NATIONAL RESEARCH, DEVELOPMENT AND INNOVATION OFFICE HUNGARY
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2025 Redox Chemistry / Biochemistry Graduate Summer Course

Summary of presentations

UNIVERSITY of DEBRECEN

Karolinska Institutet

MUSC Medical University of South Carolina

University of Nebraska Lincoln

National Tumor Biology Laboratory

Society for Free Radical Research Europe

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Lectures

Lecture 1	
Title	**Redox cycling and basic principles of redox biochemistry**
Speaker	Prof. Elias Arnér, MD, PhD
Affiliation	Division of Biochemistry, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden Department of Selenoprotein Research and National Tumor Biology Laboratory, National Institute of Oncology, Budapest, Hungary
Summary	This introductory lecture covers the fundamentals of redox reactions and their importance in biochemistry. One- and two electron transfer processes are presented, resulting in radicals or molecules with closed-shell electron structure. The concepts of electrochemical equilibria and redox potentials are introduced, along with parameters affecting the latter, incorporated in the Nernst-equation. Redox cycling are illustrated through biological examples. The production of reactive oxygen species (ROS) as part of oxygen-based life is explained, along with fundamental notions as oxidative stress and antioxidant enzyme families. Finally, redox regulation by oxidative modification is presented and potential methods for the quantification of ROS are briefly mentioned.

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Lecture 2	
Title	**Oxidative stress, introduction to the concept**
Speaker	Prof. Oleh Khalimonchuk, PhD
Affiliations	Department of Biochemistry, University of Nebraska-Lincoln, Lincoln, NE, United States Nebraska Redox Biology Center, Lincoln, NE, United States Fred & Pamela Buffett Cancer Center, Omaha, NE, United States
Summary	The presentation outlines the major aspects of oxidative stress, starting with primary production pathways of reactive oxygen species. ROS arise from endogeneous sources, such as mitochondrial respiration, or originate from exogeneous exposure. Elevated ROS production is associated with various detrimental effects, leading to tissue damage and ultimately cell death. Cells operate an arsenal of antioxidant strategies, including low molecular weight scavengers (such as glutathione, GSH) and intricate enzymatic machineries. Kinetic and mechanistic features of antioxidant protection are presented. ROS-associated signaling capacities are also discussed. H_2O_2 is an emerging second messenger with implications on redox regulation. Thiol- and metalloprotein-based peroxide sensing and transcriptional consequences are shown.

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Lecture 3	
Title	**The thioredoxin system**
Speaker	Prof. Edward E. Schmidt, PhD
Affiliations	Department of Microbiology and Cell Biology, Montana State University, Bozeman, MT, USA Department of Anatomy and Histology, HUN-REN-UVMB Laboratory of Redox Biology, University of Veterinary Medicine, Budapest, Hungary
Summary	NADPH represents a common source of electrons (i.e. reducing power) for the main intracellular disulfide reducing pathways, the thioredoxin (Trx) and the glutathione (GSH) systems. NADPH arise primarily from glucose metabolism and used directly as cofactor by thioredoxin reductase (TrxR) and glutathione reductase (Gsr), two central players of redox homeostasis. A common target of the two enzyme systems and, indeed, the system that originally led to their discovery, is ribonucleotide reductase (RNR), a critical enzyme that provides the precursors for DNA synthesis. Subsequently, it was discovered that these systems reduce an array of disulfide substrates, with widespread effects in redox balance, cell homeostasis, and signal transduction. Genetic disruption of the members of the Trx or GSH systems are followed by redirected electron flux and potential mutual replacement of the two pathways. Finally, TRP14 is introduced, a newly described thioredoxin-like protein with unique reactivity profile, including being recently discovered as the rate-limiting cystine reductase in cells across phyla.

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Lecture 4 – Open lecture	
Title	**Selenium and selenoproteins in human health and disease**
Speaker	Prof. Elias Arnér, MD, PhD
Affiliation	Division of Biochemistry, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden Department of Selenoprotein Research and National Tumor Biology Laboratory, National Institute of Oncology, Budapest, Hungary
Summary	Selenium research has recently celebrated its 200th anniversary, dated back to 1817 with the discovery of the element by the Swedish scientist Jacob Berzelius. Selenium is an essential trace element for human health, which primarily occurs endogeneously incorporated into Selenocysteine (Sec, U), conceived as the 21st proteinogenic amino acid. The integration of Sec into target proteins requires an intricate translational machinery, including a dedicated tRNA construct. The human genome encodes 25 selenoproteins, several of which are indispensable for life. Mammalian thioredoxin reductases (TrxRs) are prime examples of selenoenzymes, making any Trx-dependent reducing functions selenium-dependent. TrxR1 is highly druggable due to the increased reactivity of Sec compared to normal cysteine. Specific inhibitors have been found against TrxR1 as potential drug candidates in anti-cancer endeavors. Its inhibition largely affects cysteine supply through TRP14. Human glutathione peroxidases (Gpx1 and Gpx4) are further important representatives of the selenoproteome, with modulatory role in ferroptosis, an iron-associated cell death.

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Lecture 5	
Title	**Glutaredoxins**
Speaker	Lucia Coppo, PhD
Affiliation	Division of Biochemistry, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden
Summary	Glutathione (GSH) play a variety of roles in redox biology, from counteracting oxidative stress by scavenging oxidants and mediating antioxidant enzyme functions (Gpxs), to the regulation of protein functions by glutathionylation (P-GSH), or supporting disulfide reduction by glutaredoxins (Grxs). Human glutaredoxins are dedicated disulfide reductases which catalyze the formation and reduction of protein disulfides or mixed disulfides between protein thiols and GSH. Based on their catalytic mechanism, they can be classified into monothiol or dithiol groups. Typical dithiol Grxs include human Grx1 and Grx2 (cytosolic and mitochondrial, respectively), and Grx3 and Grx5 belong to the monothiol type. The latter contain essential iron-sulfur clusters, affecting iron homeostasis. Pathological consequences of Grx mutations are summarized in the lecture.

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Lecture 6	
Title	**Oxygen and blindness of premature newborns**
Speaker	Prof. György Balla, MD, PhD, DSc
Affiliation	ELKH-UD Vascular Biology and Myocardial Pathophysiology Research Group, Hungarian Academy of Sciences, Budapest, Hungary. Department of Pediatrics, Faculty of Medicine, University of Debrecen, Debrecen, Hungary.
Summary	Retinopathy of prematurity (ROP), a major cause of blindness, is a severe chronic morbidity affecting preterm newborns. A deeper understanding of its mechanistic background may support more effective prevention. Pathological angiogenesis is a key factor in many complications of prematurity, with abnormal oxygen exposure being a primary risk factor for ROP. After birth, preterm infants are exposed to relative hyperoxia compared to intrauterine conditions. In the first stage of ROP, excess oxygen and reduced levels of vascular endothelial growth factor (VEGF) impair vessel growth. This is followed by hypoxia-driven pathological neovascularization in the second stage. These processes are redox-regulated and exacerbated by heme toxicity. Retinal hemorrhage releases free heme, which activates pro-angiogenic factors like HIF1α, increasing VEGF expression. Heme also acts as a pro-oxidant molecule, promoting reactive oxygen species and oxidative stress. Cells protect themselves by inducing heme-oxygenase (HO-1) and the ferritin system. Targeting these heme-mediated pathways may offer new strategies to counteract the progression of ROP.

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Lecture 7	
Title	**Reductive stress**
Speaker	Prof. Kenneth D. Tew, PhD, DSc
Affiliation	Department of Cell and Molecular Pharmacology and Experimental Therapeutics, Medical University of South Carolina, Charleston, SC, USA
Summary	Reductive stress is a condition characterized by excess accumulation of reducing equivalents (e.g., NADH, NADPH, or GSH), surpassing the activity of endogenous oxidoreductases. Excessive reducing equivalents are linked with many human pathologies, such as aging, heart disease, diabetes, neurodegenerative syndromes or cancer. The expanding understanding of reductive stress underscores its significance in these pathologies. Future goals include the development of selective biomarkers to distinguish physiological from pathological reductive stress, the optimization of therapeutic interventions that target redox imbalances without disrupting normal cell functions and exploration of the interplay between reductive stress and immune/endocrine regulation to uncover novel treatment strategies.

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Lecture 8	
Title	**Persulfidation in Redox Biology**
Speaker	Éva Dóka, PhD
Affiliation	Department of Molecular Immunology and Toxicology and the National Tumor Biology Laboratory, National Institute of Oncology, Budapest, Hungary
Summary	Hydrogen sulfide (H_2S) has long been known as a toxic molecule, due to inhibition of respiratory Complex IV (cytochrome c oxidase). The discovery of endogeneous production of H_2S by the transsulfuration enzymes and the identification of H_2S as a signalling molecule introduced an additional layer of complexity to the diverse cellular redox network. H_2S primarily signals through persulfide generation on protein cysteine (Cys) residues, interactions with metalloproteins or cross-talk with ROS or RNS. The chemical biology of reactive sulfur species (RSS), including sulfide and per/polysulfide species is a rapidly developing research field. RSS can undergo a diverse array of biological reactions, the main focus of the lecture is the persulfidation of protein Cys residues and how it relates to redox regulation. Special emphasis is put on the protective effect of persulfidation under oxidative stress conditions, and their catabolism by the thioredoxin or glutathione systems.

