Rapid #: -18521879

CROSS REF ID: **6050207**

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TYPE:	Article CC:CCL
JOURNAL TITLE:	Biology bulletin reviews
USER JOURNAL TITLE:	Biology Bulletin Reviews
ARTICLE TITLE:	Oxidative stress, rRNA genes, and antioxidant enzymes in pathogenesis of schizophrenia and autism: Modeling and clinical recommendations
ARTICLE AUTHOR:	Porokhovnik, L. N
VOLUME:	4
ISSUE:	5
MONTH:	
YEAR:	2014
PAGES:	363-373
ISSN:	2079-0864
OCLC #:	

Processed by RapidX: 1/20/2022 10:30:36 AM

ISSN 2079-0864, Biology Bulletin Reviews, 2014, Vol. 4, No. 5, pp. 363–373. © Pleiades Publishing, Ltd., 2014.
Original Russian Text © L.N. Porokhovnik, V.P. Pasekov, N.A. Yegolina, T.G. Tzvetkova, N.V. Kosyakova, N.L. Gorbachevskaya, N.K. Sukhotina, G.V. Kozlovskaya, A.B. Sorokin, N.Yu. Korovina, N.A. Lyapunova, 2013, published in Zhurnal Obshchei Biologii, 2013, Vol. 74, No. 5, pp. 340–353.

Oxidative Stress, rRNA Genes, and Antioxidant Enzymes in Pathogenesis of Schizophrenia and Autism: Modeling and Clinical Recommendations

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Received December 3, 2012

Abstract—Ribosomal genes (RG), or rRNA genes, in eukaryotic genomes are represented by numerous tandem repeats, of which only a portion are transcriptionally active. The number of active copies is a constant feature of genome genome determining the cell's ability for the rapid synthesis of proteins needed to overcome the effects of stress. A low number of active RG copies leads to reduced stress resistance and elevated risk of multifactorial diseases (MFDs). Oxidative stress (OS) in the brain cells is believed to be involved in the pathogenesis of infantile autism (IA) and schizophrenia, that is, MFDs with severe genetic predisposition. With autism, OS markers are detected almost in every study, while with schizophrenia the OS data are contradictory. In a sample of patients with schizophrenia, we previously found a significantly higher quantity of active RG copies than in the population on average. In this work, we have determined the number of active RG copies in a sample of patients with IA ($n = 51$) and revealed a significantly lower mean value than in a healthy population. A novel mathematical model of the dynamic pattern of OS has been proposed. This model was implemented as a system of ordinary differential equations and assumes the induction of antioxidant protection enzymes being mediated by reactive oxygen species (ROS), with a subsequent decrease in the intracellular concentration of ROS. The rate of synthesis of antioxidant protection enzymes is limited by the ribosome synthesis rate, which depends on the number of active RG copies. Analysis of the model showed that the system always approaches a single point of stable equilibrium via the mechanism of damping oscillations, which to a certain extent resembles the dynamics of "predator–prey" interaction in the Lotka–Volterra model. The equilibrium ROS level inversely depends on the number of active RG copies. Our study allows us to explain the inconsistency of clinical data when detecting OS in IA and schizophrenia, suggesting a novel criterion for differential cytogenetic diagnostics of schizophrenia and IA, and to assume that antioxidant therapy should be effective only for children with a low number of active RG copies.

DOI: 10.1134/S2079086414050065

INTRODUCTION

Ribosomes are cytoplasmic organelles that provide the biosynthesis of all cellular proteins. A key component of ribosomes is ribosomal RNA (rRNA), the molecules of which are transcribed from matrices of ribosomal genes (RG). RG encode 18S, 28S, and 5.8S rRNAs, which are part of large and small subunits of ribosomes. Serving the mechanism of protein synthesis, the principal features of which almost have not changed over 1.5 billion years, the coding sequences of RG are characterized by high conservatism. In the genomes of all eukaryotes, the ribosomal genes are assembled into ribosomal repeats (RRs) represented by a large number of copies. In the diploid human genome, there are about 400 copies of RRs (Bross and Krone, 1972) assembled into clusters of different sizes in the short arms of five pairs of acrocentric chromosomes (13–15, 21, and 22), which form the nucleolus organizer regions (NORs) of chromosomes. For over 20 years, the laboratory of general cytogenetics of RCMG RAMS have studied the amount of RRs in individual human genomes (Lyapunova et al, 1988; Veiko et al., 1996; Lyapunova et al., 1998, 2001) and the effects of their copy number in normal and patho-

logical cases. The data obtained suggested that the number of RR copies varies from 250 to 670 in the diploid genome of different individuals (Veiko et al., 2003), wherein only a part of them (which varies among individuals) is transcriptionally active (Lyapunova et al., 1998; Veiko et al., 2001; Lyapunova and Veiko, 2010). The ability of transcription is provided by epigenetic mechanisms and is a stable and heritable characteristic of each RG copy (Santoro, 2011). Selective staining of metaphase chromosomes with silver nitrate (Ag-staining) allows selective detection of copies of active ribosomal genes (ARGs) in each of the 10 NOR on the metaphase plate (Lyapunova et al., 1998; Lyapunova et al., 2001; Miller et al., 1976; Howell and Black, 1980). The sizes of the silver precipitate (AgNOR-sizes) reflect the number of copies of active ribosomal genes in this NOR, are a stable heritable characteristic, and can be assessed visually in gradations from 0 to 4 arbitrary units. The total size of all 10 AgNOR can serve as an indicator of the number of ARG copies in the diploid genome of the individual (Lyapunova et al., 2001). The total sizes of 10 AgNOR in a large sample of almost healthy donors and patients with various diseases of hereditary and nonhereditary nature are determined.

