Rapid #: -18473836

CROSS REF ID: **6045843**

LENDER: **COD :: Main Library**

BORROWER: **HUL :: Widener Library**

TYPE:	Article CC:CCL
JOURNAL TITLE:	Psychiatric genetics
USER JOURNAL TITLE:	Psychiatr Genet
ARTICLE TITLE:	Active ribosomal genes, translational homeostasis and oxidative stress in the pathogenesis of schizophrenia and autism.
ARTICLE AUTHOR:	Porokhovnik, Lev N
VOLUME:	25
ISSUE:	2
MONTH:	
YEAR:	2015
PAGES:	NA
ISSN:	1473-5873
OCLC #:	

Processed by RapidX: 1/10/2022 11:20:58 AM

Active ribosomal genes, translational homeostasis and oxidative stress in the pathogenesis of schizophrenia and autism

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Background **Infantile autism and schizophrenia are severe multifactorial disorders with a pronounced genetic predisposition. Their pathogeneses are often associated with oxidative stress in the brain. Previously, we established that a cell's resistance to oxidative stress depended on the copy number of transcriptionally active genes for rRNA (ribosomal genes) in the cell's genome. The feature is measured cytogenetically in cultured lymphocytes derived from patients. It varies from 120 up to 190 copies per diploid genome, with an arithmetic mean of 150 ± 4 (SE) copies in a healthy population ($n = 239$), being considerably lower, according to our previous results, in a sample of patients with rheumatoid arthritis ($n = 49$), another multifactorial disease with a proven significant role of oxidative stress in its pathogenesis: from 115 to 165 copies, with a mean of 140 ± 4 (SE). Conversely, a sample of schizophrenic patients ($n = 42$) previously showed a higher value of copy number of active rRNA genes compared with a healthy population: from 145 to 190 copies, with a mean of 170 ± 4. This fact is of special interest in the context of the well-known, but still unexplained phenomenon of the reduced comorbidity rate of schizophrenia and rheumatoid arthritis.**

Results **The copy number of active ribosomal genes was estimated in a sample of autistic children ($n = 51$). In contrast with the schizophrenic patients studied previously, we found that the values were significantly lower than those in the healthy population: from 125 to 160 copies, with a mean of 142 ± 5. In this work, we suggest a mathematical model of the oxidative stress dynamics on the basis of Lotka–Volterra's approach to predator–prey interactions. In our model, the 'prey' represents reactive oxygen species, whereas the 'predator' simulates molecules of the antioxidant enzymes. The rate of biosynthesis of the latter is limited by the number of ribosomes available, which, in turn, is determined by the copy number of active rRNA genes. Analysis of the model showed the existence of a unique equilibrium point that makes biological sense. The reactive oxygen species level oscillatory approaches this equilibrium value, which inversely depends on the copy number of active rRNA genes.**

Discussion **Our findings confirm the hypothesis of disturbance of the 'translational homeostasis' in the pathogeneses of autism and schizophrenia, and would help explain why oxidative stress markers are discovered in most autism studies, whereas similar reports related to schizophrenia are far less consistent.** ***Psychiatr Genet*** **25:79–87**

Psychiatric Genetics 2015, **25**:79–87

Keywords: autism, copy number, oxidative stress, predator–prey, ribosomal gene, rRNA gene, schizophrenia, translational homeostasis

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Received 25 November 2013 **Revised** 18 November 2014
Accepted 20 January 2015

Introduction

Schizophrenia and autism are multifactorial diseases with a pronounced genetic predisposition. Despite being studied intensively, their pathogeneses remain elusive in most cases. Environmental impacts (psychogenic stress, infections, intoxication, etc.) are considered to be external factors inducing manifestation and modulating the course of disease.

Autism is a widespread developmental disorder characterized by abnormalities of verbal and non-verbal communication resulting in impairment of socialization, stereotyped restricted interests and repetitive behavioural patterns. The 10-fold increase in the diagnosis of autism over the past two decades, now affecting about one in 150 children in the USA, has raised great public health concern (McCarthy and Hendren, 2009).

There is a large body of evidence that oxidative stress plays a significant role in the pathogeneses of autism and schizophrenia (McGinnis, 2004; Chauhan and Chauhan, 2006; Bitanihirwe and Woo, 2011). The relation between oxidative stress and neuropathology is not accidental. The brain is one of the most active oxygen consumers in the body. The basic metabolism rate in the brain cells is 10 times higher than the average rate for the entire body, and is exceeded in terms of the metabolism rate only by the heart and kidneys. The brain makes up about 2% of

0955-8829 DOI: 10.1097/YPG.0000000000000076

the body mass, and yet, it consumes 20% of metabolic oxygen (Elia, 1992).

