Organizers:
Institute of Mitoengineering
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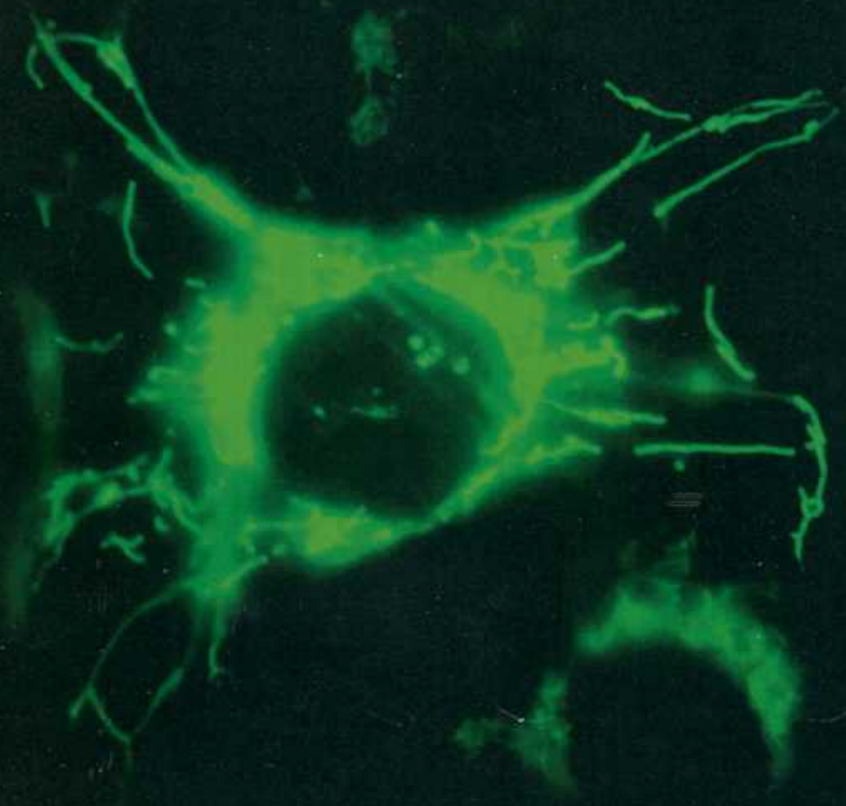
Moscow State University

From *Homo sapiens* to *Homo sapiens liberatus*

International Workshop, Moscow, MSU, May 25-26, 2010



Sponsor:
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International Workshop
"From *Homo sapiens* to *Homo sapiens liberatus*"

25 May
Conference Hall 221, Bldg. "B"

	9:15-9:45	Registration, coffee

9:45-12:00
Chairmen, G. Blobel, A. Olovnikov

V.P. Skulachev	9:45-10:00	*Introductory remarks*
V.P. Skulachev and M.V. Skulachev	10:00-10:45	*The present state of the art of the SkQ Megaproject*
	10:45-11:00	Discussion
B. Cannon	11:00-11:15	*Multiplicity of beneficial effects of mitochondrially targeted plastoquinone on ageing phenotype in premature ageing mice with increased mitochondrial DNA mutations*
	11:15-11:30	Discussion
J. Nedergaard	11:30-11:45	*Mitochondrial effects of prolonged treatment of mtDNA mutator mice with SkQ*
	11:45-12:00	Discussion
	12:00-12:30	Coffee break

12:30-14:30
Chairmen, B. Cannon, V.I. Kapelko

D.B. Zorov	12:30-12:45	*Effects of SkQs on oxidative stress-mediated injuries of kidney and brain*
	12:45-13:00	Discussion
B.V. Chernyak	13:00-13:15	*SkQ1 accelerates dermal wound healing in animals*
	13:15-13:30	Discussion
N.G. Kolosova	13:30-13:45	*Effects of SkQ on cataract and retinopathies in OXYS rats*
	13:45-14:00	Discussion
A.A. Zamyatnin	14:00-14:15	*Clinical trials of the SkQ1 drops in treatment of "dry eye"*
	14:15-14:30	Discussion
	14:30-15:45	Lunch

15:45-19:15

Chairmen, J. Nedergaard, E.I. Rogaev

V.N. Anisimov	15:45-16:00	*Effects of SkQ on lifespan and spontaneous carcinogenesis in female mice of three different strains*
	16:00-16:15	Discussion
F.F. Severin	16:15-16:30	*Two mechanisms of antioxidant activity of SkQs*
	16:30-16:45	Discussion
D.A. Cherepanov	16:45-17:00	*Possible role of the Complex III-bound cardiolipin dimer in initiation of mitochondrial lipid peroxidation*
	17:00-17:15	Discussion
S.A. Nedospasov	17:15-17:30	*Unexpected features in mice with mutant cytochrome c*
	17:30-17:45	Discussion
	17:45-18:15	Coffee break
J. Mitteldorf	18:15-18:30	*Darwinian Evolution is a Highly Evolved Process*
	18:30-18:45	Discussion
V. Gorbunova	18:45-19:00	*SIRT6 promotes DNA repair under stress by mono-ADP-ribosylating PARP1*
	19:00-19:15	Discussion

19:15-20:15

Chairmen, G. Libertini, L. Gavrilov

A. Seluanov	19:15-19:30	*Anticancer mechanisms in a longest-lived rodent, the naked mole-rat*
	19:30-19:45	Discussion
H.S. Saunders	19:45-20:00	*Non-senescence in classical evolutionary theory*
	20:00-20:15	Discussion
	20:15	Coffee break

26 May
Conference Hall 221, Bldg. "B"

	9:00-9:30	Coffee

9:30-12:00

Chairmen, C.J. Hauser, S.A. Nedospasov

K. Lewis	9:30-9:45	*Examples of prokaryotic genetic programs detrimental to individual cell*
	9:45-10:00	Discussion

N. Gavrilova	10:00-10:15	*Comparative analysis of parameters of human ontogenesis and senescence*
	10:15-10:30	Discussion
G. Libertini	10:30-10:45	*Oxidative damage and aging*
	10:45-11:00	Discussion
T. Goldsmith	11:00-11:15	*Rationale for complex programmed life span regulation in mammals*
	11:15-11:30	Discussion
	11:30-12:00	Coffee break

12:00-14:00

Chairmen, T. Goldsmith, D. Vinogradov

E.I. Rogaev	12:00-12:15	*Brain and aging*
	12:15-12:30	Discussion
C.J. Hauser	12:30-12:45	*Mitochondrial debris including mitochondrial DNA and formyl peptides that appear in the blood after major trauma can induce a syndrome resembling sepsis*
	12:45-13:00	Discussion
A.G. Ryazanov	13:00-13:15	*An enzyme whose inactivation delays aging and increases lifespan*
	13:15-13:30	Discussion
	13:30-14:00	General discussion
	14:00-15:00	Lunch
	15:00-16:00	Posters
Chairman, V.P. Skulachev	16:00-17:30	Discussion on initiation of the *Homo sapiens liberatus* (HSL) movement
G. Blobel	17:30-17:45	*Concluding remarks*
	19:00-22:00	Banquet at a Moscow river boat (starts near Radisson Royal Hotel, former "Ukraina" Hotel)

ORAL PRESENTATIONS

Mitochondrial-targeted rechargeable antioxidant SkQ inhibits the senescence program

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The crucial argument in favor of the concept of programmed senescence of the organism would be the switching off of senescence by a small molecule interrupting realization of such a program. As was shown by Lampert et al. [1], there is a correlation between the lifespan of 11 species of mammals and birds and production of reactive oxygen species (ROS) by energized mitochondria. However, a 12th species proved to be an exception. This was the naked mole-rat, a mouse-size rodent living almost 10 times longer than mice in spite of the fact that his mitochondria produce ROS faster than those of mice. According to Buffenstein, probability of the mole-rat's death is low and age-independent [2], and his cells *in vitro* could not be sent into apoptosis by adding H_20_2 [3].These and many other pieces of indirect evidence were summarized by a hypothesis considering senescence as the last step of the ontogenetic program, mediated by mitochondrial ROS and the ROS-induced apoptosis [4,5]. If this were the case, senescence could be switched off by lowering the mitochondrial ROS level. This might be done by a mitochondrial-targeted antioxidant.

To find such an antioxidant, we organized in 2005 an international project uniting several research groups in Russia, Sweden, USA, and Germany. As a result, it was shown that the SkQ-type antioxidants composed of (i) plastoquinone, (ii) a penetrating cation with delocalized charge and (iii) decane linker, meet two major requirements for a small molecule which inhibits the senescence program, i.e. (1) they prolong the lifespan of many organisms differing greatly in their systematic position and (2) they prevent age-linked decline of numerous quite different physiological functions. In the majority of experiments, a new compound synthesized in our group was used, namely, plastoquinonyl decyltriphenylphosphonium (SkQ1). It was found that SkQ1 easily penetrates into energized mitochondria, its antioxidant (reduced) form being regenerated from the oxidized form by center i of Complex III of the mitochondrial respiratory chain. In intact cells, a fluorescent SkQ derivative (SkQR1) is shown to specifically stain mitochondria. Taking into account (i) $\Delta\Psi$ values equal to 60 and 180 mV on the outer cell membrane and the inner mitochondrial membrane, respectively, and (ii) distribution coefficient in the octanol/water system of about 10^4, the SkQ concentration in the inner leaflet of the inner mitochondrial membrane is estimated to be about 10^8 times higher than in the extracellular aqueous solution [5-9]. In the following species, life-long treatment with SkQ1 resulted in an increase in lifespan: fungus *Podospora anserina*, crustacean *Ceriodaphnia affinis*, insect *Drosophila melanogaster*, fish *Nothobranchius furzeri*, and mammals (mice of various strains, dwarf hamster *Phodopus campbelli* and mole-vole *Ellobius talpinus*). In non-sterile animal houses or in outdoor cages, SkQ1 increased the lifespan of both males and females mainly due to prevention of age-linked decline of immunity [5,7,10,11]. In selected pathogen free (SPF) animal houses, the effect was specific for males [12]. Under any conditions used, both males and females showed decelerated development of many traits of senescence. Among them were osteoporosis, myeloid shift in blood cells, decline of wound healing, balding, cataract, retinopathies, some changes in behavior, appearance of β-galactosidase, phosphorylation of histone H2AX, and stimulation of apoptosis in skin fibroblasts. In females, SkQ1 prevented disappearance of estrual cycles with age [5,7,9,10,11,13]. In addition, 5 nmol SkQ1/kg per day inhibited development of lymphomas in p53$^{-/-}$ mice. A similar effect was produced by the conventional antioxidant N-acetyl cysteine at a million times higher concentration [5,14]. However, mammary carcinoma and some other tumors proved to be SkQ1-resistant [5,10]. This is why these cancers become the main reason for the death of SkQ1-treated mice.