On the basis of these materials, we formed an anonymous computer database. In a sample of $n = 715$ of almost healthy donors, the studied trait ranged from 15 to 24 arbitr. units, showing a distribution close to the normal with a mean value of 19.0 arbitr. units. In certain studies it was shown that one arbitr. unit of AgNOR corresponds to 8 ± 1 active RG copies (Veiko, 2001). Consequently, the amount of AcRG, which provides for the intensity of protein synthesis in the cell, in individual human genomes varies from 120 to 190 copies with an average value of approximately 150 copies. This is an important feature of the genome, which has a variety of phenotypic manifestations (Veiko et al., 2005; Neudakhin et al., 2008; Lyapunova et al., 2000; Morukov et al., 2008). In particular, the fewer transcriptionally active RG copies there are, the less pronounced is the cell's ability for the rapid synthesis of proteins needed to overcome the effects of stress.

According to current opinions, the effect of stress factors of any nature in competent cells causes the cessation of normal protein synthesis and a switch to the synthesis of a complex of antistress protection proteins, both specific and nonspecific (such as heat shock proteins). In in vitro experiments on human fibroblasts, we previously showed the existence of an "early" response to oxidative stress, which is induced by hexavalent potassium chromate. This response is expressed in the activation of transcription of ribosomal genes (rRNA genes) and an increase in the amount of total RNA and rRNA in the cells during the first 1.5–2 h of the experiment. It provides the emergence in the cytoplasm of new ribosomes required for the synthesis of a complex of antistress protection proteins (Veiko et al., 2005). It has been shown that the magnitude of the early response and hence the cell's ability to overcome the stress are proportional to the number of copies of active ribosomal genes in the genome of the cell.

It is known that oxidative stress plays a central role in many multifactorial diseases, including some autoimmune ones, among which rheumatoid arthritis (RA) is one of the most common and thoroughly studied. The serum and cells of RA patients showed a high level of free radicals and oxidative stress markers and reduced activity of antioxidant enzymes (Taysi et al., 2002; Kamanli et al., 2004; Maurice et al., 1999; Nagler et al., 2003; Kovacic and Jacintho, 2003; Karatas et al., 2003; Agostini et al., 2002; Ozturk et al., 1999). Our experiments studying the effects of oxidative stress on cultured cells from healthy donors and patients with RA revealed a positive correlation between the cell's resistance to the effects of oxidative stress and the number of copies of transcriptionally active rRNA genes (ribosomal genes) in its genome (Veiko et al., 2005). The number of AcRG copies in a sample of RA patients ($n = 49$) ranged from 14.4 to 20.7 arb. units with an average of 17.6 ± 0.2 (SE) arb. units. This value is significantly lower than the average in the control group of healthy donors aligned by gender and age ($n = 49$), which was equal to $19. \pm 0.2$ arb. units ($p < 0.01$) (Shubaeva, 2005). This observation allowed the authors to suggest that, even in the presence in the genome of specific genes linked to a predisposition to RA, the expressed forms of the disease develop at small and, rarely, medium (less than 150 copies) amounts of AcRG in the genome of the patient.

The multifactorial diseases with severe hereditary predisposition also include schizophrenia and early infantile autism (EIA). Despite the intensive study, the pathogenesis of these diseases is still not fully understood. Environmental influences (psychogenic-stress, infectious, intoxication, and others) play the role of factors that trigger manifestation and modulate the course of the disease. Early schizophrenia and EIA are often manifested in similar psychopathological phenomena, complicating differential diagnostics and systematization of these disorders in the absence of specific markers.

Early infantile autism is a common developmental disorder characterized by impaired communication, cognitive, and speech areas and the presence of stereotyped, repetitive behavior (*American Psychiatric Association*..., 1994). A tenfold increase in the frequency of diagnosed autism over the past decade, to a level of about one case per 150 children in the United States, arouses keen interest in the community and among medical scientists (McCarthy and Hendren, 2009).

There are numerous indications of the important role of oxidative stress in the pathogenesis of EIA and schizophrenia (Chauhan A. and Chauhan V., 2006; Bitanihirwe and Woo, 2011). The relation of oxidative

stress and neuropathology is not accidental. The brain is one of the most intensive consumers of oxygen in the body. Basal metabolism in the brain cells is 10 times higher than average for the body and is only inferior to the intensity of metabolism in the heart and kidneys. The brain amounts to only 2% of the body weight but consumes approximately 20% of the oxygen absorbed by the body (Elia, 1992).