Autistic patients often show lowered resistance to oxidative stress because of reduced levels of the antioxidant enzymes, such as superoxide dismutase, glutathione peroxidase (Golse *et al.*, 1978) and catalase (Yorbik *et al.*, 2002; Zoroglu *et al.*, 2004; Pasca *et al.*, 2006), lower levels of reduced versus oxidized glutathione (Main *et al.*, 2012) and altered homocysteine–methionine metabolism (James *et al.*, 2004). The patients also showed elevated markers of lipid peroxidation, which is the main consequence of oxidative stress (Chauhan *et al.*, 2004; Zoroglu *et al.*, 2004; Ming *et al.*, 2005), decreased levels of major antioxidant transport proteins, transferrin and ceruloplasmin, with a positive correlation between reduced levels of these proteins and the degree of regress (loss of previously acquired language skills) (Chauhan *et al.*, 2004), and significantly higher plasma levels of Zn, Ca, Fe, As, Ni, Cd and Si, and higher mRNA expression of the metallothionein isoforms in peripheral blood leucocytes compared with the controls (Vergani *et al.*, 2011).

Unlike autism, reports on the statuses of oxidative stress markers in schizophrenia are very inconsistent. Some authors report either increased or decreased activities of the main antioxidant enzymes, whereas others do not observe any significant modifications compared with the control groups. Similar facts were reported in relation to lipid peroxidation markers, although contradictions are far more reduced here than in the case of the antioxidant defence enzymes (Ciobica *et al.*, 2011).

Although reduced levels of the antioxidant enzymes are generally reported in patients with schizophrenia compared with controls (Dadheech *et al.*, 2008; Singh *et al.*, 2008; Raffa *et al.*, 2009), some studies showed either no change (Srivastava *et al.*, 2001) or the strengthening of antioxidant status in schizophrenic patients (Kuloglu *et al.*, 2002; Dakhale *et al.*, 2004; Kunz *et al.*, 2008). A recent meta-analysis indicated that there was an increase in lipid peroxidation products in schizophrenia, whereas superoxide dismutase activity was found to be significantly decreased and the activities of glutathione peroxidase and catalase showed no change in patients with schizophrenia compared with the controls (Zhang *et al.*, 2010). In other instances, researchers reported lower (Reddy *et al.*, 1991), increased (Herken *et al.*, 2001) or normal (Yao *et al.*, 1998) catalase levels in schizophrenic patients. The data published on glutathione peroxidase levels in patients with schizophrenia also appear to be inconsistent (Herken *et al.*, 2001; Ranjekar *et al.*, 2003; Gawryluk *et al.*, 2010). The results of independent measurements of superoxide dismutase are also inconsistent, showing an increase (Reddy *et al.*, 1991; Zhang *et al.*, 2003), decrease (Mukerjee *et al.*, 1996; Ranjekar *et al.*, 2003) or no change (Yao *et al.*, 1998) in enzyme activity in patients with schizophrenia compared with healthy controls.

Post-mortem examinations showed a 40% depletion of glutathione in the caudate nucleus in schizophrenic patients (Yao *et al.*, 2006). Similarly, Gawryluk *et al.* (2010) reported reduced levels of glutathione in post-mortem prefrontal cortex in schizophrenic patients. Magnetic resonance spectroscopy showed that glutathione levels were reduced by 52% in the prefrontal cortex and by 27% in the cerebrospinal fluid of drug-naive schizophrenia patients (Do *et al.*, 2000). However, later works failed to discover reduced glutathione levels in the anterior cingulated cortex (Terpstra *et al.*, 2005), the posterior medial frontal cortex (Matsuzawa *et al.*, 2008) and the medial temporal lobe (Wood *et al.*, 2009) using spectroscopy.

Thus, the data published on oxidative stress markers in schizophrenia and autism generally ascertain the presence of oxidative stress in these groups of disorders, and yet point to a quite ambiguous picture, especially with respect to the antioxidant enzymes. The ambiguity is more evident in the studies of schizophrenia rather than autism. The inconsistency in the experimental results has so far not been explained adequately. Reduced levels of antioxidant enzymes may be interpreted as a condition that causes oxidative stress. Cases in which antioxidant enzyme levels were found to exceed those in healthy controls were more difficult to estimate correctly. As a rule, the authors who encountered this condition made a reasonable assumption that the increased quantity of antioxidant enzymes was a compensatory response to an increased number of free radicals. This is why researchers usually come to a conclusion that increased superoxide dismutase, catalase or glutathione peroxidase is the evidence for oxidative stress. However, one should take into account that the opposition of scavenging enzymes to free radicals always occurs within the cell. If the balance is, at some time, shifted towards the predominance of free radicals, the cell can effectively cope with the resultant oxidative stress after launching biosynthesis of antioxidant enzymes as a compensatory mechanism. Enzyme molecules have longer lifetimes than free radicals and, therefore, remain in the cell for some time after most free radicals are eliminated and the stress condition ends. Thus, elevated antioxidant enzymes should be considered evidence of oxidative stress in the recent past, but the stress condition may disappear by the time of examination or after a while.

