3-rd International Conference

Homo sapiens liberatus

Moscow, Russia
February 20-21, 2020

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3-rd International Conference
Homo sapiens liberatus

On the occasion of the 85-th Anniversary of Director of A.N.Belozersky Institute
and Dean of Faculty of Bioengineering and Bioinformatics,
M.V.Lomonosov Moscow State University

Professor V.P.Skulachev

Abstract Book

Moscow, Russia, February 20-21, 2020.

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M.V.Lomonosov Moscow State University

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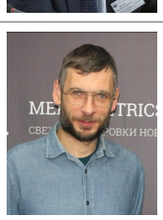
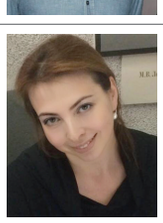
Member **B.A. Fenyuk**		assistant professor, Ph.D. Deputy Dean of Faculty of Bioengineering and Bioinformatics of M.V.Lomonosov Moscow State University
Member **M.H. Bogatyreva**		Deputy Dean of Faculty of Bioengineering and Bioinformatics of M.V.Lomonosov Moscow State University.

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Conference Programm

February, 20, 2020

Medical research and education center of Moscow State University, conference hall of the educational building (room 309)

	9:00–9.45	Registration (Educational building of Medical research and education center of Moscow State University).
Alexei Bogdanov *(Russia)*	9:45–9.55	Opening remarks.

Section 1

Non-phosphorylation respiration. Chairperson Barbara Cannon

Dmitry Zorov *(Russia)*	10:00–10:30	Non-phosphorylating respiration: production of heat and superoxide. ROS-induced-ROS-release as a chain reaction.
Jan Nedergaard *(Sweden)*	10:30–11:00	Nonshivering thermogenesis – through UCP1 or also through other means?

Section 2

Bacterial and mitochondrial electricity. Chairperson D. Zorov

Vasiliy Ptushenko *(Russia)*	11:00–11:30	Mitochondrial and bacterial electricity.
	11:30–12:00	Coffee break.

Chairperson Mikhail Vyssokikh

Yannis Kalaidzidis *(Germany)*	12:00–12:30	Polarity order in the liver tissue.
Sergei Bibikov *(USA)*	12:30–13:00	Counting nuclear encoded mitochondrial mRNA in human cells, progress and challenges.
	13:00–14:15	Lunch.

Section 3

Proton vs sodium energetics. Chairperson E. Plotnikov

Armen Mulkidjanian *(Germany)*	14:15–14:45	Bioinformatics and Expansion of the "Sodium World".
Maria Muntyan *(Russia)*	14:45–15:15	New horizons of sodium energetics.
Vasily Popov *(Russia)*	15:15–15:30	mtDNA damage detection as marker of oxidative stress and mitochondrial inhibitors genotoxicity.
Thomas Hildebrandt *(Germany)*	15:30–16:00	The mysteries of wildlife reproduction.
Michael Sherman *(Israel)*	16:00–16:30	Hsp70 on the crossroad between stress and cancer.
	16:30–17:00	Coffee break.

Chairperson Boris Chernyak

Andrei Vinogradov *(Russia)*	17:00–17:30	Internal and external electron transfer catalyzed by the mitochondrial respiratory complex I
Yuri Antonenko *(Russia)*	17:30–18:00	New uncouplers of oxidative phosphorylation: BAM15, pyrrolomycin and usnic acid.
helicon	18:00–18:10	HELICON presentation.

18:10–19:30 **Poster session**

February, 21, 2020

Medical research and education center of Moscow State University, conference hall of the educational building (room 309)

Section 4

Aging programs vs anti-aging programs. Chairperson Jan Nedergaard

Boris Chernyak *(Russia)*	10:00–10:30	Mitochondria-targeted antioxidants against inflammation.
Vladimir Marshansky *(USA)*	10:30–11:00	Structural model of a2-subunit V-ATPase and its interaction with Arf-GEF cytohesin 2: Drug development for treatment of amyotrophic lateral sclerosis (ALS) and to control the calorie restriction (CR) pathway.
Leonid Gavrilov *(USA)*	11:00–11:30	Matters of life and death: What can we learn about aging from mortality and longevity studies?
	11:30–12:00	Coffee break.

Chairperson Andrei Vinogradov

Evgeniy Galimov *(England)*	12:00–12:30	Shorter life can increase colony fitness in virtual *C. elegans*.
Giacinto Libertini *(Italy)*	12:30–13:00	TERRA sequences: the core of the core of aging mechanisms.
Josh Mitteldorf *(USA)*	13:00–13:30	A clinical trial using methylation age to evaluate current anti-aging practices.
Vadim Gladyshev *(USA)*	13:30–14:00	From mechanisms of lifespan control to longevity interventions.
	14:00–15:00	Lunch.

Chairperson Vadim Gladyshev

Leonid Margolis *(USA)*	15:00–15:30	Extracellular vesicles: a new language of cell-cell communication.
Susanne Holze *(Germany)*	15:30–16:00	Naked mole rats - myths, reality and beyond.
	16:00–16:30	Coffee break.

Assembly Hall of the Fundamental Library of Moscow State University

Section 5

A pathway to *Homo sapiens liberatus*. Chairperson A. Mulkidjanian

Vladimir Skulachev *(Russia)* 17:15–18:15 Mild depolarization of the inner mitochondrial membrane is a critical component of an anti-aging program: how this was shown?

Mikhail Vyssokikh *(Russia)* 18:15–18:45 Mild depolarization of the inner mitochondrial membrane is a crucial component of an anti-aging program: human and SkQ1 aspects.

Maxim Skulachev *(Russia)* 18:45–19:15 Clinical trials of mitochondria-targeted antioxidants. A milestone on the pathway to *Homo sapiens liberatus*?

Nonphosphorylating oxidation in mitochondria and problems associated with mitochondrial generation of reactive oxygen species

Section 1

Dmitry Zorov

A.N.Belozersky Institute of Physico-Chemical Biology,
M.V.Lomonosov Moscow State University;

E-mail: zorov@belozersky.msu.ru

DOI: 10.30826/HomoSapiens-2020-01

Regulation of the efficiency of oxidative phosphorylation is a key element in the functioning of mitochondria providing a reasonable balance between the synthesized ATP and the fraction of free energy of substrate oxidation released as heat. The share of generated chemical energy in the form of ATP directly depends on proton-motive force and consequently regulates the processes associated with this component, in particular the generation of reactive oxygen species (ROS). Sixty years ago, V.P.Skulachev presented his first proof of thermoregulatory uncoupling during short-term exposure to cold, for the first time proving the possibility of intrinsic regulation of oxidative phosphorylation responded to physiological needs. That time, the question of a possible endogenous uncoupler responsible for thermoregulatory uncoupling was resolved through the idea to consider fatty acids as this regulator. Later, the interaction of fatty acids and mitochondria took a significant part of the research in the Skulachev's group. On the one hand, it seemed that the mechanism of uncoupling is easily explained by the formation of a shuttling proton transfer through the bilayer of the inner mitochondrial membrane, but after the detection of retardation of the protonophoric action of uncouplers by inhibiting the ATP/ADP translocator (ANT) or dicarboxylate carrier, this mechanism did not look trivial anymore, because a priori it undermined the involvement of some mitochondrial proteins in the mechanism of uncoupling, in particular ANT. By the way, this indirect evidence obtained in the 20th century by Russian scientists that fatty acids provide proton conductance through the ANT was confirmed in 2020 by American scientists. It becomes clear that by providing proton leak through mitochondrial proteins, fatty acids, and other possible physiological regulators can not only regulate ATP synthesis, but also finely regulate the synthesis of ROS, which are considered as physiological and pathogenic regulators of cellular activity. This relationship was shown in the same group of Russian scientists, with the key regulator of ROS synthesis being the membrane potential ($\Delta\Psi m$) built over the inner mitochondrial membrane. It became clear that homeostasis of $\Delta\Psi m$ is one of the key components and indicators of mitochondrial

functional activity. Now, we can attribute to this factor a number of vital physiological intracellular functions, such as: to be an intermediate element of ATP synthesis, an element of the mitochondrial quality control mechanism, a control component of the transport of a number of proteins to the mitochondria, possible participation in antiviral and antibacterial defense, a driving force for the transport of cations, such as calcium, magnesium and especially potassium ions, which can provide potassium energetics, and the associated transport of water in and out of mitochondria. There are still a number of speculative assumptions about the role of ΔΨm, but the use of drugs conjugated with permeable ions traveling to mitochondria driven by ΔΨm is no longer speculative, but practically used in medical practice. Thus, the mitochondrial machine regulated by endogenous components can provide not only cellular homeostasis, but also it participates in wanted and unwanted cell death. In this process, an important role is played by ROS, the generation of which under special conditions inside the mitochondria can carry an avalanche character which was called ROS induced ROS release, and currently it considered as the fundamental basis of pathogenesis. In general, the strong shift from homeostasis of ΔΨm, the levels of intracellular ATP and intracellular and intramitochondrial ROS being a result of improper functioning of the mitochondria is the basis of pathogenesis determined by the occurrence and course of diseases and aging.

Supported by NSF grant #19-14-00173.

Nonshivering thermogenesis – through UCP1 or also through other means?

Section 1

Jan Nedergaard

Department of Molecular Biosciences,
The Wenner-Gren Institute,
Stockholm University, Stockholm, Sweden

DOI: 10.30826/HomoSapiens-2020-02

Although nonshivering thermogenesis in general has been ascribed to the activity of UCP1 in brown and brownish tissues, there are presently several discussions concerning alternative mechanisms for nonshivering thermogenesis. For several of these, the underlying data concerning mechanism, control and power will be discussed here, including the important issues of facultativeness and adaptiveness.

Excluded from this discussion is the muscle-tone thermogenesis that – although not being a shivering thermogenesis – is a mechanism where normal muscle ATP production and breakdown through muscle fibre contraction occurs. This is evidently a facultative process but it is hardly adaptive.

In muscle, several alternative mechanisms for nonshivering thermogenesis have also been discussed. One is ascribed to sarcolipin that is part of the sarcoplasmic reticulum Ca^{2+} pumping ATPase system. Ablation of sarcolipin makes mice acutely cold sensitive but it is uncertain whether this is due to the absence of nonshivering thermogenesis. The regulation of sarcolipin effects is not known. Also other aspects of Ca^{2+} metabolism in muscle have over the years been suggested to be thermogenic.

There is no doubt that UCP1 – the "holotype" of the phylogenetic family of uncoupling proteins – is an uncoupling protein (uncoupling mitochondrial respiration from oxidative phosphorylation) - but there is presently no evidence that any other protein referred to as an uncoupling protein has any such function (e.g. UCP2, UCP3 etc.).

However, even in brown and brownish adipose tissue that contain UCP1, alternative mechanisms for nonshivering thermogenesis have been suggested. They involve the use of ATP in the thermogenic process, a feature that would limit the quantitative significance of such mechanisms in theses tissues, since their capacity for ATP synthesis is low. Mechanisms involving endoplasmatic Ca^{2+} pumping, a creatine phosphate cycling process, triglyceride synthesis and breakdown, and glycerol phosphate formation and breakdown, have all been proposed.

However, physiologically the capacity of any of these processes for nonshivering thermogenesis would seem limited in that in UCP1-ablated mice in the cold, shivering

never ceases (i.e. no alternative mechanism for classical nonshivering thermogenesis seems to be able to develop), and in UCP1-ablated mice fed an obesogenic diet, no extra thermogenesis develops (i.e. no alternative mechanism for diet-induced thermogenesis seems to be able to develop).

Mitochondrial and bacterial electricity

Vasiliy Ptushenko

A.N. Belozersky Institute of Physico-Chemical Biology, M.V. Lomonosov Moscow State University, Moscow 119992, Russia

E-mail: ptush@belozersky.msu.ru

DOI: 10.30826/HomoSapiens-2020-03

Section 2

The hypothesis that coupling membranes act as power-transmitting cables at the cellular (or even at the supracellular) level was proposed in 1969 by V.P. Skulachev. The concept was subsequently confirmed and developed due to studies on both structural organization of mitochondria and its dynamics. In particular, giant mitochondria or mitochondrial reticulum were found to be characteristic of many eukaryotic cells. The thread–grain transition was shown to be a typical feature inherent in mitochondria reflecting changes in physiological state of the cell. On the other hand, the electric contact between individual mitochondria thereby forming mitochondrial bunches was also shown. A wide range of organisms manifesting the energy transmitting function of coupling membranes was discovered. Among the most spectacular examples are the giant mitochondria of some unicellular organisms (algae, fungi, protozoa) and mitochondrial reticulum in muscle tissues of animals.

In the last decade, the concept of electrical power-transmitting cables operating at the supracellular level was "rediscovered" in the works on multicellular bacteria of L.P. Nielsen and others. These bacteria form centimeter-long filaments able to transfer the electrons between the reducing compound (e.g., sulfide) and the oxygen molecules separated by centimeter distances were called "cable bacteria". It is assumed that they occur globally in marine and freshwater sediments. So, the cable functions of coupling membranes which have been proposed 50 years ago, received full appreciation, thus demonstrating great value of this idea.

Section 2

Polarity order in the liver tissue

Kalaidzidis Yannis

Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstrasse 108, 01307, Dresden, Germany; Faculty of Bioengineering and Bioinformatics, Moscow State University, Moscow, Russia;

E-mail: kalaidzi@mpi-cbg.de

DOI: 10.30826/HomoSapiens-2020-04

Liver is an organ with high regeneration capacity, which requires special mechanism to maintaining specific architecture during reparation. Most severe liver diseases are associated with tissue structure abnormalities, i.e. loss of correct architecture maintenance. Therefore, learning governing principles of tissue self-organization is an outstanding problem. First model, of liver tissue structure principles was done in 1949 by Elias. This model emphasized regular pattern in the tissue (to the regularity of crystal). However, the crystal model was in contrast with the apparent random 3D arrangement of hepatocytes on histological sections. Since then, no significant progress has been made to derive the organizing principles of liver tissue. In presented work, we utilized the computer-aided 3D histology and soft-condensed-matter-physics concepts to reconstruct liver tissue geometry and learn the basic principles which govern liver tissue self-organization. We found, that spatial organization of hepatocytes polarity follows a long-range liquid-crystal order. It was generally assumed that hepatocytes organization following the cue from endothelial cells and gradients of oxygen concentration in the blood flow. However, silencing Integrin-β1 disrupted both liquid-crystal order of hepatocyte polarity and organization of the sinusoidal network. Therefore, our results suggest that liquid-crystal order arose from bi-directional communication between hepatocytes and sinusoids underlying the self-organization of liver tissue.

Section 2

Counting nuclear encoded mitochondrial mRNA in human cells, progress and challenges

Sergei Bibikov

BioSpyder Technologies, USA

E-mail: bibikov@gmail.com

DOI: 10.30826/HomoSapiens-2020-05

Latest technologies involving various techniques of evaluating gene expression in human cells will be discussed using the MitoCarta selected nuclear encoded mitochondrial genes as a target. Detailed analysis of the current challenges and new techniques to overcome them will be presented, including gradual attenuation, intrinsic stochasticity and metabolic consequences.