Patients with EIA show reduced resistance to oxidative stress, which is caused by reduced levels of antioxidant enzymes. One of the consequences of oxidative stress is lipid peroxidation, the marker content of which is increased in the case of autism (Ming et al., 2005; Zoroglu et al., 2004; Chauhan A. et al., 2004). Changes in the composition of membrane phospholipids, the main targets of the effects of reactive oxygen species (ROS), in children with autism as compared to their healthy siblings are detected (Chauhan V. et al., 2004). The levels of major antioxidant transport proteins, transferrin (iron-binding protein) and ceruloplasmin (protein that binds copper), in the serum of autistic children are decreased, and there is a correlation between reduced levels of these proteins and the degree of regression (loss of previously acquired skills) (Chauhan A. et al., 2004). Patients with EIA show an increased content of Zn, Ca, Fe, As, Ni, Cd, and Si, as well as increased expression of metallothionein isoform mRNA in peripheral blood leukocytes as compared with the control group of children (Vergani et al., 2011). Several publications show data on the reduced activity of antioxidant enzymes such as superoxide dismutase, glutathione peroxidase (Golse et al., 1978), and catalase (Zoroglu et al., 2004; Yorbik et al., 2002; Pasca et al., 2006), decreased levels of reduced glutathione as compared to its oxidized form (Main et al., 2012), and impaired homocysteine-methionine metabolism (James et al., 2004) in autism.

However, other publications report a higher activity of superoxide dismutase in the serum (Chauhan V. et al., 2004; Vergani et al., 2011; Golse et al., 1978) with a slightly decreased catalase activity in peripheral blood leukocytes in children with EIA as compared with the control group of children (Chauhan V. et al., 2004; Vergani et al., 2011) or unmodified superoxide dismutase activity in the blood plasma and increased glutathione peroxidase activity in autism (Sogut et al., 2003).

The data on oxidative stress in schizophrenia are much more controversial. A number of studies described reduced levels of antioxidant enzymes in patients with schizophrenia as compared with the control group (Dadheech et al., 2008; Singh et al., 2008; Raffa et al., 2009). However, other studies showed either no change (Srivastava et al., 2001) or an increased antioxidant status in patients with schizophrenia (Kuloglu et al., 2002; Dakhale et al., 2004; Kunz et al., 2008). A recent meta-analysis suggests an increased level of lipid peroxidation products, significantly reduced activity of superoxide dismutase, and unchanged, as compared with the control group, glutathione peroxidase and catalase activity in the patients with schizophrenia (Zhang et al., 2010). Other studies carried out by different groups of researchers reported reduced (Reddy et al., 1991), elevated (Herken et al., 2001) or normal (Yao et al., 1998) catalase levels in patients with schizophrenia. No less controversial were the data published on the activity of glutathione peroxidase in patients with schizophrenia (Herken et al., 2001; Ranjekar et al., 2003; Gawryluk et al., 2010). Independent measurements of superoxide dismutase levels are also not consistent with one another, as different studies showed an increase (Reddy et al., 1991; Zhang et al., 2003), decrease (Ranjekar et al., 2003; Mukerjee et al., 1996) or no differences (Yao et al., 1998) in the activity of this enzyme in the schizophrenic patients as compared with normal levels.

Thus, the data published on the features of oxidative stress in schizophrenia and EIA generally favor the presence of oxidative stress in these diseases, but show a mixed picture, especially with respect to cells' antioxidant protection enzymes. This ambiguity is more pronounced in studies of schizophrenia rather than EIA. Such differences in the test results have not yet been adequately explained. The decreased levels of antioxidant enzymes can be interpreted as the conditions that lead to oxidative stress. It is more difficult to assess cases in which the content of antioxidant enzymes in the cell appeared to be elevated. When interpreting such cases, the authors tend to make the reasonable assumption that an increased level of antioxidant protection is a compensatory response to the increased amount of ROS. On this basis, it is concluded that elevated levels of superoxide dismutase, catalase, or glutathione peroxidase indicate the presence of oxidative stress. But one should not forget that the confrontation of free radicals (ROS) and enzymes, the role of which is to catalyze reactions for ROS elimination, constantly proceeds in the cell. If at some point the balance is lost in favor of free radicals, it does not yet mean that the cell was not able or will not be able to, having compensatorily generated a large amount of antioxidant enzymes, effectively cope with the occurred stress. To clarify this issue, one needs to model and (or) experimentally verify the dynamics of the cell's struggle with oxidative stress. If the intensity of oxidative stress and corresponding compensatory response of the cell is exposed to cyclical fluctuations, then a random sample of patients that have undergone a single study for oxidative stress could include patients who were at different points in the cycle of fluctuations. Therefore, due to the small sample sizes, signs of oxidative stress were found in some studies but not in others.

