Redox Biology Congress 2022

Annual Meeting of the Society of Free Radical Research - Europe
& Biannual Meeting of the Plant Oxygen Group

Oxidative stress, redox biology and antioxidants from plants to humans

+ Vitamin-E Symposium

ABSTRACT BOOK

Ghent, Belgium
Oude Vismijn
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SFRR EUROPE

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1) SFRR-E & POG Joint Keynote and Symposia Abstracts

SFRR-E & POG Joint Keynote Lectures

ROS and redox signaling in cell-to-cell and systemic responses of plants

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Reactive oxygen species (ROS) are key signaling molecules that enable cells to rapidly respond to different stimuli. In plants, ROS play a crucial role in abiotic and biotic stress sensing, integration of different environmental signals, and activation of stress-response networks, leading to the buildup of defense and acclimation mechanisms and plant resilience. Recent advances in the study of ROS signaling in plants include the identification of ROS receptors and key regulators that connect ROS signaling with other important stress-response signal transduction pathways and hormones, an increased understanding of how ROS are regulated in cells by balancing production, scavenging and transport, and the identification of new roles for ROS in organelle-to-organelle and cell-to-cell signaling. The role of ROS in cell-to-cell signaling, systemic plant responses to abiotic stress, and plant acclimation to climate change-driven multifactorial stress combination will be discussed.

Physiological redox balance: Oxidative eustress

Helmut Sies*
Institute for Biochemistry and Molecular Biology I, Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany

Maintenance of redox homeostasis is a continuously ongoing challenge. In the open metabolic system, redox-related signaling requires monitoring and fine-tuning of the steady state redox set point. The ongoing oxidative metabolism is a persistent challenge, denoted as oxidative eustress (1). This physiological and beneficial oxidative stress is contrasted to non-physiological and detrimental oxidative distress (2). H2O2 is a major redox signaling agent, targeting specific cysteines in proteins which act as molecular redox switches to activate transcription factors (3). Major functions affected by physiological redox signaling in eustress include proliferation, development, cognition, thermogenesis, impacting on global topics such as inflammation, immunity and the aging process. Current research on the control of H2O2 dynamics in subcellular, cellular and intercellular spaces is flourishing. It includes regulation of the sources and sinks of H2O2, its transport via peroxiporins, and the interaction with other signaling pathways. A major challenge is to define the borderline between oxidative eustress and oxidative distress, which is cell type-specific and fluctuates according to metabolic condition.

(1) Sies, H. (2021) Oxidative eustress: On constant alert for redox homeostasis. Redox Biol. 41, 101867
(2) Sies, H., ed. (2020) Oxidative stress: Eustress and distress, pp. 1-844, Academic Press, London
(3) Sies, H., et al (2022) Defining roles of specific reactive oxygen species (ROS) in cell biology and physiology. Nat. Rev. Mol. Cell. Biol. 23, xxx-xxx

Shining a light on ascorbate metabolism

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Plants are rich in ascorbic acid (vitamin C) and are a critical source in the human diet. It is often the most abundant primary metabolite in leaves and reaches very high concentration in some fruits. Despite its abundance, poor diets can result in vitamin C deficiency, while poor health may increase the daily requirement. Ascorbate is an effective antioxidant and free radical scavenger and readily reduces iron (for example by acting as a chaperone for 2-oxoglutarate-dependent dioxygenases), so it interacts with metabolism in multiple ways. Plants have specific ascorbate peroxidases which are important for hydrogen peroxide removal. Ascorbate concentration in leaves is strongly light responsive which reflects an important role in scavenging photosynthesis-sourced hydrogen peroxide as well as a role in other photoprotective mechanisms. Plants primarily synthesise ascorbate from L-galactose, which is derived from GDP-L-galactose via GDP-mannose. There is abundant evidence that GDP-L-galactose phosphorylase is the rate controlling enzyme in this pathway. Over-expression of the 6 enzymes on the pathway from mannose 1-P to ascorbate shows that only GDP-L-galactose phosphorylase has a significant effect on ascorbate. This is rationalised by a model of the pathway based on measured kinetic constants. The enzyme is encoded by two paralogues (VTC2 and VTC5) in Arabidopsis thaliana. VTC2 and VTC5 transcription is responsive to light and is repressed as ascorbate accumulates. Additionally, the 5'-UTR of the transcript has a conserved upstream open reading frame (uORF) which appears to have a role in controlling its translation and could contribute to feedback repression of ascorbate synthesis. CRISPR-Cas gene editing of the uORF has caused ascorbate hyper-accumulation in various species including tomato.

Thiol peroxidases in plant redox sensing and metabolic tuning

Karl-Josef Dietz*
University of Bielefeld, Germany

The cytosol, chloroplast stroma and mitochondrial matrix contain a regulatory thiol-disulfide redox network consisting of redox input elements, redox transmitters, redox targets, redox sensors and final electron acceptors like H2O2. The network structure is conserved in eukaryotes, and particularly complex in plants as indicated by large gene families encoding, e.g. thioredoxins, glutaredoxins and thiol peroxidases, namely peroxiredoxins (PRX) and glutathione peroxidase-like (GPXL) proteins. Network dynamics in plants is best explored in photosynthesizing chloroplasts. The talk will first elaborate on the role of the chloroplast 2-CysPRX as sensor and regulatory element in adjusting photosynthetic metabolism to the prevailing condition for photosynthetic CO2 fixation. The presence of oxidized 2-CysPRX is required for rapid deactivation of Calvin-Benson cycle enzymes upon lowering of light intensity or darkening. In the case of plant – but also human – 2-CysPRX, the redox-dependent conformational dynamics can be documented by single molecule mass photometry. Mathematical modelling allows for predicting network behavior. The second part will focus on the cytosolic thiol-disulfide redox network. It will be shown how reconstitution of partial networks using recombinant proteins, together with fluorescence sensors for glutathione and H2O2, allows for exploring the redox dynamics upon addition of H2O2 bursts and to explore the contribution of single elements. The talk will conclude with a personal perspective on current and future directions of research on the redox regulatory network.

Hydroxytyrosol formation from Tyrosol and its effects on the cardiovascular system; results from a randomized controlled clinical trial

Anna Boronat[1]*; Julian Andres Mateus Rodríguez[2]; Natàlia Soldevila-Domenech[1]; Jose Rodríguez-Morató[1]; Maria Isabel Covas[3]; Montserrat Fitó[1]; Rachel F Tyndale[4]; Rafael de la Torre[1]
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Background:
Hydroxytyrosol (HT) is a dietary phenol associated with several beneficial health effects in humans. HT main dietary source is extra virgin olive oil. Wine and beer are source of the phenol Tyrosol (Tyr). Pre-clinical and clinical studies had

pointed out Tyr as a potential precursor of HT. This reaction is mediated by the cytochrome P450 (CYP450) CYP2A6 and CYP2D6 polymorphic isoforms.

Aims and methods:
We performed a randomized controlled clinical trial to confirm the contribution of HT formation after the administration of Tyr and its effects on the cardiovascular system in individuals at high cardiovascular risk. The trial included 33 volunteers and had a cross-over design with three 4-week interventions: (i) white wine (WW), (ii) WW supplemented with Tyr and (iii) control intervention (no alcohol). Volunteers were genotyped for CYP2A6 and CYP2D6 to predict the activity of both enzymes.

Results:
Our results show that HT urinary recovery was significantly increased at the end of WW+Tyr intervention. CYP2A6 and CYP2D6 genotypes of the volunteers, and their predicted enzyme activity, modulated the production of HT. The following changes in the cardiovascular system were observed at the end of WW+Tyr intervention: an improvement in endothelial function, and lipid profile, an increase in antithrombin III, a decrease in homocysteine and endothelin-1 and a cardioprotective modulation of circulating levels of ceramides. Additionally, we observed a regulatory effect of Tyr supplementation on the expression on several genes associated with the cardiovascular system.
Conclusions:

Tyr was endogenously converted into HT in vivo. This conversion is mediated by polymorphic CYP2A6 and CYP2D6 isoforms of CYP450. Tyr supplementation and its following bioactivation into HT triggered several positive effects on the cardiovascular system.

Hop polyphenols for mitigating metabolic syndrome

Jan Frederik Stevens*
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Background: Our ongoing research on the health benefits of flavonoids from the hops plant (Humulus lupulus) revealed that xanthohumol (XN), the principal prenylated flavonoid in hops, has the potential to improve diet-induced dysfunctional glucose and lipid metabolism in metabolic syndrome. Sphingolipids including ceramides are implicated in the pathogenesis of obesity and insulin resistance. Correspondingly, inhibition of pro-inflammatory and neurotoxic ceramide accumulation prevents obesity-mediated insulin resistance and cognitive impairment. Increasing evidence suggests the farnesoid X receptor (FXR) is involved in ceramide metabolism, as bile acid (BA)-FXR crosstalk controls ceramide levels along the gut-liver axis.
Aims: To determine to which extent XN and structurally related flavonoids improve manifestations of metabolic syndrome through modulation of FXR.
Methods: We examined how XN and its metabolically stable hydrogenated derivatives, dihydroxanthohumol (DXN) and tetrahydroxanthohumol (TXN), improve insulin resistance and cognitive impairment. We analyzed lipid and BA profiles in the liver and hippocampus, measured sphingolipid relative abundance in the hippocampus, and linked them to metabolic and neurocognitive performance in high-fat diet (HFD) fed C57BL/6J mice. Wild-type and liver-specific FXR-null mice (FXRliver-/-) were fed HFD containing XN or the vehicle and liver tissues were examined by histological characterization, lipidomics, and BA profiling.
Results: XN, DXN and TXN (30 mg/kg body weight/day) decreased ceramide content in liver and hippocampus; the latter was linked to improvements in spatial learning and memory. In addition, XN, DXN and TXN decreased hepatic cholesterol content. HFD supplemented with XN (60 mg/kg body weight/day) resulted in amelioration of hepatic steatosis and decreased BA concentrations in FXRliver−/− mice, the effect being stronger in male mice.
Conclusions: XN, DXN and TXN may alleviate obesity-induced metabolic and neurocognitive impairments by targeting the gut-liver-brain axis. The findings indicate a sex-dependent relationship between FXR, lipids and Bas, and suggest that XN ameliorates HFD-induced liver dysfunction via FXR-dependent and independent signaling.

Novel H_2O_2 and NADPH probes to dissect subcellular redox processes in plants

Marie Mai[1]; Jan-Ole Niemeier[2]; Markus Hoffmann[1]; Jannik Zimmermann[1]; Jan Riemer[3]; Leticia Prates Roma[1]; Markus Schwarzländer[2]; Bruce Morgan[1]*
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The last 15 years has seen an explosion in our understanding of cellular and organismal redox biology, especially in our knowledge of the regulation of different redox species at the subcellular level and of redox dynamics. These insights have been made possible largely through the ongoing development of novel genetically encoded fluorescent redox probes as well as novel small chemical redox dyes. Here, we report on the development of new genetically encoded sensors of the NADP+/NADPH ratio and of basal endogenous H2O2 levels.

We have generated a series of different NADP+/NADPH ratio sensors with a range of different in vitro-determined binding affinities for NADP+ and NADPH. We have subsequently introduced these sensors to yeast, plant and mammalian tissue culture cells. We have used these sensors to monitor NADPH dynamics under both physiological conditions as well as in the context of oxidative challenge. We have utilised genetic mutants to study reductive fluxes through the thioredoxin and glutathione/glutaredoxin pathways finding a high conservation in the relative importance of these pathways across distantly related eukaroytes. In particular, our data suggest that the glutathione/glutaredoxin pathway plays a much more important role in the response to both H2O2 and diamide-based oxidative challenges than might have been expected based on the findings of other recent studies.

We have previously developed peroxiredoxin-based, ultra-sensitive H2O2 sensors, for example roGFP2-Tsa2ΔCR, which have subsequently been applied in yeast, as well as in algae and C. Elegans. However, their application in higher plants and mammalian cells has so far not been reported, possibly due to impaired functionality in these organisms. We have now developed novel peroxiredoxin-based sensors, that enable the in vivo study of peroxiredoxin activity as well as structure-function relationships and oligomerization, and which hold promise as ultra-sensitive H2O2 probes for use in higher eukaryotes including mammalian systems.

Imaging cellular biochemistry using chemical-genetic tools

Alison Tebo*
Howard Hughes Medical Institute-Janelia Research Campus, Ashburn, VA, USA

Fluorescence imaging has become an indispensable tool in cell and molecular biology. Fluorescent proteins such as GFP have revolutionized fluorescence microscopy, giving experimenters exquisite control over the localization and specificity of tagged constructs. However, to combat certain drawbacks of fluorescent proteins alternative hybrid systems based on the interaction between a small molecule fluorophore and a protein have been developed. A way to avoid unspecific background in cells and achieve high imaging contrast is to use fluorescent probes that display no fluorescence until labeling occurs. Such probes are often called fluorogenic probes to highlight their ability to generate fluorescence upon reaction/interaction with their target. A new fluorogenic hybrid system called the Fluorescence-activation and Absorption Shifting Tag (FAST) was developed, which consists of a small protein tag that reversibly and dynamically binds a small molecule chromophore, activating its fluorescence. Hybrid chemical-genetic systems such as FAST represent unique opportunities to extend the utility of the system as the protein and fluorogen present two separate opportunities for engineering. Furthermore, they also present certain advantages, such as no requirement for oxygen. I will present the development of techniques to image multiple targets and to monitor protein-protein interactions.

Quantitative imaging of signaling with genetically encoded biosensors to study signaling in plants, yeast, and human cells

Joachim Goedhart*
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Quantitative imaging of signaling with genetically encoded biosensors to study signaling in plants, yeast, and human cells.
Genetically encoded fluorescent biosensors present a powerful means to analyze cellular processes. These biosensors use fluorescent proteins, the properties of which are key to successful design and application of the biosensor. In this talk, I will discuss fluorescent protein properties, biosensors design and applications of biosensors.

Dissecting the dynamics of major cytosolic redox systems under low oxygen stress

Sophie Lichtenauer[1]; Jan-Ole Niemeier[1]; Marie Mai[2]; Christopher Bell[1]; Vincent Kaltenbach[1]; Bruce Morgan[2]; Markus Schwarzländer[1]*
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Plant metabolism is the basis for most of our food and fuel. The driver behind the vast and flexible plant metabolic network is the transfer of electrons from photosynthetic assimilates to O2 as the final acceptor. Hence, O2 limitation can become a major bottleneck for the entire metabolic network and cellular energy provision. Paradoxically O2 limitation also triggers H2O2 generation, requiring electron investment into antioxidant defence. While key adjustments in redox metabolism in response to hypoxia have been studied in detail, their dynamics, their relative significance and their regulation remain poorly understood.
We have established a biosensing setup to follow the in vivo dynamics of central cytosolic redox players, including NADH/NAD+, the glutathione redox status and H2O2 in Arabidopsis leaf tissue, while strictly controlling O2 availability to modify the paths that electrons can take through cellular metabolism. In addition, we have constructed and optimized a new biosensor family for NADPH/NADP+ status, which provides the critical link between central metabolism and the thiol redox machinery. Multiplexed live monitoring of the characteristic signatures of the different redox systems has allowed us to pinpoint a hierarchy of strategies of how the plant responds to lowered O2 concentrations. The regulation and the relative quantitative in vivo importance of different electron routes as indicated by a multiplex redox biosensing will be discussed.

SFRR-E & POG Sunrise Seminar I

How to write a great scientific paper and get it accepted

Tilman Grune[1]*, Lydia Briggs[2]
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Abstract not provided

SFRR-E & POG Sunrise Seminar II

Experimental models in science: when and how to choose the right one

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North Carolina State University, Animal Science Dept., Plants for Human Health Institute, Kannapolis Research Campus, Kannapolis, North Carolina.
Kyung Hee University, Dept. of Food and Nutrition, Seoul, South Korea.

The choice of the most appropriate model in pre-clinical research has been always an issue, especially when there is a need to translate basic science discoveries to clinical practice or, in general, to human health. Currently, the experimental models available in a research lab are represented by “in vitro”, “ex vivo” and “in vivo” approaches.
In vitro cell cultures are essential tools in medicine and biology, because they provide an insight into microorganisms and human or animal cells behavior.
Since these cell lines and microorganisms are isolated from their natural environment, these models may not completely or accurately capture the responses of an entire organism, failing to recapitulate the complexity of in vivo tissues. These methodologies, however, continue to be heavily relied upon by researchers and drug developers, presumably for the familiarity with these approaches and for their low cost.
Spheroids and organoids, which are three-dimensional, multicellular tissue culture methods, are now coming to the forefront as more representative, quickly generated, easily manipulated and bankable in vitro experimental models for research on cancer and other diseases. These methods have recently become more accessible and affordable to the laboratories. A further improvement in this area has been reached in recent years by the development of organ-on-a-chip systems. The organ-on-a-chip integrates cultured cells in a microfluidic chip. The chip simulates bioactivities, mechanics and physiological behavior of organs or organ systems, thereby generating artificial organs.
Additionally, ex-vivo approaches (*i.e.*, isolated PBMC or tissue explants) are commonly used, but also in this case, the manipulation of the tissues and the culturing environment can affect tissue responses.
The importance of choosing the model is mainly dictated by the end points that we need to evaluate and the use of more than one model is strongly recommended when translating the results into real life.

2) SFRR-E Award Lectures Abstracts in Sequence of Presentation

SFRR-E Annual Award Lecture

Redox organization of living systems

Dean Jones*
Division of Pulmonary, Allergy, Critical Care and Sleep Medicine, Emory University, Atlanta, USA

Reactive oxygen species (ROS) are key signaling molecules that enable cells to rapidly respond to different stimuli. In plants, ROS play a crucial role in abiotic and biotic stress sensing, integration of different environmental signals, and activation of stress-response networks, leading to the buildup of defense and acclimation mechanisms and plant resilience. Recent advances in the study of ROS signaling in plants include the identification of ROS receptors and key regulators that connect ROS signaling with other important stress-response signal transduction pathways and hormones, an increased understanding of how ROS are regulated in cells by balancing production, scavenging and transport, and the identification of new roles for ROS in organelle-to-organelle and cell-to-cell signaling. The role of ROS in cell-to-cell signaling, systemic plant responses to abiotic stress, and plant acclimation to climate change-driven multifactorial stress combination will be discussed.

SFRR-E Basic Science Award Lecture – Sponsored by Elsevier

Role of metabolism and bioenergetics in the pathophysiology of organ fibrosis

Santiago Lamas*
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Organ fibrosis remains a major challenge affecting 1 in 4 persons globally. Currently there exist very limited therapeutic options, none of them curative. Fibrosis results from an unbalanced response to inflammation and wound healing leading to the activation of specific subpopulations of resident mesenchymal cells, considered precursors of myofibroblasts, which generate extracellular matrix (ECM) that replace the cellular living tissue. Metabolic derangement has been identified as a key culprit in the pathophysiology of fibrogenesis. In the kidney, mitochondrial dysfunction and defective fatty acid oxidation (FAO) in tubular epithelial cells (TECs) play an important role in the development of fibrosis. We have found that conditional overexpression of the enzyme Cpt1a in kidney tubules promotes enhanced FAO, restores mitochondrial homeostasis and protects from fibrosis. In addition, we have identified specific microRNAs that are important to regulate the genesis of fibrosis by targeting specific metabolic routes. Perturbations in the circadian rhythm and the molecular clock have been associated with many human pathologies, including renal disease. We investigated the relationship between the molecular clock and renal damage in experimental models of injury and fibrosis (UUO, FAN and adenine toxicity), employing genetically-modified mice with selective deficiencies of the clock components Bmal1, Clock and Cry. We found that UUO induced a marked increase in the expression of Bmal1. In human TECs, the pro-fibrotic mediator, TGF-β, significantly altered the expression of core clock components. We further observed that the absence of Cry drastically aggravated kidney fibrosis, while both Cry and Clock played a role in the neutrophil and macrophage mediated inflammatory responses, respectively. Suppression of Cry1/2 was associated with a major shift in the expression of metabolism-related genes. Overall, our results support that kidney fibrosis is accompanied by a profound metabolic derangement and bioenergetics dysfunction, with specific molecular signatures that may constitute potential therapeutic targets.

SFRR-E Catherine Pasquier Award Lecture

Illuminating the role of compartmentalized H_2O_2 signaling in skeletal muscle stress-adaptations

Carlos Henriquez Olguín*
Molecular Physiology Section, Faculty of Science, University of Copenhagen, Denmark

Regular physical activity elicits beneficial adaptations in skeletal muscle, which counteract aging and lifestyle-related diseases, including type II diabetes and cardiovascular disease. Muscle contraction causes intracellular stress on muscle fibers, propelling them to complete molecular adaptations to maintain homeostasis. There is mounting evidence that redox signaling by reactive oxygen species (ROS) is vital for skeletal muscle exercise adaptations. Two ROS sources increasingly implicated in muscle redox signaling are mitochondria and the membrane-bound NADPH oxidase complexes (NOX). Local and discrete generation of hydrogen peroxide (H_2O_2) leads to rapid and reversible oxidation of protein cysteines, modulating a wide range of protein functions and localization.

We have studied the relative contribution of compartmentalized ROS sources in exercise's acute and chronic responses during the last decade. First, the treatment with NOX2 inhibitors and mitochondria-targeted antioxidants revealed that adaptive gene expression after acute exercise depended on NOX2 activity. In a subsequent study, we combined exercise in humans and mice with fluorescent dyes, genetically-encoded biosensors, and NADPH oxidase 2 (NOX2) loss-of-function models to show for the first time that NOX2 is a dominant cytosolic H_2O_2 source during in vivo exercise, necessary for exercise-stimulated GLUT4 translocation and muscle glucose uptake. Lastly, we demonstrated that NOX2 is required for long-term training adaptations, including increased muscle protein expression of antioxidant defense enzymes, hexokinase II, and mitochondrial network remodeling.

Our current work aims to provide novel insight into the physiological roles of compartmentalized cytosolic and mitochondrial H_2O_2 production in skeletal muscle and identify novel downstream transducers of redox signaling. Learning how to modulate localized ROS production might allow us to potentiate the redox-associated exercise hormetic responses to improve human muscle function in health and disease.

SFRR-E Leopold Flohe Award Lecture – jointly sponsored by Oxygen Club California

Multiparametric imaging with genetic biosensors and chemogenetic tools: Golden keys to redox biology

Emrah Eroğlu*
Sabanci University, Istanbul, Turkey

This lecture covers recent methodological advances in developing informative strategies for multiparametric imaging of cellular redox signaling events. Genetically encoded fluorescent protein-based biosensors for reactive molecules (i.e., ROS, RNS, and RSS) have revolutionized the field of redox biology. These informative tools permit the visualization of biological processes with high spatial and temporal resolution in vitro and in vivo. In recent years scientists have expanded the toolbox of genetically encodable tools and established novel chemogenetic enzymes to manipulate the redox tone on the cellular and tissue level. The combination of various genetic biosensors and chemogenetic devices opened new lines of investigation for multiparametric live-cell imaging of ROS and RNS signaling pathways with ultra-high precision. However, redox biologists have not exploited these approaches to their full potential. This lecture will discuss recent progress and future aspects of these toolkits, which would help fill some gaps in our understanding of redox biology.