Bioinformatics and Expansion of the "Sodium World"

Section 3

Daria V. Dibrova[1], Julia D. Belyaeva[2], Ilya M. Bushmakin[2], Olesya I. Klimchuk[2], Dmitry A. Cherepanov[3], Daria N. Shalaeva[1], Maria I. Kozlova[4], and **Armen Y. Mulkidjanian[1,2,4*]**

[1] A.N. Belozersky Institute of Physico-Chemical Biology and

[2] School of Bioengineering and Bioinformatics, Lomonosov Moscow State University, Moscow 119992, Russia;

[3] N.N. Semenov Institute of Chemical Physics, Russian Academy of Sciences, 119991 Moscow, Russia;

[4] School of Physics, Osnabrück University, 49069, Osnabrück, Germany;

E-mail: amulkid@uos.de

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In 1986, Vladimir Skulachev and his colleagues coined the name "Sodium World" for the variety of organisms with sodium-dependent bioenergetics [1]. Albeit only few such organisms had been discovered at the time, the authors insightfully noted that "the great taxonomic variety of organisms employing the Na^+-cycle points to the ubiquitous distribution of this novel type of membrane-linked energy transductions" [1]. Here we consider how the Sodium World expanded through the evolutionary time and taxonomic space.

The type of membrane bioenergetics of an organism can be inferred from the amino acid sequence of the ion translocating membrane subunit of its ATP synthase [2]. Earlier, using this approach, the occurrence of the Na-energetics in different prokaryotic lineages was traced; it was argued that the Na-energetics preceded the proton-dependent energetics in evolution [2,3]. The Na-energetics, as particularly beneficial for anoxic organisms, should have prevailed before the oxygenation of atmosphere, during the first two billion years of the Earth history [4,5].

The discovery of dozens of new prokaryotic phyla by metagenomics approach asked us to revisit the distribution of Na-energetics among prokaryotes. We have analyzed the bioenergetics of new phyla and found some that contain whole clades of organisms with Na-energetics.

In a concurrent effort, we searched prokaryotic genomes for those protein families that correlate within genomes with Na^+-translocating ATP synthases and therefore might participate in the Na^+-cycle. Expectedly, the known Na^+-translocating enzymes, the diversity of which dramatically expanded in the last years [6,7], were among such co-occurring proteins. In addition, we found that many proteins with unknown function also correlate with enzymes of Na-energetics. Hence, the whole functional diversity of prokaryotic Sodium World still awaits its explorers.

The importance of the Sodium World for eukaryotes is exemplified by G protein-coupled receptors (GPCRs) of class A. The human genome contains about 700 genes of such receptors, which are targeted by almost the half of known drugs. The class A GPCRs, when crystallized in their inactive conformation, have a Na^+ ion bound in the middle of the transmembrane seven-helical bundle. No Na^+ ions were found in same GPCRs when they were crystallized in their active state; Katritsch and colleagues suggested that the agonist-induced activation of these receptors could be promoted by electrogenic release of the bound Na^+ ion [8]. Relevant experimental data are, however, ambiguous;

membrane voltage was shown both to promote and to suppress the activation in distinct GPCRs; in addition, the voltage impact depended on the agonist nature.

Building on the previously unraveled evolutionary relatedness of GPCRs and Na^+-translocating rhodopsins [9], we modeled class A GPCRs as electrogenic carriers of Na^+ ions [10]. We showed that the Na^+-gradient on the cell membrane could promote the GPCR activation by an agonist if this activation is thermodynamically coupled with the Na^+ translocation to the cytoplasm; in this case, the GPCR operates as a kind of field effect transistor. In contrast, the retreat of the Na^+ ion to the extracellular space, against the Na^+-gradient, was shown to the receptor sensitivity. Our model quantitatively describes the available experimental data and predicts selective amplification of the signal from endogenous agonists if only they, but not their (partial) analogs, induce the Na^+ translocation to the cytoplasm. Overall, class A GPCRs appear to increase both their sensitivity and selectivity by harnessing the energy of transmembrane Na^+-potential.

The finding that the largest protein family coded by the human genome - the class A GPCRs - belongs to the Sodium World encourages exploration of other Na-dependent enzymes of eukaryotes.

Acknowledgements

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Mitochondrial ROS production and the unified theory of programmed aging: The ARS of aging

Gustavo Barja

Department of Physiology, Genetics, and Microbiology, Faculty of Biological Sciences, Complutense University of Madrid (UCM), Spain

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Aging is genetically determined because animal species differ by up to 100,000 fold in longevity (the "big effect"), many pro-aging genes exist, and are highly conserved from yeast to mammals. Therefore, aging is adaptive (for the group),1 including reaching up to the ecosystem. Life extending dietary restrictions and rapamycin signal the nucleus to specifically change gene expression of the aging program (AP) [2]. The AP, its afferent signals, and the aging effectors constitute the Cell Aging Regulation System (CARS) [3]. CARS controls the output of many different intracellular aging effectors in response to afferent signals reaching the AP, which in turn modulates longevity in the "small effect": the up to 1,4 fold increase in mammalian longevity in dietary restricted and longevity mutant mice. The AP spontaneous basal efferent activity, quantitatively different in each species, can explain the "big -interspecies- effect". Aging effectors include mitochondrial ROS production (mitROSp), fatty acid unsaturation, autophagy, apoptosis, inflammaging, telomere shortening, and likely others. MitROSp, % mitochondrial Free Radical Leak (FRL), and oxidative damage in mtDNA (not in nDNA) are low in long-lived mammals and birds and are lowered by CR, protein or methionine restriction [4] and by rapamycin [3], all of which increase longevity. MtDNA fragments increase with age in somatic tissues of yeast, mice and rats promoting aging by inserting into nDNA [3,5]. Cells with mtDNA deletions in homoplasmy (<2% of total) are far from the threshold level (70%) in most tissues of old mammals to decrease cell function, but the missing deleted fragments escape both to nucleus and bloodstream causing damage in the same or in far away situated cells and organs. This resurrects the mitochondrial free radical theory of aging, wrongly dismissed by some, because it can explain how mitROSp and mtDNA can still contribute to cause aging. On the other hand, CARS reconciles the old separated previously called "theories" of aging or "hallmarks", which in the new model correspond to effectors (executors) of the nuclear AP, which is constituted by hundreds of target genes. The expression levels of these genes are regulated by TFs, miRNAs, epigenetic marks, and other factors, and are likely hierarchically controlled by a few master genes. Identification of these AP master genes is a key strategy to achieve human negligible senescence. The AP allows the

unification of all the old "theories" of aging into a single unified theory represented by the Aging Regulating System (ARS). AP extracellular aging effectors include inflammaging due perhaps to autoimmune attack, and DAMPs including circulating mtDNA fragments (cp-mtDNA) amplifying mitROS-derived damage to other cells or organs. CARS of different cells are integrated into the whole organism level ARS through interactions between the different CARS, systemic circulating factors identified in heterochronic parabiosis experiments and, perhaps, central aging clocks.

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New horizons of sodium energetics

Section 3

Maria Muntyan

Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow 119991, Russia;

E-mail: muntyan@genebee.msu.ru

DOI: 10.30826/HomoSapiens-2020-08

Recent advances in bioenergetics have unveiled the secrecy of the prosperity of certain microbes in the environments with extreme salinity, alkalinity and buffer capacity. The combination of numerous experimental approaches, including the direct method of visualizing sodium transport using ^{22}Na-radioisotope analysis and indirect methods using the effects of ionophores and uncouplers, has led us to the solution of the problem. With their help, we found in these microbes a previously predicted new respiratory enzyme, sodium-motive cytochrome oxidase, which avoids the problem of low delta myu H^+ formed under harsh alkaline conditions and effectively converts energy to favorable delta myu Na^+.

Despite clear evidence of the activity of such an enzyme in the microbes in which it was found, the possible mechanism of its operation seemed difficult to explain. We used the latest data from genetic experiments, phylogenetic analysis, X-ray analysis and molecular dynamics calculations to solve this problem.

The data on complete operon of sodium-motive cytochrome oxidase, which we sequenced during this study, and the X-ray analysis of highly homologous proton-motive cytochrome oxidase crystals, performed by H. Michel's group (Buschmann et al., 2010), allowed us to reconstruct the 3D structure of the new enzyme. Thus, the riddle of the adjacent contacts in 3D space of amino acid residues of 13 alpha-helices of the catalytic

subunit of the new enzyme was solved. This helped us to predict the sodium-transporting pathways in the protein. Phylogenetic analysis made it possible to determine the closest homologs, namely, neighbors with a possible similar function, and to outline the region of a possible consensus sequence in the structure providing a new enzyme function. Our joint research with Dr. D. Cherepanov on the 3D-structure of the new enzyme revealed the presence of a characteristic six-coordination sphere, potentially capable of binding sodium ions in the region of the found consensus sequence. Molecular dynamics calculations showed that the center found in the new enzyme binds sodium ions in contrast to the proton-motive homolog.

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mtDNA damage detection as marker of oxidative stress and mitochondrial inhibitors genotoxicity

Section 3

Artem P. Gureev, Inna Yu. Vitkalova, **Vasily N. Popov.**

Department of Genetics, Cytology and Bioengineering, Voronezh State University, Voronezh 394018, Russia

Voronezh State University of Engineering Technologies, Voronezh 394000, Russia

E-mail: pvn@bio.vsu.ru

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Accumulation of mitochondrial DNA (mtDNA) damages is believed to be one of the causes of energy crisis in aging tissues in various types of multicellular organisms. mtDNA represents a characteristic prokaryotic-like genome lacking histones with less efficient than the nuclear one DNA repair system. mtDNA damage has been actively studied in recent years, but almost all of these studies are carried out on laboratory animals or humans, since mtDNA damage can be both a cause and a marker for the development of a wide range of diseases. Moreover, the role of reactive oxygen species (ROS) in the plant cells dysfunction is no less critical than in animals. However, now the effect of ROS on mtDNA damage induced by various external and internal factors is not clearly described for plants. The mtDNA size of plants is in the range of 200-2000 kb, while in mammals less than 20 kb. This difference in mitochondrial genomes sixe does not affect the number of genes. Most of the additional DNA found in the mitochondrial genomes of plants consists of large introns, AT-enriched non-coding repeats. Plant mtDNAs also contain a significant amount of relatively short nuclear and chloroplast genomic sequences, which, apparently, were integrated in mtDNA via evolution.

The coding sequences of the plants mitochondrial genomes have a lower mutation frequency than the coding sequences of animal mitochondria. However, plant mitochondrial genomes are often rearranged and exhibit a high mutation frequency in their non-coding sequences. The integrity of the mitochondrial genome of plants is necessary for cell survival. During evolution, plants have

developed mechanisms to replicate their mitochondrial genomes, while minimizing the effects of DNA damaging agents. mtDNA is sensitive to ROS generated by the respiratory chain, due to their close proximity.
The generation and accumulation of ROS plays a key role in the functioning of not only the vegetative parts of the plant, but also in the regulation of the metabolic and physiological state of seeds. Large changes during seed aging occur in mitochondria, because they are the main production site of ROS, therefore they are faster and more susceptible to oxidative damage than other organelles.
Thus, the search of a valid marker that will allow monitoring the state of the vegetative parts of plants and seed viability is an actual task for modern science. We believe that the analysis of the integrity of the mtDNA is a convenient marker that allows one to assess the level of oxidative stress and its effect on the functioning of mitochondria of the vegetative parts of plants and seeds.

This study was supported by Russian Scientific foundation (Grant #20-14-00262).

Advanced assisted reproduction technologies (aART) and stem cell associated techniques (SCAT) for saving critically endangered rhinoceros species

Section 3

Thomas B. Hildebrand

The Leibniz Institute for Zoo and Wildlife Research, Alfred-Kowalke-Straße 17, 10315 Berlin, Germany

E-mail: hildebrandt@izw-berlin.de

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Over decades zoo based research programmes were dominated by considerations of husbandry and enclosure design. More recently new ideas, particularly the application of assisted reproduction technologies (ART) have been incorporated into classical captive breeding programs. Progressive habitat destruction and fragmentation is causing an accelerated loss on planetary biodiversity. One of the institutional missions of the international zoo community is to counteract this process by successful captive breeding programs of endangered species and their reintroduction into the depopulated natural habitat. In this process the global management of captive breeding programmes is directed to preserve the maximum gene pool of the target species. Traditionally, this effort required the exchange of potential breeding partners between zoological institutions. However, animal transportation includes (i) high risk of disease transmission, (ii) stress-induced infertility or partner incompatibility as well as (iii) high financial and logistic efforts. The utilization of ART could serve as alternative to eliminate these problems of distance and time.
However, dealing with critical endangered species / populations such as the northern white rhinoceros or the Bornean subspecies of the Sumatran rhinoceros the effective funder population are already too small for

successful species recovery programs. Therefore, the incorporation of new genetic resources such as cryo-preserved fibroblast cultures and future production of artificial gametes using iPSC technologies will be required for saving such critically endangered species. A new strategic roadmap was outlined in "Rewinding the process of mammalian extinction." (Saragusty et al., 2016). We were capable to generate naive and primed iPSC like cells derived from cryo-preserved fibroblast cultures of several deceased northern white rhinoceros. Furthermore, it was possible to differentiate these cells into primordial germ like cells (PGC) expressing typical PGC marker genes like Blimp, Stella, Sox17 and Oct4.

These achievements provide the basis for the second phase the production of artificial oocytes and sperm cells originally derived from simple fibroblast cultures. This will open up a totally new dimension in animal conservation and will strengthen the application of ART in wildlife.

The accelerated loss off global biodiversity reflects an irretrievable loss of information and functions we haven't enlightened so far from many creatures written in the "Library of Evolution" and we may never do prior their extinction.

Hsp70 on the crossroad between stress and cancer

Michael Sherman

Ariel University, Israel

E-mail: sherma1@ariel.ac.il

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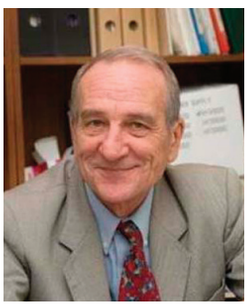
The major molecular chaperone Hsp70 can protect cells from protein damage by facilitating protein refolding or promoting degradation of damaged polypeptides. It is also highly expressed in a variety of cancers, and the common theory in the field is that cancer cells require high levels of Hsp70 because they experience massive protein abnormalities associated with the oxidative stress. We have demonstrated that this idea is incorrect, since depletion of Hsp70 in naïve cancer cells did not lead to accumulation of damaged polypeptides. Yet, Hsp70 was critical for cancer development since in different animal models knockout of this chaperone suppressed various cancer stages, including oncogenic transformation, invasion, angiogenesis and metastasis. Surprisingly the chaperone function of Hsp70 was irrelevant to its role in cancer, but rather Hsp70 could regulate a large number of signaling pathways that drive cancer, including Src, p53, MAPK, c-myc, NF-kB and others. We identified a Bag3 protein as a scaffold that links Hsp70 with components of these signaling pathways. Furthermore, in a collaborative study with a medicinal chemistry group, we identified a small molecule that dissociates of Hsp70 from Bag3. This molecule mimicked all signaling and anti-cancer effects of Hsp70 both in vitro and in vivo. Therefore the Hsp70-Bag3 protein module appears to be a central regulator of critical signaling pathways that control cell growth, motility, survival and other major functions, and targeting Hsp70-Bag3 interaction could be a promising drug design strategy.