It has long been known that one of the most reproducible epidemiological relationships characteristic for schizophrenia is the significantly reduced incidence of rheumatoid arthritis in patients with schizo-

phrenia (Gilvarry et al., 1996; Oken and Schulzer, 1999; Gorwood et al., 2004; Eaton et al., 1992, 2006). Various hypotheses were offered to explain this strong negative epidemiological relationship; however, a convincing test of these hypotheses was largely hampered by the lack of proper understanding of the genetic basis of schizophrenia and rheumatoid arthritis and possible linkages between them.

We previously examined the number of copies of active ribosomal genes in the genomes of 44 patients aged 20–60 years with a diagnosis of "schizophrenia." It turned out that the genomes of patients with schizophrenia on average contain significantly more active copies of rRNA genes than the control sample. In a sample of patients with schizophrenia, the trait ranged from 18.0 to 23.7 arb. units at a mean of 21.1 ± 0.22 (SE) as compared to 19.0 ± 0.23 (SE) in the control group ($p \pm <0.001$) (Veiko et al., 2003). Thus, rheumatoid arthritis and schizophrenia occurred to be diametrically differing in terms of the number of active copies of ribosomal genes (let us recall that RA occurs only with a low or medium copy number of active ribosomal genes—no more than 150 copies per diploid genome), thus opening up a possibility of a completely new interpretation of the known epidemiological data on low combined occurrence of these two diseases.

In light of the above data, it is interesting to note that families where children suffered from early infantile autism, by contrast, show increased occurrence of rheumatoid arthritis in the first-degree relatives. So, an increased incidence of RA was found in mothers whose children suffer from autism (Crespi and Thiselton, 2011). By interviewing parents in 61 families with children suffering from autism spectrum disorders and 46 control families with healthy children, it was noted that the average rate of autoimmune diseases is higher in families with autistic children as compared with families with healthy children (Stigler et al., 2009).

In this regard, we have obtained the first objective of this work: to determine the number of copies of transcriptionally active ribosomal genes in the genomes of children with EIA. In addition, we analyzed the dynamics of oxidative stress as a function of the number of copies of rRNA genes, which determines the rate of cell response to elevated levels of free radicals (ROS). Thus, the second objective of this work was to construct a mathematical model describing the dynamics of oxidative stress (ROS concentration) depending on the cell's antioxidant capabilities, which are largely dependent on the copy number of active ribosomal genes in the genome.

MATERIALS AND METHODS

The data on the copy number of active ribosomal genes in diploid cells of patients with schizophrenia and the control group of healthy donors were taken from an anonymous database of the Cytogenetics Laboratory of RCMG RAMS. The blood samples of anonymous patients with early infantile autism ($n = 51$) were received from the Moscow Research Institute of Psychiatry of the Health Ministry of the Russian Federation and the Mental Health Research Center of the Russian Academy of Medical Sciences (MHRC RAMS).

The study included children with autism spectrum disorders corresponding to the diagnostic criteria of early infantile autism (EIA) of the International Classification of Diseases (ICD-10) and the American Psychiatric Association (DSM-IV-TR).

The patients were selected in children's psychiatric departments using a clinical-psychopathological method that included observation of the child in various situations with psychopathological assessment of the behavior, emotional and cognitive manifestations, and social functioning features, complemented by materials of medical documentation (cards of outpatient studies provided by parents). We conducted a study of genetic factors and the early medical history of patients, taking into account peculiarities of the pregnancy and childbirth and information about the mother's diseases. Initial assessment of the status was also carried out using the Childhood Autism Raiting Scale (CARS) (Schopler et al., 1980).

The age of patients at the time of the study ranged from 2 years 8 months to 13 years at a ratio of boys and girls of 3.5 : 1. The study did not include children whose autistic manifestations were associated with other diseases, such as organic lesions of the central nervous system, epilepsy, congenital metabolic defects, etc. To eliminate these kinds of diseases, we used the method of comparative EEG mapping in addition to the clinical analysis.

The informed consents of legal representatives of the patients were obtained for carrying out the studies.

The PHA-stimulated peripheral blood lymphocytes of patients with infantile autism were cultured using the standard technique. Cell fixation and metaphase chromosome preparations were carried out also according to the standard techniques. Selective staining of the preparations with silver nitrate was conducted by the technique of Howell and Black (Howell and Black, 1980) with our modification (Lyapunova et al., 1998; Lyapunova et al., 2001). The number of active RG copies was determined by summing the rank estimates, averaged over 20 metaphase plates, of the precipitate size of metallic silver above each of the 10 NORs in arbitr. units from 0 to 4.

For mathematical modeling of the intracellular dynamics of ROS and antioxidant enzymes, we used the apparatus of ordinary differential equations. The mathematical tools used are given in the Handbook of Mathematics by I.N. Bronshtein and K.A. Semendyaev (1981). The numerical study of the model was carried out using the noncommercial software package ODE (Moscow Power Engineering Institute).