In young animals, short term SkQ treatment was found to help in a number of experimental pathologies normally developing with age, such as heart attack and arrhythmia, stroke, kidney infarction, and glaucoma [5,7,13,15]. In the progeric "mutator" mice lacking proofreading activity of DNA polymerase γ, SkQ1 prolonged the healthy lifespan [12]. In OXYS rats, also showing accelerated senescence, SkQ1 inhibited accumulation of oxidized of lipids and proteins in muscles, prevented development of cataract and retinopathies, and reversed these diseases when drops of 250 nM SkQ1 were instilled into eyes of old animals. Similar effect was found in dogs, cats, and horses [13]. Clinical trials of such drops already started

in February 2010 in two ophthalmological hospitals of Moscow. Stable forms of SkQ1 and SkQR1 applicable for preparation of drugs for *per os* administration have been developed. Clinical trials of these drugs are now in preparation. At the same time, the molecular mechanisms of the effect of SkQ are now under investigation. Special attention will be paid to its role in preventing of oxidation of cardiolipin dimers in Complex III [15,16].

References:
[1] A.J. Lamber et al., *Aging Cell*, 6, 607-618, 2007
[2] R. Buffenstein, *J. Gerontol. Biol. Sci.*, 60, 1369-1377, 2005
[3] N. Labovsky et al., *Am. J. Physiol. Heart Circ. Physiol.*, 291, 42698-42704, 2006
[4] V.P. Skulachev, in: T. Nystrom, H.D. Osiewacz (Eds.), Topics in Current Genetics, 3, Model Systems in Ageing. Springer-Verlag, Berlin-Heidelberg, 191-238, 2003
[5] V.P. Skulachev et al., *Biochim. Biophys. Acta*, 1787, 437-461, 2009
[6] Y.N. Antonenko et al., *Biochemistry (Mosc.)*, 73, 1273-1287, 2008
[7] M.V. Skulachev et al., *Curr. Drug Targ.*, 2010, accepted
[8] F.F. Severin et al., *Proc. Nat. Acad. USA*, 107, 663-668, 2010
[9] V.P. Skulachev et al., *Biochim. Biophys. Acta*, 2010, doi: 10.1016/j.bbabio.2010.03.015.
[10] V.N. Anisimov et al., *Biochemistry (Mosc.)*, 73, 1329-1342, 2008
[11] L.A. Obukhova et al., *Aging*, 1, 389-401, 2009
[12] I.G. Shabalina et al., *in preparation*
[13] V.V. Neroev et al., *Biochemistry (Mosc.)*, 73, 1317-1328, 2008
[14] L.S. Agapova et al., *Biochemistry (Mosc.)*, 73, 1300-1316, 2008
[15] L.E. Bakeeva et al., *Biochemistry (Mosc.)*, 73, 1288-1299, 2008
[16] W. Longo, J. Mitteldorf, V.P. Skulachev, *Nat. Genet. Rev.*, 6, 866-872, 2005

Multiplicity of beneficial effects of mitochondrially targeted plastoquinone on ageing phenotype in premature ageing mice with increased mitochondrial DNA mutations

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MtDNA mutator mice expressing mtDNA polymerase with reduced proof-reading activity exhibit several features of premature ageing, such as reduced lifespan, weight loss, reduced fat content, manifestation of alopecia, kyphosis and osteoporosis, anemia and reduced fertility (Trifunovic et al., 2004). A new type of mitochondrially targeted compounds (SkQs) - consisting of plastoquinone (an antioxidant moiety), triphenylphosphonium (a penetrating cation), and a decane linker - have been suggested as potential tools for treatment of senescence and age-related diseases (Skulachev et al., 2009). We have treated mtDNA mutator and wild type mice with SkQ1 added to the drinking water (0.7 - 1.0 μmol/day x kg body weight).Treated mice exhibit delayed appearance of traits of ageing phenotype such as lordokyphosis, baldness, alopecia, lowering of body temperature, torpor, body weight loss and reduced fat content. SkQ1 treatment also increased the number of estruses and the regularity of the estrous cycle in mtDNA mutator females. Notably mtDNA mutator mice treated with SkQ1 lived significantly longer than untreated littermates. However, the gross changes observed at necropsy and the main histopathological findings in tissues were identical in treated or untreated mtDNA mutator mice at death. At the end of life, the mtDNA mutator mice exhibited severe anaemia and significant leucopenia, which was not improved by SkQ1 treatment. Thus, we here demonstrate a complex of beneficial changes in ageing phenotypes in mtDNA mutator mice chronically

treated with mitochondrially targeted plastoquinone that may be therefore be suggested as a promising pharmacological treatment for premature ageing and mitochondrial diseases.

Mitochondrial effects of prolonged treatment of mtDNA mutator mice with SkQ

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We have demonstrated that chronic treatment of mtDNA mutator mice with drinking water containing SkQ1 increases their lifespan and causes delayed and less manifest appearance of ageing traits. To explore the molecular mechanism behind these improvements, we have here studied mitochondrial effects of SkQ1.The oxygen consumption rate of skeletal muscle mitochondria isolated from mtDNA mutator mice was analysed. ADP-stimulated respiration was increased in SkQ1-treated mtDNA mutator mice. Consistently with this improved function of skeletal muscle mitochondria isolated from treated animals, a restored hydrogen peroxide production rate (stimulated by a complex II substrate (succinate) or by mixed complex I and complex II substrates (pyruvate + malate + succinate)) was observed after treatment with SkQ1. Interestingly, SkQ1 treatment increased the concentration of the mitochondrial marker VDAC in skeletal muscle homogenates. Thus, SkQ1 treatment improved the function and increased the number of mitochondria in skeletal muscle. Similarly, an increased VDAC concentration per mg of brown adipose tissue (BAT) indicated an increased number of mitochondria in BAT from SkQ1-treated mice. There was a significant positive effect of SkQ treatment on interscapular BAT mass and on total protein content in BAT. Estimation of the total content of the thermogenic protein UCP1 and of VDAC per mouse implied a significant improvement in BAT thermogenic potential. The positive effects of *in vivo* treatment with SkQ1 could also be attributed to its antioxidant properties, revealed as a delayed spontaneous formation of MDA (in all 3 tissues examined: liver, kidney, brain). A lowered content of endogenously formed HNE-adducts was observed in the kidney of treated animals (in liver and brain, the content of HNE-adducts was not different between treated and untreated mice). A decreased cardiolipin content, with markedly altered fatty acid composition, in mitochondria from mtDNA mutator mice was normalised after treatment with SkQ1. These features may at least in part explain the beneficial effects of SkQ1 in-vivo. Thus, mitochondrially targeted plastoquinone may be suggested as a pharmacological treatment for premature ageing and mitochondrial diseases.

Effects of SkQs on oxidative stress-mediated injuries of kidney and brain

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Mitochondria-targeted drugs were designed to serve a tool for specific delivery to mitochondrial matrix of compounds capable to compensate the defects resulting from inherited or acquired mitochondrial malfunctioning. SkQR1 belonging to the family of mitochondria-targeted antioxidants carries rhodamine residue as a driving vehicle for direct delivery of plastoquinone moiety to the mitochondrial interior. This chimeric compound demonstrated apparent protective properties in different models of tissue pathologies with oxidative stress being involved. Its efficiency was proved in a model of kidney ischemia/reperfusion and rhabdomyolysis. In the first model, protection was demonstrated as after injection of SkQR1 prior

ischemic insult (when normalization of blood creatine and urea and survival in single-kidney animals were observed) as well as after injection in postischemic period (when almost all single-kidney animals subjected to ischemic insult survived).Two models of rhabdomyolysis were used. Firstly, the toxicity of myoglobin was studied in the culture of renal tubular cells after acute exposure. The latter induced elevation of reactive oxygen species level in cellular mitochondria with SkQR1 having inhibitory effect. Secondly, after intramuscular injection of glycerol and limitation of water access, experimental animals developed myoglobinurea with apparent signs of kidney dysfunction correlated with oxidative stress in the renal tissue. One essential indicator of developed rhabdomyolysis was an appearance of cytochrome c in the blood stream. Injection of SkQR1 significantly restored kidney functioning in rhabdomyolitic rats and decreased both the level of lipid peroxidation in renal tissue and blood cytochrome c. We found that a single injection of SkQR1 to the rat induced production of erythropoietin in the total kidney tissue and cultural kidney cells and caused elevation of phosphorylated form of glycogen synthase kinase in the renal tissue. Significant, although smaller protection was observed after injection of a SkQR1 derivative deprived of plastoquinone moiety. This compound induced some normalization of ischemic and rhabdomyolitic kidney functioning while not having any effect on the level of erythropoietin in the kidney. Protective effect of both SkQR1 and its quinone-free form was demonstrated in a model of focal brain ischemia although the second form demonstrated limited protective properties. Single i/p injection of SkQR1 diminished the size of the ischemic zone in the brain and improved performance of a test characterizing neurological deficit in ischemic animals. The study of the role of kidney in the protection of ischemic brain demonstrated significant contribution of erythropoietin produced by the kidney in protective mechanisms developed in the brain although there is some although limited endogenous production of erythropoietin in the brain cells. The SkQR1-mediated protection of the brain also goes with the involvement of glycogen synthase kinase activity. We conclude that SkQR1 affords the protection of kidney and brain tissue against oxidative stress-related pathologies with multiple mechanisms of both direct antioxidative effects involved as well as with involvement of induction of protective mechanisms in order to amplify signaling pathways and better guarantee the global and maximally effective mitochondria, cell, organ and organism defense.