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Lecture 9	
Title	**Mitochondria-borne reactive oxygen species**
Speaker	Prof. Oleh Khalimonchuk, PhD
Affiliations	Department of Biochemistry, University of Nebraska-Lincoln, Lincoln, NE, United States Nebraska Redox Biology Center, Lincoln, NE, United States Fred & Pamela Buffett Cancer Center, Omaha, NE, United States
Summary	Oxygen (O_2) is the basis of aerobic life as we know it today. Molecular oxygen has low reactivity with organic molecules due to its electronic structure. However, it undergoes a series of one-electron reduction steps in the course of respiration, orchestrated by the mitochondrial electron transport chain (ETC). Gradual reduction of O_2 produces a series of particles with elevated reactivity compared to oxygen, covered by the widely used term 'Reactive Oxygen Species' (ROS). Superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\bullet}$) are the major representatives of the group. Mitochondria are the primary source of ROS, especially Complex I and III producing superoxide with the aid of redox cofactors. NADPH oxidase is responsible for the majority of non-mitochondrial ROS production. Physiological levels of ROS play important roles in various cellular processes while accummulation of ROS is the leading cause of oxidative stress and tissue damage, therefore their concentrations are tightly controlled by various enzyme systems.

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Lecture 10	
Title	**Redox Regulation & Protein Folding: A Dynamic Interface**
Speaker	Prof. Danyelle M. Townsend, PhD
Affiliation	Department of Drug Discovery and Biomedical Sciences, Medical University of South Carolina, Charleston, SC, United States
Summary	Protein folding takes place in the endoplasmic reticulum (ER), a redox active organelle involved in the synthesis of secreted, membrane and lysosomal proteins, generation of lipid/phospholipid molecules and steroids, and calcium storage. The degradation of misfolded proteins is normally governed by ubiquitinylation, which targets them for degradation by the proteasome. However, the imbalance between the load and capacity of protein folding results in a cellular condition known as ER stress. The unfolded protein response (UPR) is the global phenomenon triggered by suboptimal folding, which is governed by the chaperone protein GRP78 (BiP) and transduced by 3 major receptors, IRE1, PERK and ATF6. Depending on the breadth and duration of the stress trigger, UPR may activate pro-survival signaling pathways through the restoration of ER homeostasis, or in severe cases, drive the cells to apoptosis. Protein glutathionylation by glutathione S-transferase pi (GSTP) plays a central role in the regulation of ER-proteins, such as protein disulfide isomerase (PDI) and impact cellular sensitivity to ER-stress inducing drugs.

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Lecture 11	
Title	**Poly(ADP-ribosyl)ation in oxidative stress signaling**
Speaker	Prof. László Virág, MD, PhD, DSc
Affiliation	Department of Medical Chemistry, Faculty of Medicine, University of Debrecen, 4032 Debrecen, Hungary HUN-REN-DE Cell Biology and Signaling Research Group, 4032 Debrecen, Hungary
Summary	Poly(ADP-ribosyl)ation (or PARylation) is a post-translational modification involving the covalent attachment of poly(ADP-ribose) chains to target proteins. This modification significantly alters the acceptor proteins' size, charge, and function. PARylation is primarily catalyzed by members of the poly(ADP-ribose) polymerase (PARP) enzyme family, with PARP-1 being the most extensively studied. PARP-1 functions as a DNA nick sensor, activated particularly by single-strand DNA breaks. Upon oxidative stress-induced DNA damage, PARP-1 activation can lead to DNA repair and promote cell survival; however, in the case of excessive damage, it may trigger cell death pathways such as apoptosis or necrosis. These outcomes are finely regulated by the dynamic balance between PARylation and its removal. PARP-1 regulation at the transcriptional level or via substrate (NAD^+) availability may enhance chemosensitivity of cancer cells. Finally, PARP-1 also functions as a transcriptional coactivator of NF-κB, linking it to the pathogenesis of various inflammatory diseases, including diabetes, arthritis, and respiratory tract infections.

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Lecture 12	
Title	**Exercise and epigenetics**
Speaker	Prof. Zsolt Radák, PhD, DSc
Affiliation	Research Institute of Molecular Exercise Science, Hungarian University of Sports Science, Budapest, Hungary
Summary	This lecture presents exercise-induced epigenetic traits, with special focus on counteracting ROS-mediated DNA damage in three main sections: the oxidation of guanine (G) into 8-oxoG, DNA methylation in aging and the role of exercise produced lactate in histone modifications and demethylation of DNA. Guanine is the most susceptible DNA base towards ROS-mediated oxidation and the level of its oxidation product 8-oxo-7,8-dihydroguanine (8-oxoG) (or its nucleoside form, 8-hydroxy-2'-deoxyguanosine (8OHdG)) is an established marker of oxidative stress as well as aging. Regular exercise was proved to salvage age-related DNA damage by lowering 8OHdG in muscle tissue. Guanine oxidation is strongly linked to cytosine methylation. 8oxoG is sensed and removed by 8-oxoguanine DNA glycosylase (OGG1) during the DNA base excision repair (BER) pathway. OGG1 harbors ten-eleven translocation (TET) 1 protein, which oxidizes adjacent 5-methyl-cytosine initiating demethylation. DNA methylation correlates with aging and it was shown that lifestyle choices strongly affect its extent, establishing a dissection between biological and chronological age. An epigenetic age assessment system called DNAmFitAge was created to monitor methylation traits as a function of time through life. This clock proved decelerated aging upon regular exercise training in general population and professional athletes as subjects. Finally, histone lactylation via lactate fermentation was shown to alter gene expression patterns and promote DNA demethylation.

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Lecture 13	
Title	**Mitochondria and their quality control**
Speaker	Prof. Oleh Khalimonchuk, PhD
Affiliations	Department of Biochemistry, University of Nebraska-Lincoln, Lincoln, NE, United States Nebraska Redox Biology Center, Lincoln, NE, United States Fred & Pamela Buffett Cancer Center, Omaha, NE, United States
Summary	Mitochondria are highly structured organelles responsible for a wide array of physiological functions, such as respiration and ATP synthesis, generation of redox cofactors and various metabolites, etc. Mitochondrial disfunctions are associated with severe pathologies, including neurodegenerative and cardiovascular diseases and cancer. Therefore, multiple mechanisms are in effect to ensure mitochondrial fidelity, i.e. maintenance of structural and functional integrity. Mitochondrial quality control (MQC) comprises two closely related levels, molecular and organellar responses. Molecular (intramitochondrial) QC largely relies on proteases and chaperones for proper protein folding etc.. Organellar MQC include dynamic processes such as fusion or fission or mitophagy to deplete damaged or dysfunctional mitochondria. Finally, mitochondria effectively communicate with nuclei by signal transduction or metabolite transport and have the capability to induce global signaling pathways or bring about epigenetic modifications.

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Lecture 14	
Title	**Mouse models for redox studies**
Speaker	Prof. Edward E. Schmidt, PhD
Affiliations	Department of Microbiology and Cell Biology, Montana State University, Bozeman, MT, USA Department of Anatomy and Histology, HUN-REN-UVMB Laboratory of Redox Biology, University of Veterinary Medicine, Budapest, Hungary
Summary	Mice provide uniquely powerful models for studying redox biology in contexts that mimic clinical situations. The availability of diverse well-characterized genetically engineered mouse (GEM) models, the relative ease of engineering one's own unique GEM models, and the extensive experiments that can be performed using mice combine to make these valuable contributors to basic and biomedical research. In this lecture, students are familiarized with the design and technologies underlying development of novel GEMs such that they might better understand, design, and evaluate work using these models. This begins with an overview of classical gene-targeting work involving homologous recombination in embryonic stem cells, use of these for producing chimeric animals, and husbandry to generate desired allelic combinations. After, they are familiarized with contemporary advances for using CRISPR/Cas9 to disrupt genes or mediate efficient recombination, use of viral vectors for targeted genetic engineering of somatic cells in adult mice, and utilization of Cre/LoxP conditional alleles and fluorescent reporters in the mice. They also are introduced to types of experiments that can be done with such models, including surgical procedures, development of cancer models, metabolic flux studies, and others. Finally, the students are presented with some of our own work which shows how strategic use of GEM models has allowed identification and characterization of potent metabolism-based redox systems that had evaded detection in other models.

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Lecture 15	
Title	**Cysteine Metabolism in Cancer**
Speaker	Prof. Péter Nagy, PhD, DSc
Affiliation	Department of Molecular Immunology and Toxicology and the National Tumor Biology Laboratory, National Institute of Oncology, Budapest, Hungary Chemistry Coordination Institute, University of Debrecen, Debrecen, 4012, Hungary Department of Anatomy and Histology, HUN-REN-UVMB Laboratory of Redox Biology, University of Veterinary Medicine, Budapest, Hungary
Summary	Altered cellular metabolism is a hallmark of cancer. Reprogramming of the transulfuration (TS) pathway, the metabolic route responsible for the generation of cysteine, is an emerging concept in tumor biology. Modified transulfuration has been observed in several agressive tumor types which are irresponsive towards traditional cancer treatment and lack modern, targeted therapeutical options. This lecture presents recent findings regarding the roles of TS enzymes in triple negative breast cancer (TNBC), pancreatic ductal adenocarcinoma (PDAC) and Braf V600E mutant melanoma. CBS and CSE provide the increased cysteine demand for uncontrolled proliferation. Furthermore, they generate reactive sulfur species (RSS) such as H_2S or persulfide species (CysSSH, GSSH) which contribute to the regulation of tumor formation and growth. Their functions affect protection against oxidative stress, bioenergetics, hypoxia, altered activity of signaling proteins and metastasis. New therapeutic targets may arise from the detailed molecular investigation of these complex processes.

