

ARMENIAN MEETING

“Climate Changes: Adaptation”



ABSTRACTS
PAPERS BY YOUNG SCIENTISTS

JOINT INSTITUTE FOR NUCLEAR RESEARCH

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“Climate Changes: Adaptation”

Yerevan, March 27–28, 2023

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ADAPTATION MECHANISMS

MECHANISMS INVOLVED IN THE FORMATION OF GENOTYPE RESISTANCE – THE CONTRIBUTION OF DSB REPAIR, CHAPERONE AND ANTIOXIDANT SYSTEMS

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Living organisms are exposed to various endogenous and exogenous stressors that may affect the structure, function, growth, and survival of cells, tissues, and organisms as a whole. Stress due to environmental pollution and climate change leads to an increased formation of reactive oxygen species (ROS) in various cellular structures. In such conditions, organic molecules such as lipids, proteins, and nucleic acids are prone to damage and/or degradation.

During the evolution, living organisms have developed numerous protective strategies at different levels of cellular organization: genetic, morphological, physiological, structural, biochemical, and molecular - changes in gene expression, morphology, physiology, stability of cell membranes, elimination of ROS, activation of the antioxidant, chaperone and DNA repair systems, etc.

Here, we will discuss the contribution of three defense systems - DSBs repair, antioxidant's and chaperone's by using mutant, hybrid and WT strains of *Chlamydomonas reinhardtii* and *Chlorella* species isolated from contrasting habitats, that differ in their response to environmentally induced stress.

Our finding would contribute to the present state of knowledge that resistant genotype's can form the genetic elite of natural populations.

GENOTOXICOLOGICAL AND MOLECULAR-GENETIC RESEARCH OF CORONAVIRUS IN ARMENIA

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The course, clinical indicators, and treatment of the COVID-19 disease are significantly influenced by the strain of the SARS-CoV-2 virus, the levels of DNA damage, and the expression of genes involved in the mtDNA-mediated inflammatory reaction. For the first time, the levels of DNA damage and the expression of YME1 and OPA1 genes, which regulate mtDNA leakage, were analyzed in groups of male and female patients with moderate and severe COVID-19 disease.

The relationship of DNA lesions with age, gender and severity of the disease was found. The levels of DNA lesions analyzed by the DNA-comet method were higher in males (9.22 ± 1.30 %) compared to females (5.78 ± 0.66 %). In the group of patients with a moderate COVID-19, a positive correlation was found between DNA damage and platelet levels ($r=0.754$). In the group of patients with a severe course of the disease, a positive correlation was observed between DNA damage and the level of aspartate aminotransferase enzyme in the blood ($r=0.875$). Positive correlations were found between DNA damage levels and the duration of hospitalization in male ($r=0.665$) and female ($r=0.572$) patients. A negative correlation was observed between DNA lesions and blood lymphocyte levels ($r=-0.901$) in males with moderate COVID-19. In women, a positive correlation was observed between DNA lesions and age ($r=0.467$) and INR ($r=0.512$).

No differences in the expression of YME1L1 and OPA1 genes were found.

The work was supported by the Science Committee of RA (#21AG-1F068).

EVALUATION OF GENOTOXIC EFFECTS IN COVID-19 PATIENTS AS A POTENTIAL MARKER OF VIRUS-HOST ADAPTATION

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Abstract – The global spreading of the SARS-CoV-2 virus with possible zoonotic origin indicates the high adaptability of the virus to its hosts and vice versa. One of the major mechanisms of the rapid adaptation of viruses is the induction of DNA damage and hijacking of the host DNA damage response apparatus. The aim of the current study was the evaluation of the levels of DNA damage in leukocytes of patients with COVID-19 using Comet assay and its correlation with levels of fibrinogen in the blood as a marker of coagulation. The results indicate elevated levels of DNA damage in leukocytes of patients with COVID-19 (10.62±4.16 %) compared to controls (3.43±1.38 %). Correlation analysis did not reveal statistically significant relationships between DNA damage and levels of fibrinogen in COVID-19 patients. The obtained results can be useful for further studies on the elucidation of the role of DNA damage in viral adaptation.

Keywords: COVID-19, comet assay, DNA damage, fibrinogen

INTRODUCTION

The outbreak of COVID-19 in 2019, caused by the SARS-CoV-2 virus, resulted in a global pandemic with over 761 million confirmed cases of infection and nearly 7 million deaths worldwide. Along with SARS-CoV and MERS-CoV coronaviruses SARS-CoV-2 is also of zoonotic origin which indicates that these viruses have been able to mutate and adapt to infect humans [1]. Thus, the identification of molecular markers of viral adaptability is of high importance to combat future pandemics.

SARS-CoV-2 is a single-stranded positive-sense RNA virus with a high potency of adaptability. One possible mechanism of adaptation of viruses to their hosts is hijacking cellular DNA damage response [2]. Recent studies indicate that SARS-CoV-2 infection can elevate host DNA damage and inflammation by impairment of 53BP1 focal recruitment and degradation of the DNA damage response kinase CHK1 [3].

Thus, we studied the levels of DNA damage in leukocytes of patients with COVID-19 using Comet assay and its correlation with levels of fibrinogen in the blood.

METHODS

Alkaline comet assay. Studies were approved by the local ethics committee, in accordance with the Helsinki Declaration and written consent was obtained from each donor. Blood samples were obtained from 10 patients diagnosed with COVID-19 and 10 healthy controls. Levels of DNA damage were analyzed by alkaline DNA comet assay [4]. The % tail DNA of comets was used as an indicator of DNA damage. The data on the levels of fibrinogen in the blood of patients were provided by the National Center for Infectious Diseases of RA. Statistical analysis was performed using a Mann-Whitney *U*-test and Pearson correlation coefficient (Statgraphics Centurion 16.2). P values <0.05 were considered statistically significant.