SFRR-E Young Investigator Award Session

Redox and inflammatory mechanisms linking urban air pollution PM2.5 exposure and cardiometabolic derangements

Timoteo Marchini[1]*; Sheu-Tijani Olawale Abogunloko[1]; Xiaowei Li[1]; Timothy Mwinyella[1]; Lourdes Caceres[2]; Mariana Garces[2]; Valeria Calabro[2]; Virginia Vanasco[2]; Natalia Magnani[2]; Silvia Alvarez[2]; Pablo Evelson[2]; Dennis Wolf [1]
[1]Cardiology and Angiology, Medical Center, University of Freiburg, Germany; [2]Universidad de Buenos Aires, CONICET, Instituto de Bioquímica y Medicina Molecular (IBIMOL), Facultad de Farmacia y Bioquímica, Buenos Aires, Argentina

Air pollution fine particulate matter (PM2.5) is a risk factor for the development of cardiometabolic disorders, by yet unclear mechanisms. In this study, we aimed to evaluate the impact of PM2.5 exposure, with a special focus on unravelling redox, inflammatory, and metabolic pathways. First, male 8-week-old C57BL/6 mice received 1 mg/kg body weight of a PM2.5 surrogate (ROFA, Residual Oil Fly Ash) or PBS (control) by intranasal instillation. A biphasic lung inflammatory cell recruitment was observed in ROFA-exposed mice with neutrophils peaking at 6 h post-exposure and macrophages peaking at 72 h, together with increased pro-inflammatory gene expression and cytokine levels (TNF-α, IL-6, CCL2). Bulk mRNA sequencing of sorted alveolar macrophages revealed a pro-inflammatory gene expression signature and altered pathways for redox and lipid metabolism. Upregulated differentially expressed genes were validated by a customised cytokine bead assay in BAL and plasma, which showed a sustained increase for up to 72 h in ROFA-exposed mice. In parallel, decreased metabolic gene expression (Ucp1, Elovl3, Adrb3) in brown adipose tissue suggests reduced lipolysis and thermogenesis, despite ongoing white adipose tissue inflammation. To further explore this observation, another set of mice were exposed to ROFA or PBS and monitored in metabolic cages for 48 h. Despite enhanced physical activity and lowered caloric intake, ROFA-exposed mice showed significantly reduced heat production. Lastly, consequences of PM2.5 inhalation were evaluated in a real-life mice model of exposure to polluted urban air for 16 weeks. Increased weight gain, impaired glucose homeostasis, and adipose tissue inflammation were observed in mice breathing urban air (27±8 µg PM2.5/m3) versus filtered air (2±1 µg PM2.5/m3), together with altered metabolic gene expression in adipose tissue. Our findings indicate that air pollution PM2.5 exposure blunts metabolic pathways in adipose tissue and promotes obesity, likely due to pulmonary and systemic inflammation.

Methodological, statistical and interpretive considerations in personalised redox biology

Nikos Margaritelis*
Aristotle University of Thessaloniki (AUTh), Greece

Emerging evidence suggests that the presence of oxidative stress per se does not rationalize the use of antioxidants indiscriminately, emphasizing the need to identify responsive phenotypes for personalized interventions. This partially originates from the fact that all people inherit and acquire different or even unique biological and behavioural characteristics. As a result, the impact of any, for example nutritional or pharmacological, redox treatment that aims to regulate our physiology may be differentiated, resulting in beneficial, harmful or neutral outcomes. On this basis, the issue of individual responsiveness attracted the interest of researchers across diverse scientific fields, while 'personalized' – also known as 'precision' or 'subject-tailored' – treatments became the ultimate translational goal. Unfortunately, the 'personalized treatment' still remains more of a buzzword than a substantive and applicable concept, at least in redox biology. A potential explanation for this issue is the lack of specificity in our methodological and statistical approaches. Noteworthy, this is in stark contrast to the great advances in analytical redox chemistry, which aims to identify and quantify the precise reactive species involved in particular settings. In fact, many studies on the topic follow suboptimal methodological approaches to quantify individual responses as well as to specify statistical or clinical thresholds of effectiveness for precise redox-dependent measures (i.e., 'minimal clinically important difference' or 'smallest worthwhile change'). Hence, the interpretational potential of any finding at the individual level commonly lies in the eye of the beholder, while any causative association between redox biology and physiology becomes tricky. Methodological and statistical practices from other fields that seem to provide a more straightforward approach for personalized studies will be presented. Hopefully, similar approaches will be applied in a redox biology context in future studies as well.

P53 triggers necroptosis through loss of sulfiredoxin in acute pancreatitis

Sergio Rius-Pérez[1]*; Salvador Pérez[1]; Michel B. Toledano[2]; Juan Sastre[1]

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Necroptosis activation is highly dependent on the generation of mitochondrial reactive oxygen species. In this study, we assess the contribution of p53 and sulfiredoxin to necroptosis in pancreas with acute pancreatitis. Acute pancreatitis was induced by seven hourly intraperitoneal injections of cerulein in C57BL6/J wild-type mice, p53 knockout mice and sulfiredoxin knockout mice. Necroptosis occurred intensely in pancreas of wild-type mice 24 h after the first cerulein injection. At this time point p53 was upregulated and translocated into pancreatic mitochondria. However, necroptosis was abrogated in p53 knockout mice with pancreatitis. In the pancreas of p53 knockout mice, PGC-1α protein levels as well as those of its transcriptional target peroxiredoxin 3 increased and remained high upon pancreatitis induction. In addition, sulfiredoxin protein levels also increased in p53-deficient mice with pancreatitis, which prevented peroxiredoxin-3 hyperoxidation. During the early stages of pancreatitis, when necroptosis was still absent in the pancreas of wild-type mice, sulfiredoxin was upregulated and located into the mitochondria, protecting peroxiredoxin 3 from hyperoxidation. The absence of sulfirredoxin caused peroxiredoxin 3 hyperoxidation, p53 mitochondrial translocation and necroptosis early in the course of acute pancreatitis. Mito-TEMPO treatment in acute pancreatitis abrogated p53 mitochondrial translocation and necroptosis in pancreas. In conclusion, p53 is required for necroptosis in pancreas during acute pancreatitis through loss of sulfiredoxin and peroxiredoxin 3.

Culturing bEnd.3 brain microvascular endothelial cells in normoxic conditions has direct consequences to hypoxia-reoxygenation injury

Gabriela Warpsinski*, Matthew J. Smith; Giovanni E. Mann

King's College London, UK

Treatments available for ischaemic stroke remain limited due to failures in clinical translation and to improve this, physiological oxygen encountered in vivo need to be considered in cell culture. As cells in vivo experience O2 levels ranging from ~13kPa to ~1kPa, cells cultured under room air – 18kPa O2 – are hyperoxic. Using an oxygen-sensitive probe (MitoXpress-INTRA, Agilent), we have identified that long-term culture under 5kPa O2 is needed to recapitulate reported intracellular O2 levels in the brain. Long-term culture under 5kPa O2 demonstrates a phenotype different to cultures under 18kPa O2, as evidenced by downregulation of specific Nrf2 target antioxidant genes. Superoxide production measured using luminescent L-012 and mitochondrial-specific superoxide indicator MitoSOX™ corroborated findings that long-term culture under 5kPa O2 prevented superoxide production associated with hypoxia-reoxygenation injury. Similarly, real-time labile Fe2+ measurements revealed less Fe2+ release following reoxygenation in bEnd.3 cells adapted to 5kPa O2. Exaggerated superoxide production in cultures exposed to hyperoxic environment may create misleading insights in screening potential therapies for pathology involving hypoxia-reoxygenation injury. The present study provides evidence that adapting cells to physiological normoxia has direct consequence for hypoxia-reoxygenation injury. Future in vitro studies should consider a paradigm shift by conducting cell culture studies under their respective physiological O2 levels to enhance translation to the clinic.

Structural and redox-metabolic remodelling of brown adipose tissue in mice lacking nuclear factor erythroid 2-related factor 2 under basal conditions and cold acclimation

Tamara Zakic[1]*; Anastasija Ninkovic[1]; Sara Stojanovic[2]; Marta Budnar Soskic[1]; Andjelika Kalezic[1]; Aleksandra Jankovic; Aleksandra Korac[2]; Vanja Pekovic-Vaughan[3]; Bato Korac[1]

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Interscapular brown adipose tissue (IBAT) is a highly metabolically active, thermogenic tissue essential for the maintenance of total energy homeostasis, with a remarkable ability for remodelling in response to exogenous stimuli. Given that nuclear factor erythroid 2-related factor 2 (NRF2) has a pivotal role in redox-metabolic homeostasis, we aimed

to investigate its role in IBAT homeostasis under basal conditions or upon cold stimulation. Therefore, we analysed structural and redox-metabolic profiles of IBAT in wild-type (WT) and mice lacking functional Nrf2 (Nrf2KO) maintained at room (RT, 24±1°C) or low temperature (4±1°C). Our results show that both WT and Nrf2KO mice appear to be acclimated to cold, showing characteristics of thermogenically active IBAT, including increased gene and protein expression of uncoupling protein 1 (UCP1). Surprisingly, light and electron microscopy revealed that Nrf2KO mice at RT displayed distinct structural features of activated IBAT, together with the presence of vasodilated blood vessels, while the expression of thermogenic marker UCP1 did not show a corresponding cold-induced change, thus indicating IBAT functional inactivity. This lack of IBAT thermogenic activity in Nrf2KO mice at RT is consistent with its altered redox-metabolic profile, whereby protein expression of the main antioxidant defence and key metabolic enzymes either remained the same or was decreased compared to WT mice at RT. Accordingly, circulatory levels of triglycerides and cholesterol were decreased while glucose, urea and creatinine remained unchanged. Moreover, gene and/or protein expression of important redox-metabolic transcriptional factors – erythroid NRF1, NFkB, PGC-1α and PPARγ, as well as eNOS and AMPKα were increased, suggesting compensatory molecular mechanisms leading to altered IBAT phenotype in Nrf2KO mice at RT. In conclusion, the lack of functional Nrf2 leads to marked structural characteristics of active IBAT in Nrf2KO mice at RT, which are only followed by its functional activation through distinct redox-metabolic reprogramming after cold stimulation.

Protective role of plasma Evs cargo released before and after endurance exercise on human iPS- derived cardiomyocytes in prooxidant conditions

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Cardiovascular diseases (CVDs) result in several conditions such as increase in ROS level that lead to decrease nitric oxide availability and vasoconstriction, promoting arterial hypertension. Physical exercise (PE) has been shown to be protective against CVD. PE can lead to a maintenance of redox homeostasis, with a decrease of ROS by increased expression of antioxidant enzymes and HSPs modulation. These molecules can be shuttled between the cells by Extracellular Vesicle (Evs). Evs are lipids bound vesicles secreted by cells with a crosstalk function, carrying bioactive molecules (i.g.proteins). Our preliminary studies, show the presence of Hsp70, Hsp27 and SOD2 in plasma Evs isolated from trained and untrained healthy young males, with higher enrichment of SOD2 in untrained respect trained. Moreover, Hsp27 phosphorylation increased by acute endurance exercise (70% Hrmax for 30'). These proteins are of particular interest for their protective role in cardiomyocyte (Vicencio et al., Bartz et al.) thus the aim of this work is to test the cardioprotective effect of plasma-Evs isolated from 2 groups of healthy young males with different VO2max levels (Untrained: 44.35 ± 3.0, Trained 52.27 ± 4.21) before and after a single bout of endurance exercise. Trained subject showed a significant decrease in Evs number ($p=0,05$) and an increase in size ($p=0,0001$) after training, the same trend is observed in untrained. Isolated small and large Evs were used to treat human iPS-derived cardiomyocyte exposed to a prooxidant condition (Doxorubicin, 0.2 uM for 3h and H2O2, 100 uM for 24h). ROS level (ROS detection kit far-red), apoptosis (caspase-3 detection) and senescence phenotype (p16 and Beta-galactosidase detection) were used as readout for cardioprotection (Milano et al.). Protein cargo of plasma Evs are also in depth characterized with proteomic (Mass spectrometry) and target specific (western blot) approach in order to identify the putative PE-related proteins involved in cardioprotection.

3) POG Invited Lecture and Talks in Sequence of Presentation

POG Opening Lecture

Lessons learned from plant evolution

Marc Van Montagu*
Department of Plant Biotechnology and Bioinformatics, Ghent University, Ghent, Belgium

Our understanding of the living world is quite recent. Advances in science and technology during the 20th century show that life is a unit that has changed our planet's environment and transformed its own history in the process. The rise of photosynthetic cyanobacteria extended between 2.9 and 3.4 billion years ago, a slow start long before the great oxygenation event that metamorphosed the Earth. The incorporation of a cyanobacterium by a heterotrophic host was a pivotal horizontal genome transfer event that sustained many ecosystems on our planet. Paradoxically photosynthesis produces reactive oxygen species that are harmful to the host cell. Plants are equipped with complex and multilevel antioxidative system that maintain a redox equilibrium and allows quick adaption to a wide array of stressors, making them the most evolutionarily successful living organisms on earth.
But plants cannot thrive alone; they co-evolve with the microbial world. About 30% of plant's energy is directed to the root zone to attract and feed surrounding soil microorganisms which, in return, make nutrients bioavailable for plants, produce chemicals to stimulate plant growth, and protect them from pathogens.
Notwithstanding the finetuned survival circuit of natural ecosystems, the plants we need to keep feeding human population while preserving the environment are nowhere to be found. Nature makes plants to survive and thrive, not to excel in productivity. Advances in plant science have enabled new tools for breeding, as well as genetic engineering, editing and synthetic biology. Technically we are ready to make new plants that will meet our needs. But science alone will not be enough for the transformative changes needed to achieve environmental, nutritional, and economic sustainability of human societies. Technological progress always triggers reactions from society, and it is essential to pay attention to how these reactions can influence the acceptance of an innovative product.

POG Session 1 - Concepts and Directions in Redox Signaling Research in plants

Roles for redox processes in adaptation to a high CO2 world

Christine H. Foyer*
University of Birmingham, UK

Increases in atmospheric CO2 concentrations (eCO2) has a strong impact on the physiology of C3 plants that goes far beyond photosynthesis and carbon metabolism. Accumulating evidence suggests that eCO2 has positive effects on the productivity and yield of C3 plants through effects on photosynthesis and negative impacts on nutrient acquisition and assimilation (see for example Ainsworth & Long, 2021 Global Change Biology 27, 27–49). In addition, eCO2 alters the redox balance and signalling of plant cells in ways that alter biotic and abiotic stress tolerance, as well as the expression of genes involved in nitrate uptake such as NRT2.1. In this talk, I will firstly address how growth under eCO2 might alter cellular redox processes and associated signalling and whether plants perceive eCO2 as a stress in itself.

POG Session 2 – ROS, RNS and redox-active gases in development and plant physiology

Integration of redox signals into hypoxia signal transduction

Romy Schmidt*
University of Bielefeld, Germany

Hypoxia, a condition of limited oxygen availability, occurs naturally in the context of flooding and negatively affects the viability of plants. Various cellular signals generated under hypoxia must be integrated in order to induce a stress-specific response. Changes in the cellular redox status in the presence of oxygen deprivation are not yet associated with important transcription cascades required for adaptation. We found that RADICAL-INDUCED CELL DEATH1 (RCD1), a redox-

sensitive interactor of several transcription factor families in Arabidopsis thaliana, is mandatory for the regulation of the most prominent transcriptional regulators under low-oxygen stress. In addition to RCD1, one of its homologues is also involved in the repression of transcription factors. To unravel the molecular mechanism of repression by RCD1, a variety of genetic, biochemical and molecular biological approaches is applied. In addition, the influence of redox signals generated under hypoxia on the function of RCD1 is examined in detail. Novel regulators of transcription under hypoxia that are controlled in an RCD1-dependent manner are also identified. Taken together, a significant expansion of our understanding of the integration of redox signals in signaling processes leading to low-oxygen stress tolerance is presented.

POG Session 3 – Redox Signaling in the abiotic stress response of plants

The cellular redox status and signal transduction in cadmium-induced acclimation responses

Ann Cuypers[1]*, Verena Iven[1], Jana Deckers[1], Isabeau Vanbuel[1], Sophie Hendrix[2]
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The environment of plants is ever-changing and as sessile organisms, plants are prone to environmental challenges, like cadmium (Cd) pollution. At the cellular level, Cd stress modifies the balance between production and scavenging of reactive oxygen species (ROS). Nevertheless, maintaining this oxidative balance is crucial for plant stress acclimation. In this regard, a central role for the antioxidant glutathione (GSH) is evident, because it is involved in neutralizing ROS but also serves as a precursor for phytochelatins (PC) chelating Cd ions.

In this study on Cd acclimation responses, acute responses (0-24 h) to Cd exposure (0-5 µM) were investigated to identify the pressure points of Cd stress in Arabidopsis thaliana. A prolonged Cd exposure (72 h) was included to obtain a first indication of acclimation, as a new steady state is typically established at this time point.

The rapid depletion of root GSH concentrations upon Cd exposure is proposed to serve as an alert response in A. thaliana. During this alarm phase no changes in the GSH redox state were observed and we suggest that a strong depletion in GSH concentrations is sufficient to alter the cell's redox potential and drive acclimation responses. Even under GSH-limiting conditions, i.e. in the GSH-deficient cad2 mutant, Cd-induced GSH depletion did not alter the redox state of the GSH pool.
Ethylene is a known mediator of the Cd-induced oxidative challenge and is closely intertwined with GSH. Investigating the reciprocal interaction between both components after Cd exposure revealed an accelerated production of the ethylene precursor ACC and ethylene signalling in GSH-deficient mutants, whereas a delayed GSH recovery was observed in mutants defective in ethylene signalling and ACC biosynthesis as compared to WT plants.

The interdependence between GSH and ethylene with oxidative challenge as a possible mediator is essential in plant acclimation responses to Cd stress.

POG Session 4 – Redox signaling in biotic stress responses

S-nitrosylation regulates immunity against infection across life kingdoms

Gary Loake*
University of Edinburgh, UK

A key feature of innate immunity in both plants and animals is the rapid engagement of a nitrosative burst leading to the accumulation of the small, redox-active signalling molecule, nitric oxide (NO). The emerging data suggests that subsequently NO has a central role in orchestrating the immune response through the reprogramming of both gene expression and protein function. The predominant route for NO bioactivity is S-nitrosylation, the addition of an NO moiety to a protein Cysteine thiol (S-H) group to form an S-nitrosothiol (S-NO). This redox-based, post-translational modification can regulate protein function in an analogous fashion to other more well-established post-translational

modifications, for example, phosphorylation. Thus, S-nitrosylation can regulate enzyme activity, protein localisation, DNA binding, protein-protein interactions and metabolite binding, among others.

We are employing a number of diverse genetic and molecular strategies to further uncover the role of NO and cognate S-nitrosylation in the establishment of plant immunity in both model and crop plants. More recently, we have also begun to explore the role of this redox control mechanism in a model animal system. We will report some of our latest findings.

POG Session 5 – Oxidative stress in algae

New insights into the redox regulation of autophagy in the single-cell microalga Chlamydomonas reinhardtii

M. Esther Perez-Perez[1]*, Luis G. Heredia-Martinez[1], Manuel J. Mallen-Ponce[1], Jose L. Crespo[1,2]
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Autophagy is a major catabolic process by which eukaryotic cells degrade and recycle intracellular material including protein aggregates or dysfunctional organelles to maintain cellular homeostasis and to cope with stress. A hallmark of autophagy is the formation of autophagosomes, double-membrane vesicles in which the cargo that will be finally degraded in the vacuole is engulfed. The ATG8 lipidation system, which includes the ubiquitin-like ATG8 protein, the ATG4 protease and the E1- and E2-activating enzymes ATG7 and ATG3, is essential for autophagosome formation since these proteins catalyze the conjugation of ATG8 to the lipid phosphatidylethanolamine. ATG8 lipidation has been widely used as a molecular autophagy marker in many eukaryotes, including the model microalga Chlamydomonas reinhardtii. Our previous studies have demonstrated that autophagy is a redox-regulated process in Chlamydomonas. Indeed, there is a strong connection between different ROS-generating stress conditions and the activation of autophagy in this microalga. We have previously shown that ATG4 is regulated by the intracellular redox potential through the formation of a disulfide bond. At present, we are investigating whether other ATG proteins respond to redox signals and the underlying mechanism of this redox regulation. Moreover, we are analyzing an atg8 mutant strain under ROS-generating and autophagy-activating conditions. Our results indicate that this mutant is hypersensitive to oxidative stress an displays an altered global response to chloroplast damage in Chlamydomonas.

POG Session 6 – Reactive oxygen species and organellar signaling

The different ways in which plants (try to) keep a happy mitochondrial pool

Olivier Van Aken*, Sylwia Kacprzak
University of Lund, Sweden

Selective degradation of mitochondria by autophagy (mitophagy) is thought to play an important role in mitochondrial quality control, but our understanding of which conditions induce mitophagy in plants is limited. Here, we developed novel reporter lines to monitor mitophagy in plants and surveyed the rate of mitophagy under a wide range of stresses and developmental conditions. Especially carbon starvation induced by dark-incubation causes a dramatic increase in mitophagy within a few hours, further increasing as dark-induced senescence progresses. Natural senescence was also a strong trigger of mitophagy, peaking when leaf yellowing became prominent. In contrast, nitrogen starvation, a trigger of general autophagy, does not induce strong increases in mitophagy. Similarly, general stresses such as hydrogen peroxide, heat, UV-B and hypoxia did not appear to trigger substantial mitophagy in plants. Additionally, we exposed plants to inhibitors of the mitochondrial electron transport chain, mitochondrial translation and protein import. Although short-term treatments did not induce high mitophagy rates, longer term exposures to uncoupling agent and inhibitors of mitochondrial protein import/translation could clearly increase mitophagic flux. These findings could further be confirmed using confocal microscopy. To validate that mitophagy is mediated by the autophagy pathway, we showed that mitophagic flux is abolished or strongly decreased in atg5/AuTophaGy 5 and atg11 mutants, respectively. Finally, we observed high rates of mitophagy in etiolated seedlings, which remarkably was completely repressed within 6 h after light exposure. In conclusion, we propose that dark-induced carbon starvation, natural senescence and specific mitochondrial stresses are key triggers of mitophagy in plants.

POG Session 7 – ROS/RNS signaling in the nucleus

Nuclear targeting of catalase in Arabidopsis

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Catalase localisation and/or activity is manipulated by pathogen effectors. Catalase is a well-known peroxisomal enzyme, but its presence or activity in other cell compartments and evidence of interactions with an increasing number of non peroxisomal proteins has been reported. The mechanism of catalase targeting to peroxisomes has remained unclear as catalase lacks a canonical PTS1 peroxisome targeting signal, multiple mutations affect peroxisomal targeting and in some cases localisation appears to be dependent on expression levels.
Catalase targeting was reinvestigated using an in vivo system that allowed assessment of catalase location and catalase function. The cat2-1 mutant was transformed with the wild type CAT2 (CAT2PSI), a C terminal deletion (CAT2 WQSV) and a variant with a canonical PTS1 (CAT2ARL), all untagged and under the control of the native CAT2 promoter. All variants targeted to peroxisomes and restored the growth defect, redox balance, oxidative signalling and photosynthetic defects of the cat2-1 mutant. However specific activity was reduced in the CAT2ARLvariant.
These variants were tagged at N- and C-terminus with the 11th β-strand of GFP and co expressed with protoplasts made from plants expressing GFP1-10 targeted to different subcellular compartments. All 3 N terminally tagged proteins localised to peroxisomes AND nucleus. C terminally tagged proteins showed variation in their location. CAT2PSI was nuclear and peroxisomal, CAT2 WQSV was peroxisomal only, while CAT2ARL was not detected in either compartment.
Collectively these results show 1. The C terminus is dispensable for catalase activity, targeting to peroxisomes and recovery of cat2-1 deficient phenotypes 2. Catalase can be targeted to the nucleus independent of any pathogen effectors and tagging and/or manipulation of the C terminus interferes with this localisation and 3. That forcing catalase to use a canonical PTS1 pathway reduces catalase activity, though not sufficiently to prevent complementation of the cat2-1 phenotypes under the conditions tested.