SkQ1 accelerates dermal wound healing in animals

To *Homo sapiens liberatus et invulnerabilis*

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It was shown that the novel mitochondria-targeted antioxidant SkQ1, (10-(6'-plastoquinonyl) decyltriphenylphosphonium) stimulated healing of full-thickness dermal wounds in mice and rats. Treatment with nanomolar doses of SkQ1 in various formulations accelerated wound cleaning and suppressed neutrophil infiltration at the early (7 h) steps of inflammatory phase. The effect was observed when rats were daily injected subcutaneously around the wound with 200 nM SkQ1 solution (0.05 nmol/kg) or the wounds were covered with the synthetic fil contained SkQ1 (0.019 μg/g). Local treatment with SkQ1 stimulated formation of granulation tissue and increased the content of myofibroblasts in the beginning of regenerative phase of wound healing. At the later steps SkQ1 accelerated accumulation of collagen fibers produced by myofibroblasts and re-epithelization of the wound. Lifelong treatment of mice with 30 nmol SkQ1/kg per day supplemented with drinking water strongly stimulated wound healing in old (28 months) animals. The similar effect was observed if SkQ1 was supplied only during the last 4 months. In the *in vitro* model of wound in human cell cultures SkQ1 stimulated movement of epitheliocytes and fibroblasts into the "wound". Myofibroblast differentiation of subcutaneous fibroblasts was stimulated by SkQ1. It is suggested that SkQ1

stimulated wound healing by suppression of the negative effects of oxidative stress in the wound and also by induction of the cell differentiation.

Effects of SkQ on cataract and retinopathies in OXYS rats

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Age-related macular degeneration (AMD) and cataracts are common causes of blindness in the elderly. The number of persons with vision loss from AMD is expected to increase dramatically during the next few decades. Cataract remains the most common successfully treated by surgery of blindness but has a large impact on healthcare budget. Some pieces of evidence suggest a stimulating effect of cataract surgery on the progression of early AMD. Therefore, creation of effective anticataract therapeutic and prophylactic agents is greatly needed. We found that senescence-accelerated OXYS rats are useful animal model for study of pathogenesis and design of new therapeutic targets of AMD and cataract since it reproduces the clinical and morphological manifestation of pathologies and positively responses to standard therapies. Development of retinopathy in OXYS rats, like humans, is associated with changes in gene expression of vascular endothelial growth factor (VEGF, an angiogenic factor) and pigment epithelium-derived factor (PEDF, an antiangiogenic factor). VEGF and PEDF are produced by retinal pigmented epithelial cells (RPE) and play critical role in vascular homeostasis in retina. Cataracts development in OXYS rats, again like in humans, is associated with reduced expression genes of α-crystallins in the lens epithelium. Using OXYS rats, we have conducted a study on therapeutic potential of mitochondria-targeted antioxidant SkQ1 for treatment of cataract and AMD. According to our data, addition of SkQ1 to the food completely prevents development of cataract and retinopathy in OXYS rats as well as decelerates the age-dependent decline of the immune system. SkQl is effective also in the drops being competent not only in preventing but also in reversal of already developed pathological changes of retina and lens in OXYS rats. SkQ1 effects were associated with improvement of gene expression: α-crystallins in the epithelial cells of lens and VEGF in RPE. Action of SkQ1 was accompanied with preservation of intact structure of choroidal vessels and retinal pigment epithelium in OXYS rats.

Clinical trials of the SkQ1 drops in treatment of 'dry eye'

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Pilot clinical trials (phase I-II) of SkQ1-based eye drops were finished recently. A total of 80 subjects with dry eye disease were involved in the open comparative study in two top-rated Moscow ophthalmological hospitals. The first aim of the study was to confirm the safety of the formula during 21 day course. The second aim was to compare the efficacy of SkQ1-based eye drops with 'Tears Naturale', Alcon in the 21 day therapy of the dry eye syndrome (light form).
Safety data were obtained. No adverse events were detected during the whole period of the study. Obtained data on the efficacy of SkQ1-based eye drops on 40 subjects (80 eyes) with dry eye disease (light form) showed statistically significant positive dynamics of all values commonly measured for the diagnosis of dry eye. These tests include Schirmer test, thickness of the tear film, and tear break-up time. Further perspectives of SkQ1-based eye drops in therapy of ocular diseases will be discussed.

Effects of SkQl on life span and spontaneous carcinogenesis in female mice of three various strains

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According to the Free Radical theory, the oxidative damages to lipids, proteins and nucleic acids increase with age, explaining the mechanism of ageing and age-related processes including cancer. The effect of mitochondria-targeted antioxidant SkQ1 on longevity and spontaneous carcinogenesis had been studied in outbred SHR, inbred 129/Sv and transgenic HER-2/neu female mice. Animals were treated with SkQ1 (0.5 - 2,500 nmol/kg/day) added to the drinking water. Age-related dynamics of the body weight and temperature, the amount of drinking water and consumed food, estrous function as well as parameters of the life span and carcinogenesis were measured.
As compared with controls, no difference in body weight or amount of consumed food and water in all mice strains was found. In SHR mice living in an old non-sterile animal house, SkQ1 treatment increased median life span (by 92% at the optimal dose of 5 nmol), maximal life span (by 6% at the dose of 50 nM) and mean life span by 32%, 45% and 35% in 0.5, 5 and 50 nmol SkQ1 groups respectively ($p<0.05$). In a new animal house, the lifespan of SHR females without SkQ1 was as long as with the optimal SkQ1 in the old animal house and SkQ1 additions failed to induce further lifespan increase. However, SkQ1 prevented the age-dependent disappearance of estrous cycles in SHR mice kept in either old or new animal house. In females of long-lived 129/Sv mice 250 nmol SkQ1 does not change the median lifespan but increased the maximal lifespan by 64 days. No effect of SkQ1 on parameters of lifespan in short-lived HER-2/neu mice dying because of mammary carcinoma was revealed. There was no significant difference in incidence of tumors in SkQ1-treated SHR mice as compared with the control. The tendency to the decrease of uterine, ovarian and liver tumors incidence has been observed in 129/Sv mice exposed to 5 nmol SkQ1.The treatment with 250 nmol of SkQ1 did not influence number of tumor incidences in this strain. The drug had no effect on mammary carcinogenesis in transgenic HER-2/neu. The treatment with SkQ1 significantly ($p<0.05$) inhibited the incidence of age-associated non-tumor pathologies of SHR mice. These pathologies (various infection diseases) were the main reason of death in the old animal house. Our data suggest geroprotective activity of SkQ1 and safety of its long term use.

Two mechanisms of antioxidant activity of SkQs

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SkQ-type molecules consist of plastoquinone and positively charged hydrophobic "tail". SkQs display two basic activities: they deliver the rechargeable antioxidant to mitochondria and they also act as mitochondrially-targeted protonophores. The latter activity depends on the non-quinone part and is self-inhibitory ("mild") because the molecules are positively-charged. The hydrophobic "tails" of SkQs can transfer protons across membranes in two ways. It was found that the molecules can catalyze the free fatty acid–driven proton flux across the membranes. At least one molecule of this type, C12-Rh19, can also act as a cationic protonophore, i.e. to cross membranes in both neutral and charged (protonated) forms. As both hyper-polarization and deep depolarization of mitochondria were shown to cause reactive oxygen species (ROS) accumulation, mild protonophoric activity of SkQ may contribute to its antioxidant properties. To explore this, we studied the antioxidant effects of the hydrophobic tails *in vitro* and *in vivo*. It was found that both the conventional protonophore (FCCP) and C12-Rh19 improve the survival in the model of yeast cell death driven by mitochondrial hyperpolarization. As expected, the concentration window for C12-Rh19 was much (approximately twenty times) wider than for FCCP.
There are established experimental models of death of rats which are mediated by mitochondrial ROS accumulation, e.g. the ones caused by infarctions of kidney or brain. It was found that SkQ-Rh19 significantly improves the survival in these models. Here we show that the non-quinone part of this molecule (C12-Rh19) also active as the death protector: its effect is approximately two-fold lower than that of SkQ-Rh19.

Importantly, the quinone moiety of SkQs can act as pro-oxidant when overdosed. Therefore, hydrophobic tails of SkQs are medically-promising antioxidant compounds. It is tempting to speculate that they also can be used as mimetics of caloric restriction.

Possible role of the Complex III - bound cardiolipin dimer in initiation of mitochondrial lipid peroxidation

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The bottleneck of chain free-radical oxidation of lipid membranes is the hydrogen atom transfer in the course of radical propagation reaction

$LOO^{\bullet} + LH \rightarrow LOOH + L^{\bullet}$ (1)

It is a slow process, for polyunsaturated linoleate acid its rate constant is as small as 60 $M^{-1}s^{-1}$. The mitochondrial membrane contains a large amount of native antioxidant ubiquinone, the rate constant of its action

$LOO^{\bullet} + UQH_2 \rightarrow LOOH + UQH^{\bullet}$ (2)

is as high as $3x10^5$ $M^{-1}s^{-1}$. Because the concentration of ubiquinone in mitochondrial membrane is comparable with the concentration of polyunsaturated lipid chains, ubiquinone protects the peroxidation of lipids with high efficiency.

The main source of polyunsaturated hydrocarbon chains in the inner mitochondrial membrane is cardiolipin (CL), one molecule of CL carries usually four linoleate chains (eight unsaturated bonds). A large fraction of CL in the inner mitochondrial membrane is not free but tightly bound to various proteins. We analyzed molecular structures of membrane protein complexes containing CL, namely, ADP/ATP carrier, II complex (succinate dehydrogenase), III complex (cytochrome bc1 complex), IV complex (cytochrome c oxidase), and formate dehydrogenase. In all cases, with the exception of III complex, CL is bound at the protein/membrane interface where it is easily reached for the direct interaction with ubiquinone in the lipid phase. The exception is the structure of III complex, where the CL binding site is deeply buried into proteins in the vicinity of the Qi site. In the structure of bovine cytochrome bc1 complex (1PP9), the site contains two CL molecules and one molecule of phosphatidilcholine, whereas in the structure of yeast enzyme (3CX5), the site contains one CL molecule and one molecule of phosphatidilcholine. An essential difference in the two structures is the accessibility of the interior of CL binding sites for the external lipid phase. In the case of bovine enzyme, the binding site is completely covered by protein and represents a well, where the bound CL is protected from the direct interaction with the lipid phase. To the contrary, in the structure of yeast enzyme, the binding site is open and CL is reached for the interaction with ubiquinone diffusing in the lipid phase.