There are two possible nonalternative explanations for the inconsistency in the above experimental data: (a) oxidative stress is not intrinsic to schizophrenia and autism generally, except for some individuals and/or disorder varieties, or (b) the level of oxidative stress and that of the compensatory response is subject to oscillations. If the latter supposition is correct, then a randomly selected

sample of patients subjected to a one-time test for oxidative stress may include individuals at different stages of the oscillations. Therefore, taking into consideration the small sample sizes, the signs of oxidative stress may have been found randomly in some experiments and not in others.

To prove or disprove the above suppositions, one must have enough information as to the time dynamics of oxidative stress.

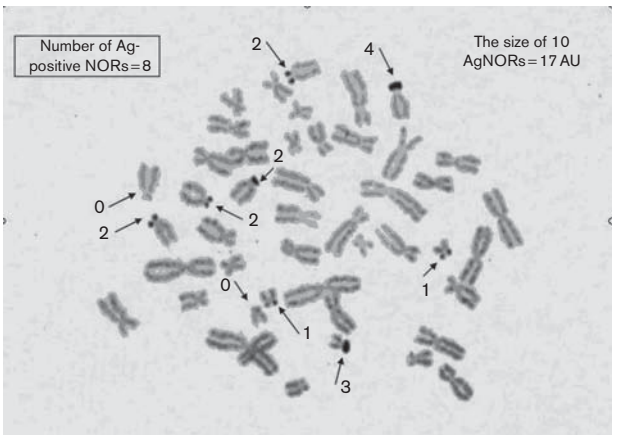
According to modern conceptions, exposure to stress factors of various natures induces the competent cells to abort the ongoing protein biosynthesis and switch to synthesizing a set of protective proteins, both specific and nonspecific (such as heat-shock proteins). In our earlier in-vitro experiments on human fibroblasts, we registered the 'early' response to oxidative stress, which included the activation of transcription of ribosomal genes (genes for rRNA) 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 cell cytoplasm with new ribosomes necessary for the synthesis of the set of antistress protective proteins (Veiko *et al.*, 2005). Moreover, the intensity of the early response and, hence, the cell's capability to cope with stress was found to be proportional to the copy number of active ribosomal genes in the cell's genome.

Human ribosomal genes (genes for rRNA) encode 18S, 28S and 5.8S rRNA, the basic components of the large and small ribosome subunits. These three genes are integrated into the transcribed regions of rDNA. The rDNA units, also termed ribosomal repeats (RR), are duplicated many times, with an average copy number of about 400 copies per human diploid genome (Bross and Krone, 1972), forming clusters of various size on the short arms of five pairs of human acrocentric chromosomes (13–15, 21 and 22) acting as nucleolus organizer regions (NOR) of these acrocentrics. Over the past 25 years, we have studied the copy number variation of the RR in human individual genomes (Veiko *et al.*, 1996; Lyapunova *et al.*, 1998) and the phenotypic effects of various copy numbers in health and pathology (Lyapunova *et al.*, 2012). We found reliable evidence to the effect that various individuals have 250 to 670 copies of RR per diploid genome (Veiko *et al.*, 2003), and only portions of the copies, numerically different in different individuals, are transcriptionally active, that is either transcribed or poised for transcription (potentially active or reversibly inactive) (Lyapunova *et al.*, 1998; Lyapunova and Veiko, 2010; Santoro, 2011; Lyapunova *et al.*, 2012). The remaining copies are stably inactive. The stably inactive copies do not contribute towards the cell metabolism, which makes it important to learn the copy number of the active (potentially active) fraction only. The stably inactive and reversibly inactive copies differ from each other in their chromatin structure, replication timing and different epigenetic marks, including histone modifications (Santoro, 2011). Because of the fact that the two fractions have the same primary DNA sequence, no sequence-based molecular technique such as PCR or FISH can distinguish between them. Any molecular technique developed so far can only count the total number of RR, including both active (potentially active) and stably inactive. The only way to selectively count the active copies is a cytogenetic method based on the staining of metaphase chromosomes with silver nitrate (Lyapunova *et al.*, 2012).