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Lecture 16	
Title	**Application and limitations of tools for redox analysis in live cells**
Speaker	Karoline Scholzen
Affiliation	Division of Biochemistry, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden
Summary	Detection method development is a crucial ongoing endeavor in redox biology. Traditional methods used for monitoring cellular redox processes include the ratio of well-known redox couples (i.e. $NADH/NAD^+$, GSH/GSSG), assessment of protein redox states on Western blots, enzyme expression etc. and most of them operate with cell lysates, which is an artificial redox milieu. However, these methods provide static information rathen than follow dynamic changes that take place during actual redox reactions. Current methods are built on live-cell analysis to gain realistic data on dynamic redox systems. Addressing live cell cultures raise their own challenges, such as uptake, selectivity or toxicity issues of the applied probes. Currently available ROS-reactive probes include fluorescent proteins such as HyPer and roGFP, and small molecule agents containing a biosensor and a reporter moiety. Advantages and limitations of modern tools are discussed in the lecture, emphasizing that detailed understanding of the studied system and the applied probes is primordial for reliable measurements and technological caveats call for cautious data interpretation in the assessment of biological redox systems.

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Lecture 17	
Title	**Recent developments in recombinant selenoprotein studies**
Speaker	Qing Cheng, PhD
Affiliation	Division of Biochemistry, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden
Summary	Selenocysteine (Sec, U) is the 21st naturally occurring proteinogenic amino acid. Sec is an analogue of cysteine (Cys, C), with the sulfur atom replaced to selenium, bestowing the molecule with stronger acidity and nucleophilicity, which make Sec highly reactive to most electrophilic agents. Selenoproteins are found in all three domains of life, human cells have 25 of them, including thioredoxin reductases, glutathione peroxidases, iodothyronine deiodinases, formatedehydrogenases, glycine reductases and some hydrogenases. Sec is encoded by a UGA codon, which is usually a stop codon, resulting in halted translation. Therefore, selenoprotein expression requires additional genetic information, in the form of a species-specific selenocysteine insertion sequence (SECIS) element in the mRNA. Recombinant expression systems of human selenoproteins in have been accomplished in E.coli strains, with both terminal and internal Sec incorporation. Mammalian thioredoxin reductase 1 (TrxR1) and glutathione peroxidase 1 (Gpx1) have been successfully produced in bacteria, with high purity, yield and activity. Ongoing approaches aim to implement Sec incorporation on multiple codons, expanding the genetic code to facilitate further studies of various selenoproteins.

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Lecture 18	
Title	**Glutathione homeostasis & Glutathione S-Transferases**
Speaker	Prof. Kenneth D. Tew, PhD, DSc
Affiliation	Department of Cell and Molecular Pharmacology and Experimental Therapeutics, Medical University of South Carolina, Charleston, SC, USA
Summary	Glutathione (GSH) is a tripeptide consisting of glutamate, cysteine and glycine, which plays a leading role in cellular redox balance. GSH is very abundant (~5mM) in most cells and its Cys thiol side chain undergoes reversible oxidation to disulfide (GSSG). GSH/GSSG ratio is a marker of cellular redox state. This ratio is regulated by multiple enzyme systems, such as glutathione peroxidases (Gpxs) which use GSH to reduce H_2O_2, and glutathione reductase (GR), reducing GSSG. The GSH/GSSG couple may assist protein folding through thiol-disulfide exchanges and GSH reacts with various electrophiles, oxidizing agents, radicals and metal ions to preserve the redox homeostasis. GSH is conjugated to drug molecules during Phase II detoxification by glutathione S-transferases (GSTs). Glutathione S-Transferase P (GSTP) is a special representative of the GST superfamily, with high expression in tumor and drug resistant cells. It regulates C-jun kinase (JNK) by non-covalent binding, reactivates oxidized peroxiredoxins and mediates widespread glutathionylation, playing central roles in the cells' redox network.

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Lecture 19	
Title	**Metals and redox stress**
Speaker	Prof. Jaekwon Lee, PhD
Affiliation	Department of Biochemistry, Redox Biology Center, University of Nebraska-Lincoln, Lincoln, NE, USA
Summary	Metal ions play a plethora of biological roles, acting as abundant biological elements with fundamental functions (Na^+, K^+, Ca^{2+}, Mg^{2+}), essential trace elements (mostly 3d metals), environmental toxins ($As^{3+/5+}$, Pb^{2+}, Hg^{2+}, Cd^{2+}) or even therapeutic agents (Pt^{2+}, Au^{3+}, Ag^+, Cu^{2+}). More than a third of the human proteome contains one or more metal ions. Metalloproteins cover a wide range of functions, such as electron transfer, oxygen transport and storage, neurosignaling, catalytic functions and structural stabilization, ion homeostasis as well as signal sensing and transduction. Toxicity of metal ions may originate from ROS generation through Fenton-type reactions, thiol oxidation or non-specific binding to sulfur species. Both surplus and deficiency of bioactive metals are correlated to disease conditions, therefore metal ion homeostasis (uptake, storage, delivery and detoxification) is tightly controlled to avoid toxification and provide sufficient supply simultaneously. Iron and copper are transition metal ions with utmost importance in redox biology, given their involvement in the respiratory chain and ROS removal (superoxide dismutase). Their bioprocessing is discussed in details, along with recent findings on metal transport, and potential roles in immune defense, lipid metabolism and anticancer efforts.

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Lecture 20	
Title	**Trace elements and redox signaling**
Speaker	Prof. Anna Kipp, PhD
Affiliation	Friedrich Schiller University Jena, Jena, Germany
Summary	The lecture focuses on the diverse redox signaling capacities of trace elements (TE), with special focus on iron (Fe), copper (Cu), selenium (Se) and zinc (Zn) and their cross-talk. While Fe and Cu are directly involved in redox reactions due to their changing redox state, Se and Zn rather modulate the cellular redox landscape as selenoproteins or via increased binding affinity to thiol proteins, respectively. The Keap1/Nrf2 axis is a master regulator of redox homeostasis. Trace elements may induce Nrf2 activation by various mechanisms, while activated Nrf2 promotes the uptake and storage of TE under oxidative stress, supporting antioxidant functions. Widespread interactions of trace elements in redox signaling, including Nrf2 activation, has been addressed and presented herein, along with potential pathophysiological implications.

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Lecture 21	
Title	**Vascular calcification - What the clinician sees and does**
Speaker	Prof. József Balla, MD, PhD, DSc
Affiliations	Department of Internal Medicine, Division of Nephrology, Faculty of Medicine; HUN-REN–UD Vascular Biology and Myocardium Pathophysiology Research Group, Hungarian Academy of Sciences, University of Debrecen, Debrecen, Hungary
Summary	Chronic kidney disease (CKD) is a prevalent health issue in Hungary. End-stage patients have a high likelyhood to develop fatal cardiovascular complications, such as atherosclerosis and increased vascular calcification. It was shown that calcification entails osteoblastic trans-differentiation of smooth muscle cells. Atheromatic lesions release highly oxidative heme intermediates aggravating tissue damage. It was found that H_2S inhibits vascular calcification and thus counteract CAVD (calfified aortic valve disease) by multiple means, i.e. capture of inflammatory cytokines. Interestingly, bioavailable H_2S level is decreased in calfified aortic valve compared to healthy valves, despite the increased expression and activity of cystathionine gamma lyase (CSE). Mitochondrial H_2S oxidation is in overdrive in the system, yet mitochondria targeted H_2S donors salvage the calcification phenotype. These findings suggests additional roles of sulfide oxidation products in this pathology and a bi-phasic adaptation sequence or H_2S metabolism to the calcification signal.

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Lecture 22	
Title	**Integration of Signaling Pathways: Free Radical, Protein Phosphorylation and Steroids in Carcinogenesis**
Speaker	Prof. Ming-Fong Lin, PhD
Affiliation	Department of Biochemistry and Molecular Biology, University of Nebraska Medical Center, Omaha, NE, USA
Summary	Cellular signaling is a complex network in which a primary signal (such as hormone release) is linked to the downstream cellular responses (growth, differentiation, cell death etc.) by multiple biochemical pathways. Reversible phosphorylation reactions represent the most thoroughly studied signaling events, catalyzed by phosphatases and kinases. Serine, threonine and tyrosine residues are the primary targets of protein phosphorylation. Steroid hormones comprise a large class of signaling molecules that are classically known to regulate gene expression by binding to intracellular receptors, and recently found in regulating non-genomic actions such as activating membrane receptors and modulating ion channel activity or ROS production. Free radicals (O, N, C or S-centered) are now recognized as signaling agents, not only oxidizing species. The p66Shc (Src homologue and collagen homologue) adaptor protein via interaction with cytochrome C serves a signaling hub that integrates the above mentioned pathways induced by diverse stimuli. Its downstream responses include apoptosis, cell growth and survival, thus leading to carcinogenesis, tumor progression and other aging-related pathologies.

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Lecture 23	
Title	**Nitric Oxide signaling**
Speaker	Prof. Danyelle M. Townsend, PhD
Affiliation	Department of Drug Discovery and Biomedical Sciences, Medical University of South Carolina, Charleston, SC, United States
Summary	Nitric oxide (NO) is a small gaseous signaling molecule with widespread physiological roles. It was first described as an endothelium derived relaxation factor (EDRF) and the Nobel-prize was awarded in 1998 for discoveries of its cardiovascular actions. NO is a lipid soluble, membrane permeable compound with a free radical character. It is endogenously produced from arginine by three nitric oxide synthases (NOS), depending on cell type, or from inorganic nitrogen species (nitrate, nitrite) by reduction. NO exerts its biological functions by direct or indirect approaches. Direct effects include coordination with metal complexes, such as guanylyl cyclase (GC) or cytochrome enzymes. GC is the primary NO sensor and the increased production of cGMP is crucial in NO-mediated vasodilation. Indirect effects are attributed to the formation of reactive nitrogen species (RNS) such as N_2O_3 or peroxynitrite (ONOO-), S-Nitrosation on Cys residues, DNA strand breaks and nitration on Tyr residues. S-nitrosation is a highly studied thiol protein modification, affecting the activity of a large number of signaling and metabolic proteins. Altered NOS levels contribute to pathophysiology of cardiovascular or neurodegenerative diseases. NO-related drug development efforts led to the introduction of PDE5 inhibitors and various donors are in use as heart medication.

