

# MEETING IN NOR AMBERD



JOINT INSTITUTE FOR NUCLEAR RESEARCH



# MEETING IN NOR AMBERD

*Fifth International Conference,  
Dedicated to N. W. Timofeeff-Ressovsky  
and His Scientific School*

«MODERN PROBLEMS OF GENETICS,  
RADIOBIOLOGY, RADIOECOLOGY,  
AND EVOLUTION»

Nor Amberd, 5–10 October 2021

*ABSTRACTS OF PRESENTATIONS  
MEMORIES & DISCUSSIONS  
LECTURES*

Dubna • 2021

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ББК 28.071.2я434+28.081.28я434  
М47

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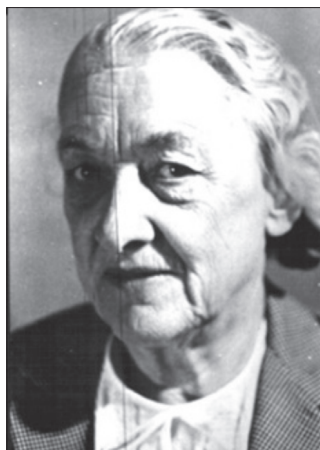
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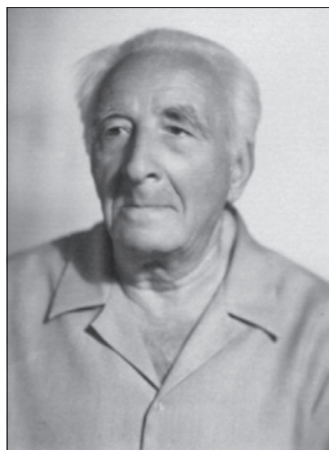
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E. A. Timofeeva-Ressovskaya  
(1898–1973)



N. W. Timofeeff-Ressovsky  
(1900–1981)



Summer School in Miassovo, 1958



The first visit of N. W. Timofeeff-Ressovsky  
to Moscow, his lecture at MSU. 1956.



A. Zurabyan, 1960s



N. W. Timofeeff-Ressovsky  
and R. Atayan, 1970s



N. Simonyan, 1970s



Ts. M. Avakyan, 2000s



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## TOPICS OF THE CONFERENCE

### Genetics

- Genome universe
- Regulation of mutation rate
- Protein variability and its regulation
- Environmental impact on the genome and epigenetic inheritance
- Site-directed mutagenesis
- Cancer and neurodegenerative diseases

*Discussion: System features of cells*

### Radiobiology

- Genetics and epigenetics of radiation-induced genome instability
- Non-targeted radiation effects
- Influence on proteomics mechanisms
- Space radiobiology
- Nuclear medicine
- Biological dosimetry

*Discussion: Effects and mechanisms induced by low dose irradiation at cell, tissue and population levels*

### Radioecology

- Characteristics of natural and anthropogenic radiation
- Radiation effect on populations and ecosystems
- Degradation of species community and formation of species
- Influence of environment conditions on radiation effects
- Epidemiology of radiation

*Discussion: System response of biogeocenoses on radiation effects including low dose radiation*

### Evolution

- Self-organization and basic laws of evolution
- Origin of life
- Evolution of the genomic universe
- Organization and evolution of the virus world
- Evolution of ethological structure of populations
- Human evolution

*Discussion: Evolution of biosphere*



**ABSTRACTS  
OF PRESENTATIONS**

# **GENETICS**

## ***Mechanisms of genetic processes***



## MOLECULAR NATURE OF THE VESTIGIAL NO WING MUTATIONS IN *DROSOPHILA MELANOGASTER*

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The product of the pleiotropic *vestigial* (*vg*) gene of *Drosophila melanogaster* is responsible for 4 traits in adult fly: wings and halteres development, normal position of postscutellar bristles, and female fertility. Radiation-induced *vg* mutations lead to variation in these traits in different degree. Classical spontaneous *vestigial no wing* (*vg<sup>nw</sup>*) null-mutation displays a deep reduction of the wings and halteres, the erected postscutellar bristles, and female sterility and it results from 3 kb-long deletion at the 3'-end of the gene. This indicates that different gene domains may be responsible for different traits. Our large collection of radiation-induced *vg* mutations (about of 170 mutants) consists of 50% of the *vg<sup>nw</sup>* mutants. It was interesting to analyze the nature of our mutations and find out if the 3'-end of the gene really is a “hot region” for intragenic mutagenesis. To study this issue, the analysis of 7 *vg<sup>nw</sup>* mutants was performed using PCR and sequencing methods. As the results showed, different DNA changes (base substitutions, small deletions and insertions) in exons 3, 4, 5, as well as in the intron 4 were found. It is important to note, that one mutant with changes in only 2 traits from 4 had a large deletion of intron 2. This data indicate that different regions of the *vg* gene may be responsible for *vg<sup>nw</sup>* phenotype. In the light of the data obtained, the functional importance of introns is discussed.

## ROLE OF POL ETA IN HIM1-DEPENDENT MUTAGENESIS

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During their life, cells are exposed to various agents of a physical and / or chemical nature, which lead to DNA damage, stopping the replication fork and further movement along the cell cycle. If the cell does not repair the damage in a timely manner, it can lead to its death. To avoid this, cells have developed a mechanism that allows you to bypass DNA damage in the shortest possible time, which allows you to start the replication fork and further advance along the cell cycle, and thereby prevent cell death. This mechanism is called DNA damage tolerance (DDT). The DDT is divided into two branches, error-free and error-prone (TLS (TransLesion Synthesis)).

The *HIM1* gene of the yeast *Saccharomyces cerevisiae* operates in the unmistakable branch of the DDT. Its main functions are not yet reliably known. It is known that the product of the *HIM1* gene is involved in the stabilization of the D-loop and can affect the synthesis of dNTPs. Deletion of the *HIM1* gene leads to an increased frequency of UV-induced and spontaneous mutagenesis.

In turn, polymerase  $\eta$  (a product of the *RAD30* gene) belongs to the Y-family of TLS DNA polymerases and works in the error-prone branch of DDT, it is able to insert two adenines opposite thymine dimers. Deletion of the *RAD30* gene does not particularly affect the frequency of UV-induced mutagenesis.

We have selected mutants resistant to canavanin of two strains *him1 $\Delta$*  and *him1 $\Delta$  rad30 $\Delta$* . Further, the *can<sup>R</sup>* locus (800 bp) was sequenced from the selected mutants and the sequence was analyzed. Based on the analysis of the sequences of the two strains *him1 $\Delta$*  and *him1 $\Delta$  rad30 $\Delta$* , we have shown that the frequency of him1-dependent mutagenesis in a single *him1 $\Delta$*  mutant is affected by the work of polymerase  $\eta$ .

## CLE GENES IN POTATO DEVELOPMENT

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*CLE* genes encode a group of small secretory peptides which involved in regulating stem cell proliferation and differentiation in plants. *CLE* peptides have been studied in many plants, but little is known about this gene family in potato. In potato *Solanum tuberosum*, we identified 37 *CLE* genes (*StCLE*), among which, using expression analysis, we identified regulators of stem thickening (*StCLE8* and *StCLE12*) and participants in the response to nitrogen (*StCLE4* and *StCLE10*). We have shown that the promoters of the *StCLE8* and *StCLE12* genes are active in the cells of the conductive bundles of stolons and stems, as well as in tubers. Next, we studied the effect of overexpression of the *StCLE8* and *StCLE12* genes and found that changes in the expression levels of the *StCLE8* and *StCLE12* genes affect the structure of the vascular bundles in the stem and stolon, tuber growth, and internode length. We also studied the activity of the promoters of the *StCLE4* and *StCLE10* genes in the roots of potatoes growing in a medium with and without nitrogen. We have shown that the promoters of these genes are active in a medium with nitrogen. We analyzed root growth and the ability to form tubers in plants with overexpression of the *StCLE4* gene.

The article was made with support of the Ministry of Science and Higher Education of the Russian Federation in accordance with agreement № 075-15-2020-922 date 16.11.2020 on providing a grant in the form of subsidies from the Federal budget of Russian Federation. The grant was provided for state support for the creation and development of a World-class Scientific Center “Agrotechnologies for the Future”.



## **LARGE INTRON 4 REGULATES THE EXPRESSION OF THE VESTIGIAL GENE OF *DROSOPHILA MELANOGASTER***

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The precise phenotypic and genetic analysis of a large sample of radiation-induced recessive point mutations in the *vestigial* gene of *D. melanogaster* allowed to detect 9 out of 115 (7,8%) analyzed mutants with phenotype resembling the classical *vestigial-strap* (*vg<sup>s</sup>*) mutation: partially reduced wings with deep incisions and erected posterior scutellar bristles. Complementation analysis using microdeletions of the gene showed the localization of *de novo* mutation in intron 4 in one of these 9 mutants. This finding allowed to suggest that intron 4 could play an important role in the regulation of the *vg* gene expression. To elucidate the nature of this mutation, sequence analysis of entire intron 4 from this mutant and two wild-type laboratory high-inbred lines (Oregon and D32) was carried out. Comparison of the obtained results showed the presence of a unique cluster of 7 base substitutions at the 5'-end of intron 4 as well as several single-base substitutions throughout the entire intron in this mutant as compared with control lines. The DNA context of exons in all three lines studied was identical. Therefore, it can be assume that mutant phenotype *vg<sup>s</sup>* resulted from mutational lesion located in 5'-end of a large intron 4. The functional significance of the cluster and other base substitutions established in intron 4 is discussed in the light of our earlier data on the important functional significance of another large intron 2 of this gene.

## **SITE-DIRECTED MUTAGENESIS**

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The seamless gene editing technologies like CRISPR/Cas system and others represent advanced methods of mutagenesis. Unlike irradiation and chemically induced mutagenesis, these new approaches are precise site-directed mutagenesis techniques (SDM). They allow desired modification of target genes, although some off-target effects still remain a problem. In the lecture, opportunities given by SDM for basic research and practical application will be discussed. For basic research, SDM represent breakthrough technology in reverse genetics for validation of candidate genes, studying of genes functions and their pleiotropic effects, for reconstruction of gene networks in many species. In the context of practical application, such as next-generation breeding of plants and animals or novel approaches in medicine, both biological restrictions and regulatory hurdles for wide practical application of the SDM will be considered. First of all, the SDM is efficient for improvement of genotypes by mono- or oligogenic traits, while most of the important traits are polygenic. For next-generation animal breeding, off-target effects and in some cases low efficiency are the main restrictions. For plant breeding, off-target effects are not so essential, however there are such problems as the low number of target genes discovered for most of the main crops as well as the species and genotype-specific dependence of transformation amenability and regeneration efficiency. The achievements in research to overcome these restrictions will be discussed. For practical medicine, potential off-target effects are an essential disadvantage. Also the ethical issues arise in respect to application of SDM in medicine. The appearance of seamless gene editing technologies boosted reconsidering of legal regulation in the field of genetic engineering application. Generally, the regulation is split to the process- and product-based approaches. These issues as well as ethical and biosafety ones will be raised in the lecture.

## THE GENE UNIVERSE

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The rapid advances of genome sequencing now allow us to explore the principles of the organization of the virtual space of genes, or gene universe. The elementary objects in the gene universe are pangenomes, that is, the entirety of the genes in a species. Pangenomes, especially those of prokaryotes, are highly dynamic, with genes rapidly gained and lost. Pangenomes consist of at least two classes of genomes: a small core of highly conserved genes and a vast 'cloud' of genes that are rapidly replaced in each individual genome. The gene universe has a self-similar organization: these two classes of genes are identifiable at all scales of evolutionary distances. Knowing the fraction of the rapidly replaced genes provides for a rough estimate of the size of the gene universe. It is vast, including, most likely, many billions of genes.

## STRUCTURAL ORGANIZATION OF *TFLI*-LIKE GENES OF *VIGNA* SPECIES

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Species of the genus *Vigna* Savi cowpea, mung bean and adzuki bean are included in the human diet as basic dietetic products for millions. Stem growth habit is one of the key features in determining of plant architectonics. This trait is an economically important because it interconnects with flowering duration, yield, stem length, resistance to plant lodging and suitability of mechanical cultivation. *Terminal Flower1* (*TFLI*) gene maintains the indeterminacy of the activity of the shoot apical meristem and it delays the plant transition to flowering. The group of *TFLI*-like genes includes *TFLI*, *ATC* and *BFT*. The aim of the present study was the characterization of the structural organization of *TFLI*-like genes in genome of cowpea, mung bean and adzuki bean. We analyzed nucleotide sequences, predicted elements in promoter regions, predicted amino acid sequences, putative functional domains and 3D protein structures. We characterized *TFLI* (one gene for adzuki bean and two copies for other species), *ATC* (one gene for all studied species) and *BFT* (one gene for all investigated species). The most of identified protein sequences had all critical amino acid residues. Based on the obtained results of the sequence analysis of identified *TFLI*-like genes, including the *cis*-regulatory regions, it can be assumed that most identified genes are maintained in a functional state. Additional studies to establish the possible role of the VrTFL1.2 and VrATC mung bean proteins in the transition to the reproductive stage are needed. Identification and analysis of genes responsible for type of stem growth and control of the transition to flowering are required for the successful selection of modern varieties. The obtained results confirm the high evolutionary conservatism of genes involved in the molecular genetic control of the flowering initiation. Obtained data based on *in silico* analysis is necessary for next step of studying of the molecular genetic mechanisms of the stem growth habit.

The present review was carried out within VIR project No. 0481-2019-0001.

## **BIOCHEMICAL STUDY OF THE CHARACTERISTICS OF VARIETIES OF WHEAT AND ITS WILD RELATIVES**

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Strategy of modern breeding is directed on creation of varieties and hybrids of agricultural crops, combining high productivity and quality of the crop with increased adaptability to unfavorable environmental conditions. The success of breeding work largely depends on the availability of the source material for breeding, as well as effective methods of their estimation and knowledge of physiological-biochemical mechanisms that determine the quality of the harvest and resistance to stress factors of different nature. The objects of study were the wild species of the genus *Aegilops* L. (*Ae. cylindrica*, *Ae. crassa*, *Ae. tauschii*, *Ae. triuncialis*) and four wheat cultivars (varieties Pandaki, Bobilo, Safedak and Marvi). The content of starch and protein in wheat grains depends on genotypic and natural climatic factors. However, strict laws are not observed, although it is planned some tendency to increase of content of starch and protein under more optimum conditions. The biochemical composition of species of *Aegilops* L., has not been adequately studied. Here is of great scientific interest in the study of some species of *Aegilops* L., collected in different ecological zones of Tajikistan. The results showed that in all studied four samples *Aegilops* L., the content of starch in the grain varied in limits from 20.2 to 25.7 respectively. On protein content in grain of wild species have higher limits of variability from 24.0 till 34.5%. The relatively low value of this indicator set of sample *Ae. tauschii* and *Ae. cylindrical* growing in conditions of Faizabad district (24.0 - 25.2%), while the greatest of *Ae. triuncialis* grown in conditions Ecanbai district (34.5%).

It is interesting to note that the studied of species of *Aegilops* L., accumulation level of the main components of the grain, starch and protein) compared with the studied cultivars of wheat has the opposite tendency. The species of *Aegilops* L., in all cases, the protein content in grain was more than starch content. Thus, the study of starch and their protein content in grain of different wheat varieties and species of *Aegilops* L., revealed ambiguous in the manifestation of these indicators depending on the species composition of plants and conditions. It is shown that environmental conditions of the habitats, along with the genetic characteristics of each species, the impact on the biosynthesis, and the level of accumulation of the main components of the grain.

## EXPLORING THE GENETIC DIVERSITY OF ARMENIAN GRAPEVINE RESOURCES

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Armenia is a unique grapevine diversity hotspot in South Caucasus where the viticulture and winemaking dating back to the beginning of the IV Millennium BC. The long-standing history of grape cultivation during centuries proffer the existence of a huge genetic diversity involving a wide range of autochthonous varieties and wild species that are well adapted to different eco-geographic and climatic conditions of the country. The investigations of grapevine genetic diversity in Armenia was carried out to identify and document the genetic heritage of country, mainly focuses the attention of unknown or yet not described grapevines and neglected cultivars in order to conserve them from extinction. The determination of almost 300 grapevine genotypes identity requires a combination of molecular fingerprinting, morphological description and exhaustive bibliographic analysis. The trueness to type of each accession was defined based on the analysis of 24 SSR markers and comparison of genetic profiles versus the *Vitis* International Variety Catalogue (VIVC) and bibliography.

The presented research of *V. vinifera* L. diversity in traditional viticultural regions across Armenia provided insights not only about the existing genetic diversity but also highlighted the relatedness of autochthonous varieties with new bred cultivars and unknown grapevine genotypes. It is so far the most representative and precise analysis of Armenian grape germplasm.

The realized study was depicted the abundance of discrete and unique genotypes and clones and indicated that more Armenian varieties can exist and there is a strong need for proper analysis of bibliography and ampelographic characterization.

On the base of realized in-depth investigation a true-to-type inventory of Armenian grape germplasm was carried out and documented in Armenian *Vitis* Database ([www.vitis.am](http://www.vitis.am)) and in *Vitis* International Variety Catalogue ([www.vitis.de](http://www.vitis.de)).

## THE PROANTHOCYANIDIN REGULATORY *R2R3-MYB*, *BHLH-MYC* AND *WDR* GENES IN *GOSSYPIMUM* GENUS

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Cotton is an important crop due to a valuable fiber raw. Naturally colored cotton fiber are mostly preferable for textile industry because of its hypoallergenic and eco-friendliness characteristics. It was established that proanthocyanidins are responsible for fiber with various brown shades. Structural genes of flavonoid biosynthesis are under control of TFs related to the *R2R3-Myb*, *bHLH-Myc* and *WDR* families that form together ternary MBW complex. The search of *R2R3-Myb*, *bHLH-Myc* and *WDR* families members was based on available sequences in the CottonFGD and NCBI databases using BLASTN algorithm in diploid and allotetraploid cotton species. *In silico* analysis revealed potential involvement of identified gene copies in PAs biosynthesis. Evolutionary studies of identified paralogous and homoeologous gene copies showed that all duplications occurred in the genome of the common diploid ancestor of the genus *Gossypium*. Moreover, the *GhTT2/GhMYB10* and *GhTT8* genes occur to be more variable in comparison with the *WDR* genes so that they represent of particular interest for further investigations. Resequencing of selected gene alleles allows finding out some significant differences between contrasting genotypes. These achievements can be potentially appropriated for creation of naturally colored cotton cultivars using marker-assisted selection. This work was conducted within the framework of the state task regarding to Theme № 0481-2019-0001.

## **SOMATIC EMBRYOGENESIS REGULATORS AMONG NF-Y FAMILIES IN *MEDICAGO TRUNCATULA***

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Somatic embryogenesis (SE) is widely used in biotechnology as a regeneration process conditioning many plant transformation methods, artificial seeds obtaining and zygotic embryogenesis studying. The main objective of this study is to identify new SE regulators among transcription factors from NF-Y families and to analyse the mechanisms of their functioning in *Medicago truncatula*, a legume model object. MtNF-YB10 belongs to the NF-YB family and it is homologous to *Arabidopsis thaliana* LEC1, which has a lot of different functions, related to embryogenesis. Heterotrimeric NF-Y transcription factor includes NF-YA, B and C subunits and binds to the specific DNA motifs. The interactions of NF-Y subunits, demonstrating high expression levels during SE, were analyzed using the yeast two-hybrid system. Our next step will be to confirm the revealed interactions *in planta* by conducting a BiFC experiment. We are obtaining *MtNF-YB10* loss of function plants using CRISPR/Cas9 technology and developing combined liquid and solid cultivation methods for somatic embryos production to analyse *MtNF-YB10* regulatory effect on SE. The research was made with support of the Ministry of Science and Higher Education of the Russian Federation in accordance with agreement № 075-15-2020-922 date 16.11.2020 on providing a grant in the form of subsidies from the Federal budget of Russian Federation. The grant was provided for state support for the creation and development of a World-class Scientific Center “Agrotechnologies for the Future”.



**TIMOFEEFF-RESSOVSKY'S VOICE:  
FROM CHILDHOOD MEMORIES AND FAMILY RELATIONSHIPS  
TO WORK ON GENE TARGETING AND GENE REPAIR IN COLOGNE  
AND BERLIN-BUCH**

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Timofeeff-Ressovsky worked in Berlin-Buch from 1925 to 1945. With Delbrück and Zimmer, in 1935, he published an early, pioneering paper on the nature of gene mutation and structure. I knew him through family relationships. Choosing immunology and genetics as my scientific subjects, I started my own research group at the Genetics Institute in Cologne, which Max Delbrück and Josef Straub had founded after the Second World War, in 1962. When in the end of the 1980ies targeted mutagenesis through homologous recombination became a reality, my group developed new techniques to delete or modify genes in mouse cells in a conditional manner, and we became fascinated by the new insights into gene function gained through this approach. After a subsequent stay of 10 years at Harvard Medical School I moved, in 2011, to the Max-Delbrück-Center in Berlin-Buch, the very place where Timofeeff-Ressovsky had done his original work. This was the time of the most recent revolution in the field of gene editing, brought about by the use of RNA-guided DNA nucleases. We immediately adopted this new tool and set out to modify and repair genes in the hematopoietic system of mouse and human. I will exemplify the unprecedented power of this approach through recent experimental work of my group.

## THE EFFECTIVENESS OF PRE-SOWING TREATMENT OF CHICKPEA SEEDS BY BIOSTIMULANTS

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Agricultural crop diversification especially the legumes will help to provide the population with proteins of plant origin and initiate the transition to organic agriculture. Last decades the natural plant biostimulants (PBs) have been used in agriculture for improvement the ecological state of agrophytocenosis and increasing the abiotic stress *resistance* of plants. The optimal doses and exposures of some agrochemicals have been determined. Despite the achievements, creation and research of new biostimulants and the testing of their effectiveness are ongoing.

The aim of our research was to study the effectiveness of pre-sowing treatment by natural biostimulants agrimitin and melanin on the seeds of spring-planted and fall-planted chickpea varieties *Cicer arietinum* L. grown in Ararat valley of Armenia. For the field experiments the pre-treatment of the seeds of the legumbres by 2.5nM agrimitin solution during 12 hours and by 0.035% melanin solution with exposure 24 hours were used. The effectiveness of pre-sowing treatment was detected by studying the biological and yield indicators of treated plants. The sowing norm was 130 kg/ha.

Two-year field experiments with pretreatment on chickpea seeds of three studied varieties with agrimitin and melanin showed the enhancing of the field germination compared to the control from 75.7% to 78.4% and from 75.2% to 77.2%, respectively. The yield was increased under the treatment by agrimitin about 32.4-40.9 c/ha and by melanin about 31.3-39.4 c/ha.

Our results suggest that agrimitin and melanin agrochemicals has a great potential and can be recommended for pre-treatment the grains which will promote the increasing the yield of legumes and the expanding the sown areas of these crops for sustainable agricultural production.

## APPLICATION OF BIOINDICATORS FOR ASSESSMENT OF HEAVY METALS' GENETIC AND EPIGENETIC EFFECTS IN DIFFERENT REGIONS OF ARMENIA

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Assessment of genetic and epigenetic effects of environmental pollutants in natural species is essential for estimation of risk to public health. Combination of comet assay, which is highly used for monitoring of environment genotoxicity in fish (*Carassius auratus gibelio*), crayfish (*Astacus leptodactylus*), lizards (bisexual *Darevskia raddei* and parthenogenetic *Darevskia armeniaca*) and field mouse (*Apodemus uralensis*) sampled in different locations of Armenia and Artsakh (Lesser Caucasus) and chemical analysis of water and soil was applied for assessment of genetic effects of environmental heavy metals. Also, for *Darevskia* lizards global DNA methylation analysis was performed for assessment of epigenetic effects, and the role of gender and age in response to environmental pollutants was studied. The obtained results showed intersite differences in DNA damage as well as a direct relationship between the levels of DNA damage and heavy metals in corresponding locations for all bioindicator organisms. While, levels of global DNA methylation in *D. raddei* and *D. armeniaca* from contaminated areas were significantly lower compared to reference sites, and a negative correlation was observed between global DNA methylation and heavy metal content. The levels of comets and global DNA methylation did not depend on sex but decreased with age of lizards. *D. armeniaca* showed a higher sensitivity toward environmental pollution than *D. raddei* in their common habitat. To summarize, all applied wide range animals could be sensitive bioindicators of heavy metal pollution of water and soil. DNA damage and global DNA methylation are valuable biomarkers for assessing the genetic/epigenetic effects of environmental pollutants.

## MOLECULAR GENETIC STUDIES OF BARLEY IN THE 12<sup>th</sup> CENTURY

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Modern molecular genetic methods research of DNA possible to study the genomes of extinct and modern organisms. Foreign and Russian scientists are studying aDNA obtained from bones of fish, mammalian's tissues and organic residues present in various systems of organs. Fossil remains of plants are of particular importance: seeds, pollen and fossilized parts of plants. The interest in paleogenetics is aimed at studying the origin and domestication of monocotyledonous and dicotyledonous plants.

In our work, we present the results of a study of fossil remains of cereal plants using morphological analysis of the microrelief of caryopses and modern molecular genetic methods. The carbonated caryopses were the material of the work, which were found in 2019 during the excavation of the Usvyat settlement in the North-West of Russia. Studies of charred material indicate the poorest preservation of the genetic material with a low probability of recovery. However, we managed to isolate ancient DNA and confirm its species identity. According to the results of the data's analysis on the territory of the Usvyatsky settlement in the North-West of Russia in 1021 (XIIth century), *Hordeum vulgare* husk barley was cultivated.

## MOLECULAR-GENETIC ANALYSIS OF HISTORICAL PATTERNS OF STURGEONS FROM THE GULF OF FINLAND AQUATIC SYSTEM

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Reviving the Baltic (Atlantic) sturgeon and obtaining its self-reproducing population in the Baltic Sea is one of the prioritized tasks of HELCOM, where an international program is currently being launched to solve this complicated and persistent problem through joint efforts of the interested Baltic countries, including Russia. Since the data on species-specific attribution of the sturgeons that previously inhabited the target waters are scanty and contradictory enough, a molecular-genetic analysis was performed on the museum specimens of sturgeons from the collections of the St. Petersburg State University, St. Petersburg State Agrarian University, Atlantic Branch of the Russian Federal Research Institute of Fisheries and Oceanography (Kaliningrad), as well as from the kitchen leftovers retrieved from the Menshikov Palace during archeological excavations in 2019.