RESULTS AND DISCUSSION

In the control group, the % tail DNA was 3.43 ± 1.38 % while in COVID-19 patients % tail DNA was 10.62 ± 4.16 % (Fig. 1). Pearson correlation analysis revealed a negative correlation ($r = -0.502$) between % tail DNA and fibrinogen levels which did not reach statistical significance ($p = 0.16$).

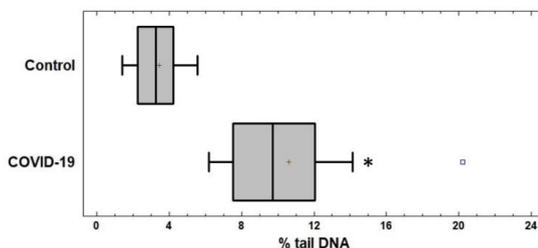


Fig. 1. % tail DNA in controls and COVID-19 patients. * $p < 0.05$ – significant difference compared to control.

CONCLUSIONS

Patients with COVID-19 have elevated levels of DNA damage in blood leukocytes. Although the tissues of the respiratory tract are the main targets of SARS-CoV-2 the negative impact of the infection can be detected in leukocytes too. Further studies are

required for elucidation of the role of DNA damage in the pathogenesis of SARS-CoV-2 in patients with COVID-19.

ACKNOWLEDGMENTS

The work was supported by the Science Committee of RA (#21AG-1F068).

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DNA DAMAGE INDUCED BY ULTRASHORT PULSED ELECTRON BEAM IRRADIATION IN HUMAN CANCER AND NORMAL CELLS

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Abstract

Recently developing laser-driven accelerators allow the generation of ultrashort accelerated particle beams. Nevertheless, the radiobiological effects of ultrashort pulsed electron beam irradiation are still not commonly studied. This work aims to compare comet formation in human cancer and normal cells after ultra-short pulsed electron beam (USPEB) irradiation. The levels of DNA damage after USPEB depended on radiation dose and the post-radiation time.

Keywords: irradiation; laser-driven accelerators; AREAL; ultrashort pulsed electron beam; Comet assay; DNA damage; MRC5; A549

INTRODUCTION

The technology of laser-generated acceleration has been developing rapidly during the last decade and gained attention for medical applications [1]. Laser-generated beams are characterized by ultrashort duration (from femtoseconds to picoseconds) and high peak dose rate (up to tens of GGy/s during pulse) [2], which create the possibility of a highly precise influence on tumors. However, the biological effects of this type of irradiation are not studied enough.

In the present study, we aim to investigate the extent of DNA damage caused by the ultrashort pulsed electron beam irradiation in human MRC-5 (human embryonic lung fibroblasts) normal cell culture and A549 (human adenocarcinoma alveolar basal epithelial cells) cancer cell line.

MATERIALS AND METHODS

Cell culture. The human lung fibroblast MRC-5 and the human lung cancer A549 cell lines were cultured in DMEM (Sigma Aldrich, Germany), supplemented with 10 % fetal bovine

serum (HyClone, UK), 2 mM L-glutamine (Sigma Aldrich, Germany), and antibiotics (1% penicillin, streptomycin) (Sigma Aldrich, Germany), at 37°C, 5 % CO₂. Prior to irradiation, 2 ml of cells were seeded in Petri dishes at a density of 0.5×10^5 cells/ml.

Irradiation. The irradiation of cells was carried out by an electron beam generated by the AREAL-Advanced Research Electron Accelerator Laboratory (AREAL) accelerator based on a laser radiofrequency gun [3] at the CANDLE Synchrotron Research Institute (Armenia). The AREAL laser-generated electron beam parameters are beam charge 30 pC, electron energy 3.6 MeV, pulse duration 450 fs, beam spot size 15 mm, RMS energy spread <1.15 %, online dose information- Faraday cup, the repetition rate of 20 Hz. For cell irradiation, each sample was placed in a sample holder facing toward the horizontal beam coming from the direction of the vacuum window. Cells were irradiated in the exponential growth phase at cell density population 70-80 %. The cells were irradiated with doses of 0.5, 2, 4, and 8 Gy, and non-irradiated cell cultures were used as a control. Cells were incubated for 0 h, 0.5, 1, and 24 h after irradiation. The doses of irradiation and durations are based on our previous studies on DNA damage and repair induced by AREAL radiation [4].

Comet Assay. The alkaline comet assay was performed according to the previously described method [5]. Briefly, the cells at the concentration of 0.5×10^5 cells/ml were mixed with low-melting-point agarose (0.5 %) (30:70 μ l v/v) at 37°C, placed on slides pre-coated with a layer of normal melting agarose (0.5 %), and allowed to solidify at 4°C for 5 min. Afterward, the slides were placed in lysis solution (1 % Triton X-100, 10 % dimethyl sulfoxide, and 2.5 M NaCl, 100 mM EDTA-Na₂, and 10 mM Tris, pH > 10) at 4 °C for 1 h, followed by incubation in a fresh alkaline (pH 13.0) electrophoresis buffer (300 mmol/L of NaOH and 1 mmol/L of EDTA) for 20 min to allow for the unwinding of DNA. Then electrophoresis was run at 25 V and 300 mA for 20 min at 4°C. After neutralization in 0.4 mol/L of Tris (pH 7.5), DNA was stained with 20 μ L of 20 μ g/mL ethidium bromide. The cells were analyzed using an IScope fluorescent microscope (Euromex, Netherlands). Images of at least 100 cells were evaluated using the CASP software package [6]. The Olive tail moment of each cell was determined.