POG Session 8 – Plant antioxidative systems

Peroxisome-derived retrograde signalling and dynamics

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Peroxisomes, which are small organelles found in most eukaryotes, are bounded by a single lipid membrane and have a close relationship with other organelles such as chloroplasts and mitochondria. Initially, these organelles were regarded as a H2O2 sink produced from different sources both inside and outside peroxisomes and degraded by catalases and other ROS-inactivating enzymes. However, in recent years, biochemical, transcriptomic and proteomic techniques have demonstrated that these organelles are much more complex and perform functions hitherto unknown. In fact, the metabolic diversity and plasticity of peroxisomes are remarkable, and new unexpected functions of plant peroxisomes continue to be discovered. The biosynthesis of the phytohormones jasmonic acid (JA), auxin indole-3-acetic acid (IAA) and salicylic acid (SA), together with ROS/RNS metabolism, makes peroxisomes a source of signaling molecules which are essential for the regulation of development processes and plant responses to stress.
Reactive oxygen species (ROS) act as secondary messengers that can be sensed by specific redox sensitive proteins responsible for the activation of signal transduction culminating in altered gene expression. The subcellular site, in which modifications in ROS/oxidation state occur, can also act as a specific cellular redox network signal. Although the identification of other organelle/compartment-dependent signaling communication with the nucleus, termed retrograde signaling, which plays a key role in cell responses to environmental cues, organelle assembly and metabolism, has improved, research into peroxisome-dependent retrograde signaling is in its infancy. We examined both in-house and publicly available data sets derived from the profiling of Arabidopsis gene expression in mutants, as well as treatments with peroxisomal-dependent ROS being altered, in order to identify a data set of common and specific genes regulated by peroxisomal ROS under different conditions. Latest analysis about peroxisome-dependent signalling and dynamics under stress will be discussed.
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4) VESS3 Invited Speakers Abstracts – Sorted Alphabetically

100 Years of vitamin E – A historical tour throughout the scientific milestones of vitamin E and its antioxidant paradigm

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The name vitamin E, was given by Barnett and Sure who suggested that the factor proposed by Evans and Bishop as substance "X," be termed vitamin "E" as the next vitamin after the A, B, C and D vitamins had been already described. The identification of vitamin E with a-tocopherol was made in 1936 by Evans' group. One year later β-tocopherol and 11 years later δ-tocopherol were isolated. Tocotrienol (named zetatocopherol) was first described in 1957 and later isolated in 1961. The antioxidant property of tocopherols was reported by Olcott and Emerson in 1937. Inherited vitamin E deficiency, AVED, characterized by a form of neuromyopathy was first described in 1981. The disease was localized to chromosome 8q and found to be caused by a mutation of the a-TTP gene. Later work has been carried out mostly in form of clinical studies on inherited vitamin E deficiency, AVED, immune protection by vitamin E, anti-inflammatory action, especially to protect against NAFELD. The atherosclerosis prevention studies, numerous and expensive, have been successful in animal models but not in humans. As to the molecular mechanism of action of vitamin E, for many years it has been stated that it would act only against oxidative damage. This view has been challenged by indicating that in vitro and in vivo vitamin E modulates gene activity. More recently, potential activated forms of vitamin E have multiplied, including tocopherol phosphate and metabolic products of vitamin E. As to the molecular targets, kinases, phospholipids and microRNA have been considered. Unfortunately, science has not clarified in 100 years all the facets related to the action of this important molecule. Fortunately for researchers, many questions are still unanswered, and a great amount of work remains to be done**.**

Vitamin E metabolism, inflammatory pathways and gut microbiota

Qing Jiang
Purdue University, United States

Natural forms of vitamin E, i.e., tocopherols and tocotrienols, are metabolized via ω-hydroxylase (CYP4F2)-initiated side chain oxidation to form 13'-hydroxychromanol and 13'-carobyxychromanol (13'-COOH), which are subsequently metabolized to terminal metabolite carboxyethyl-hydroxychroman (CEHC) and sulfated analogs. This presentation will focus on the impact of vitamin E metabolites including 13'-COOHs on inflammation in mechanistic, cellular and preclinical studies. In particular, 13'-COOHs have been shown to have anti-inflammatory effects, including inhibition of cyclooxygenase-1/-2 and 5-lipoxygenase activities. 13'-COOHs have also been documented to inhibit cancer cell growth, modulate cellular lipids and decrease nitric oxide synthase expression. Consistent with anti-inflammatory activities, δTE-13'-COOH and αT-13'-COOH suppressed colitis-associated colon cancer and peritonitis, respectively, in mice. Potential interactions between δTE-13'-COOH and gut microbes will also be discussed.

Emerging new understanding of the regulatory role of vitamin E in ferroptotic cell death.

Valerian E. Kagan*, Hülya Bayir
University of Pittsburgh, USA

Hundred years of studies of vitamin E and members of this family leave no doubts that effective and safe scavenging of injurious radicals, particularly lipid peroxyl radicals represents its major biological function. Over the last decade a new understanding of the regulatory mechanisms through which vitamin E exert its biological function has emerged. This relates to the ability of vitamin E to prevent/suppress a new type of regulated cell death, ferroptosis. There are two major mechanisms through which the anti-ferroptotic role of vitamin E is realized. 1. Vitamin E acts as a potent inhibitor of 15-lipoxygenase and its complexes with phosphatidylethanolamine (PE) binding protein 1 (PEBP1) and suppresses the production of the major pro-ferroptotic signal, 15-hydroperoxy-arachidonoyl-PE. 2. By interacting with coenzyme Q, vitamin E one-electron oxidation product, chromanoxyl radical, gets reduced back to its phenolic state and blocks the

formation of the 15-hydroperoxy-arachidonoyl-PE signal. This is a part of the mechanism identified as ferroptosis suppressor protein 1 (FSP1).
Supported by NIH U01AI156924, U01AI156923, AI145406, CA165065, CA243142, AI068021, GM113908, HL114453, AI156924, NS076511, AI156923, and NS061817.

Vitamin E metabolites as bioactive molecules: Is it time to rethink the modes of action of vitamin E?
Stefan Lorkowski*
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Although the 100th anniversary of the unravelment of vitamin E as an essential nutrient is celebrated, there are still more questions than answers regarding its role for human health. During the past hundred years, vitamin E research was characterized by great hopes but also disappointments, which finally resulted in a decline of interest in this research area. But, vitamin E research is currently experiencing a 'renaissance', which is among others due to the unraveling of vitamin E metabolism in humans and the elucidation of putative biological functions of the metabolites formed during hepatic degradation of vitamin E. Since the initial observation of the long-chain metabolites of vitamin E (LCM) in human blood, the LCM appeared as a class of molecules with putative physiological relevance in humans. Research on these molecules has made progress over the last decade and was predominantly focused on the advancement of this new promising field of vitamin E research. Published work of my group significantly contributed to the progress in the field of LCM research, i.e. elucidation of biological functions, identification of signaling pathways and comparison of LCM functionality with their vitamin precursors. These studies contributed to fundamental emerging hypotheses: (i) Do the LCM have physiologically relevance for human health? (ii) Do the LCM represent functional and therefore active forms of their vitamin precursors? (iii) Is essentiality of vitamin E for human health linked to the LCM as active mediators of vitamin E function? Validation of these hypotheses could generate valuable information on the essentiality of vitamin E and it's LCM for human health as well as on potential clinical benefits of the use of these molecules. The presentation will summarize our current knowledge on the LCM and discuss the evidence for the hypothesis that the LCM are the active form of vitamin E.

Structures, activities and analytical determination of Vitamin E and its metabolites
Alexander Maxones, Marc Birringer*
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Plant and marine organisms developed numerous natural products based on a 6-hydroxychromal ring structure. Most of the molecules are diterpenoids, among them tocopherols and tocotrienols; the biological active forms of vitamin E. The structural variability of the compounds is remarkable, since side chain modifcations by oxidation and/or cyclization occur widely, especially in marine organisms.
Cytochrome P450 enzymes are most likely responsible for the initial oxidation to epoxy-, hydroxy- and carboxy-derivatives, respectively. Besides the well investigated tocopherols and tocotrienols, side chain modified 6-hydroxychromanols are rarely studied for their biological activities and might have a high potential as anti-cancer and/or anti-inflammatory drug lead structure. A specific class of side chain modified 6-hydroxychromanols belong to the human vitamin E metabolism. Long chain metabolites of alpha- and delta-tocopherol has been investigated in the recent years and open new avenues in vitamin E research. Besides chemical syntheses approaches toward the long chain metabolites, we developed analytical methods to determine the metabolites in human plasma and cell cultures. Recently, a stable isotope dilution method was established to simultaneously determine tocopherol long chain metabolites and tocopherols via LC-MS. We were able to quantify nanomolar metabolite concentrations of metabolites besides micromolar concentrations of tocopherols in the same chromatographic run.

Vitamin E and immunity: α-tocopherol supplementation of allergic mothers blocks neonate lung microbiome dysbiosis during initiation of neonate allergy

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In animals and humans, neonates of allergic mothers have increased responsiveness to allergens and lung microbiome dysbiosis. In mice, maternal supplementation with α-tocopherol (αT) blocks but γ-tocopherol (γT) enhances neonate development of allergy. In adults and children, lower plasma γT and higher plasma αT associates with better lung function. It is not known whether αT alters neonate development of lung microbiome dysbiosis or whether neonate lung dysbiosis modifies development of allergy. To address this, adult female mice were sensitized and challenged with the allergen chicken egg ovalbumin (OVA) or saline. Then these allergic mothers received αT-supplemented diets or control diets during pregnancy. Neonates from allergic mothers with control diet had lung bacteria microbiome dysbiosis as compared to offspring of nonallergic mothers with control diet or allergic mothers with αT-supplemented diets. It was next determined whether the neonate lung microbiome dysbiosis regulates neonate responsiveness to allergen. To do this, the neonate airway microbiome was collected and transferred intranasally to recipient neonates which were then challenged with allergen. There was development of allergy and lung microbiome dysbiosis in neonates from allergic mothers (regardless of donor microbiome) and in pups of non-allergic mothers that received donor lung microbiome from pups of allergic mothers. Interestingly, transfer of dysbiotic lung microbiome from neonates of allergic mothers to neonates of non-allergic mothers was sufficient to confer recipient neonate responsiveness to allergen. In contrast, neonates of allergic mothers were not protected from development of allergy by transfer of control donor lung microbiome from either neonates of non-allergic mothers or neonates of αT-supplemented allergic mothers. This suggests that the dysbiotic lung microbiome is dominant and sufficient for enhanced neonate responsiveness to allergen. In summary, lung microbiome dysbiosis enhances neonate responses to allergen and this is blocked by αT-supplemented maternal diets.

Emerging clinical applications of vitamin E: non-alcoholic fatty liver disease (NAFLD)

Maren Catherina Podszun*
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Non-alcoholic fatty liver disease (NAFLD), a disease characterized by excessive accumulation of hepatic lipids, is becoming the most common liver disorder in the Western world. Currently, there are no FDA or EMA approved treatments for NAFLD and the lipid soluble vitamin E, specifically alpha tocopherol (αT), has been investigated as potential treatment. αT improves histological features in biopsy proven non-diabetic adults (PIVENS trial), diabetic adults (Bril et al. 2019) as well as pediatric (TONIC trial) NAFLD patients. Furthermore, αT is currently the only treatment that increases transplant free survival in NASH.

Mechanistically, αT reduces hepatic oxidative stress which may be responsible for exacerbating hepatic de novo lipogenesis (DNL) and thereby steatosis. αT also inhibits DNL in vitro and preliminary clinical data also supports this notion. Besides inhibition of DNL, and reduction of hepatic oxidative stress, αT may also positively influence inflammation, although further studies are needed.

Response to αT in NAFLD is variable and might dependent on genetic factors. A promising candidate is a copy number variation in haptoglobin which is associated with diminished antioxidative function as well as increased severity of NAFLD (Hp 2-2). Data from the PIVENS and TONIC cohort has confirmed an association of response with patients carrying the Hp 2 allele showing improvement while those having the Hp 1 allele show none. This association is currently investigated in a large cohort in China where the Hp 2 allele is highly prevalent (NCT02962297).

Overall, αT is a beneficial treatment in NAFLD that is cheap and has relatively few side effects, although it only benefits a subset of patients. Future research should focus on identifying these patients as well as the most effective dose to further improve the risk-benefit ratio of this treatment.

Vitamin E: gene regulation effects and lipid metabolism

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Vitamin E (VitE) is recognized to have a significant role in fetal development. We use the alpha-tocopherol-deficient zebrafish embryo (E– embryos) to study VitE implications in fetal development, especially neurogenesis. Early development gene expression networks are highly conserved between the zebrafish and humans. We showed in zebrafish embryos that the alpha-tocopherol transfer protein (TTP) gene (Ttpa) increases 7-fold by 12 hours post-fertilization (hpf) and remains elevated. Ttpa knockdown is lethal within 24 hpf. It is found in in the yolksac, the developing brain, eyes, and tail bud. Since TTP is needed for trafficking alpha-tocopherol, these data emphasize VitE's importance in critical tissues. Using mass spectrometry (lipidomics and metabolomics), we discovered the occurrence of increased lipid peroxidation (LPO) especially of phosphatidyl choline with docosahexaenoic acid (DHA-PC) in E– embryos. VitE specifically prevents LPO by acting as a peroxyl radical scavenger swiftly terminating LPO chain reactions. However, this process consumes both energy (NADPH) and glutathione, as well as dysregulating phospholipid metabolism as evidenced by altered lysophospholipid, choline and betaine levels. E– embryos also display altered gene expression associated with 1-carbon metabolism, energy metabolism, anabolic reactions, and gene transcription. Thus, VitE deficiency likely impacts choline and its interactions with the methionine and folic acid cycles resulting not only in impacts in neurogenesis, but also potential impacts on epigenetic regulation in the developing embryo. We also found that mTOR, which is critical in regulating metabolic function, embryonic patterning and neural stem cell differentiation, is also dysregulated in E– embryos. Our findings provide a critical link to understanding the downstream consequences of increased LPO and how VitE acts as a lynchpin to prevent the metabolic dysregulation caused by DHA-PC depletion in embryos. These data are also applicable to other cellular environments and suggest why VitE is a critical nutrient for humans.

5) SFRR-E Symposia Abstracts in Sequence of Presentation

SFRR-E Symposium 1 – Bench to bedside transition for pharmacological regulation of NRF2 in non-communicable diseases

The role of NRF2 for mitochondrial adaptation in inflammatory macrophages

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The transcription factor Nrf2 nuclear factor erythroid 2 p45-related factor 2 (Nrf2) and its negative regulator, Kelch-like ECH associated protein 1 (Keap1), regulate the expression of complex networks of genes encoding cytoprotective proteins that provide adaptation to oxidative, electrophilic, inflammatory, and metabolic stress. Using quantitative high-resolution mass-spectrometry approaches, we characterized the proteomes of bone-marrow derived macrophages with graded Nrf2 transcriptional activity at resting and activated (with lipopolysaccharide, LPS) states. We found significant differences among the genotypes in the abundance of proteins that participate in numerous cellular processes, including redox, amino acid, carbohydrate and lipid metabolism, and innate immunity. Complementary metabolomics and respirometry studies supported these findings. Analysis of oxygen consumption rates (OCR) in resting macrophages confirmed a role for Nrf2 in regulating mitochondrial respiration: Nrf2 activation increased the basal respiration rates associated with ATP production, whereas Nrf2 disruption had the opposite effect. Analysis of extracellular acidification rates (ECAR) identified a role for Nrf2 in promoting glycolysis in resting and activated macrophages. In addition to changes in metabolism, we also observed an enrichment in mitochondrial fusion with Nrf2 activation, including the mitochondrial fusion proteins, Opa1, Mfn1 and Mfn2, and a significant increase in the mitochondrial fission factors, Mff and Mief2, with Nrf2 disruption in activated macrophages, suggesting that Nrf2 may play a role in mitochondrial adaptation during inflammation. Confocal microscopy analysis of mitochondrial morphology following immunofluorescence staining of the outer mitochondrial membrane protein Tom20 further showed that prolonged stimulation with LPS caused a switch in mitochondrial morphology, from intermediate to fused/elongated, which was enhanced by Nrf2 activation and suppressed by Nrf2 disruption. Together, these findings show that Nrf2 is a critical factor governing redox and intermediary metabolism and facilitating mitochondrial adaptation in macrophages encountering pro-inflammatory stimuli.

Identifying therapeutic vulnerabilities in NRF2 dependent cancers

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The NRF2 pathway is often constitutively activated in various cancer types, particularly in non-small cell lung cancer (NSCLC), contributing to the malignant phenotype and drug resistance. Unfortunately, direct targeting of NRF2 has remained a challenge, as it is a transcription factor with many structural homologs and functions. Therefore, indirect means to inhibit NRF2 activity by for example targeting factors affecting transcriptional regulation of NRF2 or downstream pathways generating cancer-specific vulnerabilities may provide alternative ways to treat NRF2 overactive cancers. Herein, various multi-omics approaches are used to discover targetable co-dependencies in cancer with constitutive NRF2 activation. It is demonstrated how publicly available genetic and drug repurposing screens can reveal specific vulnerabilities, and how NRF2 activation affects the efficacy of immune therapies. Also, systems proteomics approaches are used to study proteins interacting with the KEAP1-NRF2 system, potentially identifying novel coregulators that can be targeted for therapy.

Targeting the NRF2/betaTrCP axis in liver disease

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Inflammation plays an important role in the pathology of most diseases, including non-alcoholic steatohepatitis (NASH) for which there is no currently approved drug to stop disease progression. Transcription factor NRF2 has been proposed recently as a promising target to stop NASH, but the most widely analyzed compounds are electrophiles that, in addition to inhibiting its main repressor KEAP1, display many off-target effects and also elicit a very strong supra-physiological NRF2 activation. As an alternative, we screened a chemical library of ~ 1 M small molecules to identify disrupters of the interaction between NRF2 and the E3 ligase adapter beta-TrCP. In vitro ubiquitination and cell culture experiments demonstrated that our hit compound is a beta-TrCP/NRF2 interaction inhibitor that activates NRF2 within physiological levels. This compound is specific for NRF2, as it does not disrupt the interaction between beta-TrCP and other substrates, such as beta-catenin. Pharmacodynamics studies demonstrated selective exposure and NRF2 activation in the liver. In mice submitted to LPS-induced acute liver inflammation, the compound greatly attenuated Kupfer cells' activation and the NFkB-mediated inflammatory response. We further analyzed the effect of this compound in the STAM model of progressive liver damage by NMR, measuring the fat/water ratio, and histochemistry of oil red (fat) and Sirius red (fibrosis), and correlated inflammatory and metabolic parameters with NRF2 activation. We found that this PPI inhibitor prevented NASH onset. Importantly, mice which were allowed to develop NASH and were then submitted to chronic treatment with this compound for 4 weeks exhibited a significant protection against the development of fibrosis. Our results report an innovative mechanism to activate NRF2 and protect the liver from NASH and fibrosis.

SFRR-E Symposium 2 – Post-translational modification of proteins in health and disease

Characterization and quantification of post-translational modifications on proteins in cardiovascular disease

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Cardiovascular disease (CVD) is a leading cause of morbidity and mortality worldwide. Atherosclerosis, which is characterized by cholesterol and lipid accumulation in the artery wall, is the major underlying cause of CVD and is often asymptomatic for decades. Unfortunately, destabilization and rupture of atherosclerotic lesions can be sudden and give rise to an acute myocardial infarction or stroke. Despite the importance of lesion stability in CVD, the mechanisms underlying lesion rupture are poorly understood, though there is considerable evidence for extracellular matrix (ECM) alterations and a weakening of lesion structure. Compared to stable lesions, rupture-prone lesions typically contain higher levels of activated inflammatory cells that generate potent oxidants, such as hypochlorous acid and nitrating species which can both damage ECM proteins directly, or activate proteases that degrade ECM components.
In this presentation, data consistent with alterations to the nature and type of materials in lesion ECM will be presented, together with modifications to these materials, as determined by immunocytochemistry, immunoblotting and liquid-chromatography (LC-MS) studies. Analysis of materials present in, or extracted from carotid lesions, has allowed identification of 890 differentially-abundant proteins between soft (rupture-prone) and hard (stable) lesions. Many of the overabundant proteins in soft lesions are involved in inflammatory responses and ECM remodeling. Detailed LC-MS analyses have shown the presence of chlorinated, nitrated and oxidized species on multiple ECM components, together with a marked increase in cleaved proteins, as judged by the N-terminal proteomics that has allowed detection of 837 cleaved peptides. The detection of these species is consistent with marked protein damage. The protein identities and the sites of cleavage have been characterized in some cases. These species are present at significantly higher abundance in unstable compared to stable lesions. Together, these data offer a unique insight into the inflammatory and proteolytic mechanisms of lesion destabilization in CVD.

The proteasome as a major player against ageing and proteinopathies

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Ageing is a physiological inevitable process that represents a high risk factor for the progression of age-related diseases. It is regulated by environmental, genetic and epigenetic factors. Several molecular pathways deteriorate with ageing or with age-related proteinopathies, including the proteasome system. Proteasomes are constituents of the proteostasis network that maintain protein homeostasis through regulated proteolysis of normal and abnormal (in any way) proteins. Using the replicative senescence model of human primary fibroblasts in combination with the organismal model Caenorhabditis elegans, we were able to achieve proteasome activation either through genetic overexpression of β5/pbs-5 proteasome subunit or through specific compounds with proteasome-activating properties. Proteasome activation promotes lifespan extension at both cellular and organismal level. Moreover, elevated proteasome function confers lower paralysis rates in various Alzheimer's disease (AD) nematode models accompanied by decreased Aβ deposits, thus ultimately decelerating the progression of the AD-like phenotype. Similar results have been shown in human cells of neuronal origin challenged with increased concentrations of Aβ peptide. In total, our results demonstrate the pivotal role of the proteasome and its regulation in the progression of ageing and proteinopathies.

Macromolecular crowding and micro-domains as modulators of protein oxidation

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Biological systems are characterized by being highly-packed and heterogeneous environments. Such crowded milieus have been shown to modulate different aspects of protein structure and function including protein folding, protein diffusion, protein aggregation and protein-protein interactions. Therefore, we hypothesized that these highly crowded and heterogeneous systems, which contain micro-domains, may also modulate protein oxidation and modification reactions both inside and outside cells.

We have recently reported the occurrence of short-chain reactions that lead to propagation of oxidative damage in concentrated solutions of intrinsically disordered proteins (casein proteins) and in solutions containing high concentrations of inert crowding agents such as dextran. These short chain reactions involve the formation and propagation of tryptophan-derived species (i.e. tryptophanyl radicals or tryptophan-derived peroxyl radicals). In addition, enhanced damage to other susceptible residues such as methionine and tyrosine residues has also been detected. The latter is supported by kinetic studies that demonstrated that 60 mg mL-1 dextran enhanced the rate of oxidation of free Trp, and peptide Trp, elicited by AAPH-derived peroxyl radicals. For free Trp, the rates of oxidation were 15.0 ± 2.1 and 30.5 ± 3.4 μM min−1 without and with dextran, respectively. LC-MS studies also confirmed enhanced amino acid loss in casein proteins, and altered formation of protein cross-links as observed by SDS-PAGE analysis. Moreover, recent studies on the glycation of human serum albumin and human transferrin induced by methylglyoxal and glyoxal under crowded conditions has shown that crowding modulates the formation of protein carbonyls and the formation of protein oligomers. Overall, these data indicate that molecular crowding, as commonly encountered in biological systems affect the rates, and extents of oxidation, and particularly of Trp residues, illustrating the importance of appropriate choice of in vitro systems to investigate oxidative processes.

SFRR-E Symposium 3 – Redox mechanisms in neurodegenerative disorders - From genes to proteins quality and propagation

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Effect of APOE ε4 allele and redox signature in circulating extracellular vesicles in cognitively impaired patients converted to Alzheimer's disease

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Alzheimer's disease (AD) is an age-related brain disorder and the leading cause of dementia. Oxidative stress is a unifying paradigm in the pathophysiology of AD and the presence of the apolipoprotein E4 variant (APOE ε4) is assumed to stimulate oxidative damage and enhance AD risk.
Exosomes or extracellular vesicles (EVs) (50-150 nm) are released by all cell types in the body. We have determined the impact of APOE ε4 on the level of apolipoproteins with antioxidant activities (apoE, apoJ, and apoD) along with oxidative markers in circulating extracellular vesicles (cEVs) and plasma from cognitively impaired-not demented (CIND) individuals converted to AD (CIND-AD).
Methods: EVs were isolated using the Total Exosome Isolation reagent and characterized according to the ISEV guidelines. Apolipoproteins E, J, and D and antioxidant response markers were determined in cEVs and plasma using immunoblotting, electrochemical examination, and spectrofluorimetry.
Results: We observed a significant decrease in the total antioxidant capacity (TAC) in the CIND-AD group. Levels of apoD in plasma and cEVs were higher in CIND-AD participants. Interestingly, protein carbonyls content and apoJ/D ratio were statistically different in cEVs but not in plasma from CIND-AD. Our data also indicate that TAC, cEVs protein carbonyls, cEVs apoJ/D levels were correlated with the neurocognitive Mini-Mental State Exam (MMSE) scores and are APOE ε4-dependant.
Discussion: Our results demonstrate that cEVs redox signature is more relevant than plasma for reflecting specific brain and systemic changes in early AD onset and particularly in APOE ε4 carriers.
Conclusion: Our findings support the pathological redox linkage between APOE ε4 and AD onset and suggest the use of cEVs oxidative signature in early AD diagnosis.