The silver staining of metaphase chromosomes derived from cultured lymphocytes (Ag-staining) enables the selective detection of the copies of active ribosomal genes in each of the 10 NORs on a metaphase plate (Miller *et al.*, 1976; Howell and Black, 1980; Lyapunova *et al.*, 1998). The size of the silver deposit (AgNOR size) correlates with the copy number of active ribosomal genes in the particular NOR. It is a stable (within the life span) and a heritable feature of each NOR capable of visual semiquantitative estimation ranking from 0 to 4. The summarized size estimation of all the 10 AgNORs (Fig. 1) can serve as an index of the active ribosomal gene copy number in the individual diploid genome under investigation (Lyapunova *et al.*, 1998, 2012).

We previously determined the summarized size estimations of the 10 AgNORs in a large sample ($n = 1191$) including both healthy donors and patients variously afflicted with diseases of a hereditary and a nonhereditary nature. The feature varies from 15 up to 24 AU, being normally distributed, with a mean of 18.9 AU. Advanced studies showed that one arbitrary unit of AgNOR

Fig. 1



Selective staining with silver nitrate (Ag-staining) of nucleolus organizer regions (NORs) of human chromosomes. The arrows point to short arms of the five pairs of acrocentric chromosomes. The digits near the arrows indicate the ranking size estimates of the silver deposits in arbitrary units (AU). This cell has two 0 (silent or deleted) NORs. The total size of 10 Ag-NORs is 0 + 0 + 1 + 1 + 2 + 2 + 2 + 2 + 3 + 4 = 17 AU. For more information, see the text.

corresponds to 8 ± 1 copies of rDNA (Lyapunova *et al.*, 2012). Consequently, the number of active ribosomal genes, limiting the top level of the cell protein biosynthesis, varies within a range from 120 up to 190 copies, with a mean of ~ 150 copies in various individual human genomes.

According to our previous data, the total copy number of active (transcribable) rRNA genes remains constant within the lifetime. A comparison between a group of newborn ($n = 99$) and that of senile individuals (80–100 years old, mean age 83 ± 3, $n = 100$) showed no difference in the mean summed Ag-NOR size (unpublished). This fact corroborates the correctness of comparing different age groups by this characteristic because age itself does not affect the feature within the lifetime of each individual.

Oxidative stress is known to play the principal role in many autoimmune diseases. These include rheumatoid arthritis (RA), one of the widest spread and best studied. Blood serum and cells of RA patients show high levels of free radicals and oxidative stress markers, and reduced activity of antioxidant enzymes (Maurice *et al.*, 1999; Ozturk *et al.*, 1999; Agostini *et al.*, 2002; Taysi *et al.*, 2002; Karatas *et al.*, 2003; Kovacic and Jacintho, 2003; Nagler *et al.*, 2003; Kamanli *et al.*, 2004). Earlier, we conducted a number of experiments aimed at studying the impact of oxidative stress on cultured cells derived from both healthy donors and RA patients. This study showed a close relation between the resistance of cells to the impact of oxidative stress and the copy number of transcriptionally active rRNA genes (ribosomal genes) in the cell genome (Veiko *et al.*, 2005). The number of active ribosomal gene copies in a test sample of RA patients ($n = 49$) varied from 14.4 to 20.7 AU, with a mean of 17.6 ± 0.2 (SE) AU. This value was significantly lower than the mean of the control group of sex-matched and age-matched healthy donors ($n = 49$), which was 19.0 ± 0.2 AU ($P < 0.01$) (Shubaeva, 2005).

One of the well-known and best-replicated immunological correlates of schizophrenia is an apparently reduced incidence of RA in individuals with schizophrenia (Gilvarry *et al.*, 1996; Oken and Schulzer, 1999; Gorwood *et al.*, 2004; Eaton *et al.*, 2006). The low rates of schizophrenia and RA comorbidity were initially reported in 1936 (Nissen and Spencer, 1936) and the first large-scale review found reduced rates of RA in schizophrenics across 12 of 14 studies (Eaton *et al.*, 1992). Diverse hypotheses have been proposed to explain this strong, inverse epidemiological association, but reliable tests of the hypotheses have thus far been precluded because of limited understanding of the genetic bases of infantile autism, schizophrenia and RA and their possible relations.

Previously, we studied the copy number of active ribosomal genes in the genomes of 42 adult patients 18–60 years of age diagnosed with schizophrenia. The genomes of schizophrenic patients were found to contain, on average, markedly more active copies of rRNA genes compared with the controls. In the sample of schizophrenic patients, the quantity varied from 18.0 up to 23.7 AU, with a mean of 21.1 ± 0.2 (SE), in comparison with 19.0 ± 0.2 in the control group ($P \ll 0.001$) (Veiko *et al.*, 2003). Thus, RA and schizophrenia appeared to be opposite in relation to the copy number of active ribosomal genes. This observation should provide an opportunity for a novel interpretation of the well-known epidemiological data on the decreased comorbidity rates of the two disorders.