Statistical analysis. The statistical analysis was performed using Graph Pad Prism 9.5.0 (Graph Pad Software, USA). The results were presented as the means of three independent experiments \pm standard error. Differences between treatments were evaluated using the

non-parametric Mann–Whitney test, and $P < 0.05$ was considered to indicate a statistically significant value.

RESULTS AND DISCUSSION

Levels of DNA damage were evaluated in MRC-5 (Fig. 1) and A549 (Fig. 2) cell lines irradiated with 0.5, 2, 4, and 8 Gy doses of UPEB after different time points (0, 0.5, 1, and 24 h).

Irradiation of MRC-5 cells with 0.5, 2, 4, and 8 Gy doses significantly increased the levels of DNA damage compared to corresponding controls (0 Gy) at all time points.

A significant decrease in the levels of DNA damage was detected in 0.5, 1, and 24 h post-irradiation variants compared to all irradiation doses at 0 h. However, the DNA repair was most prominent in 24 h post-irradiation cells irradiated with 0.5 Gy (9.26 ± 3.07 a.u.) and 2 Gy doses (10.27 ± 2.58 a.u.) compared with variants irradiated with 4 Gy (22.78 ± 6.36 a.u.) and 8 Gy (79.52 ± 13.15 a.u.) doses. Significant differences between levels of DNA damage induced by 0.5, 2, 4, and 8 Gy irradiation doses at 0 h of post-irradiation were not observed ($p > 0.05$).

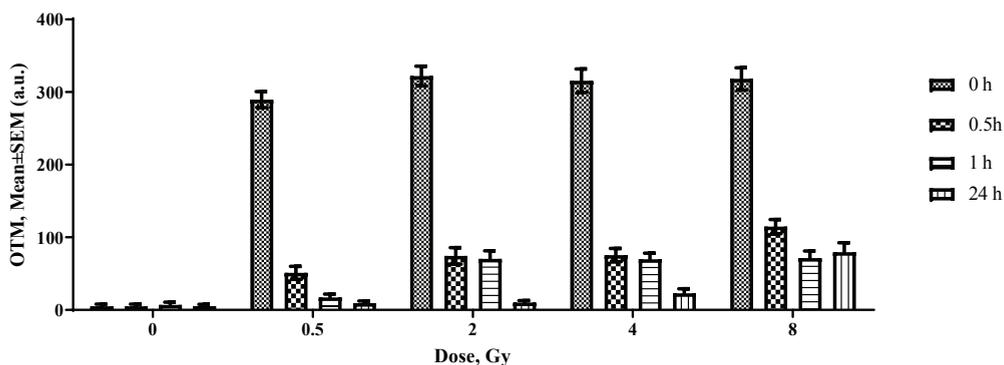


Figure 1. The level of DNA damage in MRC-5 cells after 0h, 0.5, 1, and 24h of UPEB irradiation by various doses.

Irradiation of A549 cells with 0.5, 2, 4, and 8 Gy doses significantly increased the levels of DNA damage compared to corresponding controls (0 Gy) at all time points. The highest level of DNA damage was observed in cells irradiated with 8 Gy in 0.5, 1, and 24 h post-irradiation variants compared to cells irradiated with 0.5, 2, and 4 Gy. Significant decrease in the levels of DNA damage was detected in 0.5, 1, and 24 h post-irradiation variants compared to 0 h irradiated with 2, 4 and 8 Gy doses. The most prominent DNA damage repair was observed in 24 h post-irradiated cells with 2 and 4 Gy doses.

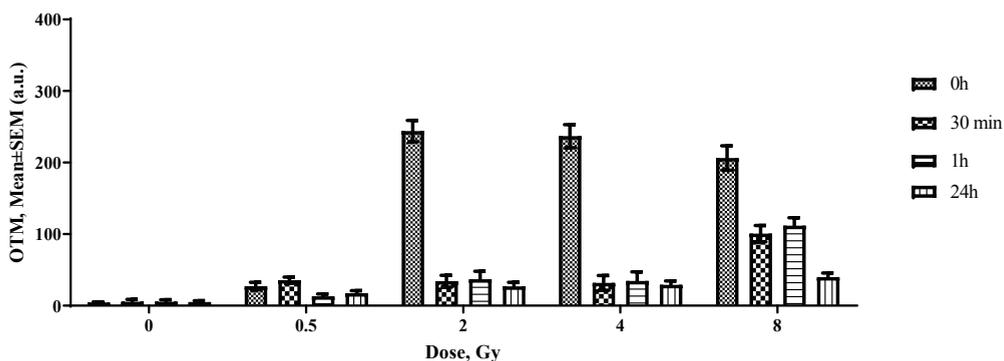


Figure 2. The level of DNA damage in A549 cells after 0h, 0.5, 1, 4, and 24h of UPEB irradiation by various doses.

CONCLUSION

The analysis of DNA damage caused by the UPEB irradiation in human cancer (A549) and normal (MRC5) cell lines revealed that the overall level of DNA damage at 0h, 30 min, 1, and 24 h after irradiation was significantly higher in MRC5 than in the A549 cell line at all doses and time points and in both cell lines exceeded the control levels.

