumulation) and combined radioactive-toxic components. Correlations of environmental

changes with primary morbidity of infantile population are analyzed (from 1998 till 2007). Radioactive pollution density of the territories by ^{137}Cs is from 18,5 kBc/m^2 to 2149,7 kBc/m^2. The total technogenic pollution density (kg/person/year) by fluid and gas toxicants is from 1,1 to 149,9 kg/person/year, from 0,1 to 6,6 out of them are carcinogen 3,4 benzapyrene, from 0,1 to 60,5 – nitrogen oxides, from 0,1 to 28,0 – sulfur dioxide, from 0,7 to 57,8 – carbon oxide.

We pointed out the following territories (table 1): 1) the territories with low level of radioactive pollution density (ecologically successful); 2) the territories with high level of radioactive pollution and with low technogenic toxic pollution density(radioactive isolated) 3); the territories with low level of radioactive and high level of technogenic toxic pollution density (toxic); 4) the territories with high level of radioactive and technogenic toxic pollution density (combined radioactive toxic).

Results

1. We have made analysis of environmental structure and it indicates sharp variety of primary infantile morbidity in ecologically successful, radioactive-isolated, toxic-chemical and radioactive-toxic regions that prove the rightfulness of our assessment.

2. Primary infantile morbidity level in ecologically successful areas of Bryansk region is lower in comparison with All-Russian morbidity on 35,5%, in toxic-chemical regions of Russian Federation, that are inherent mostly to our country, is lower in comparison with All-Russian morbidity on 17,7%, in combined radioactive- toxic it`s higher in comparison with All-Russian morbidity on 28,0%, that indicates the growth of infantile morbidity in radioactive regions, especially when we include in this structure background technogenic-toxic metabolites (3,4 benzaphyrene, nitrogen oxides, sulfur dioxide) .

3. At the same time correlation link with radioactive pollution density by ^{137}Cs on radioactive-isolated territories has no any influences, achieving imprudent (r=-0,12) negative meanings in comparison with radioactive – toxic regions, where we can observe sharp link of infantile morbidity with sulfur dioxide (r=0,99), as well as with radioactive pollution density by ^{137}Cs (r=0,56).

4. It`s also observing correlation link of main toxic substances with primary infantile morbidity in toxic regions, comprising 0,66 by 3,4 benzaphyrene, 0,95 by nitrogen oxides and sulfur dioxide, that confirm negative influence of toxic environmental substances on infantile organisms.

Tabl. 1. Territories' divisions of Bryansk region according to technogenic toxic and radioactive environmental pollution

№	Territory	Toxic substances (1998-2007)			Pollution density, ^{137}Cs, (кВс/м^2)	Primary infantile morbidity (1998-2007) n·1000/year
		3,4 ben zaphyrene	Sulfur dioxide	Nitrogen oxides		
		Average annual loadings on person, kg/person/year				
I.	**Territories with low degree of radioactive and pollution density**					
	Pogarsky	0,0	0,0	0,4	37,0	1216,0
	Pochepsky	0,0	0,0	0,4	37,0	906,0
	Rognedinsky	0,0	0,0	0,4	37,0	926,2
	Sevsky	0,0	1,0	0,5	37,0	756,0
	Mglinsky	0,0	0,1	0,3	37,0	554,7
	Suzemsky	0,0	0,2	0,7	37,0	815,7
	Surashsky	0,0	0,2	1,0	37,0	781,8
	Kletnyansky	0,0	0,4	0,3	40,7	736,0
	Starodubsky	0,0	0,0	0,6	151,7	1249,9
	Komarichsky	0,0	0,5	0,6	88,8	950,6
	Dubrovsky	0,0	0,1	0,9	77,7	940,7
	Zhiryatinsky	0,0	0,0	0,7	77,7	485,4
	Trubchevsky	0,0	0,1	0,4	55,5	1436,6
	Average		0,20	0,55	57,8	904,3±60,15
	Corr. koef.		r_2=-0,23	r_3=0,34	r_4=0,32	-498,4*
II.	**Territories with average degree of radioactive density and low degree of toxic pollution**					
	Krasnogorsky	0,0	0,1	0,3	2149,7	1544,3
	Gordeevsky	0,0	0,0	0,7	917,6	1906,9
	Klimovsky	0,0	0,0	0,2	362,6	1502,5
	Average		0,03	0,40	1143,3	1651,2±90,2
	Corr. koef		r_2=-0,42	r_3=0,99	r_4=-0,12	+248,5*
III.	**Territories with high degree of radioactive density and low degree of toxic pollution**					
	Bryansky	0,4	2,0	5,6	18,5	1153,8
	Vygonichsky	0,1	0,0	0,6	18,5	1068,0
	Karachevsky	0,2	0,1	0,8	40,7	1241,3
	Navlinsky	1,0	1,0	1,0	37,0	1242,2
	Brasovsky	0,8	1,4	1,2	88,8	1007,2
	Dyatkovsky	1,3	28,0	60,5	77,7	1892,4
	Zhukovsky	0,4	0,1	0,8	77,7	1025,0
	Unechsky	0,1	0,5	1,0	55,5	1169,2
	Average	0,54	4,14	8,94	51,8	1224,9±68,1
	Corr. koef	r_1=0,66	r_2=0,95	r_3=0,95	r_4=0,20	-177,8*
IV.	**Territories with low degree of radioactive density and high degree of toxic pollution**					
	Klinsovsky	0,2	0,1	1,3	684,5	1179,5
	Novozybkovsky	0,1	1,0	1,0	717,8	2101,7
	Zlwnkovsky	0,2	1,2	0,4	1135,9	2106,2
	Average	0,17	0,76	0,90	846,1	1795,8±90,2
	Corr. koef	r_1=-0,5	r_2=0,99	r_3=-0,76	r_4=0,56	+393,1*

Note* – difference between primary infantile morbidity of Bryansk region and All-Russian morbidity (1998-2007).

UNUSUALLY THE SAME γ-RAY- AND NEUTRON-INDUCED MOLECULAR CHANGES REVEALED BY PCR AT THE *DROSOPHILA BLACK* GENE

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The priority N.W. Timofeeff-Ressovsky's works on *Drosophila* germ cells aimed to study the nature of heritable gene/point mutations induced by low-LET radiation [1], on the one hand, and to assess the efficiency of neutrons as high-LET radiation in gene/point mutation induction [2], on the other hand, were primarily responsible for the development of the most fundamental and, at the same time, applied fields not only in a classical radiation genetics of animal genes, but in the present-day molecular radiobiology of eukaryotic genes as well [3]. Unfortunately, replacing the animal germ cells with somatic cells in mutation researches, as it had been happened in the latter half of the 20th century, has confined our knowledge in these fields by data for some suitable gene-reporters in animal somatic cells whereas the molecular bases of radiomutability of genes in animal germ cells are remained to be solved.

On this basis and as the advancement of N.W. Timofeeff-Ressovsky's works on *Drosophila* germ cells within the framework of these fields, the large-scale project aimed to study the dependence of molecular picture of gene radiomutability on varying factors such as the size of the gene, its exon-intron organization and position on chromosome in condition of action of different quality radiation was conducted using 5-locus experiments. The first result of PCR-assay of γ-ray- and neutron-induced gene/point mutations at *Drosophila black* (b) gene, as a mini-target, are presented below.