In the light of the above, it is interesting to note that families with autistic infants, conversely, showed a higher incidence of RA and other autoimmune conditions in first-degree relatives. Thus, higher rates of RA were found in mothers of autistic offspring (Crespi and Thiselton, 2011). Using self-report questionnaires in 61 families of autistic children and 46 normal controls, the mean number of autoimmune diseases was found to be increased in families with autistic children compared with families with healthy children (Stigler *et al.*, 2009). Unfortunately, no direct data are available on the comorbidity of autism and RA.

Taking the above into consideration, our first objective in this work was to estimate the copy number of transcriptionally active ribosomal genes in the genomes of autistic children. Next, we analysed the dynamics of oxidative stress depending on the copy number of active rRNA genes, a characteristic that determines the rate of the development of a cell's response to an increased concentration of free radicals (reactive nitrogen and oxygen species). Thus, the second objective of this work was to create and to interpret a mathematical model describing the dynamics of oxidative stress (the concentration of free radicals) depending on the antioxidant capabilities of the cell, which, in turn, depend considerably on the number of copies of active ribosomal genes in the genome.

Materials and methods

The data on the copy number of active ribosomal genes in schizophrenic patients ($n = 42$) and healthy controls ($n = 293$) were obtained previously and then extracted from the anonymous database of the Cytogenetics Laboratory of the Medical Genetics Research Centre. The sampling criteria for healthy controls were 'apparently healthy' conditions and when available, familial histories without mental disorders. The schizophrenic patients selected for the study underwent in-patient medical treatment. They were 18–60 years of age, and their disease duration ranging from 1 month to 20 years.

Blood samples from anonymous patients with autism ($n = 51$) aged 3–9 years were supplied by the Moscow

Research Institute of Psychiatry and the Mental Health Research Centre.

Children were included in the test group if they fulfilled the diagnostic criteria for autism of the International Classification of Diseases (ICD-10) and the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV-TR), which included the following:

(1) Qualitative impairment of social interaction manifested by an inappropriate response to social and emotional cues such as eye contact, touching, communication of feelings, as well as poor use of social cues;
(2) Qualitative communicative impairment manifested by the lack of appropriate spoken language skills used for communication, abnormalities of pitch and intonation, delay or arrest of the speech development, verbal stereotypy, lack of an age-appropriate role or imitative play;
(3) Unusual interests and stereotyped behaviour, lack of flexibility and insistence on routines, distress over changes, preoccupations with specific nonfunctional routines;
(4) Delay or abnormal development, with onset before 3 years of age.

The patients were diagnosed as in-patients in psychiatric institutions using the psychopathological procedures, which included observation in various situations to assess behaviour, emotional and cognitive features and the peculiarities of social functioning. Medical records provided by the parents were also analysed. Genetic factors and case histories of pregnancy, birth and early development were taken into consideration. The Childhood Autism Rating Scale was used to perform initial assessments (Schopler *et al.*, 1980).

The patients' ages ranged from 2 years 8 months to 13 years, with a 3.5–1 male to female ratio. Children in whom autism was comorbid to other conditions, such as organic brain disorders, epilepsy or congenital metabolic diseases, were excluded. EEG mapping was used as an additional diagnostic method.

All three groups were uniform socially, ethnically and racially, and were from middle-class Moscow Caucasian Russian-speaking families.

An informed consent for participation in the scientific research was provided by the parents or legal guardians of all the patients. The institutional ethics board approval was obtained for the study.

Phytohaemagglutinin-stimulated peripheral blood lymphocytes obtained from autistic children were cultivated using the standard procedure. Cell fixation and the preparation of metaphase chromosomes were also carried out using the standard procedure. Selective staining with silver nitrate was performed according to the methodology described by Howell and Black (1980), with modifications (Lyapunova *et al.*, 1998). The determination of the genomic dose (copy number) of active ribosomal genes was carried out by the summation of the arithmetic means (averaged over 20 metaphase plates) of rank estimates of metallic silver deposit sizes on each of the 10 NORs in AU ranging from 0 to 4.

