3-rd International Conference

Homo sapiens liberatus

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

Professor V.P.Skulachev

Abstract Book

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

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

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

The results obtained point to a new possibility by which hormones might affect oxidative phosphorylation: direct interaction with CcO, a key enzyme of the respiratory chain modulating the respiratory activity of mitochondria.

References:

[1] L. Buhrow, C. Hiser, J.R. Van Voorst, S. Ferguson-Miller, L.A. Kuhn. (2013) Biochemistry, 52 6995-7006

[2] T. Vygodina, C. Pecoraro, D. Mitchell, R. Gennis, A. Konstantinov. (1998) Biochemistry, 37 3053-3061

New bioinformatics methods for identification of lost genes and protein isoforms

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The high regenerative potential of poikilotherms relative to homeotherms remains not well understood. An original algorithm and software were used to identify a short list of lost genes of *Xenopus laevis*, one of which («c-answer») was used in experimental tests. The c-answer gene encodes a previously unknown specific signaling (FGF and ADP) modulator, a transmembrane protein factor of regeneration and telencephalon development in poikilothermic vertebrates that was lost in homeotherms including human. Experiments on embryos of *X. laevis* have demonstrated that c-answer controls the regeneration of body appendages and telencephalon development by binding FGRF and P2ry1 receptors and inducing the MAPK/ERK and purinergic pathways. One can propose that the loss of c-answer in homeotherms decreased the activity of at least two signaling pathways, which consequently contributed to the alteration of the control pathways of regeneration and telencephalon development. (This study was performed in cooperation with the Laboratory of Molecular Bases of Embryogenesis, Institute of Bioorganic Chemistry.)

We believe that the long (relative to body weight) species-specific lifespan (LS) in rodents and primates can be, among other things, due to the loss of certain genes found in short-lived representatives of the same order. The software has identified the short list of genes present in short-lived primates and rodents but missing in long-lived ones including humans, Colombian white-faced capuchin, and naked mole-rat. Other Euarchontoglires species have been considered as well. The expression level of the identified murine genes was analyzed in different tissues. The obtained results can be interpreted in terms of the

well-known theory of Williams: such genes improve the fitness of species with short LS but are neutral or harmful for those with long LS.
The program can efficiently identify the genes lost or acquired at any stage of evolution.
Cardiolipin can be considered as a functional integrator of components of the mitochondrial respiratory chain providing for the efficient transfer of electrons and protons. The enzyme tafazzin is the main if not the only factor of cardiolipin maturation. Altered proportions between tafazzin isoforms can cause severe abnormalities such as Barth syndrome. One can think that unconventional tafazzin isoforms contribute to the optimal balance between increased biochemical activities of mitochondria resulting from specific environmental or nutritional conditions and longevity and that the functional role of such isoforms is due to the altered C-terminal primary and secondary structure of the protein.
These isoforms correspond to the omission of exon 9 or retention of the intron between exons 10 and 11, both of which cause a frameshift; as well as to the emergence of an exon 5 or retention of the intron between exons 8 and 9. These isoforms can be found in many mammalian orders including animals living under low-oxygen conditions. The emergence of these isoforms in placentals correlates with the increased species-specific lifespan. For instance, E5 is found in marsupials (e.g.Tasmanian devil) and many (e.g.electric eel) but not all fishes (e.g., it is missing in the zebrafish).
This study was supported by the Russian Foundation for Basic Research (18-29-13037).

Molecular mechanisms of neighboring gene effect in the yeast knockout library

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The *S. cerevisiae* gene deletion collection is widely used for genome-wide annotation of function and study of genetic interactions. However, the standard G418-resistance cassette used to obtain knockout mutants introduces strong regulatory elements into the target genetic loci. However, their effects on the expression of neighboring genes have never been systematically assessed.
Here, using Ribo-Seq and RNA-Seq data for several *S.cerevisiae* knockout strains, we analyzed transcriptional and translational changes induced by the KanMX cassette within the modified genomic loci. In many cases, we observed significant alterations in gene expression, including severe impairment of translation. These changes could be attributed to shifted transcriptional start sites or activation of alternative polyadenylation signals. The most dramatic changes were observed when a deleted gene was arranged "head-to-head" with the neighboring gene, where a shift of transcription start site of the latter expanded the 5' untranslated region, and the appearance of upstream AUG codons inhibited translation of the main open reading frame. In some cases, these events caused false genetic interactions of the deleted genes, the so-called neighboring gene effect.
Our data describe the interactions of the KanMX cassette with neighboring genes and provide mechanistic insights into the molecular mechanisms involved. They also suggest that caution is needed in interpreting the results of deletion screens, especially those using strong regulatory elements.

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

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