Materials and Methods

A random sets of 17 γ-ray- and 13 neutron-induced gene/point (in a classical meaning) *b* mutations were obtained simultaneously with the *vestigial* gene/ point mutations at the same large-scale experiments details of which were described earlier [4]. Here, it is essential to point out that doses of γ-rays (5-60 Gy ^{60}Co) and fission neutrons 0, 85 MeV (2,5 -20 Gy) employed were iso-effective with relation to the survival of F_1 flies up to imagoes. Genomic DNA were isolated from viable b^x/ b^x homozygotes or from b^y/Df *b*81*l*42 single-locus hemizygotes, if b^y/ b^y homozygotes were sterile or lethal associated with independent point mutations elsewhere at the same chromosome, using Diatom ^{TM} DNA Prep 100 Kit (Lab. "Isogene", Russia). For PCR-analysis, the sequence of the *b* gene (2L: 34D-E; 2692 bp., 3 exons, 2 introns) was subdivided into 3 overlapping fragments to which the unique PCR praimer pairs were designed to obtain relevant products-amplicons (1068, 1063 and

859bp). PCR-products were separated in 1% agarose gels stained with ethidium bromide (0,5 mg/ml) and photographed using a charge-coupled device camera under UV transillumination. The absence of PCR product for a fragment was considered as a partial deletion of the gene and all such negative reactions were repeated.

Results and Discussion

As the combined (for all doses studied) results of PCR-assay shown, 12 out of 17 (70,6%) γ-ray- and 12 out of 13 (92,3%) neutron-induced *b* gene/point mutants showed no change in the fragment pattern which is peculiar for the wild-type *b* gene testifying that DNA alterations underlying these mutants are sufficiently small for detection by PCR. Five out of 17(29,4%) γ-ray-induced *b* mutations had lost either of gene fragment (so-called a "single-site" deletion mutations). These deletions involved 5′- fragment (one case after 20 Gy), a middle fragment (two cases after 5 Gy and a case after 40 Gy), or 3′-fragment (one case after 10 Gy) (Fig1).

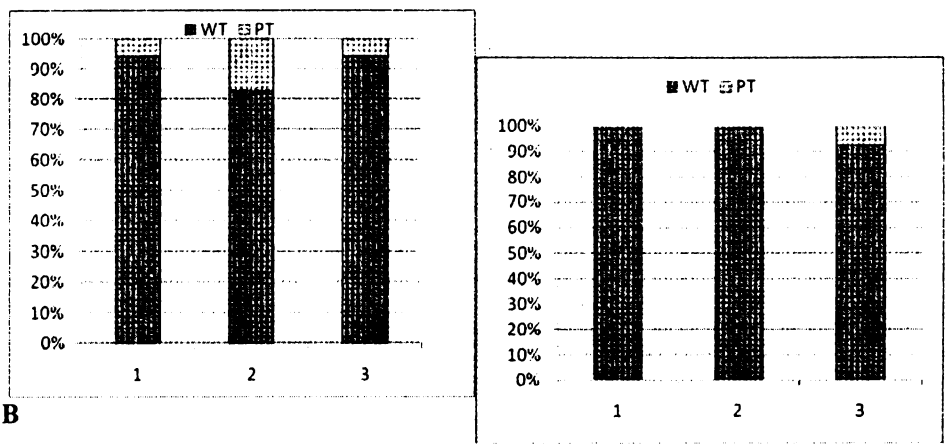

Figure 1. A share of the *b* wild-type PCR fragment pattern (WT) and with partial deletion (PT) of either fragment (1, 2 or 3) after γ- (A) and neutron (B) irradiation.

In neutron series, only one case of loss of 3′-fragment (after 10 Gy) was established. Thus, the principal mutational changes underlying both γ-ray-and neutron-induced gene/point *b* mutations in *Drosophila* male germ cells (sperms) are represented by micromolecular DNA lesions not detected by PCR. Such unusual molecular picture of radiomutability of mini-gene *b* under study in *Drosophila* is drastically differed from that for a small gene-target *aprt* (about 3 kbp) in human cells where a massive and total deletions of the gene are dominant [5].

Conclusions

A predominance of micromolecular DNA alterations not detected by PCR in a full spectra of mutational changes at the *b* gene after action of both γ-rays and neutrons is quite unexpected finding of our studies particularly when taken into account a wide spread notion that neutrons as a high-LET radiation are more effective in induction of massive and total gene deletions than low-LET radiation. The equal efficacy of γ-rays and neutrons in induction largely of a micromolecular DNA changes within a small gene-target in germ cells might be explained within the framework of the theory of track structure (action of δ-electrons) and/ or of the highly specific structural organization of the gene and genome as a whole in animal germ cells in comparison with that in animal somatic cells.

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ROLE OF GENES IN DNA DAMAGE RECOGNITION AND REPAIR IN *DROSOPHILA MELANOGASTER* LIFE SPAN REGULATION IN RESPONSE TO LOW DOSE IRRADIATIONS

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During lifetime organisms primarily deal with the action of chronic low dose factors, in particular, with ionizing radiation. As a rule, the effects caused by the influence of such environmental factors have nonlinear character, resulting from the interaction of damage genesis and accumulation processes, and resisting mechanisms of stress response. To assess the mechanisms of the complex influence of factors on living organisms it is necessary to investigate the dynamics of such an integrated parameter as life span. It depends on many factors, including behavior characteristics of cellular and physiological processes, In particular, the system of DNA damage recognition and repair is of great importance in this context.

This research work is aimed at investigating the life span changes at *Drosophila melanogaster* individuals with defects or overexpression of genes of DNA damage recognition and repair.

Materials and Methods

Drosophila melanogaster strains. The following laboratory strains of *Drosophila melanogaster* were used: *Canton-S* as a wild type strain; *mei-9/mei-9* (genotype: w^1 ,*mei-9*^{A1}/w^1,*mei-9*^{A1}) and *mei-9/+* (genotype: *mei-9*^{a},*sw*$^{20\text{-}14}$/*FM7c,ct*^{S}) with the mutation of homolog of mammalian excision repair *XPF* gene [1]; *mei-41/mei-41* (genotype: *w,mei-41*^{D5}/*w,mei-41*^{D5}) and *mei-41/+* (genotype: w^-,*mei-41*^{D5}/*Basc;cn*^{1},*bw1*) with the mutation of homolog of gene of protein kinase ATR which is responsible for DNA damages recognition [2]; *p53/p53* (genotype: y^1 w^{1118};*p53*$^{5A\text{-}1\text{-}4}$/y^1 w^{1118};*p53*$^{5A\text{-}1\text{-}4}$) with defect of gene of transcription factor p53 which regulate cell cycle and activate apoptosis in response to DNA damage [3] (were kindly provided by Bloomington Stock Center, USA). To study the influence of DNA damage repair gene overexpression on *Drosophila* resistance to action of chronic radiation the following laboratory strains were used: *UAS-D-GADD45* contains the additional copy of *D-GADD45* gene under UAS promotor control (was kindly provided by Dr. Uri Abdu, Ben-Gurion University, Israel); *GAL4[1407]* (genotype: *w*;P{GawB}1407/ w*;P{GawB}1407*) with the GAL4 driver in *Drosophila* nervous system (was kindly provided by Bloomington Stock Center, the USA). For *D-GADD45* overexpression in *Drosophila* nervous system *UAS-D-GADD45* females had been crossed with *GAL4[1407]* males.

Experimental conditions. Researches were performed in the same conditions (the 12-hour light regime, temperature of 25 °C, the agar/semolina/sugar/yeast medium). Flies of each genotype were divided into control and exposed groups. Exposed individuals were chronically irradiated by the gamma emitter with Ra^{226} in a dose of 60 cGy for generation on preimaginal development stages.

Life span assay. 150-250 flies were selected for each variant. Males and females were studied separately. Mean, median, maximum life span and other parameters were estimated. Non-parametric Gehan-Breslow-Wilcoxon and Kolmogorov-Smirnov tests were used to estimate the reliability of differences between samples.