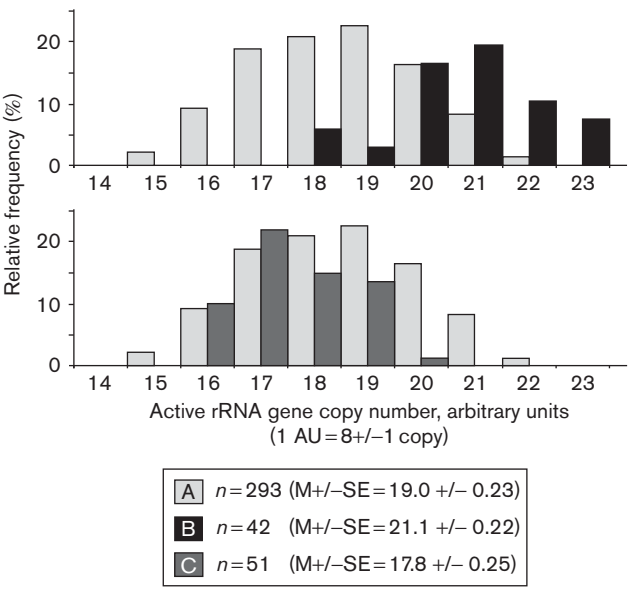
Ordinary differential equations were used for modelling the intracellular dynamics of reactive oxygen species (ROS) and antioxidant enzymes. Phase trajectories (Fig. 3) were plotted using the ODE free software pack.

Results

Number of copies of active rRNA genes in autistic children

In the genomes of children with autism spectrum disorder ($n = 51$), the number of transcriptionally active copies of rRNA genes per diploid genome varied from 15.5 up to 20.1 AU, with a mean of 17.8 ± 0.25 (SE). It differed significantly from the mean values in the samples of both healthy controls ($P < 0.01$) and schizophrenic patients ($P < 0.001$) (Fig. 2). Intriguingly, the parameters of distribution of the copy number of transcriptionally active rRNA genes in the genomes of autistic children appeared to coincide with the parameters of the sample of RA patients.

Fig. 2



Normalized distributions of the copy number of active rRNA genes in healthy controls (A) and patients with schizophrenia (B) and autism (C). Abscissa: the copy number of active ribosomal genes (arbitrary units); Ordinate: relative frequency (%). M, mean; *n*, sample size.

The mathematical model of oxidative stress dynamics depending on the copy number of active rRNA genes

In the model, we describe the intensity of oxidative stress in the cell by a nondimensional reduced total concentration of ROS without considering their nature. For the purpose of modelling the dynamics of ROS and antioxidant enzyme concentration, we used the same approach as that of Volterra (1931) in his 'predator–prey' model. Numerous applications of this model at molecular and cellular levels are known in biology and medicine: 'antigen–antibody', 'bacterium–macrophage', etc. In our model, ROS are considered 'preys', whereas enzymes, which quench them, are considered 'predators'. Let x denote the concentration of ROS in the cell and y be the total concentration of antioxidant enzymes. We propose the following system of ordinary differential equations describing the rates of change in these quantities:

$$\begin{aligned} x' &= ax - bxy, \\ y' &= cy + dx, \end{aligned} \tag{1}$$

where a, b, c and d are positive coefficients.

The first term of the equation for x' describes the exponential growth of the 'prey' (ROS) concentration at a 'birth rate' a in the absence of 'predators', whereas the second term describes the opposite tendency: a decreasing population of 'preys' because of the neutralization (quenching) of ROS as a result of their collisions with the molecules of antioxidant enzymes (predators) in accordance with second-order reaction kinetics, with an efficiency equalling b. The first equation that describes the dynamics of ROS concentration fully corresponds to the equation for the 'prey' in the Lotka–Volterra model.

The second equation also describes two opposed processes. The first term $(-cy)$ describes the disintegration and excretion of antioxidant enzyme molecules at a specific rate c (an equivalent of natural mortality for 'predator' individuals in Volterra's model), whereas the second term shows the synthesis of new molecules on ribosomes. In Volterra's model, the last term has a form of $+dxy$ and shows the reproduction of predators y in proportion with the number of preys x. In our case, the model takes into account that the antioxidant enzyme molecules y do not reproduce themselves, but are synthesized in proportion with oxidative stress x. Indeed, under the conditions of oxidative stress induced *in vitro* by potassium chromate, as we reported earlier (Veiko *et al.*, 2005), the amount of newly synthesized rRNA and, hence, the number of ribosomes engaged in the protective protein translation, within certain limits, increased linearly as the concentration of potassium chromate increased. In the same earlier work, we showed that the rate of the survival of cells after an oxidative stress impact ('late' response) depended directly on the copy number of active rRNA genes in the cell line genome. This implies that the coefficient of proportionality between the intensity of oxidative stress and the rate at which antioxidant enzymes are synthesized on ribosomes (coefficient d in our model) largely depends on the copy number of active ribosomal genes in the individual's genome.