Internal and external electron transfer catalyzed by the mitochondrial respiratory complex I

Section 3



Andrei D. Vinogradov

Department of Biochemistry, School of Biology, M.V. Lomonosov Moscow State Universiry, Moscow 119234, Russian Federation

E-mail: adv@biochem.bio.msu.su

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Mitochondrial NADH:ubiquinone oxidoreductase operationally called respiratory complex I and its prokaryotic homologue NDH-1 perform several functions of vital importance for the cellular energetics. These are: (i) maintaining of the steady-state NAD+/NADH ratio in the mitochondrial matrix or bacterial cytoplasm as is physiologically required for the oxidative metabolism; (ii) redox-coupled vectorial proton translocation thus creating proton motive force (p), the primary energy source for ATP formation; (iii) unification of the redox equivalents from a number of the ubiquinone reducing dehydrogenases by the p-depended ubiquinol:NAD+ oxidoreduction (reverse electron transfer) RET; (iv) generation of the respiratory chain linked so-called reactive oxygen species (ROS). One of our current interest in operation of the mitochondrial complex I and its simpler NDH-1 is to find out the sites and mechanisms of ROS production as catalyzed by these multisubunits and multiredox components membrane-bound complexes. Several relevant findings are reported and discussed [1–3]. The rate of ROS production follows simple first-order kinetics in oxygen concentration dependence thus showing absence of any specific oxygen binding sites in complex I [1]. Both succinate- and NADH-supported reactions produce superoxide and hydrogen peroxide and the partitioning between them depends on the concentrations of the electron donating substrates [2], indicating most possible reaction sites at flavin and iron-sulfur 2-quinone junction. The flavin-independent, rotenone-sensitive site accessible for hexaammineruthenium reduction is identified in the succinate supported energy-linked RET reaction [3].

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New uncouplers of oxidative phosphorylation: BAM15, pyrrolomycin and usnic acid

Yuri N. Antonenko

A.N.Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow 119991, Russia

E-mail: antonen@belozersky.msu.ru

DOI: 10.30826/HomoSapiens-2020-13

Transmembrane electrochemical potential difference of hydrogen ions generated at the inner mitochondrial membrane as a result of oxidation of respiratory substrates is the primary source of energy for ATP synthesis. This was proven mainly by the action of uncouplers - compounds causing dissipation of membrane potential by facilitating transmembrane diffusion of protons and thereby leading to uncoupling of oxidation and phosphorylation in mitochondria. A series of studies indicated that excessive generation of membrane potential may cause the development of pathological processes in cells. It became clear that a moderate degree of uncoupling may be useful for therapeutic purposes. However, currently known uncouplers are highly toxic. To overcome this problem, a thorough study of mechanisms of protonophoric uncoupler operation in mitochondria and whole cells is required. A substantial body of evidence allows us to assume that the action of uncouplers on mitochondria significantly differs from their effect on the conductance of artificial bilayer lipid membranes. For example, according to some reports, the uncoupling action of dinitrophenol is mediated by interaction with the inner mitochondrial membrane protein ADP / ATP translocator. On the other hand, the detailed mechanism of the uncoupling effect of fatty acids, which is mediated by the mitochondrial uncoupling protein, has not been clarified yet. Previously, it was found that the uncoupling action of fatty acids is also associated with the operation of ADP / ATP translocator. The so-called penetrating cations, e.g., tetraphenylphosphonium, multiply accumulated in mitochondria due to the driving force of the membrane potential. Several promising new compounds conjugated to penetrating cations were synthesized and studied that demonstrated protonophoric properties and uncoupled oxidative phosphorylation in isolated mitochondria. One of the most significant achievements of our study was design and synthesis of mitoCCCP, a conjugate of decyl triphenylphosphonium cation with a conventional protonophore, carbonyl cyanide m-chlorophenylhydrazone (CCCP). For the first time, a mitochondria-targeted cationic group was attached to a classical protonophoric uncoupler without losing its uncoupling activity. The success we have achieved is a result of thorough work on the development and structure-function analysis of several series of uncouplers, namely alkyltriphenylphosphonium conjugates with proton-donating fluorescent dyes, such as fluorescein and the amino derivative of nitrobenzoxadiazole (NBD). These compounds, along with their anticipated uncoupling activity, demonstrated the ability to accumulate in the

energy-dependent manner in mitochondria, which could be estimated by their bright fluorescence. Synthesis of aromatic NBD derivatives and study of their effect on mitochondria allowed us to detect the involvement of one of the integral mitochondrial proteins, the aspartate-glutamate transporter, in the uncoupling activity. We studied also several new uncouplers of oxidative phosphorylation, namely BAM15, pyrrolomycin and usnic acid.

Mitochondria-targeted antioxidants against inflammation

Section 4

Nina V. Vorobjeva, Ivan I. Galkin, Anastasia S. Prikhodko, Maria A. Chelombitko, Sergei A. Golyshev, Konstantin G. Lyamzaev, Vlada V. Zakharova, Olga Yu. Pletjushkina, Ekaterina N. Popova, Roman A. Zinovkin, **Boris V. Chernyak**

A.N.Belozersky Institute of Physico-Chemical Biology, and Biology Faculty, Lomonosov Moscow State University, Moscow 119992, Russia

E-mail: Bchernyak1@gmail.com

DOI: 10.30826/HomoSapiens-2020-14

Inflammation is a complex defense reaction to pathogens and other harmful stimuli involving immune cells as well as blood vessels. Reactive oxygen species produced by mitochondria (mtROS) can be involved in the regulation of inflammatory responses in both types of cells. We have verified this hypothesis on various models of inflammation using the new mitochondria-targeted antioxidants of SkQ family, introduced by V.P. Skulachev and co-workers two decades ago. SkQ1 has been shown to accelerate the resolution of the acute inflammatory phase of dermal wounds in old or diabetic mice [1-3]. The anti-inflammatory effect of SkQ1 contributed to a marked acceleration of wound healing in these models. The suppression of acute inflammation with SkQ1 was also demonstrated in mouse models of subcutaneous air pouch [4,5] and pyelonephritis [6]. In a systemic inflammatory response syndrome (SIRS) model, SkQ1 prevented acute mortality in mice caused by intravenous injection of TNF [7,8]. Endothelium appeared to be one of the primary targets of SkQ1 in the models of acute as well as chronic inflammation. In both old and diabetic mice increased expression of the adhesion molecules (selectins, ICAM1, VCAM) in aortas was strongly suppressed by prolonged treatment with SkQ1 while elevation of the major proinflammatory cytokines TNF and IL-6 in serum was not affected [9]. The similar effect was observed in the model of SIRS [7].

In endothelial cell culture, SkQ1 attenuated TNF-induced increase in ICAM1, VCAM, and E-selectin expression and prevented neutrophil adhesion to the endothelial monolayer. Moreover, SkQ1 rescued from TNF-induced endothelial permeability, disassembly of cell contacts and VE-cadherin cleavage by the matrix metalloprotease 9 [7, 9, 10]. Both endothelial responses are critical for the extravasation of neutrophils from the blood into the inflammatory lesions in the tissues. We have demonstrated that the effects of SkQ1 in endothelial cells are mediated by a decrese in NF-κB activation due to inhibition of phosphorylation and proteolytic cleavage of the inhibitory subunit IκBα.

Neutrophils are the most abundant blood leucocytes which are the main participants in the first line of inflammatory defense against invading pathogens. At the sites of infection, neutrophil protective weapons are activated, in particular phagocytosis, oxidative burst, exocytosis of various granule types (degranulation), and release of DNA-based extracellular traps (NETosis). Using SkQ1, we demonstrated that mtROS are involved in the oxidative burst caused by activation of NADPH oxidase (Nox2), as well as in the exocytosis of primary (azurophil) and secondary (specific) granules. SkQ1 also significantly accelerated apoptosis of activated neutrophils [11].

NETosis is the last suicidal resource of neutrophils in the fight against infection. NETosis plays also an important role in the pathogenesis of various autoimmune and inflammatory diseases. Using SkQ1 and specific inhibitors of NADPH oxidase, we showed that both sources of ROS are critical for NETosis induced by Ca^{2+}ionophore A23187 in human neutrophils [12]. Interestingly in neutrophils from patients with chronic granulomatous disease (CGD) lacking Nox2, A23187-induced NETosis also was sensitive to SkQ1. We concluded that Ca^{2+}-triggered mtROS production contributes to NETosis either directly (CGD neutrophils) or by stimulating NADPH oxidase.

Our data indicate that mtROS play a critical role in signal transduction, which mediates major inflammatory responses in both endothelial and immune cells. The anti-inflammatory effect of SkQ1 probably underlies its therapeutic action in pathologies of various origin.

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Structural model of a2-subunit V-ATPase and its interaction with Arf-GEF cytohesin 2: Drug development for the treatment of amyotrophic lateral sclerosis (ALS) and to control the calorie restriction (CR) pathway

Section 4

Vladimir Marshansky

Neuro-Horizon Pharma, Sharon, MA, 02067, USA

E-mail: vlad@neuro-horizonpharma.com

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We have previously identified the interaction between mammalian V-ATPase a2-subunit isoform and cytohesin-2 (CTH2) and studied molecular details of binding between these proteins. In particular, we found that six peptides derived from the N-terminal cytosolic domain of a2-subunit ($a2N_{1–402}$) are involved in the interaction with CTH2 [1]. However, the actual 3D binding interface was not determined in that study due to the lack of high-resolution structural information about a-subunits of V-ATPase. Here, using a combination of homology modeling and NMR analysis, we generated the structural model of complete $a2N_{1–402}$ and uncovered the CTH2-binding interface. First, using the crystal structure of the bacterial M. rubber Icyt-subunit of A-ATPase as a template [2], we built a homology model of mammalian $a2N_{1–352}$ fragment. Next, we combined it with the determined NMR structures of peptides $a2N_{368–395}$ and $a2N_{386–402}$ of the C-terminal section of $a2N_{1–402}$. The complete molecular model of $a2N_{1–402}$ revealed that six CTH2 interacting peptides are clustered in the distal and proximal lobe sub-domains of $a2N_{1–402}$. Our data indicate that the proximal lobe sub-domain is the major interacting site with the Sec7 domain of first CTH2 protein, while the distal lobe sub-domain of $a2N_{1–402}$ interacts with the PH-domain of second CTH2. Indeed, using Sec7/Arf-GEF activity assay we experimentally confirmed our model. The interface formed by peptides $a2N_{1–17}$ and $a2N_{35–49}$ is involved in specific interaction with and regulation of Sec7 domain, which is essential for an understanding of the cross-talk between V-ATPase and CTH2. Moreover, these data are critical for drug development using: i) in silico computer-aided drug design (CADD) and/or ii) TR-FRET based high throughput screening (HTS) assay [3,4]. In particular, it was shown that CTH2 interacts with mutant superoxide dismutase 1 (SOD1), a known cause of familial amyotrophic lateral sclerosis (ALS) [5]. Inhibition of CTH2 activity by small molecules protects against ER stress, enhances autophagic flux and reduces the burden of misfolded SOD1. These data indicate that targeting of cytohesins in motor neurons may be beneficial for the treatment of ALS. Cytohesins are

also components of the calorie restriction (CR) pathway and are directly involved in the regulation of the signaling of IR and IGFR-1 receptors [3]. Thus, targeting of CTH2 in CR pathway of normal postmitotic cells by peptides or small molecule compounds, may also decelerate aging, extend life expectancy, and delay age-related neurodegenerative diseases.

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Matters of life and death: What can we learn about aging from mortality and longevity studies?

Leonid A. Gavrilov, Ph.D.,
Natalia S. Gavrilova, Ph.D.

Academic Research Centers, NORC at the University of Chicago, Chicago, IL, 60637, USA

E-mail: gavrilov@longevity-science.org

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Section 4

Fundamental biological theories of aging can be tested using mortality and longevity data. For example, traditional evolutionary theory explains aging by a declining force of natural selection with age. According to this mutation accumulation theory suggested by Peter Medawar, the equilibrium frequency of deleterious mutations is higher for later acting mutations (LAM), because selection against LAM is weaker and mutation-selection balance is shifting to higher LAM levels. One testable prediction from this theory is a prediction of a fundamental change in age dynamics of mortality at very old post-reproductive ages, when the force of natural selection becomes negligible and there is no room for its further decline. For example, a prediction could be made that mortality dynamics at reproductive ages (20-40 years in humans) should be fundamentally different from mortality dynamics at extreme post-reproductive ages (90-105 years).

In this study we tested this hypothesis using data from the U.S. Social Security Administration Death Master File (DMF). The study of ten single-year extinct birth cohorts born in 1886-1895 with good data quality found that the rate of mortality growth with age at advanced post-reproductive ages (up to 105 years) is exactly the same as at younger reproductive ages following the Gompertz law. This finding was further supported by additional studies of mortality in 22 single-year U.S. birth cohorts of men and women born in 1890-1900 based on data from the Human Mortality Database and analysis of mortality trajectories in 8 cohorts of laboratory mice, and 10 cohorts of laboratory rats.

This finding represents a challenge to many aging theories, including the evolutionary theory that explains senescence by declining force of natural selection with age. New ideas are needed to explain why exactly the same exponential pattern of mortality growth is observed not only at reproductive ages, but also at very old post-reproductive ages (up to 105 years), long after the force of natural selection becomes negligible (when there is no room for its further decline).
Wide applicability of the Gompertz law to almost all adult ages leads to another burning research question for future studies: How is it possible for different diseases and causes of death to «negotiate» with each other in order to produce a simple exponential function for all-cause mortality (given that contribution of different causes of death in all-cause mortality changes dramatically with age)?

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Shorter life can increase colony fitness in virtual *C. elegans*

Section 4

Evgeniy Galimov

Institute of Healthy Ageing, Research Department of Genetics, Evolution and Environment, University College London, London WC1E 6BT, UK

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In the nematode *C. elegans* loss of function of many genes causes increases in lifespan, sometimes of a very large magnitude. Could this reflect the occurrence of programmed death that, like apoptosis of cells, promotes fitness? The notion that programmed death evolves as a mechanism to remove worn out, old individuals in order to increase food availability for kin is not supported by classic evolutionary theory for most species. However, it may apply in organisms with colonies of closely-related individuals such as *C. elegans* in which largely clonal populations subsist on spatially-limited food patches. Here we ask whether food competition between non-reproductive adults and their clonal progeny could favor programmed death by using an in silico model of *C. elegans*. Colony fitness was estimated as yield of dauer larva propagules from a limited food patch. Simulations showed that not only shorter lifespan but also shorter reproductive span can increase colony fitness, potentially by reducing futile food consumption. Early adult death was particularly beneficial when adult food consumption rate was high. These results imply that programmed, adaptive death could promote colony fitness in *C. elegans* through a consumer sacrifice mechanism. Thus, *C. elegans* lifespan may be limited not by ageing but rather by apoptosis-like programmed death.