Results and Discussion

Influence of chronic low dose gamma irradiations on life span of Drosophila melanogaster mutants by genes of DNA damage recognition and repair. The median life span of *Canton-S* wild type strain increased after chronic influence by low doses of ionizing radiation both at males and females (by 10-27 %) ($p<0.001$).

In all experimental variants life span of *Drosophila melanogaster mei-9, mei-41* and *p53* mutants decreased (by 4-38 %) ($p<0.001$) after irradiation. These genes determine the processes of sensing and repair of DNA damages caused by influence of environmental and physiological factors including gamma irradiations. Therefore mutations of these genes disturb the functioning of DNA damage recognition and repair systems and inhibit the appropriate response of live systems to the action of a damaging agent. As a result we observed the decrease of anomals' life span under the conditions of chronic low dose gamma irradiations.

Change of life span of Drosophila melanogaster individuals with D-GADD45 overexpression after chronic low dose influence of ionizing radiation. Overexpression of *D-GADD45* gene leads to life extension of *Drosophila melanogaster* individuals. Median life span at males with *D-GADD45* overexpression in comparison with median life span of males of parental lines increased by 73-77 % ($p<0.001$), at females – by 22-46 % ($p<0.001$). GADD45 proteins determine the organism reaction on environmental and phisiological influences and are involved in the processes of damaged DNA molecules recognition, excision repair of nucleotides and bases, cell cycle arrest and apoptosis in response to DNA damages [4, 5]. *D-GADD45* overexpression in *Drosophila* nervous system apparently had led to a more efficient recognition and elimination of DNA damages and revealed itself as life extension.

Under conditions of chronic irradiation life span of *Drosophila melanogaster* individuals with *D-GADD45* overexpression remained 17-37 % ($p<0.001$) higher compared to the life span of individuals of parental lines.

Thus, it is shown that *D-GADD45* overexpression in the nervous system extends the life of *Drosophila* individuals both under control conditions and in the conditions of chronic low dose gamma irradiation.

Conclusions

It has been shown that *Drosophila melanogaster* individuals with defects of genes of DNA damage recognition and repair (in particular *mei-9, mei-41* and *p53*) have the less life span and the hypersensitivity to chronic influence of low dose ionizing radiation in comparison to *Canton-S* wild type strain. *D-GADD45* overexpression leads to life extension of *Drosophila melanogaster* individuals compared to individuals of the parental lines, which is preserved under conditions of chronic irradiation and is an evidence of their better adaptation to the influence of spontaneous and induced environmental factors. Thus, the obtained experimental data demonstrate the role of mechanisms of DNA damage recognition and repair in the life span regulation and determination of the response to the influence of chronic low dose gamma irradiation.

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LOW DOSES OF DIFFERENT PHYSICAL AND CHEMICAL AGENTS INDUCE THE CYTOGENETIC ADAPTIVE RESPONSE AND DECREASE THE GROWTH OF SOLID TUMOR IN MICE IN VIVO

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Abundant data on the mechanisms and regularities of high dosed ionizing radiation action on living objects that have been accumulated in radiation biology are insufficient to explain some biological effects of low doses such as hypersensitivity, adaptive response (AR), genomic instability in offspring, bystander effect and hormesis. The phenomenon of radiation-induced AR has aroused considerable interest among specialists concerned with the problems of radiobiology, cancer radiotherapy and ecology and considered as the form of the biological defense of the cells against the mutagenic action of oxidative stress induced not only by ionizing radiation and chemical agents but also by somatic diseases. Moreover, there is substantial evidence for the occurrence of cross-adaptive response when the adaptive and challenging exposures are the factors of different nature. Probably, the phenomenon of adaptive response is one of the possible manifestations of radiation hormesis.

Materials and Methods

Two-month-old white mongrel SHK male mice were used and were kept under standard conditions. For the induction of AR, the animals were *subjected to adaptive treatments with doses of 0.1 Gy of X-radiation, 0.16 Gy* (0.43 cGy/day) of chronic high-LET radiation in the field behind the upper concrete shield of the Serpukhov accelerator of 70 GeV protons, infrared light by IRL device at wavelength 850 nm modulated by a frequency of 101 Hz with mode of power 22 mW/cm^2 and 100 μM hydrogen peroxide (i/v), and then were additionally irradiated with a challenging dose of 1.5 Gy of X-rays performed on RUM device with a voltage of 200 kV at a dose rate of 1 Gy/min. For determining the level of cytogenetic damage, mice were euthanized 28 h after the challenging irradiation by the cervical dislocation method, and bone marrow specimens for calculating micronuclei (MN) in polychromatophil erythrocytes (PCEs) were prepared by a conventional method. The influence of the adaptive treatments on the growth of solid tumor of Ehrlich ascite carcinoma was estimated by measuring the size of the tumor at different times after the inoculation of ascitic cells s.c. into the femur.

Results and Discussion

Fig. 1 shows the results of mice treatment with low dose of X-radiation. It is seen that in animals irradiated with X-rays initial dose of 0.1 Gy, after not less than 5 hours the yield of micronucleated PCE after subsequent irradiation with X-rays challenging dose of 1.5 Gy was lower than in animals that were not subjected to the initial irradiation; so, a radiation-induced AR was observed, with consequent maintenance up to 17 months.

The results of study of the Ehrlich carcinoma growth in mice irradiated with the X-rays dose of 0.1 Gy are shown in Fig. 2. It was found that the mean size of the tumor in males irradiated with dose of 0.1 Gy was decreased as compared to unirradiated males.

Fig. 3 presents the data on determining whether IRL can induce AR in mice. It can be observed that the preliminary exposure of mice with IRL led to a considerable decrease in the level of cytogenetic damage compared with mice exposed only to a dose of 1.5 Gy, indicating the induction of cross-adaptive response, which was also occurred not earlier than after 5 hours, with consequent maintenance for more than 2 months. In mice that received a dose of 1.5 Gy after sham-irradiation with IRL, the level of cytogenetic damage was not differ from that in mice irradiated with a dose of 1.5 Gy alone. It should be noted that the level of cytogenetic damage in animals exposed to IRL and in sham-irradiated animals does not differ from the level of spontaneous lesions. So, IRL-induced AR is not differed in magnitude and dynamics from the X-radiation-induced AR.

It can be seen from Fig. 4, a statistically significant suppression of cumulative tumor incidences up to 24 days after ascitic cells injection was observed in the group irradiated with IRL mice as compared to unirradiated males.

On the next step were performed the analysis of combined action of hydrogen peroxide and the dose of 1.5 Gy X-rays by the scheme of radiation AR. It can be seen from Fig. 5, in preliminary treated animals the level of cytogenetic damage induced by the challenging X-rays was decreased, so the cross adaptive response was occurred. It appeared that the rate of protection from H_2O_2 administration did not differ from that in mice X-rays- and IRL-irradiated males. In this case CAR induction was also observed not earlier than 5h after the adaptation; also it was found that CAR persists over 17 months, which is similar to our previous observations in mice irradiated with low doses of X-rays and IRL.

The influence of mice treatment with hydrogen peroxide on the growth of tumor is presented in Fig. 6. On this data shown that the suppression of tumor incidence was also observed in males that were subjected to adaptive treatment with hydrogen peroxide as compared to unirradiated males.

The results presented in Fig. 7 indicate that, in contrast to all examined adapting agents, the exposure of mice to the dose of 0.16 Gy of high-LET radiation induced no AR.

Fig. 8 shows the results of Ehrlich carcinoma growth study in mice irradiated with the dose of 0.16 Gy of high-LET radiation. It can be seen that the mean size of the tumor in males irradiated with dose of 0.16 Gy was increased as compared to unirradiated males.