Trisomy 21 and aberrant redox homeostasis: a synergistic path to Alzheimer disease

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Down syndrome (DS) is the most common genetic form of intellectual disability that results from the triplication of the entire portion or part of chromosome 21 (Trisomy21). DS represents a unique population for studying changes of brain aging across the lifespan; individuals with DS are the largest population under 60 years of age characterized by the early appearance of AD neuropathological features and are currently classified as early onset AD (EOAD).
The complexity of DS neurodegeneration involves multiple molecular mechanisms, similar to what observed in AD brain, including the deposition of beta-amyloid (Aβ) into senile plaques and tau hyperphosphorylation in neurofibrillary tangles. Intriguingly, several trisomic genes in addition to being primarily linked to Aβ pathology, are responsible for increased oxidative stress (OS) conditions, including SOD1, BACH-1, CBS among others. Indeed, our studies support the hypothesis that OS contributes to neurodevelopmental defects, neuronal dysfunction as well as the accelerated aging phenotype of DS population. We have demonstrated that oxidative damage to proteins occurs in young DS individuals, before the onset of AD-like neurodegeneration, and is associated to dysfunction of several cellular processes such as energy production, protein quality control, stress response, cytoskeleton network and synaptic function. Further, the OS phenotype is closely associated with mitochondrial defects, that likely sustains a vicious cycle further exacerbating ROS production and mitochondrial dysfunction with aging. Redox proteomics studies contributed to highlight that the impairment of energy metabolism is a key pathological feature of DS brain, showing aberrant activity of several enzymes involved in glycolysis, Krebs cycle and oxidative phosphorylation.
Collected results support the idea that mitochondrial defects, increased OS levels and impaired glucose metabolism lead to reduced ATP production, thus catalysing a synergistic path to accelerated aging and dementia.

Deciphering the noxious relationship between oxidative stress and the unfolded protein response in Alzheimer-like neuropathology

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Increasing evidence demonstrated that increased oxidative stress (OS), associated with mitochondrial dysfunction and antioxidant responses failure, is an early signature of alzheimer-like pathology, promoting protein oxidation and the deposition of toxic aggregates. In turn, protein quality control systems, involved in the surveillance of protein synthesis/folding/degradation, result impaired in AD and DS brain leading to altered proteostasis. Accordingly the redox proteomic analysis of DS and AD brain performed in our laboratory identified the increased oxidation of components of the unfolded protein response (UPR) that may promote the escape of unfolded/misfolded proteins from protein quality control favoring aberrant proteostasis and increased OS. In particular, we observed that the oxidation of BiP n DS and AD human brain may lead to the chronic selective induction of the PERK branch of the UPR. The dysregulation of PERK was associated with increased eIF2α, suggesting the reduction of translation, and with the increased expression of ATF4 and CHOP transcription factors. Interestingly, the eIF2α negative feedback loop, exerted by GADD34, was lost in brain samples contributing to the sustained activation of the PERK pathway. Surprisingly, we also detected in an early time point in DS and in a late time point in AD the uncoupling between PERK and Nrf2 response, respectively as effect of Bach1 overexpression or of aging. Data collected in human brain were corroborated by the analysis of DS murine models and of blood-derived primary and cultured cells. The pharmacological manipulation of PERK was able to rescue proteostasis, reduce the build-up of oxidative damage and improve neuropathological alterations. Our results suggest that the failure to regulate the PERK pathway is both cause and effect of increased OS and may represent an essential step in promoting aberrant proteostasis in AD-like pathologies. Further, the exploration of PERK inhibition may represent a therapeutic option for AD neurodegeneration.

SFRR-E Symposium 4 – Oxidative stress in the pathogenesis and clinical management of COVID-19

Understanding and targeting inflammation in SARS-CoV-2 and HIV infections

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Systemic inflammation following infection with SARS-CoV-2 is predictive of disease severity; yet, the immune mediators driving inflammation contributing the host antiviral defense versus those driving immune-mediated pathology are complex and not fully understood. Therefore, utilizing well-characterized models of inflammation with viral diseases, such as SIV-infected rhesus macaques (RMs), may inform therapeutic strategies to target immune-mediated pathology. For example, our recent data shows that interleukin (IL)-10 signaling regulates T cell survival, differentiation, and exhaustion, and its neutralization in combination with PD-1 blockade following antiretroviral therapy (ART) interruption synergized to improve the homeostasis of the immune system and to induce viral control. Immunological features of COVID-19 progression include an influx of innate and adaptive immune cells to the lung, with severe cases having elevated levels of pro-inflammatory cytokines and chemokines. This pro-inflammatory state can arise due to sustained, uncontrolled type-I interferon (IFN-I) responses, which have also been shown to interfere with lung repair following viral infection. RMs treated in the early acute phase of SARS-CoV-2 infection with baricitinib, a selective JAK 1/2 inhibitor, exhibit reduced inflammation, T cell immune activation, neutrophil NETosis activity, and lung pathology. Importantly, baricitinib treated RMs exhibited potent suppression of alveolar macrophage-derived cytokines and chemokines, which are responsible for the recruitment of neutrophils and pro-inflammatory monocytes. In a follow-up study, by directly modulating IFN-I signaling via the prophylactic administration of a mutated IFNalpha-2 (IFN modulator, IFNmod), we have observed a highly significant and consistent reduction in SARS-CoV-2 viral loads in the lower and upper respiratory tract. Treatment with IFNmod also potently reduced soluble markers of inflammation in bronchoalveolar lavage (BAL), the expansion of inflammatory monocytes, and lung pathogenesis. These data indicate a vital, early role of JAK and IFN-I in regulating COVID-19 progression and emphasize the importance of understanding pro-inflammatory pathways for the development of targeted therapeutic strategies.

The importance of zinc and selenium for COVID-19 survival and SARS vaccination success

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The pandemic highlighted once again the vulnerability of the human organism and the threat of viral infections. The immune system needs to be prepared and supported optimally to react appropriately and avoid damage and death. Among the trace elements, selenium (Se) and zinc (Zn) have proven of high diagnostic value, as the decline in their serum concentrations allowed some reliable prediction on disease course and fate. Potential candidates for the molecular effectors for Se are mainly restricted to the family of selenoproteins, many of which with catalytic activity and relevance for the innate and adaptive immune system. The role of Zn for the immune system is well established, albeit the key molecular processes are diverse as many more proteins depend on Zn for structure and activity.
In our analyses of clinical trials, we have determined that both trace elements decline drastically in infection, reaching states of severe deficiency (as far as can be judged from blood concentrations). Supplemental Se and Zn intake may avoid such severe deficiency when taken as prophylactic measure. In how far infected and diseased subjects profit from supplementation is unknown at present. With respect to vaccination response, supplemental intake of Se, Zn or vitamin D proved without positive effect on humoral antibody response in a small study of healthy adult health care workers. However, chronically ill, very young or elderly subjects have not been studied, yet.
Collectively, the data indicate that an avoidance of habitual low Se or Zn intake constitutes a meaningful measure in face of the current pandemic, and certain risk groups prone to develop Se or Zn deficiency may profit from a personalized supplementation regimen.

Long term outcomes after a critical COVID-19: Clinical and biological perspectives

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In this presentation, the consequences of a severe COVID-19 pneumopathy and of an intensive care unit (ICU) stay will be described. Survivors of a critical illness may experience medium- and long-term morbidities related to the critical illness, the treatment and organ support received, and the unique ICU environment. These disorders have been labeled "post-intensive care syndrome" (PICS). The princeps definition of PICS refers to new or worsening physical, mental and neurocognitive disorders that negatively affect daily functioning and quality of life of critically ill survivors.
More than 60 critical COVID-19 survivors attended our post-ICU follow-up clinic. They benefited from a multidisciplinary consultation investigating the components of the PICS. In particular, we observed significant alterations of their functional capacities, with altered cardiopulmonary exercise testing. These findings will be described, in light of the biological data, including oxidative stress.

SFRR-E Symposium 5 – Oxysterols in health and disease

Oxysterols-induced oxidative stress, mitochondrial and peroxisomal dysfunctions: potential consequences on age-related diseases

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Cholesterol oxide derivatives (also named oxysterols) are 27-carbons molecules formed by the addition of oxygen to the cholesterol molecule. This addition of oxygen on cholesterol can be achieved by non-enzymatic and/or enzymatic reactions. Among these oxysterols, some of them such as 7-ketocholesterol (7KC), 7β-hydroxycholesterol (7β-OHC), 24(S)-hydroxycholesterol and cholesterol-epoxides have cytotoxic activities characterized by an induction of oxidative stress, mitochondrial and peroxisomal dysfunctions, autophagic criteria and induction of a caspases independent or dependent mode of cell death. The caspases dependent mode of cell death activated by cytotoxic oxysterols is defined as oxiapoptophagy (OXIdative stress + APOPTOsis + autoPHAGY). As 7KC and 7β-OHC are identified at increased levels in the body fluids and/or tissues of patients with cardiovascular, neurodegenerative and ocular diseases, their contribution in these age-related diseases is widely suspected. Reducing the toxicity of these oxysterols opens up new therapeutic

perspectives. Thus, cytoprotective activities against 7KC- and 7β-OHC-induced cell damages have been observed with oils (argan, olive, milk thistle and pistacia lentiscus seed oils) as well as natural molecules associated with the Mediterranean diet such as alpha-tocopherol, omega-3 and omega-9 fatty acids (docosahexahenoic acid, oleic acid, respectively), polyphenols (resveratrol, quercetin, apigenin) but also with monomethyl and dimethyl fumarate (synthetic molecules). A better understanding of the biological activities and signalling pathways associated with the oxysterols involved in age-related diseases should lead to the identification of new therapeutic targets and innovative treatments.

Focus on the controversial Role of 24-hydroxycholesterol in Alzheimer's disease

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Cholesterol metabolism in the brain plays a major role in Alzheimer's disease (AD) development. Indeed, maintenance of brain cholesterol homeostasis is essential for neuronal functioning and brain development. To maintain the steady-state level, excess brain cholesterol is converted into the more hydrophilic metabolite 24-S-hydroxycholesterol (24-OHC) by the neuron-specific enzyme CYP46A1.
A growing bulk of evidence suggests that cholesterol oxidation products, named oxysterols, are the link connecting altered cholesterol metabolism to AD. It has been shown that the levels of some oxysterols, including 27-hydroxycholesterol, 7β-hydroxycholesterol and 7-ketocholesterol, significantly increase in AD brains contributing to disease progression; in contrast, 24-OHC levels decrease, likely due to neuronal loss. The concept is now widespread that, during AD development, certain oxysterols accumulating in the brain can act as friends and/or foes. Among the different oxysterols, 24-OHC, which is the dominant in the brain, is certainly the one whose role is most controversial: on the one hand it promotes neuroinflammation, Aβ peptide production, oxidative stress, and cell death in neuronal cell lines; on the other hand, it has been reported to be a main player of the regulatory loop between astrocytes and neurons to maintain brain cholesterol homeostasis, and to exert several beneficial effects against AD progression, such as preventing tau hyperphosphorylation, suppressing Aβ production, and regulating synaptic function.
Considering the emerging evidence supporting a positive role of 24-OHC in AD pathology, one can assume that the physiological presence of this oxysterol in the brain is fundamental to guarantee brain health, as highlighted by the up-regulation of the enzyme CYP46A1 activity, whose levels are also reduced in the AD brain. This suggests the importance of preventing the loss of 24-OHC in the brain during the course of AD; thus, its targeting could be useful for the disease prevention or, at least, in slowing down its progression.

Sulfated oxysterols in ageing and neurodegeneration

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Cholesterol levels in the brain are tightly regulated for physiological brain function, but increasing evidence indicates that changes to cholesterol metabolism and accumulation of oxidised cholesterol may drive age-related neurodegeneration. Cholesterol and oxysterols are being sulfated by sulfotransferases (SULT) to form cholesterol sulfates (CS) or oxysterol 3-sulfates. CS is a highly abundant sterol sulfate in human plasma and in the brain, cholesterol sulfate is a substrate for the synthesis of neurosteroids which display neuroprotective properties. Over expression of sulfotransferases could increase a range of oxysterol sulfates and have shown antagonistic effects on oxysterol sensing liver X receptors. It is therefore important to understand how further changes to oxysterol/oxysterol sulfate homeostasis can contribute to healthy ageing and age-related disease. The aim of this project is to develop and validate a multiple reaction monitoring based liquid chromatography-mass spectrometry method for the absolute quantification of oxysterol sulfates; 26-hydroxycholesterol sulfate (26OHCS), 25-hydroxycholesterol sulfate (25OHCS) and 7-keto cholesterol sulfate (7KS) in healthy adults. Total oxysterols were extracted from plasma (50µl) spiked with internal standards (1ng of 25OHCS-d7, 26OHCS-d6, and 7KS-d5) by adding 450µl of 100% methanol containing 50µg/ml BHT, followed by alternate vortexing and sonication for 10min. Oxysterols were enriched using two-step solid phase extraction to remove excess phospholipids (HLB PRiME, Waters). A reverse phase LC method using a methanol/water to methanol gradient was systematically developed for optimal separation of oxysterol sulfates. The assay was validated using quality control (QC) plasma samples, and freeze-thaw stability of oxysterols was evaluated. Method validation was performed to establish linearity,

sensitivity, recovery, and accuracy. The method described has been validated for three oxysterol sulfate measurements in human plasma and is suitable for studies investigating the oxysterol sulfate levels in human health and disease.

SFRR-E Symposium 6 – Natural bioactives in redox homeostasis: from food to health

Metabolism of polyphenols (by mammalian cells and host microbiota); significance in the production of physiological relevant metabolites

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(Poly)phenols or phenolic compounds are a vast group of phytochemicals with recognized features in the prevention of multiple non-communicable diseases. Phenolic compounds are metabolized by phase II enzymes in mammalian cells (mainly at intestinal level and hepatocytes, but not only), as well as by the gut microbiota. They are turned into bioavailable molecules able to impact on different biological processes related to human health. The elucidation of the metabolic fate of phenolics and their bioavailability is a tipping point to fully unravel the molecular forms responsible for their preventive actions in the framework of cardiovascular diseases, metabolic syndrome, neurodegenerative disorders, and certain kinds of cancer. However, although much has been reported on this regard, there is still a lot of work to be done. For instance, a major conundrum is related to the inter-individual variability existing in the bioavailability and physiological response to (poly)phenols, as it can impact their true efficacy.
Adhering to physiological conditions when performing (poly)phenol research should always be kept in mind at all research levels, from cell studies to human interventions. Dosages, molecules, and experimental designs should all be built over physiological assumptions in order to make research translatable and really useful. This presentation will deal with some gold standards to be considered for the application of realistic research on (poly)phenols. It will focus on recently published and ongoing experiments targeting the bioavailability and bioactivity of phenolic compounds by using, as much as possible, physiologically-plausible scenarios. Fruitful discussion is expected considering the need to better understand the contribution of phytochemicals to the health effects of plant-based foods.

Potential of Natural Products to target senescent endothelial cells Role of the eNOS/NO-ROS pathway

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Cardiovascular disease remains the leading cause of death worldwide mostly due to ischemic heart disease and stroke. The underlying mechanism involves the development of atherosclerotic lesions starting already at childhood and affecting initially atheroprone arterial sites such as bifurcations and curvatures that are exposed to disturbed blood flow and low shear stress. As a consequence the shear stress-induced endothelial formation of nitric oxide (NO, a major vasoprotective factor) is insufficient to protect such atheroprone sites promoting their progression into mature plaques under the control of the combined cardiovascular risk factors, that can ultimately lead to an atherothrombotic event following plaque erosion or rupture. Atheroprone sites are covered prematurely by senescent endothelial cells characterized by a pro-oxidant state and the acquisition of pro-atherosclerotic, pro-inflammatory and pro-thrombotic markers, promoting endothelial dysfunction and the development of atherosclerotic plaques. Thus, senescent endothelial cells appear as an interesting target to regenerate the protective function of endothelial cells on the cardiovascular system. It is now well established that a poor healthy diet score is a major contributor of cardiovascular disease, and that regular intake of polyphenol-rich fruit and vegetables contributes to improve the cardiovascular health status. Berry-, cocoa- and green tea-derived polyphenols can enhance the formation of NO subsequent to the activation of endothelial NO synthase by the PI3-kinase/Akt pathway and the MAP kinases pathway, and increase its expression level thereby promoting a sustained formation of NO. More recently, it has been shown that several natural products including berry-derived anthocyanins are able to prevent premature senescence of endothelial cells thereby perpetuating the endothelial formation of NO. The beneficial effect of anthocyanins is dependent on their structure and the nature of the osidic moiety. Thus, natural products appear as an interesting approach to preserve and/or rejuvenate endothelial cells to protect the cardiovascular system.

Lifestyle and epigenetics in metabolic disorders: participation of redox signaling and actions of anthocyanins

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The widespread consumption of high fat/high carbohydrate diets and the frequently associated obesity is directly linked to the increased worldwide burden of chronic diseases, including type 2 diabetes (T2D). Unhealthy diets also promote epigenetic changes occurring actively during early development, but also throughout life, constituting a critical link between the environment, e.g. nutrition; gene expression; and human health. While Western style diets are associated to obesity and T2D, dietary flavonoids, e.g. anthocyanins (AC), are linked to improved glucose homeostasis and insulin sensitivity. Redox-regulated mechanisms are involved at different levels in the association between diet and the triggering or prevention of insulin resistance and T2D. A central player in this link are the NADPH oxidases (NOX). Thus, upregulation of enterocyte NOX1 causes intestinal barrier permeabilization and endotoxemia, leading to systemic inflammation and insulin resistance. Upregulation of liver/adipose tissue NOX2, NOX3 and NOX4, causes oxidation of lipids and proteins and activation of redox-sensitive signals, i.e. NF-κB and mitogen activated kinases ERK1/2, p38 and JNK1/2. The activation of these cascades not only causes tissue inflammation, but IKK and JNK1/2 directly inhibit the insulin cascade leading to insulin resistance. In a mouse model of HFD-induced insulin resistance, supplementation with AC (cyanidin and delphinidin), mitigated NOXs upregulation, inhibited IKK and JNK1/2 activation and improved insulin sensitivity. In humans, consumption of AC with a single high fat meal (HFM) improved postprandial hyperglycemia and hyperlipidemia, prevented HFM-triggered NOX4 upregulation, and altered parameters of inflammation and insulin resistance in peripheral blood mononuclear cells. Additionally, recent findings showed that experimental diabetes cause the epigenetic-mediated upregulation of NOXs. Overall, deregulation of NOXs and of downstream redox signaling are central in the development and progression of T2D and on the protective effects of dietary AC. Supported by NIFA-USDA.

SFRR-E Symposium 7 – The elusive roles of nitric oxide in the brain: From signaling to neurodegeneration

The role of nitric oxide in autism spectrum disorder

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ASD is a neurodevelopmental disorder associated with impaired communication, deficits in social skills, and repetitive behavior. SHANK3 and CNTNAP2 gene mutations are among the most prominent ASD-associated mutations. Several reports on the Shank3 and Cntnap2 knockout (KO) mouse models showed defects in biochemical, electrophysiological, and behavioral phenotypes. However, the molecular mechanisms are still not revealed. Nitric oxide (NO) is one of the most important signaling molecules in the body and specifically in the brain. We have previously reported, for the first time, the involvement of NO in ASD pathology (in the Shank3 model). To examine the pathological role of NO in the brain, we treated wild-type (WT) mice with an NO donor, HU-52. Interestingly, HU-52 caused behavioral and molecular abnormalities similar to those observed in the mutant mice. In both mutant models, the protein levels of the glutamatergic and GABAergic biomarkers were significantly reduced. We also found a reduction in cortical spine density (Golgi staining) in both mouse models. To validate the causal effects of NO, we used a selective nNOS modulator, HU-53, to assess whether modulating the NO level can rescue NO-mediated changes in the brain of the mutant mice. Treatment of Shank3 KO and Cntnap2 KO mice with HU-53 reversed both molecular and behavioral abnormalities. Collectively, the results of our study show that NO plays a major role in ASD pathology. We also showed that NO alters the glutamatergic and GABAergic systems in the cortex and striatum, which may result in the disruption of neuronal processes manifested in ASD-like behavioral deficits. Finally, this work will lead to the discovery of novel NO-related drug targets for the treatment of ASD.

Nitric Oxide deficiency linking a defective minipuberty to the appearance of comorbidities: new therapeutic possibilities

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Prematurity is associated with alterations in the maturation of the Hypothalamic-Pituitary-Gonadal (HPG) axis, and specifically with its transient activation during infancy, i.e. minipuberty. Minipuberty, and the resulting surge in gonadotropin levels (LH, FSH) influences neuronal network maturation, growth, blood pressure, body composition, and lipid and glucose metabolism. Indeed, hyperandrogenism or altered follicular development, both occurring because of aberrant FSH levels at minipuberty, contribute to the risk of developing many noncommunicable diseases. Nitric oxide (NO) has long been recognized as a key player in the central hormonal regulation of ovulation. However very little is known about its role in the minipubertal activation of the HPG axis.

Extensive hormonal characterization of minipuberty, reproductive, metabolic and cognitive assessment was carried out in both a newly developed mouse model of premature birth, as well as a Nos1-deficient mouse model. In vivo pharmacological manipulation of minipubertal NO levels was used to identify the timely regulation of minipuberty and its association with cognitive and non-cognitive comorbidities. Therapeutic options targeting the restoration of minipuberty have been pre-clinically validated.

Nos1 deficiency results in dose-dependent defects not only in sexual maturation but also olfaction, hearing and cognition. In vivo pharmacological manipulation of NO levels revealed a critical time window during which Nos1 activity shaped infantile and adult physiology. Preterm mice phenocopied the effects of Nos1-deficiency, revealing a crucial role of minipubertal NO in the maturation of the neuroendocrine brain and the physiological outcomes in adulthood.

Our work is the first to associate the maturation of the neuroendocrine axis with brain development (including development of higher brain functions), identifying NO's key role in the underlying mechanism. By identifying a targetable cause for the deficits linked to premature birth (and altered minipuberty) our work aims to creating novel therapeutic avenues. miniNO is financed by the EU Research Council in 2020 (No847941).

NO-mediated neuroinflammatory pathways as treatment targets in neurodegeneration

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Numerous neurodegenerative diseases associated with protein misfolding (e.g. Alzheimer's and Parkinson's disease) exhibit enhanced oxidative and nitrergic stress conditions following initiation of neuroinflammatory pathways. The underlying activation of microglia within the central nervous system is responsible for release of pro-inflammatory molecules associated with nitric oxide (NO) production, a potent contribution to cytotoxic redox signaling. NO-mediated post-translational protein modifications impact upon protein functions and can exacerbate pathological processes. In addition, non-enzymatic and irreversible glycation signaling has been implicated as a pathway that promotes protein misfolding via the generation of advanced glycation end-products (AGE). Following activation of specific receptors recognizing AGEs (RAGE) further oxidative stress and cytokines production induces an upregulation of inflammatory mediators. However, the direct interactions between both, NO-mediated neuroinflammation and RAGE signaling remain poorly understood.

We investigated the therapeutic potential of suppressing NO signaling during early prion disease progression. To study the impacts of NO on the pathology, prion-diseased mice were injected daily with a NO synthase (NOS) inhibitor during disease onset. Neurophysiology and disease marker properties during early pathology were analysed.

Strong neuroinflammation characterized by enhanced nitrergic and oxidative stress was associated with a decline in hippocampal neuronal function in diseased mice during 6 to 10 weeks post inoculation (w.p.i.) with scrapie prion protein. Daily i.p. administration of the NOS inhibitor L-NAME between 6 and 9 w.p.i. prevented the functional degeneration of hippocampal neurons. We further found that this intervention reduced 3-nitrotyrosination of triose-phosphate isomerase (TPI), an enzyme involved in the formation of disease-associated glycation and AGE formation. Furthermore, L-NAME application reduced the degree of TPI-nitrotyrosination and the expression of RAGE. This work concludes that NO mediated post-translational modifications of TPI may enhance glycation signaling which contributes to further cytotoxicity and accumulation of misfolded prion proteins and thus illustrates an interaction between glycation and NO signaling.