RESULTS

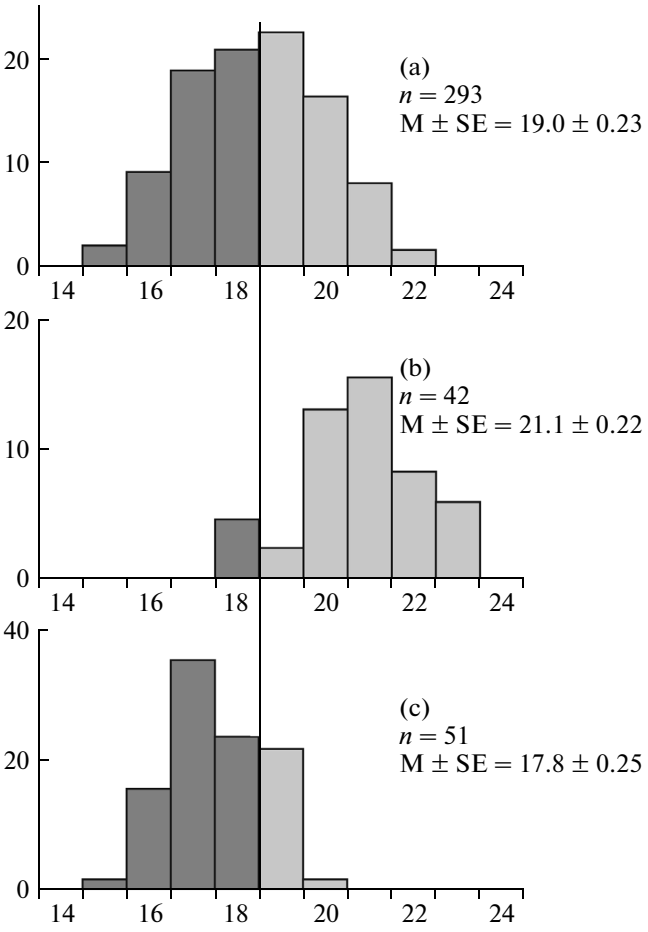
a. Copy Number of Active rRNA Genes in Children with Autism

In the genomes of children with EIA and autism spectrum disorders ($n = 51$), the number of transcriptionally active copies of rRNA genes ranged from 15.5 to 20.1 arb. units with a mean of 17.8 ± 0.25 (SE), which is significantly different from the mean value in both the control sample ($p < 0.01$) and the sample of patients with schizophrenia ($p < 0.001$) (see figure).

b. Mathematical Modeling of the Dynamics of Oxidative Stress Depending on the Number of Copies of Active rRNA Genes

As the intensity of oxidative stress in the model, we adopted the dimensionless reduced total concentration of reactive oxygen species (ROS) regardless of their nature. For mathematical modeling of the dynamics of ROS and antioxidant enzymes, we used ordinary differential equations and an approach similar to the one that Vito Volterra (Volterra, 1931) applied in his "predator–prey" model. There are numerous applications of this model in biology and medicine at the molecular and cellular levels: models "antigen–antibody," "bacterium–macrophage," etc. (Marchuk, 1980). In the proposed model, ROS are considered as an analogue of "prey," while the enzymes-catalysts of reactions of ROS neutralization act as "predators." The model is given by the rates of change of ROS and antioxidant enzymes (AE) that catalyze the ROS neutralization reaction in the cell (see Appendix). We believe that these rates are made up of components that correspond to the processes of the isolated growth of ROS and AE and the processes of their interaction.

In the absence of AE (AE is quantitatively described by variable y), ROS (variable x) increases in the simplest case at a rate proportional to the current presence of ROS x. Thus, the first component of the growth rate of ROS can be written as k_1x, where k_1 is some positive coefficient (constant). This component corresponds to an exponential growth of prey in the absence of predators in the ecological Lotka–Volterra model. It should be emphasized that the exponential growth of the number of free radicals was adopted in this model according to the same formula by which the number of the biological species increases under unlimited reproduction of individuals, despite the fact that for antioxidant molecules there are no mechanisms that provide their autocatalytic "reproduction." The fact is that the formation of ROS in cells is a self-accelerating process because of a variety of molecular mechanisms. An example is the process of lipid peroxidation, which is a chain reaction with the formation of lipid hydroperoxides according to the following formulas:



Histograms of the number of copies of active rRNA genes in samples of healthy donors (a) and patients with schizophrenia (b) and autism (c). Abscissa, number copies of active ribosomal genes, arbitr. units; ordinate, frequency, %. Black color shows values below average in the control, gray shows values above average in the control; n, sample size; M, arithmetic mean; SE, standard error.

$$\begin{aligned} R^{\cdot} + O_2 &= RO_2^{\cdot}, \\ RO_2^{\cdot} + RH &= ROOH + R^{\cdot}, \\ R^{\cdot} + O_2 &= RO_2^{\cdot} \text{ etc,} \end{aligned} \qquad (1)$$

where $R^{\cdot}$ is the radical; $RO_2^{\cdot}$ is the superoxide radical; RH is the lipid molecule; and ROOH is the lipid hydroperoxide.