Conclusion

The results of the experiments indicate that the regularities of the formation of CAR induced by treatment of mice with low-dose of IRL and hydrogen peroxide do not differ from those of RAR induced by low doses of γ-rays and X-radiation. This fact leads to the assumption that these responses are formed by common mechanisms, opening the new possibilities for the enhancement of protective responses of the organism.

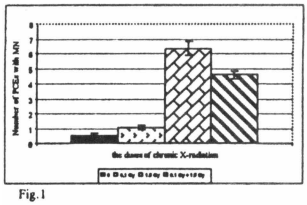
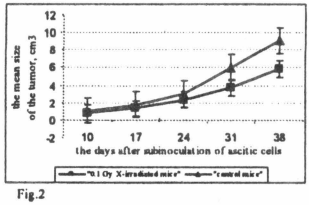


Fig.1



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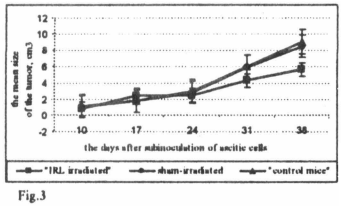
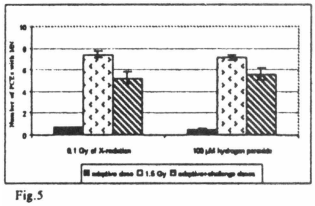


Fig.3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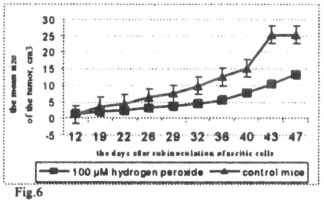

Fig.6

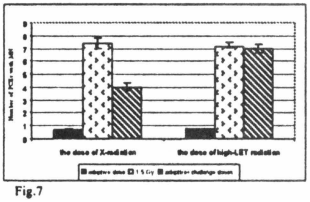
Fig.7

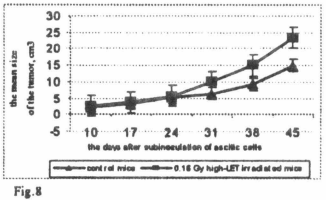

Fig.8

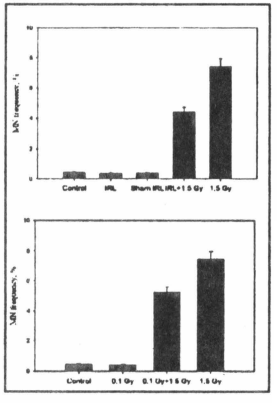

Fig.4

It seems that low doses of radiation convert the organism to a new stable state characterized by increased resistance to radiation-induced cytogenetic damage, which is retained practically till the end of the life, and decreased growth of solid tumor. The long-term maintenance of the effect resembles the immune form of the response of the organism, and one may even suggest the appearance of a new phenotype. In contrast, the irradiation of mice with low dose of high-LET radiation induced no AR. These results are in line with in vitro data demonstrating inability of high-LET radiations to induce AR, since

it was shown that high-LET radiation induces hardly- and non-repairable cluster DNA damages and, to certain extent, inhibits DNA repair by itself.

The study may be of potential importance for clinical application of low-dose exposures in the therapy of certain kinds of tumors.

RADIOECOLOGY

INVESTIGATION OF BARLEY PLANTS GENETIC POLYMORPHISM IN CONNECTION WITH LEAD TOLERANCE

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Lead is a major pollutant in aquatic and terrestrial ecosystems [1]. Despite the worldwide importance of Pb contamination, it remains unclear mechanisms of lead tolerance in different plants, particularly of separate varieties of agricultural crops. The objective of the current work was determination of the critical lead activity associated with a reduction in growth of 3 barley varieties from N.I. Vavilov Research Institute of Plant Industry (VRIPI) world collection.

Materials and methods

Seeds (100 seeds per each sample) of 3 spring barley *(Hordeum vulgare M.)* samples were exposed to 9 lead concentrations in range of 1 to 9 mg/ml taken to determination of lead critical dose. Seeds were placed in rolls of filtering paper and put in vessels with 200 ml of $Pb(NO_3)_2$ solution. Germination was proceeded 7 days at 20^0 C [2]. Germination, length of roots and shoots, weakness and strength of seedlings was measured. Seedlings of 8-10 mm in length were fixed in ethyl alcohol and acetic acid mixture (3:1) and cell division activity was examined. Total number of cells, number of prophases, metaphases and ana-telophases were counted at squashed preparations, stained with acetic orcein.

Results and discussion

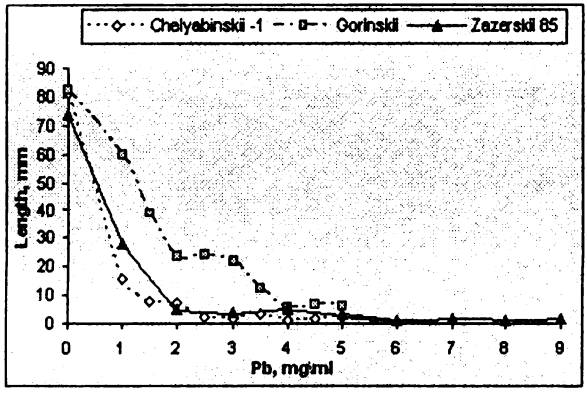

Fig. 1 Root length of 3 barley samples seedlings

Fig. 1 shows dose curves for 3 barley samples of 7-th days seedlings root length, treated with different lead concentrations. Reaction of roots was strongly pronounced. Root length decreased sharply already at 1 mg/ml of lead. Shoots length shows light stimulation at the same conditions and was more tolerant to lead. Thereat shoots length data don't present at this paper. Fig. 2 shows almost full depression roots growth at 5 mg/ml Pb^{2+} and more. It's observed at all 3 barley samples. Therefore, concentration range was decreased to 5 mg/ml for samples Chelyabinskii 1 and Gorinskii and starting with 1 mg/ml intermediate

doses 0,5 mg/ml were added. Gorinskii variety was the most tolerant. Two remaining samples show almost identical tolerance.

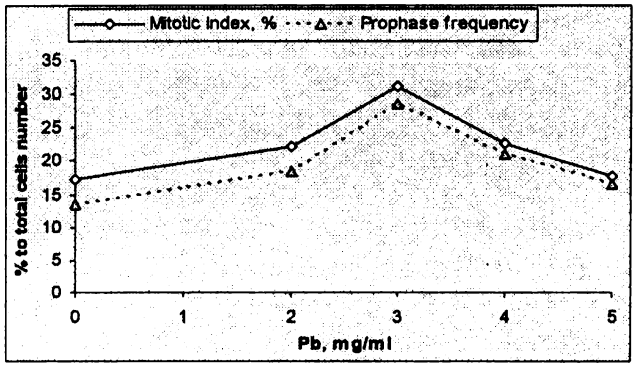

Fig.2.Mitothic index and prophase frequency in root tips

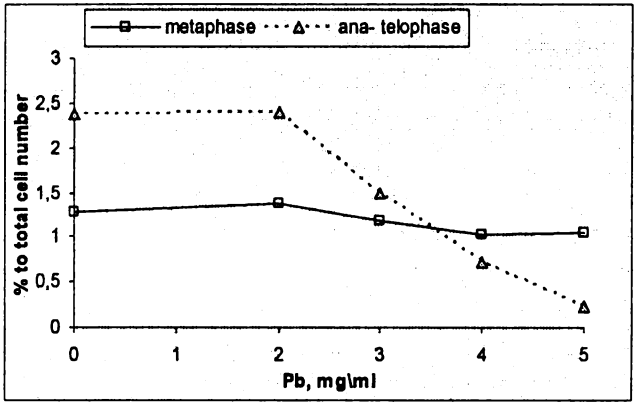

Fig.3.Metaphase and ana-telophase frequency in root tip cells

As received data showed fierce slowdown of seedlings in total and especially in root growth it was interesting to study the cell division in root apical meristem. Fig. 3 shows changes of mitotic index (MI) and prophase frequency in these cells. MI increased with the rise of lead concentration, but it sharply decreased at 3 mg/ml and more. Further increasing of lead dose caused almost full suppression of cell division. It is fair to say practically full identity of dose curves of MI and prophase frequency. It is obvious that prophase enter the main contribution in total frequency of dividing cells. This is evidence of mighty mitosis blocking at prophase intensified with increasing of lead concentration. Analysis of dose dependences of meta- and ana-telophase frequencies didn't show influence of lead concetration on metaphase frequency. However, frequency of ana-telophases remains constant to 2 mg\ml, but after this dose sharply decreases. Perhaps there is the second phase of cell division blocking connected with disruption of spin division apparatus.

