

Cosmic Rays as Bio-regulator of Deep Time Terrestrial Ecosystems

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Abstract. This paper discusses the role of cosmic factors in evolutionary processes. The variability of reaction of metachromasy of polyphosphates of volutin granules in yeast cells was studied in connection to some cosmic factors activities due to the ancient origin of these biomolecules, presence in biological objects of different organization levels and their multifunctional role in cellular processes. A hypothesis about the sol-gel phase transitions in a state of the system discusses.

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Introduction

The study of biotrophic effects of space weather is impossible without answers to the question of the formation of evolutionary and adaptive scenario of ancient biosystems functioning. In this case, inorganic polyphosphates, so-called "fossil molecules", are of special attention as they are the most ancient molecules arisen in inanimate nature and accompanying biological objects at all stages of evolution (Brown and Kornberg, 2004). Changing of the state of these biomolecules, included into volutin granules of microorganisms, is seen in their reaction with methylene blue (metachromasy reaction).

In bacterial cells the volutin granules were found in the cytoplasm, near DNA, in cyanobacteria they are close to the cell structures that are capable to photosynthesis, while in yeast - in the cytoplasm and vacuoles (Kulacov et al., 2005). The most known location of volutin granules is at the cell poles of *Corynebacterium*. "Coryne" from Greek means "club", representatives of which are causative agents of diphtheria. Studying the growth and staining of corynebacteria cells in the '30s of the XX century the physician of the Kazan Hospital for Infectious Diseases S.T. Velkhover established a link between a behavior of microorganisms and the cosmic factors activity by comparing the results of the long-term observations of the agents of diphtheria with well-known works of A.L. Chizevskiy (Velkhover, 1935; Chizevskiy, 1976). In a history of science, this phenomenon is known as bio-astronomical effect of Chizevskiy-Velkhover.

Materials and methods

Yeast *Saccharomyces cerevisiae* strain Y-517 from the Ukrainian Collection of Microorganisms were used in the study. Yeasts were cultivated on a solid medium (wort-agar) in glass tubes with cotton-gauze in a thermostat at 28°C during 24 h. Every day during the passages of cells onto fresh medium, a part of cells

was resuspended in a drop of distilled water directly on the microscopy glass slide surface to form a monolayer. After drying in open air at room temperature, cells were fixed over the lab burner flame and stained with methylene blue by Leffler (Egorov, 1976). Color of volutin granules was examined by microscopy observation using bright-field method and lens ×100/1.30. Digital images of observed cells were captured with Carl Zeiss Primo Star microscope equipped with a digital camera Canon PowerShot A460 and Zeiss AxioVision image analysis microscope software. The presence of metachromasy and its degree was performed in the following code: "1" - no metachromatic reaction (volutin granules stained in the blue color), "2" - in the color of the volutin granules predominant saturated violet color, "3" - in the color of the volutin granules dominates purple (violet-red) color.

Automatic processing of digital images of the metachromasy of volutin granules was carried out in three stages: at first stage an algorithm of selection (segmentation) of volutin granules was implemented. During the second stage an individual index of metachromasy of volutin granules was evaluated. Finally, at the third stage, the integral assessment of the degree of metachromasy of the entire image was carried out. Details of this automated algorithm are described in (Ragulskaya, 2010).

To determine stable periods in rhythmic of metachromasy, a spectral Fourier analysis and cosinor analysis were used. For studying of dependence of metachromasy from cosmophysical factors ANOVA was used.

Results and Discussion

Assessment of visual information can be done subjectively during microscopy of colored objects. In

this case, the authors made the ranking of the effect where number 1 (a blue color) means absence of metachromasy, while numbers 2 and 3 represent different degrees of metachromasy.