TERRA sequences: the core of the core of aging mechanisms

Giacinto Libertini

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Any theory suggesting an adaptive meaning for aging implicitly postulates the existence of specific mechanisms, genetically determined and modulated, causing the progressive decline of the organism.

The mechanism that appears to be the most documented and likely is the one described by the so-called subtelomere-telomere theory. According to it, each telomere is covered by a hood that is formed in the first cell of an organism and then maintains the same length at each subsequent duplication. Telomere shortening, which is quantitatively different according to telomerase regulation, causes the hood to slide on the adjacent portion of the DNA molecule, i.e., on the subtelomere. At this point the theory postulates the existence of regulatory sequences whose progressive inhibition should cause all aging manifestations.

However, these hypothetical sequences have already been described and documented in their effects. They are the [sub]TElomeric Repeat containing RNA (TERRA) sequences (here, for brevity, t-sequences) of which two types, TelBam3.4 and TelSau2.0, are well known (without excluding the possible existence of other subtelomeric sequences of this type).

The repression of t-sequences causes in a progressive way: (i) down-regulation of other regulatory sequences placed in the chromosome traits adjacent to the inhibited subtelomeric parts; (ii) up- or down-regulation of other regulatory sequences placed in other parts of the chromosomes; (iii) increase in the probability that cell senescence program is activated (blockage of the ability to replicate and alterations at the highest degree of cellular functions, probably due to maximum inhibition of the t-sequences).

So, the repression of t-sequences causes the dysregulation of many other regulatory sequences determining a progressive alteration of a large number of cellular functions. When the repression is partial, i.e. when cell senescence program has not been triggered, such alterations are easily reversible by telomerase activation.

In terms of natural selection, the location of sequences that are extremely important for the whole cellular functionality in the chromosomal part that is the most vulnerable to repression by the telomeric hood is unjustifiable if aging is not adaptive: it is necessary to admit that this location is adaptive and has the specific function of determining the aging of the cell and consequently of the whole organism.

The existence and the actions of these t-sequences,

which are the core of the core of aging mechanisms, constitute what could be called the smoking gun for the proof of specific aging mechanisms. Proponents of any non-adaptive aging theory should firstly find a plausible explanation for the meaning of such sequences, as a necessary premise for continuing to propose that their theories are plausible.

A clinical trial using methylation age to evaluate current anti-aging practices

Josh Mitteldorf

654 Carpenter Ln, Philadelphia, PA 19119, USA

E-mail: aging.advice@gmail.com

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Recent advances in the technology of «aging clocks» based on DNA methylation suggest that it may be possible to measure changes in the rate of human aging over periods as short as a year or two. To the extent that methylation (and other biomarkers) are valid surrogates for biological age, the testing of anti-aging interventions has thus become radically cheaper, faster, and more practical. Together with colleagues at UCLA and McGill University, I am organizing a clinical trial to evaluate some of the most popular anti-aging strategies currently deployed by «early adopters» in the lay community of personal health activists. We are recruiting 5,000 subjects, age 45-65, and interviewing them in detail about their diet, drugs and supplements, exercise, social, and other practices that plausibly contribute to modulate the rate of aging. They agree to submit blood samples for analysis of methylation age at the beginning, middle, and end of a two-year test period, together with 23-and-me or equivalent genetic data. Primary endpoint is the difference in methylation age over the course of two years. We are in the process of developing a specialized clock, optimized for individual differences over time. Results will be viewed as an exploratory study to identify synergistic combinations of age-retarding treatments. It is our expectation that there is a great deal of redundancy in the strategies that have been researched and promoted to the aware public; thus, most combinations can retard the rate of aging by only a few percent, consistent with the best-known single measures. However, it is our hope that among the many strategies that our subjects have adopted, there will be some combinations that synergize and achieve age retardation by 25 percent or more. A mock-up analysis of computer-generated data has been used to fix parameters of the study and confirm that we will be able to detect such combinations with good probability, should they exist. All data (redacted for privacy) will be open-sourced, available to the scientific community and to the public.

From Mechanisms of Lifespan Control to Longevity Interventions

The rate of aging varies significantly across species and cell types and is further modified by genetic and environmental factors. We employ this diversity to shed light on general principles and mechanisms of lifespan control. Specifically, we apply comparative genomics approaches to short- and long-lived species and carry out analyses across panels of mammals. We further perform analyses of gene expression, metabolites and elements across mammals, cell types and interventions. These studies point to both private and common adaptations to longevity involving central metabolic pathways and other processes. They also identify patterns of gene expression and metabolite levels, which we term longevity signatures, that characterize the potential to live a shorter or longer life, as well as the effects of interventions that adjust this potential. Using these signatures, one may identify new pharmacological, dietary and genetic interventions that affect lifespan. To experimentally characterize longevity interventions, we also developed blood and multi-tissue DNA methylation clocks in mice. Together, these genomics approaches and tools offer a platform for unbiased discovery and validation of longevity interventions in mammals.

Vadim Gladyshev

Brigham and Women's Hospital, Harvard Medical School, Boston, USA, Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow, Russia;

E-mail: vgladyshev@rics.bwh.harvard.edu

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Extracellular vesicles: a new language of cell-cell communications

For many years, double-layer phospholipid membrane vesicles, released by most cells, were not considered to be of biological significance. This stance has dramatically changed with the recognition of extracellular vesicles (exosomes) as carriers of biologically active molecules that can traffic to local or distant targets and execute defined biological functions. The dimensionality of the field has expanded with the appreciation of diverse types of extracellular vesicles and the complexity of vesicle biogenesis, cargo loading, release pathways, targeting mechanisms, and vesicle processing. The emerging and quickly expanding science of extracellular vesicles penetrates all the biomedical fields, including virology, immunology, cardiovascular diseases and general aging. Presentation will be focused on the role of extracellular vesicles in human pathophysiology.

Leonid Margolis

National Institute of Child Health and Human Development

National Institutes of Health, Bethesda, MD 20892, USA

E-mail: margolil@helix.nih.gov

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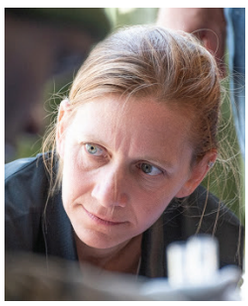
Naked mole rats - myths, reality and beyond

Section 4



Susanne Holtze[1], M. Vyssokikh[2], S. Braude[3], V. Skulachev[2], T.B. Hildebrandt[1]

[1] Department of Reproduction Management, Leibniz Institute for Zoo and Wildlife Research, 10315 Berlin, Germany

[2] Belozersky Institute of Physico-Chemical Biology and Institute of Mitoengineering, Moscow State University, Moscow

[3] Biology Department, Washington University, St. Louis, MO, 63130 USA;

E-mail: holtze@izw-berlin.de

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The naked mole-rat (*Heterocephalus glaber*) is a rodent that has gained popularity for its unique social system, unusual thermoregulatory adaptations and, more recently, for its remarkably healthy longevity, cancer-resistance and capability to survive extremely long periods of hypoxia. Although the number of studies addressing these issues has increased dramatically over the past years, the mysteries of their superpowers have not yet been fully understood. Concomitant to the accumulation of knowledge on the various aspects of their secrets of longevity, a vast body of plausible (but unsupported) hypotheses, unrepeatable experiments and myths has accumulated, to some extent impairing the search for truly relevant explanations.

Such myths include non-ageing, inbreeding, pain insensitivity, resistance to disease and oxidative stress, exposure to a constantly hypoxic environment, cancer resistance and the role of hyaluronic acid, indeterminate growth of the queen, and the organization of their caste system. These partial truths bear the risk of deviating ongoing research from questions that may lead to important discoveries. By giving clarity to some of these myths based on the literature and new results may help to avoid wasting time and money and to define some future prospects for research gaps to be addressed.

Mild depolarization of mitochondria as a mechanism of an anti-aging program which is opposed to aging program

The mitochondria of various tissues from mice, naked mole rats (NMRs) and bats possess two mechanistically similar systems to prevent the generation of mitochondrial reactive oxygen species (mROS). The systems in question are (i) hexokinases I+II and (ii) creatine kinase bound to mitochondrial membranes. Both systems operate in a manner such that the kinase substrate (mitochondrial ATP) is electrophoretically transported by the ATP/ADP-antiporter to the catalytic site of mitochondria-bound hexokinase or creatine kinase without ATP dilution in the cytosol. One of the kinase reaction products (ADP) is transported back to the mitochondrial matrix via the antiporter, again through an electrophoretic process without dilution. The system in question continuously supports H^+-ATP-synthase with ADP until glucose or creatine is available. Under these conditions, the membrane potential ($\Delta\psi$) is maintained at a lower level than the maximal one (mild depolarization of mitochondria). This $\Delta\psi$ decrease is sufficient to completely inhibit mROS generation. In 2.5-year-old mice, mild depolarization disappears in the skeletal muscles, diaphragm, heart, spleen and brain and partially decreases in the lung and kidney. This age-dependent decrease in the levels of bound kinases is not observed in NMRs and bats for many years. As a result, ROS-mediated protein damage, which is substantial during the aging of short-lived mice, is stabilized at low levels during the aging of long-lived NMRs and bats. It is suggested that the mitochondrial mild depolarization represents a crucial component of an mitochondrial anti-aging program opposed to the aging program.

Section 5

Mikhail Yu. Vyssokikh[a], Susanne Holtze[b], Olga A. Averina[a], Konstantin G. Lyamzaev[a], Alisa A. Panteleeva[a], Maria V. Marey[c], Roman A. Zinovkin[a,d,e], Fedor F. Severin[a], Maxim V. Skulachev[a,d], Nicolas Fasel[f], Thomas B. Hildebrandt[b] and **Vladimir P. Skulachev**[a]

[(a)] Lomonosov Moscow State University, Belozersky Institute of Physico-Chemical Biology, Vorobyevy Gory 1, Moscow 119991, Russia

[(b)] Leibniz Institute for Zoo and Wildlife Research, Berlin, Germany

[(c)] Research Center for Obstetrics, Gynecology and Perinatology, 117198 Moscow, Russia

[(d)] Lomonosov Moscow State University, Institute of Mitoengineering, Vorobyevy Gory 1, Moscow 119991, Russia

[(e)] Institute of Molecular Medicine, Sechenov First Moscow State Medical University, 119991 Moscow, Russia

[(f)] Department of Ecology and Evolution, University of Lausanne, Lausanne, Switzerland

E-mail: mikhail.vyssokikh@gmail.com; skulach@belozersky.msu.ru

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Section 5

Clinical trials of mitochondria-targeted antioxidants. A milestone on pathway to *Homo sapiens liberatus*?

M.V. Skulachev[1,2], A.D. Petrov[2], E.M. Karger[1,3], N.A. Popeko[3], N.V. Perekhvatova[2]

[1] Mitotech LLC, Vorobyevy Gory 1, Moscow 119991, Russia

[2] Mitotech S.A. Mitotech S.A., 42, rue de la Vallee, L-2661 Luxembourg

[3] Lomonosov Moscow State University, Institute of Mitoengineering, Vorobyevy Gory 1, Moscow 119991, Russia

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Mitochondrial dysfunction is involved in the pathogenesis of numerous diseases, thus development of mitochondrially targeted drugs remains a promising area of pharmaceutical research. In the last 15 years several projects and companies managed to start clinical trials of mitochondrial drugs and the number of such clinical stage projects is growing, however only one mitochondrially targeted pharmaceutical was given regulatory approval so far. These projects can be divided into two groups – specific mitochondrial disorders (usually hereditary) and other indications where mitochondrial dysfunction is usually caused by organism aging, inflammation, autoimmune processes etc.

Here we present clinical results of our drug development project that falls into the second group. Preclinical studies of our lead compound – mitochondrially targeted antioxidant SkQ1 - produced very promising results in several animal models of age-related diseases including models of eye-diseases, neurodegeration and inflammation. We chose local administration of SkQ1 (in the form of eye-drops) to speed up the process of clinical development and to get the first human data faster. At the moment this pharmaceutical Visomitin is approved and successfully marketed in Russia. In the US Visomitin (SkQ1 eye drop solution) successfully passed phase II of clinical development and phase III clinical trials are underway. First phase IIb/III trial (named Vista-1) demonstrated statistically significant superiority of SkQ1 eye drops to artificial tear solution. Systemic oral form of SkQ1 successfully passed phase I clinical trials in Russia and completed preclinical program in US and Canada. We consider our project a valuable attempt to create anti-aging pharmaceutical by targeting mitochondria.

Poster session

The *bd*-type and *bo*-type quinol oxidases have different susceptibility to CO

V.B. Borisov[1], E. Forte[2], S.A. Siletsky[1], M. Petrosino[2], A. Giuffre[3]

[1] Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Leninskie Gory, Moscow 119991, Russian Federation.

[2] Department of Biochemical Sciences, Sapienza University of Rome, Rome, Italy.

[3] CNR Institute of Molecular Biology and Pathology, Rome, Italy

E-mail: bor@belozersky.msu.ru

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CO, like NO and H_2S, is an important signalling molecule. It is endogenously produced by heme oxygenase and plays a role in human physiology. Bacteria, including pathogenic species, are likely to encounter CO in nature specifically produced by host macrophages. The bacterial aerobic respiratory chains can be the target for CO. We have studied in detail the inhibitory effects of CO on the terminal part of the branched respiratory chain of Escherichia coli represented by three different quinol oxidases, cytochromes *bd*-I, and *bd*-II and *bo*. The *bd*-type oxidases contain no copper site and generate a membrane potential without proton pumping, whereas the bo oxidase belongs to the heme-copper superfamily and is a proton pump [1]. We have observed that the oxygen reductase activity of each of the three enzymes is reversibly inhibited by CO but to different degrees. The apparent half-maximal inhibitory concentration values (IC50) for CO inhibition of each oxidase at four different concentrations of oxygen (50, 100, 150 and 200 μM) appeared to be proportional to the oxygen concentration, suggesting CO inhibits the three oxidases in competition with oxygen. The calculated inhibition constants (Ki) for cytochromes *bd*-I, and *bd*-II and bo are 0.04, 0.2 and 2.4 μM CO, respectively [2]. Therefore, we conclude that the bd-type oxidases are more susceptible to inhibition by CO than the bo-type oxidase. These results may open up the possibility to develop CO-releasing compounds as new antimicrobial agents. This work was supported by the Russian Foundation for Basic Research (research project № 19-04-00094).