6) SFRR-E Oral Presentation – Abstracts in Sequence of Presentation

SFRR-E Early Career Research Symposium

[YIA] Changes in NADPH oxidase activity in E-cigarette vapor condensate exposed cultured cells and the role of acrolein

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Even though electronic cigarettes (Ecig) are marketed as a healthier substitute for tobacco cigarettes, evidence is arising, that Ecig vapor could cause adverse health effects. It is assumed that Ecig liquid components that are degraded thermally are the ones responsible for the observed effects on human health, with toxic aldehydes being the most prominent group. The aim of this study is to evaluate the mechanistic effects of Ecig vapor toxicity on NADPH oxidase activation in immune and vascular cells, as well as to test if acrolein, a byproduct of Ecig liquid heating, is the main malefactor. My group previously showed that Ecig vapor exposure causes oxidative stress, inflammation, apoptosis, endothelial dysfunction and high blood pressure in a mouse model, through activation of NADPH oxidase [Kuntic et al. Eur. Heart J. 2020]. To understand the mechanism of NAPDH oxidase activation better, we have now exposed cultured endothelial cells (EA.hy 925) and macrophages (RAW 264.7) to condensed Ecig vapor (EcigCon). We observed that incubation of EA.hy 925 and RAW 264.7 cells with EcigCon leads to concentration-dependent cell death. In both EA.hy 925 and RAW 264.7 cells, EcigCon incubation promoted the transfer of the cytosolic NADPH oxidase subunits (p47phox, p67phox and Rac1) to the plasma membrane, hence showing the activation of the enzyme complex. Recent studies have shown that among toxic aldehydes found in Ecig vapor, acrolein plays a prominent role. Thus, we incubated both EA.hy 925 and RAW 264.7 cells with increasing concentrations of acrolein. It was again observed that p47phox, p67phox and Rac1 translocate to the plasma membrane. These data show that acrolein could be a significant part of Ecig vapor induced oxidative stress and cell death. More understanding is still needed to disclose the full mechanism of the negative effects of consumption of Ecig and the possible long-term toxicity.

[YIA] Proteasome activation in C. elegans causes mild mitochondrial defects; Is this the link to lifespan extension?

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Protein homeostasis is extensively regulated by a variety of proteostatic mechanisms that are considered the guardians of the proteome and ensure precise synthesis, maintenance, function and elimination of proteins. These mechanisms tend to fail with ageing, thus contributing to loss of proteostasis, one of the ageing hallmarks. Our group and others have previously shown that activation of the proteasome, that is responsible for approximately 80% of protein degradation, can prolong the lifespan of C. elegans and D. melanogaster. However, how exactly proteasome activation facilitates lifespan extension, remains unknown. In this study, we have attempted to shed light onto this open question by focusing on the underlying molecular effects of proteasome activation. A proteomic analysis of C. elegans with an activated proteasome revealed differentially regulated glycolysis, a metabolic shift compatible with mitochondrial deficiency. Evaluation of various mitochondrial parameters showed that mitochondria were depolarized and fragmented in nematodes with an activated proteasome compared to control. Although further investigation is needed, our data may suggest that a mild mitochondrial defect accounts for the lifespan extension found in nematodes with an activated proteasome. Our future work will focus on determining how proteasome activation causes mitochondrial defects and if indeed the latter are responsible for the observed lifespan extension.

[YIA] Evidence of ferroptosis involvement in Rett syndrome pathogenesis

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Rett syndrome (RTT) is a rare neurodevelopmental disorder caused in 90% of the cases by mutation in the X-linked gene encoding for MeCP2, an important epigenetic regulator. RTT patients show compromised metabolic processes including redox imbalance, dysfunctional mitochondrial bioenergetics and altered lipid metabolism. Since several molecular aspects involved in the pathophysiological mechanisms of RTT could suggest a possible role of ferroptosis, an iron-dependent cell death characterized by excessive lipid peroxidation, the aim of our study was to evaluate RTT susceptibility to this type of cell death using primary fibroblasts obtained from RTT patients. As a first step, we observed an increase of cell death rate in RTT compared to controls after treatment with several concentrations of two ferroptosis inducers: erastin (GPX4 inhibitor) or RSL3 (inhibitor of the cystine/glutamate antiporter). At the same time, the co-treatment with ferrostatin-1, a well know inhibitor of ferroptosis, reduced the levels of cell death. In addition, we found changes in GPx and GR activity after 3h treatment with 10 μM erastin or 5 nM RSL3, while Western blot analysis also showed an alteration in GPX4 protein levels and in formation of 4HNE protein adducts, after the treatment with the same doses of erastin and RSL3 for 3 and 6h. Finally, both the mitochondrial ROS production and lipid peroxidation levels were higher in RTT after the induction of ferroptosis with the two molecules, while ferrostatin-1 co-treatment significantly prevented these processes. In conclusion, our results indicate an increased vulnerability of RTT cells to ferroptosis that could contribute to the clinical features of RTT phenotypes, also suggesting this process as a possible therapeutic target to improve the quality of life of RTT patients.

[YIA] SC-Nanophytosomes formulation promotes benefits on Parkinson's disease models: a mitochondria-targeted therapy approach

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Mitochondrial dysfunction is a common pathological hallmark of many degenerative diseases, including Parkinson's disease (PD). Thus, developing therapeutic strategies to modulate mitochondrial function is a great challenge. In this work, SC-Nanophytosomes, assembled with polar lipids from Codium tomentosum Stackhouse and elderberry anthocyanin-enriched extract (EAE-extract) from Sambucus nigra L, were used to address this challenge. The algae polar lipids were chosen considering their richness in the anionic phosphatidylglycerol (cardiolipin precursor) and omega-3 polyunsaturated fatty acids, while the elderberry anthocyanins were by their mitochondriotropic properties and ability to overcome the complex I-related mitochondrial dysfunction. The competence of SC-Nanophytosomes to modulate the mitochondria functionality, by improving the activity of the mitochondrial respiratory chain complexes and protecting the cells against mitochondria-specific toxic and/or oxidant stimuli (e.g., rotenone and glutamate) was unveiled in cellular assays. SC-Nanophytosomes, engineered with 600 μM algae phospholipids and 0.5 mg/L of EAE-extract, are nanosized vesicles with high negative surface charge and versatile shapes that preserve their properties under conditions that mimics the gastrointestinal tract pH changes. The oral administration of SC-Nanophytosomes (delivery by drinking water for 3 weeks at the concentration of 3 μM in phospholipid) to a rotenone-induced PD-like pathology rat model showed positive outcomes disabling motor symptoms associated with rotenone neurotoxicity. Ex vivo brain biochemical assays showed that SC-Nanophytosomes-treatment induced benefits on mitochondrial functionality, revealed by respiratory control index and by redox complexes activities. Cell redox state also shows benefits, indicated by the activity of superoxide dismutase, catalase, and glutathione reductase enzymes. While these data point out SC-Nanophytosomes as promising tool to support a mitochondria-targeted therapy for neurodegenerative diseases, additional assays with other doses and animal models are needed to evaluate their effectiveness.

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[YIA] Generation of genetically-encoded redox biosensors for super-resolution imaging of hydrogen peroxide production

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In cells, hydrogen peroxide (H2O2) is deliberately produced and is key for a healthy cellular metabolism, and an adequate H2O2 production and delivery is required for correct cellular and tissue functions. The majority of this H2O2 production occurs in cellular microdomains, and the signalling actions in these microdomains are restricted to a very confined area. Most of these discoveries were made possible thanks to the use of genetically-encoded fluorescent biosensors that detect the changes in H2O2 concentration, including the HyPer family of biosensors. However, the nanoscale details of these microdomains are missing due to the spatial resolution limitation (~200nm) of conventional fluorescence microscopy methods. To overcome this resolution limitation, we have generated HyPer variants that can be reversibly switched off and on upon illumination with cyan and violet light, respectively, while keeping their H2O2-sensing abilities. The photoswitching ability allows the use of super-resolution microscopy techniques, such as photochromic stochastic optical fluctuation imaging (pcSOFI) or reversible optically-linear fluorescence transitions (RESOLFT). Combining these techniques with the photoswitchable HyPers has enabled a high-resolution mapping of mitochondrial H2O2 production upon hypoxia-reoxygenation.

[YIA] Avenanthramides: antioxidant and anti-inflammatory activities from oats to human therapy

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The Avena sativa is a cereal known since antiquity as a useful grain with abundant nutritional and health benefits. It contains distinct molecular components with high antioxidant activity, such as tocopherols, tocotrienols, and flavonoids. Among them, avenanthramides (Avns), polyphenols found exclusively in oats, are particularly emerging as promising therapeutic candidates for the treatment of several human diseases. Avenanthramides are phenolic amides containing anthranilic acid and hydroxycinnamic acid moieties and endowed with major beneficial health properties because of their antioxidant, anti-inflammatory, and antiproliferative effects. Several studies have clearly shown the beneficial biological activities of avenanthramides, including analogs produced in recombinant yeast (YAvns), with a major focus on the therapeutic potential of these secondary metabolites in the treatment of oxidative stress-related human diseases. In particular, we have demonstrated that the antioxidant and anti-inflammatory activities of avenanthramides were effective in rescuing major molecular and cellular dysfunctions underlying the pathogenesis of cerebral cavernous malformation (CCM) disease, a major cerebrovascular disorder affecting up to 0.5% of the human population. Indeed, the treatment of cellular and animal models of CCM disease with avenanthramides has been shown to enhance the expression level of fundamental scavengers of reactive oxygen species (ROS), such as superoxide dismutase 2 (SOD2), through the upregulation of the master transcription factor FoxO1, as well as to reduce the NF-κB signaling pathway. Using nanotechnology methods, we have recently implemented these findings by developing cytocompatible nanosystems loaded with avenanthramides, which were specifically designed for the treatment of CCM disease and neurological comorbidities though targeted administration routes that bypass the systemic circulation, such as the nose-to-brain delivery route. This nanomedicine approach may allow the selective delivery of avenanthramides to the brain, thus increasing their local therapeutic effectiveness and reducing their potential systemic side-effects and is currently being tested in cellular and animal models of CCM disease.

[YIA] Assessment of enzyme-like activity in metal-free nanomaterials

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Antioxidant nanozymes are promising therapeutic tools for pathologies associated with oxidative stress. Fullerenes, carbon nanotubes, graphene, and carbon dots show the ability to mimic antioxidant enzymes, but most of these nanomaterials have low water solubility and only exhibit antioxidant enzyme-like activities after functionalization and/or doping. Electrogenerated hydrophilic carbon (EHC) nanomaterials produced from graphite in one single step may emerge

as a potential alternative to overcome these limitations. This work aimed to evaluate if three sets of EHC nanomaterials, synthesized using three biological compatible buffers, namely, citrate, malate, and tartrate, could mimic the activity of superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD). TEM and AFM analysis demonstrated that all nanomaterials exhibit a string-assembly organization dominated by amorphous carbon nanoparticles. Cyclic voltammetry indicated that they also possess electron-donating properties in the same potential range. The SOD-like activity was assessed using the hypoxanthine-xanthine oxidase system as the source of O2•- and NBT as the detector. The results revealed that, under physiological-like aqueous buffer conditions, only EHC@malic exhibited SOD-like activity. The CAT-like ability was appraised in the presence of H2O2 following the rate of O2 formation using a Clark-type electrode system. Results showed that all nanomaterials have a semi-CAT-like activity since they can react with H2O2 without O2 generation. The POD-like activity appraised spectrophotometrically by the TMB method indicated that none of the EHC nanomaterials exhibit POD-like activity. Since EHC@malic is the only nanomaterial that exhibits enzyme mimic ability, its effects on cell viability were investigated. Results showed that EHC@malic displays no toxicity for human neuronal and keratinocyte cells as well as for mouse preadipocytes cells. Overall, despite the similar structure and redox behavior, only EHC@malic emerge as a new SOD mimic with the potential to be considered for therapeutic applications development.

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[YIA] Regulation of metastasis suppressor NME1 by a key metabolic cofactor coenzyme A

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The metastasis suppressor protein NME1 is an evolutionarily conserved and multifunctional enzyme that plays an important role in suppressing the invasion and metastasis of tumour cells. The nucleoside diphosphate kinase (NDPK) activity of NME1 is well recognized in balancing the intracellular pools of nucleotide diphosphates and triphosphates to regulate cytoskeletal rearrangement and cell motility, endocytosis, intracellular trafficking, and metastasis. In addition, NME1 was found to function as a protein-histidine kinase, 3'-5' exonuclease and geranyl/farnesyl pyrophosphate kinase. These diverse cellular functions are regulated at the level of expression, post-translational modification, and regulatory interactions. The NDPK activity of NME1 has been shown to be inhibited in vitro and in vivo under oxidative stress, and the inhibitory effect mediated via redox-sensitive cysteine residues. In this study, affinity purification followed by mass spectrometric analysis revealed NME1 to be a major coenzyme A binding protein in cultured cells and rat tissues. NME1 is also found covalently modified by CoA (CoAlation) at Cys109 in the CoAlome analysis of HEK293/Pank1β cells treated with the disulfide-stress inducer, diamide. Further analysis showed that recombinant NME1 is efficiently CoAlated in vitro and in cellular response to oxidising agents and metabolic stress. In vitro CoAlation of recombinant wild type NME1, but not the C109A mutant, results in the inhibition of its NDPK activity. Moreover, CoA also functions as a competitive inhibitor of the NME1 NDPK activity by binding non-covalently to the nucleotide binding site. Taken together, our data reveal metastasis suppressor protein NME1 as a novel binding partner of the key metabolic regulator CoA, which inhibits its nucleoside diphosphate kinase activity via non-covalent and covalent interactions. Further studies on the functional modulation of NME1 by CoA will be presented.

Cardio-Pulmonary Consequences of Synergistic Exposure to Particulate Matter and Noise pollution

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An excess of 8.79 million deaths worldwide have recently been attributed to air pollution, and particulate matter (PM), a constituent of air pollution, has lately been shown to have detrimental effects on human health. Noise is a problem of a modern urbanised world, but unlike air pollution, it has not been recognized widely as a serious risk factor. In order to probe the synergistic effect of PM and noise, we used a custom exposure system from TSE Systems (Germany). For the combined noise/PM exposure, C57BL/6 mice were acutely exposed to ambient PM (fully characterised particles obtained from the NIST, USA) and noise (aircraft landing and take-off sounds at random intervals), separately, as well as to both stressors simultaneously. Uptake and adverse health effects of PM were documented by endothelial dysfunction, increased blood pressure and successful uptake and body distribution was documented by IVIS imaging of fluorescent particles and MRI of iron oxide particles for nano-/micro-dimension particles. We observed that blood pressure was significantly increased in all exposed groups. An additive impairment of the endothelial function was observed in isolated aortic rings. Upregulation of the NOX2 protein was observed in the immunohistochemistry staining of aortic sections, accompanied by the increase in 3-nitrotyrosine positive protein. Increase in oxidative stress markers, such as dihydroethidine (DHE) staining and 3-nitrotyrosine, was observed in both aortic and lung sections. Activation of the antioxidant system in the combined exposure was demonstrated through an increase in protein expression of heme oxygenase-1 in the lung and in the vasculature. In addition to oxidative stress, markers of inflammation, such as COX2 and MCP1 were also found to be upregulated. Oxidative stress and inflammation seem to be aggravated by exposure to both factors, but more data are needed to fully elucidate the mechanism of the combined PM and Noise exposure.

[YIA] Amplification of ROS/NET formation induces resolution of inflammation

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Although neutrophil granulocytes are usually regarded as archetypical pro-inflammatory cells, they also can exert anti-inflammatory and immune-regulatory functions. In previous studies, we showed that reactive oxygen species (ROS)-dependent formation of aggregated neutrophil extracellular traps (aggNETs) is crucial for resolution of inflammation of experimental arthritis and lupus. The underlying mechanism involves neutrophil serine proteases that degrade locally released cytokines and chemokines and thereby interrupt ongoing inflammatory processes. In this project we aimed to employ restoration and amplification of NET formation for inducing resolution of innate- and autoimmune-driven inflammation. To this end we made use of NADPH oxidase (NOX) 2-activating sulfonamides and aminoferrocene-based NOX2-independent ROS amplifiers. Preliminary data suggest that the NOX2-activator RE-02 induces (agg)NET formation and alleviates experimental lupus and that the aminoferrocene-based prodrug MIS43 triggers (agg)NET formation and prevents chronification of gouty arthritis in mice with non-functional NOX2. Treatment with MIS43 was associated with lower local levels of inflammatory cytokines/chemokines. Furthermore, serological analysis of WT and NOX2 defective mice and transcriptomic analysis on paws from MIS43-treated mice support our findings and show induction of inflammatory resolution. Taken together, the studies suggest a therapeutic efficiency of ROS-induction in chronic inflammatory syndromes that occur in the context of unsufficient ROS production from NOX2.

Mapping the modification of histones by the myeloperoxidase-derived oxidant hypochlorous acid (HOCl)

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Histones are highly basic nuclear proteins that are important to package and stabilise DNA. Histones are rich in Lys and Arg residues, which facilitate the tight wrapping and packaging of DNA in chromatin. These residues play a key role in the regulatory function of histones and are the site of a range of different post-translational modifications. Histones are

also present in the extracellular environment and are a key component of neutrophil extracellular traps (NETs). In addition to histones, NETs contain myeloperoxidase, which retains its enzymatic activity and produces hypochlorous acid (HOCl). This localised production of HOCl could result in oxidative modification of the NET components, particularly the abundant histones. Therefore, in this study, we examine the reactivity of HOCl with a mixture of linker (H1) and core (H2A, H2B, H3 and H4) histones. HOCl induced histone modification in a dose- and time-dependent manner, resulting in fragmentation, aggregation, and the formation of both unstable and stable oxidation products. Under the conditions used in this study, histone H1 was the most susceptible to modification. Exposure of the histones to HOCl resulted in the formation of unstable N-chloramines together with the formation of Lys products, including nitriles and carbonyls (aminoadipic semialdehydes), at multiple on each of the histones. Chlorination and dichlorination of Tyr residues, but not Trp, were also observed, together with Met sulfoxide and Met sulfone, though relatively high yields of Met sulfoxide were also present in the non-treated histones. The formation of these oxidative products was observed on exposure of the histones to < 20-fold molar excess HOCl, which is readily achievable under patho-physiological conditions. Given that histones comprise ca. 70% of NET-associated proteins, these results could have implications for the development of diseases where aberrant NET release is dominant.

Gain of function effects of oxidized phospholipids make them pharmacological targets and leads

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Upon oxidation phospholipids acquire a number of biological activities that were not characteristic of their non-oxidized precursors. Recent in vivo studies have convincingly demonstrated causative role of endogenously generated oxidized phospholipids (OxPLs) in a number of pathologies, including those associated with acute and chronic inflammation. Our data suggest that in addition to direct induction of inflammatory mediators, an important mechanism of proinflammatory action of OxPLs is their ability to amplify effects of low concentrations of inflammatory cytokines. In particular, OxPLs act synergistically with low concentrations of TNFα thus shifting its dose-response to the left. We have identified several known non-antioxidant drug-like molecules, as well as a novel molecular scaffold, that were capable of inhibiting proinflammatory action of OxPLs. The data suggest that in addition to direct or indirect antioxidant therapy, inflammation induced by oxidative stress and lipid peroxidation can be inhibited by targeting intracellular signaling mechanisms. Paradoxically, OxPLs also demonstrated protective effects in certain types of pathology. For example, OxPLs inhibited inflammation induced by Toll-like receptors and protected lung endothelial barrier. We have synthesized non-electrophilic phospholipase-resistant alkyl-amide-OxPLs demonstrating anti-LPS and anti-edemagenic protective activity in vitro and in animal models. In summary, OxPLs represent potential targets for treatment of inflammation associated with oxidative stress. On the other hand, molecular leads mimicking OxPLs structure can be applied for pharmacological inhibition of Toll-like receptors and prevention of lung edema.

[YIA] Myeloid cell derived Interleukin-6 causes vascular dysfunction, inflammation and endothelin-1 expression in mice

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Objective: The exact contribution of the pro-inflammatory cytokine Interleukin-6 (IL-6) to cardiovascular disease is poorly understood. As myeloid cells are an important source of IL-6, we aimed to analyze the influence of myeloid cell-derived IL-6 on vascular function. Methods: We generated a mouse strain overexpressing IL-6 in lysozyme M+ myeloid cells (LysM-IL-6OE mice) which are one main source of this cytokine. For bone marrow transfer studies, BM cells from LysM-IL-6OE or control mice were transplanted into recipient mice in different concentrations. We assessed systolic blood pressure and vascular function. Furthermore, we measured ROS/RNS in whole blood after incubation with L-012 and phorbol 12,13-dibutyrate (PDBu). For visualization of reactive oxygen species in the aortas, aortic sections were stained with dihydroethidium (DHE). Vascular infiltration of inflammatory cells was investigated by flow cytometry. For in vitro studies, human pulmonary arterial endothelial cells (HPAECs) were cultivated and stimulated with IL-6/soluble IL-6 receptor with subsequent protein and RNA analyses. Results: LysM-IL-6OE mice had normal blood pressure, but

significantly impaired endothelium dependent aortic relaxation and increased aortic reactive oxygen species (ROS). Bone-marrow-transplantation studies indicated that vascular dysfunction and ROS formation correlated with IL-6 levels. Vascular dysfunction was accompanied by accumulation of neutrophils and Ly6C+ monocytes and macrophages in the aortic wall and by vascular fibrosis. Furthermore, we detected elevated endothelin 1 (ET-1) expression in LysM-IL-6OE aortas, a known mediator of vascular dysfunction. We could reproduce IL-6 driven vascular ET-1 expression in in vitro studies. Conclusion: Myeloid cell-derived IL-6 induces vascular dysfunction mediated by vascular inflammation, increased oxidative stress, vascular fibrotic remodeling, dysregulated nitric oxide, and elevated vascular ET-1 expression. Notably, these findings were not linked to arterial hypertension. Funding: DFG Grant KA4035-1.

Erythrocyte catalase S-nitrosation as a sensor of chronic subclinical oxidative stress and metabolic complications associated with childhood obesity

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Chronic subclinical oxidative stress suffered by obese children is characterized by an alteration of redox homeostasis and a depleted antioxidant capacity of their erythrocytes. We previously observed that obese children with metabolic complications present difficulties in activating erythroid antioxidant responses when facing compromised metabolic situations such as an acute glucose ingestion. Among others, erythroid catalase was postulated as a good sensor of such metabolic dystress in children with evidence of insulin resistance (prediabetes), in contrast to eustress in metabolically healthy obese children. Our aim was to study the nitrosation of the enzyme as possible cause of the enzyme inhibition. The study included 95 prepubescent children divided into 3 groups according to their clinical and anthropometric profile: metabolically healthy and unhealthy obese children (Ob.IR- and Ob. IR+, respectively), versus a lean children group. Fasting blood was collected in all three groups, and at different points after an acute glucose intake only in obese children. Catalase levels and activity were analyzed by western blotting and spectrophotometry, respectively. Catalase nitrosation was studied by the biotin-switch method and combined by a final analysis of the erythrocyte nitrosylated protein profile by LCS-MS/MS. Catalase levels were similar among groups, but the baseline activity of the enzyme was partially inhibited in the obese ones. Nevertheless, an induction in catalase activity was showed after glucose intake in healthy obese children, but not in those with metabolic disorders, in which the enzyme activity remained unchanged. The enzyme activity inhibition was proportional to the increased level of its nitrosation in the obese IR+, and incubation of erythrocytes with a nitric oxide donor had detrimental effects over catalase activity in every group. We have showed that erythrocyte catalase nitrosation levels is a good indicator of metabolic complications associated with chronic stress in obese prepubescent children. [Funding codes: PI18/01316 ISCIII; PI-0209-2019; LII19/16IN-CO24].