According to (1), the concentration of radicals in the environment remains constant; only the amount of hydroperoxides (ROOH) increases. Chain peroxidation reactions occurring in nature, however, are more complex and are characterized as a spontaneous accelerating reaction because of the degenerate branching of the chain. Chain branching occurs with the participation of ferrous ions constantly present in the cell. The basis of this process is the emergence of new free radicals in the decay of hydroperoxides, as a

result of which the total concentration of free radicals in the system increases and the rate of oxidation increases continuously (Vladimirov and Archakov, 1972):

$$\begin{gathered} ROOH + Fe^{2+} = Fe^{3+} + OH^- + RO^\cdot, \\ RO^\cdot + RH + O_2 = ROH + RO_2^\cdot. \end{gathered} \quad (2)$$

There are also other processes that lead to an autocatalytic increase in the level of ROS, such as oxidative modification of xanthine dehydrogenase (Boldyrev, 2001), but they are beyond the scope of this article.

The second component of the ROS rate corresponds to the ROS and AE interactions and describes the ROS decline, which is proportional to the current values of x and y, and is written as $-k_2xy$, where k_2 is a positive constant. This component is an analog of descending victims because of their destruction by the predator.

Let us now refer to the rate of change of AE in the cell. In the simplest case, regardless of the presence or absence of ROS, AE molecules degrade at a constant speed so that their decline is proportional to the current presence of AE in the cell, that is, the corresponding component of the rate is written as $-K_2y$, $K_2 > 0$. The other component of the rate of change of AE indicates the influence of ROS and complies with the production of AE in response to oxidative stress. This component is proportional to the current presence of ROS and does not depend on the presence of AE in the cell. Therefore, let us write it as K_1x, $K_1 > 0$. The coefficient (K_1) of the AE synthesis rate is dependent on the copy number of active ribosomal genes, as the number of copies of the ribosomal repeat determines the concentration of ribosomes in the cell and, as has been repeatedly shown previously, thereby modulates the overall rate of translation. There is no overlap with the corresponding addend in the Lotka–Volterra model, but both models describe "plus–minus" interaction, in which one of the considered types suppresses the other and the latter favors the growth of the former. In the environmental context, such interactions correspond to the relations of predator and prey.

As a result of the assumptions made, we have the following model of the dynamics of ROS and AE in the cell (the prime denotes the derivative with time):

$$x' = k_1x - k_2xy, \quad y' = K_1x - K_2y. \quad (3)$$

Of course, the proposed scheme of interaction of the rates of the ROS and AE dynamics is greatly simplified; in reality, there are interactions not only between but also within the two types of molecules considered. Theoretically, the components of the rates should be limited and so on, but let us start by considering a simpler case. Since the components of the rates are not known numerically for ROS and AE, let us study just the qualitative properties of the model: we will investigate issues of the existence of equilibria and their stability, the presence of oscillations and their nature under a single assumption—positive and constant coefficients k_1, k_2, K_1, and K_2.

In the proposed model, we neglect mitochondrial mechanisms for oxidative stress repression, which consist in uncoupling of respiration and phosphorylation under a deficiency of adenosine diphosphate (ADP) or when ROS levels increase, resulting in the reduction of intracellular concentration of O_2 (Skulachev, 1996). Apart from considerations of reasonable simplification of the model, we assume that an active metabolism in infancy makes a permanent deficit of ADP unlikely. In addition, there are indications of the spread of mitochondrial diseases in children with autism (see review by Rossignol and Frye, 2012). Consequently, the mitochondria of patients with EIA can conversely be a constant source of ROS in abnormally high quantities and in the presence of ADP. For healthy individuals, by contrast, there is a described mechanism of positive feedback between the concentration and rate of formation of ROS in the mitochondria (Zorov et al., 2000) upon energy starvation (ADP excess), but the implementation of such conditions, such as ischemia in infancy, is also unlikely .

An analysis of model (3) is presented in the APPENDIX; here we provide only its main results. The analysis consists of three stages: a search for equilibrium points, a study of local behavior of the system near the equilibrium points (stability analysis of equilibria), and an analysis of the global behavior of the system. If the equilibrium is unstable in the mathematical sense (that is, under small deviations from it, simply put, their significant growth is possible), one should not expect implementation of such an equilibrium due to random disturbances that are common to natural environment. In other words, the system deviates from a point of unstable equilibrium and tends to a sustainable solution. The model always (that is, for any values of the coefficients) have two equilibria. The first is a zero balance with the complete absence of both free radicals and antioxidant enzymes: $x = 0$, $y = 0$. Analysis of this equilibrium showed its instability. Consequently, in a living cell such a condition cannot be realized, since respiration inevitably generates free radicals (small deviations from zero in terms of the model) and the system deviates from the unstable equilibrium.

In addition to the zero equilibrium, there is always one more equilibrium with positive values of the variables (x, y) of the form

$$(x^*, y^*) = \left(\frac{k_1K_2}{k_2K_1}, \frac{k_1}{k_2}\right). \quad (4)$$

From (4), it is seen that the higher the rate K_1 of the antioxidant enzyme synthesis on ribosomes is, the lower the equilibrium level $\frac{k_1K_2}{k_2K_1}$ is for ROS.