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THE MICROBIAL DIVERSITY OF HIGH-ALTITUDE GEOTHERMAL SPRINGS OF ARMENIA AND NAGORNO-KARABAKH

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The microbial diversity of nine high-altitude geothermal springs (T 25.8–70°C, pH 6.0–7.5, total mineralization of 0.5 mg/L) located on the Armenian Highland has been recently assessed to explore their biotechnological potential. All these geothermal springs are at altitudes ranging from 960–2090 m above sea level and are located on the Alpide (Alpine–Himalayan) orogenic belt, a seismically active region. The taxonomic diversity of hot spring microbiomes has been examined using culture-independent approaches, including denaturing gradient gel electrophoresis (DGGE), 16S rRNA gene library construction, 454 pyrosequencing, and Illumina HiSeq. The bacterial phyla Proteobacteria, Bacteroidetes, Cyanobacteria, and Firmicutes are the predominant life forms in the studied springs. Archaea mainly include the phyla Euryarchaeota, Crenarchaeota, and Thaumarchaeota, and comprise less than 1% of the prokaryotic community. Temperature seems to be an important factor in shaping the microbial communities of these springs. Overall, the diversity and richness of the microbiota are negatively affected by increasing temperature. Other abiotic factors, including pH, mineralization, and geological history, also impact the structure and function of the microbial community. More than 130 bacterial and archaeal strains (*Bacillus*, *Geobacillus*, *Parageobacillus*, *Anoxybacillus*, *Paenibacillus*, *Brevibacillus*, *Aeribacillus*, *Ureibacillus*, *Thermoactinomyces*, *Sporosarcina*, *Thermus*, *Rhodobacter*, *Thiospirillum*, *Thiocapsa*, *Rhodopseudomonas*, *Methylocaldum*, *Desulfomicrobium*, *Desulfovibrio*, *Treponema*, *Arcobacter*, *Nitrospira*, and *Methanoculleus*) have been reported, some of which may be representative of novel species (sharing 91–97% sequence identity with their closest matches in GenBank) and producers of thermozymes and biomolecules with potential biotechnological applications. Whole-genome shotgun sequencing of *T. scotoductus* K1, as well as of the potentially new *Treponema* sp. J25 and *Anoxybacillus* sp. K1, were performed. Detailed characterization of thermophilic isolates indicate the potential of the studied springs as a source of biotechnologically valuable microbes and biomolecules.

THE *SACCHAROMYCES CEREVISIAE* RESPONSE TO VARIOUS STRESSORS STRONGLY DEPENDS ON THE GENOTYPE CHARACTERISTICS

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As it was already discussed, that key players in the formation of the genotype resistance are DNA repair, chaperone, and antioxidant repair systems as well as high levels of SH-groups, the presence of cell wall, stability of cells ultra-structural compartments, phases of the mitotic cycle, the energy provision, etc.

Here, we have addressed two questions: whether organisms with similar sensitivity to certain stressors measured as cell survival as well as primary induced levels of double-strand breaks would differ both: in their response depending on the physiological state; in their repair capacity?

Our work revealed that the *Saccharomyces cerevisiae* haploid strain BY4741 responds differently depending on the stressors and the physiological state. Cells in quiescent phase are more prone to Menadione treatment than to Zeocin. Additionally, the diploid strain D7ts1 is more sensitive to Zeocin when cells are in the beginning of stationary phase.

Concerning the other question, our results revealed the role of repair capacity depending on the genotype, and the recovery time.

In strain BY4741 increasing the recovery time to up to 60 min led to increase in the repair capacity of logarithmic cells. In strains 551 (haploid) and D7ts1 the most appropriate recovery time was found to be 30 min. After that a decrease in the repair capacity was observed.

Nevertheless, genotype differences have been obtained after combined treatment with *Clinopodium vulgare* plant extract and Zeocin. A well expressed decrease in the DSBs after the treatment and 30 min recovery time was calculated in D7ts1 while no effect was observed in strain 551.

By providing this experimental evidence, we aim to provide basis for further consideration of the physiological state and repair capacity in evaluation of the mechanisms of adaptation of *Saccharomyces cerevisiae*.

**BIOLOGICAL RESEARCH AT BAKSAN NEUTRINO OBSERVATORY
IN THE FIELD OF LOW BACKGROUND RADIOBIOLOGY,
DEEP UNDERGROUND MICROBIOLOGY AND ASTROBIOLOGY**

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Baksan Neutrino Observatory (BNO INR RAS) and deep underground low radiation background laboratory (DULB-4900), located in the Elbrus region (North Caucasus, Russia) beneath the peak of Andyrchy mountain, were founded on purposes of nuclear physics and astrophysics, but possess a unique potential for interdisciplinary research. Molecular genetics group of DLNP JINR initiated cooperative studies at BNO facility, those link tasks of biophysics, radiobiology, astrobiology, microbiology and medicine.

Studies of effects of low background radiation on biological systems is a rapidly evolving field of radiobiology in 2010s. In our work [1,2], we aimed to register for the first time the response to reduced radiation background of DULB-4900 of complex multicellular organism (*D. melanogaster*) at the transcriptome level by RNA-seq profiling and to analyze obtained results in terms of the impact of different types of stress including radiation treatment. Obtained data indicates non-specificity of biological response in DULB-4900 conditions, which involves down-regulation of primary metabolic processes, up-regulation both of the immune system process and the response to stimuli.

Another branch of our studies is devoted to investigation of extremophile organisms and deep underground microbiome of saline warm springs at unused part of BNO tunnel, emerged due to high hydrothermal activity of Elbrus volcanic region. In recent work [3], we present the characterization of the novel bacterium *Cytobacillus pseudoceanisediminis* sp. nov. isolated from the deep underground spring at the depth ~2.5 km. We obtained complete bacterial genome at Oxford Nanopore MinION, analyzed the metabolism, performed chemotaxonomic characterization and approved methylotrophy of the organism and it's high heavy metal resistance. Currently ongoing experiment will enable to validate one of few deep underground metagenomes of the North Caucasus and Elbrus region, isolated from deep underground saline spring.