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Effect of membrane environment on ligand-binding properties of cytochrome *bd*-I from *Escherichia coli*

V.B. Borisov

Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Leninskie Gory, Moscow 119991, Russian Federation

E-mail: bor@belozersky.msu.ru

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Cytochrome *bd*-I is one of the three terminal quinol: O_2-oxidoreductases of the aerobic respiratory chain of Escherichia coli. The enzyme is encoded by the cydABX operon and is expressed under oxygen-limited growth conditions. Although cytochrome *bd*-I lacks proton pumping capability, it is able to produce a proton motive force with an efficiency of one proton per electron. The enzyme consists of four subunits, two large - CydA and CydB, and two small - CydX and CydH. CydA carries all redox-cofactors: a quinol binding site called "Q-loop" and three hemes - b_{558}, b_{595} and *d*. The low-spin hexacoordinate heme b_{558} is ligated by H186 and M393. The high-spin pentacoordinate hemes b_{595} and d have E445 and His19 as the axial ligand, respectively. We investigated the effect of lipid environment on ligand-binding properties of cytochrome *bd*-I. As exogenous ligands, cyanide and CO were used. Parameters of cyanide and CO binding by cytochrome *bd*-I in bacterial membranes, in isolated form or in proteoliposomes were obtained. The optical changes caused by the addition of CO to the enzyme solubilized in detergent micelles are heterogeneous and much more intense in amplitude compared to those in cytochrome *bd*-I of bacterial membranes. When cytochrome *bd*-I is solubilized, an additional CO reactive site appears, as evidenced by the increase in the ligand-induced absorption change. Apart from heme d, CO reacts with part of heme b_{558} (~20%). This additional reactivity is observed even if a zwitterionic detergent is simply added to the bacterial membranes. The effect manifests as additional spectral changes in the Soret region: an intensive band with a maximum at 420 nm and a minimum around 430-440 nm. This is not a trivial denaturation of the isolated hemeprotein since its incorporation into liposomes returns CO-binding properties to those observed in intact bacterial membranes. Cyanide reacts with the membrane-bound cytochrome *bd*-I causing a red shift in the Soret band and decrease in the 650-nm absorption, indicating the decay of the heme *d* oxy-complex. A small magnitude of optical changes in the Soret region in the presence of oxidants allows us to exclude interaction of the ligand with a *b*-type heme. Both isolation of the enzyme and a simple treatment of the bacterial membranes with the detergent causes additional absorption changes in the Soret band which are not reversed in the presence of oxidants. These changes indicate the appearance of the cyanide-reactive fraction of heme b_{558} (about 20%). Incorporation

of the isolated cytochrome *bd*-I into azolectin liposomes restores cyanide-binding properties to those observed with the native membranes: the ligand no longer reacts to heme *b* and binds only to heme *d*. These novel data on the influence of lipid environment on the interaction of the *E. coli* cytochrome *bd*-I with ligands/inhibitors will help us toward a better understanding of the mechanism of regulation of *bd*-type terminal oxidases by modulators/ effectors of different nature. This work was supported by the Russian Science Foundation (project 19-14-00063).

Time-resolved generation of membrane potential by ba_3 cytochrome c oxidase from *Thermus thermophilus* coupled to the single electron injection into the OH state

S.A. Siletsky[1], I.N. Belevich[2], N.P. Belevich[3], T. Soulimane[4], R.Gennis[5], M. Wikström[3]

[1] Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow, Russian Federation

[2] Electron Microscopy Unit, Institute of Biotechnology, P.O. Box 56, FI-00014, University of Helsinki, Finland

[3] Helsinki Bioenergetics Group, Institute of Biotechnology, P.O. Box 65, FI-00014, University of Helsinki, Finland

[4] Materials and Surface Science Institute, University of Limerick, Ireland

[5] Department of Biochemistry, University of Illinois at Urbana-Champaign, Urbana, IL 61801.

E-mail: siletsky@genebee.msu.ru

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The 4-electron reduction of oxygen to water is catalyzed by heme-copper terminal oxidases. The catalytic cycle of cytochrome oxidase includes 4 single-electron transitions (OH → EH → R(P) → F → OH), during which a proton-moving force is formed, due to: a) the transfer of electrons and substrate protons from different sides of the membrane, and *b*) a redox-dependent proton pump. The ba_3 cytochrome oxidase from *Thermus thermophilus* belongs to the B family of heme-copper oxidases and is characterized by a decrease in the average stoichiometry of proton pumping across the membrane, by differences in the catalytic center and proton-conducting pathways. The kinetics of membrane potential generation coupled to the single-electron injection into the activated catalytically competent OH state of ba_3 oxidase from *T. thermophilus* has been resolved. The OH→EH transition of ba3 is accompanied with electrogenic reduction of heme b/heme a_3 pair by CuA through the «fast» phase (~21 mks) and transfer of protons in the «middle» and «slow» electrogenic phases (~0.15 ms and ~0.8 ms) coupled to the electron redistribution from the heme *b*/heme a_3 pair to the CuB [1]. The "middle" and "slow" electrogenic phases seem to be associated with transfer of protons to the proton-loading site (PLS) of the proton pump. When all injected electrons reach CuB the electronic charge is compensated by back leakage of the protons from the PLS into the binuclear site, probably due to the formed membrane potential in the experiment. T315V mutation in the K channel affects the proton steps in the kinetics of membrane potential generation, which indicates the functional role of this proton pathway in the reductive half-reaction of the catalytic cycle of ba_3 oxidase. The work was

carried out with the financial support of the RFBR (project no. 18-04-00503).

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Investigation of electron transfer through the heme centers of aa_3 cytochrome oxidase from *R. sphaeroides* under conditions of ultrafast reduction by a photoactive ruthenium complex with real-time spectrum registration

S.A. Siletsky[1], N.P. Belevich[2]

[1] Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow, Russian Federation

[2] Helsinki Bioenergetics Group, Institute of Biotechnology, P.O. Box 65, FI-00014, University of Helsinki, Finland

E-mail: siletsky@genebee.msu.ru

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A study of the fast kinetics of electron transfer through the redox centers of cytochrome oxidase aa_3 from *R. sphaeroides* was performed with registration of spectra in the visible region with microsecond time resolution. The spectra of transition states that occur during the single-electron ultrafast reduction by the photoactive ruthenium complex of cytochrome oxidase are characterized. In the kinetics of changes in the absorption spectrum, three main transient processes of electron transport through the hemes are resolved for the single-electron reduction of the F state of cytochrome oxidase. The spectrum of the first process (~12 mks) reflects the reduction of heme a from the CuA, the "input" redox center. The position of the maximum and half-width of the absorption band in the spectrum of this kinetic phase are ~607 nm and ~18 nm, respectively. The spectra of the other two processes (~0.21 ms and ~2.2 ms, respectively) are characterized by minima at 605 nm and at 580 nm. The spectra of both components reflect the oxidation of heme a when an electron is transferred to the catalytic center and the reduction of heme a_3 from the F state to O. Heme a is completely oxidized by the catalytic center. In parallel electrometric measurements of cytochrome oxidase membrane potential generation, 4 stages of electrogenic charge transfer are resolved (~10 mks, ~40 mks, ~0.4 ms, ~1.6 ms). The transfer of charges in three of the four electrogenic stages is accompanied by parallel stages of electron transfer through the heme centers. The reduction of heme a occurs at an identical rate (~10 mks) in electrometric and spectral measurements. No spectral changes parallel to the ~40 mks electrogenic phase were detected. The electron transfer stages from heme a to the catalytic center (~0.2 ms and ~2.2 ms) are close in time to the electrogenic proton transfer stages (~0.4 ms and ~1.6 ms), indicating that they are directly

coupled to each other. This work was supported by the Russian Science Foundation (project 19-14-00063).

The direct interaction of hormones with cytochrome c oxidase from bovine heart

I.P.Oleynikov, N.V. Azarkina, T.V. Vygodina, A.A. Konstantinov

A.N. Belozersky Institute of Physico-Chemical Biology, M.V. Lomonosov Moscow State University

E-mail: oleynikov.biophys@gmail.com

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Cytochrome c oxidase (CcO) is a terminal enzyme of the respiratory chain of mitochondria and aerobic bacteria. In the last decades, structure and molecular mechanism of terminal oxidases have been solved in considerable detail and current studies are directed more towards physiological regulation of the enzyme adjusting CcO turnover rate to the energy needs of the cell. The work presented aims to explore a recently revealed mechanism of CcO regulation based on direct reversible interaction of the enzyme with hormones and other physiologically active compounds of steroid or similar structure.

It is shown for the first time that steroid sex hormones estradiol and testosterone as well as progesterone and dehydroepiandrosterone, secosteroid vitamin D_3 and also thyroid hormone triiodo-thyronine (T3) suppress the activity of cytochrome c oxidase (CcO), purified from bovine heart with inhibitory constants around 10^{-5} – 10^{-4} M, All these ligands represent a typical amphiphilic compounds, capable to bind with the special amphipathic groove, discovered by Ferguson –Miller's group in 3D-structure of CcO from *Rhodobacter sphaeroides* and CcO from bovine heart and located near the entry of K^- proton channel [1]. According to data of Ferguson –Miller's group this groove contains bind bile acids so it was called Bile Acid Binding Site (BABS). BABS also binds dodecyl-maltoside (DM), mild detergent added to buffer to keep CcO in solubilized state. All examined hormones compete with DM for binding with CcO with the 1:1 ratio. Estradiol and vitamin D_3 induce spectral shift of the Soret band in absorption spectrum of oxidized CcO with minimum at 415 and maximum at 437 nm of difference spectrum which is typical for the "red shift" induced by strong ligands of heme a_3 such as cyanide. Molar extinction of spectral change induced by 1mM estradiol (ca 10$мM^{-1}$) corresponds to about 20% of maximal spectral change induced by cyanide. While cytochrome c oxidase activity of CcO is strongly inhibited by steroid hormones partial peroxidase activity of the enzyme appeared to be not sensitive to its effect. Such a special type of CcO inhibition was described earlier as an effect of mutations inside the K proton channel [2] and is in agreement with a hypothesis of Ferguson –Miller's group that amphiphilic ligands of BABS might impair proton transport via K channel as BABS is located near its entrance. In contrast to

steroid hormones triiodo-thyronine (T3) suppresses both oxidase and peroxidase activities of CcO and possibly the mechanism of its effect is different.
The results obtained point to a new possibility by which hormones might affect oxidative phosphorylation: direct interaction with CcO, a key enzyme of the respiratory chain modulating the respiratory activity of mitochondria.

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New bioinformatics methods for identification of lost genes and protein isoforms

V.A. Lyubetsky, G.A. Shilovsky, O.A. Zverkov, A.V. Seliverstov, L.I. Rubanov

Institute for Information Transmission Problems of the Russian Academy of Sciences (Kharkevich Institute), 127051, Russia;

Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, 119991, Russia

E-mail: lyubetsk@iitp.ru

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The high regenerative potential of poikilotherms relative to homeotherms remains not well understood. An original algorithm and software were used to identify a short list of lost genes of *Xenopus laevis*, one of which («c-answer») was used in experimental tests. The c-answer gene encodes a previously unknown specific signaling (FGF and ADP) modulator, a transmembrane protein factor of regeneration and telencephalon development in poikilothermic vertebrates that was lost in homeotherms including human. Experiments on embryos of *X. laevis* have demonstrated that c-answer controls the regeneration of body appendages and telencephalon development by binding FGRF and P2ry1 receptors and inducing the MAPK/ERK and purinergic pathways. One can propose that the loss of c-answer in homeotherms decreased the activity of at least two signaling pathways, which consequently contributed to the alteration of the control pathways of regeneration and telencephalon development. (This study was performed in cooperation with the Laboratory of Molecular Bases of Embryogenesis, Institute of Bioorganic Chemistry.)
We believe that the long (relative to body weight) species-specific lifespan (LS) in rodents and primates can be, among other things, due to the loss of certain genes found in short-lived representatives of the same order. The software has identified the short list of genes present in short-lived primates and rodents but missing in long-lived ones including humans, Colombian white-faced capuchin, and naked mole-rat. Other Euarchontoglires species have been considered as well. The expression level of the identified murine genes was analyzed in different tissues. The obtained results can be interpreted in terms of the

well-known theory of Williams: such genes improve the fitness of species with short LS but are neutral or harmful for those with long LS.

The program can efficiently identify the genes lost or acquired at any stage of evolution.

Cardiolipin can be considered as a functional integrator of components of the mitochondrial respiratory chain providing for the efficient transfer of electrons and protons. The enzyme tafazzin is the main if not the only factor of cardiolipin maturation. Altered proportions between tafazzin isoforms can cause severe abnormalities such as Barth syndrome. One can think that unconventional tafazzin isoforms contribute to the optimal balance between increased biochemical activities of mitochondria resulting from specific environmental or nutritional conditions and longevity and that the functional role of such isoforms is due to the altered C-terminal primary and secondary structure of the protein.

These isoforms correspond to the omission of exon 9 or retention of the intron between exons 10 and 11, both of which cause a frameshift; as well as to the emergence of an exon 5 or retention of the intron between exons 8 and 9. These isoforms can be found in many mammalian orders including animals living under low-oxygen conditions. The emergence of these isoforms in placentals correlates with the increased species-specific lifespan. For instance, E5 is found in marsupials (e.g.Tasmanian devil) and many (e.g.electric eel) but not all fishes (e.g., it is missing in the zebrafish).

This study was supported by the Russian Foundation for Basic Research (18-29-13037).

Molecular mechanisms of neighboring gene effect in the yeast knockout library

A.A. Egorov, A.I. Alexandrov, V.N. Urakov, R.O. Edakin, D.S. Makeeva, V.N. Gladyshev, I.V. Kulakovskiy, S.E. Dmitriev

Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow, 119234 Russia; School of Bioengineering and Bioinformatics, Lomonosov Moscow State University, Moscow, 119234 Russia; Bach Institute of Biochemistry, Federal Research Center of Biotechnology of the Russian Academy of Sciences, Moscow, 119071 Russia; Division of Genetics, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston MA 02115, USA

E-mail: artyom.egorov@belozersky.msu.ru

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The *S. cerevisiae* gene deletion collection is widely used for genome-wide annotation of function and study of genetic interactions. However, the standard G418-resistance cassette used to obtain knockout mutants introduces strong regulatory elements into the target genetic loci. However, their effects on the expression of neighboring genes have never been systematically assessed.

Here, using Ribo-Seq and RNA-Seq data for several *S.cerevisiae* knockout strains, we analyzed transcriptional and translational changes induced by the KanMX cassette within the modified genomic loci. In many cases, we observed significant alterations in gene expression, including severe impairment of translation. These changes could be attributed to shifted transcriptional start sites

or activation of alternative polyadenylation signals. The most dramatic changes were observed when a deleted gene was arranged "head-to-head" with the neighboring gene, where a shift of transcription start site of the latter expanded the 5' untranslated region, and the appearance of upstream AUG codons inhibited translation of the main open reading frame. In some cases, these events caused false genetic interactions of the deleted genes, the so-called neighboring gene effect.
Our data describe the interactions of the KanMX cassette with neighboring genes and provide mechanistic insights into the molecular mechanisms involved. They also suggest that caution is needed in interpreting the results of deletion screens, especially those using strong regulatory elements.