For all values of the coefficients of the model, this equilibrium is asymptotically stable, that is, both pos-

itive and negative small deviations from equilibrium decay with time. Interestingly, when $K_2 < 4k_1$, deviations decay in an oscillatory manner, that is, with a change of sign. Given the fact that antioxidant enzymes ("predators") are large protein molecules with a long half-life in the cells and that free radicals ("prey") are very short-lived and reactive species, it becomes obvious that the rate of the chain growth of free radicals is higher than the rate of excretion from the cell or the decay of protein enzymes, that is, $k_1 > K_2$, and all the more so $K_2 < 4k_1$. Therefore, we have an oscillatory nature of approaching equilibrium.

Model (3) can be interpreted as a model describing dynamics of oxidative stress in some individual with his inherent properties (individuality is expressed through the values of model coefficients). Thus, each individualized model, regardless of other properties of the individual (except for the state (0, 0), which is inaccessible in reality), has a stable equilibrium, and the main characteristic of oxidative stress—the concentration x of free radicals—tends toward the equilibrium value (4). In it $x = \frac{k_1 K_2}{k_2 K_1}$, the denominator contains coefficient K_1, which shows the rate of generation of antioxidant enzymes by the cell under stress and reflects, as was mentioned above, the number of transcriptionally active RG copies of the individual. Consequently, the more copies of active ribosomal genes are found in the genome of the individual, the lower the ROS concentration, and therefore the level of oxidative stress, is in this individual.

In addition to fading oscillations, the models of the type "predator–prey" theoretically may have continuous oscillations with constantly sustained periodic changes in ROS and AE (*cycles*). The analysis shows that the dynamics of this type is not possible in model (3). An unlimited increase in ROS and AE in the model is also not possible, that is, all the trajectories with positive initial values of ROS and AE tend to a single stable equilibrium position.

DISCUSSION

In patients with schizophrenia, the genomic dose of active ribosomal genes (the number of copies of rRNA genes) is above the population mean, whereas in children with early infantile autism the copy number of active rRNA genes, in contrast, was below the healthy population mean. The mean and variance in the sample of patients with infantile autism were almost the same as in the sample of patients with rheumatoid arthritis.

This fact is of considerable practical importance, since the analysis of the number of copies of active rRNA genes can be used as a new objective criterion for differential diagnostics of early schizophrenia and EIA. Furthermore, on the basis of this fact and the simulation results, it can be suggested that the contribution of oxidative stress is more pronounced in the pathogenesis of early infantile autism than in the pathogenesis of schizophrenia.

As was shown by mathematical modeling, the dynamics of oxidative stress is oscillatory. These fluctuations in the ideal model are damping. However, the cell with a varying frequency is in reality constantly exposed to external stress influences that bring it out of equilibrium. The system then tends toward equilibrium by oscillatory trajectory. It is impossible to predict in which particular phase of the cycle (ascending or descending) an individual would occur at the time of measurements. Shown by the model, the cyclical dynamics of the process can explain the contradictory data on the presence of oxidative stress in schizophrenia and childhood autism. Typically, the published papers studied oxidative stress a single time in a small sample of patients who probably were at different stages of cyclic oscillations—both at peak stress and on the decline.

We believe that the increased content of antioxidant enzymes, which was observed in some experiments in the context of the absence of lipid peroxidation markers, is characteristic for the downward phase of the cycle of the cell's struggle with oxidative stress.

Based on the above assumptions, we can outline the following directions for future research.

First, it seems important to estimate the average level of oxidative stress in patients, that is, the equilibrium point by multiple definitions of some indicators at regular long enough intervals of time with subsequent averaging. Such an averaged indicator should be negatively correlated with the indicator of the genomic dosage of active ribosomal genes, which should be determined for each patient participating in the study. The presence of such a correlation would be evidence in favor of the correctness of our assumptions.

Second, the literature has some indications of positive results of antioxidant therapy in early childhood autism (Chez et al., 2002; Dolske et al., 1993; Morris and Agin, 2009; Yui et al., 2012; Hardan et al., 2012). It is of interest to study the impact of antioxidant therapy in a sample of children who have undergone assessment of the genomic dose of active ribosomal genes. If our hypothesis about the relationship of the copy number of active ribosomal genes and oxidative stress as a mechanism of EIA pathogenesis is correct, then the therapeutic effect of antioxidant therapy should be most pronounced in children with a low copy number of active ribosomal genes (<17 arbitr. units), for which the intensity of oxidative stress in the cells is maximal. The presence of a negative correlation between the effectiveness of antioxidant therapy and the copy number of active rRNA genes in a patient would also be a convincing confirmation of our hypothesis.

The study of relationships between the copy number of ribosomal genes, oxidative stress, and various

pathologies may shed light on the role of the meaning of free radicals and oxidative stress for the body. It can also help to answer the question of whether oxidative stress is only an inevitable price to pay for the evolution-based possibility of aerobic utilization of chemical energy sources or whether it provides signaling and safety functions, protecting the cell from a hostile (chemically or microbiologically) environment. The latter view is reflected in the interesting concept of "oxidative shielding." From the viewpoint of this concept, oxidative stress is considered as the most evolutionarily ancient response to adverse effects in the framework of the innate immunity (Naviaux, 2012), which is activated under a variety of pathological conditions of the organism but does not cause them. Based on this, supporters of the concept of "oxidative shielding" conclude the uselessness of antioxidant therapy, which eliminates only the consequence of the disease. Further studies should clarify this issue.