Cytochrome bc1 complex is a major producer of superoxide radical in the mitochondrial membrane, the radicals are generated at the Qi site just in the vicinity of the CL binding site. Some features of the CL binding site in the bovine enzyme point out that it might function as an initiator of the mitochondrial membrane oxidation: (1) eight linoleate chains are tightly packed in the site so that the local concentration of polyunsaturated bonds is an order of magnitude higher than that in the lipid phase on the average (increased oxidation rate); (2) the chains of bound CL could not directly interact with ubiquinone coming from the lipid phase (decreased antioxidant protection). As a consequence, appearance of the first peroxide radical in the CL binding site would result in a fast oxidation of all eight linoleate chains to their peroxide derivatives. It is noteworthy, that in yeast mitochondria such function of the CL binding site is apparently missing, that correlates with the absence of polyunsaturated acids in yeast membranes.

Oxidation of CL in the binding site of the bc1 complex might induce some conformational changes, which could increase the superoxide production by III complex. In such a case peroxidation of CL in III complex is the primary trigger of apoptotic cascade coordinated by mitochondria.

Unexpected features in mice with mutant cytochrome c

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Cytochrome c released from mitochondria is critical for apoptosome-dependent pathway of the programmed cell death. Because of its critical involvement also in electron transport, mice lacking this gene and gene product die at embryonic stage. Certain mutations in cytochrome c, including K72 position, may dissociate electron transfer functions of this protein from proapoptotic one (e.g., in the K72W mutant, the latter is inhibited while the former remains intact). Using Cre-LoxP and Flp/Frt technologies, we have engineered mice with K72W mutation introduced into the germline in a conditional fashion. In particular, we have generated mice with homozygous K72W mutation in all cells. Although fraction of these mice displays anatomical abnormalities and die early, some mice are grossly normal and can live up to 1.5 years. Cells isolated from these mice show partial deficiency in apoptotic responses *in vitro*. *In vivo* studies demonstrated some inhibition of response of organism to bacterial lipopolysaccharide. Moreover, abnormalities certain in brain/behavioral functions, were revealed. Overall, this study revealed both expected and unexpected consequences of cytochrome c mutation.

Darwinian Evolution is a Highly Evolved Process

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The 'Modern Synthesis; or 'Population Genetics' has been the standard quantitative version of Darwin's theory for eighty years. A foundational hypothesis of the theory is that the primary target of natural selection is immediate reproductive success. Indeed, the standard population genetic definition of fitness refers to an increase in gene frequency from one generation to the next. But there is diverse evidence that this hypothesis is false. The most compelling evidence comes from the phenomenology of aging, which detracts strongly from individual fitness, and from the implementation of genetic exchange via separate sexes, which costs a full factor of two in individual fitness. How can it be that natural selection can strongly oppose individual reproductive fitness? The resolution of this paradox begins with an appreciation of population dynamics. Dependence on a common food pool ties together the fate of a population community, and creates a powerful selective force for restrained reproduction in order to avoid unstable population cycling and local extinctions. The full resolution involves appreciation of evolution as a highly-evolved process. Natural selection in the short term has been shaped by natural selection in the long term, so that dead ends such as unrestrained reproduction are consistently avoided. Programmed death and sexual reproduction, among other less obvious features of the biosphere, exist for the purpose of enhancing evolvability.

SIRT6 promotes DNA repair under stress by mono-ADP-ribosylating PARP1

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SIRT6 is a mammalian homolog of the yeast Sir2 deacetylase that promotes longevity in yeast and invertebrates. Mice deficient for SIRT6 exhibit premature aging and genome instability. However, the mechanisms of SIRT6 function in genome maintenance and lifespan regulation are unclear. Here we show that in mammalian cells subjected to oxidative stress SIRT6 is recruited to the sites of DNA double-strand breaks (DSBs) and strongly stimulates DSB repair. SIRT6 physically associates with PARP1 and mono-ADP-ribosylates it, leading to stimulation of PARP1 poly-ADP-ribose polymerase activity. Our results suggest that SIRT6 promotes genome stability by stimulating PARP1 and enhancing DSB repair under oxidative stress. We propose that SIRT6 functions as a regulator integrating oxidative stress signaling and

DNA damage response. The theory of hormesis proposes that mild doses of stress may have beneficial effects on the organism by stimulating stress and survival pathways. This theory has been explored extensively by biogerontologists, and was also used to explain life extending effects of food restriction. We hypothesize that SIRT6 serves as a mediator of hormetic response, promoting longevity by stimulating DNA repair under stressful conditions.

Anticancer mechanisms in a longest-lived rodent, the naked mole-rat

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The naked mole-rat is the longest living rodent with a maximum lifespan exceeding 28 years. In addition to its longevity, naked mole-rats have an extraordinary resistance to cancer as tumors have never been observed in these rodents. Furthermore, we demonstrated that naked mole-rat fibroblasts require more "hits" for malignant transformation than the mouse cells. Interestingly, naked mole-rat cells constitutively express telomerase and do not become senescent in culture. Replicative senescence is an important anticancer mechanisms that limits cell proliferation. The presence of active telomerase may provide an advantage by allowing for better cell renewal in the naked mole-rat tissues, but on the other hand, active telomerase is often associated with tumorigenesis. These observations make the cancer resistance of naked mole-rats even more intriguing, and suggest that naked mole-rats evolved alternative telomere-independent tumor-suppressor mechanisms. We identified one such mechanism, termed early contact inhibition (ECI). Contact inhibition is a key anticancer mechanism that arrests cell division when cells reach a high density. In cell culture, naked mole-rat fibroblasts arrest at a much lower density than those from a mouse. We demonstrate that early contact inhibition requires the activity of p53 and pRb tumor suppressor pathways, and is associated with upregulation of p16INK4A. Recently, we identified that ESI is triggered by an extracellular signal, a very high molecular weight hyaluronic acid (HA) secreted by naked mole-rat cells. Cancer-prone mouse models are valuable for development of cancer treatments. However, to find ways to prevent cancer before it occurs it would be extremely useful to study cancer-resistant models such as the naked mole-rat. We anticipate that these unusual rodents have evolved multiple novel anticancer adaptations which would pave the way for development of novel therapies for cancer treatment and prevention.

Non-senescence in Classical Evolutionary Theory

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Traditional statements of the classical evolutionary theory of senescence claim that senescence is a virtually inevitable result of the fact that genes affecting survival or fecundity only early in life have a greater selective impact than genes that express only late in life.
However, it can easily be shown that the standard theory allows for non-senescent genotypes to dominate and displace senescent genotypes under a variety of conditions. This presentation will show quantitative modeling results demonstrating the dominance of non-senescent genotypes across specific domains of certain inputs used in standard theories, namely fecundity rates, mutation rates, and fidelity and accuracy of breeding.
The quantitative model, a dynamic systems model, adheres to exacting theoretical standards, depicting different genotypes within a population exhibiting different tradeoffs between intrinsic lifespan and fecundity, and permits the accumulation of mutations. The use of dynamic systems theory allows the extraction of system eigenvectors and eigenvalues that provide a clear picture of genotype structure and enables compact extensions of the classical Euler-Lotka equation and its derivatives. This methodology also provides insight into the field studies of Reznick et al. on predator-mitigated senescence in Trinidadian guppies, *Poecilia Reticulata*. It further generates predictions conforming to standard theory with respect to

population fecundity trends with age, population mortality trends with age, Williams' Hypothesis, and the evolution of semelparity and iteroparity.
The significance of these results is that standard theory does not disallow the evolution of non-senescent populations.

Examples of prokaryotic genetic programs detrimental to individual cell

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Programs that replicate more efficiently are selected for, and this also applies to cases when the program appears to be counter-productive, at least to an individual. We then consider how such individually counterproductive programs may benefit a population.
Perhaps the most wide-spread program that limits the reproduction of individual cells is one that leads to the formation of dormant persister cells. In all bacterial species, a small subpopulation of persisters plays the role of a safeguard against threats. Persisters are formed in response to increased expression of toxin/antitoxin proteins such as HipA, a kinase of Ef-Tu, orTisB, a membrane-acting peptide. Persisters are highly tolerant to antibiotics and other lethal factors, but this comes at a price of forfeiting propagation. In the case of persisters, at least in the presence of a lethal factor, there is direct individual benefit. This is not so obvious in a seemingly bizarre strategy of "unculturable" microorganisms that actually make up the vast majority, 99% of all bacterial species. Unculturable bacteria in a marine sediment biofilm evolved to lose their genes for making siderophores, iron chelators. They now depend stringently on the presence of neighboring culturable bacteria. Their growth is then restricted to familiar environments, and they have lost the ability to colonize new territory.
Programmed cell death is one of the more striking examples of counter-productive behavior, and in bacteria, formation of a fruiting body by Myxococcus depends on the lysis of members of the community, which provides nutrients to the rest of the population. Apart from this isolated example, a more widespread PCD occurs during formation of biofilms by a number of species, where cells lyse and release DNA, which serves to physically bind together and strengthen the community. Finally, PCD occurs during DNA damage, when expression of TisB causes part of the population to differentiate into persisters, and the same TisB peptide kills about half of the cells. It appears that cells which are less fit are not able to sustain damage caused by TisB and dye, a possible case of phenoptosis aimed at clearing the population of individuals that will ultimately be unable to repair, and will only draw resources from their healthier kin.