The mitochondrial-targeted compounds C_{12}TPP and DNP decrease ICAM1 expression in EA.hy926 cells and cause CpG hypermethylation in its promoter region

C.I. Makievskaya[1], V.V. Sidlyarchuk[1], L. A. Zinovkina[1], R.A. Zinovkin[2]

[1] Faculty of Bioengineering and Bioinformatics, Lomonosov Moscow State University;

[2] Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University.

E-mail: roman.zinovkin@gmail.com

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The inflammatory processes in the endothelium may result in the development of cardiovascular diseases.
Proinflammatory cytokines stimulate the expression of cell adhesion molecules on the surface of endothelial cells thus promoting adhesion and transmigration of leukocytes. It has been previously shown that the mitochondria-targeted compounds SkQ1, C_{12}TPP, and DNP lowered the expression of endothelial proinflammatory cytokines and adhesion molecules including ICAM1. Noteworthy decreased expression of ICAM1 sustained for many days indicating the possible involvement of epigenetic modification(s) in the ICAM1 promoter. We hypothesized that the long-term effect of the studied compounds on ICAM1 mRNA expression could be achieved via the modulation of CpG methylation level of its promoter. The aim of our work was to study the effect of SkQ1, C_{12}TPP and DNP on the methylation of the ICAM1 gene promoter. Our results indicate that both C_{12}TPP and DNP increase CpG methylation in the ICAM1 promoter in EA.hy926 cells. This increase in the CpG methylation coincides with the decreased ICAM1 mRNA expression. The results suppose that the modulation of mitochondrial function in the endothelial cells leads to the epigenetic regulation of ICAM1 gene expression via CpG methylation of ICAM1 promotor. The study was supported by the RFBR grant No. 18-04-01110.

Diversification of functions between cells in filamentous cyanobacteria

Potapova T.V., Koksharova O.A.

AN Belozersky Institute of Physical-Chemical Biology, Lomonosov Moscow State University

E-mail: koksharova@genebee.msu.ru

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Multicellularity is a form of organization in living systems, in which a group of cells fulfils more complex functions than any individual cell. Filamentous heterocysts-forming cyanobacteria are the prototype of a multicellular organism, because their cells are not just clustered into agglomerates but represent a functional association unified by exchange of metabolic products and regulatory molecules as well as by electrical communications through highly permeable intercellular contacts (PIC) [1]. The separation of functions between neighboring cells in any tissue allows some cells to receive an "energy subsidy" from neighbor cells by virtue of ion fluxes through PIC. The energy acquired by this means corresponds to the energy needed for the operation of primary ion pumps (up to one-third of the total cell energy production). The cell-to-cell transmission of energy by means of intercellular electrical communication through permeable intercellular contacts should apparently be regarded as one of the oldest natural technologies of biological systems. The hyperpolarization of cell membranes generated by the cyanobacterial photosynthetic apparatus can perform a number of energy-dependent functions: it promotes the functioning of ATP synthases, transport mechanisms, the motor apparatus, etc., both at the point of illumination and at a distance of tens of cells from the illuminated site. In filamentous cyanobacteria featuring the separation of functions between heterocysts and vegetative cells, the intercellular electrical connections within the trichome ensure the operation of plasma-membrane ATP synthases in heterocysts that are lacking photosynthetic systems of their own. Thus, the heterocysts remain functional and well supplied with ATP resources without the increase in oxygen concentration.

Multicellularity is an evolutionary innovation that represents a new level of organization and is a necessary tool for sophisticated adaptation techniques. From the viewpoint of modern molecular genetics, a group of filamentous cyanobacteria featuring separation of functions between neighboring cells is a prototype of multicellular organization and provides a convenient model for elucidating the mechanisms of regulation of multicellularity, which, apparently, appeared more than once during the evolution in different phylogenetic groups. It is highly important that the ability of bioenergetic cooperation in electrically interconnected cells appeared at the dawn of evolution, billions of years

ago, as a structural and functional basis for the division of labor in trichomes of filamentous cyanobacteria, the first multicellular organisms of our planet.

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Electrical heterogeneity role in structural and functional organization of *Neurospora crassa* hyphal tips

Potapova T.V.

A.N. Belozersky Institute of Fisico-Chemical Biology
Lomonosov Moscow State University

E-mail: tvpotapova@gmail.com

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Clarification of the laws determining the organization of cell–to–cell and intracellular interactions in living systems is one of the most important problems in modern biology. Tip growth of *Neurospora crassa* mycelium can be a suitable experimental model for studying patterns of such interactions, since tip growth is a typical system function which involves coordinated interactions of different cells as well as different intracellular structures. The growing hyphae of *N. crassa* drive longitudinal proton currents through themselves. The pattern of the current flow may be described as a spatially extended chemiosmotic system, with proton pumps and proton leaks separated in space [1]. Thus, the growing tip of the *N. crassa* vegetative hyphae can be characterized as a natural system realizing the technology of energetic cooperation, i.e. the division of labor between the more adult cells of the hypha for energy accumulation as V_m with expenditure of own ATP resources and the apical cells (possessing a reliable electric connection with mature cells of the hyphal stem) for spending the energy of V_m to perform the work required for accumulation of metabolites from the environmental medium. The inevitable consequence of separation of labor between the neighboring cells is maintaining significant local electric currents and local electric fields determining the relocation rate and orientation of intercellular structures in the growing hyphal tip. According to data of electrophysiological measurements and theoretical model analysis, we can assess the electric field strength along the hyphal front end: E=100 V/m. Studies performed in recent years revealed a specific structural patterning of the anterior end of the hypha at a distance of 120 — 150 μm from the tip. Recently, research attentions are careful focused on describing the interactions between intracellular structures in the tip growth and the elucidation of molecular and genetic mechanisms for the implementation of this process as well as its reaction to events in the external environment. On the basis of the comparison of the

behavior of mitochondria and microtubules and the data on the electrical heterogeneity of the hyphal apex, a hypothesis is proposed about a possible supervisory role of the longitudinal electric field in the structural and functional organization of the growing tips of the *N.crassa* hyphae [2]. Thus, it can be assumed that electrical gradients in the hyphal tips are at least a part of the control system, which monitors the hyphal growth regulation, including its power supply.

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MitoNBD — a new fluorescent mitochondrial uncoupler with high antibacterial activity

I.R. Iaubasarova[1,2]; L.S. Khailova[1]; T.I. Rokitskaya[1]; E.A. Kotova[1]; G.A. Korshunova[1]; Y.N. Antonenko[1]

[1] Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Leninskie Gory 1/40, Moscow 119991, Russia;

[2] Faculty of Chemistry, Lomonosov Moscow State University, Leninskie Gory 1/3, Moscow 119991, Russia

E-mail: maililuza@mail.ru

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Appending lipophilic cations to various small molecules has been widely used as an approach to obtain mitochondria-targeted compounds with specific activity. By applying this approach at our lab, we have previously synthesized fluorescein decyl(triphenyl)phosphonium ester (mitoFluo) that proved to be a rather effective mitochondria-targeted uncoupler of oxidative phosphorylation (Denisov et al. Chem. Commun., 2014) exhibiting neuro- and nephroprotective properties in rats (Antonenko et al. BBA-General Subjects, 2016). Here, we prepared a series of derivatives of the well-known fluorescent dye 7-nitrobenz-2-oxa-1,3-diazole (NBD). According to our earlier study (Denisov et al. Bioelectrochemistry, 2014), alkyl NBD derivatives are able to uncouple isolated mitochondria at concentrations of tens of micromoles, despite the very high pK_a (about 11) of their proton-dissociating group. We have synthesized a series of (triphenyl)phosphonium derivatives linked to NBD via hydrocarbon spacers of various lengths — C_5, C_8, C_{10}, and C_{12} (mitoNBD analogues). These compounds showed energy-dependent accumulation into mitochondria. NBD-C_{10}-TPP (C_{10}-mitoNBD) displayed protonophoric activity on artificial lipid membranes and uncoupled the isolated mitochondria at micromolar concentrations, while a derivative with a short linker, NBD-C_5-TPP (C_5-mitoNBD), practically did not exert the protonophoric and uncoupling effects. In line with these results, C_{10}-mitoNBD was much more effective than C_5-mitoNBD in suppressing the growth of Bacillus subtilis. Of the mitoNBD analogues studied here, C_{10}-mitoNBD exhibited the maximal antibacterial potency.

Proton-coupled electron transfer reaction between the primary quinone acceptor Q_A and the non-heme iron in photosystem II

Fedorenko E.[1], Liya Vitukhnovskaya[1,2], Alexey Semenov[1,2], Mahir Mamedov[1]

[1] A.N. Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow, Russia;

[2] N.N. Semenov Institute of Chemical Physics, Russian Academy of Sciences, Moscow, Russia

E-mail: ms.ekaterina.fedorenko@mail.ru

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An electrometrical technique was used to study the effect of synthetic electron acceptors, such as 2,6-dimethylbenzoquinone (DMBQ) and p-phenylbenzoquinone (PPBQ) on the light-induced voltage changes (ΔΨ) in photosystem II (PS II) core complexes reconstituted into liposomes. In the absence of exogenous acceptors, the fast kinetically unresolvable phase (< 0.2 μs) of ΔΨ generation induced by the laser flash was due to electron transfer between the redox-active tyrosine Yz and the primary quinone acceptor Q_A. In the presence of PPBQ, besides the fast kinetic phase of ΔΨ generation, an additional electrogenic phase (τ ~ 220 μs, relative amplitude ~ 10% of the overall amplitude) was observed upon the second laser flash. The sensitivity of this phase to diuron, an inhibitor that binds to the site of the exchangeable plastoquinone (Q_B), slowing down of this phase in the presence of D_2O instead of H_2O suggests that the submillisecond electrogenic component is due to vectorial proton transfer from external aqueous phase to an amino acid residue(s) in the vicinity of the non-heme iron, oxidized upon the first flash by the semiquinone form of DMBQ. Similar data were obtained in the presence of DMBQ. All these data are important for understanding the mechanisms of ΔΨ generation in the presence of artificial electron carriers of PS II.

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Fibroblasts of hypoxia-tolerant, long living and cancer resistant blind mole rat *Spalax* produce high amounts of ATP at extremely low mitochondrial membrane potential

Chaban, D.Miskevich, I. Shams

University of Haifa, The Department of Evolutionary and Environmental Biology

E-mail: anastasia@chaban.su

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Blind mole rat *Spalax* is an obligatory fossorial rodent, which inhabits open fields in the Western Mediterranean. In its natural habitat it is frequently exposed to severe hypoxia (down to 7% oxygen) and hypoxia-reoxygenation cycles during rainfalls and digging activities. *Spalax* has been studied for decades in the lab and was found to be long-lived (about 20 years in captivity) and cancer resistant.

In the present study we aim to explore bioenergetic strategies in the species harboring these outstanding traits in the background.

In our work we tested mitochondrial membrane potential (MMP) in rat and *Spalax* cultured skin fibroblasts, their ATP contents and mitochondrial superoxide production. We estimated MMP levels by TMRE staining under normal conditions and respiratory complexes I, III, IV and V blockage. ATP concentrations were measured by luciferase reaction-based kit under normal conditions, glycolysis or ATP-synthase inhibition. Mitochondrial superoxide production was assessed using MitoSOX staining. Rat cells were used for comparison as both animals belong to the same order Rodentia, have about the same weight, which is important for metabolic and longevity studies; however, rat significantly differs from *Spalax* in longevity, hypoxia-tolerance and cancer-resistance.

We found that MMP formation differs significantly in *Spalax* and rat fibroblasts. *Spalax* mitochondria show about two-fold lower MMP than rat and it seems to be almost unsusceptible to standard respiratory complexes I, III and IV blockers (MMP dropped not more than 10-15% under each treatment, while in rat it decreased by 75-80%). Oligomycin was found to significantly increase MMP in both *Spalax* and rat fibroblasts. Mitochondrial superoxide production was 2 times lower in *Spalax* cells than in rat; nevertheless, ATP concentrations were about 2 times higher in *Spalax* fibroblasts under normal conditions. Both oligomycin and 2DG application to inhibit OXPHOS or glycolysis resulted in two-fold decrease in ATP concentrations in *Spalax*. Meanwhile, in rat cells ATP production decreased under OXPHOS (-20%) or glycolysis (-35%) blockage not so dramatically.

Summing up this data we may conclude that *Spalax* mitochondria produce ATP efficiently at very low MMP. We assume that some alterations in the ETC in *Spalax* cells result in low mitochondrial superoxide production to reduce possible ROS damage during hypoxia and hypoxia-reoxygenation cycles. These alterations may also cause such poor susceptibility of MMP to ETC complexes blockers in Spalax fibroblasts. Based on the present experiments and preliminary tracing data, we suppose that *Spalax* cells fine-tuned its energy production machinery and evolved alternative bioenergetics strategies to withstand harsh environmental conditions. Together these adaptive mechanisms may contribute to *Spalax* longevity and cancer resistance.

Modulation of human neutrophil survival and oxidation status by synthetic CpG-oligonucleotides

E. Golenkina, G.Viryasova, G. Sud'ina

A.N. Belozersky Institute of Physico-Chemical Biology, M.V. Lomonosov Moscow State University

E-mail: gali.inimitable@yandex.ru

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Neutrophils are the major population of circulating white blood cells, that provides the first line of defense against invading microorganisms. These cells carry out their main task due to the ability to phagocytize the pathogen, produce reactive oxygen species (ROS), and release lytic enzymes and proinflammatory mediators. An important feature of neutrophils is their ability to apoptosis, that is necessary to resolve the inflammatory process.

Active host protection against bacterial infections is initiated not only directly by the infectious agent but also by exposure to pathogen associated molecular patterns (PAMPs), which are conserved molecular structures expressed by invading microorganisms. Stimulation by PAMPs normally triggers immune cells to destroy intruding pathogens and successfully overcome infectious inflammation. At the same time, pro-inflammatory mediator production by PAMP-stimulated host cells underlies a variety of disorders. Bacterial DNA also belongs to pathogen associated structures, actively studied as potential therapeutic agents. The immunostimulatory activity of bacterial DNA can be mimicked by synthetic oligonucleotides that contain unmethylated CpG sequences plus flanking regions (CpG oligonucleotides, CpG ODNs). We studied the effects of a synthetic CpG oligonucleotide 2006 (5'-tcgtcgttttgtcgttttg tcgtt-3') on neutrophil survival and oxidant status.

CpG ODN2006 showed a dose-dependent effect on the apoptosis of resting neutrophils. Without affecting the viability of resting cells, low concentrations of CpG ODN2006 interfered with *Salmonella typhimurium* (S147) mediated viability prolongation and increased neutrophil apoptosis to control levels. CpG-ODN2006 stimulated neutrophil apoptosis by enhancing ROS generation. Even small doses of ODN could induce the production of intracellular superoxide anions. The high superoxide reactogenicity, including with respect to nitrogen oxide, led to increased levels of intracellular ROS and RNS, which ultimately caused apoptosis. The pro-oxidant effect of low concentrations of CpGODN2006 was not sufficient to trigger irreversible pro-apoptotic mechanisms. However, the sensitivity of PMNLs to ODN2006, a modulator of apoptosis, increased significantly under conditions of infectious inflammation. Inactivated *S. typhimurium* proved to be suitable for simulating inflammatory conditions in vitro. The effect of CpG ODN2006 on neutrophil survival was abolished by MyD88 blockade indicates the involvement of TLR9-mediated pathways in its pro-apoptotic actions.