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Lecture 24	
Title	Structural Basis of Iron-Sulfur Proteins in Redox Regulation
Speaker	Limei Zhang, PhD
Affiliations	Department of Biochemistry; Redox Biology Center; Nebraska Center for Integrated Biomolecular Communication, University of Nebraska-Lincoln, Lincoln, NE, USA
Summary	Iron-sulfur cluster (ISC) proteins are a structurally and functionally diverse family with broad involvement in redox processes. Fe-S clusters emerged very early on the evolutionary timescale and they are essential in all domains of life. ISCs have various structures. The three most common types are 2Fe-2S, 3Fe-4S and 4Fe-4S core units and more elaborately built clusters also occur. Carrying proteins generally harbor ISCs via their Cys-S, His-N, Asp-O/Thr-O/Ser-O/Glu-O coordination sites, or through non-protein ligands, such as glutathione-S. The redox potentials of ISCs span over 1V range and their composition allows charge distribution, which accounts for colorful reactivity and substrate selectivity. Fe-S proteins play versatile functions in electron transfer processes, substrate activation, Fe and S supply, translational and post-translational regulation etc. ISC biogenesis requires an intricate assembly line with multiple core elements. ISC-based regulators of gene expression in various organisms may respond to O_2, ROS or NO signals. Recent technological innovations in structural biology, spectroscopy and omics promise new insights into the redox regulation by Fe-S sensors.

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Lecture 25	
Title	**Oxidative stress-inducing bacterial metabolites regulating breast cancer behavior**
Speaker	Prof. Péter Bay, PhD, DSc
Affiliations	Department of Medical Chemistry, Faculty of Medicine; HUN-REN Cell Biology and Signaling Research Group; The Hungarian Academy of Sciences, Center of Excellence; MTA-DE Lendület Laboratory of Cellular Metabolism; Research Center for Molecular Medicine, Faculty of Medicine, University of Debrecen, Debrecen, Hungary
Summary	Anomalies of the resident microbiome (dysbiosis) are associated to an array of pathologies. Oncobiosis refers to dysbiosis supporting tumor progression. Breast cancer patients experience oncobiosis at multiple sites, such as the GI tract, blood, breast tissue, milk ducts and urinary tract. However, it is unclear whether oncobiosis is the source of malignancy or rather an accompanying issue. Tumors are intertwined with the host microbiome, and it was shown that bacterial metabolites exert hormone-like effects in breast cancer models, with cytostatic and/or proliferative outcomes. A subset of cytostatic bacterial metabolites are redox-sensitive and elicit redox-associated outcomes, likely shifting tumoral reductive stress towards oxidative of nitrosative stress. For example, litocholic acid (LCA), a bile acid derivative, suppresses Nrf2 activation in tumor cells, but not in non-transformed fibroblasts. Understanding the anti-progression and anti-metastatic features of (tumor) cytostatic metabolites holds promise in future therapeutic interventions.

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Lecture 26	
Title	**Using stable sulfur isotopes for tracking redox metabolism**
Speaker	Colin Miller, PhD
Affiliations	Panosome GmbH, Heidelberg, Germany
Summary	NADPH is the shared cofactor of two central disulfide reductase enzymes, thioredoxin reductase (TrxR) and glutathione reductase (GR). As such, it acts as the common fuel of disulfide reduction machineries, the thioredoxin (Trx) and the glutathione systems (GSH), which are responsible for redox homeostasis of the cells, nucleotide synthesis etc. Full-body knockout of both TrxR1 and GR is lethal due to the lack of reduction power, yet conditional hepatocyte-specific knockout TR/GR-null mice are fully viable, which suggests altered routes of sulfur amino acid (Cys, Met) metabolism. In order to implement a reliable monitoring approach to follow these pathways, we have synthesized stable ^{34}S-labeled methionine and cysteine with in-house apparatus and set up mass spectrometry-based analysis to gain quantitative information on their fluxes. Prolonged, continuous delivery of these compounds to our rodent models was realized through catheterization of the jugular vein, which was proved superior to common administration methods, such as tail injections. Our findings suggest additional roles of the transsulfuration pathway in the maintenance of sulfur supply in the reductively impaired hepatocytes. Our experimental methods provide affordable and accessible means to track redox metabolism with ample potential for future applications.

PROJECT FINANCED FROM THE NRDI FUND

Lecture 27	
Title	**Peroxidasin: a link between reactive oxygen species and extracellular matrix**
Speaker	Prof. Miklós Geiszt, MD, PhD, DSc
Affiliation	Department of Physiology, Faculty of Medicine, Semmelweis University, Budapest, Hungary
Summary	Peroxidasin (PXDN) is a somewhat undercharacterized mammalian heme-peroxidase whose primary function lies in the synthesis of extracellular matrix (ECM). PXDN is a large protein with a homotrimeric structure, where subunits are linked by disulfide bonds. Peroxidasin contributes to the establishment of collagen IV network in the basement membrane through the creation of sulfilimine (S=N) crosslinks between Met and Lys residues. This step requires hypobromite (HOBr) as oxidizing agent which is produced by PXDN from H_2O_2 and bromide. Hydrogen peroxide for this activity originates from currently unknown sources, other than well-established NADPH-oxidase mediated production. PXDN activity may be monitored via the analysis of the crosslinking state of the NC1 domains of collagen IV, following the collagenase-mediated digestion of cell culture lysates. Furthermore, a signal amplification was observed in the Amplex Red assay upon the addition of increasing bromide, which allowed determination of PXDN activity in live cells. PXDN biosynthesis involves a proteolytic step by, which is largely dependent on the enzyme's peroxidase activit, therefore subject of redox regulation. Peroxidasin was shown indispensible for normal eye development in human patients and rodent models and the lack of cross-linking capacity may result in other pathologies.

PROJECT FINANCED FROM THE NRDI FUND

Young Investigators' Satellite Day - Student Group Presentations

Group 1 - Thiol redox systems	
Speakers	
Name	**Affiliation**
Rachelle Nelson	UNL
Katalin Fruzsina Magi	UD
Daniella Dörgő	NIO
Bohdana Sokolova	KI
Thomas Dempster	MUSC
Balázs Gombos	UVMB
Questions / Viewpoints	

1. What is the function of thioredoxin? How many thioredoxin isoforms are in humans and where are they localized in the cell? Describe the structure of thioredoxin. Name at least three protein targets of thioredoxin.
2. What is the function of glutaredoxin? How many glutaredoxin isoforms are in humans and where are they localized in the cell? Describe the structure of glutaredoxin. Name at least three protein targets of glutaredoxin.
3. Describe the enzyme systems responsible for recycling thioredoxin and glutaredoxin by showing the structures and catalytic mechanisms. Where are these recycling enzymes localized in mammalian cells? In plants?
4. Describe the biosynthesis pathway for glutathione and the system for maintainining the intracelullar GSH/GSSG ratio. What are the concentrations of glutathione in different intracellular compartments (e.g., cytosol, mitochondria, endoplasmic reticulum). How is glutathione imported into cells?

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Group 2 - Metals and Trace Elements in Redox Biology	
Speakers	
Name	**Affiliation**
Emma Bergmeyer	UNL
Yuchao Ding	UD
Martina Serrano Álvarez	US
Andrea Cecília Badari, PhD	UVMB
Mattia Russel Pantalone	KI

Questions

1. Which metals are the major contributors to hydroxyl radical formation in cells? What are the intracellular concentrations of these metals in mammals? Show the reaction by which these metals catalyze the formation of hydroxyl radicals. Provide an example of how mammalian cells import/export metals?
2. How do mammalian cells import/export metals? How are import/export systems used to protect against stress? Provide specific examples.
3. Give an example of a metalloprotein that is involved in oxygen or redox sensing? Describe the mechanism of response.
4. Give some examples on how Zinc, Copper and Iron are linked to different redox processes in cells.
5. Give four examples each of selenoproteins from bacteria, archaea and eukaryotes, as well as cysteine orthologues of each of these selenoproteins from other organisms. Provide some hypotheses on why or how selenoproteins have evolved, and why not all organisms have them.

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Group 3 - Redox regulation	
Speakers	
Name	**Affiliation**
Brett Hilbers	UNL
Njomza Gashi	UD
Viola Varga, PhD	SU
Alexander van Deventer	KI
Kathleen Klinzing	MUSC

Questions

1. Describe the mechanism by which OxyR responds to hydrogen peroxide and regulates gene expression. Include structures if available. Describe at least five genes regulated by OxyR.
2. Describe the mechanism by which Yap1p is involved in the response to oxidative stress in S. cerevisiae. What target genes are activated by Yap1p?
3. Describe the Keap1-Nrf2-ARE signaling pathway. How does it help cells respond to oxidative stress? What genes are regulated by this system?
4. Give at least four levels of redox control that affect cellular responses to insulin.

