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P.3.2-031
Structure of complexes of DNA with HMGB1 chromosomal protein in presence of linker histone H1

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The chromatin of the eukaryotic cells comprises of DNA and variety of nuclear proteins. DNA interacts with histones and non-histone proteins and forms nucleoprotein complex. Among such proteins, the most interesting are the linker histone H1 and the protein HMGB1. H1 histone is a conservative and tissue-specific protein. H1 interacts with linker DNA and plays an essential role in the post nucleosomal level of the structural organization of the chromatin. Non-histone chromosomal protein HMGB1 is the member of a large family of High Mobility Group proteins. Despite the fact that HMGB1 presents in the cells of all investigated organisms its functions remain unclear. The interest in HMGB proteins is also explained by the fact that structural motifs similar to its DNA-binding domains (HMGB domains) were found in many regulatory proteins. Similar to histone H1, HMGB-proteins bind to linker DNA regions. Sometimes HMGB1 is regarded as "architectural" factor of transcription. It participates in the assembly of transcriptionally active multi-protein complexes on DNA. The presence of histone H1 may inhibit the formation of such complexes. However, it is still not clear what kind of interplay between these two proteins takes place in the chromatin. We investigated tertiary complex DNA-H1-HMGB1 on the different stages of formation of complexes using UV Circular Dichroism (CD) and IR/UV absorption spectroscopy, atomic force microscopy. The combined influence of HMGB1 and H1 on the structure of formed complexes with DNA is not a result of competitive interactions between proteins. Structural organization of such complexes depends not only on DNA–protein interactions but also on the interaction between the protein molecules bound to DNA. The observed interaction between the HMGB1 and the H1 stimulates DNA condensation forming large DNA–protein complexes. The work was supported by Russian Foundation for Basic Research (15-08-06876) and Russian Science Foundation (17-14-01407).

Single molecule conformational dynamics of the ABC transporter BtuCD

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In all kingdoms of life ATP Binding Cassette (ABC) transporters translocate a diversity of molecules across cell membranes. They are involved in most physiological processes and are tightly linked to human disease and to multidrug resistance (MDR) of tumor and bacterial cells. Structural studies of ABC transporters have provided an invaluable context for understanding their mechanism of action. However, since crystal structures provide static pictures of highly dynamic machines, almost nothing is known of the conformational dynamics of ABC transporters. Herein, we studied the dynamics of an ABC transporter using single molecule FRET (smFRET). We observed that in each of its hydrolysis intermediate states, the vitamin B_{12} ABC transporter BtuCD, adopts a single dominating conformation with very little population heterogeneity. We also saw that a single molecule is conformationally stable, and does not spontaneously fluctuate between different conformations. These observations are very different from those made with secondary transporters, and describe for the first time an ATP-driven system that shows such conformational plasticity.

Redox Regulation of Biological Activities

P.3.3.A-001
Oxalomalate reduces expression and secretion of VEGF in the retinal pigment epithelium and inhibits angiogenesis

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Clinical and experimental observations indicate a critical role for vascular endothelial growth factor (VEGF), secreted by the retinal pigment epithelium (RPE), in pathological angiogenesis and the development of choroidal neovascularization (CNV) in age-related macular degeneration (AMD). RPE-mediated VEGF expression, leading to angiogenesis, is a major signaling mechanism underlying ocular neovascular disease. Inhibiting this signaling pathway with a therapeutic molecule is a promising anti-angiogenic strategy to treat this disease with potentially fewer side effects. Oxalomalate (OMA) is a competitive inhibitor of $NADP^+$-dependent isocitrate dehydrogenase (IDH), which plays an important role in cellular signaling pathways regulated by reactive oxygen species (ROS). Here, we have investigated the inhibitory effect of OMA on the expression of VEGF, and the associated underlying mechanism of action, using *in vitro* and *in vivo* RPE cell models of AMD. We found that OMA reduced the expression and secretion of VEGF in RPE cells, and consequently inhibited CNV formation. This function of OMA was linked to its capacity to activate the pVHL-mediated HIF-1α degradation in these cells, partly via a ROS-dependent ATM signaling axis, through inhibition of IDH enzymes. These findings reveal a novel role for OMA in inhibiting RPE-derived VEGF expression and angiogenesis, and suggest unique therapeutic strategies for treating pathological angiogenesis and AMD development.

P.3.3.A-002
Sigma-1 receptors are involved in modulation of Ca2 + responses induced by glutoxim and molixan in macrophages

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Sigma-1 receptors, ligand-regulated molecular chaperones, are located in endoplasmic reticulum membranes at the interface with mitochondria. Their ligands are endogenous steroids, antidepressants, antipsychotics, anticonvulsants, and analgesics. Sigma-1 receptors interact with target proteins, including ion channels and receptors, and modulate many cellular processes. It was found that interacting with inositol 1,4,5-trisphosphate receptors, sigma-1 receptors modulate Ca^{2+} signaling processes in cells.

Earlier, we have shown that disulfide-containing immunomodulators glutoxim® (disodium salt of oxidized glutathione with d-metal at nanoconcentration, PHARMA VAM, Saint-Petersburg)

and molixan® (complex of glutoxim with nucleoside inosine) cause biphasic intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) increase due to Ca^{2+} mobilization from thapsigargin-sensitive Ca^{2+} stores and subsequent store-dependent Ca^{2+} entry in rat peritoneal macrophages.