Comparative analysis of parameters of human ontogenesis and senescence

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The idea of this study is to compare standard deviations for parameters, which are known to be determined by the developmental program (such as ages of sexual maturity) with variation of characteristics related to aging (such as menopause and death).
One of the arguments used by the opponents of programmed aging is a too high variation in individual lifespans compared to the observed variation of programmed events (such as the age of sexual maturation). The main objective of this study is to test the validity of this argument.
Presentation provides the first results on this topic of scientific studies. In particular, data available in the scientific literature on variability of ages at sexual maturation (menarche), menopause and death are compared to results obtained from the nationally representative survey of adult population of the United States (MIDUS) as well as official life table data.
It is shown that standard deviations for age at onset of menarche are about 10 times smaller than standard deviations for ages of death. Such a difference corresponds well to a difference in mean values of ages when

menarche and death occur. Thus, the adjusted variability (coefficient of variation) for age at death is of a similar order of magnitude as that for ages at onset of menarche.

Oxidative Damage and Aging

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The risk of cardiovascular diseases is positively related to hypercholesterolemia, hypertension, diabetes, smoking, age, etc. and lowered by preventive lifestyle measures and by anti-hypertensive, hypoglycaemic and anti-dislipidemic drugs.
The common interpretation is that modifiable risk factors increase oxidative damage while preventive lifestyle measures and lowering-risk-factors drugs reduce this harm. Moreover, aging, interpreted as consequence of cumulative oxidative damage, is necessarily the cause of age-related cardiovascular increasing risks and is not modifiable with preventive measures and drugs.
Statins, ace-ACE-inhibitors and sartans ("protective drugs") are known to be effective in reducing the cardiovascular risk even without acting on risk factors, namely with a direct action on atherogenesis but this is compatible with the above-said general interpretation.
These ideas are challenged by the observation that the number of circulating endothelial progenitor cells (EPC) is negatively related to the cardiovascular risk and to the increasing age and that the intake of protective drugs is associated with higher values of EPC.
A likely deduction is that: 1) Excessive stress (oxidative or of other types) increases the apoptotic rate of endothelial cells (which show continuous cell turnover ensured by EPC) and quickens their turnover, so lowering renewal capacities and reducing EPC count; 2) Older endothelial cells, which suffer by cell senescence, increase the probability of atherosclerosis; 3) In old individuals, with or without excessive stress, EPC are reduced because of EPC stem cell exhaustion by telomere shortening: diseases derived from compromised blood circulation are a common end to the life of healthy old individuals with no particular risk factor.
In short, oxidative damage is important in atherogenic process and in aging but the key actor is the progressive failure of cell turnover caused by cell duplication limits, which are determined by the genetic regulation of telomere-telomerase system.
The scheme proposed for endothelial cells and atherogenesis is likely valid for other organs and tissues and for the whole organism.
This stimulates a general view where: I) Organism shows a continuous renewal of its cells; II) Aging is the consequence of the progressive slackening of this turnover and can be described as the progressive atrophy of each tissue and organ; III) Many diseases are the effect of the acceleration of the physiologic turnover of some cell types and the consequent exhaustion of their renewal capacities; IV) Many risk factors and many drugs contrasting these factors act by increasing or reducing, respectively, the turnover acceleration.
However, a well-founded objection needs a sound justification: some cells or tissues (as muscle and heart myocytes, eye crystalline lens, photoreceptor cells and neurons of central nervous system) appear to have no turnover and so should not be included in this scheme, thus greatly weakening it.
But: A) Muscle and heart myocytes are cells with turnover; B) The functionality of crystalline lens depends on lens epithelial cells that show turnover; C) Photoreceptor cells, particularly exposed to oxidative damage, and neurons of central nervous system, which have high metabolic activity, both depend from specialized types of gliocytes that show turnover. Turnover decline of these cells is a likely cause of age-related macular degeneration (ARMD) and of Alzheimer disease (AD), respectively; D) Smoking, diabetes, and obesity are risk factors for these diseases while "protective drugs" lower the risk.
Cures for ARMD and for AD that try to contrast ARMD and AD by lowering the oxidative damage or reducing the accumulation of metabolic substances result ineffective. A rational cure should contrast the decline of gliocyte turnover by the activation of telomerase, a possibility that is well documented *in vitro* by important experiments.
This type of cures for ARMD and AD would be very important *per se* but would be as much important in a more general perspective: ARMD and AD are the pivotal expression of aging for the nervous system and the control of these diseases would be an important step in the control of aging.

Rationale for Complex Programmed Life Span Regulation in Mammals

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Arguments are presented suggesting that aging in mammals is the result of a complex life span regulation system that evolved because life span limitation produces direct evolutionary benefit. This concept, if valid and pursued, could have a significant effect on efforts to combat aging processes by providing additional targets for potential intervention.
A complex life span regulation system implementing purposely programmed (adaptive) aging provides a better match to experimental evidence than the more popular non-programmed theories. The primary objection has historically been that adaptive aging is "impossible" because it is not supported by the mechanisms of the evolution process. This argument was once generally accepted. However, more recently a number of alternatives to classical evolutionary mechanics theory have been proposed that support purposely programmed aging. These alternatives were developed in response to observed issues other than aging and include group selection, kin selection, evolvability, and gene-oriented evolutionary mechanics theories.
This paper shows how one of the alternatives, evolvability theory, supports adaptive aging, and also presents arguments showing how evolvability theory can overcome specific objections put forward by proponents of classical evolutionary mechanics theory.
The underlying issue, the evolutionary value of life as a function of age relative to reproductive maturity, has now endured unresolved for 150 years. Four different concepts still have adherents and each has corresponding dependent theories of biological aging. Lack of resolution is clearly interfering with efforts toward understanding aging and producing treatments for age-related conditions. Arguments are presented to the effect that many non-science factors, unique to this discipline, have acted to inhibit advances in this area.

Brain and aging

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Abstract is not submitted.

Mitochondrial debris including mitochondrial DNA and formyl peptides that appear in the blood after major trauma can induce a syndrome resembling sepsis

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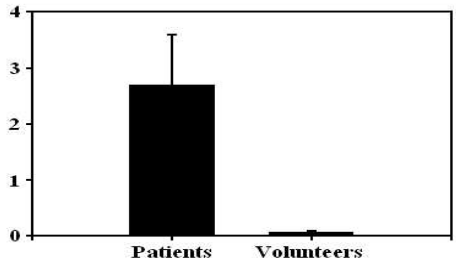
Injury causes a systemic inflammatory response syndrome ("SIRS") that is clinically much like sepsis, and predisposes to organ failure, infection critical illness and death. SIRS is responsible for more than half of intensive care morbidity and costs in the USA. It is known that microbes present "pathogen-associated molecular patterns" (PAMPs) to the immune system that activate innate immune cells through "pattern-recognition receptors" (PRR). But it has recently been noted that cellular injury (especially non-apoptotic cell death) can release endogenous "damage associated molecular patterns" (DAMPs) from cells that also act on innate immunity. Part of this similarity is due to mitochondria being evolutionary endosymbionts descended from bacteria.
We recently showed (Zhang et al., *Nature*, March 4, 2010) that mitochondria bear bacterial molecular motifs including bacterial-style CpG-DNA and formylated peptides that act upon human innate immune cells via Toll-Like Receptor-9 (TLR-9) and G-protein coupled formyl peptide receptors. We have also showed that

major injury releases mitochondrial detritus into the circulation (Figure, left, shows qPCR for Cytochrome B DNA) with mitochondrial DNA (mtDNA) reaching levels in the circulation that activate immune cells. Mitochondrial peptides and mtDNA signal through Formyl Peptide Receptor-1 and TLR9 respectively, promoting neutrophil (PMN) cytosolic calcium ([Ca2+]i) release and entry as well as causing phosphorylation of P38- and P44/42-mitogen activated protein kinases (MAPK).These signals cause PMN migration to and degranulation in the lung and liver, initiating PMN-mediated oxidative organ injury there. Thus cellular disruption by major trauma releases mitochondrial DAMPs into the circulation that have evolutionarily conserved molecular signatures similar to bacterial PAMPs. Release of such mitochondrial 'enemies within' locally is likely a necessary step in the initiation of inflammation, which then leads to wound healing. In overwhelming injuries however, the same pathways become activated systemically. In that case, widespread activation of innate immunity leads to a SIRS syndrome that is similar to sepsis and has a high lethality even with modern care. Thus innate immune activation is likely to be beneficial at the local level in the case of minor, survivable injuries. But conversely, global innate immune activation appears to promote early organ failure and death in the case of major injuries that would not have been survivable early in the human evolutionary process. Trauma surgeons who support these patients now with intensive care must accept the SIRS response as a consequence of survival. But for early Man, it is likely that in the case of major injuries selection pressures at the societal level would have favored early death over prolonged illnesses that could have immobilized the entire social group or exhausted its resources.



Quantitative PCR (qPCR) for Cyt B DNA in plasma from trauma patients (µg/mL)

An enzyme whose inactivation delays aging and increases lifespan

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Abstract is not submitted

Homo sapiens liberatus

It is well known for a long time that genomes contain numerous biological programs. The great majority of them are useful and even quite necessary for the organism. However, some genetic programs are obviously counterproductive for the individual. In certain cases, their operation results in the death of the organism. By analogy with apoptosis and mitoptosis (programmed elimination of cell and mitochondrion, respectively), programmed suicide of organisms can be defined as *phenoptosis*. For organisms that reproduced only once, death usually occurs soon after reproduction is over, being in animals sometimes the final step of copulation. Such an event can be exemplified by the death of bamboo in the middle of the summer immediately after formation of seed, as well of *Arabidopsis thaliana* on day 25 of life, also when formation of seeds is completed. In the latter case, removal of seeds prevents the death so that the plant can survive for many months. Similar relationships were shown in soy. An attempt was undertaken to identify a lethal poison that is produced by soy beans. It proved to be an organic acid of unusual structure. Again, removal of beans greatly prolongs the lifespan of soy, which is converted in this way from annual to perennial plant. Ejaculation of the male praying mantis occurs only after his decapitation which is carried out by the female

at the very end of copulation. The male *Australian marsupial* mouse dies two weeks after the rut, being poisoned by his own pheromones that attracted females during the rut. The evolutionary advantage of such dramatic events is rather clear. Death of one of the parents (sometimes even both of them, as happens, e.g., in certain squids) results in an increase in diversity of progeny, an effect certainly increasing evolvability of the species. Males of the marsupial mouse or praying mantis can play the role of father only once in life, so the next litter of the same female will require another father.