Conclusions

Results of current work showed the strong suppression of lead at all barley vital processes at different plant arrangement levels. This effect especially expressed at seedling roots. Lead harms them first of all [3]. Apart numerous violations of root morphology: curvature of roots tips (it's likely caused by disruption of the length decreasing, we observed geotropism), flatus formation, tissue compression, coloration changes, etc. The depositions of some lead compounds were observed in intercellular spaces at cytological preparations with the increasing of lead concentration.

Stimulation of seed germination took place at lead concentrations of 1-2 mg/ml. High lead tolerance polymorphism was found at 70 studied barley samples from VRIPI world collection. In future work we plan investigation of 100 barley samples tolerance and study of their genetic differentiation at oxidative stress tolerance by isoenzyme analysis.

Acknowledgements

We appreciate very much the help with seed of barley reception to Ph. Dr. L.V. Kozlenko.

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ENVIRONMENTAL-IMPACT STUDY OF RADIONUCLIDE-SPECIFIC REMOVAL FACTORS OF THE LHC SITE PA3

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CERN Large Hadron Collider (LHC) tunnel is almost entirely embedded in impermeable molasse except for a sector between points 4 and 3. The water flow has been rather constant over the years: 22 litres per second. The water is pumped from the tunnel on the surface of the site PA3 where it is retained by an existing decantation basin and will be retained by another decantation basin in the future. The two basins will also decant particles and release water into a small stream Le Gailloux which supplies a lake in a commune of Crozet, in France. The technical note describes the decantation basins and hydrological parameters of the stream on the LHC site PA3 and presents the removal factors due to radioactive decay for radionuclides of interest in the discharged water.

Materials and Methods

The transit time of the stream have been measured by introducing concentrated solution of NaCl in output of the decantation basin and by observing the water conductivity linearly proportional to the NaCl concentration above the baseline level. Three portable instruments WTW Multiline P4, 340i and 315i had been installed at the outlet of Le Gailloux into the lake. Environmental impact studies for radionuclide-specific removal factors of the two decantations basins require an estimation of activity of few radionuclides, namely ^{3}H, ^{7}Be, ^{11}C, ^{13}N, ^{14}O, ^{15}O and ^{24}Na [1]. The removal factor R is defined from the ratios of the activity densities in water when entering a basin and when leaving a basin: $R = C_{out}/C_{in}$ [2]. If there are m compartments in a series within a decantation basin, the total removal factor will be the product of the individual removal factors: $R = \prod_{i=1}^{m} R_i$

The removal factors of the first and second decantation basin, R_1 and R_2 respectively, as well as of a cascade made of the two basins R_C was assumed for each radionuclides.

Results

The conductivity peak of NaCl lasted from 1800 s to 4410 s. The measured mean transit time $\tau = 2892$ s. The conversion factor from the *net* conductivity to the NaCl concentration was determined in the laboratory: 1953 (μS cm)/(g/l). Figure 1 shows the resulting net NaCl concentration signal. The calculated flow rate of the stream Le Gailloux before reaching the lake is 29.8 l/s.

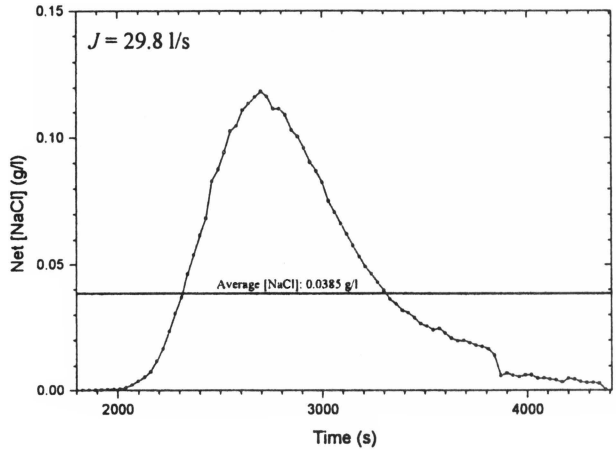

Figure 1: Net [NaCl] signal during the passage of the conductivity peak (13/08/2007).

The removal factors of the first and second decantation basin, R_1 and R_2 respectively, as well as of a cascade made of the two basins R_C are listed in Table 1 for the assumed radionuclides.

Table 1: Removal factors of the first and second decantation basin, R_1 and R_2 respectively, and of the cascade R_C for the assumed radionuclides.

Radionuclide	**Half-life**	R_1	R_2	R_C
^{3}H	12.32 y	1.00E+00	1.00E+00	1.00E+00
^{7}Be	53.22 d	9.99E-01	9.98E-01	9.97E-01
^{10}Be	1.51E+06 y	1.00E+00	1.00E+00	1.00E+00
^{11}C	20.39 min	1.08E-01	8.23E-03	8.86E-04
^{14}C	5'700 y	1.00E+00	1.00E+00	1.00E+00
^{13}N	9.965 min	3.73E-02	2.17E-04	8.10E-06
^{14}O	1.177 min	3.29E-04	5.81E-13	1.91E-16
^{15}O	2.037 min	1.35E-03	2.56E-10	3.45E-13
^{24}Na	14.959 h	8.97E-01	8.77E-01	7.87E-01

Conclusion

Without the second decantation basin, ^{11}C and ^{13}N will be identifiable in water released from the LHC site PA3 by commonly available radio-analytical

instruments and methods. With the second decantation basin, the activity densities of all radionuclides will be well below the usual detection limits except for ^{11}C, which be very close to its detection limit in an instrument optimized for measuring short-lived positron emitters like ^{11}C. These statements apply to the operation of the LHC with the ultimate beam intensity. The hydrological parameters of the stream in the sector from the site PA3 until the lake are stable and there is no significant infiltration into ground of the water of Le Gailloux while flowing from the PA3 site into the lake.

References

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INVESTIGATION OF GENOTOXIC EFFECTS OF FIPRONIL ON MAMMALS

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Kazakh National University named al-Farabi

Currently, in Kazakhstan against large number of grasshoppers new classes of insecticides, which comprise the class of phenylpyrazole and main active component of which is fipronil, are used [Information bulletin RK, 2000; Sagytov at al., 2002]. In natural conditions and in mammalian organisms it can degrade and form toxic metabolites, which are characterized by high toxicity and stability in contrast to former chemical [Hainzl, 1998]. Data on mutagenic effects of fipronil on mammals are quite controversial and there is almost no data on its effects on organism during the postnatal ontogenesis.

The purpose of the given work was to study cytotoxical effects of fipronil on large-toothed gopher *(Citellus fulvus)* from fipronil polluted biotopes of South Kazakhstan.

Materials and Methods

Large-toothed gopher (*Citellus fulvus*) from pesticides-polluted and conventionally non-polluted biotopes of South Kazakhstan served as research objects.