To elucidate the possible involvement of sigma-1 receptors in the effect of glutoxim and molixan on $[Ca^{2+}]_i$ in macrophages we used sigma-1 receptor antagonist neuroleptic haloperidol, widely used for treatment of schizophrenia.

Using Fura-2AM microfluorimetry we have found that macrophage preincubation with 30 μg/ml haloperidol for 6 min before 100 μg/ml molixan addition leads to a significant suppression of both Ca^{2+} mobilization (on average, by 49.3 ± 8.1%) and subsequent Ca^{2+} entry (on average, by 47.6 ± 9.7%), induced by molixan. Similar results were obtained in experiments with glutoxim.

Thus, we have demonstrated for the first time that sigma-1 receptor antagonist haloperidol inhibits both phases of the Ca^{2+} response induced by glutoxim or molixan, which indicates the possible involvement of sigma-1 receptors in signaling cascade triggered by these immunomodulators in macrophages. Our results also indicate that it is inadvisable to use glutoxim or molixan in combination with antipsychotic haloperidol in clinical practice.

P.3.3.A-003
Stizolophus balsamita Lam. isolated culture extracts as the antioxidant agents

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Plant cell redox homeostasis is formed as a result of the balance between the accumulation of reactive oxygen species and functioning of the antioxidant enzymes or non-enzymatic antioxidants. The production level of the last ones by plant tissue cultures is of importance also taking into account their value for the pharmaceutical and food industry. So, the aim of this investigation was to study the antioxidant activity (AOA) of *Stizolophus balsamita* Lam. medicinal plant tissue culture extracts (ethanol, methanol, water, hexane, acetone, chloroform). *S. balsamita* isolated culture was obtained, using Murashige and Skoog (MS) mineral-based nutrient media: MS1 – supplemented with indole-3-acetic acid (IAA) (2.0 mg/l) and kinetine (0.2 mg/l); MS2 – 6-benzylaminopurine (BAP) (2.0 mg/l) and IAA (0.5 mg/l).

According to the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay results the highest radical scavenging activity possessed methanol and acetone extracts of *S. balsamita* tissue culture, growing on MS2 medium (both of cultures were tested at the end of their exponential growth phase). Their IC_{50} values were reached 250–300 μg/ml. Further analyses were carried out with the most active fractions. TBARS (thiobarbituric acid reactive substances) assay data showed that malondialdehyde-equivalent quantity was approximately 4-fold lower against control under the influence of 1 mg/ml extracts. AOA of *S. balsamita* extracts was investigated also by microbe test systems, using *Escherichia coli* GC 4468 wt; *E. coli* GC4468 sodA49 (sodA-lacZ) and *E. coli* BN 407 (iucC-lacZ) mutant strains. It was tested the extracts capability to support aerobic growth of *E. coli* strains in the presence of 4 mM H_2O_2. The obtained data showed that pretreatment of the bacteria with the extracts (50 mg/ml in DMSO) 20 min before adding H_2O_2 increased the resistance of bacteria against oxidative stressor.

The results show the great potential of *S. balsamita* isolated culture extracts as antioxidant agents.

P.3.3.A-004
Decrease of external oxygen concentration as an early response to cell wall injury of Chara corallina

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Molecular oxygen plays a crucial role in plant metabolism. O_2 is a source of reactive oxygen species (ROS) and oxidative stress. The excessively generated ROS plays both signaling and protective function in mechanically stressed plants. Generation of ROS in microwounded cells is presumably mediated by the plasma membrane NADPH-oxidase that transfers electrons from cytoplasmic NADPH to extracellular oxygen with a concomitant production of ROS, H_2O_2 in particular. Microscopic injuries associated with ROS generation might be accompanied by oxygen concentration changes in the apoplast. Appropriate methods for oxygen measurements on the cellular and subcellular levels are necessary to reveal O_2 alterations. In the last decades significant progress has been made in the development of miniature sensors, including oxygen- and ROS-sensitive sensors. Recent invention of nanoscale electrochemical probes provides the opportunity to gain a deeper understanding of oxygen and ROS metabolism upon mechanical stress in plant cells. Our data obtained by applying carbon-filled quartz micropipettes with platinum-coated tips (oxygen nanosensors) showed a considerable drop in oxygen concentration at *Chara corallina* cell surface in response to microperforation of the cell wall (CW). The oxygen gulp activated by mechanical stress is dependent on stretching of plasma membrane, calcium fluxes across plasmalemma and dynamical rearrangements of micritubules. We tested possible involvement of the suppression of photosynthesis, the enhancement of respiration, and the activation of the plasma membrane NADPH oxidase as an origin of oxygen decline upon CW microwounding. The results provide evidence for major role of plasmalemmal NADPH-oxidase in the discovered local drop of O_2 content.

P.3.3.A-005
Development of sensitive SERS-based approaches to study redox state of cytochrome C in living mitochondria

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The redox state and the conformation of cytochrome C (cyt C) depend on interaction with complexes of the electron transport chain (ETC), the electron transfer efficiency and properties of mitochondria intermembrane space (IMS). Impairment of electron transfer leads to overproduction of reactive oxygen species and cytochrome C-related apoptosis. This implicates mitochondria in several human diseases, including hypertension and cardiac dysfunction. Therefore, methods for direct investigation of cyt C redox state and its conformational dynamics in living mitochondria are essential for understanding of mechanisms