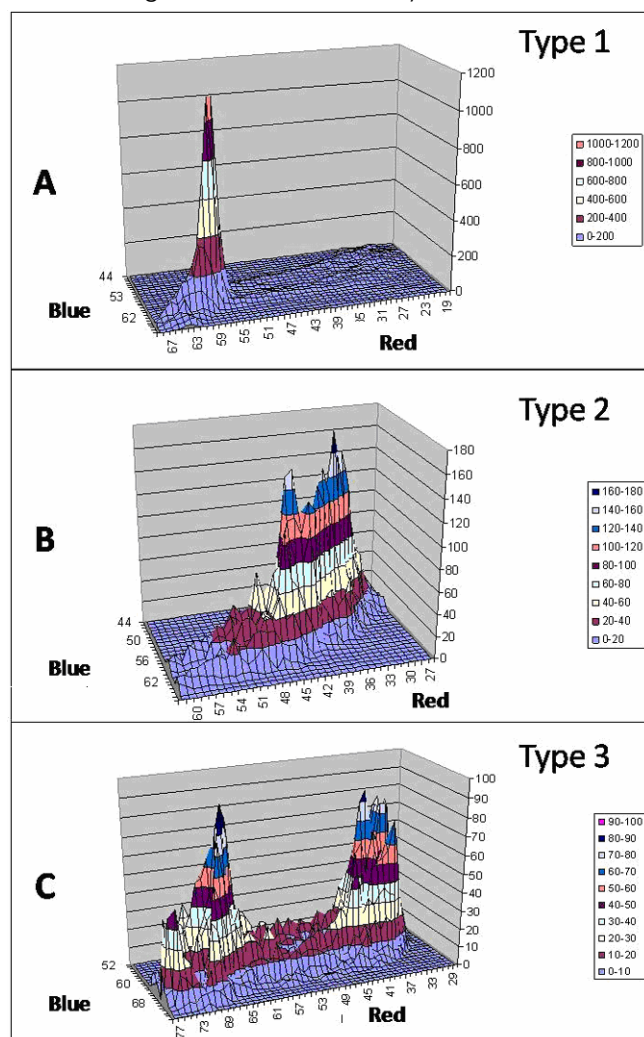


Fig. 1. The figure A shows the typical histogram of pixels in the grain volutin red-blue plane RGB-image model for the phenomenon of lack of metachromasy. On the figure B and C the similar histograms for the most obvious manifestation of metachromasy of volutin granules are presented

Visual assessment of the effect characterized with a large range of color perception, however, at the present stage the development of automated methods of working with digital photos of the effect, which would eliminate subjectivity, is required.

It is interesting to note that our first attempts at computer-based image analysis of the metachromasy allow us to propose the constructive hypotheses about the mechanism of reaction.

We made an analysis of histograms of color distribution of images pixels of the volutin granules without effect and with pronounced metachromasy effect (Fig. 1)

The figure shows that during metachromasy the polyphosphates of volutin granules exhibit at least two stable states. In our opinion this may be due to the sol-

gel phase transitions that lead to conformational changes of polyphosphates.

Interest in the study of this phenomenon is currently linked with the new data on the composition and functional role of volutin granules, intracellular inorganic polyphosphates and advanced technical capabilities in space weather studying.

Processing of the data of time series of metachromasy reaction (10 years of daily observations (2001-2011)) by means of spectral Fourier analysis and cosinor analysis let to reveal pronounced periods (Table 1).

Table 1. Comparison of the rhythms of the metachromasy and variations of solar activity indices (in days).

Stable rhythms of solar activity by Vladimirskiy et al. (1994)	Metachromasy periodicity	
	Cosinor analysis	Fourier analysis
3.5	-	3.2
5.0	5.0	5.3
7.0	-	7.4
9.0	9.0	10.5
13.5	14.0	13.7
17.0	-	18.0
21.0	22.0	20.0
23.0	-	23.0
26.0-29.0	27.0	28.5
30.0	-	-
34.0	35.0	33.8-35.9
44.0	46.0	44.0-46.5
53.2	51.0	52.9
-	59.0	59.0
87.0	89.0	84.6
114.0	108.0	103.0-119.0
120.0	124.0	121.0
152.0-155.0	253.0	153.0-157.0

Both of these methods yield similar results. As can be seen from the data shown in Table 1, the rhythmicity of metachromasy is almost identical to the known cosmic periods (Vladimirskiy et al., 1994). This fact can not testify about the causal relationship between these events, but clearly demonstrates a specific type of Solar-biosphere interactions: the synchronization of biological rhythms with variations of cosmic factors activity (Vladimirskiy and Temuryants, 2000). A similar rhythmicity was marked for emergence of abnormal bacterial colonies from aeroflora and of *Staphylococcus aureus* (Faraone et al., 2005).