The aim of the investigation was the studying fossils of the research object for further molecular-genetic analysis; disclosing possible similarities between ancient fish and extant sturgeons using molecular-genetic techniques. The DNA isolated from archival sturgeon specimens was significantly degraded (ca. 300 bp), but its concentration was high (on average, about 200 ng/μL). We studied regions of nuclear and mitochondrial DNA.

For the first time, ancient DNA from sturgeon samples has been isolated. Modern molecular-genetic techniques were used to identify a considerable diversity of sturgeon species among the samples from the kitchen leftovers excavated in the Menshikov Palace: *A. gueldenstaedtii*, *A. sturio*, *A. ruthenus* and *A. persicus* were identified. Analysis of the sequenced mitochondrial DNA fragment sequences showed differences in the nucleotide sequences of the *cytb* region in the archaeological samples of the Menshikov Palace from extant representatives of *A. oxyrinchus* and other sturgeon species, including *A. sturio* (according to the NCBI database and the reference material).

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**DWARFISM GENES IN WATERMELON *CITRULLUS LANATUS*  
(THUNB.) MATSUM. & NAKAI**

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The melon crops are characterized by the development of long vine, which makes harvesting difficult. Using methods of molecular genetics and marker-assisted selection, it is possible to create dwarf varieties growing in more northern latitudes, including beyond the Arctic Circle. The aim of this work is to identify and mark genes in the genome of watermelon *Citrullus lanatus* responsible for the formation of dwarf plant forms based on the unique collection of melon crops of the Federal Research Center N. I. Vavilov All-Russian Institute of Plant Genetic Resources (VIR). The allelic polymorphism of watermelon dwarf genes *dw-1* (ABC transporter), *dsh* (gibberellin 20-oxidase) and *df* (gibberellin 3-beta-hydroxylase) was analyzed in genomes of VIR watermelon varieties. A total of 23 varieties and lines and 28 hybrids of *C. lanatus* were studied. A significant change in the motives, which could lead to changes in gene function, has been identified during sequencing in varieties with short and ultra-short vine. To identify candidate dwarfism genes in genomes of *Cucurbitaceae* species, the search of homologous sequences of watermelon dwarf genes was performed. In genomes of *Benincaseae* species except for *C. melo*, one copy of *dw-1* was identified, and in genomes of *Cucurbiteae* species and *C. melo*, two copies were found. The *dsh* gene was identified in two copies in *C. lanatus*, in four copies in *B. hispida*, and in one copy in other analyzed species except for *C. maxima* and *C. moschata*, in which the gene was not identified. In *C. lanatus*, *L. siceraria*, and all *Cucurbita* species, and in *B. hispida* and all *Cucumis* species, two and three copies of *df* were found, respectively. The present study was supported by the VIR project No. 0481-2019-0001.

## THE SEARCH OF SOMATIC EMBRYOGENESIS REGULATORS IN *MEDICAGO TRUNCATULA*

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Somatic embryogenesis (SE) is a way of plant regeneration consisting in the development of embryo-like structures from somatic cells. Like usual zygotic embryos, somatic embryos are able to develop into new plant. SE is used in biotechnology for genetic modification of plants, and the search of SE regulators may be useful for the development of new methods of plant transformation.

A number of transcription factors were found, whose ectopic expression can induce SE, including WUSCHEL homeodomain-containing protein from the *WOX* family, as well as *BABY BOOM*, *LEAFY COTYLEDON1* proteins etc. We found recently, that overexpression of *MtWOX9-1* gene from the *WOX* family can also stimulate SE in *Medicago truncatula*. Now we are trying to find the regulatory factors, functioning together with *MtWOX9-1* during SE. We used CRISPR/Cas9 technology to obtain plants with *MtWOX9-1* loss of function and to evaluate their SE capacity. Using phylogeny and expression analysis, we found other *WOX* genes which can possibly act redundantly with *MtWOX9-1* during SE. *WOX* genes are known to be regulated by small peptides from *CLE* family. We searched for *CLE* peptide which could suppress or activate *MtWOX9-1* expression and, therefore, to suppress or stimulate SE itself. We found one possible suppressor of SE among *CLE* genes. We also use EMSA and ChIP analysis to find *MtWOX9-1* targets.

The research was supported by the RFBR (20-016-00124).

**PHENOANALYSIS AND PHENOGENESIS OF RECOMBINANTS OBTAINED  
BY CROSSING OF MORPHOLOGICAL MUTANT *CLA* WITH ALLELIC  
CHLOROPHYLL MUTANTS *FLAVI* OF *A. THALIANA***

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The results of genetic analysis showed that mutations in different subunits of the same *flavi 1* gene in three allelic states, that determine chlorophyll deficiency acting pleiotropically on all morphological and quantitative traits, lead to the specificity of the expression of the fascial trait and depend on the genotypic background. Depending on the variant of crossing mutant *cla* with three from the *flavi1* series, a pronounced specificity of fasciated stem and inflorescence types was manifested. Fasciated plants have a ribbon-like (sometimes in the form of a spiral) or cruciform stem with inflorescences in the form of a basket, ridge and panicle.



# THE EMERGENCE OF CELL TYPES IN THE *DROSOPHILA* NERVOUS SYSTEM

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Embryonic neurogenesis in *Drosophila* is a highly coordinated sequence of cell fate decisions that bears many similarities to nervous system development in vertebrate organisms. These cell fate decisions are coordinated in space and time and depend on the immediate cellular environment of neurogenic cells. In the *Drosophila* embryo neuroblasts delaminate, divide and give rise to stereotypic lineages, which are defined in terms of the cell types, positions and connections they establish. While decades of genetics have uncovered factors necessary for many of the differentiation decisions, a genome-level understanding of specific cell types as neurogenesis unfolds has been impossible in vivo until recently. Single cell mRNA sequencing now allows a thorough understanding of the transcriptomes of neurogenic cells as neurogenesis unfolds in embryos.

We explore transcriptome dynamics in neurogenic stem cells and their decedents over the course of early neurogenesis to decipher the mechanisms that direct cellular specification and differentiation. The aim is to develop predictive models for the molecular mechanisms that drive early nervous system development. Early results yield unprecedented insights into molecular drivers that endow specific cells with their neuro-glial potential. Uncovering characteristic repertoires of signaling, guidance and adhesion molecules now allows us to predictively modulate developmental trajectories of neurogenic lineages in vivo by targeted perturbation.

## ***Protein variability and its regulation***

## ENVIRONMENTAL STRESS AND PROTEIN-BASED INHERITANCE

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Self-perpetuating protein aggregates (amyloids) and their transmissible variety (prions) are associated with some human diseases. Yeast self-perpetuating protein isoforms (yeast prions) control heritable traits. Metastable prions and non-transmissible aggregates (mnemons) control cellular memory of environmental or physiological changes. Stress-inducible chaperones control prion propagation. De novo prion formation is counteracted by the ribosome-associated chaperone complex. Environmental stresses and proteotoxic agents lead to massive accumulation of damaged aggregated proteins. Toxic effects of stress-induced protein aggregation are counteracted by the disaggregating chaperone machinery and by the spatial control apparatus, assuring asymmetric accumulation of aggregated proteins in the mother cell after cell division. Prions hijack the chaperone machinery for the purpose of their own propagation and escape the spatial control, that enables them to be inherited by daughter cells. Our data show that while environmental stress promotes formation of some prions, it also counteracts prion propagation via influencing the proteostasis networks and activating the spatial control machinery. Depletion of the ribosome-associated chaperone Hsp70-Ssb stabilizes some prions during stress and promotes both de novo formation and propagation of the protein-based determinants of cell memory. Defect of the spatial control machinery in the cells lacking the protein deacetylase Sir2 also results in prion stabilization under stress conditions. Cell aging impacts prion formation and propagation in a manner similar to environmental stress. Overall, the stress- and aging-related protein quality control machineries of the cell plays an important role in the processes of prion formation and propagation in yeast. An impact of environmental and aging-related stress on heritable protein aggregation, detected in yeast, could be applicable to human amyloid diseases. (Supported by NSF, RSF and RFBR.)

## **PROTEIN BIOSYNTHESIS DURING TUBER FORMATION IN DIFFERENT POTATO GENOTYPES *IN VITRO***

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The hormones and carbohydrates can activate the expression of various groups of genes responsible for biosynthesis of specific proteins that act on initiation of tuberization and growth of tubers of potato plants. It was suggested that among these proteins can present regulatory proteins that affect the translational activity of ribosomes.

Protein synthesis was studied in stress tolerant potato genotypes in presence of sucrose and phytohormones *in vitro*. Potato regenerated plants were cultivated in Murashige - Skoog medium supplemented with phytohormones kinetin and NAA.

Using the method of gel electrophoresis, the proteins biosynthesis were analyzed under denaturing conditions. Protein analysis showed a change in the intensity of protein components from the presence of kinetin or NAA in the culture medium.

The electrophoresis clearly distinguished protein components with molecular weights of 22, 27, 50, 70, and 78 kDa. Differences were observed in the intensity of protein zones depending on the phase of plant development. These proteins are more intense in the tuberization phase and less intense in the plant growth phase. It should be noted that in the tuberization phase, a number of other protein components appear that are absent in the growth phase (45, 29, and 57 kDa). That is, the set of protein components of the plant growth phase differs from the set of proteins of the tuberization phase.

It should be noted that there is a stable synthesis of two protein components related to 53 kDa and 27 kDa both in the tuberization phase and in growth phase. Apparently, the protein with a molecular weight of 53 kDa belongs to the large subunit of the key enzyme of photosynthesis RUBISCO and the protein with a molecular weight of 27 kDa belongs to the potatin protein.

A protein with a molecular weight of 70 kDa is also present in the tuberization phase and in the growth phase in all studied potato genotypes, but in the tuberization phase the intensity of this protein is more pronounced than in the growth phase.

# USING THE IDENTICAL PROTEIN GROUP CONTENT FOR QUALITY ASSESSMENT AND PROPER TAXONOMICAL ATTRIBUTION OF THE RESEQUENCED GENOME ASSEMBLIES

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The ongoing progress in next generation genome sequencing techniques have resulted in the emergence of numerous genome assemblies available in public access with many organisms being subjected to iterative genome or transcriptome sequencing. Protein sequences inferred from these assemblies may be further clustered based on their sequence similarity, up to total sequence identity, as implemented in the NCBI Identity Protein Groups (NCBI IPG) database. Most of the gene content in the resequenced genomes are expected to coincide with those reported in the previous assemblies for the organism. The percentage of the shared sequences within the total gene pool of the assembly may serve as an indirect measure of relatedness between novel assemblies and the pre-existing pool of verified entries. In this work we propose a genome assembly quality metric based on the genome IPG content and a respective bioinformatic tool to measure it. First, we estimated the average non-unique IPG content for over 200 species. The resulting intervals were further used as a baseline to which the novel assemblies of the respective species were compared to in term of their IPG content. To facilitate the estimation of the assembly IPG repertoire, we implemented a rapid string comparison tool based on locality-sensitive hashing, which allowed the incorporation of a distance calculating facility providing means for a less stringent sequence matching. All in all, the presented tool enables a rapid, resequencing-based analysis of assembly quality and gene content adequacy.

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## AMYLOID PROTEINS OF PLANTS

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Amyloids represent protein fibrils with highly ordered spatial structure. These protein fibrils are known to be involved in the development of dozens of incurable disorders in humans and animals. However, growing number of recent studies demonstrate that amyloids represent an important variant of quaternary protein structure implementing different biological functions in a great variety of species from archaea and bacteria to humans. Plants remained unexplored group of organisms in the field of amyloid biology.

Our bioinformatic study demonstrated that proteins bearing potentially amyloidogenic regions are abundant across proteomes of the land plants [1]. Notably, seed storage proteins containing CUPIN-1 domains belonging to the ancient  $\beta$ -barrel CUPIN domain superfamily contained QN-rich potentially amyloidogenic regions in most of the analyzed land plant species. Based on this observation we hypothesized that amyloid formation could play role in protein storage in plant seeds [1].

Experimental verification of our idea performed using seeds of garden pea *Pisum sativum* L. revealed amyloid formation by the seed storage protein vicilin *in vivo* and *in vitro* [2]. This protein contains two CUPIN-1 domains that have been demonstrated to form amyloids *in vitro* thus representing important amyloidogenic determinants of the full-length vicilin amyloid formation. We found that vicilin amyloids accumulate in the pea seeds during maturation and disassemble upon germination. Importantly, vicilin amyloids are resistant to treatment with digestive enzymes (pepsin, pancreatin). Thus, amyloid formation by vicilin and, probably, other storage proteins can reduce the nutritional quality of plant seeds [2].

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## PROTEOMIC INSIGHTS INTO *BACILLUS THURINGIENSIS* VIRULENCE FACTORS REVEAL LIMITATIONS OF SEROTYPING CLASSIFICATION

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A spore-forming bacterium, *Bacillus thuringiensis* (*Bt*), has tremendous agricultural potential for it produces specific and efficient pesticidal toxins. Conventional classification of multiple *Bt* strains implies serotype attribution via the agglutination reaction; however, this technique does not reflect phenotypic traits and phylogenetic relationships. The current research applies two-dimensional difference gel electrophoresis (2D-DIGE) with liquid chromatography/tandem mass spectrometry (LC-MS/MS) to investigate whether bottom-up proteomics allows strain identification. In this study, virulent and avirulent spores coupled with vegetative cells belonging to the *israelensis* serovar as well as virulent spores of two other commonly used serovars, *darmstadiensis* and *thuringiensis*, were proteomically analyzed. In total, 21 non-redundant proteins dispersed across 45 DIGE spots have been identified. Most of the proteins detected in the vegetative cells' culture are involved in constitutive cell metabolism, whereas in spores, Cyt1-like and ETX/MTX2-like Cry toxins were observed. While the proteomics approach revealed differences between spores and vegetative cells, it failed to distinguish distinct serovars. Given that, for the proteins identified, the distribution of respective genes in 104 serovar-attributed genomes was characterized to check if presence/absence patterns reflect the serotype attribution. Pangenome-wise phylogeny was reconstructed for the same purposes. As a result, genes encoding 15 proteins of 21 were observed in all assemblies analyzed using a 67% identity threshold; thus, they represented core genes. It is noteworthy that none of the phylogenetic approaches applied, namely, core genes single loci-based phylogenies, phylogenomics reconstruction, or flagellin-based trees, reflected serotype attribution. As phylogenetic inferences did not support the serotyping-based division, the re-evaluation of the established classification based on the large-scale phylogenetic approaches is highly demanded. This research was supported by the Russian Foundation for Basic Research (grant No 20-316-70020).

## **INTRACELLULAR AMINOACYLASE OF THE RECOMBINANT STRAIN OF *ESCHERICHIA COLI* LGE 36**

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The method for obtaining (isolation and purification) of the recombinant intracellular aminoacylase of *Escherichia coli* LGE 36 by two-fold ion exchange chromatography on DEAE-cellulose was developed. The enzyme was obtained with 30% activity yield and 32-fold purification level.

Comparison of physicochemical properties of obtained ferment with the literature data showed, that the recombinant intracellular aminoacylase of *E. coli* was similar to investigated aminoacylases from different microorganisms.

Similar to the abovementioned aminoacylases, investigated ferment has high stereo specificity, more effectively hydrolyses methionine derivatives, is activated with  $\text{Co}^{2+}$  ions and inhibited under SH reagents.

The study of the obtained data about substrate specificity of the recombinant intracellular aminoacylase of *E. coli* and acylases with microbial and animal origin shows, that the investigated ferment with its catalytic activity is similar to other microbial ferments (e.g. *Aspergillus oryzae*) and significantly surpasses the aminoacylase I from pig kidney by suite of hydrolysing substrates and comparative rate of hydrolysing. The essential peculiarity of the recombinant intracellular aminoacylase of *E. coli* is its unusual high catalytic activity and ability to high velocity hydrolyse N-acetyl derivatives of diamino acids, lysine and ornithine.



# *Human genetics*

## **SITE-DIRECTED ENGINEERING OF THE SUBSTRATE SPECIFICITY OF A THERMOSTABLE D-HYDANTOINASE (DIHYDROPYRIMIDINASE)**

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D-hydantoinase catalyzes an enantioselective opening of 5- and 6-membered cyclic structures and therefore can be used for biocatalytic production of optically pure precursors for biomedical applications. Enzymes of thermophilic bacteria in comparison with their mesophilic counterparts have higher biological and chemical stability, which is a valuable operational criterion for the development of biocatalysts.

We studied thermostable D-hydantoinase from *Geobacillus stearothermophilus* ATCC 31783 and showed that it is a manganese-dependent enzyme with low activity towards bulky hydantoin derivatives. Homology modeling with a known 3D structure (PDB code: 1K1D) allowed us to identify the amino acids to be mutated at the substrate binding site and in its immediate vicinity to modulate the substrate specificity. Both single and double substituted mutants were generated by site-directed mutagenesis at appropriate sites located inside and outside of the stereochemistry gate loops (SGL) involved in the substrate binding. Substrate specificity and kinetic constant data demonstrate that the replacement of Phe159 and Trp287 with alanine leads to an increase in the enzyme activity towards D,L-5-benzyl and D,L-5-indolylmethyl hydantoins. The length of the side chain is an essential parameter to consider when designing the substrate binding pocket for bulky hydantoins. The hydrophobicity in the proximity of the enlarged pocket in mutant D-hydantoinase is also important to facilitate the recognition of bulky substrates or the binding to the active site. A long inverse PCR was developed to clone a large region of DNA around D-hydantoinase gene. The sequence analysis revealed close location of three genes encoding for dihydropyrimidine dehydrogenase, dihydropyrimidinase (D-hydantoinase), and ribonucleotide reductase, all transcribed in the same direction. Therefore, D-hydantoinase is an authentic dihydropyrimidinase involved in the catabolic pathway of pyrimidine reduction in moderate thermophiles.

## DOXORUBICIN-INDUCED MTDNA INSERTIONS IN NUCLEAR GENOME OF HUMAN LYMPHOCYTES

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Over 750 nuclear DNA sequences of mitochondrial origin have been identified in human reference genome. Recent data indicate that de novo occurrence of mitochondrial DNA (mtDNA) in the nuclear genome may contribute to development of pathological conditions. Moreover, elevation of nuclear mtDNA was observed in tumor cells of patients with different types of malignancies such as breast and colon cancer. It was suggested that DNA double strand breaks (DSBs) may contribute to occurrence of mtDNA in the nuclear genome. Thus we hypothesized that DSB-inducing mutagens can influence the frequency of mtDNA insertions in the human chromosomes. To test our hypothesis whole blood cultures from two male and two female healthy volunteers were treated with DSB-inducing antitumor drug doxorubicin (DOX) at concentrations 0.025, 0.035 and 0.05 µg/mL for 24 h. Insertions of mtDNA in the chromosomes were identified using fluorescence in situ hybridization with application of mtDNA specific probes. Levels of chromosome damage were analyzed by cytokinesis-block micronucleus (MN) assay. DOX significantly elevated the frequency of metaphases with mtDNA insertions. In the control group, six mtDNA insertions were identified. In cells treated with DOX at concentrations of 0.025, 0.035 and 0.05 µg/mL in total 21, 18 and 16 mtDNA insertions were identified, respectively. Positive correlations between mtDNA insertions induced by DOX at concentrations of 0.025 and 0.035 µg/mL and chromosome length ( $r = 0.631$  and  $r = 0.502$ , respectively) were revealed indicating the randomness of DOX-induced mtDNA insertions. Positive correlations between mtDNA insertions and levels of MN induced by DOX at concentrations of 0.025 and 0.05 µg/mL were revealed ( $r = 0.985$  and  $r = 0.956$ , respectively) indicating the potential influence of chromosome damage on insertion of mtDNA in the nuclear genome. Overall these results indicate that DOX can promote mtDNA translocation in the nuclear genome of human lymphocytes which correlated with the level of DOX-induced chromosome damage determined by MN assay.

## **GENERATION OF NOVEL DISEASE MODELS USING CRISPR/CAS9 MEDIATED GENE EDITING IN RATS**

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CRISPR/Cas9 has revolutionized site-directed mutagenesis in animal models. We have used this method in the rat for functional genomics and for the creation of novel disease models since this species is preferred for cardiovascular and behavioral studies.

In a first novel disease model, we created a targeted mutation within a 15 bp region of the gene encoding phosphodiesterase 3A (*PDE3A*), which has been shown to be genetically linked to autosomal-dominant hypertension with brachydactyly (HTNB). This inherited disease clinically resembles salt-resistant essential hypertension and causes death by stroke before the age of 50 years. The CRISPR/Cas9-generated rat model carries a 9 bp deletion in the *PDE3A* gene analogous to a deletion also found in HTNB patients. Like in humans this deletion leads to a dominant disease characterized by hypertension and brachydactyly and thereby recapitulates HTNB. The mutated *PDE3A* gene drives mechanisms that increase peripheral vascular resistance causing hypertension. This new animal model will serve to elucidate the underlying mechanisms further and will facilitate the search for novel antihypertensive treatments.

## BIO-INSPIRED RANDOM SEARCH METHOD WITH EARLY STOP

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Very powerful classification and optimization technique are the mathematical models of Neural Networks (NN). Training of NN is performed with multivariate data of large dimensionality (big data). A common complaint of NN training techniques is the dependence of the final classification scheme on the purity and representability of the training data (lack of the generalization). For selecting the best training scenarios, we use a different modification of the random search method. These methods have roots in evolutionary biology and we will discuss a few examples of connected concepts of random search method and population genetics.

**Random search method with return on unsuccessful step and adaptation of random step.** In multivariate space of NN parameters, randomly is chosen set of parameters and then a random increment is added to them. After the calculation of the quality function, the increment is subtracted if quality function decreased and a new step is generated.

About 70 percent of mutations have damaging effects, and the remainder mostly being either neutral or weakly beneficial. Thus, the randomness of increment and subtractions of increment is biologically justified.

**Multiple random search and early stop.** For selecting important discriminants in 2 multidimensional populations are randomly selected a limited number of features and Mahalanobis distance between populations is calculated. Then randomly one of the features is exchanged with other features from the huge population pool and so on until the number of iterations expired. The best features are stored. Then, another cycle of iteration started and so on. As a final step best features from all samples are selected.

Integration of best variables from the independent search trials is biologically justified as a gene flow, i.e., the transfer of alleles from one population to another population through immigration of individuals. Gene transfer between species includes the formation of hybrid organisms that can introduce new potentially successful genetic variants.

**Early stop.** There is usually a geographic range within which individuals are more closely related to each other; thus, several sub-populations show significant genetic isolation from. However, if we plan to use the gene flow mechanism, we have to stop isolation relatively soon, not allowing the genome to change too much.

## **CALCULATION OF OPTIMAL VALUES OF MRI EXPERIMENTAL PARAMETERS FOR RESEARCHING THE HUMAN BRAIN ANATOMICAL STRUCTURE**

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Nuclear magnetic resonance imaging (NMR), also called magnetic resonance imaging (MRI), is considered the most relevant method of neuroimaging today. The results of MRI are used for research purposes and medical practice. For example, for the early diagnosis of Alzheimer's disease or for tracking the dynamics of the size of a brain tumor during proton therapy. The MRI experiment is based on the principle of nuclear magnetic resonance and is performed using a tomograph. During an MRI experiment, the nuclei of hydrogen atoms, protons, are disturbed by a radio frequency pulse of the Larmor frequency in order to receive a response signal. This signal is then encoded numerically for the purpose of studying and visualizing it. MRI examination is applicable to all organs of a person or animal. In this talk, we will discuss the MRI experiments of the human brain.

Before starting each MRI experiment, it must be set a value for each of the experimental parameters (such as repetition time (TE), echo time (TR), inversion time, and others). The set of values of all parameters of the experiment is called the tomograph mode. The main task of this work was to determine the degree of influence of changing the mode of the tomograph on the results of the MRI experiment of the human brain. For this goal, it was a comparative analysis of a group of sets of experimental MRI data of the human brain, performed in various tomography modes. In particular, a comparative description of the real data of two MRI experiments performed in different modes for one subject was prepared: two 3D models of the brain of one subject were obtained and it was a comparative analysis of their anatomical structures.

The study used MRI data from open sources and MRI data obtained on a Tomograph Siemens Verio Magnetom 3T (NRC KI Resource Center "Cognimed"). Data processing and analysis were performed in the System "Neuroimaging" based at the "Digital Lab" platform NRC KI, with involvement of the HPC4 cluster of the supercomputer NRC "Kurchatov Institute". This works was supported by the Kurchatov Institute research activities on the project «Creation of a distributed modular research and development platform "Digital laboratory"» approved by order of the Kurchatov Institute on July 02, 2020, No. 1055.

## **DETERMINATION OF EPIGENETIC MARKERS OF HUMAN PSYCHOEMOTIONAL STATUS**

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Assessment of the methylation status of specific loci of genomic DNA is one of the most effective ways to study psychoemotional human activity. We have developed and constructed the unique panel for next generation sequencing analysis of the methylation profile of epigenetic loci associated with the psychoemotional status of a person. The designed panel allows to determine the quantitative ratio of methylated and demethylated target CpG sites. The advantage of this approach is the ability to study the degree of methylation of tens and hundreds of loci during the analysis of one sample, as well as the ability to test hundreds of samples within a single run of the sequencer. Psychoemotional phenotypes were determined using the questionnaires "Perceived Stress Scale" and "Hospital Anxiety and Depression Scale" in 1250 representatives of three groups of the Belarusian population - the special forces group of the Internal Affairs Ministry of the Belarus Republic (special forces), the group of patients with personality disorders and the control groups. Significant differences between the groups had been revealed: the special forces group had been shown to be the most stress-resistant and the group of patients - the most emotionally unstable. Methylation levels of 25 gene loci of the brain neurotransmitter systems were analyzed with Illumina MiSeq in 144 DNA samples of the studied groups. The results showed that the ratios of methylated / demethylated sites of the target loci differed significantly in the DNA samples of groups with psychologically various phenotypes: most of the sites in the control group samples were less methylated than those in the samples of other groups. Noteworthy is the fact that the methylation profiles of different epigenetic loci of the same gene have very similar values. Obviously it is explained by the uniform methylation / demethylation process of the total promoter region of the gene.