Role of the Thioredoxin-1 in heart failure

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Thioredoxin-1 (Trx-1) is a 12 kDa protein with antioxidant and anti-inflammatory properties. It is a highly protective protein against myocardial infarction in murine model. Whether this protective phenomenon can be attributed to cardiomyocyte regeneration, reduced levels of ROS, inflammatory cytokines, protein oxidation or another phenomenon is not known. Nevertheless, its cleavage leading to the truncated pro-inflammatory and pro-oxidant isoform, Trx-80, could compromise its therapeutic use. To circumvent this problem, Trx-mimetic peptide called CB3 has been developed. The aim of the study is to evaluate the impact of Trx-1 and CB3 on heart failure in mice model and to identify the mechanisms involved in their effects. For this purpose, we analyzed the impact of Trx-1, Trx-80 and CB3, on cultured neonatal mice cardiomyocytes and in vivo on adult mice with experimental myocardial infarction. To overexpress Trx-1 and Trx-80, we generated different AAV with a TnT chicken cardiac promoter which is specific to the cardiomyocytes. Our results showed that Trx-1 and CB3 improved cardiac functions such as cardiac contractility and dilatation. In contrast, Trx-80 aggravated such parameters. Also, Trx-1 and CB3 reduced the infarct size. These results suggest that both Trx-1 and CB3 have beneficial effects and in all cases the effect of CB3 is greater than Trx-1 indicating that the effect of Trx-1 is probably attenuated by the cleavage process. In vitro studies showed that CB3 decreases intracellular level of ROS which could be linked to the observed reduced protein carbonylation. As a consequence, an increase of cell proliferation was found. In conclusion, Trx-1 plays an important role against heart failure through probably a reduction of protein oxidation involved in cell cycling. Nevertheless, such beneficial effect is attenuated by the cleavage process. In this context, the use of CB3 for therapeutic purposes could constitute a way to overcome this obstacle.

A chemoproteomic approach to identify redox-active methionines in secreted proteins of saprophytic fungi during biomass degradation

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Saprophytic fungi degrade lignocellulosic polymers present in plant cell walls using dedicated enzymes called 'Carbohydrate Active enZYmes' (CAZYmes). These enzymes are secreted in the extracellular environment together with reactive oxygen species (ROS), shown to participate actively in vegetal biomass degradation. Numerous CAZYmes are routinely used in biotechnology approaches and the understanding of their functioning is of crucial importance to improve and create new ways of biomass valorization. ROS can oxidize proteins on sensitive amino acids leading to oxidative post-translational modifications, the consequences of which range from damaging effects to fine-tuned regulation of protein functions and fates. For example, the reversible oxidation of methionine into methionine sulfoxide (MetO) can act as redox switch to regulate proteins involved in several cell functions. If the effects of ROS on numerous proteins of bacteria, plants or animals have been studied, almost nothing is known about fungal proteins and specifically during biomass degradation. Our study aims to identify and characterize proteins carrying redox-sensitive Met, that are secreted during biomass degradation in fungi. We chose Pycnoporus cinnabarinus as a model fungus for its capacity of producing a large arsenal of lignocellulosic enzymes. We developed a chemoproteomic approach using an oxaziridine probe targeting redox-sensitive Met. The probe has been synthesized and validated in a model protein, for which increased concentration of oxaziridine allowed ranking of the different Met according to their redox-sensibility. Thanks to coupled mass spectrometry analysis, and along with the use of 18O labelled hydrogen peroxide, this probe will allow us to identify proteins carrying redox-active Met in fungi. The effect of redox modification of Met will then be elucidated on recombinant proteins. Additionally, compared analysis of intracellular fungal proteins could give insight into mechanisms set up to protect extracellular proteins against oxidative stress. This approach should help to uncover redox regulated CAZYmes.

[YIA] Identification of 11'-α-tocomonoenol in the microalgae Monodopsis subterranea and effect of nitrogen depletion on its tocomonoenol content

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Introduction. α-Tocomonoenols (αT1) are tocochromanols differing form α-tocopherol (αT) and α-tocotrienol due to the presence of a single double bond in the side chain. Two αT1 isomers have been reported, 11'-αT1, mostly reported in land plants, and 12'-αT1, mainly found in marine organisms. Recently, it has been reported that 11'-αT1 and not 12'-αT1 is the major αT1 congener in microalgae and that it is metabolized in human liver cells similar to αT. The aim of the present study was to evaluate the effect of nitrogen depletion in Monodopsis subterranea on tocochromanol content with particular emphasis on αT1 isomers. Methods. M. subterranea was obtained under nitrogen-repleted and -depleted conditions for determination of the tocochromanol profile. Presence and identity of αT1 was determined by LC-MSn and GC-MS and quantification of tocochromanols was done by HPLC-FLD. Results. 11'-αT1 is the predominant αT1 congener in M. subterranea. Nitrogen depletion increased tocochromanol content with no significant effect on absolute αT1 concentration, but with a significant relative increase when normalized to chlorophyll content. Conclusions. 11'-αT1 is the major αT1 congener in M. subterranea in agreement with previous reports. Nitrogen depletion does not affect absolute αT1, despite significant increases in total tocochromanols.

Characterization of the copper chaperone PcuC as substrate of the periplasmic methionine sulfoxide reductase MsrP in Cereibacter sphaeroides.

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In proteins, methionine (Met) residues can be oxidized by various oxidants and converted to methionine sulfoxide (MetO). Depending on the affected protein, the consequences of Met oxidation range from uncontrolled damage to fine regulation of function or activity. Almost all characterized organisms possess methionine sulfoxide reductase (Msr) enzymes to reduce MetO to Met. Msrs play an important role in cellular functions by protecting against oxidative stress: they reduce oxidized proteins and scavenge reactive oxygen species through cyclic oxidation and reduction of Met. Moreover, reversible Met oxidation can act as a post-translational modification responsible for the activation of enzymes and transcription factors or the regulation of protein-protein interactions. Bacteria have specific molybdenum-containing Msrs, such as the periplasmic MsrP. We previously characterized the substrate specificity of MsrP from the purple bacterium Cereibacter (Rhodobacter) sphaeroides and identified potential protein targets by proteomics. Among these, we found the copper chaperone PcuC, involved in copper loading of cytochrome oxidase. PcuC has an unusually high Met content (5.8% instead of 2.4% for most proteins) and 5 Met are potentially involved in copper binding. In this study, we determined that an oxidized PcuC is efficiently reduced by MsrP. In particular, we mapped the sensitivity of each Met to oxidation using 18O-labeled hydrogen peroxide and oxaziridine probes coupled to proteomics. Solving the three-dimensional structure of PcuC helped to understand the sensitivity of Met to oxidation. We showed that PcuC can bind two copper ions and that, apparently, oxidation barely affects its ability to bind the metal. Experiments are underway to evaluate the effect of oxidation on the ability of PcuC to deliver copper to the cytochrome oxidase and to determine if MsrP can affect this process. The results should help in understanding the role of MsrP in the protection of bacteria against oxidative stress.

SFRR-E Oral Presentation Session 3 – Redox biology of neurodegenerative and infectious diseases

Abnormalities of the cellular redox associated with the cytopathic and proinflammatory effects of SARS-CoV2 infection

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SARS-CoV-2 infection can cause a severe respiratory distress syndrome and major inflammatory and thrombotic complications. The risk of severe symptoms and mortality increases with patients' age and with the presence of comorbidity. The age-depended alterations of physiological functions that may increase the risk of severe COVID-19 could be many, including a defect of homeostatic mechanisms that govern the tissue redox, leading to increased susceptibility to oxidative stress and poor control of inflammatory pathways. Viral infections may take advantage of such age and disease-related defects. Alterations of the thiol redox balance in the extracellular fluids and lung tissue may influence the risk of infection and the host capability to respond to the pathogen and to avoid severe complications. We demonstrated that SARS-CoV2 likewise other viruses (such as HIV, HSV and influenza virus), sustains its replication cycle actively promoting a pro-oxidant environment in the host cell. In the case of SARS-CoV2, this occurs through infection-specific alterations of cellular glutathione and Cys metabolism. Protein glutathionylation and ER stress pathway activation were associated with these alterations to sustain the cytopathic activity of the virus. Nrf2 and NFkB transcription factors are involved in the transcriptional mechanisms that stand behind the defects of GSH metabolism and the inflammatory response to SARS-CoV2 infection of the host cell. Cytoprotection and chemoprevention strategies to limit the pro-oxidant effects of SARS-CoV2 and to recover tissue homeostasis, are also presented. These aspects are discussed in this paper which is proposed as an ancillary presentation to the SFRR-E conference Symposium 4 "Oxidative stress in the pathogenesis and clinical management of COVID-19".

APP mutations give rise to redox related mitochondrial alterations

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From several factors that are purposely involved in Alzheimer's disease (AD) initiation and progression, redox regulation has been highlighted as a relevant process that mediates significant protein changes affecting neuronal function. Thioredoxins (Trx) and Glutaredoxins (Grx) are of utmost importance for the maintenance of the reduced state of proteins involved in relevant cellular processes; being key factors in redox regulation. Changes in redoxins levels have been associated with oxidative distress in AD. To test the association of increased oxidation and intracellular dynamic defects in the progression of AD we developed human brain organoids from iPSC control and with the APP Swedish mutation (APPSwe). Our characterization of AD pathology showed, in APPSwe organoids, an increase in Aβ reactive area, as well as an increase in p-Tau levels using western blot (WB). WB analysis of thioredoxin protein levels showed a significant decrease of Grx2 and a significant increase of the exclusively mitochondrial Trx2, in APPswe organoids. These results go hand in hand with an increase in superoxide anion levels, increased mitochondrial oxidation and loss of mitochondrial membrane stability, as well as changes in mitochondrial morphology and motility. These alterations were observed using redox and mitochondrial probes along with live imaging techniques in organoids with the APPswe mutation. Our results highlight the relevance of modeling neurological diseases using complex tissue arrangements and point to a deregulation in redox pathways in AD, potentially related to mitochondrial homeostasis. This pathway, if modulated, could be used as a therapeutic strategy for the treatment of abnormal oxidation in AD.

[YIA] Development of ELISA for the detection of nitro-oxidative stress biomarkers

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Enzyme linked immunosorbent assay (ELISA) for detection of 3-nitrotyrosine (NO2Y), NO2Y containing fibrinogen (NO2YFg), and fibrinogen (Fg) in human plasma were developed. The nitration of tyrosine is a consequence and a marker of nitro-oxidative distress, a condition that occurs when there is an imbalance between the production of reactive nitrogen and oxygen species and the antioxidant system, in favor of the first. This condition plays an important role in the pathogenesis of many clinical conditions (cardiovascular diseases, chronic obstructive pulmonary disease, chronic kidney disease, neurodegenerative diseases, cancer) and aging. Nitro-oxidative distress biomarkers may provide important information about the severity and degree of the disease, efficacy of the treatment, and their presence shed light into the pathophysiological mechanisms of it. Enriched by affinity chromatography, rabbit polyclonal anti-fibrinogen immunoglobulins were obtained and used as capture antibodies in sandwich ELISA for the determination of Fg and NO2YFg. Commercial mouse monoclonal anti-NO2Y biotinylated immunoglobulins were used as detection antibodies in sandwich ELISA for the determination of NO2YFg and in competitive ELISA for the determination of NO2Y; then, after the addition of avidin-peroxidase, substrate and chromogen, development of color was read at 450 nm. For the detection step in the ELISA for the determination of Fg, a peroxidase conjugated rabbit polyclonal anti-fibrinogen immunoglobulin was used, revealed with substrate and chromogen and development of color was read at 450 nm. As standards, polyethylene glycol enriched fibrinogen from human plasma were nitrated in different levels, with in house synthesized peroxynitrite. A small volume of peroxynitrite solution was added to a tube containing the fibrinogen solution, followed by vigorous vortexing. For testing the assays plasma samples with the addition of NO2YFg were used. In conclusion, three different immunoassays, two in a sandwich format and one in a competitive format, were successfully developed for the analysis of human plasma.

A possible role for the loss of brain Scavenger Receptor B1 (SR-B1) in Rett syndrome

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Loss-of-function mutations in the X-linked MECP2 gene cause Rett syndrome (RTT), a severe neurodevelopmental disorder mainly affecting females. In addition to neurological defects, a variety of multisystem symptoms (i.e. respiratory, gastrointestinal, cardiovascular, skeletal, muscular and urinary problems) associated with metabolic abnormalities (i.e. altered redox homeostasis, impaired immune response and mitochondrial dysfunction) add further complexity to RTT pathology. Interestingly, an important metabolic component of RTT involves a perturbed cholesterol metabolism. Our previous studies in patient-derived cells demonstrated that a loss of scavenger receptor B1 (SR-B1), a cell-surface HDL receptor, due to oxidative post-translational modifications, could play a relevant role in RTT altered plasma lipid profile. Based on this, main aim of this study was to determine whether an altered expression of SR-B1 could also affect the brain in RTT.
A decreased SR-B1 immunofluorescent signal was observed in brain of Mecp2-y mice (P50–60) compared to wildtype mice. The loss of SR-B1 expression in mutant brains was also associated with increased levels of 4-hydroxynonenal-protein-adducts, a marker of lipid peroxidation and protein oxidative damage. Furthermore, double-immunofluorescence staining showed that SR-B1 was strongly expressed in neurons and oligodendrocytes of wildtype mice and almost absent in the same cells in Mecp2-null brains. Finally, when early (P30–35) and late (P50–60) symptomatic Mecp2-y mice were analyzed, there was no significant difference in SR-B1 levels between wildtype and Mecp2–/y mice at P30–35. Our data suggest that loss of SR-B1 expression with disease progression may affect brain cell function and contribute to RTT pathophysiology.

Overexpression of the tau protein alters the redox equilibrium of SH-SY5Y cells

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Tau protein is a microtubule-associated protein, predominantly expressed in the neurons, involved in the microtubule assembly and associated with the proper functioning of the cytoskeletal network. Abnormally phosphorylated tau protein

is the principal component of neurofibrillary tangles, accumulating in the brain especially in Alzheimer's disease, a disease accompanied by oxidative stress. The aim of this study was to examine whether overexpression of the tau protein leads to changes in the redox status of SH-SY5Y cells. SH-SY5Y cells transfected with the human 4-repeat tau isoform, hTau40, subcloned into pcDNA3.2/V5/DEST, and cells transfected with the empty vector were provided by Dr. M. Fahnestock (McMaster University) and grown in the presence of 250 μg/ml G418. The level of reactive oxygen species was elevated in tau-overexpressing cells as compared with cells transfected with the empty vector (by 15±6% when estimated by 2',7'-dichlorodihydrofluorescein (DCFDA) and by 119±19% when estimated with dihydroethidine). The level of glutathione was increased in the tau-overexpressing cells by 71±36% (apparently overproduction of glutathione to compensate for oxidative stress). The tau-overexpressing cells were more sensitive to hydrogen peroxide (c50 = 191±5 μM) than cells transfected with the empty plasmid (c50 = 278±13 μM). The doubling time of tau-overexpressing cells was significantly slowed down (65 vs 34 h). These results indicate that overexpression of the tau protein imposes oxidative stress on the cells resulting in the growth impairment. Nitroxide-containing nanoparticles (NNPs) mitigated oxidative stress in tau-overexpressing cells, decreasing the level of ROS estimated with DCFDA (150 μM NNPs) and increasing the level of glutathione in both tau-overexpressing cells and cells transfected with the empty plasmid (100 and 150 μM NNPs). This study was performed within the Project SONATA BIS 6 2016/22/E/NZ7/00641 "Nanomolecular antioxidants: biological basis of targeted therapy of neurodegenerative diseases" financed by the National Science Centre of Poland.

[YIA] Expression signatures associated with oxidative stress sensitivity in 30 human cancer cell lines

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Expression signatures associated with oxidative stress sensitivity in 30 human cancer cell lines
Oxidative stress is described to have physiological, pathological, and therapeutic consequences in nearly all types of cancer. Yet, the expression signatures associated with oxidative stress sensitivity in cancer cells are understudied. To this end, we cultured more than 30 tumor cell lines and analyzed their basal expression on several levels: 1. Flow cytometry screening of 33 receptors and redox-related enzymes (e.g., catalase, NOS, NOX, MPO, aquaporins 1-10); 2. whole-genome gene expression transcriptomic analysis; 3. mass spectrometry and proteomics, including oxidative post-translational modifications (oxPTMs). In parallel to cell pellet collection, we exposed the cultured cell lines to oxidative stress induced via hydrogen peroxide, hypochlorous acid, or multi-ROS/RNS generating gas plasma technology at different concentrations. Subsequently, the metabolic activity was assessed, and for each cell line and oxidative stress-inducing agent, the IC25 was calculated to reflect oxidative eustress and distress elements. Next, these IC25 values were correlated with expression levels determined in flow cytometry, transcriptomic, and proteomic data to identify expression objects that correlate with oxidative stress sensitivity and resistance across the 30 cancer cell lines. For gas plasma treatment, NOX3 and AQP1 cell surface expression as well as cholesterol content and baseline metabolic activity correlated with exposure resistance. For hydrogen peroxide exposure, cell cycle-related genes but not antioxidant genes were associated with sensitivity to the treatment in the tumor cell lines. For hypochlorous acid exposure, proteins involved in cell migration and cytoskeleton correlated positively with resistance. Overall, gas plasma exposure with the atmospheric pressure argon plasma jet kINPen and hydrogen peroxide treatment correlated well, while hypochlorous acid did not. Expression assays and oxidative stress treatments were crossed analyzed to identify targets and pathways expressed in resting cells that predict their sensitivity (but not regulation) to subsequent oxidative stress induced by different agents.

The role of microRNA and oxidised microRNA-133 in muscle wasting during ageing and cachexia

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miRNAs are short non-coding RNAs that play a central role in post-transcriptional gene regulation. microRNAs have been shown to play important roles in muscle development and homeostasis in adulthood. We hypothesized that microRNAs are oxidised and become pathological in muscle during ageing and cachexia, and this oxidation can alter their function. The aim of this study was to evaluate the therapeutic potential miR-133 overexpression and/or inhibition of oxidised miR-133 for muscle wasting through establishing changes in grip strength, muscle mass and fiber size in mice treated with miR-133 and/or antagomir to oxidised miR-133. Our data show that miR-133 and oxidised miR-133 inhibitor restore grip strength and lead to increase in fibre diameter in aged mice. Moreover, in adult mice, oxidised miR-133 led to increased abundance of mitochondrial complexes I and II in adult mice, mitochondrial biogenesis- and ER stress-related genes. In old mice, miR-133 and oxidised miR-133 inhibitor led to decrease in abundance of mitochondrial complexes I and V and modified ER stress-associated genes. Oxidised miRNAs were associated with an increase of senescence markers mainly in adult mice. Downregulation of muscle atrophy protein markers was detected with following oxo-miR-133 inhibitor treatment in aged mice. In cancer cachexia animal model, inhibitor to oxidised miR-133 led to a decrease in tumor weight in male and female mice. Cachectic male and female mice treated with inhibitor to oxidised miR-133 showed changes in p21 senescence marker, mitochondrial porin VDAC and p62 autophagy marker and mitochondrial complex I. Together, these data for the first time show functional consequences of microRNA oxidation in muscle and warrant further investigation in miR-133 overexpression and oxidised miR-133 inhibition therapeutic potential for caxechia and sarcopenia

[YIA] Insights into the mitochondrial functions of glutaredoxin 2 in cellular and mouse models

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Glutaredoxin 2 (Grx2) is a redox enzyme endowed with glutathionylation/de-glutathionylation activity, which can coordinate a [2Fe-2S] cluster in an enzymatically inactive holo-dimer. Different Grx2 splicing variants are present: the mitochondrial Grx2a, the nuclear Grx2b and the cytosolic Grx2c. Little is known about the different roles of these isoforms hence we studied the functions of Grx2a exploiting both cellular and animal models. In HeLa cells, we observed that, following oxidative stress, mitochondrial Grx2 acts as a sensor of the cellular redox state and releases the [2Fe-2S] cluster promoting the consequent activation of cell death pathways. [1] Basing on this result and to further explore the role of Grx2 in cancer, in collaboration with Dr. Marcus Conrad (MCD, Helmholtz Zentrum München) we generated Glrx2 knock out cell lines. The consequences of Grx2 deprivation on the cellular redox state, mitochondrial functions and the overall metabolism of cancer cells has been dissected especially in relation to the glutathionylation status of mitochondrial proteins, highlighting different consequences of Grx2 depletion in the various cell types. In order to determine the importance of Grx2 in vivo, we also characterized a whole-body mitochondrial Grx2 depleted (mGD) mouse model. These animals become overweight and show increased plasmatic and hepatic lipid levels when fed a standard chow diet. In addition, mGD liver presents lower capacity of glycogen storage, changed expression of enzymes involved in lipid metabolism and dysfunctional mitochondria. Moreover, an altered glutathionylation pattern of mitochondrial proteins was associated to the observed phenotype leading us to the conclusion that the altered glutathionylation of mitochondrial proteins in Grx2 deficient mice affects the mitochondrial physiology especially in the liver, leading to the development of a phenotype resembling the metabolic associated fatty liver disease (MAFLD). [2]

[1] Scalcon V. et al. Metallomics. 2019; 11:1241-1251. [2] Scalcon V. et al. Redox Biol. 2022; 51:102277.

Identification and quantification of nitration and oxidation sites in extracellular matrix from human coronary artery smooth muscle cells treated with peroxynitrous acid (ONOOH)

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Peroxynitrous acid (ONOOH) is a powerful oxidizing and nitrating agent generated at sites of inflammation by the diffusion-controlled reaction of nitric oxide (NO.) with superoxide radicals (O2.-). Extracellular matrix (ECM) proteins are major targets as these are highly abundant, react rapidly and poorly-protected by antioxidant defenses. The resulting modifications have been associated with multiple pathologies. Previous studies have examined specific ECM components, but data on native ECM targets is lacking. We hypothesized that LC-MS/MS methods could detect and map nitration/oxidation sites on intact ECM generated by human coronary artery smooth muscle cells. Cells, and associated ECM, were exposed to ONOOH (0, 50-, 500- and 5000 μM) before protein extraction, digestion to peptides, and analysis by LC-MS/MS. Nitration occurred on both cell and ECM species in a site-specific manner with modifications detected on multiple proteins. Some ECM proteins were modified to a greater extent (e.g. fibronectin and collagens) than others (laminins), and the extent of alteration increased with higher oxidant concentrations. Nitration occurred mainly at Tyr and Trp residues, with oxidation detected at Met and His. Met oxidation (to the sulfoxide) was facile, and detected even at the lowest ONOOH doses. Tyr nitration predominated over Trp nitration, with 23 Tyr, and only 1 Trp nitrated on fibronectin. The extent of modification at individual residues on individual ECM proteins was quantified, with some proteins showing both large numbers of sites, and high extents of modification (e.g. 20 sites in fibronectin with >10% conversion). The nitration of particular residues did not increase in a linear manner. These data indicate that LC-MS/MS is a powerful tool to unveil, in an unbiased manner, the sites and extents of protein modification in complex systems. The modified species may be useful biomarkers of ongoing inflammation and damage, as these may be released into plasma and urine.