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Group 4 - Oxidative Stress and Antioxidant Enzymes	
Speakers	
Name	**Affiliation**
Adedotun Adefolalu	UNL
Kristóf Guy	UD
Maja Mikolás	UD
Krisztina Veszelyi	SU
Kia Liermann-Wooldrik	UNMC
Linglong Huang	KI

Questions / Viewpoints

1. How many superoxide dismutase (SOD) genes are in E. coli, B. subtilis, S. cerevisiae, and humans? What metal cofactors are utilized by the different SODs and where are the SODs localized? Describe the overall reaction catalyzed by SOD. Does SOD have a role in redox signaling? If so, how?
2. How many catalase genes are in E. coli, B. subtilis, S. cerevisiae, and humans? What cofactor does catalase require? Where are the different catalase enzymes localized? Are there any diseases or phenotype associated with lack of catalase?
3. How do peroxiredoxins protect cells against oxidative stress? Describe the reaction catalyzed by peroxidredoxin. Where are peroxiredoxins localized in mammalian cells? Do peroxiredoxins have a role in redox signaling? If so, how?
4. What are the biochemical functions of glutathione peroxidases? Describe the reaction catalyzed by glutathione peroxidase. Where are glutathione peroxidases localized in mammalian cells?
5. Describe DNA and lipid modifications that are found in cells exposed to oxidative stress agents? How do these modifications affect cell viability and contribute to disease progression?
6. What are some of the most common protein modifications that occur under oxidative stress? Describe how oxidative modification of SOD is associated with a disease.
7. How do cells repair oxidatively damaged DNA and proteins? Describe a specific example of one DNA and one protein repair enzyme.

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Group 5 - Reactive sulfur species in redox biology	
Speakers	
Name	**Affiliation**
David Obe	UNL
Katalin Éva Sikura, PhD	UD
Zoe Seaford	MSU
Xueping Jiang	KI
Kathryn Glorioso	MUSC
Avia Simmons	MSU
Questions / Viewpoints	

1. Describe sources of biological sulfur. Briefly present metabolic routes of amino acids cysteine and methionine.
2. Mention some pathophysiological roles of transsulfuration enzymes in cancer models and metabolic diseases (i.e. homocystinuria)
3. Reactive sulfur species arising from cysteine metabolism. Give 3 examples for the biological roles of H2S and/or cysteine persulfidation. What is the role of selenoproteins in the metabolism of persulfide species.
4. How RSS may contribute to mitochondrial bioenergetics?

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Posters

P1

Potential Protective Effect of Hydrogen Sulfide and Persulfidation Against Overoxidation of Peroxiredoxin 2

Presenting author: Daniella Dörgő

Affiliations:

Synthetic, Organic and Biomolecular Chemistry Program, Hevesy György PhD School of Chemistry, Eötvös Loránd University, Hungary

Department of Molecular Immunology and Toxicology and the National Tumor Biology Laboratory, National Institute of Oncology, Budapest, Hungary

Supervisor: Prof. Péter Nagy, PhD, DSc

Brief summary of research:

Since the discovery of the biological significance of hydrogen sulfide, which was previously known only for its toxic effects, it has become a major player in redox biology. Now it is considered as the third gaseous signaling molecule besides nitric oxide (NO) and carbon monoxide (CO). It is thought to be involved in protection against oxidative stress, a process that causes biological damage, including aging and diverse pathological events. Oxidative damage is caused by reactive oxygen species (ROS), against which one possible defense mechanism is a direct reaction by antioxidant enzymes. One important representative of ROS is hydrogen peroxide, which can be broken down by the peroxiredoxin (Prx) protein family in a fast and selective reaction. However, in the presence of an excessive quantity of peroxide, these proteins become overoxidized, thereby losing their enzymatic function because the cells are unable to rapidly reduce the overoxidized form back to the active species. The overoxidized persulfide form of Prxs, on the other hand, can be reduced by the body, allowing the enzyme to regain its antioxidant activity. According to some theories, sulfide is involved in the persulfidation of thiol proteins to protect their functional cysteine residue from overoxidation. I investigated this phenomenon in my present work for one member of the Prx family, peroxiredoxin 2 (Prx2).

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P2

Hydrogen sulfide as an anti-calcification stratagem in human aortic valve: Altered biogenesis and mitochondrial metabolism of H_2S lead to H_2S deficiency in calcific aortic valve disease

Presenting author: Katalin Éva Sikura, PhD

Affiliation:

Division of Nephrology, Department of Internal Medicine, Faculty of Medicine, University of Debrecen, Hungary; ELKH-UD Vascular Pathophysiology Research Group, University of Debrecen, 11003, Hungary; Kálmán Laki Doctoral School, University of Debrecen, Debrecen, Hungary

Brief summary of research:

Background and Purpose Calcification of heart valves (CAVD) is a frequent pathologic finding in CKD (chronic kidney disease) mostly in the elderly. Hydrogen sulfide (H_2S) has been suggested to possess various anti-calcific actions. We aimed to investigate the precise role of H_2S in valvular calcification and to identify its targets in the pathogenesis.

Experimental approach Potential of H_2S for regulating mineralization/osteoblastic transdifferentiation of valvular interstitial cells (VIC; isolated from human aortic valves) focused on mithocondrial biogenesis.

Key results The expression of mitochondrial enzymes involved in H_2S catabolism including sulfide quinone oxidoreductase (SQR), the key enzyme in mitochondrial H_2S oxidation, persulfide dioxygenase (ETHE1), sulfite oxidase (SO) and thiosulfate sulfurtransferase (TST) were up-regulated in calcific aortic valve tissues, and a similar expression pattern was observed in response to high phosphate levels in VICs. AP39, a mitochondria-targeting H_2S donor, rescued VICs from an osteoblastic phenotype switch and reduced the expression of IL-1β and TNF-α in VICs. Importantly, we also showed that IL-1β and TNF-α provided an early and transient inhibition of VICs calcification and osteoblastic differentiation in healthy cells and that effect was lost as H_2S levels decreased. The benefit was mediated via CSE induction and H_2S generation.

Conclusion and implications We conclude that decreased levels of bioavailable H_2S in human calcific aortic valves result from an increased H_2S metabolism that facilitates the development of CAVD. CSE/H_2S represent a pathway that reverses the action of calcifying stimuli.

PROJECT
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P3

Ferryl hemoglobin generated by hemoglobin oxidation inhibits osteoclastic differentiation of macrophages in hemorrhaged atherosclerotic plaques

Presenting author: Erzsébet Zavaczki, PhD

Affiliations:

Department of Internal Medicine, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

HUN-REN-UD Vascular Biology and Myocardial Pathophysiology Research Group, Hungarian Academy of Sciences, Debrecen, Hungary

Brief summary of research:

Pathogenesis of vascular calcification is an active, finely tuned process with many similarities to the mechanism of skeletal bone formation. Arterial calcium deposits represent a unique scenario which might favor the formation of osteoclast-like cells (OLCs) from hematopoietic precursors. Within the intramural compartment of the arteries, OLCs might degrade mineral deposits, thereby attenuating calcification and counterbalancing the activity of VSMCs-derived osteoblasts. Intraplaque hemorrhage frequently occurs in atherosclerotic plaques resulting in cell-free hemoglobin, which is oxidized to ferryl hemoglobin (FHb) in the highly oxidative environment. The purpose of this study was to investigate whether the compensatory effect of OLCs in vascular calcification is influenced by products of Hb oxidation. FHb, but not ferrohemoglobin, decreased bone resorption activity and inhibited osteoclast-specific gene expression induced by RANKL. In addition, FHb inhibited osteoclastogenic signaling pathways downstream of RANK (receptor activator of nuclear factor kappa-B), it prevented the nuclear translocation of NFATc1 (nuclear factor of activated T-cells, cytoplasmic 1). Importantly, FHb competed with RANK for RANKL binding suggesting possible mechanisms by which FHb impairs osteoclastic differentiation. In diseased human carotid arteries, OLCs were abundantly present in calcified plaques and co-localized with regions of calcium deposition, while the number of these cells were lower in hemorrhagic lesions exhibiting accumulation of FHb despite calcium deposition. We conclude that FHb inhibits RANKL-induced osteoclastic differentiation of macrophages and suggest that accumulation of FHb in calcified area of atherosclerotic lesion with hemorrhage retards the formation of OLCs potentially impairing calcium resorption.

PROJECT
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P5

Chronic inflammation in skeletal muscle induces ER stress and activation of the IRE1a pathway

Presenting author: Alexander van Deventer

Affiliation:

Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden

Supervisor: Johanna Lanner, PhD

Brief summary of research:

Chronic inflammation in skeletal muscle is characterized by upregulation of inflammatory cytokines and oxidative stress. How these factors interplay and impact skeletal muscle function, is largely unclear. In my research project, I focus on how oxidative stress impairs muscle function, with a focus on oxidative protein folding in the sarcoplasmic reticulum (SR). Various pathway related to protein folding were analyzed so access how oxidative stress impairs SR homeostasis.

PROJECT
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P6

The Anti-Tumor Efficacy of TXNRD1 Inhibitor and Selenium via STAT3 Pathway on Cancers

Presenting author: Xueping Jiang

Affiliation:

Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden

Supervisor: Prof. Elias Arnér, MD, PhD

Brief summary of research:

The thioredoxin (Trx) system, comprising Trx reductase (TrxR), Trx, and NADPH, is a key antioxidant system that counteracts oxidative stress in mammalian cells. It has been proved that selenium (Se) can upregulate thioredoxin reductase 1 (TR1). TrxR1 inhibitors were also able to inhibit STAT3 transcriptional activity. STAT3 is an important family member of the STAT transcription factor family.

Our research will systematically characterize the molecular interplay between the TrxR1 inhibitor (TRi-1) and Se, particularly examining how this combination modulates STAT3 signaling pathway. However, the influence of Trx system inhibitors on the tumor immunosuppressive microenvironment remains unclear. We will further investigate their combinatorial effects on tumor microenvironment (TME) remodeling.