There is much evidence that programs favorable for evolvability but counterproductive for the individual exist also in repeatedly multiplying organisms. Senescence of organism seems to be one of the most important cases of this kind. Concerted decline of many physiological functions with age may be regarded as slow phenoptosis. This process also increases evolvability due to the fact that the pressure of natural selection on an organism rises when this organism becomes weaker and weaker due to progression of senescence. A new small trait that is not essential for survival of a strong young individual may appear essential (and hence, can be recognized by evolution) in weak old individual.

Impressive examples of phenoptotic programs have been found in studies of interactions of animals with pathogens. In rats, a protein is present in the blood that recognizes the plague bacterium and, after such recognition, induces septic shock killing the infected animal before it passes infection to all its relatives. Septic shock is operative also in humans, but the rat-type protein is replaced by a less efficient one. As a result, phenoptosis appears to be much slower, and pandemic becomes possible. This is why rats rather than humans serve as a reservoir of plague infection.

There are some reasons to assume that both other animals and humans have a special phenoptotic program preventing uncontrolled genome modifications under conditions of severe stress. Apparently, there are sensors monitoring the level of certain crucial parameters changing under stress conditions. If deviations of these parameters from normal appear to be larger than some critical level, a signal for "biochemical suicide" is generated. As a result, the ill individual dies even if the crisis is over. Perhaps, an example of this situation was quite recently described by Carl Hauser (see his abstract). It was found that receptors recognizing bacterial DNA (or formyl methionine, which is specific for bacteria) and inducing sepsis can be actuated by mitochondrial DNA and formyl methionine originating from mitochondrial proteins. Large trauma always results in appearance of these mitochondrial compounds in the blood, entailing a syndrome quite similar to septic shock. A possible physiological function of this mechanism is to beat completely an individual weakened by a large trauma ("enemy within", C. Hauser) and, hence, to purify the population from such organisms becoming a burden for the community. Interestingly, buffalos solve the same problem by allowing lionesses to follow the herd in its rear guard, to kill those buffalos that constantly remain behind the others.

Generally speaking, any contradictions between interests of genome evolution and individual are solved in favor of the genome, a rule increasing evolvability. However, humans no more rely on their evolution, which is too slow and unpredicted. If they need to fly, they construct a plane instead of waiting for millions of years for a moment when wings occasionally appear on their back. This is why programs that were invented by evolution to increase evolvability but proved to be counterproductive for the individual have no more sense for humans, who should try to abolish such atavisms. Senescence, septic shock, mechanisms of sudden death at the moment when a crisis is, in fact, over – this is an incomplete list of "enemies within" It seems possible that the list should include cancer if this disease represents a program eliminating an organism with large mutational load.

In any case, it seems obvious that cancellation of genetic programs counterproductive for the human organism would be the great achievement of XXI century biology and medicine. This will mean prolongation of youth and disappearance of age-related diseases. Such an event will be a sort of "Rise of the machines" to stop genome tyranny and to convert *Homo sapiens* into *Homo sapiens liberatus*.

V.P. Skulachev

POSTERS

Role of mitochondria in cell senescence-induced death of yeast *Saccharomyces cerevisiae*

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It is known that mammalian cells with heavily damaged DNA and/or mitotic apparatus may undergo long term cell cycle arrest. This arrest is called senescence and, unlike G0/G1 delay (quiescence), eventually leads either to cell death or cancerous transformation. It was shown that *S. cerevisiae* cells arrested with non-replicated telomeres undergo senescence-like cell death which can be rescued by either antioxidant or rapamycin treatment [1]. Importantly, the same treatments prolong the survival of senescent mammalian cells. We asked first whether this type of yeast cell death is specific to telomere replication defect or can be induced by other factors arresting cells in the division phase. It appeared that long-term mitotic arrest induced by cdc15-1 and cdc26delta mutations in Anaphase-Promoting Complex (APC) displays the same features: causes rapamycin- and antioxidant-sensitive cell death.
Why the prolonged arrest causes death? Unlike higher cells, yeast lack specialized proteins (p53, etc.) which trigger cell senescence-dependent apoptosis. One possible reason for the arrest-induced death can be multiplication of mitochondria in the absence of nuclear DNA replication, resulting in the imbalance between mitochondrial and nuclear-encoded proteins in mitochondria. Supporting this, we found that mitochondrial translational inhibitor chloramphenicol or *rho0* mutation rescue the senescence-induced death while mutations in mitochondrial retrograde signaling reduce the survival. Together it suggests a novel conservative pathway for senescence-induced cell death.
This work was supported by RFBR grant 09-04-01704 and Grant of President of Russian Federation MK-4628.2009.4

References:
[1] Qi H, Chen Y Fu X, Lin C-P, Zheng XFS, et al. (2008) TOR Regulates Cell Death Induced by Telomere Dysfunction in Budding Yeast. *PLoS ONE* 3(10): e3520. doi:10.1371/journal.pone.0003520

Induction of multidrug resistance P-glycoprotein inhibits the antiapoptotic action of mitochondria-targeted antioxidant SkQR1

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Mitochondria-targeted antioxidants of the SkQ family, being accumulated in energized mitochondria, protect cells from oxidative stress by increasing the level of reduced glutathione and decreasing the cell-damaging effect induced by reactive oxygen species. Using various human transformed cell lines and SkQR1 (a fluorescent member of the SkQ family), we show that SkQR1 is ejected from chemotherapy-resistant cells by P-glycoprotein, one of the main transport proteins determining multidrug resistance typical for many neoplastic cells. It is also shown that SkQR1 ejection is neutralized by P-glycoprotein inhibitors (verapamil and pluronic L61). In experiments on K- 562 cells, it was found that the subline sensitive to chemotherapy is protected by SkQR1 from apoptotic action of oxidative stress. Protection of the resistant subline occurs only after inhibition of P-glycoprotein.

Demographic Consequences of Defeating Aging

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A common objection against starting a large-scale biomedical war on aging is the fear of catastrophic population consequences (overpopulation). This fear is only exacerbated by the fact that no detailed demographic projections for radical life extension scenario have been conducted so far. This study explores different demographic scenarios and population projections, in order to clarify what could be the demographic consequences of a successful biomedical war on aging. A general conclusion of this study is that population changes are surprisingly slow in their response to a dramatic life extension. For example, we applied the cohort-component method of population projections to 2005 Swedish population for several scenarios of life extension and a fertility schedule observed in 2005. Even for very long 100-year projection horizon, with the most radical life extension scenario (assuming no aging at all after age 60), the total population increases by 22% only (from 9.1 to 11.0 million). Moreover, if some members of society reject to use new anti-aging technologies for some religious or any other reasons (inconvenience, non-compliance, fear of side effects, costs, etc.), then the total population size may even decrease over time. Thus, even in the case of the most radical life extension scenario, population growth could be relatively slow and may not necessarily lead to overpopulation. Therefore, the real concerns should be placed not on the threat of catastrophic population consequences (overpopulation), but rather on such potential obstacles to a success of biomedical war on aging, as scientific, organizational, and financial limitations.

The novel antioxidant SkQ1 as an effective protector of rat eye tissues during long-term organotypic cultivation

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Cells posses by an array of the antioxidant mechanisms to clean harmful oxidants represented mainly by reactive oxygen species (ROS). Despite the antioxidant defense, ROS can cause serious damage to the neural retina, retinal pigment epithelium (RPE), Bruch membrane and choroid, and finally brings to the development of different visual abnormalities. Among numerous sites of ROS generation in the cell, mitochondrial electron transport is of crucial importance. Recently, a novel strong antioxidant SkQ1, with the aim to clean the matrix space of mitochondria, "the dirtiest place in the cell" in respect of ROS, has been invented. The present study examines the SkQ1 effects upon tissues of the adult rat eye.
Preparations of the eye posterior sector, consisting of the complex RPE-choroid-sclera with or without the neural retina, were obtained from eyes of narcotized adult (2 – 3 months) albino rats and cultivated in a roller with or without of 20 nM SkQ1. After fixation of the cultivated sectors, their cross-sections were then investigated using methods of routine histology, immunohistochemistry and computer morphometric analyses.
It has been demonstrated that under *ex vivo* conditions of long-term roller cultivation of the eye posterior sector, 20 nM SkQ1 effectively prevents the layers of the neural retina from destruction and conserves its neurons. SkQ1 also decreases death of RPE and choroid cells and protects the RPE layer from disintegration and exhaustion caused by withdrawal and macrophagal transformation of RPE cells. In our *ex vivo* model, degenerative processes in the central part of the retina are much more pronounced than in its peripheral region that resembles the *in vivo* situation in which the central part of the retina, macula, is particularly susceptible to injury and degeneration of a different nature. Remarkably, the protective effects of SkQ1 upon the eye tissues during long-term roller cultivation are much more pronounced in the central part of the eye posterior sector in comparison with the sector periphery.
Therefore, under *ex vivo* conditions of long-term roller cultivation of the rat eye posterior sector, consisting of the complex RPE-choroid-sclera with or without the neural retina, 20 nM SkQ1 acts as a strong protective agent, preventing degenerative processes in the neural retina, RPE and choroid.