The analysis of model 1 yielded two equilibrium points from the system:

$$\begin{aligned} ax - bxy &= x(a - by) = 0, \\ -cy + dx &= 0. \end{aligned} \tag{2}$$

The first solution of Eq. (2) is $(x, y) = (0, 0)$, which indicates a complete absence of both free radicals and enzymes of antioxidant protection. In a living cell, this is an impossible situation; thus, the solution makes no biological sense.

The other solution is as follows:

$$\begin{aligned} -ca/b + dx = 0,\ x = ac/bd, \\ \text{i.e. } (x, y) = (ac/bd,\ a/b) \end{aligned} \tag{3}$$

The linearization matrix at this nonzero equilibrium is as follows:

$$L(ac/bd,\ a/b) = \begin{bmatrix} 0 & -ac/d \\ d & -c \end{bmatrix} \tag{4}$$

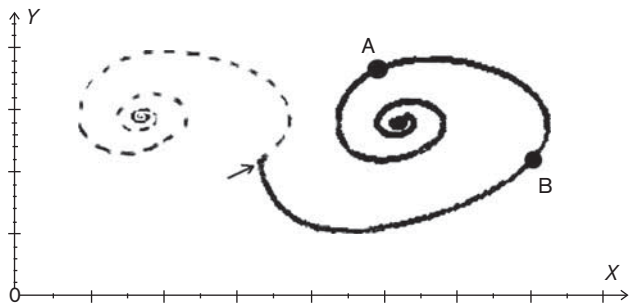
The eigenvalues of this matrix satisfy the quadratic equation $\lambda 2 + c\lambda + ac = 0$. As the coefficients of the resulting quadratic equation are positive, it is easy to show (using Vieta's theorem) that the real parts of eigenvalues λ are negative, that is equilibrium (3) is asymptotically stable. There are two types of perturbed state convergence on the equilibrium.

(1) If roots λ are real, that is the discriminant $c^2 - 4ac \geq 0$ then deviations from equilibrium converge on 0 as decreasing exponentials.
(2) If roots λ are complex, that is $c^2 - 4ac < 0$ then deviations converge on 0 as damped oscillations (Fig. 3).

Free radicals are very short-living compounds compared with the protein molecules of enzymes. Thus, $c < a$; hence, $c^2 - 4ac < 0$ and the oxidative stress intensity should show damped oscillations.

The numerical study shows that the key index of an oxidative stress, the concentration of free radicals x, converges on equilibrium $x = ac/bd$ irrespective of the initial state for all individuals, except for the state (0, 0). Here, the denominator has the factor d, the specific rate of antioxidant enzyme generation by a cell under stress conditions. As pointed out above, d is the genomic dose (the number of copies) of active ribosomal genes of the individual. Therefore, the more the copies of active

Fig. 3



Phase trajectories for two systems (1) with different values (d_1 and d_2) of coefficient d corresponding to individuals with different quantities of active ribosomal gene copies (the dashed line is for d_1 and the continuous line is for d_2, $d_1 > d_2$). All the other coefficients are identical in both systems. The coordinates of spiral focuses were obtained using formula (4). The initial point is indicated by an arrow. Abscissa axis: concentration of reactive oxygen species (ROS), x; ordinate axis: concentration of antioxidant protection enzymes, y. At point A, the oxidative stress (ROS level) is lower (but the level of antioxidant enzymes is higher) than that at point B. As the coordinates of perturbed system (1) cannot be predicted at the sampling time, a single measurement of antioxidant enzyme concentration does not provide an accurate estimation of equilibrium oxidative stress level.

ribosomal genes in the genome of an individual, the lower his/her equilibrium level of oxidative stress indicated by the number of free radicals.

Discussion

In adult patients with schizophrenia, the copy number of rRNA genes was higher than the average value in the healthy population, whereas in children with infantile autism, the copy number of active rRNA genes was, conversely, lower than the mean of the healthy controls. The mean and dispersion in the sample of autistic children were almost identical to those in the sample of RA patients.

In this context, distinct pathogenetic mechanisms underlying infantile autism and early-onset schizophrenia may be suggested.

Recently, a promising concept of 'translational homeostasis' (Yanagiya *et al.*, 2012) has emerged and very interesting reports have been published, providing evidence that a disturbance in the translational homeostasis, namely, an increased translation rate because of a mutation in the translation initiation factor 4E (eIF4E) may cause autism (Neves-Pereira *et al.*, 2009; Santini *et al.*, 2013). Another mechanism of disturbed translational homeostasis occurring because of a missense mutation in the ribosomal protein gene RPL10 was reported earlier (Klauck *et al.*, 2006).