In this report, the potential of deep underground facilities of Baksan Neutrino Observatory INR RAS for biological studies will be discussed and ongoing cooperative experiments of JINR and INR RAS will be reviewed.

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**CLIMATE CHANGES:
INFLUENCE ON POPULATIONS**

**CHANGE OF DISTRIBUTION AND MIGRATORY PATTERNS OF BIRDS,
POSSIBLE RESERVOIRS OF VECTOR-BORNE DISEASES,
UNDER INFLUENCE OF CLIMATE CHANGE**

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Armenia is located at the crossroad of three global migration flyways: Black Sea – Mediterranean, East Asian – East African, and Central Asian. Majority of the migrants, which fly through Armenia, belong to raptors, water birds, and passerines, most of which have stopover points in the country, where the birds get rest and sometimes food. Thus, raptors stop in the grasslands and scrublands, waterbirds stop at the shorelines and wetlands, passerines stop in scrublands and wetlands. Regular observations, conducted since 1995, allowed documenting the changes in occurrence status and migratory patterns for a number of species. For example, Shikra (*Accipiter badius*) expanded its distribution to the north-west and begun breeding in Armenia since 2009. See-see Partridge (*Ammoperdix griseogularis*) expanded its distribution to the north and started breeding in Armenia since 2003. Spur-winged Lapwing (*Vanellus spinosus*) expanded its distribution to the north, becoming frequently recorded in Armenia since 2016, year-round visitor since 2019, and a breeding bird since 2022. White-tailed Lapwing (*Vanellus leucurus*) was full migrant but starting from 2021 it was recorded staying in the country year-round, thus changing its migration pattern. The subspecies of Siberian Stonechat *Saxicola maurus variegatus* (by some authors considered as *S.m. hemprichii*), was migrating through the country, but since at least 2010s it stays in the country overwinter, thus shrinking its migration distance. Masked Shrike (*Lanius nubicus*) expanded its distribution to north-east, becoming a regular visitor 2007, with possible breeding. Desert Finch (*Rhodospiza obsoleta*) expanded its distribution to the north, starting breeding in the country since 2013. The current examples show that many birds are entering Armenia, expanding their distribution range from the south, which is most-probably conditioned by the climate change, as it is observed in Europe and North America. Change of the birds' distribution is strengthening the link between tropical countries and Armenia thus bringing all the risks of possible tick-borne diseases to the country.

CLIMATE CHANGE IN HIGH-MOUNTAIN REGIONS OF ARMENIA

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For 80 years Cosmic Ray Division (CRD) of Yerevan Physics Institute has investigated galactic and solar cosmic rays, solar-terrestrial connections, high-energy atmospheric physics, and space weather. Recently, Aragats physicists enlarged research topics by installing modern facilities detecting the electrical and geomagnetic fields, radio emission from atmospheric discharges, rain rate, temperature, relative humidity, and other meteorological parameters. The adopted multivariate approach of investigations allows for connecting different fluxes, fields, and lightning occurrences and finally establishing a comprehensive model of the electrified lower atmosphere. The adopted methodology gives exciting results on the vertical and horizontal profiles of the atmospheric electric fields and explains radiation bursts within one framework and many others. Research on Aragats in 2019-2022 allows the discovery of substantial ionizing fluxes incident upon the terrestrial atmosphere during thunderstorms and vast electric fields (up to 200 kV/m) nearby the earth's surface (50-150m), which can have significant consequences on the safety of launching rockets and operation of the aircraft during thunderstorms. However, the vast meteorological information from 4 measuring locations contained in the databases of CRD can serve for climate change research. Mountain and polar regions are very sensitive to geospace changes and can be an early forewarning of upcoming climatic catastrophes. An example of the data available from CRD databases is shown in Table 1.

Table 1. Climate change patterns measured on Aragats mountain 3200 m asl

	Mean outside temperature (C°)	Variance of the outside temperature (C°)	Minimum of outside temperature (C°)	Maximum of the outside temperature (C°)	Mean wind speed (m/sec)	Variance of the wind speed (m/sec)	Maximum of wind speed (m/sec)
2011	-9.2	5.59	-23.5	4.9	3.03	2.5	16.5
2012	-7.8	4.8	-24.1	6.6	2.97	2.58	21.5
2013	-6.72	4.87	-21.5	4.6	2.58	2.39	19.7
2014	-6.94	5.32	-28.3	8.1	3.31	2.46	20.6
2015	-6.9	5.49	-28.3	8.1	3.25	2.47	16.1
2016	-9.01	5.84	-25.5	3.7	2.43	2.11	18.8
2017	-5.78	4.23	-18.2	4.3	0.45	1.22	14.8
2018	-7.55	4.65	-18.9	4.6	2.53	2.46	18.8
2019	-6.09	5.09	-27.8	7.2	1.98	2.05	21.9
2020	-6.62	4.77	-21.9	3.9	2.04	2.09	20.1
2021	-7.63	5.81	-24.7	6.8	2.35	2.33	23.2
2022	-7.6	5.66	-23.0	4.8	1.72	1.73	16.5



Aragats research station, 3200 asl, in the background bible Mt. Ararat, 100 km apart

ASSESSMENT OF THE IMPACT OF CLIMATE CHANGE ON THE ECOLOGY OF THE COMMON VOLE IN ARMENIA

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Introduction. According to the latest forecast, by 2030 the temperature in the territory of Armenia is predicted to rise by 1°C. According to the latest data, this increase has already taken place. According to the «Third National Communication on Climate Change», by 2100, climate changes are expected to cause the average annual temperature to increase to 10.2°C in Armenia. This exceeds the baseline by 4.7°C and precipitation is expected to decrease by 23%. Our study aimed to use climate forecasts made by the Intergovernmental Panel on Climate Change under the A2A scenario for 2050 for the country of Armenia to assess how these changes will affect the habitat and populations of the common vole as the main carrier of especially dangerous infections, and hence the epizootic potential these territories.