PROJECT
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P8

Study of glutathione- and thioredoxin-reductase function in mouse hepatoma cells

Presenting author: Balázs Gombos

Affiliation:

Department of Anatomy and Histology, HUN-REN-UVMB Laboratory of Redox Biology, University of Veterinary Medicine, Budapest, Hungary

Supervisor: Prof. Edward E. Schmidt, PhD; Prof. Péter Nagy, PhD, DSc

Brief summary of research:

Cellular processes such as DNA replication, respiration, metabolism, and inflammation generate reactive oxygen species (ROS) that oxidize cytosolic thiols to disulfides, threatening cellular redox balance. To counteract this, cells depend primarily on two major antioxidant systems: the thioredoxin (Trx1) and glutathione (GSH) pathways. Both systems utilize reducing equivalents from NADPH to drive the activity of thioredoxin reductase (TrxR1) and glutathione reductase (Gsr), respectively. These enzymes are essential for maintaining thiol redox homeostasis, protecting protein function, and minimizing oxidative damage. However, under conditions of elevated oxidative or toxic stress, the capacity of TrxR1 and Gsr can be overwhelmed, resulting in redox imbalance, protein dysfunction, and potentially cell death. Our research focuses on better understanding how cells adapt to the loss of these key disulfide reductases. Specifically, our objective is to isolate and characterize viable mouse hepatoma cells that are deficient in both TrxR1 and Gsr (TR/GR-null). Creating and studying these double knockout cell lines will allow us to explore how cells compensate for the absence of the primary NADPH-dependent disulfide reductase systems. We aim to investigate alternative redox and metabolic pathways that may be activated under these conditions. The TR/GR-null cell lines will serve as a powerful model to study compensatory mechanisms, including altered sulfur amino acid metabolism and redox buffering strategies. Ultimately, these studies will provide insights into cellular redox regulation and may inform the development of therapeutic strategies to either protect healthy cells or sensitize pathological cells, such as cancer cells, to oxidative stress.

PROJECT
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P9

Working Redox Biology: From Mouse Models to Liver Tumors

Presenting author: Andrea Cecília Badari, PhD

Affiliation:

Department of Anatomy and Histology, HUN-REN-UVMB Laboratory of Redox Biology, University of Veterinary Medicine, Budapest, Hungary

Brief summary of research:

The Redox Biology Laboratory, operating since 2021 at the University of Veterinary Medicine, is a dynamically developing research group specializing in the investigation of the physiological and pathological roles of redox systems at the cellular level, with a particular focus on cancer.

The aim of the research is to understand how cells can maintain redox homeostasis in the absence of classical disulfide reductase enzymes, such as TrxR1 and Gsr. The central hypothesis posits that cysteine persulfide (Cys-SSH), produced by PLP-dependent transsulfuration enzymes (CBS and CSE), may serve as an alternative reductive source under these conditions.

RBL operates modern, well-equipped molecular and cell biology laboratories and also maintains a dedicated SPF-status mouse care facility. This animal facility includes a cage-cleaning room, a large climate-controlled, HEPA-filtered vivarium for housing and general procedures, a surgical area, and new state-of-the-art Techniplast caging systems. The laboratory currently maintains mouse lines carrying more than 12 different engineered alleles and holds all the required ethical approvals for its animal experiments.

The lab's research heavily relies on advanced molecular, genetic, and in vivo experimental techniques, including genetically engineered mouse models (GEM), somatic CRISPR/Cas9 gene editing, whole-body metabolic labeling with stable isotope-labeled amino acids for metabolic flux experiments, primary cell explants, and the development of parallel GEM cell culture models.

Members of the group possess extensive practical expertise in surgical jugular cannulation, stable isotope labeling, and various microsurgical procedures. Tissue samples obtained from live animal experiments are transported on dry ice to partner institutions for further molecular and metabolic analyses.

PROJECT
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P10

Conversion of Cystine Into Cysteine Via Biochemical Reactions Using WT or TR/GR-Null Liver Models

Presenting authors: Zoe Seaford[1,2], Martina Serrano Álvarez[2,3]

Affiliations:

[1]Department of Microbiology and Cell Biology, Montana State University, Bozeman, MT, USA

[2]Department of Anatomy and Histology, HUN-REN-UVMB Laboratory of Redox Biology, University of Veterinary Medicine, Budapest, Hungary

[3]University of Seville, Seville, Spain

Supervisor: Prof. Edward E. Schmidt, PhD; Prof. Péter Nagy, PhD, DSc

Brief summary of research:

The NADPH-dependent enzymes thioredoxin reductase-1 (TrxR1) or glutathione reductase (Gsr) support basal homeostasis and protect against oxidative stress. Early work in our lab found that mice with livers lacking both TrxR1 and Gsr ("TR/GR-null") remain viable and maintain liver redox homeostasis, by rewiring sulfur amino acid metabolism to supply the thiol amino acid cysteine. Isotope tracing studies in whole mouse models suggested that a major source of cysteine was coming from cystine not via disulfide reduction, but by C-N bond cleavage to yield cysteine-persulfide (CSSH), which subsequently undergoes exchange reactions leading to cysteine. In work presented here, we established robust primary hepatocyte cultures from WT and TR/GR-null livers. These cultures allow us to test cell-autonomy and reaction mechanisms.

PROJECT
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P11

The Protective Role of Neddylation in the Vascular Endothelium

Presenting author: Rachelle Nelson

Affiliation:

Department of Biochemistry, University of Nebraska-Lincoln, Lincoln, NE, USA

Supervisor: Xinghui Sun, PhD

Brief summary of research:

Neddylation is a post-translational protein modification that attaches a ubiquitin-like NEDD8 molecule to a lysine residue of the substrate protein. The most well-known neddylation substrates are the cullin family of proteins, which are important in the ubiquitin pathway. When neddylation is inhibited in vascular endothelial cells, we see an increase in cell death and vascular dysfunction. Specifically, we see evidence of pyroptosis, a gasdermin-mediated inflammatory cell death. This project studies the pathway and mechanism of gasdermin E (GSDME) activation in our HUVEC and mouse models upon neddylation deficiency. This includes looking at possible regulatory mechanisms of GSDME-mediated pyroptosis, including redox sensitivity. The more well-studied pyroptosis mediator, gasdermin D, is known to have redox-sensitive residues, and it is likely GSDME is redox-sensitive as well. Additionally, upstream activators of the pyroptosis pathway such as NLRP3 inflammasomes can be activated by redox stress. It is also known that neddylation deficiency inhibits cullin-mediated ubiquitination of Nrf2 and its degradation. This increases the levels of Nrf2 that interferes with cellular redox homeostasis. We are especially interested in how neddylation inhibition and redox stress may intersect to contribute to GSDME activation, cell death and impaired vascular integrity.

PROJECT
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P12

Mitochondrial protein Afg1 and its role in human health and aging

Presenting author: Emma Bergmeyer

Affiliation:

Department of Biochemistry, University of Nebraska-Lincoln, Lincoln, NE, USA

Supervisor: Prof. Oleh Khalimonchuk, PhD

Brief summary of research:

Afg1 (ATPase Family Gene 1) is a mitochondrial protein associated with the inner mitochondrial membrane, which acts to maintain mitochondrial protein homeostasis. While Afg1 is a relatively understudied protein, a few key characteristics have been determined such as it having general refoldase activity, playing a role in the TIM22 import pathway, and playing a role in oxidative stress tolerance. As declining mitochondrial function is a characteristic of many neurodegenerative disorders, studying Afg1 has applications in improving human health, as Afg1 has been shown to be essential in maintaining mitochondrial and overall cellular health. My current work aims to further explore the role that Afg1 (and its human homologue LACE1) plays in the mitochondria, by attempting protein purification and structural determination, and identification of interacting partners and pathways that Afg1 is involved in.

PROJECT
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P13

Crosstalk Between Ubiquitin-Proteasome System and Protein Quality Control in Mitochondria

Presenting author: Brett Gehrig Hilbers

Affiliation:

Department of Biochemistry, University of Nebraska-Lincoln, Lincoln, NE, USA

Supervisor: Prof. Oleh Khalimonchuk, PhD

Brief summary of research:

As central hubs of cellular metabolism, mitochondria integrate a variety of functions spanning bioenergetics, metabolite anabolism/catabolism, and signaling, and therefore rely on robust mechanisms to sense and respond to diverse cellular insults. The mitochondrial protein quality control (MPQC) network maintains mitochondrial proteostasis by ensuring import fidelity, clearing damaged or misassembled proteins, and activating adaptive stress responses that preserve organelle function. Complementing the MPQC, the mitochondria-associated ubiquitin-proteasome system (UPS) selectively targets misfolded and aggregated proteins for degradation and marks severely compromised mitochondria for mitophagy, preventing further cellular damage from accumulating. Together, these systems resolve threats to proteostasis while dynamically shaping organelle composition and behavior – including fusion-fission balance, membrane remodeling, interorganelle contacts, and selective organelle turnover.

My research aims to define how the MPQC and the mitochondrial UPS mechanistically interact, and to determine how MPQC perturbations reshape the mitochondrial ubiquitin landscape and downstream organelle physiology. To address these questions, I am using biochemical fractionation of yeast knockout and rescue strains combined with mass spectrometry-based proteomic profiling to map changes in mitochondrial ubiquitination following MPQC/UPS perturbation. By linking specific alterations in MPQC function to discrete changes in the UPS and resulting mitochondrial ubiquitinome, I seek to uncover causal circuits that exist between these two systems that drive mitochondrial function and dysfunction. Because defects in MPQC and related ubiquitin pathways are implicated in many neurodegenerative and musculodegenerative disorders, clarifying these mechanisms may reveal conserved molecular targets that have translational potential in the context of human health and disease.