Our findings also suggested that a relationship existed between the pathogenesis of mental disorders and the translation rate, but through a different mechanism. The copy number of active ribosomal genes, determining the maximum possible concentration of ribosomes synthesized within a certain time after a stress impact, obviously exerts an effect on translation intensity, which is especially important under poststress conditions and during the critical stages of ontogenesis. Apparently, the current concentration of ribosomes, translation units of the cell, directly determines the total translation rate.

Thus, translation homeostasis can be disturbed by altered translation initiation rates (mutated eIF4E), or by altered ribosomes with the normal translation initiation (mutated ribosomal protein RPL10), or by an increased/decreased amount of normal ribosomes with the normal translation initiation (low or high copies of active ribosomal genes), and the effect of these seems to be similar or the same. Further studies are required on this subject.

As shown by the mathematical model, oxidative stress dynamics are of an oscillatory nature. The oscillations are damped in our ideal model. However, the real cell is regularly exposed to stress impacts, knocking it out of equilibrium. After becoming imbalanced, the cell would return to the steady point along an oscillation trajectory. When measuring the oxidative stress markers, it is impossible to determine in which cyclical phase (ascendant or descendant) the individual is at the time of sampling. The periodical dynamics of oxidative stress found on analysing the results of the modelling might explain the inconsistency in the experimental data on the existence or absence of oxidative stress during the course of schizophrenia and infantile autism. The authors of most published works tested for oxidative stress by a single measurement of enzymes in a small sample of patients, who, apparently, were going through different stages of periodic oscillations, being either at the top or at the bottom of the stress condition.

We believe that the experimental picture of increased antioxidant enzymes together with the absence of lipid peroxidation markers is characteristic of the declining phase of the cycle of cell struggle with oxidative stress (in our model, x is behind y in phase; see Fig. 3, points A and B).

On the basis of the above assumptions, we can point to the following lines for future investigations.

First, the average oxidative stress level in patients, that is, the steady point, can be determined by multiple determinations of an unstable parameter (or parameters) over certain sufficiently long-time intervals, with subsequent averaging. This averaged index is expected to correlate negatively with the genomic dose of active ribosomal genes, which should be measured in every patient participating in the research. The existence of such a correlation will serve as evidence that our assumptions

are true. Alternatively, focusing on stabile parameters may yield more reliable information on the averaged oxidative stress level in terms of its long-term time dynamics. These 'stabile' compounds are those that are accumulated over a long period of time, such as 8-oxo-guanosine, which is a DNA oxidation marker, or thio-barbituric acid and malondialdehyde, which are lipid peroxidation markers. The concentration of such markers should correlate negatively with the copy number of active ribosomal genes in the genome if our model is correct.

Second, there are a number of published reports on the beneficial effect of antioxidant therapy in the treatment of infantile autism (Dolske *et al.*, 1993; Chez *et al.*, 2002; McGinnis, 2004; Morris and Agin, 2009; Hardan *et al.*, 2012; Yui *et al.*, 2012). It would be interesting to study the effectiveness of antioxidant therapy in a sample of autistic children whose copy number of active ribosomal genes has been preliminarily estimated. Should our hypothesis on the association between the copy number of active ribosomal genes and oxidative stress as a pathogenesis mechanism of infantile autism be true, the curative effect of antioxidant therapy must be most evident in children with low copy number of active ribosomal genes (<140 active copies), that is, with the maximum intensity of oxidative stress. The successful detection of a negative correlation between the effectiveness of antioxidant therapy and the copy number of active rRNA genes in a patient might be a convincing corroboration of our hypothesis.

In the future, our assumptions might be validated by observing nonpsychotic siblings of cases to estimate the heritable susceptibility of individuals with too low or too high copy number of active ribosomal genes.

We believe that further investigation into the correlations between the copy number of ribosomal genes, oxidative stress and various kinds of pathology might shed light on the role of free radicals and the biological importance of oxidative stress and may help answer the question of whether oxidative stress is just the inevitable payoff for the evolutionarily formed possibility of aerobic utilization of sources of chemical energy, or whether it performs signal and protective functions, safeguarding the cell in a hostile, chemical or microbiological, environment. The latter viewpoint, not shared by us, is the essence of a peculiar concept of 'oxidative shielding'. According to this concept, oxidative stress is an evolutionarily primordial reaction to injurious impacts in the context of inborn immunity (Naviaux, 2012), activated in various pathological conditions, but not causing such conditions. The adherents of the 'oxidative shield' concept arrive at the conclusion that antioxidant therapy is ineffective, being capable of eliminating the effects, but not the causes of the disease. Further studies should clarify this concept.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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