Cytogenetic analysis of bone marrow cells were performed using standard protocol [Grafodatsky, Radgably, 1988]. Rodent chromosomes were stained with Giemsa stain. Slides were analyzed and photographed by Axioscope-40 (Zeiss) light microscope.

Statistical analysis for evaluation of all quantitative data standard Student's test were used. In order to obtain mean values of parameters were treated by standard methods of variational statistics [Rokitsky, 1978].

Results and Discussion

To determine biological state of rodents from fipronil exposed lands (South Kazakhstan) male large-toothed gopher (Citellus fulvus) were collected from Arys and Shardara regions.

The results of cytogenetical analysis of bone marrow cells of Citellus fulvus from fipronil exposed biotopes demonstrated higher frequency of structural chromosomal aberrations (Table 1).

Frequency of aberrant bone marrow cells in rodents from Arys and Shardara areas was high 2,5 ($p<0.05$) and 3 times ($p<0.01$), respectively, than the same rates in rodents from Kazygurt areas. Additionally, the level of polyploid cells increased significantly in those animals ($p<0.01$).

Table 1 - The frequency of structural chromosomal aberrations in bone marrow cells Citellus fulvus

Biotopes	Studied cell numbers	Frequency, aberrant cells (M ± m), %	Chromosomal aberrations number, 100 metaphases			Frequency, polyploid cells (M ± m), %
			All types	chromosome	chromatid	
Kazygurt	1028	2.44 ± 0.49	2.62 ± 0.41	0.29 ± 0.12	2.33 ± 0.45	0.19 ± 0.12
Arys	814	6.18 ± 1.03*	7.20 ± 1.14**	0.76 ± 0.25	6.44± 0.31***	1.47 ± 0.30**
Shardara	921	7.25 ± 1.18**	8.47 ± 1.32**	0.82 ± 0.40	7.65 ± 1.04**	1.68 ± 0.49*
Note: * - p<0.05, ** - p<0.01; *** - p<0.001 in comparison with control						

Chromosomal aberrations were presented by pair chromosomal deletions and pair point deletions; chromatid – cromatid deletions, chromatid point fragments and acentric fragments. Polyploid cells were presented by tetraploid, hexaploid and octaploid cells (fig. 1).

Thus, the cytogenetical study of bone marrow cells of rodents from fipronil exposed biotopes revealed significant increase of chromosomal aberrations frequency in comparison with the same rates in rodents from Kazygurt areas. Significant increase of structural mutations number resulted from chromatid aberrations. High levels of chromatid aberrations in rodents from Arys and Sharadar areas indicate presence of chemical pollutants in those biotopes.

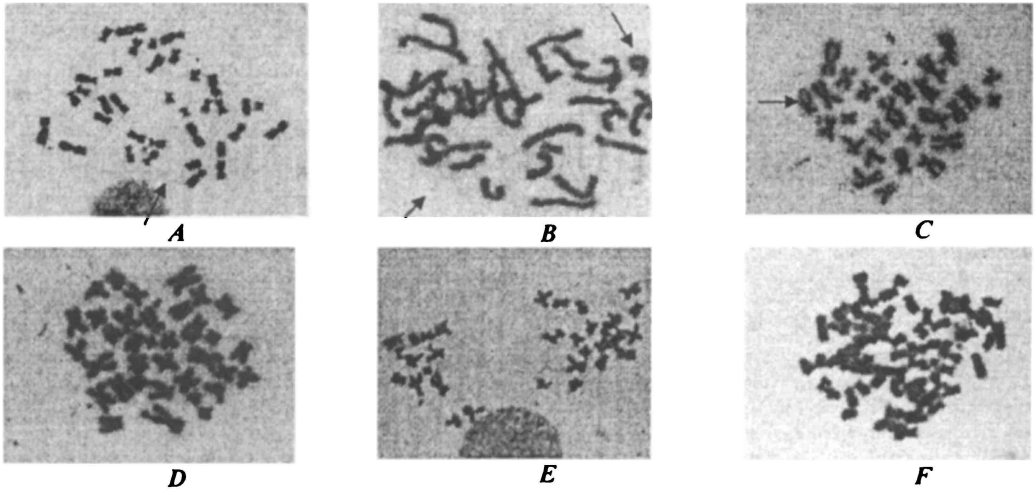

Figure 1. Chromosomal aberrations and changed chromosome sets: A - normal chromosome set (2n=36); B - entric ring; C - double fragment; D – end deletion; E - acentric ring; F - polyploid metaphase (4n=72)

The results demonstrate the increase of genetical burden in rodents from natural population, subjected to man-made chemical pollutants. The present structural changes in animal organs and high frequency of chromosome

aberration in Citellus fulvus indicatives of the presence of toxic and genotoxic factors in studied environment and can be considered as indication of general disorders in rodent organisms from polluted biotopes.

Conclusions

The cytogenetical study revealed significant increase that frequency of chromosomal and genomic mutations in bone marrow cells of Citellus fulvus from Arys and Shardara areas were significantly higher ($p<0.01$) than in rodents from Kazygurt region. High level of chromatid aberrations are indicative for the presence of chemical mutagens in studied environment.

CYTOGENETIC EFFECTS IN CRESTED HAIRGRASS POPULATIONS FROM SEMIPALATINSK NUCLEAR WEAPONS TEST SITE

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Semipalatinsk test site (STS) was a main site for testing nuclear weapon in the former Soviet Union. In 1949–1989, 456 nuclear tests were conducted at STS. As a result of this, a large-scale and non-uniform radioactive contamination of STS and adjacent territory was formed. Up to now, within the STS there are areas with very high level of radioactive contamination. The set of main dose-forming radionuclides at the STS territory is different from what we get used to in numerous studies at the East-Ural radioactive trial, territories contaminated after the Chernobyl accident, and areas with the enhanced background radiation. This makes unique any studies of biological effects at the examined area. STS is located in steppe zone with a strong continental climate. Climatic conditions of region, biological species are very different from what usually radioecologist deals with. This also emphasizes an importance of biological studies at the STS.

Up to now most investigations at the STS have been devoted to an assessment of radioactive contamination of the environment, studies of radionuclides behavior, and estimation of doses and health effects in population of nearby settlements. Information about effects of radioactive contamination on non-human biota is few and far between. Organisms inhabiting the STS now are progeny of plants and animals that had experienced the acute radiation impact in the course of nuclear weapon tests and then have been exposed to chronic irradiation during many generations. So, studies of current state of plant and animal populations at the STS should give unique information about biological consequences of long-term radiation exposure.

The objective of this study was to determine if exposure to radionuclides causes cytogenetic effects in populations of typical for Kazakhstan wild cereal a crested hairgrass (*Koeleria gracilis* Pers.) inhabiting contrasting in level of radioactive contamination plots within Semipalatinsk nuclear test site.

Materials and Methods

Four contrasting in level of radioactive contamination plots within STS area were chosen in 2005 and five - in 2008. The exposure dose rate in the air at the 1m level and at the ground surface as well as α- and β-particles flux density were measured at each plot. For all experimental plots chestnut soil is typical. Climate is arid and sharply continental.

Soil samples were collected separately by 0-5 cm and 5-10 cm layers at each experimental plot. specific activity of ^{40}K, ^{60}Co, ^{137}Cs, ^{152}Eu, ^{154}Eu, ^{226}Ra, ^{232}Th, ^{238}U and ^{241}Am γ-ray spectrometric analysis. ^{90}Sr activity concentration was determined by radiochemical method of isotope separation with spectral measurements of activity. Total concentrations as well as water-soluble, mobile and acid-soluble forms of heavy metals (Mn, Cu, Zn, Cd, Pb, Cr, Ni and Co) in soil samples were determined by atomic absorptive method.