## ALLOSTERIC DEGRADERS IN THE FIGHT AGAINST CANCER

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New polyfunctional heterocyclic compounds have been synthesized, which bind to the allosteric pocket in the immediate vicinity to the catalytic site of Epidermal Growth Factor Receptor (EGFR) and provide weak inhibition to tyrosine phosphorylation but increased receptor degradation (Iradyan et al., 2019). The anticancer action of allosteric compounds relies on two distinct routes. First, binding to EGFR allows rapid endocytic degradation of the receptor. Second, depletion of EGFR leads to sequestration of the sensor protein Bim with subsequent degradation of cytoskeletal proteins  $\beta$ -actin and  $\alpha$ -tubulin, causing cell detachment from extracellular matrix, and ultimately cancer cell death. Allosteric degraders also remarkably suppressed Ser69 phosphorylation in BimEL, associated with decreased EGFR phosphorylation, regardless of the addition of EGF ligand, the amino acid glutamine, or both to the growth medium. Glutamine starvation leading to ATP deficiency accelerates and enhances autophagic protein degradation. Given the multiple roles of glutamine in cells, including supplying a large number of phosphate groups to EGFR to support phosphorylation of downstream signaling pathways, this relationship can be summarized as "no glutamine, no EGFR signaling". Allosteric EGFR degraders have been shown to be active in triple negative breast cancer, prostate cancer and non-small-cell lung-cancer cells. Tumor growth inhibition has also been demonstrated in mice treated with these agents.

Chemotherapeutic interruption of phosphorylation flux is considered a promising means of reducing cancer progression. The EGFR tyrosine kinase inhibitors described to date block protein phosphorylation to provide a therapeutic effect in cancer cells. In contrast to these agents, allosteric EGFR degraders, while weak inhibitors, cause less dramatic disruption of interrelated signaling and metabolic pathways and open up an apoptotic pathway leading to more selective destruction of cancer cells. Therefore, allosteric degraders of proteins are of great interest for the development of patient-friendly therapies to reduce drug resistance, decrease harmful side effects, and prevent metastatic spread of aggressive tumors.



# **RADIOBIOLOGY**

***Genetics and epigenetics  
of radiation-induced genome instability***

## **LIKE FATHER LIKE SON: RADIATION-INDUCED TRANSGENERATIONAL GENOMIC INSTABILITY**

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Mutation induction in the parental germline is regarded as the main component of the genetic risk of human exposure to mutagens, including ionizing radiation and chemical mutagens. However, according to our data, paternal acute exposure to mutagens radiation can significantly destabilise the genomes of their offspring, which is attributed to the presence of a subset of endogenous DNA lesions, including single- and double-strand DNA breaks. The abovementioned results may imply that transgenerational destabilisation could potentially affect a number of health-related traits in the offspring, thus representing another component of the genetic risk of human exposure to environmental mutagens. To this end we designed two studies aimed to establish whether relatively moderate doses of ionising radiation or doses of anticancer drugs, both similar to those used to treat cancer patients can result in transgenerational destabilisation. According to our data, paternal exposure to clinically-relevant doses of mutagenic anticancer drugs results in transgenerational destabilisation observed among their first-generation offspring. In contrast, exposure to 0.1 Gy of  $\gamma$ -rays, the maximum dose to normal tissues per single radiotherapy procedure, does not affect mutation rates in the germline and somatic tissues of their offspring. In addition, chronic paternal exposure to 1 Gy delivered over the period of 2 weeks also failed to destabilise the genomes of their offspring. Taking together the results of these studies imply that the manifestation of transgenerational instability is triggered by a threshold dose of acute paternal irradiation and that the manifestation of transgenerational effects in humans may more often be found among the children of cancer survivors treated by mutagenic anticancer drugs than those therapeutically exposed to ionising radiation.

The epigenetic mechanisms underlying the phenomenon of transgenerational instability in mammals will be presented and discussed.

## **EPIGENETIC EVENTS IN WHEAT SEEDLINGS NUCLEI ABIOTIC STRESS RESPONSES**

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Epigenetic mechanisms, including DNA methylation, chromatin dynamics and small RNAs, play an essential role in the regulation of stress-responsive gene expression. Stress induced both long-term and short-term effects on epigenetic mechanisms in plant germ cell development. In our study of plant adaptation to abiotic stress, such as emission extremely high frequent of electromagnetic irradiation (EHF EMI) by different type of electromagnetic equipment and power station of cellular phone communication, have been investigated. Influence of EHF EMI in the mm-range (45-53 GHz) on nuclear envelope PL content and DNA methylation of wheat seedlings on 3 and 4 day after irradiation have been studied. Under impact of EHF EMI we observed falling of nuclear membrane charge, due to decreasing of anionic PL in content of nuclear envelope and simultaneous increasing of this ones in nuclear soluble fraction, which can change direction of facilitate transport through nuclear envelope according to proposed by us physical model. Although the genome often depicted as a static structure upon which protein-aqueous factors bind to control turn expression, the genome is actually highly mobile and capable of exploring the complex domain architecture of the nucleus, which in turn controls genome maintenance and gene expression. As direct stress response we investigated treated by mm-waves wheat seedlings of first generations on PL content of NE and Soluble nuclear fraction, as well on DNA methylation. For revealing long-term stress responses we study alterations in DNA methylation of treated by EHF EMI seeds in next generation. As obtained by us part of methylated DNA sites preserved in the next generation. It is assumed, that symmetrical methylation pattern are stably inherited through mitotic and to some extent also through meiotic divisions, and it might facilitate the inheritance of a stress memory. So alteration in many direction of nuclear envelope and adjacent to it peripheral chromatin may alter and as we show in this study chromatin conformation may changes through DNA methylation of transposable elements of genome under EHF EMI influence.

## **RADIATION-INDUCED GENOMIC INSTABILITY AND BYSTANDER EFFECTS AS DRIVERS FOR ENVIRONMENTAL EVOLUTION**

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The "non-targeted effects" of ionizing radiation including bystander effects and genomic instability predominate after low dose exposures and dominate response outcomes. These effects are unique in that no classic mutagenic event occurs in the cell showing the effect. In the case of bystander effects, cells which were not in the field affected by the radiation show high levels of mutations, chromosome aberrations, ROS and membrane signaling changes (horizontal transmission of mutations and information which may be damaging) while in the case of genomic instability, generations of cells derived from an irradiated progenitor appear normal but then lethal and non-lethal mutations appear in distant progeny (vertical transmission). The phenomena are characterized by high yields of mutations and distant occurrence of events both in space and time. This precludes a mutator phenotype or other conventional explanation and appears to indicate a generalized form of ROS mediated stress induced mutagenesis which is well documented in bacteria. The nature of the signal travelling between irradiated and unirradiated cells and organisms is currently unknown but our recent experiments suggest that there may be a physical component such as a vibration wave involved. UV photon mediated transmission has also been documented and the latter mechanisms can induce the release of exosomes which by themselves can induce bystander effects when added to recipient cells. This review will discuss the phenomenology of non-targeted effects both in vitro and in vivo, including recent data suggesting that excitation decay-induced photons in the UVA range lead to exosome release and consequent mitochondrial malfunction and elevated ROS in recipient cells. Photons, calcium, and neurochemicals are important in signal production while the exosome cargo, and cytokine mediated pathways especially TGF $\beta$  determine response to the signal. By highlighting some key challenges and controversies, concerning the mechanisms and more importantly, the reason these effects exist, current ideas about the wider implications of non-targeted effects in evolution and biology will be discussed.

## TRANSCRIPTIONAL ACTIVITY OF GENES INVOLVED IN CELLULAR HOMEOSTASIS AND APOPTOSIS IN CHRONICALLY EXPOSED PERSONS

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The objective of the work is to study the mRNA transcriptional activity of genes of reparation (*ATM*, *XPC*, *OGGI*), cell cycle (*TP53*, *MDM2*, *CDKN1A*), apoptosis (*BCL-2*, *BAX*), and immune response (*STAT3*, *GATA3*, *MAPK8*, *NF-kB1*, *PADI4*) in chronically exposed people with an increased rate of apoptosis of peripheral blood lymphocytes (PBL) in the long-term period. 119 Techa Riverside residents with the reconstructed exposure dose to red bone marrow (RBM) were included in the study. A decrease in the number of mRNA of the *STAT3* genes and an increase in the number of mRNA of the *PADI4* gene are registered in chronically exposed persons (mean RBM dose -  $577 \pm 84$  mGy) with an increased rate of PBL apoptosis in long-term period compared to unexposed persons. The analysis of the functional relationship of the studied genes showed the involvement of the p53 signaling pathway into the regulation of cellular homeostasis in the group of chronically exposed individuals with normal rate of PBL apoptosis. Regulation of intracellular processes by PADI4 signaling pathway was detected in the group of chronically exposed individuals with an increased rate of PBL apoptosis. The objective of the work is to study the mRNA transcriptional activity of genes of reparation (*ATM*, *XPC*, *OGGI*), cell cycle (*TP53*, *MDM2*, *CDKN1A*), apoptosis (*BCL-2*, *BAX*), and immune response (*STAT3*, *GATA3*, *MAPK8*, *NF-kB1*, *PADI4*) in chronically exposed people with an increased rate of apoptosis of peripheral blood lymphocytes (PBL) in the long-term period. 119 Techa Riverside residents with the reconstructed exposure dose to red bone marrow (RBM) were included in the study. A decrease in the number of mRNA of the *STAT3* genes and an increase in the number of mRNA of the *PADI4* gene are registered in chronically exposed persons (mean RBM dose -  $577 \pm 84$  mGy) with an increased rate of PBL apoptosis in long-term period compared to unexposed persons. The analysis of the functional relationship of the studied genes showed the involvement of the p53 signaling pathway into the regulation of cellular homeostasis in the group of chronically exposed individuals with normal rate of PBL apoptosis. Regulation of intracellular processes by PADI4 signaling pathway was detected in the group of chronically exposed individuals with an increased rate of PBL apoptosis.

## TRANSGENERATIONAL RADIATION-INDUCED EFFECTS IN INVERTEBRATES

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To date, the phenomenon of radiation-induced transgenerational inheritance has been extensively analyzed by studying the progeny of irradiated *Daphnia*. According to the results of our studies, compromised survival of the directly irradiated *Daphnia magna* was observed following exposure to a wide range of doses of acute exposure to  $\gamma$ -rays. The survival of *D. magna* exposed to 10 mGy of  $\gamma$ -rays does not significantly differ from that in controls, whereas it is significantly compromised at 0.1, 1 and 10 Gy. The life expectancy of irradiated animals was 20-30% lower than in the control. According to our data, parental exposure to 1 and 10 Gy significantly compromised the first viability. The magnitude of transgenerational effects was close to the direct effects on the parental generation. On the other hand, the survival of second-generation progeny of irradiated parents does not significantly differ from that in controls.

We have shown significantly elevated cytotoxicity in *D. magna* exposed to 0.1, 1 and 10 Gy. We found that the toxic effect also manifested in the F1 progeny of irradiated *D. magna*. We also found that in *D. magna* the level of free radical is significantly elevated following exposure. It would therefore appear that the abovementioned cytotoxic effects may explain the compromised viability of irradiated *D. magna*. According to our results, parental acute exposure of *D. magna* at doses of 0.100-10 Gy significantly compromise the main components of total fertility – the number and size of broods. The effect was maintained in the F1 generation and almost leveled in F2 and remains compromised following parental high-dose exposure to 10 Gy. The decrease in the number of broods in F0 is attributed to the cytotoxic effect of radiation on the germ cells.

Given that we analyzed parthenogenetic strain of *D. magna*, we irradiated of primordial diploid eggs, the results of abovementioned studies provide a plausible explanation for the compromised fertility of irradiated exposed *D. magna*.

The described transgenerational effects can only be explained by epigenetic mechanisms. The main argument for this is the very high incidence of mortality among the progeny of irradiated parents, which cannot be attributable to a Mendelian segregation of radiation-induced mutations.

## **UNCERTAINTY CONCERNING EFFECTS OF LOW DOSE RADIATION EXPOSURE; LESSONS FROM CHERNOBYL AND FUKUSHIMA**

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Few environmental toxins cause as much anxiety as radiation. This is not just due to the fact that it is invisible and odourless but because of the great uncertainty and disagreement among scientists about the risks to human and environmental health of exposure. As was the case with the cigarette industry, the scientists who study radiation effects tend to be involved in the nuclear industry or to work for government agencies that get their advice about risks from research funded by the industry. Much of our information about low dose risk either comes from laboratory experiments or from extrapolations using modelling approaches from high acute dose data. Where actual field work has been done, results suggest far greater radiosensitivity of species exposed in the field rather than the lab. Human epidemiology is very uncertain about low dose risk and relies on extrapolation from high dose data assuming a linear dose effect relationship. Some recent data suggests this may lead to underestimates of risk while other data suggests it overestimates risk. This presentation will discuss the controversy using examples from Chernobyl and Fukushima, and from our own recent low dose experiments. We will suggest that a major reason why there is disagreement is because low dose effects are very variable and uncertain. They are influenced by lifestyle and genetic factors, which makes attribution of radiation risk very difficult.



## GENE EXPRESSION IN CHRONICALLY IRRADIATED *TRIFOLIUM REPENS*, *DACTYLIS GLOMERATA*, AND *TARAXACUM OFFICINALE* FROM THE CHERNOBYL EXCLUSION ZONE

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The study of molecular changes in plant populations, growing under high levels of sparsely and densely ionizing radiation for many generations, provides unique data on the mechanisms of adaptation to long-term radioactive impact. Differential gene expression was **studied in 3 herbaceous species with different sensitivity to acute irradiation**: *Trifolium repens*, *Dactylis glomerata*, and *Taraxacum officinale*, sampled in the Chernobyl Exclusion Zone at two control and three radioactively contaminated plots. Several genes, as possible markers of chronic radiation exposure, were suggested for each plant species. The initial choice of candidate genes was based on previous high-throughput transcriptomic experiments. To develop primers for three non-model plant species, several programs were used: model plant *Arabidopsis thaliana* portal ARAPORT.ORG; basic local alignment search tool BLAST NCBI, multiple sequence alignment Clustal Omega 1.2.2. The efficiency and specificity of the primer pairs were tested for DNA and cDNA of the experimental plants. Generalization of the data obtained for *T. repens*, *D. glomerata*, and *T. officinale* populations demonstrated that all studied species of herbaceous plants were characterized by an increase in the expression of the *CAB1* homologue (encodes the photosystem II subunit) at contaminated plots, with the exception of *T. repens* that grew under simultaneous elevated temperatures. Populations that did not experience combined abiotic stress exposure showed an increase in the expression of genes associated with the water and hydrogen peroxide transport, intensity of photosynthesis, and stress responses (homologues of *TIP1* and *CAB1* for *T. repens*; *PIP1*, *HY5*, *CAB1* for *D. glomerata*; *CAB1* and *CIPK20* for *T. officinale*). The adopted approach for studying differential expression of candidate genes, presumably involved in plant response to chronic radiation exposure, on a broad range of wild plants may be recommended for the cost-effective screening of chronic radiation effects in a wide variety of species.

This work was supported by RFBR (project No. 18-34-20012, field sampling) and by RSF (project No. 20-74-10004, laboratory analysis).

## **DSBS AS A TRIGGERING EVENT FOR THE FORMATION OF AN ADAPTIVE RESPONSE IN *SACCHAROMYCES CEREVISIAE***

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It is well known that pre-exposure to low levels of oxidative stress could increase cells resistance to higher levels of the same or different type of stress when some intertreatment time (ITT) is given between both doses. The main philosophy of this nonspecific phenomenon named “adaptive response” (AR) is based on the potential of these stressors to generate reactive oxygen species, and respectively to damage DNA. Although it is believed that small levels of DNA damage could trigger the development of an AR, the question of their extent remains still open. Earlier, it was shown by us on *Chlamydomonas reinhardtii* strains that initially induced levels of double-strand breaks (DSB) equal to/or 1.5-fold higher than in the control could trigger an AR. Based on these findings the present work aims to clarify both the triggering role of initially induced DSBs and experimental design for the formation of AR in *S. cerevisiae*. For this purpose two strains - 551 (haploid) and D7ts1 (diploid) were used due to their different DNA susceptibility to low doses of the radiomimetic zeocin (Zeo). Despite their similar Zeo sensitivity, measured as cell survival, 10 µg/ml Zeo can induce small but statistically significant higher DSB levels than those in the control only in D7ts1. This was good starting point to assess the contribution of primary induced DSBs to AR formation. Standard protocol for cultivation and treatment was applied - priming (10µg/ml) and test (100µg/ml) Zeo dose. Molecular (constant field gel electrophoresis) and microbiological (Zimmermann’s test) endpoints were used. Different ITT and recovery times in the range of 30 – 60 min were tested. Well expressed AR measured as an increased cell survival, decreased genetic events and acceleration of DSB DNA re-joining was developed by D7ts1 when cells were given 45 min ITT and the priming dose induce 1.5 fold higher level of DSBs than those in the control. Maximum acceleration of DSB rejoining was observed 30 min after the test dose. The same experimental design did not result in the formation of AR in strain 551 where the priming concentration - 10 µg/ml did not possess DNA damaging potential. Here, both triggering role of initially induced DSBs and acceleration of DSBs rejoining were confirmed as mechanisms involved in the formation of AR in *S. cerevisiae* strains.

## **INDIRECT ACTION OF 150kHz ELECTROMAGNETIC RADIATION ON REPRODUCTIVE SYSTEM OF RATS**

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Intermediate frequency non-ionizing radiation of 100-300kHz is utilized for treatment of glioblastoma and other types of cancers. There are several publications demonstrated direct effect of the electro-magnetic (EM) radiation on cell division. Our experiments in 4 cell lines of mammalian and yeast cells have shown no such effect. But we observed reduction of cysts formation induced by Estradiol Valverate and small changes in certain types of ovarian cells in rats. To study mechanism of the effect, we irradiated Sprague Dawley rats by 150kHz EM radiation for 8-12 weeks and looked at hypothalamic-pituitary-gonadal axis in female rats. A disruption in this axis may result in most common endocrine disorder known as Polycystic Ovarian Syndrome. There were changes observed in some pituitary cells and in the level of specific hormones. The changes of hormonal level were observed in male rats as well without changes in any types of cells in reproductive system. We concluded that non-ionizing radiation could affect reproductive system of rats indirectly. Most likely it is happened by interaction of EM radiation with certain parts of brain, but exact mechanism is not studied yet.



# ***Radiation effects on biological molecules***

## THE NATURE OF DIRECT RADIATION-INDUCED POINT GENE MUTATIONS IN *DROSOPHILA MELANOGASTER*

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The results of the classical radiation-genetic studies of N.W. Timofeeff-Ressovsky (early 30s of the past century) on the direct and reverse mutation of separate individual genes in *Drosophila melanogaster* should historically be considered as the first experimental substantiation of the new, in contrast to the then dominant Betson's "presence-absence" hypothesis, conception on the nature of X-ray-induced recessive gene mutations, which are based on intragenic changes. This new conception was supported and further developed in subsequent radiation-genetic studies on *Drosophila*, mice and other model test- systems, as a result of which spontaneous and radiation recessive gene mutations were isolated into an independent class of "*point*" mutations with intragenic changes. It is significant that according to the accumulating data of molecular genetics, "*point*" mutations account for almost half of human genetic diseases. The importance of this class of mutations is emphasized by the fact that they become one of the main genetic effects in assessing genetic hazard (risk) of ionizing radiation using the doubling dose method. The observed progress in DNA technologies opens up real opportunities for elucidating the molecular nature of "*point*" gene mutations. Started by us under the leadership of N.W. Timofeeff-Ressovsky (late 60s of the past century) experiments on the getting of  $\gamma$ -ray- and neutron-induced recessive "*point*" mutations at the five different individual genes, these studies have now been extended by analyzing of the molecular nature of such mutations using PCR and sequence methods. The main results obtained for the *black* gene will be presented, among which the main ones are following: (i) Unlike  $\gamma$ -rays which induce a variety of DNA changes with a predominance of base substitutions, neutrons induce mainly changes leading to gene conversion events resulting from interallelic homologous recombination in the first diploid cleavage nucleus. (ii) The gametic doubling doses for phenotypically *black* gene "*point*" mutations and for intragenic base substitution mutations were estimated as 5.8 and 1.2 Gy, respectively, showing that doubling dose at the molecular level can be much lower than that at the phenotypic level.

## FEATURES OF UV-INDUCED MUTAGENESIS OF LACTIC ACID BACTERIA

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Traditional genetic approaches for selection mutants of lactic acid bacteria (LAB) by use of physical and chemical mutagenesis is purely investigated and not widely used because of specify their genomes. Comparative genomics has already revealed important similarities and differences in strains, species, and genera and will likely identify key genetic features responsible for the beneficial properties ascribed to probiotic lactic acid bacteria. LAB couldn't synthesize numerous amino acids and nucleotides, due to loss and mutations in their biosynthetic genes. Their requirement in growth factors is higher than human or animals. Beyond about 25% important genes loss, *Lactobacillales* have clear ancestral adaptations for nutritionally rich, microaerophilic environments. Therefore, the scope of this work was to examine the sensitivity of LABs to UV light and evaluate the effectiveness of UV-induced mutagenesis by direct selection of antibiotic resistant mutants. The LABu in this work were *L. acidophilus* GH 201, *L. delbrueckii* MH10 and *L. lactis* GH 204 and *E. coli* K-12 from laboratory collection. Cells from aerobically grown log phase broth cultures were transferred into phosphate buffer (pH 6.8) and irradiated by 15 w paired UV lamps at distance of 30 cm in range of 5 – 30 s and plated on LAPTg agar with and without antibiotics.

It was revealed that LABs were 1 to 2 log more resistant to UV, than *E. coli*. The yield of rifampicin (Rif) and streptomycin (Str) resistant mutants vary depending of UV exposure. The maximum yield of both antibiotic resistant mutants about 2-3 log observed at dose 20 sec giving survival about 0.1%. The relatively high resistance to irradiation can be explained by small size of LABs genomes (2.0 – 2.3 Mb), the absence of genes for sporulation, catalase, and other key enzymes of oxidative stress response (e.g., superoxide dismutase).

## **EFFECTS OF RADIOPROTECTIVE DSUP PROTEIN (DAMAGE SUPPRESSOR) ON THE RESISTANCE OF *D. MELANOGASTER* TO VARIOUS DOSES OF IONIZING RADIATION**

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Tasks associated with studies of effects of increased radiation doses on biological organisms are relevant due to the need to minimize risks of man-made disasters at nuclear power stations and during weapon tests, as well as to solve the problem of overcoming limitations for long-term presence of living organisms in space. One of the promising areas at the intersection of molecular biology and radiobiology is the search for new mechanisms to increase radioresistance. Representatives of the genus *Tardigrada* are considered one of the most radioresistant organisms on the Earth. They are able to survive after exposure to rare and dense ionizing radiation at doses up to 5 kGy. In 2016 the genome of *Ramazzottius variornatus* was sequenced, that is one of the most extremophilic species of tardigrades. After analyzing the data and comparing the proteins of *Ramazzottius variornatus* and other organisms, the unique Damage suppressor protein (Dsup) was found, which is specific to this tardigrade. This protein is localized in the cell nucleus, binds to nucleosomes and, to a lesser extent, to protein-free DNA. Dsup-bound DNA molecules were found to be more resistant to free hydroxyl radicals, which helps preserve the integrity of the molecule. According to experimental data, the expression of *Dsup* in plants and cell cultures increases their radioresistance, suggesting that this protein increases radioresistance in other organisms, in particular, in *D. melanogaster*, which is a model object for a wide range of modern genetic methods. In our laboratory, *D. melanogaster* lines stably expressing this protein were created which were irradiated with  $\gamma$ -rays at a dose of 1000 Gy in the FLNR JINR at the MT-25 facility. In the first three days after irradiation, the survival rate of the Dsup protein-synthesizing lines increased significantly compared to the control line and it is planned to evaluate the survival of *D. melanogaster* lines expressing Dsup after exposure to protons and heavy ions. Our studies may contribute to the description of new mechanisms of radioresistance and assess the possibility of applying these mechanisms in various fields of biology and medicine.



## EFFECT OF X-RAYS AND GIBBERELIC ACID (GA<sub>3</sub>) ON WINTER WHEAT

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Radiation mutagenesis is one of the most promising approaches among breeding methods widely used to increase genetic diversity and obtain initial material for breeding. It is clearly established that mutagenesis is not a simple act and its basis a biological process that is rather lengthy and complex deeply affected the main structural and biochemical components of living organisms. The main goal of the presented research was to study the genetic effect of ionizing radiation and phytohormone on winter wheat (*Triticum aestivum* L.) cv Armyanka-60. X-ray irradiation of wheat seeds with doses of 100 and 150 Gy led to decrease the level of mitotic activity of cells in comparison with the control samples, and at a dose of 200 Gy the mitosis was almost absent. Post-irradiation treatment with GA<sub>3</sub> (0.01; 0.005%) of seeds at doses of 100 and 150 Gy have had a radioprotective effect on the mitotic activity of the meristematic root tissue, and with an increase in GA<sub>3</sub> concentration the effect of radiation protection increased. It was shown that post-irradiation treatment of wheat seeds with GA<sub>3</sub> (0.01 and 0.005%) have reduced the number of structurally damaged chromosomes induced by X-ray irradiation at doses of 100 and 150 Gy. The effectiveness of the genetic effect of X-ray irradiation and GA<sub>3</sub> depends on both the dose and the GA<sub>3</sub> concentrations have been proven. The combined effects of X-rays and GA<sub>3</sub> has increased the frequency and have expanded the range of mutations that are of significant interest in selection.

## **DOSE-RATE EFFECT IN RADIATION BIOLOGY: DNA DOUBLE-STRAND BREAKS REPAIR EFFICIENCY**

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Understanding cellular responses to DNA double-strand breaks formation and repair after a particular radiation exposure mode is an important step towards understanding the potential biological consequences of such exposure. Although repair of radiation-induced DNA double-strand breaks is well studied for acute irradiation, responses to DNA double-strand breaks produced by continuous or chronic exposures to ionizing radiation are not well characterized. Yet, most real-life scenarios of human exposures to low and intermediate doses of radiation include prolonged exposures, lasting from hours to days or even years most of the time, and possible health consequences of such exposures are of great concern. The challenges for such continuous irradiation studies include both technical ones related to irradiation facilities and difficulties in interpreting results. For example, one would need to account for i) two opposite but concurrent processes of accumulation and elimination of DNA damage during exposure, ii) cell cycle redistribution, and iii) cell proliferation during exposure, i.e. a dose is split between mother and daughter cells, etc. Our findings indicate that continuous irradiation of normal human cells (fibroblasts, stem cells) triggers DNA repair responses that are different from those elicited after acute irradiation. During continuous irradiation, accumulation of cells in S/G2 phases and associated activation of homologous recombination DNA DSB repair pathway are observed. The observed activation of the error-free DNA DSB repair pathway suggests compensatory adaptive mechanisms that may help alleviate long-term biological consequences and could potentially be utilized both in radiation protection and medical practices. This research was funded by the Russian Science Foundation, grant no. 19-14-00151.