In addition to the similarity of their rhythmic structure, a statistical correlation of metachromasy with the level of cosmic factors indexes over the same period of time may be another proof of connection of metachromasy to space weather. In this case, when the rate of metachromasy may have only the values 1, 2, 3, and time series are sufficiently long, it is reasonable to apply a one-way ANOVA.

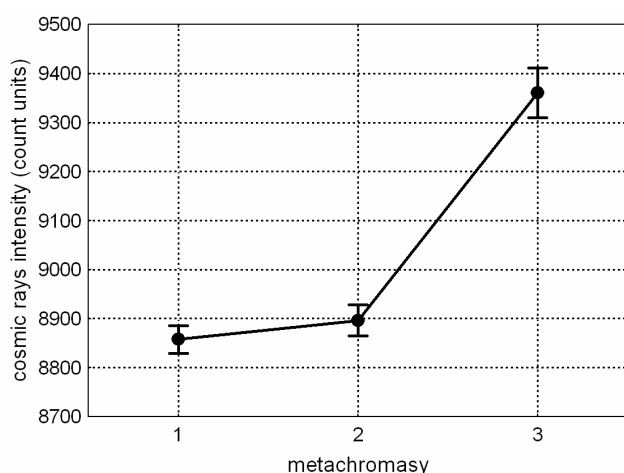


Fig. 2. Results of the one-way ANOVA of statistical dependence of the metachromasy index on the intensity of cosmic rays. On the abscissa axis is an index of metachromasy. On the ordinate axis are the mean values of cosmic ray intensity (in counts). The 95% confidence intervals are marked

The one-way ANOVA showed highly significant (close to the line) connection of the metachromasy index with cosmic rays ($F(2) = 151.14$ that corresponds to statistical significance at $p < 10^{-7}$ by Fisher test) – see Fig. 2. Connection of the metachromasy index with the parameters of factors of the Solar-Terrestrial nature was marked too: (i) the feedback with the solar activity ($F(2)=70.99$, $p < 10^{-6}$ by Fisher test), (ii) close to the inverse relationship with geomagnetic activity ($F(2)=19.90$, $p < 10^{-4}$ by Fisher test), and (iii) nonlinear coupling with the solar wind speed ($F(2)=20.01$, $p < 10^{-4}$ by Fisher test). Close to the negative correlations of the metachromasy index to the solar wind density ($F(2)=3.87$, $p=0.02108$ by Fisher test) and to the polarity of the interplanetary magnetic field ($F(2)=9.00$, $p=0.00013$ by Fisher test) were much less pronounced. (Gromozova et al., 2010)

It should be noted that the degree of statistical dependence of metachromasy on the mentioned above cosmic factors was significantly lower than those of cosmic rays, suggesting that the primary agent affecting metachromasy is directly connected with the cosmic rays (possibly with the high-energy particles). At the same time lower relationship of metachromasy with the other Solar-Terrestrial factors may be due to the fact that they are, to some extent, modulated by the intensity of cosmic rays in interplanetary space (Faraone et al., 2005). Then the logical interpretation of the results is as follows: the relationship with each of the Solar-Terrestrial factors exists, but this relationship is weaker than with the intensity of cosmic rays (mostly of galactic origin).

Thus, according to the results of ANOVA, on the background of a clear correlation between the detected rhythms of metachromasy and stable cosmic rhythms, the cosmic rays can be considered as the most significant factor in this interaction. A similar conclusion was achieved by Israel researchers after study of the dependence of the deaths in oncology (Stoupelet et al., 2002), cardiology (Stoupelet et al., 2008),

as well as the relationship of depression and suicide with space weather (Stoupelet et al., 2005). The population consequences of changing of the dose of cosmic radiation in the minima of solar activity were identified. The correlation coefficient between infant mortality and the dose of the neutron component of cosmic rays is $+0.51$ (Lozitska, 1998).