The ability of synthetic CpG ODNs to affect neutrophil functions draws attention to their use in immunotherapy. Thus, whether their modulation of PMNL functions is pro- or anti-inflammatory needs to be determined. The results of our work indicate the duality of CpG ODN2006 actions in vitro. The ability to induce ROS generation suggests a pro-inflammatory effect. On the other hand, its ability to interfere with the pro-survival effect of PAMPs can contribute to the successful resolution of inflammation.
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The effect of C-terminal domain of subunit on ATPase activity of *Bacillus subtilis* ATP synthase

V.M. Zubareva[1], D.O. Tretyakov[1], A.S. Lapashina[1,2], B. A. Feniouk[1,2]

[1] Faculty of Bioengineering and Bioinformatics, Lomonosov Moscow State University;

[2] A.N. Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University

E-mail: zubareva.valeriaa@gmail.com

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FOF1-ATP synthase is a membrane enzyme, which catalyzes ATP synthesis using the energy of transmembrane electrochemical proton gradient. The enzyme may function in a reverse mode as ATP-dependent proton pump. The ATPase activity can be suppressed by MgADP complex in an uncompetitive manner (MgADP inhibition). When ADP is bound in the catalytic site in absence of inorganic Pi, the enzyme may lapse into inactive state. In chloroplasts and some bacteria, ATP hydrolysis may also be suppressed by C-terminal domain of subunit. Among bacteria, the inhibitory effect of have been observed in *Escherichia coli, Bacillus sp. PS3* and *Bacillus subtilis*. Relationship between MgADP inhibition and -dependent inhibition is obscure. Some authors have claimed that subunit relieves ATP synthase from MgADP inhibition and activates the enzyme. Others have demonstrated that both types of inhibition support each other.

We decided to study the relationship between MgADP inhibition and -mediated inhibition in *B. subtilis* FOF1. We compared the activities of subbacterial membrane vesicles prepared from two *E. coli* strains expressing *B. subtilis* ATP synthase of the wild type or lacking the C-terminal domain of subunit (c). Rates of both ATP synthesis and hydrolysis in c strain were lower than in the wild type. ADP affected the steady-state phase of ATP hydrolysis in both strains in similar way. Sulfite which is known to relieve ATP synthase from MgADP inhibition has also affected both strains similarly. Along with that, the ATP hydrolysis kinetics of c membrane particles demonstrated a lag phase which can be due to a presence of inhibitory MgADP in the catalytic site of c enzyme. The stimulating effect of inorganic phosphate on ATPase activity was more pronounced in c strain. All these data allow us to suggest that C-terminal domain of subunit does not relieve ATP synthase from MgADP inhibition.

Mitochondria-targeted antioxidant SKQ1 prevents UV-induced keratouveitis in rabbits

V. Tiulina[1,2], E. Zernii[1], V. Baksheeva[1], O. Gancharova[1], M. Tsarkova[2], P. Philippov[1], I. Senin[1]

[1] A.N. Belozersky Institute of Physico–Chemical Biology, Lomonosov Moscow State University, Moscow, Russia,

[2] Moscow State Academy of Veterinary Medicine and Biotechnology – MVA by K.I. Skryabin, Moscow, Russia

E-mail: tyulina_nika@list.ru

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Keratouveitis is an inflammatory condition of the eyes characterized by keratitis and anterior uveitis. Also, keratouveitis refers to a clinical picture of active corneal disease associated with anterior chamber inflammation. We have developed an experimental model of UV-induced keratouveitis, which can allow us to study the therapeutic effectiveness of drugs for the treatment of this pathology. Because the inflammation diseases are triggering by proinflammatory cytokines production through promoting the production of mitochondrial reactive oxygen species, in this work, the feasibility of mitochondria-targeted antioxidant SkQ1 based therapy for enhancement of keratouveitis prevention after UV irradiation was investigated in vivo.

It was demonstrated that 312 nm light irradiation of the eyes triggers the development of corneal lesions and anterior inflammation in rabbits. In particular, loss of epithelium, stromal edema and apoptosis of keratocytes and endothelium cells were observed. It was emphasized that multiple apoptosis of stromal keratocytes can be associated with UV-induced oxidative stress, which manifested in tears, aqueous humor and cornea as elevation of MDA content. The feasibility of SkQ1 based antioxidant premedication therapy for protection against UV irradiation-induced keratouveitis was demonstrated. The therapy prevents the loss of stromal keratocytes, other corneal cells and decrease level of inflammation in the anterior. According to biochemical studies, the instillations of SkQ1 also suppresses oxidative stress in tears, aqueous humor and cornea. In conclusion, our data suggest that the mitochondria-targeted antioxidant SkQ1 is effective for protection against UV-induced keratouveitis.

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Effects of SkQN on rat liver mitochondria and yeast cells

Goleva T.N., Rogov A.G., Epremyan K.K., Shumakovich G.P., Zvyagilskaya R.A.

Bach Institute of Biochemistry, Federal Research Center "Fundamentals of Biotechnology", Russian Academy of Sciences

E-mail: goleva13@yandex.ru

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One of distinct features of cancer tissues is the elevated concentration of reactive oxygen species (ROS). Moreover, ROS and cellular oxidative stress are regulators of cancer cells (Qiu et al., 2015). At early precancerous and neoplastic stages, especially in melanoma, ovary and breast cancer, antioxidant activity decreases and increased levels of intracellular ROS appear to promote cancer initiation via inducing oxidative damage of mitochondrial and nuclear DNAs, mutations in pro-oncogenes and tumor suppressor genes, which promote cancer development and progression. ROS produced by mitochondria (mtROS) are of special importance for pro-tumorigenic signaling (Agapova et al., 2008; Sabharwal and Schumacker, 2014). The metabolic and (epi)genetic reprogramming including dysfunctional mitochondria and impaired mitochondrial metabolism subsequently contribute to cancer cell evolution towards more aggressive phenotypes (Ekoue et al., 2017) and cancer cell response to chemotherapy (Guerra et al., 2017). The accumulation of excessive levels of ROS (oxidative insults) prevents tumorigenesis and promotes the death of cancer cells via various mechanisms making scientists search for potential oxidative stress modulators as anticancer strategies (Arif et al., 2018; Gurer-Orhan et al., 2018). The mitochondria-targeted (i.e., transported exclusively to the mitochondrial interior) derivative of menadione ($MitoK_3$) was recently synthesized by conjugating cationic TPP^+ and an aliphatic linker to the C_3 position of the naphthoquinone ring (Teixeira et al., 2018). Mitochondria-targeted cationic agents offer advantages over water-soluble cationic compounds as they are electrophoretically transported into and accumulated within cells and mitochondria in conformity with the membrane potential generated on the cytoplasmic or mitochondrial membrane, respectively. Moreover, they predominantly accumulate in mitochondria of cancer cells, since these cells exhibit a considerably higher mitochondrial membrane potential than non-transformed cells (Modica-Napolitano, 2001). In attempt to improve prooxidant activity of mitochondria-targeted naphthoquinones 1,4- naphthoquinone conjugated with TPP^+ (SkQN) was synthesized.

SkQN was found to be more active redox agent according to electrochemical measurements and more reactive as electrophile than $MitoK_3$, presumably due to the absence of methyl group in naphthoquinone ring. Both SkQN and $MitoK_3$ stimulated state 4 respiration in rat liver mitochondria, inhibited state 3 respiration, decreased the

membrane potential, inhibited ATP synthesis, promoted the opening of the (permeability transition pore, with SkQN being more efficient "uncoupler", stronger inhibitor of respiration as compared to MitoK_3. SkQN was also more efficient prooxidant presumably due to significantly higher (less negative) redox potential. Both SkQN and MitoK_3 triggered an oxidative stress challenge in yeast cells and cell death with SkQN showing again higher lower efficacy in these processes than MitoK_3. Thus, the use of SkQN, a newly syntheses mitochondria-addressed prooxidant would be a promising strategy for anticancer therapy.

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Yeast model for studying the role of mitochondrial oxidative stress in the development of hepatocellular carcinoma

K.K. Epremian, R.A. Zvyagilskaya, A.G. Rogov, R.A. Zinovkin

Bach Institute of Biochemistry, Research Center of Biotechnology of the Russian Academy of Sciences, Moscow, Russia;

Lomonosov Moscow State University, Belozersky Institute of Physico-Chemical Biology, Moscow, Russia

E-mail: 7700077@mail.ru

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Numerous studies have shown that chronic hepatitis B virus (HBV) infection is a risk factor for hepatocellular carcinoma (HCC) induction. It is estimated that currently there are over 350 million HBV carriers globally. However, the underlying mechanism of HBV-associated HCC remains unclear. Protein HBx, expressed in infected cells, is known to play a critical role in HBV replication and in the pathogenesis of HCC. HBx does not interact directly with nuclear DNA, but acts as a regulator of transcription of cellular proteins. When the level of HBx expression is high, HBx binds to mitochondria and causes their dysfunction, fragmentation, clustering and abnormal distribution within the cell. Chronic hepatitis B infection is accompanied by oxidative stress, which plays an important role in carcinogenesis, but the mechanisms underlying its induction are not known. We are suggested that HBx, acting on mitochondria, induces the production of reactive oxygen species (ROS), which would lead to the development of oxidative stress provoking tumor transformation of infected hepatocytes. To verify this hypothesis, a yeast model for the heterologous expression of HBx based on *Yarrowia lipolytica* yeast was created and studied. This simple yeast model would contribute to revealing HBx-induced changes in the cell by abstracting from chronic inflammation, immune response and other factors, also implicated in oxidative stress induction. *Y. lipolytica*, an obligate aerobe with the respiratory metabolism closely resembling that of mammalian cells, vigorously growing on a variety of simple, well defined

media, amenable to both classical and molecular genetic techniques, having a long history of use as a producer of heterological proteins seems to be a promising model for such kind of investigations.

Genetic constructs carrying HBx and HBx fused with eGFP were obtained; *E. coli* were transformed by these constructions; plasmids bearing genes of interest were isolated and purified, *Y. lipolytica* were transformed by the resulting plasmids by electroporation. Fluorescent microscopy assay showed that HBx is expressed in yeast cells as small aggregates and that multiple HBx aggregates were located at the periphery of yeast cells, the prevailing location sited of mitochondria. HBx expression inhibited respiration and ATP synthesis, enhanced ROS production, reduced resistance to prooxidants, triggered fragmentation of mitochondria, decreased cell viability. Low concentrations of SkQThy, the mitochondria-targeted (i.e. transported exclusively into mitochondria) antioxidant reduced the level of oxidative stress and increased survival of the studied mutants. The obtained results indicate the adequacy of the proposed and developed yeast model for studying HBx-mediated changes in the cell, as well as the efficiency of mitochondria-targeted antioxidants in alleviating harmful effects typical for HBx-induced carcinogenesis.

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Propagation of prooxidant-induced oxidative stress within the yeast cell

A.G. Rogov, T.N. Goleva, K.K. Epremyan, I.I. Kireev, R.A. Zvyagilskaya

Bach Institute of Biochemistry, Research Center of Biotechnology of the Russian Academy of Sciences, Moscow, Russia;

Lomonosov Moscow State University, Belozersky Institute of Physico-Chemical Biology, Moscow, Russia

E-mail: lloss@rambler.ru

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Reactive oxygen species (ROS) play a dual role in biological systems. Under physiological conditions, ROS, formed as natural byproducts, are an essential element cell signaling, regulating processes of division, differentiation, growth (Dröge et al., 2002). Excessive production of ROS (oxidative stress), as a result of an imbalance between the generation and clearance of ROS, can induce significant damage to cell components, including DNA, proteins and lipids (Cooke et al., 2003) and compromised multiple cellular signaling pathways, thereby leading to the aggravation of many diseases (Lee et al., 2017; Tan et al. 2018). Oxidative stress has been shown to increase with age, being a major contributor to functional decline (Islam, 2017). Mitochondria in various eukaryotes, unlike static organelles, change size and shape by undergoing fission and fusion, processes that are orchestrated by the cellular machinery comprised of

dynamin-related proteins. It is argued that this organellar dynamics is an essential part of the mitochondrial quality system. Excessive mitochondrial fission (fragmentation) is a prominent early event, contributing to the progression of neurodegenerative diseases (Ong et al., 2019).

Although there is a general belief that mitochondria are the main source of ROS in the cell and that excessive ROS production largely derived from mitochondrial dysfunction gives rise to pathogenicity (Feniouk and Skulachev, 2017), only a few studies have investigated the direct effect of mitochondrially-derived ROS to the oxidative state of the cell (Ishii et al. 2017, Lee et al. 2017). We believe that lack of convenient, vigorously growing and low-cost models impeded such investigations on living cells.

In this study we used as a model organism the yeast *Dipodascus magnusii* having the respiratory metabolism closely resembling that of mammalian cells (Bazhenova et al., 1998), vigorously growing on a variety of simple, well defined low-cost and normally containing the highly structured mitochondrial reticulum. Recently we showed the benefits of applying these giant cells for visualization of mitochondrial fragmentation (Rogov et al. 2018). Using these cells and a combination of fluorescence microscopy, time-lapse microscopy and flow cytometry assays, we followed the temporal development of prooxidant-induced oxidative stress in mitochondria and in the protoplasm. It was established, to our the best knowledge, in the first time, that oxidative stress detectable in mitochondria with MitoSox Red, increased immediately after cell treatment and preceded emergence of generalized oxidative stress in the whole cell. Moreover, progressively increased oxidative stress in mitochondria was accompanied by gradual mitochondrial fragmentation.