Materials and Methods. The *WorldClim* temperature and precipitation database was used, which is a set of global climate layers with a spatial resolution of about 1 square kilometer. This data was used for mapping and spatial modeling in geographic information systems. For spatial analysis, the database on the location of the common vole population in Armenia from 1981 to 2021 was also used. Separate layers were created from these sources and habitat areas with extreme and favorable habitat conditions for the common vole under climate change were differentiated.

Results. It is predicted that climate change will lead to the expansion of regions that are classified as desert, semi-desert, arid, and sparsely forested, which will lead to the movement of desert ecosystems up the mountain slopes by 250-400 m. In the area of dry mountain zones and areas bordering the mountain steppe zone, populations will either have to adapt or migrate to cooler and wetter habitats, for example, with access to the northern slopes. Areas of the meadow-steppe and lower subalpine belt, most densely populated by voles, are currently found at an altitude of about 2500 m. It is possible that some isolated populations will survive in lower areas, but probably only along the banks of large rivers, where vegetation will remain in sufficient quantities to feed them. Based on the overall reduction in size and isolation of many populations of common voles, we can assume a decrease in the number of epizootics in those places where they were previously recorded,

and vice versa, an increase in the number of epizootics of especially dangerous infections in those places where the areas of the main carrier will remain.

Conclusions. Based on this study, the authors tried to predict whether climate change will affect common vole ranges and the occurrence of epizootics. The number and distribution of the main reservoir of plague, tularemia, and other especially dangerous infections are predicted to be significantly reduced. The range of the remaining vole population will decrease and will be limited mainly to high mountain zones, above 2400-2800 meters above sea level. The current mountain-steppe and meadow-steppe ecosystems will be shifted to areas that are currently subalpine and alpine. According to the authors, additional surveillance and research are needed to document changes in the ecology of zoonotic diseases, as well as closer collaboration between human and animal health authorities for risk assessment.

**CHARACTERISTICS OF THE EPIZOOTIC ACTIVITY
OF THE FOCUS OF TULAREMIA IN THE SOUTHEASTERN REGION
OF THE REPUBLIC OF ARMENIA IN 1970–2018**

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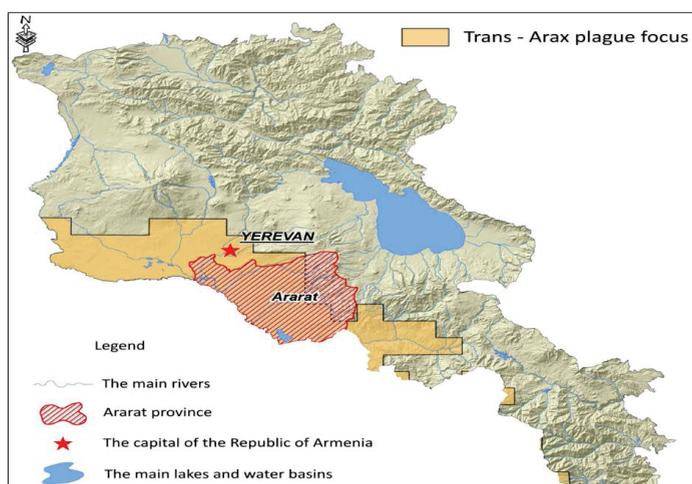
The role of the common vole population dynamics in the activity of the tularemia focus in the Southeastern Region of Armenia has been determined. The prognostic value of the dynamics of the number of voles as a factor determining the epizootic activity of tularemia focus is manifested only at high rates and is relatively small. To create a full-fledged model for predicting epizootic activity of tularemia focus, it is necessary to search for additional significant ecological factors. Almost 86, 7% of the strains of the tularemia pathogen are isolates from the common voles. Thus, the common vole is the basis of the ecosystem of the tularemia focus in the southeastern region. Throughout the entire period of the study - from 1970 to the present - there is a pronounced, statistically significant trend towards a decrease in the number of common voles. The studies carried out within the framework of this work have shown that the number of voles is declining as a result of global warming. It has been established that in the region there is an increase in the average annual air temperature with a practically unchanged amount of precipitation. Analysis using the Mann-Whitney-Wilcoxon test showed that the average annual temperature, according to the Sisian weather station, has increased by 0.85 degrees over the past 24 years (from 7.02°C to 7.87°C, $p < 0.01$), according to Meghri weather station increased by 1.18°C (from 14.17°C to 15.35°C, $p < 0.01$), Goris weather station by 1.1°C (from 8.55°C to 9.65°C, $p < 0.01$). At the same time, according to the data of the meteorological stations Sisian, Meghri and Goris, there was no increase in precipitation. In other words, against the background of global warming, aridization of the climate occurs in the focus. The arid climate is unfavorable for the life activity of common voles, its impact is especially pronounced in the mountain-steppe zone, and somewhat less in the high-mountain zone. Analysis of the data confirms that high temperatures, even in the cold months of the year, are unfavorable for the abundance of the common vole in the mountain-steppe belt, but an increased amount of precipitation has a positive effect. The adverse effect of global warming on the abundance of the common vole is less pronounced in the high mountain zone. Thus, the study showed an important fact: the aridization of the climate observed over the past 50 years leads to a decrease in the average number of voles.