Sir2-dependent daughter-to-mother transport of the damaged proteins in yeast is necessary to prevent high stress sensitivity of the daughters

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During cell division yeast *Saccharomyces cerevisiae* distribute intracellular "junk" asymmetrically: carbonylated proteins, extrachromosomal DNA circles are accumulated predominantly in mother cells (Sinclair, Guarente, 1997; Aguilaniu et al., 2003). It is also known that the number of individual divisions for *S. cerevisiae* cells is limited – a phenomenon called replicative aging. Asymmetrical distribution of the adverse factors was suggested to be responsible for "zeroing" of replicative age of the daughter cells thus preventing the clonal age-dependent decline. Our data suggest another reason why yeast maintain the asymmetry of the damaged intracellular material. We found that acidic stress was about three-fold more harmful for the recently formed daughter cells than for their mothers. A relatively lower stress resistance of the daughters was observed in cells subjected to heat-shock and in cells expressing a fragment of human protein huntingtin with polyglutamine expansion. SIR2 is known to act to maintain the asymmetry between the mother and the bud. Interestingly, under our experimental conditions sir2 knockout appeared to increase the percentage of the inviable daughter cells and to increase the stress resistance of the mothers. We speculate that daughter cells are born more vulnerable to stress conditions than the mothers and that Sir2-dependent asymmetrical distribution of the intracellular "junk" is necessary to diminish the risks for the daughter cells.
The work is supported by Grant of President of Russian Federation MK-4628.2009.4 and RFBR grant 09-04-01704

References:
Sinclair, D.A., Guarente, L. (1997), *Cell* 91(7):1033-42.
Aguilaniu, H., Gustafsson, L., Rigoulet, M., Nyström, T. (2003) *Science* 299(5613):1751-3

Comparison between two paradigms about aging

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According to the current prevailing interpretations, the age-related fitness decline shown by many species in natural conditions, commonly defined as "aging", is an effect of:
1) the age-related decline of natural selection (mutation accumulation hypothesis);
2) a balance between possible advantages at a younger age and the disadvantages of fitness decline (antagonistic pleiotropy hypothesis);
3) limited "resources" - not better defined - which are used preferentially for reproduction and not for soma maintenance (disposable soma hypothesis).
This interpretation ("first paradigm") is challenged by a different paradigm ("second paradigm") that explains aging as an adaptive phenomenon and, in shorts, maintains:
1) Age-related decline of natural selection cannot explain age-related fitness decline;
2) There is no evidence for antagonistic pleiotropic genes or for limited "resources" causing age-related fitness decline;
3) The first paradigm predicts a direct relation between environmental mortality and the proportion of deaths caused by aging. The second paradigm predicts the opposite. Observational data falsify the prediction of the first paradigm and confirm that of the second.

4) The limitations in cell turnover determined by telomere-telomerase system are a plausible mechanism underlying senescence. This is hardly explainable by the first paradigm. On the contrary, this is compatible with the second paradigm and, in fact, the adaptive hypothesis predicts and requires the existence of specific mechanisms causing the fitness decline.
5) For the first paradigm aging is only a common term for many age-related different diseases: aging as a distinct entity does not exist and, in principle, cannot be mastered. On the contrary, for the second paradigm, all manifestations of aging have common mechanisms: aging is a distinct entity and, in principle, can be mastered.
The coexistence of the two paradigms or the formulation of intermediate hypotheses appears impossible. Therefore, a choice based on scientific data is indispensable.

Are *C. elegans* and *D. melanogaster* valid animal models for studies on aging?

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C. elegans and *D. melanogaster* are common animal models for studies on aging, but there are strong arguments against the validity of these models for this type of studies:
I) Many bird and mammal species - our species included - show an increment of mortality with increasing chronological age in natural conditions. This phenomenon ("A" phenomenon) is well documented and, being existent in the wild, is influenced by natural selection. On the contrary, animals as *C. elegans* and *D. melanogaster* show in natural conditions a constant mortality rate but, in artificial protected conditions, they display an age-related mortality increment starting from ages not existing in the wild. In fact, in natural conditions: 1) the longevity of *C. elegans* is reduced up to 10 fold compared with standard laboratory culture conditions and few individuals of this species remain fertile in the wild after 10 days; 2) *D. melanogaster* has a reported adult life span in the wild of 10-12 days. Therefore, the mortality increment for these two species ("B" phenomenon), being a laboratory artefact, cannot be influenced by selection. "A" and "B" phenomena are radically different in their possible evolutionary determinants and so the results of experiments on "B" phenomenon are not automatically applicable to "A" phenomenon.
II) *C. elegans* and *D. melanogaster* (and in general the adult insects) are composed by cells with no turnover, while birds and mammals have cells and tissues with turnover. If, as it seems likely, the slowdown and later the stopping of cell turnover, and the correlated cell senescence, are pivotal elements in the age-related fitness decline of birds and mammals, it is rather doubtful to use experiments on animals with no cell turnover to explain the fitness decline in animals with cell turnover.
III) Animals as *C. elegans* and *D. melanogaster* have life cycles thoroughly different from those of bird and mammal species. Studies on aging that use these animal models implicitly assume that their adult stages are equivalent to the postnatal stages of birds and mammals for the extension of their results to these species. But this assumption is not proved and seems quite doubtful.
The appropriateness of *C. elegans* and *D. melanogaster* as animal models for aging is a problem that cannot be neglected in aging studies. Unfortunately, in renowned texts and very influential journals, the issue is not considered and it is frequent that experiments modifying – in laboratory conditions and at ages non-existent in the wild - the modifications of *C. elegans* and *D. melanogaster* life tables are presented as meaningful advances in the understanding of human aging!

Arguments against telomere-telomerase system as general defence against cancer

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Telomere-telomerase system, which is genetically determined and regulated, causes cell senescence and limits cell replication capacity. The existence of life-limiting mechanisms is specifically predicted by

adaptive aging theories while the contrary is true for non-adaptive aging theories. Therefore, for this second type of theories, it is essential an adaptive function to justify the existence of these life-limiting mechanisms.
A popular interpretation is that they are a general defence against cancer but there are strong arguments against this hypothesis:
1) It does not justify: a) the existence of animals that show in the wild no observable increase in age-specific mortality rate, cancer mortality included; b) the great differences of duplication limits and of cell overall functionality decay from species to species, unless cancer risk is postulated as varying from species to species in direct correlation with the limits imposed to cell duplication capacities and to cell overall functionality by the genetic modulation of telomere-telomerase system;
2) Shortened telomeres increase vulnerability to cancer as a consequence of dysfunctional telomere-induced instability;
3) The decline of duplication capacities and of overall cell functionality weakens immune system efficiency, which is inversely related to cancer incidence;
4) The role of the telomere in chromosomal stability argues that telomerase protects against carcinogenesis.
5) In yeast, an eukaryotic species, replicative senescence and cell senescence, although not caused by telomere shortening but by another mechanism related to the number of duplications, cannot be a consequence of an impossible cancer risk. Moreover, these phenomena and others strictly associated observed in yeast have been interpreted as adaptive.
6) *Dyskeratosis congenita*, an inherited human disease, is characterized by an altered telomerase. Problems tend to occur in tissues in which cells multiply rapidly and there is a higher rate of cancer that can likewise be explained by the lack of telomerase, which results in unstable chromosomes.
But: 7) in rodents, telomerase activity is not related to maximum lifespan while is inversely related to body mass and this has been interpreted as a fact in support of the defensive role against cancer risk of telomere-telomerase system, as a greater body mass presumably increases cancer risk.
In short, with the important exception of point (7), which stimulates further data and discussions, there are not specific arguments and experimental tests in support of the hypothesis that telomere-telomerase age-related limiting actions on cell turnover is a general defence against cancer. Therefore, telomere-telomerase age-related limits on cell turnover are hardly justifiable as a defence against cancer risk and, lacking other plausible explanations, only the adaptive hypotheses of age-related fitness decline appear a rational cause for their existence.

A proposal: Project "*Homo sapiens liberatus II*"!

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Ageing is commonly considered not a physiological event but a mixed set of diseases with age-related increasing frequency and severity: ageing manifestations are empirically treated for their dysfunctions, in analogy with diseases showing the same alterations, and the cures allow often an increase in survival time in conditions of low quality of life.
But, it is indispensable to acquire the awareness that ageing is something other than a disease and that it needs specific measures. It is possible to conceive an ambitious project for the solution of the problem, with a possible honorific name indicating the inspiration of Prof. Skulachev's great design.
It the first phase, same preliminary targets will be pursued, that is a better understanding of: a) telomere-telomerase system; b) apoptosis phenomenon; c) cell turnover of all tissues and its effect on the function of the organs in each age of the life; d) morphogenetic mechanism, in particular for dentition. Another important goal of the same phase is the development of genetic techniques for: a) the effective and precise insertion of a genetic sequence in a point of the genome not causing dangerous alterations; b) the effective and precise substitution of a genetic sequence with another sequence.
In the second phase, with experiments on animals, genetic sequences will be inserted or substituted with the aim of modifying the modulation of telomere-telomerase system for increasing longevity. The same techniques will be applied for the treatment of severe genetic diseases and for age-related severe diseases such as Age-Related Macular Degeneration and Alzheimer’s disease. In the same phase, on animals, experiments will be performed with the same techniques to obtain multiple dentitions and other experiments for testing possible drugs with increasing longevity qualities.

In the third phase, on man, first experiments of gene therapy (not on germinal cells) will be performed and possible drugs with increasing longevity qualities will be tested, with the verification of the results and progressive widening of the experiments.
In the fourth phase, the project plans possible experimentation and application of gene therapy on human germinal cells and applications on a large scale of safe and tested techniques and drugs.
For the extreme weight of the argument, it could be useful the creation of an apposite international agency, adequately funded, with the specific aim of controlling ageing and, as a very important corollary, genetic diseases, following the example and the wonderful outcomes of NASA.
But, to go on the moon or to live one thousand years must not be a foolish attempt to compete with the Infinite but just another way to contemplate It.

Altered content and fatty acid composition in cardiolipin of mitochondria from mtDNA mutator mice

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We have recently demonstrated that the assembly and turnover of the mitochondrial respiratory chain complexes I, III and IV, (but not complexes II and V) are altered in mitochondria from mtDNA mutator mice [1]. Since it is known that cardiolipin is essential for assembly and stability of respiratory chain complexes, we studied this phospholipid in mitochondria from mtDNA mutator mice. The content of mitochondrial phospholipids was analysed by two-dimensional high performance thin layer chromatography (2D-HPTLC). Content of cardiolipin was found significantly lower in liver and skeletal muscle mitochondria from mtDNA mutator mice as compared with wild-type mitochondria. To analyse fatty acid composition of cardiolipin, gas chromatography/flame ionization detection or electron ionization - mass spectrometry (GC/FID or El MS) was applied. Content of n6 fatty acids were remarkable lowered in cardiolipin from skeletal muscle and liver mitochondria of mtDNA mutator mice as compared with wild-type mice. As to content of saturated fatty acids, it was increased. Mitochondrial phospholipids were also studied in mice chronically treated with SkQ1, mitochondria-targeted plastoquinone linked by decane to triphenyl phosphonium cation. Content of cardiolipin was significantly increased in both wild-type and mtDNA mutator mitochondria. Alteration in fatty acid composition of cardiolipin from mtDNA mutator mitochondria was reversed after treatment with SkQ1. It consisted in an increase in the amount of 6n polyunsaturated fatty acids and in decrease in amount of saturated fatty acids.