Ears of crested hairgrass were collected in July-August of the years 2005-2008. On each plot, 25-40 plants were taken. The coleoptile in the stage of first mitosis were fixed in acetic acid: alcohol (1:3). Temporary squashed preparations of coleoptile apical meristem were made and stained with carmine. All preparations were coded and examined blindly. In each preparation, all the ana-telophase cells (4800-11900 cells from 30-90 seedlings at each site) were scored to determine the fraction of cells with alterations. Chromatid (single) and chromosome (double) bridges and fragments, as well as multipolar mitoses and lagging chromosomes were identified.

A portion of the seeds collected in 2005-2006 was acutely irradiated with γ-rays at a dose of 68,8 Gy (2790 Gy/h), whereas portion of the seeds collected in 2007 at a dose of 50 Gy (39 Gy/h).

Results

Experimental plots do not differ from each other on concentration of heavy metals, but differ on a level and a spectrum of radioactive contamination. ^{152}Eu, ^{137}Cs, ^{154}Eu, ^{241}Am, and ^{90}Sr give the main contribution to radioactive contamination of plots. The greatest total specific activities was found in an epicenter of nuclear tests and on a site where experiments with uncompleted nuclear reaction were carried out. Plants from these plots have received the greatest absorbed doses of 265 and 228 mGy/year, respectively.

The frequency of cytogenetic disturbances in coleoptiles of germinated seeds increases proportionally to the dose absorbed by plants (Figure). The agreement between findings from four successive years of study (2005-2008), different in weather conditions, suggests the leading role of radioactive contamination in an occurrence of cytogenetic effects. Severe disturbances of single and double bridges and laggard chromosomes contribute mainly to the observed cytogenetic effect. In spite of the fact that the crested hairgrass populations have occupied radioactively contaminated sites for a long time, the data analysis fails to reveal radio-adaptation effect, although acute γ-irradiations of seeds has been carried out at different doses and dose rates.

Dose rate in the epicenter of nuclear tests amounts to 36 μGy/h, which is more than 3 fold of the predicted no-effect dose rate of 10 μGy/h derived in the EC ERICA project. It is, however, well below the threshold for statistically significant effects (100 μGy/h) derived at the FASSET Radiation Effects

Database analysis. It is not surprising, than, that in the STS study there are found significant cytogenetic effects in crested hairgrass populations but no morphological alterations have been registered. Thus, the findings obtained are in agreement with the benchmark values proposed in the FASSET and ERICA EC projects to restrict radiation impact on biota.

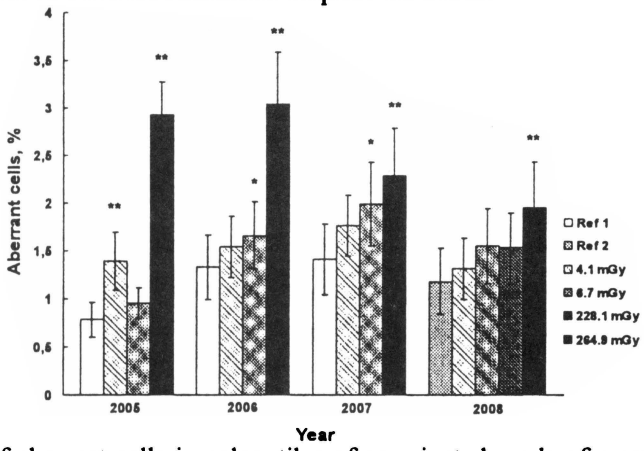

Figure. Frequency of aberrant cells in coleoptiles of germinated seeds of crested hairgrass collected in the Semipalatinsk test site, Kazakhstan in 2005-2008 in dependence on annual dose absorbed. Ref 1 and Ref 2 are the reference sites in 2005-2007 and 2008, respectively. Significant difference from the corresponding reference site: * - $P < 0.10$; ** - $P < 0.05$

A basic level of concern within a newly developing system for radiological protection of the environment is a population. Of special importance in this context are studies of plant and animal populations inhabiting sites with contrasting levels and spectra of radioactive contamination. Special attention should be paid to population-level effects such as radioadaptation, changes in sexual, age and genetic structure of populations, since knowledge of elementary mechanisms of the radionuclides' impact is insufficient to predict them. Corresponding studies are likely to increase in importance as the rate at which we change the environment worldwide continues to accelerate. The findings presented here clearly indicate that plant populations growing in areas with relatively low levels of pollution are characterized by an increased level of cytogenetic disturbances. Finally, in spite of the wealth of information collected so far, much more still remains to be explained in order to fully understand the basis of plant populations' adaptation to a harmful environment.

RADIOACTIVITY OF DRAINAGE SYSTEMS AND THEIR ROLE IN CLEARANCE OF OVERGROUND BIOGEOCOENOSIS OF KYIV

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Kyiv is the world's only city with a multimillion population that was contaminated by artificial radionuclides during the Chernobyl accident. And despite the fact that the accident's been almost 23 years, the relevance of its treatment of radioactive remains have not lost its importance. The main way of surface contamination is the sedimentation of radionuclides from the air with dust particles [3]. In addition, radioactive materials entered Kyiv with transport, which in those days went from Chernobyl and Pripyat. Dust remains mainly on rough surfaces - and the city, with its dense buildings and heterogeneous relief is a good holder for radionuclides. That is why, among other measures for decontamination of surfaces, during the first weeks after the accident watering of roads, sidewalks, courtyards, walls took place [1]. Scrubbing water washed down to the drainage system in and through them fall into the sewage treatment stations. But aerosol particles had been confining on the asphalt, concrete, wood, metal surfaces tightly, so some radioactivity remained and in process of time, by erosion and redistribution had been washing down to underground communications [2]. This facts allowed making assumptions about accumulation of certain number of radioactive substances in the silt of city's drainage systems.

Material and Methods

Research was conducted in the territory of Klov drainage collector, which is situated under Kyiv during autumn 2007 spring 2008. Silt was taken from six points from the flow of the collector bed and three points on the slack water, following standard "envelope" technique.

Taking into account the width of an object – 5 m (exactly this distance was taken for the envelope's side) we took 15×15 cm to 10 cm depth layer. We set "envelopes" on the selection points at an angle of 30 degrees to the walls of the collector, as it was necessary for the representation of all possible areas of silt accumulation. Control points had been set outdoors, according to collector's run. Samples of soil were selected on the flower beds and lawns in the 5-10 m from the roadway, homogenized, measured and received results were used as control data. In the drainage system we measured the thickness of accumulated silt layer, the radioactivity of silt samples, which was determined via a RUB-01-P6. Exposure dose measurement in the reservoir and outdoors was provided via dosimeter-roentgenometer "Pripyat" [3].

Technical radionuclides accumulates mainly in organic fraction of soil, so we determined the content of organic compounds in silt by the solid residue method.

Specificity of research object required as much variability of conditions as possible, that's why sampling points were elected so that they can give a representation of drainage system contamination (Table 1).

Table 1. Characteristics of sampling points

Point #,	Distance from entrance, m	Conditions characteristic
1	2100	pit formed by turbulent flows at junction of two collector pipes, high sediment thickness
2	1883	Change of the shape and material of pipes (parquet floor), deepening in the center; lamellar flow with individual jets; about 30 cm pipes raising, low thickness of sedimentation
3	1432	flat bottom; lamellar flow, almost no sediment is observed
4	1162	channel split, turbulent flow, some fragments of concrete structures are observed, what leads to intensive sediment accumulation;
5	756	bend channel, deepening in the center of the tunnel, turbulent flow, small thickness of sediments
6	300	direct channel, deepening in the center, turbulent flow, sampling was made under the drip hatches, high thickness of sediments, some polyethylene and glass particles had been observed
exit	0	outgate, flow into Lybid-river; lamellar flow, turbulence comes near outgate, some sediment accumulates.