THE POSSIBLE IMPACT OF THE CLIMATE CHANGE ON THE TRANS-ARAX PLAGUE FOCUS

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Introduction. The polyhostal, polyvector Trans-Arax plague focus (TAPF) in Armenia covers semi-deserts, dry mountain steppes, and mountain steppes with a total territory of 450,000 hectares (Fig. 1). The major carriers of the plague in the TAPF are rodents Vinogradov's jird (*Meriones vinogradovi*) and the Persian jird (*Meriones persicus*), while the main vectors are fleas *Xenopsilla conformis* and *Ctenophtalmus iranus*. The objective of this survey is to observe the density dynamics of carriers and vectors of plague over the last 5 years, clarify the influence of climatic conditions on carrier and vector density dynamics, and estimate biological risks in region.

Figure 1. Map of the Trans-Arax Plague Focus.



Materials and Methods. Gero-type traps and live traps were used for trapping Vinogradov's jirds, and aspirators were used to trap *X. conformis* and *C. iranus* in three regions of Ararat Province, located within the TAPF. Trapping locations were selected based on vector and carrier density. About 500-600 mammals and 700-800 vectors were trapped annually in early spring and late autumn. Trapping location coordinates and type of material collected were entered into the Reference Laboratory's database of TAPF data. Blood and tissue samples from collected organisms were tested to identify *Yersinia pestis* (polymerase chain reaction, indirect immunofluorescence assay, classical bacteriological

method, infection bioassays, microscopy, etc.). The SPSS Statistics program was used to analyze the correlations between temperature and vector density.

Results. Density of both carriers and vectors depended on climate conditions (Table 1): Vinogradov's jirds and their associated vectors increased in density in the seasons following hot summer and warmer winter conditions (spring and autumn, respectively). As shown in Table 2, summer 2017 was particularly warm (absolute high temperature of +42.5° C and dry (absolute absence of precipitation). High summer temperatures were strongly correlated with *X. conformis* density (correlation coefficient=0.93, p-value <0.01). Of all the carrier and vector samples tested from 2013-2017, zero were positive for *Y. pestis*.

Table 1. Density of *M. vinogradovi*, *X. conformis*, and *C. iranus* per hectare, by year and season.

	2013		2014		2015		2016		2017	
	Spring	Autumn								
<i>M. vinogradovi</i>	-	2,8	6,15	3,3	3,05	2,3	3,1	4,1	5,8	19,3
<i>X. conformis</i>	2,8	5,2	5,9	3,1	10,3	8,7	6,9	8,1	24,3	47,7
<i>C. iranus</i>	10,4	17,6	11,3	4,7	5,3	3,7	2	3	30,5	65,5

Table 2. Average temperature, by year and season

	2013	2014	2015	2016	2017
spring	+17,2	+14,3	+15,5	+13,4	+9,3
summer	+24,3	+27,5	+30	+26	+37
autumn	+10,2	+8,4	+11,2	+9,1	+14,4
winter	-12,2	-13,5	-9,1	-10,6	-6,3

Conclusions. Notable increases in the densities of both carriers and vectors of plague in the spring and autumn of 2017 directly followed an unusually mild winter and warm summer that year. The TAPF of Armenia is mainly located in Ararat valley, where most of the population spends the year working in the fields, where direct contact with both carriers and vectors of plague is possible. Additionally, Ararat province is located within the boundaries of greater Yerevan, which increases the epidemiological and epizootological danger to the capital and its population. While none of the tested isolates were positive for *Y. pestis*, systematic epizootological studies should be carried out to track the density of and *Y. pestis* prevalence among plague carriers and vectors to allow a timely forecast of the epizootological situation.

CLIMATE CHANGE INFLUENCE ON RANGE EXPANSION OF INVASIVE *Aedes albopictus* MOSQUITO IN ARMENIA

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Ae. albopictus Scuse, 1895 (syn. *Stegomyia albopicta*; Asian Tiger mosquito) is an invasive mosquito species native to tropical and subtropical areas of Australia and Oceania, the Mediterranean region, Africa, North America and South America. This mosquito is known vector of Dengue, Zika and Chikungunya viruses, as well as the roundworms *Dirofilara immitis* and *D. repens*. It is also conceivable for the mosquito to transmit the West Nile fever virus and a group of mosquito-borne encephalitis viruses.

In Armenia, the studies on mosquito fauna have been conducted for almost a century on a regular basis, however the medically significant *Ae. albopictus* was not detected until 2016, when it was trapped by NCDPCP entomological team from a single locality in vill. Bagratashen (Armenia-Georgia border). Further recordings of *Ae. albopictus* dated 2017, 2018, 2020 are known from the same Tavush region, not far from the first registration point, and include towns Ayrum, Noyemberyan, and Ijevan (L. Paronyan et al., 2020). It could be therefore concluded that the range of this mosquito has been expanded southeastward, although in 2020 was still limited by subtropical areas of Tavush marz, at elevation range between 380 to 750 meters above sea level.

While conducting a mosquito field study in Lori Marz, in late September of 2022, our team managed to capture two adult females of *Ae. albopictus* mosquitoes by net in the daytime, in Marts River Canyon, which is situated at an elevation of 1,135 meters above sea level. The registration of *Ae. albopictus* from new locality, with a temperate climate and a higher elevation than its typical habitats, needs to be emphasized as evidence of the further areal expansion of the mosquito species, now to the southwest. This finding could possibly indicate an influence of climate change on the distribution shift of *Ae. albopictus*, a mosquito species with a large capacity for the transmission and distribution of several arboviral and parasitic infections that may now occur in Armenia. In this context, routine field surveillance of mosquito populations, particularly those of arthropod/insect vectors should be carried out continuously and serve as a tool for early disease detection and prompt response to emerging disease.

Research has been performed within the frames of 20RF-163 Arm-Rus joint project.