## RADIOPROTECTIVE POTENTIAL OF CREATINE

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The study of the radioprotective properties of natural compounds becomes relevant due to certain disadvantages of synthetic radioprotectors, such as high toxicity and limited duration of effect. Radiation causes oxidative stress (OS) in cells [1]. The creatine-creatine phosphate-creatine kinase (Cr-PCr-CK) system provides cell energy and calcium homeostasis, as well as ensures the structural and functional integrity of the mitochondria. These functions altogether determine the protective properties of Cr against OS [2]. The purpose of this work was to assess the radioprotective potential of Cr by using various models. As a dietary supplement, creatine monohydrate (Sigma) was used in a 0.9% glucose solution per os at the 1 g/kg of rat weight ratio two weeks prior and two weeks after the total one-time irradiation of 6.5 Gy=LD<sub>70/30</sub>. The post-radiation damage of the DNA was assessed by using the alkaline version of the DNA comet method [3]. The survival rate of rats was calculated according to the Kaplan-Meier method. The activity of CK was determined by the accumulation rate of Cr [4]. The DNA amount and cytomorphometry of the nuclei determined based on the television method on a cytospectrophotometer [5]. **The results have demonstrated** that Cr: 1) stimulates the tissue-specific adaptive capabilities of the rat brain and liver CK-Cr system; 2) reduces the radiation-induced genomic instability of hepatocytes; 3) has a radioprotective effect, affecting the survival rate of rats (up by 67%), as well as life expectancy (up by 39%); 4) halves the level of post-radiation DNA damage of peripheral blood mononuclear cells; 5) levels out the post-radiation alterations in the composition of the peripheral blood cells. Due to all the listed effects, the Cr can be recommended as a potential radioprotector.

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## **$\gamma$ -RADIATION AND LEAD IMPACT ON BARLEY SEED GROWTH DYNAMICS**

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The industrial toxicants contamination of the environment has become one of the factors hindering the agriculture development and posing a threat for the population health. Some of the main dangerous industrial pollution components are heavy metals and radionuclides. The purpose of the research was the study of microphenological germination phases of spring barley when exposed to ionizing radiation and lead. The subject of our research is Nur barley seeds (*Hordeum vulgare L.*), the first reproduction of 2019. The experiment lasted for 85 hours, with detailed observation from the 18th to 70th hours.

Seeds were placed in Petri dishes, 20 seeds in each, on double layer of filter paper. In total 800 barley seeds were examined. The germination took place in MIR - 254 thermostat (Sanyo, Japan) in the dark and at  $20^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ . To assess the plants response to stressors they were exposed to 20 Gy dose at 60 Gy/hr, where  $^{60}\text{Co}$  was used as a source of  $\gamma$ -rays (using GUR-120, RIRAE, Obninsk), and concentration of lead ( $\text{Pb}(\text{NO}_3)_2$ ) of 2 mg/ml. Concentration was converted into  $\text{Pb}^{2+}$ . To assess the microphenological germination phases the method by Kazakova was used (Kazakova, 2009).

Results: Separate exposure to ionizing radiation of 20 Gy wasn't significant for seeds development whereas lead contamination slowed it down. When the seeds were exposed to the combined action of radiation and lead the toxic effect reduction was observed.

All the seeds having crossed the "dot" phase, reached the terminate development phase, but in different time.

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Kazakova, A.S. & Kozyaeva, S.Y. (2009). Scale of microphenological phases of germination of summer barley seeds. *Agricultural Biology*, 3, 88-92.

## **PCR-ANALYSIS OF DIRECT RADIATION-INDUCED MUTATIONS OF WHITE GENE IN *DROSOPHILA MELANOGASTER***

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PCR-analysis of vast range of mutations (54  $\gamma$ -ray- and neutron-induced in sum.) of *white* gene of *Drosophila melanogaster* was performed. We have shown linear dependency between mutation frequency and applied dose for both radiation types. This shows one-hit mechanism of mutation induction. PCR-analysis have shown four discrete groups of introgenic DNA changes underlying mutations induced by both radiations. These groups are (i) PCR+; (ii) one missing fragment; (iii) extending deletions of several neighboring missing fragments; (iv) cluster changes of several independent missing fragments in the same mutant. Proportion for each group is different between  $\gamma$ -ray- and neutron-induced mutations. In particularly PCR+ mutations are mostly characteristic for  $\gamma$ -rays comparing to neutron-induced ones (86,4 and 33,3% respectively), one fragment loss is less present for  $\gamma$ -rays than for neutrons (12,8 and 33,3% respectively), mutants with extended deletions arise more rarely after  $\gamma$ -rays than those after neutrons (2,6 and 26,6% respectively), whereas cluster mutations occur with similar frequency for  $\gamma$ -rays and neutrons (2,6 and 6,6% respectively). Thus according to our results one-hit mutational events can be induced as a result of different introgenic changes at the DNA level.

## **Cu AND Cs IMPACT ON GENOME STABILITY OF SOIL BACTERIA**

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The threat of heavy metals pollution to public health and wildlife has stimulated close attention to developing systems, which could remove or suppress their toxic effects. Bioremediation with the bacterial mixtures of isolated strains have been demonstrated to be an effective method for heavy metal removal from contaminated environments. For developing efficient methods of metal bioremediation, along with the ability of microorganisms to mobilize or to immobilize heavy metals, their toxic effect should be estimated. Accordance to the proposed strategy it has been studied the potentially toxic metals and abundant contaminants (Cu and Cs) impact on genome structure of soil bacteria *Arthrobacter oxydans* isolated from the highly polluted area and known as the chromium-reducing bacteria with high potential for heavy metal detoxification. Differential scanning calorimetry (DSC) method has been applied. DSC technique allows detecting the early cell reactions to the metal presence *in situ*. Thermal analysis has revealed the metals influence on the DNA–protein (DNP) complex stability of *A. oxydans* in short-time action (2 h) of 1.0 mM and 0.5 mM copper. DSC analysis demonstrated the cell damages by multi-hit mechanism, resulting in the multiple disorders in the pattern of melting processes of bacterial cell population. It has been identified the shift of DNP melting temperature from 97.4 to 103.9°C and the decrease of the total heat capacity. The concentration of 0.1 mM copper affected membrane and protein thermostability and did not influence on the DNP complex of the studied bacteria. As concern caesium action, it was demonstrated that *A. oxydans* cells were tolerant to the presence of Cs in the range from 0.3 mM to 6.0 mM, as the thermostability of the DNP complex of the studied bacteria was not changed. Whereas the 10 mM concentration affected the intracellular processes, changing the character of cell population thermal profile, particularly affecting the regions of ribosome (60-85°C) and DNP complex, shifting DNP melting temperature from 97.4 to 98.6°C. Both metals increase DNP complex stability at their higher studied concentrations. The significant decrease of the cell populations at 0.5 and 1.0 mM copper concentrations has been confirmed by the colony forming units viability assay.

# **BIOPHYSICAL INTERPRETATION OF UNEXPECTED DEPENDENCE OF SYNERGISTIC EFFECTS ON THE INTENSITY OF PHYSICAL AGENTS OR THE CONCENTRATION OF HEAVY METALS SALTS**

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From many radiobiological studies devoted to the study of the effect of dose rate of ionizing radiation, UV light intensity or concentration of chemical pollutants on various biological objects, it follows that with a decrease in the intensity of these agents, biological efficiency decreases mainly due to the recovery processes occurring during the irradiation itself.

The report will present original results demonstrating the bell shaped dependence of synergism on dose rate of ionizing radiation, UV light intensity, or concentration of chemical compounds. These data indicate the existence of an optimal intensity of the agents used, at which the greatest synergistic effect is recorded. Any deviation of the intensity of the applied agents from the optimal value leads to a decrease in the of the synergistic enhancement ratio.

According to the mathematical model of synergism, at a relatively small dose rate of ionizing radiation or a concentration of chemicals due to prolonged exposure, the number of lethal damage and corresponding sublesions from hyperthermia is high. A further increase in the intensity of the agents used leads to a decrease in the number of thermal sublesions due to a reduction in the duration of exposure, which results in an increase in the synergistic enhancement ratio. This increase continues until the greatest synergy is achieved. Further, the effectiveness of synergism is reduced due to a decrease in the number of thermal damage and the duration of action at high dose rates or concentrations of heavy metal salts.

The results of the study indicate the existence of an unexpected dependence of synergistic effects on the intensity of physical agents or the concentration of chemical compounds, which is qualitatively explained by the use of a biophysical model of synergism.

# ***Radiation medicine***



## **RADIOBIOLOGICAL ASSESSMENT OF THE PHYSICAL PROTECTION OF COSMONAUTS BY THE ELEMENTS OF THE SPACESHIP**

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The urgency of the problem of ensuring the radiation safety of cosmonauts in long-distance space flights is quite obvious. Protons account for more than 50% of the absorbed dose. An additional contribution to the radiation dose of astronauts is made by secondary radiation formed during the interaction of cosmic radiation with the wall, structures and equipment of the spacecraft, as well as the astronaut's body. Of course, one should take into account the induced radioactivity inside the spacecraft. Purpose of the study: to conduct a radiobiological assessment of the radioprotective properties of the shell of the projected transport ship (PTS). The experiments were performed on 106 outbred mice. Mice were irradiated with 170 MeV protons at a dose of 1.0 Gy at an average dose rate of 0.5 Gy / min. For the study, a three-layer structure of the PTS aggregate compartment frame was provided, consisting of a layer of thermal protection on a carbon-fiber substrate. Additionally, an aluminum plate with a thickness of 4 mm, plexiglass with a thickness of 50 mm, and water phantoms with a thickness of 160 mm and 250 mm were used. It was found that the PTS shell, together with an aluminum plate, minimally protects the body of animals (mice) from the damaging action of 170 MeV protons. The use of protection in the form of 50 mm plexiglass also further weakened the damaging effect of protons in the chromosomal aberration test. Additional water protection with a water layer thickness of 250 mm proved to be effective. The experimental results showed the high importance of secondary radiation and its significant contribution to the development of radiobiological effects, even under conditions of complete screening from the primary proton flux. In the course of the experiment, it was found that in the medical cabin of the phasotron during the sessions of the accelerator operation, induced radioactivity is formed. In conclusion, it is worth noting that in radiobiological experiment established important evidence of the exceptional complexity of the problem of radiation safety of cosmonauts.

## **RADIOSENSITIVITY OF PEOPLE PROFESSIONALLY EXPOSED TO IONIZING RADIATION**

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The main problem in interpreting of the results of individual dosimetry comes down to different reactions of people to irradiation, which dictates the need for its quantitative determination and consideration when calculating the radiation dose. 137 people, who professionally contacted with ionizing radiation ( $\leq 0.2\text{mSv/y}$  physical dosimetry data) were examined. The frequency of chromosomal aberrations (FCA) in professionals was  $4.11 \pm 0.17\%$  (control -  $0.87 \pm 0.1\%$  -42 p) ( $p \leq 0.01$ ). Based on the total frequency of dicentrics and rings, the average cumulative radiation dose for the group was 0.103-0.145Sv. To study radiosensitivity, lymphocytes of "professionals" in vitro were irradiated with 0.5, 1 and 2Gy of  $\gamma$ -radiation (0.1 Gy/min) at the  $G_0$  stage of the cell cycle. The ratio of the FCA in "professionals" to the average FCA in control (using similar doses) is designated as the "coefficient of radiosensitivity" (CR). The FCA professional at a dose of 0.5Gy was lower than the average ( $9.79 \pm 1.04\%$  and  $12.0 \pm 4.4\%$ ,  $p \leq 0.05$ ), CR 0.33-2. Irradiation with 1Gy showed a radiosensitivity of  $18.38 \pm 0.66\%$  at the level of control  $17 \pm 0.84\%$ , CR 0.55-2.26. 2Gy irradiation induced an average  $34.25 \pm 0.6\%$  of CA ( $31.0 \pm 1.03\%$ ,  $p \leq 0.01$ ), CR 0.43-2.55. 95% of professionals develop an adaptive response or a standard response to average doses, but exposure to large doses of radiation causes radiosensitization in 30% of persons. In each variant there are individuals who are radiosensitive and radioresistant. Average radiosensitivity was 55-60%. People with average radiosensitivity observed a significant correlation ( $+0.683$ ;  $n=67$ ,  $\beta \geq 0.99$ ) between the values of doses determined by the results of cytogenetic analysis and effective dose of external radiation for the year. The cytogenetic method of biodosimetry evaluates the body's response to radiation, mediated by specific characteristics of the body. Therefore, when assessing individual doses, it is important to take into account individual radiosensitivity using certain correction factors.

## CHANGES IN CYTOGENETIC INDICATORS DURING THE APPLICATION OF COMPOUNDS OF THE COPPER-ORGANIC COMPLEXES

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One of the priorities of the contemporary radiobiology is the search for new efficient radioprotective compounds. In this field metal-organic complexes of high antioxidant activity are of special interest. Such complexes have low toxicity and obvious radioprotective properties. With a view to relieve possible radiation protective actions, we studied compounds of copper-organic complexes:  $[\text{Cu}(\text{PTA})_4]\text{BF}_4$  and  $[\text{Cu}(\text{PCN})(\text{HBP}_{23})]\text{BF}_4$ , in which legands are: PTA-1,3,5-triaza-7-phosphaadamantane and PCN-tris(cyanoethyl)phosphine  $\text{HBP}_{23}$ -trispyrazolilborato (hereinafter referred to as "PTA" and "PCN") which were synthesized in Camerino University (Italy) under the supervision of Professor Carlo Santini. The experiments were carried out on reproductive, white, outbred rats with middle-weight of 180-200gr. An hour before general, single-shot radiography of the animals with dose 5,3Gy and 7,0Gy, by PYM-17 equipment, PTA and PNC Cu-organic complexes were injected abdominally with dose of 20mg/kg in the form of an aqueous suspension. Since ionizing radiation is essential disturbing factor of cytogenetic status of the organism, we analyzed radiation-induced clastogenesis of the bone marrow cells (BMC) as one of the most informative indicators. Generally accepted method of cytogenetic analysis of white rats' BMC indices was used. Proliferative activity (mitotic index-MI), chromosome aberration (CA), cell ploidy (CP) was taken into consideration. We also performed hematological analysis of peripheral blood: red blood cell count, leucocyte count, level of hemoglobin. Basing on the survival results, cytogenetic and hematological indicators, it can be concluded that study complexes demonstrate noticeable radioprotective properties. Both complexes showed identical effects on experimental animals according to all observed criteria. In eruptive phase, on early dates of analysis (3<sup>rd</sup> and 7<sup>th</sup> days) both compounds soften the damaging effects of radiation with both doses which is especially obvious in significant shifts of mitotic index and CA in comparison with control data. On the last dates of observation (14<sup>th</sup> and 30<sup>th</sup> days) many data of analysis (MI, red blood readings) approached the standard data (norm), CA and number of leucocytes in comparison with the control data, demonstrate statistically significant difference. Based on the results obtained, it can be assumed that the studied Cu-ligands effectively promote reparative processes in bone marrow cells and have the properties of pronounced radiomodifiers.

## **CHROMOSOMAL MUTAGENESIS IN MAMMALIAN CELLS FOLLOWING IRRADIATION AT THERAPEUTIC BEAMS OF DIFFERENT QUALITY IN THE LOW DOSE RANGE**

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Radiobiological advantages of high-LET radiation (carbon ions, neutrons), possible synergism when they act simultaneously or sequentially with low-LET radiation are the rationale for developing fractionation schemes of combined irradiation. Before implementing such an innovative approach in clinical practice it is necessary to assess long-term effects of combined irradiation. Radiation-induced cancers and other hereditary genetic pathologies, arising from mutations, are the most dangerous types of them, the RBE-LET relationships for cell survival, induction of chromosomal aberrations (CA) and mutations being similar. Besides, decrease of absorbed dose per fraction <1 Gy in normal surrounding tissues may results in irregular dose-effect curves (phenomena of hypersensitivity (HRS) and induced radioresistance (IRR)).

We investigated the cytogenetic effects in Chinese hamster CHO-K1 cells induced by a scanning proton beam, a  $^{12}\text{C}$  ion beam with a LET of 10-12 and 25-27 keV/ $\mu\text{m}$ , 14.5 MeV neutrons which are considered for use in combined therapy, and  $^{60}\text{Co}$  standard  $\gamma$ -radiation. The irregular dose curves were observed for all types of radiation studied indicating the HRS/IRR phenomenon (a sharp increase in the CA yield to ~0.15 Gy and a plateau in the dose range of ~0.2-0.6 Gy) and LET-dependent increase in the structural mutations frequency in the region of IRR. The data obtained for radiations differing in LET suggest the general feature of the HRS/IRR effect. It should be noted that the effect is not observed in some cell cultures; therefore, its presence must be established for specific cell lines. Concerning the risk of radiation carcinogenesis, it is important that the dose curves of the CA yield following exposure to all types of radiation studied are irregular at doses below 1 Gy, i.e. in the range of absorbed doses in normal tissues when the daily fraction of the dose is applied to the tumor.

## CLINICAL AND EPIDEMIOLOGICAL ANALYSIS OF LENS CHANGES IN PATIENTS EXPOSED TO CHRONIC RADIATION EXPOSURE

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Probability of cataract development after chronic radiation exposure is a subject of radiobiology and radiation medicine.

**Objective:** to study peculiarities of lens opacity in exposed persons at later time after chronic radiation exposure with due account for dose impact and nonradiation factors.

**Material and Methods:** In the clinical department, an ophthalmologist examined 1,377 patients from the URCRM registry of exposed population. The same technique of medical examination with photofixation of lens opacity was applied to all examined individuals. A case-control technique was used to conduct the study. Individual exposure doses to lens were calculated on the basis of TRDS-2016 for the first time within the framework of the present study.

**Results:** As a result of the performed study among persons exposed to long-term ionizing low-dose radiation we have determined an exposure dose impact on risk growth of opacity in a posterior capsule and lens nucleus. No reliable statistical dependence of lens change with an increasing exposure dose in anterior capsule and cortical layers as well as colour change of a lens nucleus was obtained. Belonging to different ethnical groups, smoking, alcohol consumption, and obesity showed no impact on priority opacification development in any lens layers.

## HEAVY CHARGED PARTICLES IN RADIATION THERAPY AND RADIOBIOLOGY

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Radiotherapy with beams of heavy charged particles has significant advantages over photon and electron beams. The main advantage of using proton and ion beams in radiotherapy is the possibility of forming spatial dose distributions, the maximum dose area of which most closely coincides with the shape of the irradiated target and sharply falls beyond its borders.

In addition, under the influence of radiation on biological objects, the most important characteristic is the magnitude of the linear energy transfer (LET), on which the mechanism of the action of radiation on living cells depends. The LET spectra of proton beams, carbon ions, and heavier ions were measured. These results allow studies of the effects of radiation on biological objects.

It is also discussed how great are the advantages of carbon ion beams relative to proton beams and the possibility of using heavier ions. The main advantages of carbon ion beams are a weak lateral scattering and a high LET value, especially in the Bragg peak region, which leads to high values of the relative biological efficiency (RBE) of the beam reaching a value of 3 to 3.5. However, such a high RBE is observed only in the Bragg peak region, and upon irradiation of large targets, this advantage decreases significantly. In addition, a fragmentation region forms beyond the Bragg peak, giving an undesirable contribution to the dose distribution.

## INCREASED MAMMALIAN CELLS RADIOSENSITIVITY DUE TO THE PRESENCE OF GOLD NANOPARTICLES AND UNDER THE INFLUENCE OF $\gamma$ -RADIATION

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Increasing the efficiency of radiation therapy is important for the treatment of cancer. Therefore, it is crucially to ensure the efficiency of radiation in the tumor while maximizing the preservation of normal surrounding tissues. It can be achieved by using the modifiers of the dose, in particularly metal nanoparticles of a high atomic number. Such particles have a large value of cross section of interaction with photons. Gold nanoparticles are one of the most suitable for this purpose. **Methods and Materials:** *Cell culture:* In experiments we used a cell line of mouse fibroblasts C3H10T1/2. Cells were grown in Eagle MEM medium with addition of FBS (10 %), penicillin, streptomycin (50 un /ml). *GNP:* Gold nanoparticles (30-50 nm, with PEG) were treatment for 10 minutes with ultrasound. Then they were added to cells with concentration of 50  $\mu\text{g/ml}$  24 hours before irradiation.  *$\gamma$  – irradiation:* The cells were irradiated with a dose of 2 and 10 Gy with  $\gamma$  - rays, with the source  $^{60}\text{Co}$  and dose rate  $\sim 0.47$  Gy/min. *Cell fixation, antibody staining:* Fixation of samples were performed 0.5;6; 24 hours after irradiation. Samples were incubated with antiphospho Histone H2AX Clone JBW301, Alexa Fluor 488. **Results:** There were no significant differences in expression of  $\gamma$  -H2AX foci after 30 minute of irradiation with a dose of 0 Gy and 2 Gy with or without gold nanoparticles. We were not able to obtain data about absolute number of  $\gamma$ H2AX foci after 30 minutes of irradiation with a dose of 10 Gy. Significant increase in radiosensitivity of cells in presence of GNPs is observed after 6 hours of irradiation with a dose of 2 Gy and 10 Gy . The factor enhancement dose (FED) is 1.7 for 2 Gy and 1.4 for 10 Gy. **Conclusion:** We observe a significant increase in cell radiosensitivity of cells irradiated with a dose of 2 Gy in the presence of GNPs after 6 hours of irradiation. This may be due to the fact that during this time period, the mechanism of homologous repair is triggered and cells that have included GNPs and thus received more severe radiation damage require more time to repair DNA damage.

## **EXPERIENCE IN THE USE OF CONFORMAL PROTON RADIATION THERAPY AT THE PHASOTRON FACILITY**

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At the JINR Laboratory of Nuclear Problems, a program for the development of proton radiation therapy using synchrocyclotron beams was launched back in 1967.

The present work received a new round of development when a specialized radiological department was opened in Dubna. Since 2000, in the Medical - technical complex, sessions have been held to treat patients with different tumors. For the first treatment room of the complex, a three-dimensional conformal irradiation technique was developed for deeply buried tumors of complex shape.

The main methodological and technological stages of preparation:

- Immobilization of treatment area;
- Diagnostic and topometric imaging of an area to be irradiated. X-ray computed tomography (CT), Magnetic Resonance Imaging (MRI);
- Three-dimensional computer treatment planning;
- Manufacturing of individual beam modifying devices: complex shape collimators, compensating boluses;
- Realization and verification of treatment plans.

The main requirement when conducting precision radiotherapy is the full compliance of the position of the irradiated area in the diagnosis and in each of the subsequent sessions of fractionated irradiation of the patient.

The proton irradiation itself is carried out, as a rule, fractionated. Before the start of the radiation session, the therapeutic proton beam is removed into the treatment room and its careful dosimetry is performed. The beam profile, its depth-dose distribution, and dose rate are measured. These parameters are then monitored directly during irradiation of patient.

From 2000 to the present, 1299 patients have undergone a course of precision radiation proton therapy on JINR Phasotron beams.

Clinical and imaging results demonstrated that developed treatment technique provides precise dose deliverance to the prescribed target volume.



## THE BIOLOGICAL EFFICIENCY OF U-70 ACCELERATOR IN CARBON ION SOBP

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Radiobiological and physical advantages of carbon ions are well documented in radiation therapy worldwide. The first ion therapy center in Russia is planned for use in 2022 at the Institute of High Energy Physics. Physical, dosimetric and radiobiological studies are under way at the carbon ions beam of U-70 accelerator. To produce a spread-out Bragg peak (SOBP), a dedicated ripple filter was applied based on Geant4 radiation field calculations and the modified MKM model. Biological verification of the modified Bragg curve was the next experimental step in the ions beam studies.

Chinese hamster cells B14-150 in the stationary growth stage were irradiated in 2 ml microcentrifuge tubes. The tubes with cell suspension (30 000 cells/ml) were set in a water phantom at 6 cm SOBP with a step of 5 mm. One of the tubes was placed at the Bragg curve plateau. The energy of carbon ions at the water phantom entrance was ~ 450 MeV/n, irradiation dose was 2 Gy. The results obtained showed that in the plateau area the experimental ( $0.54 \pm 0.04$ ) and the calculated (0.61) cell survival fractions were in good agreement. Calculated values were underestimated on average by ~20% at SOBP. When averaging 12 experimental points, calculated and experimental survivals were ~0.36 and ~0.44 respectively. The disagreements observed may be due to the uncertainties in the Geant4 modeling of secondary charged particles contribution to dose, as well as the discrepancies in the expected  $\alpha$  and  $\beta$  coefficients in the linear-quadratic model at high LETs. Furthermore, the simplification of the irradiated object geometry or methodological errors of the dose monitoring can also be sources of differences.

In conclusion, although SOBP survivals were underestimated in calculated model, the filter provides flat biological effect throughout the spread-out Bragg peak.

## APPLICATION OF ULTRASHORT PULSED LASER TECHNOLOGIES IN BIOMEDICINE

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Nowadays, there is a raising interest in ultrafast lasers applications in a broad range of fields, including accelerator technology, medicine, biology and imaging. Particularly, ultrashort laser pulses can be used in the technology of two photon laser scanning microscopy and generation of ultrashort electron pulses in laser driven radio-frequency gun. It is assumed that accelerators of this type can make a revolution in radiation therapy of tumors by delivering an ultrashort low energy electron pulses.

In our studies, using two photon microscopy technique, we demonstrated a novel approach for the investigation of oxidative stress in human living red blood cells (RBCs) that could efficiently be applied in clinical research and testing of antioxidant compounds. We showed that two-photon laser scanning imaging is a valuable tool for studying oxidative stress in living RBCs not only under oxidative stress related different pathological conditions, including aging and radiation exposure on the organism, but also in the studies of the effects of different natural or chemically synthesized compounds.