APPENDIX: STUDYING THE OXIDATIVE STRESS MODEL

The proposed model of the dynamics of the dimensionless reduced total concentration (x) of reactive oxygen species (ROS), irrespective of their nature, and antioxidant enzymes AE (y) in the cell is of the form

$$x' = k_1x - k_2xy = x(k_1 - k_2y),$$
$$y' = K_1x - K_2y,\ k_1, k_2, K_1, K_2 > 0, x, y \geq 0. \quad \text{(A1)}$$

Here the prime denotes the derivative with time, constant coefficients k_1, k_2, K_1, and K_2 are the specific growth rates of ROS, neutralization of each ROS unit catalyzed by AE, AE production as a response to ROS, and decay and/or removal of AE from the cell, respectively. Since the coefficients are generalized characteristics and their exact values are not yet known, assuming the roughness of the model let us investigate the system (A1) qualitatively, defining the behavior of trajectories without solving the equations of dynamics. Since the derivatives x' and y' turn to zero on the coordinate axes, then the trajectories that start at the nonnegative quadrant forever remain in it. Such trajectories either converge to equilibrium or to a limit cycle, or go to infinity. There are no regular methods for a full qualitative analysis of the behavior of trajectories in the general case of plane systems. However, in this case it is possible to determine the qualitative behavior of the trajectories of (A1).

First, let us show the absence of limit cycles in (A1) by applying the Dulac's criterion with the function $B(x, y) = 1/x$. Within the positive quadrant we have

$$\partial(Bx(k_1 - k_2y))/\partial x + \partial(B(K_1x - K_2y)/\partial y$$
$$= \partial(k_1 - k_2y)/\partial x + \partial(K_1 - K_2y/x)/\partial y = -K_2/x < 0,$$

which means no closed trajectories.

Second, the trajectories cannot go to infinity. To prove this, let us check the inequality $(x_2 + y_2)' < 0$ for sufficiently large values of x and y:

$$(x^2 + y^2)' = 2x \cdot x(k_1 - k_2y) + 2y(K_1x - K_2y)$$
$$= 2x^2\left(k_1 - k_2y + \frac{y}{x}\left(K_1 - K_2\frac{y}{x}\right)\right).$$

Let us suppose that y is sufficiently large. Then $k_1 - k_2y < 0$. The value of $\frac{y}{x}$ may be different, but the remaining member is bounded from above (as an arched up parabola from $\frac{y}{x}$). Therefore, $(x^2 + y^2)'$ is negative for large values of y.

It remains to consider the situation with equilibria. They are found from the system of equations

$$x(k_1 - k_2y) = 0, \quad K_1x - K_2y = 0. \quad \text{(A2)}$$

The obvious solutions of this system are points (0, 0) and $\left(\frac{k_1K_2}{k_2K_1}, \frac{k_1}{k_2}\right)$. Let us investigate the local stability of these equilibria in the linear approximation. The linearization matrices (A1) in these equilibria and the corresponding characteristic equations have the following form

$$\begin{bmatrix} k_1 & 0 \\ K_1 & -K_2 \end{bmatrix}, (k_1 - \lambda)(K_2 + \lambda) = 0, \begin{bmatrix} 0 & -k_1K_2/K_1 \\ K_1 & -K_2 \end{bmatrix},$$

$$\lambda^2 + K_2\lambda + k_1K_2 = 0.$$

Obviously, the first (zero) equilibrium is unstable, because the eigenvalues λ of the linearization matrix are real and of opposite signs. This saddle and coordinate axes are separatrices. All the coefficients of the characteristic equation for the second equilibrium are positive, which implies that the real parts of its roots are negative. Therefore, the model (A1) for all positive values of the coefficients not only has equilibrium with the presence of ROS and AE but is also asymptotically stable. Approaching it occurs with damping oscillations at $K_2 - 4k_1 < 0$, or deviations from the equilibrium that decrease exponentially with $K_2 - 4k_1 \geq 0$. The trajectories converge to this equilibrium from any point within the positive quadrant, that is, behave therein in a similar manner.

Like any other model, ours is a rather rough simplification of the real situation. More adequate (but less accessible to infer) is a model that takes into account interactions of molecules of the same type for AE, the enzyme kinetics by the Michaelis–Menten scheme, and the boundedness of specific rates:

$$x' = k_1x - k_2\frac{x}{k + x}y - k_3x^2, \quad y' = K_1\frac{x}{K + x} - K_2y,$$
$$k_1, k_2, k, K_1, K_2, K > 0, x, y \geq 0.$$

Theoretically, there could be up to four equilibria, not all of which may have biological meaning. The numerical analysis of particular cases of this model did not reveal any limit cycles, but for some values of the coefficients it is possible for the trajectories to go to

infinity. It is also possible that there are no equilibria with biological sense.

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Translated by K. Lazarev