PROJECT
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P14

Radiation-induced adipose tissue dysfunction drives cancer migration

Presenting author: Kia Liermann-Wooldrik

Affiliation:

Department of Biochemistry and Molecular Biology, University of Nebraska Medical Center, Omaha, NE, USA

Supervisor: Rebecca Oberley-Deegan, PhD

Brief summary of research:

One of the main cancer treatments prescribed clinically is targeted radiation therapy. During the course of radiation, healthy tissue found in the region of the tumor is often not spared. Hence, in the context of pelvic cancers (prostate and colorectal), adipose tissue is often times exposed to high doses of radiation causing damage to the typical metabolic, redox, and immune status of this tissue. Within my research, I aim to address the implications that irradiated adipose tissue can have cancer progression. So far, we have found that irradiated adipocytes are able to induce an epithelial to mesenchymal transition in cancer cells leading to an enhancement in migration/metastasis. We know that lipolysis is elevated in adipocytes exposed to radiation and believe that through CD36, cancer cells are taking up adipocyte-derived lipids as an energy source to drive cellular migration. We are currently working on investigating a mechanism that links CD36 and migration in cancer cells, as well as establish ways to protect adipose tissue health in an attempt to mitigate cancer progression.

PROJECT
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P15

Investigating Complex Formation, Substrate Channeling, and Redox Regulation in Proline Catabolism in *Thermus thermophilus*

Presenting author: Adedotun Adefolalu

Affiliation:

Department of Biochemistry, University of Nebraska-Lincoln, Lincoln, NE, USA

Supervisor: Prof. Donald Becker, PhD

Brief summary of research:

Proline metabolism plays a critical role in cellular energy production and stress responses. The conversion of proline to glutamate is mediated by two enzymes: proline dehydrogenase (PRODH) and glutamate semi-aldehyde dehydrogenase (GSALDH). In many bacteria, these enzymes are fused into a single bifunctional unit, the 'proline utilization enzyme A' (PutAs). This fusion enables coupling, enhances metabolic efficiency and prevents the release of labile intermediates into the bulk solvent. However, in *Thermus thermophilus*, the enzymes are expressed as separate monofunctional proteins. Previous studies have provided evidence for substrate channeling between PRODH and GSALDH in *T. thermophilus*, suggesting that transient interactions facilitate efficient intermediate transfer. In this study, we hypothesize that PRODH and GSALDH form a dynamic complex to mediate substrate channeling in *T. thermophilus*. Using cryo-electron microscopy (cryo-EM) and biochemical assays, we investigate the structural basis and functional dynamics of this interaction. Also, since PRODH, a flavin-dependent enzyme, directly contributes to the electron transport chain through $FADH_2$ oxidation, potentially generating reactive oxygen species (ROS), the study will also investigate the role of this redox process in enzyme coordination and substrate channeling, to determine how redox regulation influences their interaction and activity.

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P16

Structural Insights into the Engineered Oxygen-Tolerant THI4 Metallozymes for Enhanced Efficiency Under Sulfur-limiting Conditions

Presenting author: David Akintayo Obe

Affiliation:

Department of Biochemistry, University of Nebraska-Lincoln, Lincoln, NE, USA

Supervisor: Prof. Mark A. Wilson, PhD

Brief summary of research:

Thiazole synthase 4 (Thi4) is a metalloenzyme that catalyzes the formation of thiazole, a precursor of the thiamine diphosphate (TDP) cofactor. Thi4 enzymes can function either as single-turnover sacrificial proteins, employing an active-site cysteine as the sulfur donor, or as multiple-turnover true catalysts that utilize inorganic sulfur sources under low-oxygen conditions. Our long-term goal is to evolve oxygen-tolerant catalytic Thi4s for plant bioengineering applications. In this study, we structurally characterized Saccharicrinis fermentans (Sf) Thi4, a catalytic enzyme selected for enhanced oxygen tolerance through continuous directed evolution. High-resolution X-ray crystallography and cryo-EM revealed distinct ligands at the enzyme active site, representing key intermediates in its catalytic cycle. At pH 4.5, a 1.3 Å resolution X-ray crystal structure captured a germinal diol at the active site, while a cryo-EM structure at 2.9 Å resolution at pH 7.5 revealed a bound adenosine diphosphate (ADP) molecule. These ligands represent off-pathway intermediate species stabilized by distinct experimental conditions. Additionally, we resolved a 1.9 Å resolution structure of D168G SfThi4 mutant at pH 7.7, a variant identified during our directed evolution campaign. These structural insights is fundamental to understanding the mechanisms underlying catalytic Thi4 function and provide a rationale for how mutations, such as D168G, improve Thi4 function in model organism.

PROJECT
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THE NRDI FUND

P17

Inhibition of JNK with Inhibitor SP600125 Induces Memory Surface Markers on T lymphocytes in vitro

Presenting author: Kathleen Klinzing

Affiliation:

Medical University of South Carolina, Charleston, SC, USA

Supervisor: Shikhar Mehrotra, PhD

Brief summary of research:

One barrier to adoptive cell therapy (ACT) for the treatment of solid tumors is lack of persistence of tumor-specific T cells. Increasing administration of minimally differentiated memory cells improves persistence of antigen-specific T cells. c-jun N-terminal kinase (JNK) is an intracellular mediator of stress-induced pathways leading to cell death. Here, we show that addition of JNK inhibitor SP600125 in vitro to both murine and human T cells results in expression of extracellular memory markers. Washout caused effector-like secretion of IFN-γ. Induction of a memory phenotype with SP600125 could be a useful strategy to increase persistence T cells administered by ACT. Given the memory phenotype found here, we also plan to explore the role of JNK in regulating the oxidative metabolic phenotype typically seen in memory cells and its role in ROS signaling in the context of T cell memory.

PROJECT
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P18

RNA Expression Profiles of CD8+ T-cells Post-MI

Presenting author: Thomas Dempster

Affiliation:

Department of Medicine, Medical University of South Carolina, Charleston, SC, USA

Supervisor: Kristine DeLeon-Pennell, PhD

Brief summary of research:

My research project involves examining the effects of cytotoxic CD8+ T-cells at different time points after acute myocardial infarction (heart attack). Our lab uses a novel mouse model in which CD8+ T-cells will be eliminated at various days during the inflammatory stage. Then we will phenotype the left ventricular structure and function as well as the expression profiles of the T-cells. This will include echocardiography, stress/strain measurement of the replacement scar, interactions with other immune cells and fibroblasts, and metabolic activities. A large portion of the project will include single-cell RNA sequencing and TCR sequencing, which is what I am currently working on. Cardiomyocytes prefer to oxidize fatty acids for energy, so we are especially interested if this activity is compromised with or without the presence of CD8+ T-cells, and if the metabolite secretion of other immune cells, such as macrophages, changes in the time course.

PROJECT
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THE NRDI FUND

P19

Regulation of PTEN Oxidation and Akt signaling by Peroxiredoxins in β-Cells

Presenting author: Kathryn Glorioso

Affiliation:

Department of Biochemistry and Molecular Biology, Medical University of South Carolina, Charleston, SC, USA

Supervisor: Jennifer S. Stancill, PhD

Brief summary of research:

Diabetes mellitus (DM) is a class of diseases that are characterized by a dysregulation of blood glucose levels. This dysregulation is due to a decrease in secreted insulin, the hormone responsible for efficient glucose transport into cells. Decreased insulin secretion is caused by destruction or dysfunction of the insulin-secreting β-cells found in the pancreatic islets. β-cells have a carefully balanced redox environment, relying primarily on peroxiredoxin-1 (Prdx1) for protection against hydrogen peroxide (H_2O_2). Prior studies in other cell types have demonstrated that peroxiredoxin-1 may have a role in regulating the oxidation state of PTEN (Phosphatase and TENsin homolog deleted on chromosome 10) by direct protein-protein interaction. PTEN is inactivated by H_2O_2 oxidation, but whether peroxiredoxins regulate PTEN in β-cells is unknown. We expected that inhibition of peroxiredoxin would increase PTEN's susceptibility to oxidation and lead to an increase in downstream Akt phosphorylation. We found that inhibiting Prdx1 and 2 by Conoidin A sensitized PTEN to oxidation in response to continuously generated H_2O_2. However, while we saw an increase in p-AKT (S473) with increasing H_2O_2, we saw a decrease in p-AKT with Conoidin A treatment. This decrease is in opposition to the canonical PI3K-AKT pathway, where increased PTEN oxidation would correlate with increased p-AKT. The result needs to be further scrutinized and perhaps raises important questions about the use of Conoidin A as a common inhibitor.

PROJECT
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P20

Endoplasmic Reticulum Redox Homeostasis - the Potential Absence of the Thioredoxin / Thioredoxin Reductase System in the Lumen

Presenting authors: Viola Varga, PhD; Krisztina Veszelyi

Affiliation:

Institute of Translational Medicine, Semmelweis University

Brief summary of research:

The unique redox balance within the endoplasmic reticulum (ER) is essential for its diverse functions. Due to its role in protein folding, the ER lumen has long been regarded as a uniformly oxidative environment. However, it also serves as a site for reductive processes, suggesting the separate functioning of the two main redox systems, the thiol/disulfide and reduced/oxidized pyridine nucleotide systems. There are two enzymes capable of coupling them directly, glutathione reductase (GR) and the thioredoxin (Trx)/thioredoxin reductase (TrxR) system. The absence of GR in the ER lumen has already been described. Therefore, our aim was to investigate the presence of the Trx/TrxR system in the lumen.

