различных R- и T-конформациях белка, что является ключевым моментом для понимания кооперативной аллостерической реакции связывания Hb и O_2.

В заключении, автор предполагает обсудить возможные направления будущих исследований фотоиндуцированных и фотосенсибилизированных процессов с участием молекулярного кислорода.

UBIQUITOUS $[Na^+]_i/[K^+]_i$-SENSITIVE TRANSCRIPTOME IN MAMMALIAN CELLS: EVIDENCE FOR $Ca^{2+}{}_i$-INDEPENDENT EXCITATION -TRANSCRIPTION COUPLING

Koltsova S.V.[1,2], Trushina Y.[3], Haloui M.[1],Akimova O.A.[2,3], Tremblay J.[1,4], Hamet P.[1,4] and Orlov S.N.[1-4]

[1]Centre de recherche, Centre hospitalier de l'Université de Montréal (CRCHUM) – Technopôle Angus, Montreal, PQ, Canada; [2]Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, Moscow, Russia; [3]Faculty of Biology, M.V. Lomonosov Moscow State University, Moscow, Russia; [4]Department of Medicine, Université de Montréal, Montreal, PQ, CanadaMontréal; e-mail:sergei.n.orlov@umontreal.ca

Gene expression is regulated by diverse stimuli to achieve tissue-specific functional responses via coordinate synthesis of the cell's macromolecular components [1]. Electrochemical gradients of monovalent cations across the plasma membrane (high intracellular potassium, $[K^+]_i$ vs low intracellular sodium, $[Na^+]_i$) are created by the Na^+,K^+-pump and determine a large variety of physiologically important processes. These processes include maintenance of resting and action electrical membrane potentials, regulation of cell volume, secondary transport of mono- and divalent ions (such as chloride, calcium and phosphate), and accumulation of nutrients (glucose, amino acids, nucleotides) and other relevant molecules [2]. More recent studies demonstrated that side-by-side with the above-listed "classic" $Na^+{}_i,K^+{}_i$-dependent cellular processes, sustained elevation of the $[Na^+]_i/[K^+]_i$ ratio causes differential expression of *c-Fos* and other immediate response genes, as well as cell type-specific late response genes [3;4]. According to the generally accepted paradigm $Na^+{}_i/K^+{}_i$-sensitive mechanism of excitation-transcription coupling is driven by changes in intracellular $[Ca^{2+}]$ and activation of several Ca^{2+}-sensitive pathways – a phenomenon termed excitation-transcription coupling [5-7]. Indeed, it is well-documented that elevation of the $[Na^+]_i/[K^+]_i$

ratio typically leads to increases in $[Ca^{2+}]_i$ via activation of the Na^+/Ca^{2+} exchanger and/or voltage-gated Ca^{2+} channels. It has also been shown that promoters of different genes including *c-Fos* contain serum response element and Ca^{2+}+cAMP response element activated by $[Ca^{2+}]$ increments in the cytoplasm and nucleus, respectively. In contrast to the aforementioned mechanistic view, we found that in vascular smooth muscle cells from the rat aorta (RVSMC) and the human adenocarcinoma cell line (HeLa) the ouabain-induced changes in the c-Fos expression were preserved in the presence of Ca^{2+} channel blockers and extra- and intracellular Ca^{2+} chelators [8;9]. These results made us conclude that along with canonical $Ca^{2+}{}_i$-mediated signaling, sustained elevation of the $[Na^+]_i/[K^+]_i$ ratio affects gene transcription via unknown $Ca^{2+}{}_i$-independent mechanism(s) [3].

To further examine this hypothesis, we identified ubiquitous and tissue-specific $[Na^+]_i/[K^+]_i$-sensitive transcriptomes by comparative analysis of differentially expressed genes in RVSMC, HeLa, and human umbilical vein endothelial cells (HUVEC). To augment $[Na^+]_i$ and reduce $[K^+]_i$, cells were treated for 3 hrs with the Na^+,K^+-ATPase inhibitor ouabain or placed for the same time in the K^+-free medium. The whole hybridization procedure was conducted with the Affymetrix GeneChip® system. The hybridization results were evaluated with Affymetrix GeneChip® Command Console Software (AGCC). Quality of the chips was determined using Affymetrix Expression Console. To validate genome-wide Affymetric results, we estimated changes in the gene expression for several selected transcripts by qRT-PCR, which was performed using Express SYBR GreenER qPCR Supermix kit. Data analysis was performed within Partek Genomics Suite. The data were initially normalized by Robust Multichip Average (RMA) algorithm, which uses background adjustment, quantile normalization and summarization. Then, normalized data were analyzed by principal component analysis (PCA) to identify patterns in the dataset and high-light similarities and differences among the samples. Major sources of variability identified within the dataset by PCA were used as grouping variabilities for analysis of variance (ANOVA) with n=4 for each group of samples. The calculated *p*-value and geometric fold change for each probe set identifier were imported into Ingenuity Pathway Analysis (IPA, Ingenuity Systems, http://www.ingenuity.com) to ascertain networks, biological functions and their pathophysiological implications.

Using this technology, we detected changes in expression of 684, 737 and 1839 transcripts in HeLa, HUVEC and RVSMC, respectively, that were highly correlated between two treatments ($p<0.0001$; $R^2>0.62$). Among these $Na^+{}_i/K^+{}_i$-sensitive genes, 80 transcripts were common for all three types of cells. To establish if changes in gene expression are dependent on increases in

$[Ca^{2+}]_i$, we performed identical experiments in Ca^{2+}-free media supplemented with extracellular and intracellular Ca^{2+} chelators. Surprisingly, this procedure elevated rather than decreased the number of ubiquitous and cell-type specific Na^+_i/K^+_i-sensitive genes. Among the ubiquitous Na^+_i/K^+_i-sensitive genes whose expression was regulated independently of the presence of Ca^{2+} chelators by more than 3-fold, we discovered several transcription factors (*Fos, Jun, Hes1, Nfkbia*), interleukin-6, protein phosphatase 1 regulatory subunit, dual specificity phosphatase (*Dusp8)*, prostaglandin-endoperoxide synthase 2, cyclin L1, whereas expression of metallopeptidase *Adamts1*, adrenomedulin, *Dups1, Dusp10* and *Dusp16* was detected exclusively in Ca^{2+}-depleted cells. Overall, our findings indicate that Ca^{2+}_i-independent mechanisms of excitation-transcription coupling are involved in transcriptomic alterations triggered by elevation of the $[Na^+]_i/[K^+]_i$ ratio. There results likely have profound implications for normal and pathological regulation of mammalian cell functions, including sustained excitation of neuronal cells, intensive exercise and ischemia-triggered disorders.

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