Regarding the application of ultrashort electron pulses, currently we study their biological effect on the whole body rat irradiation to understand the effect of ultrashort pulsed electron beam on the organism, which will serve as a good basis for future cancer treatment studies. Overall first results indicate that the LD50 for electron beam whole body rat irradiation is 2 Gy with 2 Hz repetition rate, which we used for the main experiments to maintain the optimal survival rate. After the whole body rat irradiation by the low energy ultrashort-pulsed electron beam, pathological processes in animals' immune system increase up to the 3<sup>rd</sup> day, and the processes of recovery start from the 7th day of exposure continuing up to 14th and 28th days, demonstrating partial recovery of immune system in shorter period than in case of irradiation with X-rays or gamma-rays.

## **REACTOR-PRODUCED RADIOISOTOPES FOR DIAGNOSTICS AND THERAPY**

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Several radioactive nuclides can be produced with greatest efficiency in nuclear reactors. Examples are Mo-99, Tb-161, Ho-166, and Lu-177. Lu-177, e.g., emits low energy electrons (0,5 MeV) and photons (0.2 MeV), and it can be produced without admixtures of other elements and isotopes, i.e., ultra-pure and carrier-free. The half-life is 6.71 d which optimally allows for production, transport, and clinical administration, and at the same time avoids problems with long-living isotopes. The energy of the beta-radiation leads to a range in tissue of about 2 mm which leads to an optimum safety for the patient. The clinical product is made by the company ITM (Isotope Technology Munich) the labs of which are situated on the ground of the research reactor FRM II. Of course, there are about ten other reactors all over Europe including Russia from where the raw Lu-177 is delivered in order to guarantee a constant availability in the hospitals. Another field of increasing importance is the production of Mo-99 because there were already shortfalls in availability in Europe and world-wide. At the high-flux research reactor FRM II, an irradiation facility of uranium pellets has been constructed and awaits “hot” testing. Mo-99 is a fission product and will be extracted in Belgium. Latest results will be reported.

## POSSIBLE APPLICATIONS OF *SACCHAROMYCES BOULARDII* IN SPACE MEDICINE

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During space flight, the cosmonaut's body is exposed to various stress factors that can lead to the development of infectious diseases due to impaired immune regulation and changes in the microbiome. The health of the crew members can be maintained by special probiotic therapy. Ground-based research has shown that probiotics have the potential to improve certain conditions that manifest during space travel, such as gastrointestinal disorders, dermatitis, and respiratory infections. However, it is not known how probiotic strains will behave in space flight conditions.

The aim of this work is to study the effect of cosmic ionizing radiation as one of the stress factors during space flight on the stability of the probiotic properties of the yeast *Saccharomyces boulardii*. In clinical practice, drugs based on it, Enterol (France) and Cosm-o-tentic (Belgium), have proved to be highly efficient and safe probiotic agents that can prevent and treat a number of diseases of the gastrointestinal tract. These drugs have a pronounced stimulating effect on the immune system of the macroorganism, ensure the early destruction of pathogenic microorganisms, and contribute to the rapid restoration of normal intestinal microbiota. We have carried out laboratory studies of the biochemical, genetic, and microbiological properties of the strains isolated from these preparations. An analysis of the sensitivity of probiotics to  $\gamma$ -rays and high-energy proton irradiation has been carried out. The mutability of the strains was also assessed by the frequency of antibiotic resistance mutation induction.

# **RADIOECOLOGY**

***Characteristics of natural and  
anthropogenic radiation***

## TRITIUM — EFFECTS ON AQUATIC LIVING ORGANISMS, DOSE-FORMING EFFECTS

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Tritium is one of the most important biologically significant radionuclides. Being a part of the water molecule,  $^3\text{H}$  is an ideal label for studying the exchange processes between the stratosphere and the troposphere when studying air-mass transport and the water cycle in nature. It is a part of many organic compounds, including biologically important ones.

Tritium is a by-product of many radiochemical reactions in the nuclear industry, and its effects on water organisms, particularly low-dose effects, are worthy of special attention. The low-dose effects of tritium on aquatic microbiota have been intensively studied, using luminous marine bacteria as a model microorganism. Low-dose activation has been demonstrated and has been explained by the signalling role of reactive oxygen species, through the “bystander effect” in bacterial suspension. The activation of microbial functions in natural reservoirs by the low content of tritium might result in unpredictable changes in food chains and a misbalance of the natural equilibrium. The transformation of tritium from free tritium water into organically bound compounds occurs mainly in the dark and at a temperature of 25 °C. When tritium enters the fish body with food, up to 56 % of tritium accumulates in the muscle tissue and up to 36 % in the liver. About 50 % of tritium in the liver is bound in non-exchangeable forms. The use of fish fillets containing tritium as food products and the use of drinking water with a tritium content at background levels (2-4 Bq·L<sup>-1</sup>) does not significantly contribute to the total dose load on the human body.

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## ORIGIN OF ENHANCED GAMMA RADIATION DURING THUNDERSTORMS

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Natural gamma radiation (NGR), one of the major geophysical parameters directly connected with cloud electrification and lightning initiation, is highly enhanced during thunderstorms. At low energies below 3 MeV, the enhancement of NGR is due to natural isotope radiation, and for energies up to 50 MeV, it is due to the operation of the newly discovered electron accelerators in the thunderclouds. For the first time, we present a comprehensive model of the enhanced fluxes of radiation incident on the earth's surface during thunderstorms. In addition to the already explained minute-long fluxes of high-energy electrons and gamma rays from relativistic runaway electron avalanches (RREA), we clarify also the origin of hour-long isotropic fluxes of low-energy gamma rays from the Rn-222 progenies. Natural radioactivity is a source of continuous exposure of human beings to radiation. Radiation protection of living organisms requires an understanding of all sources and possible ways of enhancement of the radiation levels that can double for several hours in the energy domain of hundreds of keV. Therefore, individual irradiation doses can be exceeded during thunderstorms. The models used for the forecasting of thunderstorms and other severe atmospheric phenomena need an accurate account of the ionizing radiation in the atmosphere.



## **EVALUATION OF THE SPECIFIC VOLUMES RATE OF RADIOACTIVE WASTE FOR COMPLIANCE WITH THE BASIC PRINCIPLE OF THE INPRO METHODOLOGY ON THE EXAMPLE OF RUSSIAN NPPS**

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According to the basic principle of the INPRO (IAEA) methodology, new NPP projects should be safer for personnel, the public and the environment than comparable currently operational NPPs. Increased safety can be achieved by reducing the generation of radioactive waste during normal operation of NPPs, since radioactive waste is one of the most significant environmental factors continuously generated as a result of the nuclear energy using [1]. A convenient criterion for assessing the compliance of NPPs with the basic INPRO principle [2] is the specific waste rate ( $\text{m}^3/\text{Gw}\cdot\text{h}$ ). To implement the basic principle of the INPRO methodology, it is necessary that the values of the specific waste rate of the radioactive waste annual generation of all designed power units categories do not exceed the values of the specific ones of the "best" practices of currently operational NPPs.

This work considers data on the volumes of radioactive waste at ten Russian nuclear power plants for the period 2008-2018. The average and median values of the specific RW formation index, expressed in  $\text{m}^3/\text{Gw}\cdot\text{h}$  are calculated. The medians for solid radioactive waste of the categories VLLW, LLW, OLW and HLW are equal  $1,5\cdot 10^{-2}$ ,  $3,3\cdot 10^{-2}$ ,  $3,3\cdot 10^{-3}$  and  $2,8\cdot 10^{-4}$   $\text{m}^3 / \text{GWh}$ , respectively; for liquid radioactive waste of the LLW and ILW categories -  $1,4\cdot 10^{-3}$   $\text{m}^3/\text{GW}\cdot\text{h}$  and  $2,5\cdot 10^{-3}$   $\text{m}^3/\text{GWh}$ , respectively. The median volumes of solid radioactive waste and liquid radioactive waste were compared among three types of reactor plants operating in the Russian Federation – RBMK, VVER, and BN. The studied nuclear power plants are categorized using the calculated specific volume of RW formation.

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## MONITORING OF LONG-TERM AND LARGE-SCALE DEPOSITION OF AIR POLLUTANTS BASED ON MOSS ANALYSIS

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A brief historical review is given on the development and milestones of the moss biomonitoring technique used to study atmospheric deposition of trace elements, nitrogen, persistent organic pollutants (POPs);d radionuclides of technogenic and natural origin in Europe, as well as cosmic dust. The relevance of these studies to the UNECE Convention on Long-range Transboundary Air Pollution (LRTAP) is shown. Examples of the long-term activity of the ICP Vegetation (International Cooperative Programme on Effects of Air Pollution on Natural Vegetation and Crops) established in 1987 are given to illustrate the tendencies in behavior on a large scale of air pollutants such as heavy metals, nitrogen and persistent organic pollutants. In agreement with the long-term strategy of the LRTAP Convention to enhance participation and improve air quality in Eastern Europe, the Caucasus, Central Asia, and South Eastern Europe, efforts to extend the moss survey to former republics of the USSR such as Armenia, Azerbaijan, Georgia, Moldova, Kazakhstan, and Uzbekistan were successfully undertaken. Around 15 teams were formed in Russia to cover moss sampling in Northern and Central Russia, Western Siberia, and the Far East of Russia (Kamchatka and Sakhalin). To date, 42 countries, including Bulgaria, expressed their desire to participate in the 2020-2021-2022 moss survey. Analytical methods and approaches to data interpretation are reviewed.

**Keywords:** moss biomonitoring, trace elements, nitrogen, POPs, radionuclides, cosmic dust

## **RISK ASSESSMENT OF RADIOECOLOGICAL POLLUTION IN THE AREA OF OPERATING COAL TPP**

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Coal is one of the most important fuel resources. However, using coal in coal-fired thermal power plants (TPP) due to emissions of relatively large amounts of gaseous and solid pollutants (SO<sub>2</sub>, NO<sub>x</sub>, CO<sub>2</sub>, hydrocarbons, fly ash), chemically hazardous microelements (arsenic, mercury, cadmium, etc.) and uranium and thorium series radionuclides creates a risk of air pollution and a threat of radiological impact on the environment. This problem is typical for such countries, for example, Mongolia, where coal-fired TPP are dominant in the structure of electricity production.

The study of radioactivity in coals and the first estimates of the release of radionuclides into the environment after burning coal appeared in the 60s of the last century [1], however, there is still a required more detailed assessments of the radiological impact of operating coal-fired TPPs, depending on their territorial location and the quality of the coals used. The average content of uranium and thorium in coals is at the level of 1-10 mg/kg, however, coals from Mongolia or South Africa, for example, have content up to 100 mg/kg for Th and U, and it should be taken into account when assessing the local the influence of coal-fired power plants. Also during the combustion of coal reactions with components of flue gases are occurred and the formation of volatile compounds of individual radionuclides, in particular <sup>210</sup>Pb and <sup>210</sup>Po, is possible. In this regard, research to determine the content of radionuclides of the radioactive series Th and U in environmental objects (soil, plants) around operating coal-fired power plants have particular relevance.

Thermal power plant No.4 (TPP-4) is the largest power plant in Mongolian energy sector which generates over 67.8 percent of electricity for central region and 60.3 percent of heat for consumption of the Ulaanbaatar city. The main fuel of the TPP-4 is brown coal of the Baganaur and Shivee-Ovoo coal mines. A year usage of coal is about 2.4 million tones. To assess the risk of radioecological pollution of the environment the content of elements in samples of coal, ash, as well as soil and plants around TPP-4 was determined by gamma-spectrometry, X-ray fluorescence and gamma activation analysis. *J. Tadmor* Radioactivity from Coal-Fired Power Plants: A Review //J. Environ. Radioactivity. N 4. 1986. P.177-204.

## **DYNAMICS OF $^{90}\text{Sr}$ AND $^{137}\text{Cs}$ IN ICHTHYOFAUNA OF THE CHERNOBYL NPP COOLING POND DURING 2010–2020**

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The Chernobyl NPP (CNPP) cooling pond (CP) is an artificial water body. Before the start of the drawdown of water level in the CP its length was 11 km, average width - 2 km, surface area - 22.7 km<sup>2</sup>, prevailing depth - 4-7 m but on separate areas - up to 18-20 m and the amount of 149 million m<sup>3</sup>. In late 2014, after the cessation of pumping water into the CP from the Pripyat River, the natural decline in the water level has begun. In place of the CP three water bodies with different size and volume were formed. We compare the average specific activity of  $^{90}\text{Sr}$  and  $^{137}\text{Cs}$  for two periods: the first one is 2010-2014 - before the drawdown of the water level, and the second one is 2019-2020 - when the process of drawdown of the water level was completed. Thus the drawdown of water level in the CNPP CP resulted in an increase of  $^{90}\text{Sr}$  in fish, which reached 359-2084 (875±317) Bq/kg w.w. during 2019-2020 for the pray fish species and 175-375 (394±117) Bq/kg for the predatory fish compared to 41-285 (136±53) and 43-139 (84±28) Bq/kg during 2010-2014, respectively. The specific activity of  $^{137}\text{Cs}$  in this case was 530-1720 (1020±275) Bq/kg for the pray fish of the CP during 2019-2020, and 1220-4160 (2715±904) Bq/kg for predatory fish compared to 608-2327 (1073±338) and 1740-8340 (3273±649) Bp/kg during 2010-2014, respectively. The  $^{137}\text{Cs}$  concentration in fish during the period of studies with some oscillations within the measurement error remained at the same level, while the specific activity of  $^{90}\text{Sr}$  since 2016 increased. The specific activity of radionuclides in fish during the period 2019-2020 exceeded the permissible levels, according to the standards adopted in Ukraine for fishery products in 5.0-59.5 times for  $^{90}\text{Sr}$  and in 3.5-27.7 times for  $^{137}\text{Cs}$  concentration. This study was partly supported by the the SATREPS Project “Strengthening of the environmental radiation control and legislative basis for the environmental remediation of radioactively contaminated sites” and by National Research Foundation of Ukraine (Project No 2020.02/0264).

## **FUKUSHIMA AND CHERNOBYL: SIMILARITIES AND DIFFERENCES IN BEHAVIOUR OF RADIONUCLIDES IN THE ENVIRONMENT**

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The ease with which radionuclide moves through the environment and is taken up by plants and animals is determined by its chemical forms and site-specific environmental characteristics. The peculiarities in climate, geomorphology and  $^{137}\text{Cs}$  speciation in the fallout were demonstrated to lead to differences in migration rates of  $^{137}\text{Cs}$  in the environment and rates of its natural attenuation. It has been revealed that in the exclusion zone the Fukushima-derived  $^{137}\text{Cs}$  is strongly bound to soil and sediment particles, which reduces potential bioavailability of this radionuclide. Up to 30% of the deposited  $^{137}\text{Cs}$  on soil of the exclusion zone were found to be incorporated in hot glassy particles (“Cs balls”) insoluble in water. These particles are decomposing in the environment essentially slower as compared with Chernobyl derived fuel particles. Wash-off from the slopes of contaminated catchments and river transport are key long-term pathways for radionuclide dispersal from contaminated areas after the Fukushima accident. The climate conditions for the Fukushima Prefecture of Japan are characterized by higher annual precipitation (1300-1800 mm/year) with maximum rainstorm events during typhoon season. Typhoons Etou in 2015 and Hagibis in 2019 demonstrated the substantial redistribution of  $^{137}\text{Cs}$  on river watersheds and floodplains and in some cases natural self-decontamination occurred. Steep slopes of Fukushima catchments are conducive to higher erosion and higher particulate r-Cs wash-off. Irrigation ponds in Okuma and Futaba towns demonstrated persistent behavior of  $^{137}\text{Cs}$  similar to the closed lakes in Chernobyl, its concentration is decreasing slowly and showing regular seasonal variations: the  $^{137}\text{Cs}$  concentrations tend to grow in the summer and decrease in the winter.

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## **ACCUMULATION OF ARTIFICIAL RADIONUCLIDES IN *CALLISIA FRAGRANS* UNDER OPEN-AIR HYDROPONIC CONDITIONS OF ARARAT VALLEY**

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Production of ecologically safe medicinal raw material is one of the most challenging issues of plant biotechnology and pharmaceutical industry. Meanwhile, poor ecological conditions of the environment affect negatively on living organisms and the balance of ecosystem at all. Among the pollutants of the environment radionuclides are considered to be one of the most dangerous. These contaminants penetrate into the plants through atmosphere, water, soil and cause internal irradiation of the consumers by trans-locating in various parts of the plants. Therefore, the controlling over their content in the environment remains an urgent problem. Taking into account the above mentioned, in the frame of this study an assessment of  $^{90}\text{Sr}$  and  $^{137}\text{Cs}$  artificial radionuclides in various parts of valuable medicinal plant *Callisia fragrans* has been done for the first time under open-air hydroponic conditions of Ararat Valley. The plants were grown hydroponically in the experimental field, which is located in territory of the Institute of Hydroponics Problems after G.S. Davtyan (National Academy of Sciences, Yerevan, Armenia). Determination of the artificial radionuclides in the medicinal raw material was conducted according to the established radiochemical methods in the Laboratory of Radiation Agrochemistry of the Institute. The obtained results were compared with the established safety requirements. The results of the experiments shown, that both in the leaves and lateral sprouts of *Callisia fragrans* grown hydroponically under open-air conditions of Ararat Valley the content of  $^{90}\text{Sr}$  and  $^{137}\text{Cs}$  is much lower than MAC.

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## **FEATURES OF THE RATIO OF CONCENTRATIONS OF ARTIFICIAL RADIONUCLIDES $^{137}\text{Cs}$ AND $^{90}\text{Sr}$ IN THE ECOSYSTEMS OF SALT LAKES OF THE CRIMEA AT THE PERIOD 2016-2018**

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It is known that the ratio precipitated on the water area of the Crimean water bodies and on the Black Sea of  $^{137}\text{Cs}$  to  $^{90}\text{Sr}$  was 3: 1 in 1986 (Polikarpov et al., 2008), by 2018 in the Black and Azov Seas this ratio was 1.5:1 and 1:1, respectively.

According to our data, by 2018 the average concentration of  $^{90}\text{Sr}$  in the water of Crimean salt lakes exceeded that for  $^{137}\text{Cs}$  and their ratio varied from 1: 1.3 to 1: 21.9. All this testifies to a different pathway of radionuclide entry into the Crimean salt lakes. If for  $^{137}\text{Cs}$  the main path is atmospheric transport and precipitation immediately after the accident at the Chernobyl nuclear power plant, then a significant input of  $^{90}\text{Sr}$  into the studied water bodies occurred after the accident at the Chernobyl nuclear power plant with Dnieper water through the North-Crimean canal until 2014 (Mirzoyeva et al., 2018). In addition, all the lakes studied by us are drainless, i.e. are natural reservoirs that accumulate radioactive substances that fall into them over time.

It was determined that in 2016-2018 the distribution of  $^{90}\text{Sr}$  and  $^{137}\text{Cs}$  between bottom sediments and water in the studied lakes was insignificant, but had a different character for these radionuclides. The main factors of the  $^{137}\text{Cs}$  and  $^{90}\text{Sr}$  concentration levels in the Crimean salt lakes are: sources of radionuclides entry, and their distribution in the components of lake ecosystems is influenced by the hydrochemical and hydrological characteristics of the lakes, first of all, the salinity level of the environment and the lack of drainage of water bodies.

In 2016–2018 the  $^{137}\text{Cs}$  and  $^{90}\text{Sr}$  concentrations in water of all investigated Crimean salt lakes and control sampling stations did not exceed the Control Levels for these radionuclides in seawater (R52.18.852-2016, 2017).

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## **RADIONUCLIDES DISTRIBUTION AND ASSOCIATED ECOLOGICAL RISK IN THE ENVIRONMENT OF ARMENIAN MOUNTAINS**

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Mountain regions are of great interest for radioecological studies, first of all, serving as a barrier for pollutants from air-migration flow, such as global artificial pollutant Cs-137, as well as being an object to reveal the altitudinal distribution and lateral migration of naturally occurring and artificial radionuclides. The purpose of the work was to estimate the activity concentrations of natural Ra-226, Th-232 and artificial Cs-137 radionuclides and assess the associated ecological risk for non-human biota in the environment of Armenian mountains. Soil samples from areas of the highest mountains (Aragats Mountain, Syunik Highland, Pambak, Geghama, Vardenis, Bazum, Meghri, Khustup-Katar Ridges) were sampled within the two-phase project of Radioecological monitoring (REMA I and II), implemented during 2016-2020. The altitudes of sampling profiles were within the ranges of 900-3200 m above sea level. A gamma-ray spectrometer by CANBERRA (HpGe detector, DSA-1000 multi-channel analyser, Genie 2K and LabSOSC software) was applied for radionuclides identification and determination of their specific activities. The activities of natural terrestrial Ra-226 and Th-232 were calculated from the activities of daughter products and artificial Cs-137 was measured directly from 661.7 keV energy line. The radionuclides' distributions in soil were identified: Cs-137 has altitudinal-distribution patterns, for natural radionuclides, such patterns did not observe.

ERICA Tool (Version 1.3) based on ERICA Integrated Approach (Environmental Risk from Ionising Contaminants: Assessment and Management) was used for assessment of ecological risk to non-human biota of studies regions. The assessment was implemented by using the ERICA Tier 1 approach based on activity concentrations of radionuclides in soil and reference organisms from FREDERICA database. The average and highest activities of radionuclides were used for the assessment. The Risk Quotients (RQ) were evaluated and potential exposed non-human organisms were revealed: for mean values the RQ was 1.03, that is the values are below the 10  $\mu\text{Gy/h}$  reference dose rate, for maximal values the RQ was 4.5 indicating that one of the radionuclides, in particular, Ra-226 is above the 10  $\mu\text{Gy/h}$  reference dose rate and the limiting reference organisms are Lichen and Bryophytes.



## **MONITORING OF RADIOACTIVITY IN NORWEGIAN FOOD AND FEED – LORAKON**

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Radioactive pollution caused by the Chernobyl accident of 1986, reaching Norway, also put at risk the quality of locally produced food and feed. So, one of the priority issues became controlling and ensuring the safety of Norwegian food and feed via continuous monitoring of radioactivity in it. To this function serves LORAKON - Local Radioactivity Control for Food and Feed - a monitoring network set up after the Chernobyl accident and initially consisted of a few laboratories in Norway's regions affected by the Chernobyl fallout. Last upgraded in 2015, the Network presently comprises 11 laboratories and 7 organizations across Norway and is equipped with identical NaI detectors for gamma-activity measurements. The Network is coordinated by the Norwegian Radiation and Nuclear Safety Authority (DSA) and the Norwegian Food Safety Authority. The upgraded LORAKON pursues a dual goal: 1) Conducting local monitoring of radioactivity levels in food and feed, controlling thus the possible radioactive contamination intake. This is also important to ensure the safety of exportable Norwegian foods and goods and those imported to the country. To monitor radioactivity levels in daily intakes of staples, a program 'Radioactivity in Food Basket Products' is initiated covering purchase and measurements of foods from groceries or food markets. The laboratories are tasked with food purchase, sample treatment and preparation, radioactivity analyses, and reporting the results to DSA. 2) Maintaining a good reputation and analytical strength of the Network to be used in case of a nuclear incident that would cause radioactive contamination in Norway's regions. In such a case, it is important to mitigate the consequences as soon as possible. One of measures to be then taken is measurements of local foods to offer reliable recommendations on what food is safe to eat or implement restrictions for human health to protect. The Network can also be used in case of a nuclear incident in neighboring states that would cause a cross-border spread of radioactive contamination rather than a direct impact on the Norwegians. For food safety-related doubts and panic to avoid, measurement-based documentation of radioactivity levels will also be required. So, the LORAKON built up through years of monitoring of radioactivity in food and feed, is an important part of emergency preparedness and response system in Norway that can be used to properly and timely handle nuclear incidents.

## **DETERMINATION OF SOIL-TO-PLANT TRANSFER FACTORS IN LOCAL VEGETABLES, USING STABLE ISOTOPES OF CAESIUM AND STRONTIUM IN UAE – A PILOT STUDY**

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Following the recent advancement of the UAE nuclear programme, the necessity for a radioecological database for local conditions has become urgent. None or few relevant work for radiological environmental impact assessment and modelling for arid regions has been published or is available, especially for the UAE. The major pathway of radionuclides via food to humans begins with their uptake through roots by plants. Transfer factors (TF) are key pre-requisites to better estimate contamination levels in the relevant media and depend on several factors including soil and plant type, climate, and the type of nuclides. There is currently no established standard protocol for determining soil-to-plant TF values in the UAE. Therefore, an extensive pilot root uptake experiment is performed at ENTC to estimate local TF values. The characteristics of the soil, climate, local biodiversity, agricultural and pastoral practices, as well as dietary habits of the population were considered for the protocol development of the study. A majority of UAE soils have only limited suitability for agriculture due to high salinity. Torripsamments (75% of UAE), also known as sweet sand, is a red sandy desert soil enriched with minerals and used with bio-compost to increase the fertility and water retention capacity in agricultural soils and thus yield of crops. Some of the most locally produced agricultural vegetable crops namely, tomatoes, cucumbers, and eggplants, were cultivated outdoors and measured. They were potted in a prepared mix of sweet sand, bio-compost, and fertilizers (representative of local agricultural practices) and watered daily for 20 weeks with distilled water for the control group and with prepared solutions of  $\text{Sr}(\text{NO}_3)_2$  and  $\text{Cs}(\text{NO}_3)$  respectively, for the experimental group. These stable isotopes (Sr-88 and Cs-133) simulate long-lived fallout radionuclides, Sr-90 and Cs-137, and are assumed to exhibit the same transfer kinetics as their radioactive counterparts. The transfer factor values will be determined by the ratio of concentrations in the vegetables & leaves to that in the soil. This substantiated protocol will be extended in the future to other agricultural crops and desert plants and in a larger scale experiment with radionuclide spiking.

## PLUTONIUM ISOTOPES IN THE SEVASTOPOL BAY ECOSYSTEM (THE BLACK SEA)

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As a result of global fallout after nuclear weapon testing in open environments and atmospheric fallout caused by Chernobyl NPP accident, the Black Sea and Crimean Peninsula ecosystems received significant amount of long-lived plutonium isotopes  $^{239+240}\text{Pu}$ . The Sevastopol Bay is the largest and the most widely used in economic and recreational purposes one on the Crimean Black Sea coast. The aim of the study was to analyze the modern (2010-2019) levels of plutonium radioisotopes in water, suspended matter, bottom sediments and biota (fish, mollusks and algae) of the Sevastopol Bay.