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Mitophagy in yeast cells

D.V. Mamaev, A.G. Rogov, T.N. Goleva, R.A. Zvyagilskaya

Bach Institute of Biochemistry, Research Center of Biotechnology of the Russian Academy of Sciences, Moscow, Russia

E-mail: dmamaev_inbi@mail.ru

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Mitochondria, functionally versatile organelles, are critical for cell survival and death. Apart from the best-known function of mitochondria to supply most of the cellular ATP through oxidative phosphorylation, they are tightly integrated into the general cell metabolism, play a key role in global processes such as cell signaling, proliferation, inflammation, immune response, activation of endoplasmic reticulum (ER)-stress response, regulation of apoptosis, and generation of reactive oxygen species (ROS) as byproducts of respiration (see, Mamaev and Zvyagilskaya, 2019). Mitochondrial dysfunction is associated with aging and various human diseases, such as neurodegeneration, metabolic disorders, diabetes, and cancer (see, Goleva et al., 2017). Therefore, the number of existing mitochondria should strictly correspond to energy demands of the organism. The amount of mitochondria should not be excessive, and the redox state of mitochondria should be carefully controlled by the cell. Mitophagy, the selective removal of mitochondria by means of autophagy, serves to this task in parallel with mitochondrial biogenesis and other processes. The goal of this work was to investigate mitophagy in the *Saccharomyces cerevisiae* yeast. For this purpose a collection of *S. cerevisiae* mutants with deletions of genes coding for proteins involved in mitophagy process in yeasts was assembled. It was found that oxidative stress induced by the prooxidant tert-butylhydroperoxide (t-BHP) caused mitophagy in *S. cerevisiae* cells grown on galactose. Mitochondrial fragmentation is the necessary stage in mitophagy in mammalian cells. For yeast, this conclusion is not so unambiguous. For all mutants tested, 3D-models of mitochondrial structures under normoxia and t-BHP-mediated oxidative stress were obtained. Deletion of *ATG32* did not affect t-BHP-triggered mitochondrial fragmentation. Mutants with deleted Fis1, Dnm1 and Mdv1 proteins responsible for mitochondrial fission, expectedly preserved well-developed mitochondrial reticulum, oxidative stress did not induce full fragmentation, while changing mitochondrial structure. Mutants without atg1, atg8 and atg11 proteins involved at the various stages of phagophore formation, possessed well-developed mitochondrial reticulum, but, in contrast to the control strain, did not lose it in the presence of t-BHP. Strain with deletion of *ATG5* initially (under normoxia) showed fragmented mitochondria. So, some unknown facts on the cross-talk between mitophagy and structure and dynamics of mitochondria were revealed. For identification a role of ER in mitophagy in yeasts, we investigated S.

cerevisiae mutants without proteins of the ERMES complex (endoplasmic reticulum–mitochondria encounter structure) Mmm1, Mdm10 and Mdm34. Some new data were obtained pointing to the impact of ERMES proteins on mitochondrial dynamics in yeasts and on interrelations between these processes and mitophagy and oxidative stress.

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Mitochondria-targeted antioxidant SkQ1 as a promising tool for suppression of Alzheimer's disease

N.G. Kolosova, M.A. Tyumentsev, N.A. Stefanova

Institute of Cytology and Genetics SB RAS, Novosibirsk, Russia.

E-mail: kolosova@bionet.nsc.ru

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Mitochondrial dysfunction is called the missing link between brain aging and Alzheimer's disease (AD), the most common type of senile dementia worldwide, but the impact of mitochondrial dysfunction (MD) on the transition from healthy aging to AD remains elusive. We explored impact of MD on the initiation or progression of pathological molecular cascades of AD using accelerated-senescence OXYS rats, which spontaneously develop all the major signs of AD and largely reproduce the stages of the disease. We conclude that MD appears to mediate or possibly even initiate pathological molecular cascades of AD-like pathology in OXYS rats. The key role of MD in the pathophysiology of AD we confirmed by the ability of mitochondria-targeted antioxidant SkQ1 to alleviate the neurodegenerative alterations via improvement of structural and functional state of mitochondria in OXYS rats. We have shown that supplementation with SkQ1 starting at the preclinical stage of AD-like pathology (at age 1.5 months) reduces the age-related alterations in behavior and the spatial memory deficit and slows down pathological accumulation of Aβ and tau protein hyperphosphorylation in 23-month-old OXYS rats. Later, we demonstrated that SkQ1 can delay progression of AD signs in OXYS rats starting from the disease stage that we can define as an analog of the predementia phase of AD in humans. Through improvement of the structural and functional state of mitochondria, treatment with SkQ1 from age 12 to 18months prevented the neuronal loss and synaptic damage, enhanced neurotrophic supply, and decreased Aβ1–42 peptide levels and tau hyperphosphorylation in the hippocampus of OXYS rats, thus improving the learning ability and memory. Moreover, we showed that SkQ1 can alleviate some signs of AD-like pathology in OXYS rats even at the stage of severe neurodegenerative damage. Prophylactic and therapeutic effects of SkQ1 in all cases

were associated with improvement of the mitochondrial apparatus. SkQ1 is an antioxidant and has been developed as such to counteract oxidative damage in mitochondria. One would expect that the effects of SkQ1 are mediated by the suppression of ROS production. Nevertheless, we did not detect enhanced production of ROS by brain mitochondria when the AD-like pathology developed and progressed, and well-pronounced structural disturbances in hippocampal mitochondria were observed in OXYS rats. We explored the mechanisms of the anti-AD effects of SkQ1 through deep RNA-seq and focused upon the cell-specific gene expression alterations in the hippocampus. OXYS rats had 1,159 differentially expressed genes (DEGs) relative to Wistar rats (control), and 6-month treatment with SkQ1 decreased their number twofold. We found that 10.5% of all DEGs in untreated (control) OXYS rats were associated with mitochondrial function, whereas SkQ1 eliminated differences in the expression of 76% of DEGs (93 from 122 genes). Using transcriptome approaches, we found that the anti-AD effects of SkQ1 are associated with an improvement of the activity of many signaling pathways and intracellular processes. SkQ1 changed the expression of genes in neuronal, glial, and endothelial cells, and these genes are related to mitochondrial function, neurotrophic and synaptic activity, calcium processes, immune and cerebrovascular systems, catabolism, degradation, and apoptosis. Thus, we assume that MD may be considered a predictor of the early development of the late-onset form of AD in humans and the repair of the mitochondrial apparatus by SkQ1 is a promising strategy to maintain brain health and to treat AD. A RSF (project 19-15-00044) and RFBR (project 18-015-00320) supported this work.

Variations in coloration in ants as a characteristic of the direction of natural selection

G.A. Shilovsky [1,2,3], T.S. Putyatina [2]

[1] Belozersky Institute of Physico-Chemical Biology;

[2] Faculty of Biology, Lomonosov Moscow State University, Moscow 119991, Russia; 3Institute for Information Transmission Problems, RAS, 127051 Moscow, Russia

E-mail: tsergput@gmail.com

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The variability of the trait serves as one of the important characteristics of its determinism. If the genetic component in the phenotypic variability of the trait is small (this is equivalent to low heritability), then it is the phenotypic plasticity and the width of the reaction norm that determine the adaptive capabilities of populations. The ants variability in coloring of the head and the alitrunk is used to distinguish species and intraspecific populations. In phenetics, an approach was developed that avoided subjectivity and provided easily comparable results (Gilev, 2002). This is the unification and cataloging of phenes, which is a necessary stage in population research. In this

work we studied the color variations of the head and three parts of the alitrunk of the ants *Formica exsecta* of the Kuzokotsky peninsula (Karelia, 66.5^0 N, 33.6^0 E). According to Dlussky (1967), from 3 to 11 classes (variants) of coloratio n were usually distinguished in Formica ants. The samples were compared using the phenotypic diversity (average number of phenotypes), the proportion of rare morphs, and phenotypic distances. In the most common (76.6%) variant of head coloration found in *F. exsecta*, the pigment occupies the entire occipital area and reaches the front edges of the eyes. There is also a variant of the spot on the forehead (20.6%) and in 2.8% of specimens the head is not pigmented. Among the coloration variants of the prothorax, the most common variant was Pn2 (85.6%) with one central spot. The next most frequent cases (14.4%) were Pn1 (lack of pigment, 7.2%), Pn3 (one large central spot and two smaller lateral spots, 3.4%), and Pn4 variant (so-called "crown", 3.8%). The complete pigment filling characteristic of species of the *F. rufa* group was found very rarely. The most common patterns for mesothorax are Mn1 (no pigment, 44.4%) and Mn4 (single dorsal spot, 38.3%). The next most prevalent (15.3%) was a new morph (Mn3) - a pigment collected in the form of a compact dark dot. Metathorax: the En1 variant is the most numerous - the complete absence of pigment in the propodeum (95.2%). Based on the proposed characteristics, a scheme of color morphs of *F. exsecta* is described. Analysis of the new scheme of coloring morphs showed that a shift towards lighter coloration is observed. Such non-invasive methods based on the assessment of color variability can be used to assess the speed and direction of selection. The proposed method can be useful for environmental and population studies, to compare the degree of relatedness between neighboring populations and in laboratory conditions to assess the effects of diet and other rearing conditions.

Gilev A.V. (2002) Zool. Zhurn. (in Russian).-V. 81.-№3.-P. 336-341.

Detection of progerin mRNA in peripheral blood, buccal epithelium, and human dermal fibroblasts

S.Y. Kurchashova[1], L.I. Kutueva[1], T.V. Gasanova[2], P.A. Ivanov[2], I.D. Strazhesko[3], V.V. Ashapkin[1], I.I. Kireev[1]

[1] Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow 119991, Russia;

[2] Department of Virology, Faculty of Biology, Lomonosov Moscow State University, Moscow 119234, Russia;

[3] Russian Clinical and Research Center of Gerontology, Pirogov Russian National Research Medical University, 129226, Moscow, Russia.

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The morphological and functional analysis does not allow estimating the degree of aging of the human cardiovascular system with necessary accuracy in the presence of one or more risk factors. Thus, searching for the markers that reflect the biological age remains relevant. Lamin A represents one of the structural components of the nuclear lamina – a fibrous structure underlying the inner nuclear membrane. Lamins define the structure, morphology and size of the nucleus. Lamin A plays an essential role in nuclear retention of proteins that interact with chromatin. Progerin, which is the product of aberrant splicing, incorporates into the lamina and changes the mechanical properties of the nuclear envelope. Numerous studies confirmed the age-associated accumulation of progerin in nuclei of cardiomyocytes and human fibroblasts. Since progerin affects the properties of nuclear lamins and proteins belonging to LINC complexes, we can suggest this protein to be one of the aging markers. In the present study, we have developed a system for the comparative evaluation of the amount of lamin A and progerin mRNA by quantitative PCR. Beta-actin and GAPDH were used as internal controls. The primers for progerin and lamin A detection were designed taking into consideration the peculiarities of splicing of mRNAs encoding both proteins. Each pair of primers contributed to the amplification of only one of the alternatively spliced variants of the lamin A gene. The expected lengths of progerin and lamin A-based PCR products were 222 bp and 361 bp respectively.

Total RNA was prepared from peripheral blood, leukocytes, dermal fibroblasts, and buccal epithelium cells with "Qiagen Mini Kit" (Germany) according to the manufacturer's protocol. Reverse transcription was carried out with RevertAid cDNA First-Strand Synthesis Kit ("Thermo Fisher Sci Inc.", USA). We used 100 ng of total RNA for each reaction. Then one µL of synthesized cDNA was used per a 25-µL PCR reaction. Real-time PCR was performed using a "DNA Technology" (Russia) equipment and the qPCR mix-HS SYBR+ROX kit ("Eurogene," Russia). PCR products were analyzed in 2% agarose gels to confirm the absence of cross-reactions. Lamin A and progerin product lengths coincided with predicted sizes. It was found that the amount of progerin mRNA was increased during the continued cultivation of dermal fibroblasts in cell culture. The cell aging appears to be accompanied by a gradual increase in splicing error rate, and the aberrant progerin transcripts may serve as a quantitive aging marker.

Dlussky G.M. (1967) Ants of the genus Formica (in Russian). Nauka, Moscow.

Naked mole rat mitochondria in skeletal muscles resemble those in cardiomyocytes

L.E. Bakeeva, V.B. Vays, I.M. Vangeli, C.M. Eldarov

A.N.Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow 119991, Russia

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The authors examined the ultrastructure of mitochondrial apparatus of *m. gracilis* and medial ventrum of *m. quadriceps femoris* of naked mole rats (*Heterocephalus glaber*) at the age of 1-2 weeks, 6 months, 5 years, 7 years and 11 years. The obtained results have demonstrated that the chondriome in skeletal muscles of naked mole rats aged < 5 years is not well-developed and represented by few separate small mitochondria. Mitochondrial reticulum is absent. Starting from the age of 5 years, a powerful mitochondrial structures are develop. By the age of 11 years, it become obvious that, the mitochondrial apparatus formed differs from that in the skeletal muscle of adult rats but resembles that of cardiomyocytes of rats or naked mole rats. From the age of 6 months to 11 years, percentage area occupied by of chondriome in the skeletal muscle of naked mole rat is increasing by almost five times (from 4.8 ± 0.4 % to 25.8 ± 3 % of the total size of muscle fiber). The growth of chondriome is mainly driven by increased number of organelles; the number of mitochondrial sections per 1 μm^2 of skeletal muscle fiber is increased from 0.23 ± 0.02 items/μm^2 to 0.75 ± 0.07 items/μm^2. Such significant growth of chondriome is not associated with any abnormal changes in mitochondrial ultrastructure.

We suppose that specific structure of mitochondrial apparatus developed in the skeletal muscle of naked mole rats by the age of 11 years is necessary for continual skeletal muscle activity of these small mammals burrowing very long holes in stony earth, resembling continual activity of heart muscle. In any case, ontogenesis of naked mole rat skeletal muscles is much slower than of rats (one more example of neoteny).

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Cryo-EM structure of Saccharomyces cerevisiae Respiratory Supercomplex composed of Complexes III and IV

E. Mileykovskaya[1], S. Azinas[1], M. Baker[2], V.K. Mallampalli[1], G. Fan[1], I. Serysheva[1], J. Nguyen[3], C.K. Chan[4], A. Singharoy[3], W. Dowhan[1]

[1] UTHealth McGovern Medical School,
[2] Baylor College of Medicine,
[3] Arizona State University,
[4] University of Illinois at Urbana Champaign;

E-mail: eugenia.mileykovskaya@uth.tmc.edu

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In our earlier work, we showed that both the assembling of yeast respiratory supercomplex (SC) consisting of one dimeric Complex III (CIII or III2, cytochrome bc1) and two Complexes IV (CIV, cytochrome c oxidase), and the channeling of cytochrome *c* between CIII and CIV within the SC are dependent on the mitochondrial phospholipid cardiolipin (CL). Recently, using electron cryo-microscopy (Cryo-EM), we determined a 3D structure of this SC at 4 Å resolution (FSC-gold standard) by employing a FEI Titan Krios microscope and cryo-SPARC software to generate the density map. The density map for CIV was generated by applying a soft mask around III2IVa and III2IVb for image processing. To build the SC structure, the individual subunits derived from the crystal structure of CIII (PDB: 3CX5) as well as models for the CIV subunits generated by SWISS- MODEL, were fitted using UCSF Chimera and Flex-EM into the density map followed by Phenix real-space refinement and model optimization in COOT. The dimeric CIII is flanked on each side by one CIV; the yeast CIV subunit Cox5a is located at the interface with CIII, and its matrix facing domain interacts with Cor1 subunit of CIII. The densities in the space between CIII and CIV can be fitted with molecules of CL confirming its involvement in association of CIII with CIV to form the SC. Our gentle method of SC purification (digitonin extraction followed by sucrose gradient centrifugation) yielded the SC with less than stoichiometric amounts of bound cytochrome *c* (verified by Western blot), permitting a low level of ubiquinol-oxidase activity by the SC to be registered without adding external cytochrome *c*. Importantly, an additional density in the vicinity of the Qcr6 subunit was observed, which hypothetically is due to cytochrome *c* adjacent to Qcr6 subunit near the CIII cytochrome *c* binding site. Molecular dynamics simulation for binding of the cytochrome *c* to our SC structure is in progress.

Notes