Results and Discussion.

Obtained results did not confirm assumption that were stated previousely. It turned out that the value of specific activity fluctuate within 84,3-137,9 Bq/kg, while the control sample specific activity was 297,6 Bq/kg (Table 2), surface activity ranged from 14,9 to 25,3 kBq/m^2.As had been expected, results correlated with the content of organic matter in silt, entailing exposition dose fluctuations – from 9 to 18 mcR/h.

One can see that almost all indicators (except the maximum content of organic matter) reaches its maximum at point # 5, where lot of drip hatches are situated, and therefore the statement that radioactive particles accumulates in the fan is fair also for drainage systems.

The explanation of the fact that drainage collector has not accumulated considerable activity should be sought in the powerful and turbulent flows that occur after every significant rainfall and take with almost everything – even rather big formations – that was accumulated during the relatively dry period.

Table 2. Results of performance measurements of the radiation situation in Klov drainage collector

Sample #	Exposure, mcR/h	Radioactivity, Bq/kg	Density of contamination Bq/m^2	Organic, mg/100 g soil
6	14,3±2	90,6±2	16,1±0,2	0,15±0,01
5	18,0±2,0	137,9±2	25,3±0,2	0,21±0,01
4	16,5±1	99,4±2	16,9±0,2	0,18±0,01
3	9,1±1	84,3±6	15,4±0,6	0,15±0,01
2	14,9±1	95,2±6	16,7±0,6	0,20±0,01
1	17,0±2	119,2±3	17,6±0,3	0,18±0,01
0 (exit)	13,8±2	98,7±2	14,9±0,2	0,18±0,01
Control	14,7±2	297,6±3	46,0±0,3	0,27±0,01

The next question studied which was part of the radionuclides that was washed down from outdoors to the city rain sewer. After comparing data with the one from control, it appears that this part ranges from 54,89 to 32,40% depending on the point of sampling. So one can make a conclusion that city surface treatment processes with have notable intensity.

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INFLUENCE OF MAN-MADE DUST POLLUTION ON FORMATION OF PHYSIOLOGICAL AND BIOCHEMICAL ADAPTATIONS AND RADIOSTABILITY OF SEED PROGENY *LEPIDIUM APETALUM*

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It is known that dust pollution has a dual effect on organisms - it is a mechanical impact and effect of chemical substances contained in the dust. Man-made pollution causes the accumulation of a large number of ions of heavy metals in a soil-plant system. Prolonged influence of pollutants on plants growing in this territory can induce not only the toxic effects, but adaptive physiological, cytological and biochemical responses [1, 2].

Nevertheless, studies on the impact of various stress factors of the environment on change of the physiological, biochemical characteristics and radiostability of plants that exist in extreme permafrost conditions are insufficient.

Materials and Methods

The criteria for assessing the physiological, cytological and biochemical characteristics were: the survival of seedlings at the stage of real foliage, mitotic activity (MA) of meristematic cells in roots of seedlings (%), total content of low molecular antioxidants (LMAO), enzymes activity of superoxide dismutase (SOD) and peroxidase (POX), intensity of lipid peroxidation (LP) by malondialdehyde (MDA) concentration, rate of incorporation of ^{3}H-thymidine and ^{14}C-leucine in the metabolism of seedlings cells. Coefficient of reparative ability, general relative stability and activity of the genome of seedlings cells were estimated by using an integrated cytological and biochemical methods [3].

To determine radiostability, air-dried seeds were exposed to γ-radiation from the source ^{60}Co in the dose range from 10 to 500 Gy. The level of radiostability of seeds was characterized by value of median lethal dose for a 30-day observation ($LD_{50/30}$)

Results and Discussion

Seeds of *L. apetalum* exposed to man-made dust were collected from plants on the territory of Yakutsk city, at a 10-m distance from the carriageway (point 2), where the level of dust pollution was 6.9 ± 0.69 g/(m^2·day). As a control, we have taken seeds of plants growing as far as 10 km from the city at a sufficient distance from the carriageway (point.1) with the level of dust pollution equal 0.3±0.03 g/(m^2·day).

Analysis of the heavy metals content in the investigated dust shows that in the roadside dust (point.2) there was an increased copper content by 2 times,

zinc by 2.7 times, molybdenum by 3.4 times, lead by 2 times as compared with the content of these elements in a control sample of dust.

Table 1 presents physiological, cytological and biochemical characteristics of seed progeny of *L. apetalum* whose parent plants grew under different conditions of dust pollution. It was established that seed progeny of plants growing in conditions of chronic man-made dust pollution (point.2), had a higher activity of all studied antioxidant systems as compared with control (point.1).

Table 1 Physiological, cytological and biochemical characteristics of seed progeny of *L. apetalum* depending on the degree of dust pollution

Points	LMAO, μg-equival$_{querc}$ /g	activity of enzymes, μmol / min·g		^{3}H-thymidine, fmol / day·g	^{14}C-leucine, pmol / day·g	MA, %	MDA, μmol / g
		SOD	POX				
1	1.7±0.1	0.8±0.2	1.2±0.1	2.4±0.3	1.8±0.2	1.8±0.2	2,4±0.2
2	3.6±0.1*	1.5±0.2*	1.6±0.2*	3.1±0.5	2.1±0.3	1.7±0.1	1,4±0.1*

* - Significantly different from control, p <0.05 (Mann–Whitney U-test)

Based on the data obtained coefficients (table 2) were calculated. We found that prolonged growth of *L. Apetalum* parental plants under chronic man-made dust pollution resulted in a 3–time increase of the value of antioxidant protection $C_{aop/lp}$, which, in turn, increased the overall relative stability of the genome (C_{ogs} = 2.0) of their seed progeny in these conditions of growth.

Table 2 Coefficients of antioxidant protection, overall activity of the genome, reparations, overall relative stability of the genome, cells of seed progeny (normalized with respect to control)

Points	$C_{aop/lp}$	C_{oag}	C_{rep}	C_{ogs}
1	1.00	1.00	1.00	1.00
2	1.79	1.00	1.00	1.79

However, the variability of biologically important coefficients in seed progeny of plants grown under conditions of man-made dust pollution tends to decreasing as compared with the control (table 3). In this way the chronic man-made pollution by dust of *L. apetalum*, caused a decrease in the intra-population diversity. Perhaps the reduction of variability is associated with the action of heavy metals contained in the dust.

Additional impact of γ-rays on the seeds of *L. apetalum* allowed to determine their radiostability. It is shown that a higher degree of man-made dust pollution in the habitat of parent plants did not influence upon radiostability of seed progeny (table 3), despite higher value of their antioxidant status (Table 1, 2).

Table 3 Values of variability (CV,%) coefficients of antioxidant protection, overall activity of the genome, reparations, overall relative stability of the genome of cells and values of median lethal dose of *L. apetalum* seeds

Points	$C_{aop/lp}$	C_{oag}	C_{rep}	C_{ogs}	$LD_{50/30}$, Gy
1	41.1	28.4	59.8	60.5	331.3±35.4
2	27.5	27.9	48.6	24.4	361.9±25.7

Conclusion

It was established that long-term growth of *L. apetalum* parental plants in conditions of chronic pollution by dust containing heavy metals increased the total content of low molecular and activated enzymatic antioxidants which in turn led to increased stability of the genome of their seed progeny in these conditions of growth. Growth of *L. apetalum* in conditions of chronic man-made dust pollution does not influence on radiostability of their seed progeny, despite the high value of their antioxidant status, which may be a consequence of reducing the variability of the investigated characteristics.

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