$^{239+240}\text{Pu}$  activity concentration in the Sevastopol Bay water was  $1.10 \pm 0.21 \text{ mBq} \cdot \text{m}^{-3}$ . This value was slightly lower than in the western deep Black Sea area and in the Sea of Azov where  $^{239+240}\text{Pu}$  activity concentrations were  $1.66 \pm 0.21$  and  $3.17 \pm 0.37 \text{ mBq} \cdot \text{m}^{-3}$ , respectively.  $^{239+240}\text{Pu}$  content in suspended form in the coastal waters –  $0.59 \pm 0.05 \text{ mBq} \cdot \text{m}^{-3}$  – was an order of magnitude higher than in open sea areas with the values of  $0.040 \pm 0.009 \text{ mBq} \cdot \text{m}^{-3}$ . The values of  $^{239+240}\text{Pu}$  activity concentration in the surface (0-5 cm) bottom sediments ranged from  $110 \pm 20$  to  $993 \pm 90 \text{ mBq} \cdot \text{kg}^{-1}$ . The highest  $^{239+240}\text{Pu}$  activity concentrations among the Sevastopol Bay representative biota species were obtained for brown microalgae *Cystoseira crinita* (Duby, 1830) and for the shells of bivalve mollusks *Mytilus galloprovincialis* (Lamarck, 1819) with the values varied in ranges of  $1.55 \div 11.40$  and  $3.82 \div 12.11 \text{ mBq} \cdot \text{kg}^{-1}$ , respectively. The lowest  $^{239+240}\text{Pu}$  content, ranging from 0.3 to  $1.8 \text{ mBq} \cdot \text{kg}^{-1}$ , was observed in fish – *Trachurus mediterraneus ponticus* (Aleev, 1956), *Sprattus sprattus phalericus* (Risso, 1827) and *Merlagius merlangus euxinus* (Nordmann, 1840).

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## **DEVELOPMENT OF SCIENTIFIC METHODS FOR DETERMINING THE ENERGY SPECTRUM OF NEUTRON RADIATION TO ASSESS ITS RADIATION EXPOSURE**

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An approach to assess the radiation exposure of neutron fluxes to personnel in nuclear facilities in a wide energy range has been developed. It is based on the multisphere method, where in the process of measuring the thermal neutron counter is sequentially placed in the center of spherical moderators of various diameters. Knowledge of the sensitivity functions of such spherical detectors make it possible to reconstruct the neutron energy spectrum from the measured counting rates. The reconstruction of the initial flux spectrum is based on calculating the minimum discrepancy of the measured counting rates and obtained by convolution of the sensitivity of the neutron detector in the moderator sphere and a set of model neutron emission spectra.

The developed method for assessing the radiation exposure of personnel to neutron fluxes at nuclear facilities in a wide energy range made it possible for the first time to estimate the radiation doses to personnel of facilities using fast neutron reactors. The method was tested at Institute of Nuclear Materials (Zarechniy) and Beloyarsk NPP. The correction coefficients for individual neutron radiation dosimeters obtained by the differences of the calibration field from the neutron radiation spectrum under conditions of real measurement form the basis for improving the radiation safety of nuclear facilities at the present stage of the development of science and technology.

## TRANSFER FACTOR FIELD EXPERIMENT FOR DATE-PALMS IN ARID REGIONS

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For arid desert climates like the United Arab Emirates (UAE), date palms (*Phoenix dactylifera* L.) are the most common cultivars, grown on large scales for food products and livestock fodder, as well as for city landscaping. With over 100 gene-variants and 40 million trees, UAE produces 12% of the world's dates. We are studying the date-palms in the Sas-al-Nakhl locality of Abu Dhabi in terms of their natural radioactive content and their baseline properties for soil-to-plant transfer of radionuclides. Not only the date fruits directly, but their by-products, seeds, pedicels, palm leaves, barks and other parts are industrially processed, packaged, and sold. In past, activities on the orders of 300 Bq/kg for dried flesh and 100 Bq/kg for pits have been measured for factory produced dates. Concentration ratios (CR) of NORM ( $^{238}\text{U}$ ,  $^{232}\text{Th}$  and  $^{40}\text{K}$ ) and anthropogenic radionuclides between soil and palm trees have never been measured and is part of this pilot study. It becomes critical from radioecological point of view, given that the UAE is rapidly progressing towards a full-fledged nuclear energy program in this decade. We have sampled 14 trees, covering different varieties, with the soils under their canopies. The specimens for gamma-spectrometry are being prepared using standardized methods of cleaning, drying, pulverizing, sieving, and packing in Marinelli beakers. A 40% relative efficiency HPGe spectrometer is under calibration with mixed-gamma sources and IAEA standard reference materials, for measurements of isotopic specific activities in the fruits, leaves, soil, etc. We have carefully adapted the similar transfer factor estimation methods for fruits like apples, strawberries etc., and present here, a consistent and reproducible protocol for the baseline date-palm soil-to-plant CR monitoring in the UAE. Along with that, a preliminary set of output CR data from our experiments will be shown, which can be employed for other arid and semiarid regions as well as added to the international databases. We also outline the extension of this study to larger areas, like Al Ain, Al Dhafra (near Barakkah, the location of nuclear power plants), and with additional local crops, like jujube, mangoes, green vegetables, and grasses.

## **A COMPREHENSIVE DATABASE OF RADIOECOLOGICAL DATA ON ARID AND SEMI-ARID CLIMATES. IUR TASK GROUP ON RADIOECOLOGY IN ARID REGIONS**

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The behaviour of radionuclides is well investigated in regions with temperate climate, e.g., after accidents such as Chernobyl and more recently Fukushima and other releases due to e.g., nuclear weapon testing. However, limited radioecological data are available for tropical regions and especially for arid or semi-arid climates. Relevant publications are scarce; only very few laboratory experiments have been conducted to study the migration of radionuclides in soil and water, and to determine transfer of radionuclides to endemic plants and animals.

In order to investigate the influence of climate change on transfer behaviour of radionuclides, the IUR has established a task force to study the transfer in dry regions.

The objective of the IUR taskforce is to create a state-of-the-art database on the behaviour of the major radionuclides in arid regions, and to identify existing gaps including studies not yet published or available in the open literature.

Currently, our data base has about 850 entries both for Natural occurring and anthropogenic radionuclides from 14 countries located in arid zones. The Data base includes transfer factors for 37 different plants and fruits. There are both, plants to be found in temperate climate and endemic plants specific for arid climate, such as palm dates, okra, Jew's-mallow etc.

The comparison of transfer factors for arid and temperate climates (TRS472) shows essential differences.

## **ALPHA-EMITTERS $^{238,239+240}\text{Pu}$ IN THE BLACK SEA ECOSYSTEM AFTER ACCIDENT ON CHERNOBYL NUCLEAR POWER PLANT**

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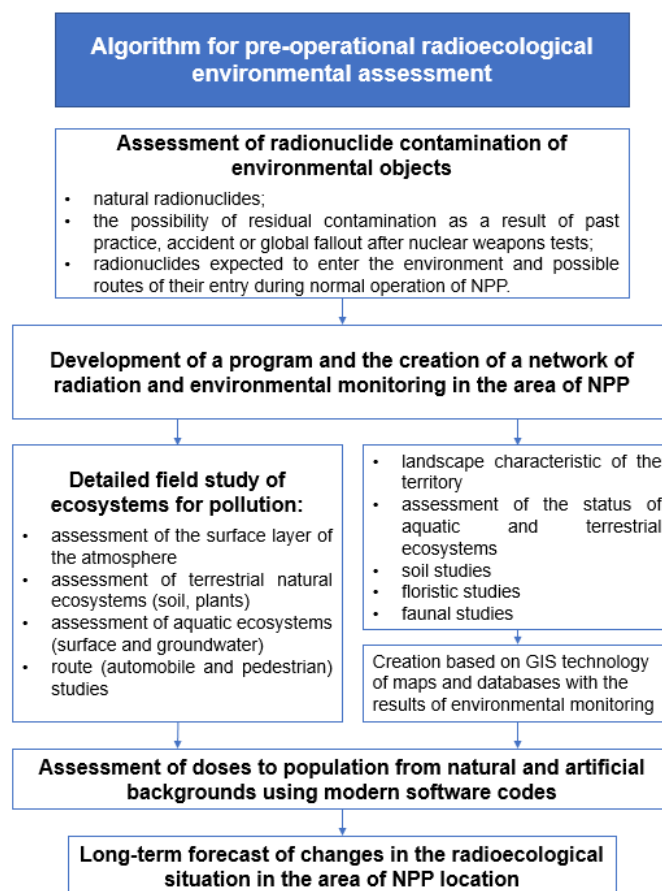
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The Black Sea received abundant amount of plutonium alpha-emitting radioisotopes  $^{238,239,240}\text{Pu}$  with global fallout after nuclear weapon testing in atmosphere and after accident on the Chernobyl nuclear power plant (ChNPP) in 1986. The aim of the study was to investigate the contamination of the Black Sea hydrobionts and marine environment by man-made plutonium radioisotopes and to use of the comparative analysis, biogeochemical and equidosimetric approach to the ecological effects assessment from  $^{239,240}\text{Pu}$  on hydrobionts in a wide range of these radioisotopes concentrations in seawater. Distribution of plutonium radioisotopes in abiotic (water and bottom sediment) and biotic (algae, mollusks, fish) components of the Black Sea ecosystems was studied during the post-Chernobyl period at different areas as a rule at western and central parts of the sea. This study was devoted to the analysis of a radioecological situation in the Black Sea. The long-term accumulation and migration trends of these radioisotopes were analyzed concerning abiotic components of the Black Sea after the ChNPP accident. The Black Sea zones were revealed that have an increased ability to accumulate these radioactive isotopes. The estimations of the elimination fluxes of the after-accidental plutonium alpha-radioisotopes into bottom sediments in open and coastal areas of the sea were obtained. Evaluation of irradiation dose rates formed with alpha-radiation of  $^{239,240}\text{Pu}$  in Black Sea hydrobionts was carried out. Important quantitative characteristics of plutonium migration in the Black Sea ecosystems were obtained and application of the comparative analysis together with biogeochemical and equidosimetric approach to the ecological effects assessment of the dose commitments from the artificial  $^{239,240}\text{Pu}$  to hydrobionts in contemporary radiological situation in the Black Sea as well as in different potential radioecological situations in wide range of alpha-emitting plutonium radioisotopes concentration activity in seawater was fulfilled.

# ALGORITHM FOR PRE-OPERATIONAL RADIOECOLOGICAL ASSESSMENT OF THE ENVIRONMENT STATE IN THE AREA OF A NEW NUCLEAR POWER PLANT CONSTRUCTION

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A methodology has been developed for carrying out a pre-operational radioecological assessment of the environment state in the area of a new nuclear power plant construction. The developed methodology allows assessing the environmental impact correctly, determining the radiation doses to the population and workers during the operation of the NPP; define the sites and methods for monitoring the radioecological situation in the area of the nuclear power plant; prevent erroneous interpretation of radiation monitoring data. A set of developed methods and approaches for pre-operational radioecological research is implemented on the example of the Belarusian NPP. Practical experience and the results of a public pre-operational study can be applied at other nuclear and radiation hazardous facilities both in Russia and abroad.





## INVESTIGATION OF THE DESTRUCTION OF CHERNOBYL ORIGIN HOT PARTICLES UNDER THE SOIL MICROMYCETES' INFLUENCE

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The most dangerous artificial radionuclides are alpha-emitting long-lived isotopes of uranium and transuranium elements. Radioactive microparticles are currently one of the common forms of transuranium nuclides in the environment. After the destruction of the Chernobyl nuclear power plant (ChNPP) 4th unit radionuclides from the reactor core fell to the environment. The studies of radionuclides migration on highly contaminated areas of the ChNPP 5 km zone demonstrated that at present, the <sup>241</sup>Am activity could be traced to the depth of 50-60 cm (Bondarkov et al., 2011, 2016). This fact indicates that intensive processes of destruction of the fuel deposition take place in the soil. According to some assumptions, these processes can be influenced by soil micromycetes (Neu et al., 2005; Dighton et al., 2008; Lukyanova et al., 2008; Zhdanova et al., 2010, Francis et al., 2015).

The influence of irradiated generations of some micromycetes strains on the destruction of Chernobyl origin hot particles with high content of <sup>241</sup>Am, <sup>90</sup>Sr, <sup>137</sup>Cs and plutonium isotopes are studied in the presented work. Studies of the interaction of "micromycete – hot particle" were performed by culturing the fungus in a liquid medium in oligotrophic conditions with hot particles for 60 days. Then all components of the system were separated for study: hot particles, the culture liquid, the mycelium. The specific activity for each system component was determined using gamma-spectroscopic measurements and alpha-spectroscopic analysis after radiochemical separation. The specific activity of <sup>90</sup>Sr in samples was determined with a beta-spectrometer "SEB-50." The amount of biomass was determined gravimetrically. The <sup>241</sup>Am and plutonium isotopes direct accumulation of micromycetes was detected for the first time. The reported study was funded by RFBR, project number 19-05-50095.

***Radiation effect  
on populations and ecosystems***

## BIOLOGICAL EFFECTS IN *BROMUS INERMIS* POPULATIONS UNDER CHRONIC RADIATION AND CHEMICAL POLLUTION EXPOSURE

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Ionizing radiation and chemical pollution are strong mutagenic factors (Mehes-Smith, Nkongolo 2015, Oudalova et al. 2002, Pozolotina et al. 2008, Słomka et al. 2011). In the course of a long existence, living organisms have adapted to the natural radiation background clarkes of heavy metals and metalloids. However, environmental pollution by radionuclides and heavy metals is associated mainly with human influence. Technogenic pollution can accelerate microevolutionary processes in natural populations. The smooth brome (*Bromus inermis* Leyss.) is a polymorphic species of the Poaceae family (Williams et al. 2011). For this species, octoploid (genome AAAAB<sub>1</sub>B<sub>1</sub>B<sub>2</sub>B<sub>2</sub>,  $2n=8x=56$ ), hexaploid ( $2n=6x=42$ ) and tetraploid (AABB,  $2n=4x=28$ ) forms have been described (Tuna et al. 2004). Investigated populations of *B. inermis* have grown under different levels of man-made pollution (heavy metals – Nizhny Tagil Iron and Steel Works; radionuclides – East-Ural Radioactive Trace) and unexposed localities. The average DNA content of *B. inermis* of  $22.61\pm0.45$  pg/2C (from 21.82 to 23.55 pg/2C), indicates that all tested populations represent the octoploid form (Antonova et al. 2020). Based on the flavonoid biosynthesis genes of *B. inermis*, one copy of the chalcone flavanone isomerase gene (*BiChi-1*) clusters with *Aegilops tauschii* (D genome), while the other two copies (*BiChi-2* and *BiChi-3*) form a separate cluster in the subfamily Pooideae, flanked by *Hordeum vulgare*. A copy of the *F3h* gene of *B. inermis* forms a separate branch in the subfamily Pooideae (Antonova et al. 2019). RAPD analysis indicates a decrease in diversity in the most polluted population, as well as the appearance of new alleles in chronically irradiated samples of the *B. inermis* (Antonova et al. 2019). Under laboratory conditions, the intra- (Antonova et al. 2015) and interannual (Antonova et al. 2014) variability in the quality of *B. inermis* seed offspring from the EURT area was estimated. This made it possible to identify the modifying role of weather conditions under the influence of technogenic factors. This study was performed as part of the state assignment of the Institute of Plant and Animal Ecology, Ural Branch, Russian Academy of Sciences (AAAA-A19-119032090023-0).

## **ASSESSMENT OF THE LIMITS OF ADAPTABILITY OF SOIL MICROORGANISMS POPULATIONS UNDER LONG-TERM IRRADIATION BY SMALL DOSES**

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Intensive anthropogenic activities of mankind in the sphere of nuclear energy have led to a change in radiation balance of the environment. In condemning this problem, usually, extreme situations such as disasters at Nuclear Power Plants or nuclear explosions are carried out at the forefront. However, long-term exposure of living organisms to low doses of radiation near a Nuclear Power Plant can also pose the serious potential hazards. Therefore, it is important to study and evaluate the consequences of irradiation by low doses on several generations of biological objects since there is a threat of death of the population when the limits of adaptability are reached. It is expedient to conduct such investigations on the objects with a high generation rate, for example, microorganisms. A fast biological response and a high rate of generation of microorganisms allow studying the limits of their adaptability during the relatively short period of time. They have an extremely labile biochemical organization of the cell, expressed in the restructuring of the enzyme apparatus or changes in the kinetic characteristics of existing enzymes, as well as the synthesis of new enzymes. Thanks to this, bacteria relatively quickly adapt to changes in environmental conditions and the emergence of new environmental risks of both natural and anthropogenic origin. Assuming that the lag-phase on the growth curve correlates with the adaptation processes of the enzymatic system of microorganisms in result of their long-term irradiation by low doses to new conditions, a theoretical description of the kinetics of microorganism growth is carried out. The analysis of the results obtained makes it possible to identify the adaptation mechanism of the reaction of the population of microorganisms to long-term irradiation by low doses of radiation, as well as to estimate the limits of adaptability of the populations of microorganisms.

## **SKELETAL ANOMALIES IN JUVENILE *LEUCASPIUS DELINEATUS* FROM AZBUCHYN LAKE IN THE CHERNOBYL ZONE**

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The skeleton abnormalities in juvenile fish of the sunbleak (*Leucaspius delineatus*, Heckel, 1843) from Azbuchyn Lake in the Chernobyl exclusion zone (CEZ) were studied. The samples were taken in 2016. All individuals were cleared and stained for bone with alizarin red by T. Potthoff's method. 30 individuals from reference Pidbirna Lake and 78 individuals from Azbuchyn Lake were selected on the stages of development of fish G (L = 15.0-18.0 mm) and F (L = 18.0-25.5 mm). The radiation exposure for fish in the CEZ water bodies is mainly formed by  $^{90}\text{Sr}$  and  $^{137}\text{Cs}$ . The average current absorbed dose rate for studied parental fish in Azbuchyn Lake was  $36 \mu\text{Gy h}^{-1}$ . The absorbed dose rate for fish from the reference lake did not exceed  $0.07 \mu\text{Gy h}^{-1}$ . Among the observed anomalies in juvenile fish the predominance of structural disorders of the elements of the tail and abdomen was registered. 22 cases of anomalies in *L. delineatus* from Pidbirna Lake and 61 from Azbuchyn Lake were identified. The predominant anomalies for fish from the reference lake are additional processes of neural arches - 68%. Such anomalies as deformations of neural processes, deformation of the last vertebra and additional neural processes prevail in fish of Azbuchyn Lake - 20, 25 and 31%, respectively. For other studied anomalies, the manifestation did not exceed 10%. In fish from the reference lake 40% of individuals with one anomaly and 17% with two per specimen were found. At the same time, for fish from Azbuchyn Lake, the number of anomalies in some individuals reached 6 per individual.

## **EFFECTS OF CHRONIC EXPOSURE TO RADIONUCLIDES IN PLANT POPULATIONS**

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Despite many studies carried out to date, the long-term effects of chronic exposure on plants and animals inhabiting the contaminated with radionuclides territories remain the subject of scientific discussions. In order to obtain a comprehensive view on the consequences of chronic radiation exposure, a response of natural plant populations at different levels of biological complexity has been characterized. The results of our field studies carried out on different plant species in various radioecological situations (nuclear weapons testing, the Chernobyl and Fukushima accidents, uranium and radium processing) will be presented. Populations growing for many years under chronic radiation exposure are characterized by the enhanced mutation rates, increased genetic diversity, changes in the gene expression, oxidative and phytohormonal status, as well as in the level of genome-wide methylation, alterations in the temporal dynamics of cytogenetic abnormalities and genetic structure of populations. In plant populations inhabiting heavily contaminated territories cytogenetic damage could be accompanied by a decrease in reproductive capacity. However, in less contaminated sites, significant changes at the genetic level had no effects on enzymatic activity, morphological abnormalities, and reproductive ability of plants. Under radioactive contamination of the plant's environment, a population's resistance to exposure may increase. However, there are radioecological situations where an enhanced radioresistance has not evolved or has not persisted. The results presented increase our understanding of the long-term effects of chronic radiation exposure on plant populations in the wild nature and provide important information for the management and monitoring of radioactively contaminated territories.

## TOTAL BETA-RADIOACTIVITY OF MEDICINAL PLANTS IN OPEN-AIR HYDROPONIC AND SOIL CULTIVATION CONDITIONS OF ARARAT VALLEY

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As a result of the radiochemical studies it was revealed that in hydroponics the controlled technogenic radionuclides (RN), ( $^{90}\text{Sr}$ ,  $^{137}\text{Cs}$ ) penetrate into medicinal plants (artemisia annua - *Artemisia annua* L., common chicory - *Cichorium intybus* L.) from the nutrition solution and in soil from the irrigation water and soil. According to the received data the radioactivity of cultivated plants is low, thus it may be proposed that the penetrated amount of the RN from the air pool (precipitations, smoke) in the overground mass of cultivated plants has been insignificant. An important indicator describing the quality of the raw material is the compliance of the total  $\beta$ -radioactivity with the established criteria of radioecological safety. It was found that in hydroponics the radioecologically safer raw material was provided by the Davtyan's nutrition solution, compared with the Steiner's, Chesnakov-Bazirina's and Knop's nutrition solutions. The radioecologically safer raw material of the medicinal plants cultivated on different substrates (gravel, volcanic slag, their mixture) was received on the mixture of gravel with volcanic slag (1:1 by volume). It was revealed that overground mass of the common chicory cultivated in soil and on different substrates of classical hydroponics has exceeded the mass of roots 1.7 and 3.0 times. Regardless of the cultivation way and conditions, raw material of the artemisia annua and common chicory is radioecologically safe in Ararat Valley (zone of technogenic influence of Armenian NPP with 30 km radius), because its total  $\beta$ -radioactivity hasn't prevail the limit of 1.0 Bq/g. To receive radioecologically safer raw material of the artemisia annua and common chicory it is proposed to use cultivation hydroponic way – nutrition solution offered by Davtyan and mixture of gravel with volcanic slag.

## **TRANSGENERATIONAL TRANSMISSION OF EPIGENETIC EFFECTS BY SMALL MAMMALS' MIGRANTS FROM RADIOCONTAMINATED ZONE**

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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. Patterns of transgenerational transmission of radiation exposure' biological effects insufficiently studied. This work we used the new method of small mammals' group biomarking with rhodamine B (RB) to evaluate: 1) animals' migrations beyond Eastern Urals Radioactive Trace zone (Kyshtym Accident 1957, Southern Urals) to adjacent areas; 2) to check the suitability of biomarker' transgenerational transmission to offspring through mothers' milk. Animal body structures containing keratin (claws, vibrissae, fur) are marked by this dye and detected by yellow fluorescence under green light. Received data demonstrate migrants of different ecological specialization (mice, voles, shrews) in adjacent area. First time we have revealed shrews-migrants (overwintered pregnant females) to control plot (more than 9000 m). The share of all small mammals' migrants to contiguous territories varies from 5% up to 30% in different monitoring seasons. Presence of different sex and functional status individuals in structure of migrants was shown. We revealed juvenile mice and shrews (*Apodemus agrarius*, *Sylvaemus uralensis*, *Sorex araneus*) which received RB with mother's milk. In all cases this label was treated as systemic. Transgenerational transmission of RB through mothers' breast milk in animals from natural populations is assumed as a solid evidence of real possibility of epigenetic effects' transference. The literature discusses the epigenetic effects of breastfeeding in two ways through micro RNA and genomic DNA methylation, which, in turn, can mediate a wide range of long-term effects on the development and health. Thus, the presence of labeled individuals of small mammals of different taxa, who received a biomarker with milk, is a real basis to make a conclusion about transgenerational channel for transmitting epigenetic effects, stemming from exposure, to vertebrates' offspring in adjacent areas.

This work performed as part of a State Contract of the Institute Plant and Animal Ecology UB RAS and partly supported by the RFBR (project No. 20-04-00164).



## **EFFECTS OF LONG-TERM RADIATION EXPOSURE ON AQUATIC BIOTA WITHIN THE CHERNOBYL EXCLUSION ZONE**

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The effects of chronic irradiation of aquatic biota in closed water bodies within the Chernobyl exclusion zone during 2000-2020 were studied. It is determined that the rate of chromosomal aberrations in the root meristem tissues of aquatic plants in the most radioactive contaminated lakes on average in 2-3 times, and in cells of the pond snail embryos in 4-6 times exceeding the spontaneous mutagenesis level, inherent to aquatic organisms. Analysis of leukograms of fish peripheral blood showed the decrease of lymphocyte cells, as well as the increase in the number of granulocytic cells with increase of radiation dose rate. Along with changes in leukograms an increased level of morphological damages of erythrocytes (structural and proliferation abnormalities) was determined, which is generally for pray fish in 4-12 times and for predatory fish in 7-15 times higher than in fish from reference lakes. High amount of erythrocytes with structural and proliferation abnormalities in the peripheral blood of fish from lakes with high levels of radioactive contamination allows us to assume that the qualitative indexes of red cells in blood of fish are more sensitive to chronic radiation influence in comparison with the elements of white blood. A variety of forms of pathological changes in the structure of blood cells, mainly erythrocytes, may indicate low resistance of cytogenetic apparatus of fish in the face of considerable mutagenicity and genotoxicity of environment. In this situation the ionizing radiation causes damage to the lipid structures of biological membranes (e. g. lysosomes) and violation of their barrier functions that ensure compartmentalization in the cell. This leads to disruption of spatial isolation of enzymes to their substrates and release enzymes to further destruction of macromolecules and intracellular structures. As a result, there are changes not only in the cytoskeleton, but also in functioning of all the organelles in the cell. Analysis of the viability of the seed progeny of the common reed from contaminated lakes at germination in the laboratory showed a reduction in technical germination, germination energy and seed viability with increase of radiation dose rate.

**EFFECTS OF ENVIRONMENTAL RADIATION ON THE POPULATIONS OF  
MURINE RODENTS AND EARTHWORMS LIVING IN THE AREAS WITH  
INCREASED LEVEL OF RADIONUCLIDES IN THE REMOTE PERIOD  
AFTER THE CHERNOBYL ACCIDENT**

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An assessment of the long-term consequences of radioactive contamination on murine rodents in the exclusion zone of the Chernobyl NPP is given. Monitoring of rodents was carried out in the 30 and 35 years after the accident at sites with different levels of radioactive contamination. The  $^{137}\text{Cs}$ ,  $^{90}\text{Sr}$  whole body activity concentration, the micronucleus test of bone marrow cells and the parameters of metabolism of rodents were investigated. The main stages of qualitative changes in body systems and regulation processes in generations of animals had been determined. The high variability of various parameters of the main body systems can had led to the increased level of cell mutations. Disruption of the functioning of various cellular systems was shown.