**THEORETICAL FOUNDATION
OF ADAPTATION AND EVOLUTION**

THERMODYNAMICS OF ADAPTATION AND THE LE CHATELIER PRINCIPLE

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A major limitation of many thermodynamic devices (catalysts, heat engines) is that their functioning demands external fitting between the environmental parameters and internal parameters of the device. Here we explore thermodynamic limits of adaptation, where this fitting is achieved automatically. These limits are based on the Le Chatelier principle, whose implications for adaptation will be briefly reviewed. Within our minimal model of catalysis, particles are transferred from a higher chemical potential reservoir to a lower one. The catalyst is supposed to function with the maximal current under uncertain chemical potentials: if they change, the machine tunes its own structure fitting it to the maximal current under new conditions. This adaptation is possible under two limitations: (i) The degree of freedom that controls the device structure has to have stored energy (described via a negative temperature). (ii) The device has to malfunction in a constant environment due to structural fluctuations, whose relative magnitude is controlled solely by the stored energy. We argue that several features of the adaptive machine are similar to those of living organisms (energy storage, aging).

DISORDERED POLYELECTROLYTES IN CONFINED GEOMETRIES

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DNA and RNA are charged biopolymers commonly found in confined environments, such as protein capsids, nanofibers, etc. Their primary structure is essentially disordered. They also interact short-range (disorder) and long-range (electrostatic), allowing the use of the mean-field approach. We find the conformation statistics of this biopolymer as a solution to a nonlinear Schroedinger equation, and analyze the effects of confinement size and disorder strength. At a critical size, the biopolymer undergoes a conformational transition accompanied by a change in pressure and adsorption. After this transition, the system shows higher adsorption to the confining walls and negative total pressure.

TRANSPORT PROCESSES IN BIOMOLECULES AND QUANTUM INFORMATION

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The processes of energy and charge transfer in biomolecules play an important role in the vital activity of living organisms. For example, it is believed that protein molecules are the mediators of transfer of energy generated in the hydrolysis of triphosphate (ATP) for diverse biological processes such as photochemical reactions, cross-membrane ion transfer and muscle contraction. In its turn, charge migration in the DNA molecule resulting from, e.g., the action of ionizing radiation is directly related to the damage of DNA, which, as is known, can reduce the fidelity in the replication of the DNA molecule and, consequently, it can be the source of genomic mutations. Moreover, the description of transport processes in highly molecular structures at the quantum mechanical level makes it possible to consider also the question of quantum correlations arising in such systems. It is currently believed that quantum correlations and entanglement between the electronic states of nucleic acids in DNA are responsible, in particular, for the processes of its repair and replication. At the same time, it is important to realize that the transfer of energy and charge strongly depends on the molecular environment, which makes it extremely necessary to take the environment into account when describing such processes. Therefore, by studying the response in the dynamics of transport processes to changes in the environment, one can approach the analysis of the problems of adaptation and evolution.

In the proposed polaron approach to the description of transport processes in biomolecules, the possibility of energy and charge transfer in the form of states of a stable polaron (soliton) formed due to interaction with phonons is demonstrated. The nature of the polaron dynamics and the emerging quantum correlations between the structural elements of the macromolecular chain is analyzed taking into account the influence of temperature and the basic system parameters of the chain.

A.V. Chizhov. Quantum Correlation Effects in Biopolymer Structures. In: *Nonlinear Dynamics of Nanobiophysics* (Editors: S. Zdravkovic, D. Chevzovich). 2022. Singapore: Springer Nature Singapore Pte Ltd. Chapter 9. pp. 233-262.

EVOLUTION OF COMPLEXITY

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What are the origin and routes of evolution of the enormous hierarchical complexity of life that has no precedence in inanimate matter? Arguably, this is the ultimate question of evolutionary biology if not of biology as a whole. Then, to understand the origin of life with its complexity, a more general, envelope theory is required, perhaps, one that amounts to new physics explaining evolution and emergence of complexity across all scales in the universe, with life being a special case of this universal evolution. I argue that such a theory can be developed at the nexus between evolutionary biology, theory of learning, and non-equilibrium thermodynamics. The key concept for explaining the emergence and evolution of complexity is multilevel learning. I will formulate 7 fundamental principles of evolution that appear to be necessary and sufficient to render a universe observable and show that they entail the major features of biological evolution, including replication of genetic material and natural selection. These cornerstone phenomena of biology emerge from the fundamental features of learning dynamics, such as the existence of a loss function, which is minimized during learning. I will then sketch the general theory of evolution using the mathematical framework of learning in neural networks. To demonstrate the potential of the proposed theoretical framework, I will demonstrate how a generalized version of the Central Dogma of molecular biology can be derived by analyzing the flow of information during learning (back propagation) and predicting (forward propagation) the environment by evolving organisms. I then outline a phenomenological theory of evolution and origin of life by combining the formalism of classical thermodynamics with a statistical description of learning. Within this thermodynamics framework, major transitions in evolution, such as the transition from an ensemble of molecules to an ensemble of organisms, that is, the origin of life, can be modeled as a special case of bona fide physical phase transitions that are associated with the emergence of a new type of grand canonical ensemble and the corresponding new level of description. Driving from these theoretical principles, I will finally outline a model of the origin of life in specific biological terms.

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THE SOLUTION OF THE SMERLAK'S MODIFICATION OF THE EIGEN'S MODEL

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Eigen (quasispecies) model describes the virus evolution via a system of nonlinear differential equations (nonlinear master equation). The model describes the virus evolution, the origin of life and some other phenomena of interdisciplinary research. Recently M. Smerlak suggested a method how these nonlinear models can be connected to Markov (linear) models. Here we formulate the Smerlak's version of the Eigen model and solve the dynamics exactly at the large genome limit.

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Abstracts
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