In silico predictions of ER localization showed a low probability for all isoforms (0–5%). The specific activity of TrxR in the ER was nearly zero (0,02 U/mg ± 0,01), while we measured higher activities in the cytoplasm (1,26 U/mg ± 0,11) and mitochondria (1,57 U/mg ± 0,19). We could not detect the protein expression of any Trx/TrxR isoform by Western blot analysis; moreover, immunofluorescence analysis showed no co-localization of any isoform with the ER marker Grp94. Next, we investigated ER-targeted transient overexpression of the Trx1/TrxR1 system, which led to the rapid induction of apoptosis in HeLa cells.

Our results suggest that the Trx/TrxR system is also absent from the ER lumen, which allows the parallel existence of oxidized proteins and reduced pyridine nucleotides.

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P21

Control of transcription factor regulation through the selenium-dependent thioredoxin system

Presenting author: Beáta Biri-Kovács, PhD

Affiliations:

Department of Selenoprotein Research and the National Tumor Biology Laboratory, National Institute of Oncology, Budapest, Hungary

Division of Biochemistry, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden

Brief summary of research:

Monitoring the cross-talk and regulation of signaling mechanisms of different transcription factors is of high importance in several diseases. However, tools for understanding their complex regulation within single cells and how their activities are intertwined in cellular contexts are still limited. We recently developed a new reporter (called pTRAF, for plasmid for transcription factor reporter activation based upon fluorescence) that enables simultaneous single-cell resolution monitoring of three separate redox biology related transcription factors: NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells), STAT3 (signal transducer and activator of transcription 3) and Nrf2 (nuclear factor E2–related factor 2), that is a derivative of a former variant of this reporter tool. Detection of the activation of the transcription factors is based upon measuring the fluorescence intensity of three separate fluorescent proteins linked to the activation of each transcription factors, and is measured by fluorescence microscopy, flow cytometry and live cell imaging techniques. With the help of the reporter plasmid, and other fluorescent reporter tools that enable the dynamic measurement of H_2O_2 levels in living cells, we were able to monitor the intertwined regulation of the selenium-dependent thioredoxin system (using a specific TrxR1 inhibitor) and the Nrf2 signaling pathway. In the other focus of our research is the analysis of TXNL1, a redox-active thioredoxin-like protein with chaperone functions, related to Nrf2 signaling, and how its expression modulates the answer of cells to oxidative stress. The newly described pTRAF variant can be used to simultaneously monitor the impact of diverse redox related perturbances on NF-κB, STAT3 and Nrf2 signaling in relation to the thioredoxin system at single cell resolution.

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P22

A simple method to detect trace-amount of catalase impurity in purified recombinant peroxiredoxin 2

Presenting author: Zsuzsanna Pató

Affiliations:

Department of Selenoprotein Research and the National Tumor Biology Laboratory, National Institute of Oncology, Budapest, Hungary

Division of Biochemistry, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden

Brief summary of research:

In our research, we use the overoxidation-sensitive human Peroxiredoxin 2 (hsPrx2) as a model to investigate the sensitivity of selenocysteine to (over)oxidation. Therefore, we expressed and purified various selenocysteine variants of hsPrx2 for comparing their kinetic properties to the wild type (cysteine containing) enzyme.
Using the conventional Trx-NADPH recycling system for measuring peroxidase activity, we noticed a non-stoichiometric consumption of NADPH in the assay. The reactions could be restart with the addition of a bolus of hydrogen peroxide, leading to the conclusion, that there must have happened a NADPH-independent reaction with H_2O_2.
It is already known, recombinant proteins produced in E. coli may contain trace amounts of catalase. Since catalase reacts with H_2O_2 in a very fast and NADPH-independent manner, its trace presence affects the concentration of H_2O_2 and thus the results of Prx2 kinetic measurements. To verify this hypothesis, to develop reactions without catalase activity and to purify catalase-free enzymes, it was necessary to develop an assay based on following the H_2O_2 concentration change.
For this purpose, AmplexRed, an assay system for the determination of H_2O_2 concentration in solutions, was optimized. The method was validated using two approaches: the addition of a catalase inhibitor (sodium azide) to the reaction mixtures, or using Prx2 enzymes isolated from catalase-free E.coli strains (MH1, C321.dAEG), then used for monitoring the catalase content of Prx2 fractions obtained during protein purification.
This study contributed to the development of an efficient purification method for catalase-free Prx2 isolation.

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FINANCED FROM
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P23

Catalase Free Recombinant Peroxiredoxin 2 Production and Purification from E.coli

Presenting author: Attila Andor, PhD

Affiliations:

Department of Selenoprotein Research and the National Tumor Biology Laboratory, National Institute of Oncology, Budapest, Hungary

Division of Biochemistry, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden

Brief summary of research:

Peroxiredoxin 2 (Prx2) belongs to the family of antioxidant enzymes that removes H_2O_2 using the thioredoxin (Trx)/thioredoxin reductase (TrxR)/NADPH system as reducing equivalent. Prx2 exists mostly in the cytosol as homodimer in a head to tail orientation, but also readily forms larger oligomers. It belongs to the typical 2-Cys Prxs, which contain two active site cysteines: the peroxidatic (CP) and resolving (CR) residues. In the catalytic cycle first, the CP residues are oxidized to sulfinic acid by H_2O_2, followed by the formation of an intermolecular disulfide bond with the CR of another Prx2 subunit. In the final recycling step Prx2 undergoes reduction by the thioredoxin Trx/TrxR/NADPH system.

We decided to express human Prx2 and its active site mutants in E. coli and purify them to homogeneity for in vitro kinetic study. To get rid of endogenous E. coli catalase in the purification process is very important because its turnover number is extremely high and competes with Prx2 for H_2O_2 in an NADPH independent manner, while the thioredoxin system requires NADPH for Prx2 recycling. Expression constructs encoding His-SUMO-tagged Prx2 variants were designed for protein production in E. coli. After lysis of E. coli cells containing the fusion protein special immobilized metal affinity chromatography (IMAC) was elaborated to eliminate trace amount of catalase from the purified recombinant enzyme.

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P24

Thioredoxin-related Protein of 32 kDa (TXNL1) modulates p62 function upon auranofin treatment as it is rapidly degraded by the proteasome system without being ubiquitinated

Presenting author: Mohanraj Mahendravarman, PhD

Affiliations:

Department of Selenoprotein Research and the National Tumor Biology Laboratory, National Institute of Oncology, Budapest, Hungary

Division of Biochemistry, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden

Brief summary of research:

Thioredoxin-like protein-1 (TXNL1; also known as thioredoxin-related protein of 32 kDa, TRP32) is ubiquitously expressed in eukaryotes but has yet essentially unknown roles in relation to cellular redox homeostasis. Recently, we have demonstrated that TXNL1 has dual functions as a thioredoxin-like reductase and also an ATP- and redox-independent chaperone. Here, we found that treatment with auranofin (AF), an FDA-approved thioredoxin reductase inhibitor and the strong activator of transcription factor Nrf2, very rapidly (within hours) downregulates TXNL1 in a time- and dose-dependent manner, while AF had no such effects on thioredoxin 1 (Trx1, TXN1) protein levels. Pre-treatment of A549 cells with proteasome inhibitors (Bortezomib/MG132) reversed the effect of AF on TXNL1 levels, but a ubiquitin activating enzyme inhibitor (TAK-243) did not, suggesting that TXNL1 is degraded via ubiquitin-independent proteasomal manner. Interestingly, CRISPR-Cas9 knockout of TXNL1 in 293T cells resulted in a mild accumulation of poly-ubiquinated proteins and significant decrease of p62 levels and its monomer compared to WT-cells under non-reducing condition, indicating increased p62 aggregation and/or sequestration in the absence of TXNL1. Moreover, TXNL1-KO cells showed a higher basal level of Nrf2 activation, but it was stabilized upon addition of AF treatment compared to WT-cells. Taken together, these results suggest that TXNL1 is involved in regulation of p62 and is a major target in AF-triggered proteasomal degradation, possibly providing a functional link between Nrf2 and the ubiquitin–proteasome system in responses to oxidative stress.

PROJECT
FINANCED FROM
THE NRDI FUND

P25

Role of TXN1 Gene in Redox Buffering Capacity in HEK-293T Cells

Presenting author: Attila Kolonics, PhD

Affiliations:

Department of Selenoprotein Research and the National Tumor Biology Laboratory, National Institute of Oncology, Budapest, Hungary

Division of Biochemistry, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden

Brief summary of research:

Thioredoxin-like protein-1 (TXNL1; also known as thioredoxin-related protein of 32 kDa, TRP32) is ubiquitously expressed in eukaryotes but has an unknown role in cellular redox homeostasis. TXNL1 exhibits thioredoxin-like reductase and chaperone activity functions. Auranofin (AF) - a thioredoxin reductase inhibitor – downregulates TXNL1 in a time- and dose-dependent manner and induces cell apoptosis involving selective disruption of mitochondrial redox homeostasis associated with oxidation of Prx3.

Redox buffering capacity and modulation of proliferation were examined with AF and hydrogen peroxide (H2O2) in wild-type cells (WT) and CRISPR-Cas9 knockout of TXNL1 in HEK-293T cells (TXNL1 KO) by Incucyte® SX1 live cell analysis instrument to reveal phenotypic differences. The change in ROS level was characterized using the cytoplasmic, mitochondrial matrix- and mitochondrial membrane-specific ultrasensitive hydrogen peroxide indicator HyPer7 and 2,7-dichlorodihydrofluorescein (H2DCF). AF and H_2O_2 caused a rapid burst of ROS in all organelles examined both in TXLN-1 and WT cells revealed by HyPer7 probes in the first hour.

Our results suggest that TXNL1 is an important element of redox regulation and the absence of the protein may cause a susceptible phenotype in strenuous oxidative stress conditions.