The assessment of genotoxic effects in earthworms (Lumbricidae) sampled in 35 years after the accident on the Chernobyl NPP was given in Comet-assay test.

## RODENTS OF THE EAST-URALS RADIOACTIVE TRACE AND PROBLEMS OF RADIATION POLYGONS

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Radiation problems (Timofeev-Resovsky et al., 1967) are added by specifics of radioactive polygons (Garnier-Laplace, 2013; Moller, Musso, 2016; Balonov, 2018; Dubrova, 2019; Fesenko, 2019). So, radiosensitivity of the impact ecosystem is an order higher than in laboratory; decrease irradiation don't reduce the mutation level; there is no signs of ecosystem radioadaptation in general. Rodent lesions and epigenetic metamorphoses are uniforms, often independent of differences chronic radiation doses (Lyubashevsky et al., 2002, Vasil'eva et al., 2002). It's results of high stability of hereditary shifts with same their distribution among the descendants of sublethally irradiated at accidents. Increased radiosensitivity was observed compared to laboratory data. Unique trump card of radio adaptation is the mole vole (*Ellobius talpinus* Pall.) in the zone of 1000 Ki/km<sup>2</sup> EURT. It has no pathology at an absorbed dose of up to 2.0 Gy / year. Other rodents' pathologies are 70-90%. And the viability of generations at ultra-tolerant doses is adaptive, but imperfect. Island-type polygons isolation (Krivolutsky, 1999) are ensuring adaptation. Migrationally active mole vole (Evdokimov, 2013) is isolated on EURT, migration of other rodents is reduced. Uniformity of their haplotypes with control ones (Meeks et al., 2009; Modorov, Pozolotina, 2011), rejecting genetic isolation, promotes epigenetic interpretations of rodent features (Vasil'ev et al., 2000, 2002, 2009), their pathology, morphogenesis, 90Sr metabolism (Lyubashevsky et al., 2009, 2019; Bol'shakov et al., 2012; Starichenko, 2010). Thus, epigenesis initiation in radiation accidents, epigenetic nature of population adaptation and individual metabolism at its morphogenetic indications corresponding epigenetic replacing of genetics mechanisms. Its biologic sense is population gene pool defense from "over selection" at high mutagenesis by conservation. Indication of all ecosystem conservation is pine genome hypermethylation (Kovalchuk et al., 2003; Geraskin et al., 2018). But over disruption of the normal ratio is pathogenic. Probably, accidents of protective evolution were not comparable in duration and mutagenicity to radiation polygons.

This study was performed within the frameworks of state contract with the Institute of Plant and Animal Ecology, Ural Branch, Russian Academy of Sciences.

**BIOLOGICAL CONSEQUENCES OF IONIZING RADIATION:  
WHAT HAVE WE LEARNED FROM STUDIES OF CHERNOBYL?**

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Environmental disasters sometimes offer the unique opportunity for landscape-scale ecological and evolutionary studies that are not possible in the laboratory or small experimental plots. The nuclear accident at Chernobyl (1986) allows for rigorous analyses of radiation effects on individuals and populations at an ecosystem scale. Here, the current state of knowledge related to populations within the Chernobyl region of Ukraine and Belarus following the largest civil nuclear accident in history is reviewed. There is now a significant literature that provides contrasting and occasionally conflicting views of the state of the flora and fauna and how they are affected by this mutagenic stressor. Studies of genetic and physiological effects have largely suggested significant injuries to individuals inhabiting the more radioactive areas of the Chernobyl region. Most population censuses for most species suggest that abundances are reduced in the more radioactive areas. Some reports of increased mammal abundances are likely largely the result of dramatically reduced hunting pressures combined with immigration from other regions although some field surveys show depressed population abundances in the most contaminated regions.

## **EFFECTS OF ENVIRONMENTAL LOW-DOSE ON FUNCTIONAL-METABOLIC ORGANS RESPONSE IN A NATURAL MOUSE POPULATION: EAST-URALS RADIOACTIVE TRACE**

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As a result of many years of radioecological monitoring of natural mouse populations (*Apodemus uralensis* Pallas, 1811) in EURT zone (consequence the Kyshtym accident in South Urals in 1957); it is presented the functional-metabolic response to irradiation by caused external and internal exposure to  $^{137}\text{Cs}$  and  $^{90}\text{Sr}$ . Radiation-induced effects in the myocardium: lipid catabolism, mitochondrial oxidation, and antioxidant protection are decreased. The low efficiency of energy production in cardiomyocytes by activating glycolysis, along with induction of the protein-, DNA-, RNA- synthesis, indicates the development of compensatory myocardial hypertrophy for maintaining contractile function. Adrenal reactions to chronic radiation: activation of steroidogenesis through the delipidisation of adrenocortical cells and intensification of lipid peroxidation. There is an increase in the protein mass to maintain organ hyperfunction. In the spleen: restriction of carbohydrate catabolism, prooxidant shift, along with suppression of DNA and protein-synthesizing activity of splenocytes. This indicates radiation-induced minimization of the hemato-, immune-poetic organ function. Liver: induction in the depositing function by increased lipid-forming and glycogen-synthesising activities of hepatocytes. In general, similar changes (both in level and in vector) are observed with high population density animals in areas with a natural radiation background. The combined effect of chronic exposure and overabundance population can lead to the fact that even with a dose rate of 0.1 mGy/day, the adaptive stress response (eustress) in mice from the EURT area can go to the stage of adaptation failure and development of decompensation (distress). The sexually immature yearlings mice unlike immature animals can be considered to be the more sensitive (reactive) intrapopulation group to synergy of unfavorable factors of radiation and non-radiation genesis.

**ASSESSMENT OF SEED GERMINATION, PHYTOHORMONES CONTENT AND PHOTOSYNTHETIC PARAMETERS IN CHRONICALLY IRRADIATED ECOTYPES OF *ARABIDOPSIS THALIANA* AFTER ACUTE  $\gamma$ -IRRADIATION AND HYPERTHERMIA TREATMENT**

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*Arabidopsis thaliana* seeds, collected from the radionuclide-contaminated plots Masany (**M**) and Vygrebnyaya Sloboda (**VS**) and the control plot Babchin (**B**) (Polesie State Radiation-Ecological Reserve, Republic of Belarus), have been subjected to acute  $\gamma$ -irradiation at a dose of 150 Gy (dose rate 460 Gy/h, source  $^{60}\text{Co}$ ) or treated with 50°C for 12 hours. Two independent experiments were carried out with 4 biological replicates (20 seeds/replicate) in each. Germinability and germination rate were statistically significantly lower in seeds from contaminated areas, as compared to the seeds of the control plot. The hyperthermia treated seeds from **M** (the plot with drastically increased levels of  $^{137}\text{Cs}$ ,  $^{90}\text{Sr}$ ,  $^{241}\text{Am}$ ) showed statistically significantly lower values of germinability and germination rate in relation to the untreated seeds of the same plot. The  $\gamma$ -irradiated seeds from the control plot **B** revealed statistically significantly higher levels of emergence rate index, as compared to the untreated seeds of the same plot. At the same time, acute irradiation caused a significant decrease in the average leaf area of juvenile plants, grown from **B** seeds, and affected some of their photosynthetic parameters. A higher content of zeatin was found in juvenile plants, grown from untreated seeds of both radiation-contaminated plots. The plants from **M** seeds showed the highest amount of abscisic acid (ABA), which may serve as a confirmation of an increased stress response. An increase in the ABA concentration was also revealed as a response to stressors applied to seeds from control plot **B**. Hyperthermia modulated the concentrations of salicylic acid and the indole butyric acid in plants, grown from seeds collected at **VS**. Consequently, chronically irradiated ecotypes of *A. thaliana* could act as an informative object for fundamental studies of plant response to stressors.

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## **MORPHOLOGICAL CHANGES OF ERYTHROCYTES IN PERIPHERAL BLOOD OF FISH FROM WATER BODIES CONTAMINATED WITH RADIONUCLIDES**

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During 2018-20 we studied hematological parameters of peripheral blood of the roach, common rudd, perch and Prussian carp living in water bodies within the Chernobyl Exclusion Zone (CEZ) and exposed to long-term ionizing radiation in gradient of the average absorbed dose rate (ADR) from 0.07 (reference conditions) to 84.5  $\mu\text{Gy h}^{-1}$ . The qualitative analysis of erythrocytes in blood of fish from reservoirs within the CEZ revealed the numerous structural abnormalities of erythrocytes, as well as disorders, associated with the pathology of mitotic division: deformation of the nucleus, pyknosis, parietal nuclei, cytolysis, micronuclei etc. The number of different types of erythrocytes increased from 3-5 for reference fish species, reaching 11 for fish in reservoirs with the highest levels of radioactive contamination. The increase of structural damage of erythrocytes and disorders, associated with pathology of mitotic division with increase of ADR for all types of fish in the CEZ was dose-dependent, increasing at the highest dose rates by 5.0, 5.6, 10.7 and 13.8 times for the perch, Prussian carp, common rudd, and roach, respectively, in comparison with number of violations in the blood of fish from reference water bodies. Thus the haematological research of fish in water bodies within the CEZ shows high amount of red cells with varied abnormalities. The pathology of mitotic division of erythrocytes can be a manifestation of the radiation-induced genome instability, which is one of the main mechanisms for the protection of living organisms from exposure to stressors with subsequent implementation at higher levels of organization of biological systems. Increase of the genome instability and accumulation of chromosomal and genetic defects in organisms of fish can be transmitted to future generations with high probability of appearance in the form of increased mutation rates, decreased fertility and loss of the most sensitive species.

## ACCUMULATION OF $^{90}\text{Sr}$ BY *PINUS SYLVESTRIS* AND REACTION OF RADIAL GROWTH OF TREES ON RADIATION INFLUENCE IN THE EAST-URAL RADIOACTIVE TRACE ZONE

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The East Ural Radioactive Trace (EURT) was formed in 1957 as a result of the accident at "Mayak" Production Association (Kyshtym accident). Small sites of the pine-birch forest with individual trees *Pinus sylvestris* that survived the accident were preserved on the periphery of the EURT and in sites with density of  $^{90}\text{Sr}$  contamination less than 60,000 kBq/m<sup>2</sup> at the first period after the accident. Aims of investigation: 1) study of  $^{90}\text{Sr}$  accumulation by different organs of pines (*Pinus sylvestris*) that survived the Kyshtym accident; 2) study of the influence of radioactive release on the width of annual rings of pines. The concentration of  $^{90}\text{Sr}$  in the air-dry matter of aboveground organs of pine increases with rising density of soil contamination. This dependence is approximated by a power function with high degree of certainty. The distribution of  $^{90}\text{Sr}$  in the aboveground organs of pine growing in the EURT and in the background area was similar. The maximum of  $^{90}\text{Sr}$  concentration was noted in the tree bark, and the minimum – in wood and cones. An analysis of the width of annual rings of pines survived after the Kyshtym accident showed a threefold decrease in growth in 1958. In the background territories during the same period, the natural dynamics of radial growth was preserved. The synchronization of the variability of the width of the tree rings in the background and contaminated areas was completed in 3-10 years after the accident, depending on the level of soil contamination.

This study was performed within the frameworks of state contract with the IPAE UB RAS and the Russian Foundation for Basic Research (project № 19-05-00469).



## **CYTOGENETIC MONITORING OF HIGHER AQUATIC PLANTS IN WATER-BODIES OF THE CHERNOBYL EXCLUSION ZONE**

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Radioecological monitoring of the Chornobyl Exclusion Zone (CEZ) water-bodies is carried out by the Department of Aquatic Radioecology of the Institute of Hydrobiology of the NASU since the first days of the Chornobyl accident. In particular, cytogenetic monitoring, which is one of the effective ways to control the growth of the mutagenic potential of the environment, was launched in 2000. The increase in the degree of cytogenetic damage in the apical cells of meristematic tissues of plant roots, which are characterized by rapid cell division, is a very sensitive indicator in the registration and quantification of the effect of ionizing radiation on plants. The analysis of data from 2006 to the present, obtained by anaphase rapid test, revealed a significant excess – two-six orders higher the level of spontaneous mutagenesis for the studied reference species of higher aquatic plants. While conducting cytogenetic monitoring it is very important to control the correlation of genetic disorders of different types. Last 6-7 years, the proportion of multiple aberrations in chromosomal aberration spectrum for root meristem cells has increased significantly. Among them there are chromosomal disorders indicating not only clastogenic but also aneugenic effects of small doses of chronic low-intensity ionizing radiation associated with damage of the mitotic apparatus and even cytotomy disorders. The number of multipolar mitoses and cells with unevenly divided sets of chromosomes also increased during the observations. During 2013–2019, an increase in the number of cells with severe damage to the mitotic apparatus was observed, which leads to a violation of the normal distribution of chromosomes in the cell and a violation of their segregation. This may indicate a certain deepening of the process of damage to the mitotic apparatus of cells - a violation in the formation of centrioles and achromatin spindle cells of the root meristems of helophytes, which are under the influence of chronic radiation exposure. Further study of these anomalies is an important component of complex measures related to forecasting and minimizing the consequences of the Chornobyl catastrophe for non-human biota.

# *Epidemiology of radiation*

## NEURODEGENERATIVE DISEASES IN A COHORT OF WORKERS CHRONICALLY EXPOSED TO IONIZING RADIATION

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Neurodegenerative diseases (NDD) are health conditions characterized with the progressive degeneration and/or death of neuron cells. According to WHO projections NDD incidence, including Parkinson's disease, will double by 2030. On one hand, the increase in NDD incidence is due to achievements of modern medicine and the increase in life expectancy, while on the other hand it is due to environmental factors inducing NDDs (hydrocarbons, organic solvents, pesticides, etc.).

Radiotherapy patients exposed at high radiation doses (20–50 Gy) demonstrated severe cognitive disorders, neurogenesis and neurovascular alterations.

This study was aimed to assess the risk of Parkinson's disease (PD) in a cohort of 22,377 workers chronically occupationally exposed to ionizing radiation. Over 570 294 person-years of follow-up, 300 cases of PD were registered among the study cohort.

A linear association was found between PD incidence and cumulative gamma-dose after adjusting for sex and attained age. The excess relative risk per unit dose (ERR/Gy) was 1.02 (95% confidence interval (CI): 0.59, 1.63). The ERR/Gy of external radiation for PD incidence was stable after adjusting for neutron dose (ERR/Gy = 1.03; 95% CI: 0.59, 1.67). The risk increased with increasing lag-period and decreased notably after adjusting for body mass index, smoking and alcohol consumption. Additional adjustments for hypertension, gout, gastric ulcer, head injuries with loss of awareness, and diabetes mellitus did not affect the risk estimate.

This study is the first to suggest that PD is associated with prolonged occupational external gamma-ray exposure.

## CHANGES IN CYTOGENETIC INDICATORS DURING THE APPLICATION OF COMPOUNDS OF THE COPPER-ORGANIC COMPLEXES

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One of the priorities of the contemporary radiobiology is the search for new efficient radioprotective compounds. With a view to relieve possible radiation protective actions, we studied compounds of copper-organic complexes:  $[\text{Cu}(\text{PTA})_4]\text{BF}_4$  and  $[\text{Cu}(\text{PCN})(\text{HBP}_{23})]\text{BF}_4$ , in which legands are: PTA-1,3,5-triaza-7-phosphaadamantane ("PTA") and PCN-tris(cyanoethyl)phosphine  $\text{HBP}_{23}$ -trispyrazolilborato ("PCN") which were synthesized in Camerino University (Italy).

The experiments were carried out on reproductive, white, outbred rats with weight of 180gr. An hour before general, single-shot radiography of the animals with dose 5,3Gy and 7,0Gy, PTA and PCN complexes were injected abdominally with dose of 20mg/kg. We analyzed radiation-induced clastogenesis of the bone marrow cells (BMC) as one of the most informative indicators. Generally accepted method of cytogenetic analysis of white rats' BMC indices was used. Proliferative activity (MI), chromosome aberration (ChA), cell ploidy (CP) was taken into consideration. We also performed hematological analysis of peripheral blood: red blood cell count, leucocyte count, level of hemoglobin. Basing on the survival results, cytogenetic and hematological indicators, it can be concluded that study complexes demonstrate noticeable radioprotective properties. Both complexes showed identical effects on animals according to all observed criteria.

In eruptive phase, on early dates of analysis (3<sup>rd</sup> and 7<sup>th</sup> days) both compounds soften the damaging effects of radiation with both doses which is especially obvious in significant shifts of MI and ChA in comparison with control data. On the last dates of observation (14<sup>th</sup> and 30<sup>th</sup> days) many data of analysis (MI, red blood readings) approached the norm, ChA and number of leucocytes in comparison with the control data, demonstrate statistically significant difference.

Based on the results, it can be assumed that the studied Cu-ligands effectively promote reparative processes in bone marrow cells and have the properties of pronounced radiomodifiers.

## INTEGRATED ECOLOGICAL AND HYGIENIC ASSESSMENT OF THE ENVIRONMENTAL CONDITION OF THE BRYANSK REGION (2008–2017)

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**The purpose of the study:** on the basis of official statistics for 2008-2017, we conducted a comprehensive ecological and hygienic assessment of the state of the environment in all cities and regions of the Bryansk oblast (4 cities and 27 regions) by radiation contamination (due to the Chernobyl accident), chemical pollution (due to atmospheric pollution air pollutants from stationary sources) and combined radiation-chemical contamination. **Methods:** environmental-hygienic, environmental mapping, mathematical statistics. **Results:** we found that the level of both chemical and radioactive contamination in cities and regions of the Bryansk oblast varies widely – from 9 to 28047 g/m<sup>2</sup> in terms of gross emissions of gaseous pollutants into the air in the area of the region; from 4,1 to 427,1 kBq/m<sup>2</sup> for Cesium-137 and from 0,4 to 15,0 kBq/m<sup>2</sup> for Strontium-90, while the average annual effective doses to the population from the Chernobyl component range from 0,05 to 1,9 mSv per year. Based on a comprehensive ecological and hygienic assessment of the state of the environment, 4 groups of territories of the Bryansk region were identified according to the level of radiation, chemical and combined contamination. **Conclusions:** the results obtained make it possible to assess and predict changes in the state of public health in territories affected by the Chernobyl accident, depending on the level of associated chemical pollution of the environment, taking into account additive and synergistic effects.

**Key words:** Chernobyl accident, radioactive contamination, chemical pollution, combined contamination, Cesium-137, Strontium-90, pollutants, Bryansk region.

**A COMPARATIVE ANALYSIS OF THE STILLBIRTH RATE  
IN RADIOACTIVELY CONTAMINATED AREAS OF BRYANSK REGION  
AFTER THE CHERNOBYL ACCIDENT (1986–2016)**

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**The purpose of the study:** Based on the official statistics for 1986–2016, this paper presents the findings of a comparative analysis of the rate of stillbirth in boys and girls in radioactively contaminated areas of the Bryansk region with different radioactive contamination densities by the long-lived radionuclides Cesium-137 and Strontium-90 after the Chernobyl accident. **Methods:** radioecological, mathematical statistics (Student t-test, Shapiro-Wilk test, White test, Spearman correlation analysis, regression analysis, cluster analysis). **Results:** No significant differences were found in the stillbirth rate for both the boys and girls between the radioactively contaminated and non-contaminated areas of the Bryansk region over the 31 years following the Chernobyl accident (1986-2016). The rate of stillborn boys over girls was significantly higher in radioactively contaminated areas (17%) compared with the corresponding data across the oblast (10%) and in the control areas (8%), with the most significant differences observed in the third period after the accident (52%). No considerable dependences have been identified between the density of Cesium-137 and Strontium-90 radioactive contamination and stillbirth rates among boys and girls estimated both for the Bryansk region (mean value) overall and for the radioactively contaminated South West Territories (SWT) separately over the period of 1986–2016. In contrast to the findings averaged for the entire Bryansk region and for the SWT, the linear regression revealed a statistically significant relationship between the stillbirth rates and densities of contamination by Cesium-137 and, to a lesser extent, with Strontium-90 in the most heavily radioactively contaminated areas (from 373 to 567 kBq/m<sup>2</sup> by <sup>137</sup>Cs and from 6 to 20 kBq/m<sup>2</sup> by <sup>90</sup>Sr). **Conclusions:** The results obtained may indicate a higher sensitivity of male fetuses to low-dose radiation exposure.

**Key words:** stillbirth, teratogenic effect, Chernobyl accident, Bryansk region, radioactive contamination, Cesium-137, Strontium-90.

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## **CYTOGENETIC CHANGES IN LIQUIDATORS OF THE CONSEQUENCES OF THE CHERNOBYL ACCIDENT RESIDENT IN ARMENIA**

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It is known that one of the first and direct signs of the effect of ionizing radiation on a cell is the destabilization of chromosomes. Radiation-induced instability of the genome arises, with which the long-term consequences of ionizing radiation are associated - mutagenesis, carcinogenesis, aging. Cytogenetic examination included analysis of chromosomes with Giemsa staining. The unit of proliferative activity of phytohemagglutinin-stimulated lymphocytes is the number of metaphases per 0.5 ml of culture suspension obtained after treatment with 1.2 ml of whole blood. The analysis took chromosome plates strictly at the metaphase stage of cell division (degree of chromosome helixing of at least 20%).

Studies have shown that the chromosome apparatus of peripheral blood lymphocytes in liquidators has changed significantly in dynamics. These changes also depend on the time elapsed after irradiation (the remoteness of the event). Since 1987 the frequency of chromosomal aberrations (HA) was more than 2-2.5 times higher than the spontaneous level. By 2005-2006 the average number of chromosome aberrations reached  $15.34 \pm 2.52$ .

According to the types of aberrations, chromatid and chromosomal (single and double DNA breaks) were mainly recorded, and since 1995, exchange aberrations were recorded. In liquidators, dicentrics and rings, which are considered biomarkers of ionizing radiation, were not found. Using the methods of logarithmic, exponential, polynomial regression, equations were obtained that describe the change in cytogenetic indicators in dynamics, where  $x$  is the number of years after the Chernobyl accident, and  $y_1$ ,  $y_2$ ,  $y_3$ ,  $y_4$  are chromosome aberrations (1), proliferative activity (2), single (3) and double (4) DNA breaks.

Summarizing the results of cytogenetic studies in Armenian liquidators, we can clearly state that the instability of the peripheral blood lymphocyte genome is quite pronounced and, although in the long term there is a tendency to normalization of indicators (chromosomal aberrations and proliferative activity), nevertheless, these indicators significantly differ from the control group.

# **EVOLUTION**



## **MODERN THEORIES OF HUMAN ORIGINS AND DARWIN'S "DESCENT OF MAN"**

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Charles Darwin's *The Descent of Man*, published 150 years ago, laid the grounds for scientific studies into human origins and evolution. Three of his insights have been reinforced by modern science. The first is that we share many characteristics (genetic, developmental, physiological, morphological, cognitive, and psychological) with our closest relatives--the anthropoid apes. The second is that humans have a talent for high-level cooperation reinforced by morality and social norms. The third is that we have greatly expanded the social learning capacity that we see already in other primates. Darwin's emphasis on the role of culture deserves special attention because during an increasingly unstable Pleistocene environment cultural accumulation allowed changes in life history, increased cognition, and the appearance of language, social norms and institutions. Understanding and predicting human behavior requires further integration of these insights into the social sciences.

## **FRUSTRATIONS, MEMORY, AND COMPLEXITY IN PHYSICS AND BEYOND**

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The origin of complexity remains one of the most important and, at the same time, the most controversial scientific problems. Earlier attempts were based on theory of dynamical systems (limit circles, strange attractors, etc.) but did not lead to a satisfactory solution of the problem. I believe that a deeper understanding is possible based on a recent development of statistical physics, combining it with relevant ideas from evolutionary biology, AdS/CMT, and machine learning.

Using patterns in magnetic materials as the main example, I discuss some general problems such as (a) a formal definition of pattern complexity and its relation to “holographic complexity”; (b) self-induced spin glassiness due to competing interactions as a way to interpret chaotic patterns; (c) multi-well states intermediate between glasses and ordinary ordered states and their relevance for the problem of long-term memory in complicated systems; and (d) complexity of frustrated quantum spin systems. I will also review a very recent experimental observation of self-induced spin-glass state in elemental neodymium and, in a more speculative way, the role of frustrations and competing interactions in biological evolution and origin of biological complexity.

## **THE WORLD OF VIRUSES, ITS GLOBAL ORGANIZATION AND EVOLUTION**

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Viruses and virus-like mobile genetic elements are ubiquitous parasites (and sometime symbionts) of all cellular life forms and the most abundant biological entities on earth. The recent, unprecedented advances of comparative genomics and metagenomics have led to the discovery of diverse novel groups of viruses and provide for a vastly improved understanding of the evolutionary relationships within the virosphere. Arguably, we are approaching the point when the global architecture of the virus world can be outlined in its entirety, and the key evolutionary events in each of its domains can be reconstructed.

I will present such an outline of the global organization of the virus world and the corresponding megataxonomy structure that has been recently approved by the International Committee for Taxonomy of Viruses. Although the global structure of the virus world is becoming apparent, major groups of viruses within the established realms, with unique features of genome organization and expression, are being discovered at a high pace. Examples of such new viruses will be presented including the most abundant bacteriophages in the human gut.

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# LIPID MEMBRANE COMPOSITION IN THE LAST UNIVERSAL CELLULAR ANCESTOR

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Dominant phospholipids of all modern cells are built of a glycerol phosphate moiety that links the polar headgroup with two hydrophobic chains of 15-25 carbon atoms. Still, the lipids of bacteria and eukaryotes differ from those found in Archaea in several respects, namely: (1) archaea use isoprenoid compounds as hydrophobic chains, whereas bacteria and eukaryotes mostly rely on fatty acids; (2) the stereoisomer of glycerol phosphate is sn-glycerol-3-phosphate (G3P) in bacteria and eukaryotes and sn-glycerol-1-phosphate (G1P) in Archaea; (3) hydrophobic tails are attached to the glycerol phosphate by ester bonds in bacteria and eukaryotes and by ether bonds in Archaea.