It should be noted that energy of modern cosmic rays is too weak to significantly affect the cellular structures. Therefore, to consider the modern cosmic rays as a really existing biotropic agent is at least incorrect from a physical point of view. To resolve the contradiction between theories and experiment we must go to the question of the evolutionary adaptation of ancient ecosystems. When assessing the impact of radiation of the Sun and of other cosmic factors on the formation of life on the Earth and evolutionary adaptation of ancient biosystems the "principle of actualism" should be followed (the necessity to use modern analogues during historical reconstructions). That is, in theoretical models the conditions of ancient Earth are included initially, and they are very different from today ones, while the parameters of solar activity and radiation flux by default are considered similar to those observed in the present.

In many modern works the significant differences of solar dynamics in the epoch of life origin and during its early stages of development (about 4 - 4.2 billion years ago) were shown. It is shown by Katsova and Livshits (2009) that the properties of differential solar rotation are closer to the stars with lower regular activity (typical for younger stars) than to the stars with established cycles. That is the more active non-stationary processes were observed in the young Sun, than it was previously thought. With regard to biophysical problems, this means that even undisturbed Sun of epoch of formation of life on Earth had emitted much more ultraviolet radiation (shorter than 304\AA) than the present Sun at its maximum of activity. The number of subflashes and their intensities, as well as a soft X-ray flux, was much more and stronger. These impulse phenomena were accompanied by very efficient particle acceleration up to relativistic energies. New data on the activity of the ancient sun allow authors to make some hypotheses about the evolutionary processes of adaptation of the ancient biosystems:

1 The time of life origin.

It is known that ultraviolet radiation and particles of relativistic energies are a factor of active mutations. On the other hand, the excess of their intensities above a certain level is destructive to biological systems. It is likely that the origin of life itself in the range 4 - 4.2 billion years ago, no sooner and no later, was determined by the moment of achievement of an equilibrium of the reactions of origin and destruction of new organic structures under the influence of cosmic radiation of different types. Until that time, even under favorable temperature, atmospheric and lithospheric conditions, a newly formed (or brought with meteorites) life was almost immediately destroyed with

the next pulse of the external radiation flux preventing the start of the evolution process. It is probable that the establishment of such equilibrium was due to the transition of the Sun to a more ordered type of activity.

2 *The emergence of protein structures.*

The emergence of secondary and higher order of complexity of the structures of proteins may be due to a defensive reaction of the ancient probiots to the intense ionizing radiation, which values were several orders higher than ones that usually considered in the literature today. Folding of linear forms in complex structure allows bringing the atoms, which make up the protein, closer to each other, thereby increasing the overall effective potential and the scattering cross section to increase the stability of the structure to the damaging external influences. The presence of phase transitions, marked in the present study, support this hypothesis.

3. *The emergence of system-forming evolutionary functions of cosmic factors and their inefficiency today.*

It can be assumed that the effects of adaptation of biosystems to cosmic-terrestrial factors observed today are in fact the atavisms of those ancient times when the solar radiation was much more intensive and was a real destructive factor for newly formed life on Earth. Modern solar-terrestrial and cosmic processes do not possess enough energy for such effects, however the reaction is uniquely and reproducibly marked by numerous researchers at all levels of the biosphere, including at the cellular level. After the change in the evolutionary dynamics of the Sun and the sharp decrease in the intensity of the accelerated particles and soft X-ray, the cosmic rays became not dangerous, but left some suitable as an external synchronizer of individual rhythms of organisms and biological systems in general.

Considering a polyfunctionality of inorganic phosphates in cells (bioenergetic aspects, ion transport, gene expression, etc.) and their evolutionary role

(Kulaev et al., 2005) it is possible to suggest that study of the mechanism of the metachromasy reaction of volutin granules of yeast cells may lead to the explanation of a lot of phenomena of space biology. Apparently, the continuation of this work requires the combined efforts of specialists in different fields of knowledge.

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