SELENBP1 and SEMO-1: copper-dependent H2S -generating enzymes in humans and in the model organism C. elegans

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Depending on its concentration, hydrogen sulfide (H2S) may act as a toxin, a redox regulator triggering protein persulfidation, or a substrate for the respiratory chain. Recently, selenium-binding protein 1 (SELENBP1) has been identified as a novel H2S-producing enzyme in mammals, by means of its methanethiol oxidase (MTO) activity. To assess MTO-catalyzed H2S production, we developed a coupled assay that is based on in situ-generation of methanethiol through recombinant L-methionine gamma-lyase, and subsequent detection of H2S (1). Applying this assay, we detected a strong increase in MTO activity in cultured Caco-2 cells during differentiation from a proliferating to an enterocyte/colonocyte-like phenotype, occurring in parallel with elevated SELENBP1 expression. MTO activity of recombinant human SELENBP1 required the binding of copper ions but not of selenium. In the nematode Caenorhabditis elegans, we identified an ortholog of human SELENBP1, also showing MTO activity; we therefore named it SEMO-1 (SELENBP1 ortholog with MTO activity) (2). SEMO-1 is involved in the regulation of stress resistance and life span in C. elegans (2, 3). SELENBP1 and SEMO-1 apparently exhibit a similar enzymatic mechanism: MTO activity of both recombinant proteins was copper-dependent, and the introduction of point mutations previously reported to naturally occur in human SELENBP1, causing the loss of MTO activity, also abolished MTO activity of SEMO-1 (2). Supplementation of wild-type worms with copper chloride increased their methanethiol-derived H2S production, whereas a copper chelator attenuated C. elegans MTO activity; moreover, SEMO-1-deficient worms showed suppressed MTO activity. Taken together, SELENBP1 and SEMO-1 are copper-dependent H2S-producing enzymes that degrade methanethiol, a toxic product of sulfur metabolism of bacteria living in the mammalian gut as well as in the soil habitat of nematodes. (1) Philipp et al., Redox Biol 43:101972, 2021 (2) Philipp et al., BioFactors 2022 (doi: 10.1002/biof.1836) (3) Köhnlein et al., Redox Biol 28:101323, 2020

7) POG Oral Presentations Sessions – Authors sorted alphabetically

ANAC089 transcription factor is an ABA and redox molecular player during seed germination and stress

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Seed dormancy and germination are complex traits regulated by the interaction of a plethora of signaling molecules, including phytohormones (abscisic acid, ABA) and plant growth regulators (nitric oxide, NO) (Sanz e tal., 2015). A genetic screening in 3µM (+)-S-ABA coupled to the effect of the NO scavenger (carboxy-2-phenyl-4,4,5,5-tetramethylimidazolin-1-oxyl-3-oxide, cPTIO) was performed. We have previously described the identification of two mutants gap1 and gap2 (germination in ABA and cPTIO1 and 2) showing ABA- and cPTIO-insensitive phenotypes in the transition from dormancy to germination and the characterization and positional cloning of one of them, gap2/abi5 mutant (Albertos et al., 2015). Concerning the other locus, we found that GAP1 encodes ANAC089 transcription factor, a member of the NAC (NAM-ATAF1,2-CUC2) family with a critical membrane-bound domain and extra-nuclear location. We demonstrated that mutants lacking the membrane-related domain of ANAC089 displayed ABA, salt, osmotic and cold stresses insensitivity revealing a repressor function of ABA and abiotic stresses responses. In addition, these ANAC089 truncated mutants exhibited higher endogenous NO levels avoiding the effect of NO-depletion during seed germination. Consistently, translocation of ANAC089 protein to the nucleus was directed by changes in cell redox status after NO- and redox-related compound treatments. Whole-genome transcriptional profiling uncovered the existence of different groups of ABA- and redox-related genes that are differentially regulated by ANAC089. The DNA binding specificity of the ANAC089 TF following a microarray-based approach is also fully provided demonstrating that can specifically bind to the core cis-regulatory element GCGTCAGC present in the promoters of ANAC089 regulated genes. Collectively, our results indicate that ANAC089 transcription factor integrates ABA signaling with NO levels to modulate redox homeostasis as a novel master regulator during seed germination and stresses in Arabidopsis (Albertos et al., 2021).

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Chloroplasts lacking class I glutaredoxins – Unraveling the function of GRXC5 in Physcomitrium patens

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Oxidative stress is known to induce post-translational protein modifications on cysteines such as protein S-glutathionylation. Protein S-glutathionylation is reversible and catalysed by class I glutaredoxins (GRX) belonging to a family of small oxidoreductases. Four classes of GRX are described so far, based on the active site motif. Plastids contain members of class I (GRXC5, GRXS12) and class II glutaredoxins. Class I glutaredoxins are known to be involved in protein (de)-glutathionylation while class II glutaredoxins play a role in iron sulfur cluster coordination. Phylogenetic analysis revealed that GRXC5 is the ancestral isoform and the only plastidial class I glutaredoxin in Physcomitrium patens. However, the exact function and impact of class I glutaredoxins on plastid redox processes in vivo is still unknown. Here we show that P. patens plants lacking plastid class I glutaredoxin are still viable and show alterations in stromal redox dynamics. We generated knock-out lines of GRXC5 in P. patens and introduced plastid-targeted redox-sensitive GFP2 (roGFP2) as model target for protein S-glutathionylation, into WT and mutant background (Δgrxc5). Using plate-reader based fluorometry assays, we found altered light-dependent roGFP2 dynamics compared to WT. Moreover, after oxidative challenge, the ability to deglutathionylate stromal roGFP2 was largely impaired in Δgrxc5 while plant growth under control and tested abiotic stress conditions was not distinguishable from WT. Our results suggest that P. patens Δgrxc5 plants can maintain growth without glutaredoxins catalyzing protein deglutathionylation in plastids under the tested conditions, even though removal of S-glutathionylation is retarded upon stress. Future challenges include to identify GRXC5 target proteins and to further assess S-glutathionylation dynamics in vivo.

Ascorbate peroxidase 2 of Chlamydomonas reinhardtii is involved in the regulation of the plastocyanin levels

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In the green microalga Chlamydomonas reinhardtii, APX2 is one of the four ascorbate peroxidase isoforms. These H2O2–scavenging enzymes use ascorbate for the reduction of H2O2. APX2 from C. reinhardtii and APX6 from A. thaliana, its orthologous, belong to a new class, named Ascorbate Peroxidase-Related (APX-R). The APX-R enzymes lack the essential amino acids to bind ascorbate and in vitro studies confirmed that AtAPX6 does not bind ascorbate, but several aromatic compounds [1]. In silico analyses showed that APX2 might reside in the lumen of the thylakoid. However, no differences were observed during growth in null apx2 mutants. The photosynthetic activity at increasing light intensities was only impacted when apx2 mutant cells were grown under phototrophic condition in low light. This was accompanied by a faster P700 oxidation upon a sudden increase of light and a slower re-reduction rate, a phenotype observed under all tested growth conditions. Furthermore, no H2O2 increase was detected in the apx2 mutants when they were transferred from low light to high light, suggesting that the lower photosynthetic activity would rather be due to regulation at the intersystem electron carriers than to oxidative stress damage. We then analysed soluble extracts with spectroscopic and mass spectrometry techniques, which showed a reduced levels of PC and the presence of the pre-apo-plastocyanin form in the apx2 mutants. In addition, a functional replacement of PC by cytochrome c6 under Cu2+ deficient conditions restored the wild-type phenotype of the electron transport to Photosystem I, confirming a specific role of APX2 on PC. Additionally, predicted structures with AlphaFold2 indicated a high probability of an interacting complex APX2:PC.
Our results suggest that APX2 might be involved in the regulation of the PC levels, which questions the role of APX2 during photosynthesis.

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[1] Lazzarotto et al., (2021). Antioxidants, 10(1):65

Redox regulation of histone acetylation in response to environnemental stress in A. thaliana

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High temperatures impact plant growth and survival. Chromatin modification is an essential gene expression reprogramming process during plant response to high temperature. Histone deacetylases (HDAs) that regulate histone acetylation levels have been shown to play an important role in the adaptation of plants to the environment. In animals, some HDAs are regulated by post-translational oxidation-reduction (redox) modifications involving the oxidation of conserved cysteines. In plants, the redox regulation of HDAs is very little known. A recent study demonstrated that redox modifications affect histone acetylation by inhibiting HDA activities. During my thesis, I detected a redox modification of HDA6 (a highly conserved plant HDA) under oxidative conditions, affecting its oligomerization state. I also showed that the expression of genes regulated by HDA6 is affected by the redox environment of the cell. Additionally, I discovered that HDA6 is involved in high temperature response of plants, which is known to generate cellular oxidation. Specifically, I studied two different high temperature regimes: a rise in ambient temperature from 20°C to 27°C inducing a developmental adaptation program called thermomorphogenesis, and a 37°C treatment mimicking an intense heat wave and affecting plant viability. I have shown that hda6-6 and hda6-7 (HDA6 KO mutants) are extremely sensitive to heat stress and are unable to induce thermomorphogenesis. To decipher which genes are misregulated in hda6 mutants, I performed RNA-seq analysis and found a marked deviation in genome expression in the mutant at high temperature (27°C and 37°C). Furthermore, I found that HDA6 co-localizes with the stress granule marker protein PAB2 at 37°C in a redox-dependant manner, suggesting that HDA6 is part of stress-granule complexes. Finally, I will discuss the emerging link between redox signaling and histone acetylation in response to heat stress.

Glutathione-mediated redox regulation of plant response to high temperature

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In the context of climate change, global rise of temperature as well as intense heat waves affect plant development and productivity. Among the molecular perturbations that high temperature induce in living cells is the accumulation of reactive oxygen species (ROS), which can damage macromolecules of the cell and perturb the cellular redox state. To cope with deleterious effects of ROS, plant, as other organisms, have developed strategies to scavenge ROS and to regulate their redox state. Among those, glutathione play a major role in maintaining the cellular redox state and the function of key antioxidant enzymes like peroxidases. Here, we investigated the contribution of the redox systems in plant adaptation to high temperature. We studied two different high temperature regimes: a rise of ambient temperature to 27°C inducing a plant developmental adaptation program called thermomorphogenesis, and a 37°C treatment mimicking intense heat wave and affecting plant viability. Using the genetically encoded redox marker roGFP, we show that high temperature regimes lead to cytoplasm and nuclear oxidation and impact profoundly the glutathione pool rather than the glutathione redox state. Moreover, plants are able to restore the glutathione pool within a few hours, which likely contribute to plant adaptation to high temperature. In contrast, conditional glutathione deficient mutants fail to adapt to intense heat waves or to induce thermomorphogenesis, suggesting that glutathione is involved in both of these heat adaptation mechanisms. We also evaluate by RNAseq analyses, how plant change its genome expression signature upon heat stress and identified a marked genome expression deviation in the glutathione deficient mutant which might contribute to their sensitivity to high temperature. Thus, we define glutathione as a major actor in the adaptation of plant to contrasting high temperature regimes.

Identification of intramembrane proteases that activate membrane-bound transcription factors during mitochondrial retrograde regulation

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Due to their sessile lifestyle, plants are exposed to ever-changing and often stressful environments, such as drought, heat, and pathogen assaults. To survive these harmful conditions, plants evolved to have complex mechanisms to recognize and counteract these conditions. Besides the plasma membrane, intracellular organelles such as chloroplasts and mitochondria are in a prime position for sensing and reporting stress signals to the nucleus to regulate stress-responsive gene expression. The molecular mechanisms of these organelle-to-nucleus (also referred to as retrograde) signaling networks are not well understood in plants. Our lab identified a novel mitochondrial retrograde signaling pathway, in which transcription factors of the NO APICAL MERISTEM/ ARABIDOPSIS TRANSCRIPTION ACTIVATION FACTOR/ CUPSHAPED COTYLEDON (NAC) family that are anchored to the ER -membranes through their C-terminal transmembrane domain play a key role. Upon mitochondrial perturbation by stresses, the N-terminal part of these transcription factors is released from the ER-membranes and translocated to the nucleus to regulate stress-responsive gene expression. However, the molecular mechanisms that underlie the release of these transcription factors and how mitochondria signal to the ER during plant stress responses remain not well understood. In silico and pharmacological analysis indicate that these ER-anchored NAC transcription factors are cleaved by rhomboid proteases. The aim of my PhD project is to identify the responsible proteases by activity-based-protein profiling and proximity-based labeling approaches and consequently unravel the molecular mechanisms of ER-membrane bound transcription factor activation during mitochondrial retrograde signaling of plant stress responses.

Redox properties of the plant atypical thioredoxin DCC1

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The redox state of cysteinyl residues is mainly controlled by proteins such as thioredoxins, glutaredoxins and protein-disulfide isomerases (Zaffagnini et al., 2019). These thiol-disulfide oxidoreductases are present in all kingdoms of life and carry a common thioredoxin-fold. They typically use a pair of reactive cysteines present in a CXXC motif to perform thiol–disulfide exchange reactions and control the activity or folding of their target proteins. Many uncharacterized proteins possess one or several conserved CXXC motif. In Arabidopsis thaliana and other terrestrial plants, three proteins are formed by a DUF393 domain (Pfam database), that includes a conserved N-terminal DXXCXXC motif, which led to the name DCC proteins (Ginalski et al., 2004). Some representatives are also present in algae and some archaea and bacteria. The DCC proteins are predicted to adopt a thioredoxin-fold, but with a C-terminal extension. In order to characterize the biochemical and structural properties of these atypical thioredoxins, the sequence encoding the mature form of Arabidopsis thaliana and Populus trichocarpa DCC1 proteins were expressed as recombinant proteins in E. coli, the proteins purified and their redox characteristics analyzed, .i.e. activity profiling, susceptibility to oxidative modifications, interactions with other oxidoreductases.

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Phytochrome B regulates reactive oxygen signaling during abiotic and biotic stress in plants

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Plants are essential for life on Earth converting light into chemical energy in the form of sugars. To adjust for changes in light intensity and quality, and to become as efficient as possible in harnessing light, plants utilize multiple light receptors, signaling, and acclimation mechanisms. In addition to altering plant metabolism, development and growth, light cues sensed by some photoreceptors, such as phytochromes, impact many plant responses to biotic and abiotic stresses. Central for plant responses to different stresses are reactive oxygen species (ROS) that function as key signaling molecules. Recent studies demonstrated that respiratory burst oxidase homolog (RBOH) proteins that reside at the plasma membrane and produce ROS at the apoplast play a key role in plant responses to different biotic and abiotic stresses. Here we reveal that phytochrome B (phyB) and RBOHs function as part of a key regulatory module that controls ROS production, transcript expression, and plant acclimation to excess light stress. We further show that phyB can regulate ROS production during stress even if it is restricted to the cytosol, and that phyB, RBOHD and RBOHF co-regulate thousands of transcripts in response to light stress. Surprisingly, we found that phyB is also required for ROS accumulation in response to heat, wounding, cold, and bacterial infection. Taken together, our findings reveal that phyB plays a canonical role in plant responses to biotic and abiotic stresses, regulating apoplastic ROS production, and that phyB and RBOHs function in the same pathway.

Redox-sensitive fluorescent biosensors detect the symbiotic bacteria Sinorhizobium meliloti intracellular redox changes under free-living and symbiotic lifestyles

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Reactive Oxygen Species such as hydrogen peroxide (H2O2) are key signaling molecules that control the setup and functioning of Rhizobium-Legume symbiosis. This interaction results in the formation of a new organ, the root nodule, in which bacteria enter the host cells and differentiate into atmospheric nitrogen (N2)-fixing bacteroids. The interaction between Sinorhizobium meliloti and Medicago truncatula is a genetic model to study N2-fixing symbiosis. In previous

work, several S. meliloti mutants, impaired in antioxidant defense, showed altered symbiotic properties, emphasizing the importance of redox-based regulation in the bacterial partner. However, direct measurements of S. meliloti intracellular redox state have never been performed. Here, we measured dynamic changes of intracellular H2O2 and glutathione redox potential by expressing roGFP2-Orp1 and Grx1-roGFP2 biosensors in S. meliloti. Kinetic analyses of redox changes under free-living conditions showed that these biosensors are suitable to monitor the bacterial redox state in real-time, after H2O2 challenge and in different genetic backgrounds. In planta, flow cytometry and confocal imaging experiments allowed the determination of sensor oxidation state in nodule bacteria. These cellular studies establish the existence of an oxidative shift in the redox status of S. meliloti during bacteroid differentiation, opening up new avenues for in vivo studies of redox dynamics during N2-fixing symbiosis.

Unravelling plant responses to heat stress using genetically encoded biosensors

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Temperature extremes become more prevalent as a consequence of climate change. As heat waves severely hamper crop productivity, it is crucial to unravel how plants respond to heat stress in order to develop strategies to improve plant growth under conditions of global warming. In this study, Arabidopsis thaliana plants expressing genetically encoded biosensors were used to determine the influence of heat stress on dynamics of the following parameters: Grx1-roGFP2 for the glutathione redox potential (EGSH), HyPer7 for hydrogen peroxide (H2O2), ATeam1.03-nD/nA for magnesium adenosine 5'-triphosphate (MgATP2-), YC3.6 for free Ca2+, cpYFP for pH, and Peredox-mCherry for the NADH/NAD+ ratio. Sensor responses were kinetically monitored in a plate reader upon temperature increase.

Severe heat stress (43°C) induced fast increases in cytosolic H2O2 and Ca2+ levels, whereas MgATP2- and pH rapidly decreased. These responses coincided with increases in EGSH and the NADH/NAD+ ratio. Responses to a milder heat stress (37°C) were very similar, but occurred over an extended time period. In general, responses showed a biphasic pattern in which milder effects were followed by more severe changes. Evans blue staining of seedlings at different time points after heat stress initiation demonstrated that this second phase corresponded to the occurrence of cell death. Recovery experiments revealed that all cytosolic parameters quickly recovered to their basal state when the temperature was returned to 25°C after 1 h of heat stress. Exogenous sucrose application partially mitigated heat-induced increases in cytosolic H2O2 levels and promoted the rise in free Ca2+ levels. In contrast, responses were not affected by mannitol treatment.

Our data show that genetically encoded biosensors are excellent tools to study plant heat stress responses. Biosensor analyses in different mutant backgrounds and subcellular compartments will help to further dissect these responses. However, stability and responsivity of the sensors under high temperature conditions should be verified.

Aboveground Plant-to-Plant Electrical Signaling and ROS waves regulates Network and Systemic Acquired Acclimation

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Background: An injured leaf (e.g., by insect herbivory or excess light) generates electric signals (ES) that spread to tissues, leaves, and organs of the entire plant. ES are mediated by changes in the activity of ion channels and are accompanied by waves of reactive oxygen species (ROS) and nonphotochemical quenching (NPQ). These waves are interdependent and propagate systemically throughout the plant. This process is essential for priming specific changes in gene expression and plant acclimation (e.g., cellular light memory). As a result, the entire plant enters a state of Systemic Acquired Acclimation (SAA). Question: Could ES, ROS and NPQ waves spread from an injured plant to neighbouring plants, providing their leaves touch each other? Up to now, only indirect signaling routes like underground ES mediated by mycorrhizal networks between roots of different plants, or aboveground plant volatiles, were found to connect different plants. Findings: Under humid conditions, an injured plant can directly communicate a danger signal to other plants that touch it within a community of plants, like a meadow of dandelion. ES and ROS waves serve as plant-to-plant signals propagating on and in the leaf with a velocity of several mm per s or cm per min, respectively. These signals can induce changes in NPQ that

are prerequisite for chloroplast retrograde signaling, genes expression, hormones, ROS signaling, and acclimation responses, in neighbouring plants. Majority of these complex communication responses can also be induced between two plants connected by a copper wire circuit indicating that ES is the main player of plant-to-plant communication. We show that ES, NPQ and ROS induce a new acclimation phenomenon termed "Network Acquired Acclimation (NAA)", necessary for SAA induction within a plant community. Next steps: Could plants be capable of using ES as communication or warning signals between a plant and animals (e.g. insects)?

Pollen fertility and the role of ROS and redox homeostasis in heat stress tolerance during sexual reproduction.

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To ensure reproductive success, flowering plants produce an excess of pollen to fertilize a limited number of ovules. However, in many flowering plants the male gametophyte is considered to be the most sensitive tissue to high temperatures, with even a single hot day being able to cause male sterility. Using flow cytometry approach, we recently showed that pollen grains mature into two distinct subpopulations – those that display high metabolic activity and elevated reactive oxygen species (ROS) levels immediately after hydration (high-ROS/active), and those that maintain an extended period of dormancy with low metabolic activity (low-ROS/dormant). We show that while the high-ROS pollen grains are highly susceptible to heat stress, low-ROS pollen grains may survive even extreme temperatures. We propose that the dormant pollen serves as a backup to provide a second chance for successful fertilization when the 'first wave' of pollen encounters an unpredictable growth condition such as heat stress. Pollen grains expressing redox-sensitive ro-GFP further reveal two distinct subpopulations with the majority being highly reduced. While the relationship between the redox status and the active/dormant state of the pollen awaits further investigation, a recently developed ro-mCherry, is now being employed in Arabidopsis to simultaneously determine the redox and ROS status within the pollen population. In addition, FACS-purified active and dormant pollen revealed different ribosomal RNA and transcriptional profiles, pointing to mechanisms involved in dormancy initiation/release and resilience. Our findings suggest that by regulating the activity state of pollen, thus modulating the composition of the pollen population, flowering plants may optimize their reproductive success to best-fit changes in their environment.

3-mercaptopyruvate sulfurtransferases: new actors in sulfur trafficking and H2S mediated-redox signalling in plants?

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Hydrogen sulfide (H2S) is a gaseous effector involved in a wide variety of physiological processes in most organisms including photosynthetic organisms. The oxidative modification of cysteine residues to persulfides is suggested to represent the main way by which H2S exerts its biological functions. Hence, the signaling functions of H2S likely rely on the reactions with specific proteins and cysteines leading to their persulfidation and represents a novel thiol switching mechanism comparable to nitrosylation or glutathionylation.
The widely distributed 3-mercaptopyruvate sulfurtransferases (MSTs) are implicated in the generation of persulfidated molecules and H2S biogenesis through transfer of a sulfane sulfur atom from a suitable donor to an acceptor. Arabidopsis thaliana possesses two MSTs (namely STR1 and STR2) which remain poorly characterized. To learn more about these enzymes, we conducted a series of biochemical experiments combining a variety of sulfur donors and reducing systems as acceptors. Our kinetic studies revealed that both MSTs use 3-mercaptopyruvate efficiently as a sulfur donor while thioredoxins, glutathione, and glutaredoxins all served as high-affinity sulfane sulfur acceptors. Using the redox-sensitive GFP (roGFP2) as a model acceptor protein, we showed that the persulfide-forming MSTs catalyze roGFP2 oxidation and more generally trans-persulfidation reactions. However, a preferential interaction with the thioredoxin system and glutathione was observed in competition assays mixing roGFP2 and these sulfur acceptors. Moreover, while we observed that MST catalytic cysteines are prone to overoxidation, prior persulfidation prevents the irreversible inactivation of MSTs because oxidized persulfides are reduced by thioredoxins or glutathione. This work provides significant insights into Arabidopsis STR1 and STR2 catalytic properties and more specifically emphasizes the roles of cellular reducing systems for the generation of H2S or glutathione persulfide and for the reactivation of oxidatively modified MST forms.

NO and Phytoglobins role in Arabidopsis-Fusarium oxysporum interaction

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During evolution, plants have developed different resistance mechanisms against biotic and abiotic stress. Reactive oxygen and nitrogen species (ROS/RNS) are signaling molecules being involved in many physiological processes, including defence response during plant-pathogen interactions. Especially, it is well known that NO produced after plant recognition of pathogens is part of the signaling cascades that trigger the expression of defence genes, the production of secondary metabolites and finally, hypersensitive response (HR) and systemic acquired resistance (SAR).

One of the major economically relevant plant pathogens is Fusarium spp. Root-colonizing fungi Fusarium oxysporum causes vascular wilt disease that leads to devastating yield losses. Indeed, the eradication is very tough, due to its persistence and colonizing capacity. In addition, phytoglobin 1 (Glb1) is an important player regulating NO concentration protecting organisms from oxidative and nitrosative stress. Recently, the role of Glb1 in F. oxysporum-tomato interaction has been assessed. In order to get a deeper insight onto NO signaling and Glb1 role in plant defence, in this work, we have studied the response of a model plant Arabidopsis thaliana, wild type (WT) and lines with altered levels of Glb1, to F. oxysporum. Both, the antisense and the overexpressor line showed a more resistant phenotype than WT to the fungus. The mutants also present differences in defence gene expression and RNS production among others, suggesting that Glb1 may be able to regulate NO level and to enhance the defence response.