This fundamental difference has prompted many questions on the early evolution of biological membranes and on the membranes of the common ancestor of Bacteria and Archaea. The existence of such a cenancestor, known as LUCA (Last Universal Cellular (Common) Ancestor), is supported by the universality of the genetic code and by the identification of about 100 genes common to all free-living organisms.

Here, we have applied our phylogenomic analysis pipeline to the lipid biosynthesis enzymes present among bacteria and archaea, including the representatives of recently described uncultured phyla. Our analysis suggests a vast repertoire of prenyl-based compounds in cenancestor, so that the lipids of LUCA could have both trans and cis-elongated isoprenoids as tails. The (S)-2,3-di-O-geranylgeranylglycerol phosphate (DGGGP) synthase, an enzyme attaching the second prenyl tail of Archaeal phospholipids, is unlikely in the LUCA, implying that the evidence for Archaeal-type two-tail lipids in the LUCA is thin. We would present a model of an asymmetric primordial membrane of the LUCA as built of different types of single-chain polyisoprenoid lipids.

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## **EVOLUTION OF REPLICATION AND TRANSCRIPTION ERROR RATES ACROSS THE TREE OF LIFE**

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The application of whole-genome sequencing to mutation-accumulation experiments has revolutionized our understanding of the rate and molecular spectrum of spontaneous mutations across the Tree Life, revealing a number of generalities, and leading to the development of a general theory to explain the data. The mutation rate per nucleotide site varies ~1000-fold among different lineages, with the lowest rates being found in unicellular eukaryotes followed by bacteria and then by multicellular species. This phylogenetic variation in the mutation rate is consistent with the drift-barrier hypothesis, which postulates that the ability of natural selection to maintain low mutation rates is compromised by the power of random genetic drift, which is elevated in populations of relatively small size. The theory also explains why: DNA polymerases involved in fewer nucleotide transactions have higher error rates; why secondary error-correction mechanisms are less accurate than earlier steps; and why error rates are so extraordinarily high in viruses.

Finally, recent methodological advances have allowed the estimation of the rates at which errors arise in transcripts (i.e., at the mRNA level), demonstrating these to be 1,000 to 100,000 greater than those arising during DNA replication. As transcript errors are transient, these elevated rates are also consistent with the drift-barrier hypothesis. Notably, our results suggest that the major impact of environmental mutagens may not be slowly accumulating genomic damage, but the persistent accumulation of damaged transcripts and downstream proteins causing immediate cellular stress.

# INCREASE IN THE ATMOSPHERIC PRESSURE MAY HAVE DRIVEN THE TRANSITION FROM THE FIRST REPLICATORS TO THE FIRST CELLULAR ORGANISMS

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The abiotic formation of amino acids and nucleotides was shown to be favored by high levels of cyanide/formamide, which has prompted suggestions on the origin of life in a kind of "formamide world" [1-3]. The boiling temperature of formamide (200°C) is much higher than the boiling temperature of water. Therefore, the transient removal of water and establishment of formamide/cyanide based (bio)chemistry was suggested to happen, owing to the heating, either in primordial deserts [1] or within impact craters [2,3].

Alternatively, evaporation of water could be caused by an atmosphere loss after a giant impact. At the low atmospheric pressure, liquid water would have been virtually absent being concentrated on the earth poles, with Earth resembling modern Mars. Unlike water, formamide and its derivatives would be present in a liquid state at the sites of geothermal activity. Earlier we have suggested that the first cells could have emerged in the pools and puddles of primordial anoxic geothermal fields where the elementary composition of the condensed vapor would resemble the internal milieu of modern cells [4]. Therefore, geothermal fields all over the primordial Earth could have served as cradles of life. The recovery of the atmosphere would slowly increase the amount of liquid water on the surface of Earth, which would drive the transition from the first replicators to the first cellular organisms.

**Acknowledgements:** The work was supported by the Federal Ministry of Education and Research of Germany (AYM) and the Russian Science Foundation (grant #14-50-00029).

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**MEMORIES  
&  
DISCUSSIONS**

**PROFESSOR ARMEN SAGHATELYAN:  
THE HISTORY OF RADIOECOLOGY SCHOOL IN ARMENIA**

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Professor A.K. Saghatelyan's holistic vision of the pathways and fate of chemical elements in Earth Crust and Biosphere which he inherited from USSR geochemical science, helped him to establish and promote several scientific schools in Armenia. Inter alia, Prof. Saghatelyan inspired and contributed to the Radioecology discipline development at his founded the Center for Ecological-Noosphere Studies of NAS RA (CENS).

Within the series of international research projects led by Prof. Saghatelyan, a modern laboratory was created at CENS and continuous personnel training was arranged. Initial radioecological studies were implemented with the aim to assess environmental radioactivity and its relation with the main geochemical peculiarities of Armenia's territory. In 2016 Radioecology Department was re-opened at CENS as a separate unit. Prof. Saghatelyan's conceptualization allowed establishing novel radioecological monitoring net within the frame of series of national research projects. The yielded monitoring data enabled estimating the baseline activity of naturally occurring radionuclides as well as the fallout inventory of some artificial radionuclides (e.g. Cs-137), which is essential for early warning and rapid response system within the frames of national radiation protection and nuclear security.

From 1989 Prof. Saghatelyan supervised geochemical surveys in the biggest cities and industrial zones of Armenia, which resulted in arrangement of soil sample archive at CENS. This valuable material has been using further for radioecological studies which revealed the importance of monitoring naturally occurring radionuclides and related radon hazard as well as the factors of technologically enhanced natural radioactivity.

Finally, thanks to CENS's up-to-date laboratory, established national and international consortia, and personnel continuous training policy, which are the heritage of Prof. Saghatelyan, his pupils master new areas at the interface of sciences, in particular, making a transition from Environmental Radioactivity studies to Nuclear Forensics.



## **TO THE HISTORY OF THE URAL RADIOECOLOGICAL N.V. TIMOFEEV-RESOVSKY'S SCHOOL**

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The beginning of the history of the Ural radioecological N.V.Timofeev-Resovsky's school is associated with the Laboratory "B", which worked from 1946 to 1955 in the village of Sungul, Chelyabinsk region to solve the problems of protection from radiation in the USSR Atomic Project. In 1950, the first scientific reports on radiation (experimental) biogeocenology appeared, the author of which was Elena Alexandrovna Timofeeva-Resovskaya, the leader was Nikolay Vladimirovich Timofeev-Resovsky. These works were declassified in 1954. In 1963, E.A.Timofeeva-Resovskaya summarized the results of her long-term research in the monograph "Distribution of radioisotopes by the main components of freshwater reservoirs".

The formation of the Ural radioecological school began in 1955, when N.V.Timofeev-Resovsky organized the Laboratory of Biophysics with 16 employees at the Institute of Biology in Sverdlovsk (now Yekaterinburg). At the same time, under his leadership, a biological station "Miassovo" was built in the Ilmensky state reserve near the city of Miass, Chelyabinsk region. Here radioecological studies were continued on the basis of the general doctrine of the biosphere created by V.I.Vernadsky and the biogeocenological ideas of V.N.Sukachev. In addition, the Miassovo biological station is famous for seminars that have had a significant impact on the development of biomedical sciences in our country.

From 1964 to 1993, the laboratory, created by N.V.Timofeev-Resovsky, was headed by Nikolay Vasilievich Kulikov. The successor of the biological station "Miassovo" in 1979 was the Biophysical Station (radioecological station) near the Beloyarsk NPP named after I.V.Kurchatov in the town of Zarechny. In 1982, the Department of Continental Radioecology of the Institute of Plant and Animal Ecology was organized on the basis of the laboratory. Since 1993, the Department and the Biophysical Station have been headed by Alexander Viktorovich Trapeznikov. The history of the radioecological N.V.Timofeev-Resovsky's school in the Urals continues.

## **N.W. TIMOFEEFF-RESSOVSKY IN TAJIKISTAN**

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In the early 70s of the last century, a delegation of scientists from the Academy of Sciences USSR, headed by academician N.V. Tsitsin with professors P.A. Genkel, N.N. Konstantinov (from Russian Federation) and Yu.S. Nasyrov (from Tajikistan) visited a number of research centers in the GDR. Visiting the Central Institute of Genetics and Plant Research of the Academy of Sciences of the German Democratic Republic in Gatersleben, they knew about the outstanding scientist - genetics N.W. Timofeeff-Ressovsky.

Later, N.W. Timofeeff-Ressovsky was invited by tajik scientists to Tajikistan. Visit of Institute of Plant Physiology and Biophysics of Academy of Sciences of the Republic of Tajikistan, and familiarization with the research on photosynthesis carried out in Institute made a great impression on N.W. Timofeeff - Ressovsky. When discussing the main results and prospects of research on photosynthesis the idea of new direction of Genetics was first born, it was genetic of photosynthesis. New horizons opening up in this regard prompted N.W. Timofeeff-Ressovsky to publish in 1968 an article on the topic: "Genetics and physiology of plants." In this work, in essence, the emergence of a new section - the genetics of photosynthesis, was proclaimed, aimed at studying the phenomena of heredity and variability of the system of signs of the photosynthetic apparatus at various levels of life organization.

N.W. Timofeev-Ressovsky was directly involved in the final edition of the program of the International Symposium on the Genetic Aspects of Photosynthesis. Prominent scientists from the USA, Germany, Denmark, Hungary and other countries took part in the work of the forum, which was held in Dushanbe in October 1972. Research by Tajik scientists in the field of the genetic basis of photosynthesis was highly appreciate at this symposium and Institute of Plant Physiology and Biophysics was recognized as the head one, in charge of all research on the genetics of photosynthesis carried out in the Soviet Union. Recognition of the significant contribution of Tajik scientists to the development of new direction was the adoption by the program committee of the decision to organize a separate section "Genetics of photosynthesis" at the XIV International Genetics Congress in Moscow in 1978.

## THE CHERNOBYL ACCIDENT AND GLOBAL CLIMATE CHANGE

*Gale R.P.*  
Imperial College London

People reading the popular press or even some scientific reports will surely be confused about long-term health consequences of the Chernobyl nuclear power facility accident 35 years ago this week. Some sources report thousands, hundreds of thousands or even millions of cancers caused by radiations released by the Chernobyl accident. Other sources claim few if any cancers. There are other reports claiming large numbers of birth defects, congenital abnormalities and even heritable genetic mutations. Which of these claims is true?

Much of what we know about radiation and cancer comes from data of 94,000 A-bomb survivors and 27,000 controls who were not in Hiroshima or Nagasaki when the bombs exploded. Because these persons have been carefully followed since 1950 we can reasonably accurately estimate the proportion of cancers caused by A-bomb radiations. What do these data show? First, risk of developing a radiation-related cancer is dose-dependent, the higher the dose the greater the probability cancer in a survivor was caused by their A-bomb radiation exposure. Second, blood cancers (leukaemias) occurred relatively quickly, within a few years whereas other cancers occurred after 10-20 years and remain over a person's lifetime. At a low dose (5-100 milliGray, a measure of radiation dose) the increased cancer risk to an exposed survivor is about 2 percent whereas at high doses ( $> 2$ Gray) the risk is  $> 60$  percent. Conclusions from the A-bomb survivor study are mostly confirmed by studies in other radiation-exposed populations.

Can we use these data to estimate how many persons exposed to radiation have or will develop cancer. Yes, but with caveats. For example, the A-bomb survivors were exposed to acute, high-dose, external radiations whereas the roughly 6.4 billion people exposed to radiations from the Chernobyl accident was chronic, internal and external low-dose. Another difference is genetic background. As such, one must be cautious when applying estimates based on the A-bomb survivors to persons exposed from the Chernobyl nuclear power facility accident.

The consensus amongst scientists, bearing in mind consensus does not equal truth, namely any exposure, no matter how small, increases cancer. This is not to say one photon of radiation can cause cancer but that one photon increases a person's cancer

risk. The linear, no threshold relationship, albeit controversial, is the most conservative interpretation of the data we have and the most protective of public health. Others dispute this threshold concept arguing people living in places with high and low background radiation levels have similar cancer incidences. Because of these contradictory hypotheses we need caution estimating cancer risk in persons exposed to radiation from the Chernobyl accident.

Now let's consider the impact of radiation exposures from the Chernobyl accident on millions of people living in Ukraine, Belarus and Russia and the 6.4 billion people in the Northern Hemisphere. For example, the average dose from the Chernobyl accident to the about 300,000 evacuees and 1 million people living in contaminated lands is 10-30 milliGray, a dose is equivalent to the background radiation dose received from living 3 to 10 years in Los Angeles. Based on calculations like these the UN Scientific Committee on Atomic Radiation (UNSCEAR) predicted about 4,000 excess cancers over the first 50 years. The UN Chernobyl Forum predicted 10,000 excess cancer deaths. Other sources, often without scientific credentials and/or a political agenda predict thousands, hundreds of thousands or even millions of cancers and cancer deaths. Putting aside these predictions which are fundamentally unverifiable what do the data show? First, there were about 7,000 excess thyroid cancers in children and adolescents living proximal to the accident site, an estimated 100-fold increased incidence. Tragic, but fortunately few cancer were fatal. These cancers were mostly caused by radioactive iodine-131 in milk. Except for one report there are no convincing data of increased leukaemias. The exception is a form of leukaemia which most scientists think is not radiation-related and occurred in clean-up workers receiving much higher radiation doses compared with the general population.

There are also no convincing data of an increase in solid cancers except one report of a increase in breast cancer, again in heavily-exposed women.

The bottom line is there little evidence radiation released from Chernobyl increased cancers globally. Admittedly, failing to detect an increase does not prove there was no increase. However, any increase must be relatively small and below our level of detection. We should recall about 40 percent of us will develop cancer in our lifetime. Even if there were an increase from Chernobyl related radiation it would be less than 0.1 percent using current radiation risk models. I should mention there are also no convincing data birth defects or genetic abnormalities were increased by radiations released from Chernobyl despite what you might read elsewhere.

Lastly, we need to put the Chernobyl accident and nuclear energy in the context of other energy alternatives and global climate change. There are more than 400 nuclear power reactors operating worldwide providing about 10 percent of global energy with 50 under construction. Most people understand burning fossil fuels worsens global warming but not that it releases more radiation into the environment *per* kilowatt generated than a nuclear power facility. Mining to provide copper pipes for a solar power station also brings more radiation to the earth's surface than radiation release from a nuclear power facility. As for cancer consider that construction of the Aswan high dam in Egypt caused many thousand bladder cancers caused by *Schistosoma haematobium* infection from slowed flow of the Nile. There are no simple answers.

In summary, the Chernobyl nuclear power facility accident was a global tragedy. Although I focused on cancer, we should not forget the important social, psychological and economic consequences. The Chernobyl accident was preventable resulting from human error and faulty reactor design. We now have safer nuclear reactors and have learned important lessons from the Chernobyl and Fukushima accidents. It's time for the US to re-evaluate the potential role of nuclear energy in helping us deal with global climate change.

# LECTURES

## **MODERN THEORIES OF HUMAN ORIGINS AND DARWIN'S "DESCENT OF MAN"**

*Gavrilets S.Yu.*

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Charles Darwin's *The Descent of Man*, published 150 years ago, laid the grounds for scientific studies into human origins and evolution. Three of his insights have been reinforced by modern science. The first is that we share many characteristics (genetic, developmental, physiological, morphological, cognitive, and psychological) with our closest relatives--the anthropoid apes. The second is that humans have a talent for high-level cooperation reinforced by morality and social norms. The third is that we have greatly expanded the social learning capacity that we see already in other primates. Darwin's emphasis on the role of culture deserves special attention because during an increasingly unstable Pleistocene environment cultural accumulation allowed changes in life history, increased cognition, and the appearance of language, social norms and institutions. Understanding and predicting human behavior requires further integration of these insights into the social sciences.

## **FRUSTRATIONS, MEMORY, AND COMPLEXITY IN PHYSICS AND BEYOND**

*Katsnelson M.I.*

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The origin of complexity remains one of the most important and, at the same time, the most controversial scientific problems. Earlier attempts were based on theory of dynamical systems (limit circles, strange attractors, etc.) but did not lead to a satisfactory solution of the problem. I believe that a deeper understanding is possible based on a recent development of statistical physics, combining it with relevant ideas from evolutionary biology, AdS/CMT, and machine learning.

Using patterns in magnetic materials as the main example, I discuss some general problems such as (a) a formal definition of pattern complexity and its relation to “holographic complexity”; (b) self-induced spin glassiness due to competing interactions as a way to interpret chaotic patterns; (c) multi-well states intermediate between glasses and ordinary ordered states and their relevance for the problem of long-term memory in complicated systems; and (d) complexity of frustrated quantum spin systems. I will also review a very recent experimental observation of self-induced spin-glass state in elemental neodymium and, in a more speculative way, the role of frustrations and competing interactions in biological evolution and origin of biological complexity.



## **THE WORLD OF VIRUSES, ITS GLOBAL ORGANIZATION AND EVOLUTION**

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Viruses and virus-like mobile genetic elements are ubiquitous parasites (and sometime symbionts) of all cellular life forms and the most abundant biological entities on earth. The recent, unprecedented advances of comparative genomics and metagenomics have led to the discovery of diverse novel groups of viruses and provide for a vastly improved understanding of the evolutionary relationships within the virosphere. Arguably, we are approaching the point when the global architecture of the virus world can be outlined in its entirety, and the key evolutionary events in each of its domains can be reconstructed.

I will present such an outline of the global organization of the virus world and the corresponding megataxonomy structure that has been recently approved by the International Committee for Taxonomy of Viruses. In particular, I will present the comprehensive evolutionary tree of RNA viruses and discuss the positions of the viruses that cause major human diseases in the different branches of this tree. It is of note that coronaviruses, including SARS-CoV-2, possess the largest and most complex genomes among the RNA viruses known to date, arguably, reflecting the complexity of the virus-host interactions. Although the global structure of the virus world is becoming apparent, major groups of viruses within the established realms, with unique features of genome organization and expression, are being discovered at a high pace. Examples of such new viruses will be presented including the most abundant bacteriophages in the human gut.

## **EVOLUTION OF REPLICATION AND TRANSCRIPTION ERROR RATES ACROSS THE TREE OF LIFE**

*Lynch M.*

Center for Mechanisms of Evolution, Arizona State University, Tempe, Arizona, USA

The application of whole-genome sequencing to mutation-accumulation experiments has revolutionized our understanding of the rate and molecular spectrum of spontaneous mutations across the Tree Life, revealing a number of generalities, and leading to the development of a general theory to explain the data. The mutation rate per nucleotide site varies ~1000-fold among different lineages, with the lowest rates being found in unicellular eukaryotes followed by bacteria and then by multicellular species. This phylogenetic variation in the mutation rate is consistent with the drift-barrier hypothesis, which postulates that the ability of natural selection to maintain low mutation rates is compromised by the power of random genetic drift, which is elevated in populations of relatively small size. The theory also explains why: DNA polymerases involved in fewer nucleotide transactions have higher error rates; why secondary error-correction mechanisms are less accurate than earlier steps; and why error rates are so extraordinarily high in viruses.

Finally, recent methodological advances have allowed the estimation of the rates at which errors arise in transcripts (i.e., at the mRNA level), demonstrating these to be 1,000 to 100,000 greater than those arising during DNA replication. As transcript errors are transient, these elevated rates are also consistent with the drift-barrier hypothesis. Notably, our results suggest that the major impact of environmental mutagens may not be slowly accumulating genomic damage, but the persistent accumulation of damaged transcripts and downstream proteins causing immediate cellular stress.

## **RADIATION PROTECTION OF NON-HUMANS: ISSUES AND CHALLENGES**

*Mothersill C.E.*  
McMaster University, Canada

This lecture will look at the different approaches to developing a radiation protection framework for the environment. The currently established system has identified 12 “reference organisms” chosen to represent major animal and plant groups. The objections to this approach are that while it is simple, it does not capture the complexity of ecosystems and the multiple factors including radiation which impact survival of populations in ecosystems. However it is very difficult to regulate radiation exposures at levels above the individual. The issues and challenges will be discussed as well as possible approaches.

## **BIOLOGICAL CONSEQUENCES OF IONIZING RADIATION: WHAT HAVE WE LEARNED FROM STUDIES OF CHERNOBYL?**

*Mousseau T.A.*

University of South Carolina, Columbia, SC, USA

Environmental disasters sometimes offer the unique opportunity for landscape-scale ecological and evolutionary studies that are not possible in the laboratory or small experimental plots. The nuclear accident at Chernobyl (1986) allows for rigorous analyses of radiation effects on individuals and populations at an ecosystem scale. Here, the current state of knowledge related to populations within the Chernobyl region of Ukraine and Belarus following the largest civil nuclear accident in history is reviewed. There is now a significant literature that provides contrasting and occasionally conflicting views of the state of the flora and fauna and how they are affected by this mutagenic stressor. Studies of genetic and physiological effects have largely suggested significant injuries to individuals inhabiting the more radioactive areas of the Chernobyl region. Most population censuses for most species suggest that abundances are reduced in the more radioactive areas. Some reports of increased mammal abundances are likely largely the result of dramatically reduced hunting pressures combined with immigration from other regions although some field surveys show depressed population abundances in the most contaminated regions.

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## A STRANGE, CHARM, BEAUTIFUL ... WORLD!

Nikitin V.A.

Joint Institute for Nuclear Research, Dubna, Russia

*The level of physics determines the level of understanding of the entire world around us, determines the level of intellectual maturity of humanity.*  
Academician L. B. Okun

An inexperienced reader in physics, looking at the title, will think that the author wants to intrigue him with beautiful metaphors. But these are not metaphors – they are terms used in physics to refer to classes of elementary particles. The corresponding Russian and English words (strange, charm, beautiful) are used in special literature. So, this report is about elementary particle physics. It is also called high-energy physics, since in order to obtain and study the fundamental particles of matter, it is necessary to realize the interaction of protons or atomic nuclei with a sufficiently high energy.

The types and characteristics of particles accelerators are presented. The types and characteristics of particles detectors presented. The main of them are: scintillator, semiconductor, wire chamber, drift chamber, Cherenkov counter, time projection chamber. The methods of particles identification are discussed. Typical particles detection setups at accelerators are presented.

A well-developed concept of particle physics is called the Standard Model (SM). The basis of the SM is 6 quarks and 6 leptons. The quarks are u, c, t - these are the so-called upper quarks. They have charge  $+2/3$  of electron charge. The quarks d, s, b called bottom end have charge  $-1/3$ . The leptons are: e,  $\mu$ ,  $\tau$  - charged leptons,  $\nu_e$ ,  $\nu_\mu$ ,  $\nu_\tau$  - it is neutral objects - neutrinos. They are associated with corresponding charged leptons. There are four types of particle interactions - strong, electromagnetic, weak, and gravitational. Interactions are realized by particles called interaction mediators. Here they are: g (gluon - strong force),  $\gamma$  (electromagnetic force),  $W^{+-}$ ,  $Z_0$  (weak force), graviton (hypothetical object, gravitational force). The quarks have baryon number  $1/3$ , it conserves in all types of interaction. The conservation of the baryon number guarantees the stability of our world. Quarks and leptons decay into lightest states due to weak interaction. In total, several hundred particles and antiparticles are known. Baryons are constructed from three quarks, mesons from a quark and an antiquark. For instance: proton p(u,u,d), neutron n(u,d,d), the lightest strange hyperon  $\Lambda(u, d, s)$ , the lightest

strange meson  $K^0(d, \text{anti-}s)$ . The lightest quarks  $u, d$  have a mass of  $\approx 3 \text{ MeV}$ , the heaviest  $t$  quark has a mass of  $\approx 1773 \text{ MeV}$ . It is a heaviest object in SM. Its mass is close to mass of rhenium nucleus. Life time of  $t$ - quark is  $\approx 5 \times 10^{-25} \text{ s}$ , It is ten times less of time scale of strong interaction  $\approx 3 \times 10^{-24} \text{ s}$ .

Although SM implements the particle systematics, it allows us to calculate some interaction cross-sections and of the decay modes of many particles, it is not complete. It includes about 18 unknown parameters which must be determined empirically: particle masses, interaction constants, mixing angles of different quantum states and so on. This means that experimenters and theorists will have a lot of hard work to do on the way to the truth. In particular, projects for the construction of accelerators with higher beam energy are formulated.

## **THE MULTIPLE MECHANISMS OF NON-TARGETED EFFECTS**

*Seymour C.*

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This lecture will review the current state of the art in the field of non-targeted effects of ionizing radiation. Discussion will include the various mechanisms of signal production in the targeted cell, signal transmission to the bystander cell, and responses in the bystander cell including genomic instability and adaptive response. The impacts of dose, dose rate and other stressors will also be covered.

## **GENETIC DIVERSITY OF CULTIVATED PLANTS FOR FOOD SECURITY AND FOR DEVELOPMENT OF NEXT-GENERATION BREEDING TECHNOLOGIES**

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Key-words: cultivated plants, *ex situ* conservation, genetic diversity, *in situ* conservation, wild relatives

The Vavilov Institute (VIR) has established and presently holds one of the world's largest collections (and the richest in botanical diversity) of crop genetic resources and crop wild relatives which comprises over 320,000 accessions representing 64 plant families, 376 genera, and more than 2000 species. The collection is a scientifically systemized and documented repository of live plant germplasm samples, coupled with an instrumental and methodological complex (for working with plant genetic resources in the spheres of crop breeding, seed science, plant physiology, biochemistry, genetics, immunology, molecular biology, biotechnology, etc.), incorporating low-temperature and cryogenic storage facilities in St. Petersburg and at the Kuban Genetic Seed Bank as well as a network of 11 experiment stations in major phytoclimatic zones within Russia. In addition to the seed collection maintained under operational and long-term storage conditions, there are field genebanks at the experiment stations in different geographic regions of the country, where the genetic diversity of perennial horticultural crops is preserved. Obtaining new knowledge on plant genetic resources makes it possible to produce new generations of cultivars and hybrids. However, the richest collection is not only a source of valuable traits for traditional breeding. The availability of a scientifically systematized, well-described and structured collection makes it possible to find suitable samples for comparative genetic and omics studies (genomics, transcriptomics, metabolomics, proteomics). Taking this into account, the rich genetic diversity of such a collection as the VIR collection, the availability of sources of new genes or alleles, a wide range of samples for genetic editing, in turn, provides (1) priority in the identification of new target genes (2) novelty in the choice of objects and setting practical tasks for editing, (3) priority development of approaches to protecting the germplasm from unauthorized changes using seamless technologies and (4) big data for modelling future varieties for decades to come.



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AND EVOLUTION»

*Abstracts of Presentations  
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