References:
[1] D. Edgar, I. Shabalina, Y. Camara, A. Wredenberg, M.A. Calvaruso, L. Nijtmans, J. Nedergaard, B. Cannon, N.G. Larsson, A. Trifunovic, Random point mutations with major effects on protein-coding genes are the driving force behind premature aging in mtDNA mutator mice, *Cell. Metab.* 10 (2009) 131-138.

The study of uncoupling action mechanism of SkQ-SkQ3 family compounds

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Earlier in our work it was demonstrated that under conditions of mitochondrial proton pumps activation on the outer side of inner mitochondrial membrane the special fraction of protons is formed. These protons are

nonequilibrium bounded with membrane and take part in ATP synthesis directly. Hydrogen ions of this fraction do not interact effectively with classic uncouplers. It was found out that weak Lewis bases (such as HEPES, MES, citrate) decrease the volume of the proton fraction discussed dew to catalysis of dissociation reaction of these protons from membrane surface into aqueous volume. We synthesized and tested new type of uncouplers (2,4,6-trichloro-3-pentadecylphenol,TCP-C15) with significantly increased affinity to membrane. It was shown that TCP-C15 selectively interacts with nonequilibrium membrane bounded protons. Removal of these protons by catalyst (HEPES) leads to dissipation of TCP-C15 uncoupling effect. However the uncoupling effect of classical uncoupler pentachlorophenol (PCP) remains unchanged in same conditions. In actual investigation we have demonstrated that addition of SkQ3 to mitochondria induces increase of respiration like the addition of TCP-C15 it does. This effect can be significantly decreased by higher concentration of catalyst (HEPES).The results obtained led us to preliminary conclusion that high membrane-acting properties of SkQ3 provide this substance with ability to interact with nonequilibrium membrane bounded protons effectively. In the general case SkQ3 may induce uncoupling effect by two ways: as single proton carrier and (in accordance to Severin F.F. et al.) as penetrating complex with fatty acid anions.

References:
Severin F.F., Severina I.I., Antonenko Y.N., Rokitskaya T.I., Cherepanov D.A., Mokhova E.N., Vyssokikh M.Y., Pustovidko A.V., Markova O.V., Yaguzhinsky L.S., Korshunova G.A., Sumbatyan N.V., Skulachev M.V., Skulachev V.P. Penetrating cation/fatty acid anion pair as a mitochondria-targeted protonophore. *Proc. Natl. Acad. Sci. USA*. (2010);107(2):663-8.

The impact of membrane-targeted cations on energy conversion in membrane vesicles from *Rb. capsulatus*

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We have studied the impact of mitochondrially targeted penetrating cations on energy conversion in membrane vesicles from phototrophic bacteria *Rb. capsulatus* (chromatophores). The energy-converting enzymes of purple bacteria are closely related to their mitochondrial counterparts, but can be synchronized by short flashes of light. The same membranes contain electrochromic pigments, mostly carotenoids, which can serve as internal voltmeters.
The mitochondrially targeted cations, did not affect notably the flash-triggered generation of membrane potential (at least when added at < 1 μM), but accelerated its decay. The similar uncoupling efficiency of SkQ1, MitoQ and C12TPP indicates the involvement of the TPP moiety in the proton translocation. This uncoupling action could be partly prevented by depletion of chromatophore preparation from fatty acids (via pre-washing with bovine serum albumin, BSA). Addition of palmitic acid to the BSA-treated chromatophores partially reversed the effect of BSA. We consider these data as an evidence for the transmembrane, H+-conducting fatty acid cycling mediated by penetrating cations, as suggested by Skulachev and co-workers (Severin et al. 2010).
Neither SkQ nor MitoQ affected the flash induced redox-changes of cytochrome *b* in the cytochrome bc_1 complex. Accordingly, these cations which, most likely, can interact only with the quinone-reducing center *N* of the complex (Gu et al., 2000), did not block the electron flow out of this center.
When added in concentration of > 5μM over antimycin A, SkQ1 could serve as an electron shuttle, overcoming the antimycin block. This observation explains why the addition of SkQ1 over antimycin induces a ROS burst in mitochondria.
The studies of the Zn-treated chromatophores, where the oxidation of cytochrome *b* was retarded (Skulachev et al., 1967, Klishin et al. 2002, Mulkidjanian, 2007) have shown that MitoQ and SkQ, when addded at > 1 μM slightly slowed down the reduction of heme b_h. Any slowing of this reaction, however, should be accompanied by an increase in the ROS production.

We suggest that at high concentrations MitoQ and SkQ can bind to the cytochrome bc_1 complex and affect the mechanism of ubiquinol oxidation in center *P*. This effect could account for the pro-oxidant action of the mitochondrially targeted cations.

References
Gu, L.Q., L. Yu, and C.A. Yu. 1990. *Biochim. Biophys. Acta* 1015:482-492.
Klishin, S.S., W. Junge, and A.Y. Mulkidjanian. 2002. *Biochim. Biophys. Acta* 1553:177-182.
Mulkidjanian, A.Y. 2007. *Photochem. Photobiol. Sci.* 6:19-34.
Severin FF, Severina II, Antonenko YN, Rokitskaya TI, Cherepanov DA, Mokhova EN, Vyssokikh MY, Pustovidko AV, Markova OV, Yaguzhinsky LS, Korshunova GA, Sumbatyan NV, Skulachev MV, Skulachev VP. 2010. *Proc. Natl. Acad. Sci. USA* 107:663-668
Skulachev VP, Chistyakov VV, Jasaitis AA, Smirnova EG. 1967. *Biochem. Biophys. Res Commun.* 26:1-6.

The effect of mitochondria-targeted antioxidants on normal and transformed cells in culture

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The reorganization of actin microfilaments plays a crucial role in oncogenic transformation and leads to distortion of cell polarization and motility. One of possible explanation of actin network system disorders is activation of oncogenic signaling pathways which determine cell polarization, directional motility and substrate adhesion by changing in gene expression of actin binding proteins such as myosin, gelsolin, tropomyosin. In this study we investigated the effect of antioxidants specifically addressed to mitochondria (SkQ1 and their analogs) on normal (MRC5) and transformed (MRC5-V1 and MRC5-V2) human pulmonary fibroblasts and on human fibrosarcoma HT1080 cells.
We have detected an important cell shape alteration during incubation of normal and transformed fibroblasts with mitochondria-targeted antioxidants (20-40 nM from 5 hours to 3-7 days): cells became highly spread, actin microfilaments bundles were formed. We have evaluated immunomorphologically that SkQ1 and their analogs led to formation of thick and parallel actin bundles (stress fibers containing beta actin, myosin II, alpha actinin, gelsolin, alpha smooth muscle actin) in MRC5-V1 and MRC5-V2 cultures. The effect of N-acetyl-cysteine (NAC) and Trolox on MRC5-V1 was similar to SkQ1, but working concentrations of this antioxidants were much higher: 5mM for NAC and 100μM for Trolox. Phenotype reversion by SkQ1 treatment was observed also with human fibrosarcoma HT1080 cell line.
We have revealed immunomorphologically and morphometrically that mitochondria-targeted antioxidants led to focal adhesion (FA) elongation and further reorganization with forming of mature FAs in transformed fibroblasts. Western blot analysis showed the increase of FA protein vinculin in MRC5-V1 after mitochondria-targeted antioxidants (20-40 nM, 7 days), NAC (5 mM, 7 days) and trolox (100 μM, 7 days) incubation.
We have showed the effect of SkQR1 on human fibrosarcoma cells proliferation. SkQR1 treatment (20 nM, 24 h) led to mitotic-entry delay in HT1080 cells after 18 h of serum stimulation.
Mitochondria-targeted antioxidants induced phenotypic reversion in our experimental model. The results of our present work are in concerning with the effect of antioxidants on ras-transformed cells investigated earlier (Alexandrova et. al, 2006; Popova et al., 2006).

The effects of mitochondria-targeted antioxidants on human cervical cancer cells in culture

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The disruption of cytoskeleton and intercellular adhesions is an important component of the acquisition of invasive properties in epithelial malignancies. Elevation of the reactive oxygen species (ROS) level plays an important role in the tumor development. It was proposed that changes of ROS level in various cell

compartments can differentially affect probability of neoplastic transformation and tumorigenesis. We have studied the effects of new mitochondria-targeted antioxidant SkQ1 [10-(6'-plastoquinonyl) decyltriphenylphosphonium] and its counterparts on tumor cells in culture.

To study SkQ1-dependent reorganization of actin cytoskeleton and adhesion junctions in the SiHa, C33A and HaCaT cells we used immunofluorescent microscopy and Western blot analysis. It was shown that incubation of the SiHa and C33A cells with SkQ1 (40 nM, 4 days) leads to more organized actin cytoskeleton: the microfilament bundles at the cell periphery and at cell-cell contacts were well pronounced. Immunostaining of E-cadherin revealed a formation of prolonged E-cadherin-positive contacts. Morphology of these cells and their islets became almost indistinguishable from normal keratinocytes. Treatment with SkQ1 (40 nM, 7 days) increased the total amount of E-cadherin and α-catenin in the SiHa and C33A cells. Both proteins play a causal role in the establishment and maintenance of the differentiated epithelial phenotype. Also we have found that SkQ1 inhibited the SiHa cells proliferation.

The morphology of nontransformed keratinocytes HaCaT was not significantly affected by SkQ1. Phenotype reversion (normal epithelial-like morphology restoration) was observed also with other antioxidants N-acetyl-L-cysteine (NAC, 1-5 mM) and Trolox (100 μM).

We suppose that the SkQ1-induced cytoskeleton changes and proliferation inhibition are connected with the ability of SkQ1 to affect differentiation state of neoplastic epithelial cells and, as a result, to modulate pathways whose activity, on one hand, is dependent on expression of some differentiation markers and, on the other hand, can regulate cell cycle progression.

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Cover: mitochondrial reticulum of SkQ-treated cell