Influence of nitrogen availability and form on sensitivity to heat and light stress in coral endosymbionts

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The trophic and structural foundations of coral reef ecosystems rely on the mutualistic relationship existing between Scleractinia and dinoflagellates of the Family Symbiodiniaceae. In this endosymbiosis, the photosynthetic algae reside in a symbiosome within the gastrodermal cells of the coral host, allowing reciprocal and complimentary transfers of highly energetic compounds and efficient recycling of growth-limiting nutrients in an oligotrophic environment.
Coral reef cover is declining globally because of anthropogenic stresses. Among these, high sea surface temperature accompanied by high levels of solar irradiance are known to cause coral bleaching, a phenomenon during which the host loses most of its symbionts and becomes physiologically and nutritionally compromised. Data accumulated to date indicate that the disruption of metabolic homeostasis between host and symbiont and the dysregulation of redox homeostasis are responsible for the collapse of the symbiosis.
Local-scale stressors, such as nutrient loading and sedimentation can also exacerbate the impacts of thermal stress on corals. Although numerous studies have investigated the interactive effects of elevated temperature and nitrogen availability on reef-building corals, results documented to date are inconsistent, with some showing an increase and others a decrease in the susceptibility to bleaching. In this study, we investigated the effects of nitrogen source (no nitrogen, 500 µM NH_4^+ or 500 µM NO_3^-) and temperature stress combined with light stress in Symbiodinium microadriaticum, isolated from the coral Stylophora pistillata. After 24h of treatment, we observed a significant impact of light and temperature stress on photosynthesis (lower Fv/Fm and max rETRPSII) and on photoprotective mechanisms (increased DPS of the xanthophylls but lower NPQ). This treatment also significantly increased the intra- and extracellular reactive species production. Nitrogen-deprived cells appeared more sensitive to stress and displayed a higher NPQ, DPS, and production of intracellular reactive species compared to cells grown with NO_3^- and NH_4^+.

Chloroplastic ascorbate content is a modulator of the plant metabolome

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Ascorbate is a major plant metabolite that plays crucial roles in various processes from reactive oxygen scavenging to epigenetic regulation. However, to what extent and how ascorbate modulates metabolism is largely unknown. To address this, we investigated the consequences of chloroplastic ascorbate deficiency and total cellular ascorbate deficiency, by studying a novel chloroplastic ascorbate transporter PHT4;4 mutant line, and comparing it to the ascorbate-deficient vtc2-4 mutant in Arabidopsis thaliana. Under regular short-day growth conditions, the morphological phenotypes of the mutants and the wild type were indistinguishable, and both deficiencies caused minor alterations in photosynthesis, without being compensated by the accumulation of other antioxidants. In contrast, metabolomics analysis revealed global reorganization of metabolite levels in the vtc2-4 mutant, affecting over 50% of the identified metabolites. Unexpectedly, there was a large overlap in the changes in metabolite levels between the ascorbate-deficient mutant and the chloroplastic ascorbate transporter mutant. The changes concerned the central, amino acid, and nucleotide metabolism and secondary metabolites without the typical symptoms of oxidative stress. Therefore, our data show that upon chloroplastic ascorbate deficiency, the metabolome is rewired independently of oxidative stress, thus ascorbate may possibly act as a metabolic regulator in vascular plants.

Endoplasmic reticulum oxidoreductin (ERO) provides resilience against reductive stress and hypoxic conditions by mediating luminal redox dynamics

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Oxidative protein folding in the endoplasmic reticulum (ER) depends on the coordinated action of protein disulfide isomerases and ER oxidoreductins (EROs). Strict dependence of ERO activity on molecular oxygen as the final electron acceptor implies that oxidative protein folding and other ER processes are severely compromised under hypoxia. Here, we isolated viable ero1 ero2 double mutants that are highly sensitive to reductive stress and hypoxia. To elucidate the specific redox dynamics in the ER in vivo, we expressed the glutathione redox potential (EGSH) sensor Grx1-roGFP2iL-HDEL with a midpoint potential of -240 mV in the ER of Arabidopsis plants. We found EGSH values of -241 mV in wild-type plants, which is less oxidizing than previously estimated. In the ero1 ero2 mutants, luminal EGSH was reduced further to -253 mV. Recovery to reductive ER stress induced by dithiothreitol, was delayed in ero1 ero2. The characteristic signature of EGSH dynamics in the ER lumen triggered by hypoxia was affected in ero1 ero2 reflecting a disrupted balance of reductive and oxidizing inputs, including nascent polypeptides and glutathione entry. The ER redox dynamics can now be dissected in vivo, revealing a central role of EROs as major redox integrators to promote luminal redox homeostasis.

OZ.26 is a novel receptor central for plant abiotic and biotic stress tolerance.

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Understanding plant response mechanisms to biotic and abiotic stresses at the molecular level is essential for developing cultivars that perform better in a shifting climate. Stress perception leads to reactive oxygen species (ROS) production, which activates tightly coordinated and regulated defense pathways. Despite continuous research, relevant signaling pathways are still incomplete.

Ozone (O3) exposure induces an intrinsic ROS elevation, thus activating downstream signaling pathways. In order to identify new components in plant stress tolerance regulation, we performed a mutant screen in Arabidopsis based on O3 sensitivity. I have identified and mapped OZ.26, a novel component in early biotic and abiotic stress responses. oz.26 mutants are highly sensitive to O3, Pseudomonas, Botrytis and freezing but not to drought, salt and heat treatments, showing specificity. OZ.26 is ubiquitously expressed in various plant tissues and localized to the ER. Additionally, oz.26 mutants show elevated ROS levels in response to flg22 and chitin treatments when compared to WT. Furthermore, I have shown that O3 and flg22 induce autophagy in OZ.26-YFP lines. Autophagy is a highly conserved major degradation and

recycling pathway, an interplay between ROS and autophagy has been observed previously. NADPH dependent ROS activate autophagy to degrade damaged organelles and oxidized proteins in plant cells. However OZ.26 is the first protein to be identified in O3 induced autophagy.
OZ.26 carries a putative heme binding domain. Heme plays an active role in plant metabolic pathways as well as in stress signaling. Oxidative stress leads to increased heme content in the cell and accumulating free heme molecules are capable of reacting with oxygen to generate cytotoxic ROS. Recently I have shown that OZ.26 has heme binding properties by applying Microscale Thermophoresis. OZ.26 is a novel heme receptor, degraded through autophagy to limit over accumulation of cytotoxic ROS thereby conferring resistance to biotic and abiotic stresses.

Monitoring the degree of cysteine oxidation using data-independent acquisition

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Protein cysteine residues are intrinsically reactive and readily oxidized within cells by electrophilic species such as reactive oxygen and nitrogen species (ROS/RNS). Resulting oxidative post-translational modifications (OxiPTMs) can adjust protein function and thereby act as post-translational regulators in various physiological processes. While various proteomic methods have been developed for identifying and quantifying relative levels of OxiPTMs, assessing the degree of cysteine oxidation remains challenging. Here, we describe a method that quantifies the degree of cysteine oxidation by data-independent acquisition mass spectrometry (DIA-MS). More specifically, isotopically labeled iodoacetamide is used to discriminately label native thiols and those liberated after a reduction step – allowing to monitor absolute ratios within each sample based on isotopically-labeled fragment ions in DIA-MS. Furthermore, as no enrichment step is involved, protein levels can be monitored simultaneously. Taken together, this strategy paves the way towards future reproducible and accurate redox proteome profiling using DIA-MS.

Redox-Regulation of Histone Deacetylation in Arabidopsis thaliana

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Histone acetylation affects the DNA accessibility and thereby contributes to epigenetic transcription control. The removal of acetyl groups from acetylated histone lysine residues is catalyzed by histone deacetylases (HDAs). HDAs are very important transcriptional regulators involved in a variety of physiological processes. However, it is still unknown how these enzymes are regulated. Nitric oxide (NO) is a signaling molecule with multiple regulatory functions in plant physiology and stress response. S-Nitrosylated glutathione (S-nitrosoglutathione, GSNO) has important functions as physiological NO donor. The level of GSNO is controlled by GSNO reductase (GSNOR), suggesting an important regulatory function of GSNOR in NO-signaling.
We demonstrated, that the activity of HDA6 is inhibited by NO. Moreover, chromatin immunoprecipitation sequencing and RNA-seq analyses revealed that both, GSNOR and HDA6 act in similar pathways responsible for regulating an identical set of growth/development-related genes as well as stress-related genes, suggesting that NO and GSNOR are involved in the regulation of histone acetylation.
Furthermore, using a liquid chromatography approach in combination with activity measurements, we identified HDA5 as NO-sensitive HDA-isoform. In contrast to HDA6, HDA5 is activated by NO, implying a novel role of NO as activator of HDAs. Interestingly, oxidized and reduced glutathione and H2O2 affected the activity of HDA5, too, concluding that the regulatory effect triggered by redox-dependent posttranslational modification of cysteines is not only restricted to S-nitrosylation but includes also S-glutathionylation and oxygen-dependent modifications. Structural modeling and amino acid sequence analysis suggest the presence of multiple redox-sensitive cysteine residues in HDA5. Histone modifications have been analysed in hda5-ko to identify the substrates of HDA5. Moreover, transcriptome analysis of hda5-KO plants have been done to reveal the physiological function of HDA5. In sum, our results imply a crucial role of NO as regulator of histone acetylation in Arabidopsis thaliana.

Mitochondrial retrograde signaling regulates hypoxia tolerance in rice

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Mitochondria, as the powerhouse in eukaryotic cells, are affected by various biotic and abiotic stresses. Our recent studies indicate that maintaining functional and flexible mitochondrial metabolisms via retrograde regulation of nuclear genes facilitates plant adaption to stressful environments. Alternative oxidase (AOX) is a non-ATP generating terminal oxidase in the plant mitochondrial electron transport chain. We showed that AOX expression is induced and it functions in mitochondria for metabolic and signaling homeostasis in response to hypoxia in rice. We observed that H2O2 activates the expression of AOX and genes encoding enzymes involved in H2O2 homeostasis in mitochondria, as well as genes that regulate sugar production, glycolysis, and ethylene biosynthesis essential for rice seedling development under hypoxia/submergence. We also identified many protein kinases and transcription factors that could be involved in hypoxia signaling. We demonstrated that AOX is necessary and sufficient for germination and seedling development, as well as H2O2 accumulation, in rice under both aerobic and anaerobic conditions. Moreover, ectopic expression of AOX1 enhances root aerenchyma development and lateral root emergence that enhance root system under submergence, as well as photosynthesis rates and grain yield in the field under regular and reduced irrigation conditions. Our discoveries reveal new insights into a unique regulatory mechanism for maintaining ROS homeostasis and regulating seedling development in rice under submergence, and highlight genes involved in ROS homeostasis as potential targets for crop improvement.

8) VESS3 Oral Presentations Sessions – Authors Sorted Alphabetically

Anticancer effect of the vitamin E metabolite garcinoic acid in mouse models of Her2/neu positive breast cancer

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University of Perugia, Italy

This study aims to investigate the effect of garcinoic acid (GA), a plant metabolite of vitamin E with potent PXR agonist and anti-inflammatory activity, in the prevention and therapy of Her2/neu-positive breast cancer, which is characterized by high invasiveness and metastatic potential.

GA was compared with annatto tocotrienols (A-T3) that are rich in the GA precursor delta-tocotrienol. The compounds were studied both in vitro in MCF-7 breast cancer cells and in vivo in two different transgenic mouse models that spontaneously develop breast cancer by the expression of variants of the human c-ErbB2 (Her2)/neu genotype (namely FVB-D16HER2/neu and FVBN233 HER2/neu mice).

The results of the in vivo studies demonstrated in both the transgenic models that GA and A-T3 did not significantly prevent breast cancer, but significantly reduce the volume of the tumor masses. Furthermore, in FVB-D16HER2/neu mice, A-T3 used in chemotherapy mode, significantly reduced the number of tumor masses. The same effect on this parameter was confirmed in FVBN233-HER2/neu mice when treated in chemoprevention mode with both GA and A-T3. In vitro, GA was found to be less toxic than A-T3, and both these treatments were confirmed to stimulate PXR expression in breast cancer cells.

The original findings in this project confirm the anticancer potential of GA providing first evidence of efficacy in vivo in mouse models of breast cancer employing the HER2/neu oncogene. This preliminary evidence is worth investigating further at the preclinical and molecular level to explore its therapeutic application in human breast cancer.

The antioxidant-independent actions of vitamin E on gene expression

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Vitamins A and D are lipid-soluble vitamins that bind with high affinity to nuclear receptors. The two remaining lipid soluble vitamins, vitamins E and K, are currently not known to interact with nuclear receptors but their structure does not exclude this possibility. While it has been previously established that alpha-tocopherol is capable of modulating gene expression, it is unclear if these are antioxidant dependent or independent. To address this question, we have synthesized and characterized the redox-inert analog of vitamin E, 6-hydroxymethyl alpha-tocopherol (6-HMTC), and investigated the role that this antioxidant-inert vitamin plays in modulating gene expression in vitro. The analog bound to the alpha-tocopherol transfer protein with similar affinity to that of the natural vitamin but did not show any antioxidant activity in vitro nor in cultured cells. We compared the impact of alpha-tocopherol vs. that of 6-HMTC on the transcriptome of cultured immortalized human hepatocytes using RNA-sequencing (RNA-seq). Importantly, 6-HMTC modulated the expression of some genes in efficacy and dose response similar to those exhibited by alpha-tocopherol. These findings suggest that alpha-tocopherol modulates gene expression through an antioxidant-independent mechanism that is mediated and controlled by specific members of the nuclear receptor family and ligand-activated transcription.

Industrial Synthesis of Vitamin E

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In 1922, 100 years ago, Evans and Bishop first described a "factor X", whose absence in the diet of female rats led to death of the foetus and its resorption.[1] In the following decades, this factor was renamed to "Vitamin E", isolated from natural sources, structurally elucidated, and its additional functionalities were explored. Due to its biological activity and antioxidant properties, this essential food ingredient is of great economic importance, with a market volume of more than 75.000 t/a. Its main application lies in animal nutrition, with about 85% of synthetic Vitamin E used as additives for industrially produced feed mix and only 15% going to human applications.[2]
Vitamin E is predominantly produced by total synthesis as a mixture of eight stereoisomers of its most active form, α-tocopherol. The main market product is a 50% adsorption of the corresponding (all-rac)-α-tocopheryl acetate on silica. While synthetic Vitamin E is represented in all areas of application, semisynthetic (R,R,R)-α-tocopherol from natural sources is restricted to the pharmaceutical, food, and cosmetics sector, with a total market volume of ca. 3000 t/a.[2]
The total synthesis of (all-rac)-α-tocopherol consists of three major parts: the preparation of the aromatic building block (trimethylhydroquinone), the production of the side chain component (isophytol, phytol or analogues), and the condensation of those two components.[2]
We will give an overview about the history and current production methods of synthetic Vitamin E as well as challenges and current trends.[3]

Literature

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The role of hypercortisolism, inflammation and modified redox on the functional loss of muscle with age: protective effects of vitamin E and cocoa polyphenols.

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Older adults are vulnerable to undernutrition, resulting from deficient food or nutrient intake, leading to weight loss and frailty. Loss of skeletal muscle mass and function is a major contributor to frailty and prevention of such loss is crucial. Ageing is associated with dysregulation of the hypothalamic-pituitary-adrenal axis, with an increase in cortisol levels, affecting skeletal muscle and potentially leading to sarcopenia and frailty.
This project will examine the effect of cortisol-lowering chocolate polyphenols, combined with vitamin E on modulation of the effects of physical activity in older people, potentially preventing the age-dependent declines in key cellular functions that underpin the onset of muscle wasting in older people.
Initial studies are examining the effect of vitamin E and cocoa polyphenols on activation of atrophic processes following challenge of a human muscle cell model with cortisol and/or inflammatory cytokines (IL-6, TNF-alpha and INF-gamma) and/or reactive oxygen species (eg H2O2). Analyses include myotube diameter, volume, inflammation, insulin and cortisol metabolism, cytokine mRNA and protein release, cell death and redox status of cells including changes in cytosolic peroxiredoxin oxidation seen in muscles during ageing and data will be presented on these studies.
A subsequent study (see Migni et al abstract) examines the effect of a 3-month supplementation of older individuals with dark chocolate with high vitamin E content and cocoa polyphenols, combined with physical activity in a 3-month trial. Muscle biopsy analyses will include contractile characteristics of isolated fibres, cross-sectional area, inflammatory cytokines and the proportion of peroxiredoxins 2 and 3 in the oxidised form.

We hypothesise that the cytoprotective functions of vitamin E combined with cocoa polyphenols and physical activity may help prevent the age-dependent decline of skeletal muscle via key underpinning molecular events.
Choko-agE is an EU project, funded within the Horizon 2020, ERA-HDHL ("Healthy Diet for a Healthy Life") program.

Role of vitamin E and cannabinoids in regulating cellular lipid homeostasis relevant for macrophages foam cells formation

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Background: Recently, an increased number of foam cells was detected in bronchoalveolar lavages and lungs in cases of electronic-cigarette or vaping product use-associated lung injury (EVALI) upon inhaling vapors containing cannabinoids and the vitamin E analogue, alpha-tocopherol acetate (aTA). Increased levels of intact aTA are reportedly detected in EVALI indicating insufficient conversion of aTA to aT in lungs.
Aims: We investigate whether aTA affects macrophages foam cells formation in response to cannabinoids. Dysregulation of cannabinoid receptors by aTA and cannabinoids may affect expression of fatty acids transporters (CD36/FAT, SRBI, FFAR1, FABP4/aP2, ABCA1/G1) possibly via regulating the phosphatase PP2AC leading to dysregulation of cellular lipid homeostasis.
Methods: We analyzed regulatory effects on the expression of genes involved in cellular lipid homeostasis of natural alpha-tocopherol (RRR-aT), natural RRR-aTA (aTAn), synthetic racemic all-rac-aTA (aTAr), and as control alpha-tocopheryl phosphate (aTP) with/without co-treatment with cannabinoids (cannabinol (CBD), d9-tetrahydrocannabinol (THC)) in human THP-1 monocytes and macrophages.
Results: Our results suggest robust induction of CD36/FAT mRNA expression after treatment with THC that can be prevented, albeit incompletely, by aTA or CBD. A similar response pattern was observed with PP2AC and genes involved in lipid efflux (ABCA1), but much less with SRB1, suggesting an imbalance between uptake, metabolism, and efflux of lipids/aTA as possible mechanism of foam cell formation. Interestingly, genes involved in cellular lipid export (SRBI, ABCA1) were downregulated less by aTAn when compared to aTAr possibly as result of differentially forming a complex with THC.
Conclusions: Dysregulation of lipid homeostasis genes by aTA and cannabinoids may contribute to EVALI, although intact aTA may also act as linactant and by pulmonary surfactant disruption. Investigating the molecular mechanisms by which foam cells are formed will not only be important for EVALI but also for other diseases with dysregulated lipid homeostasis such as atherosclerosis and non-alcoholic steatohepatitis (NASH).

Nutrigenomics of wheat germ oil-vitamin E in human liver cells exposed to oleic acid-induced lipotoxicity.

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Background and methods: Wheat germ oil (WGO) is a main natural source of vitamin E with proposed role in hepatoprotection. In the present study, this role was investigated together with its transcriptomics fingerprint in an in vitro model of hepatic lipotoxicity consisting of human liver cells treated with oleic acid (OA) to develop steatosis. In this model, the cytoprotection function of WGO-vitamin E (WGO-VE) was compared with RRRα-tocopherol and all-rac-α-tocopherol VE (nVE and sVE, respectively).
Results: WGO-VE and nVE were more efficient compared to sVE in reducing steatosis and lipotoxicity, as indicated by the production and efflux of cellular H2O2, and cell death levels.
Transcriptomics data demonstrated formulation-specific fingerprints for the cytoprotection effect of VE. Ingenuity Pathway Analysis of gene datasets restricted to liver-related diseases and biofunctions, highlighted that the WGO-VE cytoprotection is associated to liver carcinogenesis and steatosis biofunction, and nVE modulated genes involved in liver cell metabolism and viability, whereas sVE did not significantly modulate the genes relevant to the selected biofunctions.
Conclusions: WGO-VE provides as efficient protection as nVE against the OA-induced lipotoxicity of human liver cells. The groups of genes and biofunctions involved in this effect differ from those of pure forms of VE (either natural or synthetic), reflecting the molecular complexity of this VE-rich oil that holds great potential in preventing liver cell lipotoxicity.

The Choko-Age project: development and analytical characterization of a new chocolate product functionalized with vitamin E to combine with physical exercise in preventing malnutrion in pre-dementia eldery subjects.

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Choko-agE is an EU project, funded within the Horizon 2020, ERA-HDHL ("Healthy Diet for a Healthy Life") program, aiming to study in a randomized clinical trial the effects of a new vitamin E (VE) -functionalized (70 % cocoa) dark chocolate product (VE-Cho) combined with physical exercise and a protein-rich diet, on indices of protein-energy malnutrition (PEM) in pre-dementia elderly subjects. Muscle loss prevention will be the primary endpoint; laboratory endpoints include: 1) cortisol-levels, 2) muscle metabolism and proteomics indictors of PEM in muscle biopsies.
VE-Cho development and laboratory characterization are presented. HPLC and GC-MS analysis were used to assess epicatechin and its epimerization, and then the functional ingredient α-tocopherol acetate (α-TAC) and its transformation to free α-tocopherol (α-TOH) and its oxidation metabolite α-tocopheryl quinone (α-TQ). A matrix solid dispersion clean-up protocol of chocolate extracts was developed to assess both (+) and (-) epicatechin, and methoxamine was used for α-TQ derivatization and simultaneous determination with all VE molecules and epicatechin in GC-MS. The results demonstrated that γ-tocopherol and (-)epicatechin are the most abundant VE and polyphenol forms present in standard dark chocolate, respectively; (+)epicatechin showed a trend toward an increase while α-TAC was stable during VE-Cho production. The simulation of gastro-intestinal digestion process significantly reduced the relative content of epicatechin. Finally, the analytical procedures used for chocolate products were also validated for an application to other biological samples, including blood plasma and tissues, that will be used to verify the nutritional intervention of the Choko-agE clinical trial.

Hepatic metabolism of 11'-α-tocomonoenol and 11'-γ-tocomonoenol confirms prenyl side chain saturation as determinant of tocochromanol metabolism.

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Introduction. Tocomonoenols (T1) are structurally related to vitamin E, but with a single double bond in the prenyl side chain attached to the chromanol ring, in contrast to the fully saturated and three-fold unsaturated side chains present in tocopherols and tocotrienols, respectively. Metabolic transformation of tocochromanols influence their bioavailability and biological functions, but limited information exists regarding biotransformation of tocomonoenols. Therefore, we studied the metabolism of α- and γ-tocomonoenols compared to the corresponding tocopherols and tocotrienols in hepatocytes. Methods. HepG2 cells were incubated independently with 11'-α-tocomonoenol (11'-αT1) or 11'-γ-tocomonoenol (11'-γT1) for 72 h in order to compare their uptake and metabolism with those of α- and γ-tocopherol; and α- and γ-tocotrienol. The formation of metabolites CMBHC and CEHC was quantified in culture medium by HPLC-ECD while intracellular content of parent compounds was measured by HPLC-FLD. Results. Hepatic uptake and metabolism of 11'-γT1 is significantly higher compared to 11'-αT1, a pattern also observed for γ- and α-forms of tocopherols and tocotrienols. 11'-αT1 and 11'-γT1 in HepG2 produce CMBHC as major metabolite and CEHC was a minor end product of 11'-γT1, but not of 11'-αT1. The conversion of the different congeners into CMBHC increased with the amount of double bonds in tocochromanols sharing the same ring structure. Conclusion. Tocomonoenols are mostly metabolized to CMBHC in HepG2 cells and the degree of saturation of the side chain is a determinant of the rate of tocochromanol metabolism.

Effects of mixed tocopherol versus α-tocopherol on biomarkers of reactive oxygen and nitrogen-induced stress and low-grade inflammation in healthy male volunteers: Comparison between smokers and non-smokers

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Results of α-tocopherol supplementation studies did not hold promises of epidemiological studies suggesting protection of non-communicable diseases (NCDs) by vitamin E. Smokers exhibit oxidative and nitrosative stress, resulting in low-grade inflammation and increased risk of NCDs. γ-tocopherol is lacking a methyl group on position 5 of the chromanol ring, proposed to facilitate 5-nitro-γ-tocopherol (5-NGT) formation under cigarette smoke exposure as a possible protective mechanism.
In a double-blind placebo-controlled study, the hypothesis that responses of smokers are different when α-tocopherol is compared to an equimolar mixture of α-, β-, γ- and δ-tocopherol, was tested. Healthy male smokers (n = 38) and non-smokers (n = 44), 18-45 y, received 294 mg RRR-α-tocopherol (AT) or mixed tocopherols (MT, 60 % α-, 24 % β-, 14 % γ-, 2 % δ-tocopherol) at 294 mg/d (MT1), 147 mg/d (MT2), or 73.5 mg/d (MT3), or placebo (P) for 4 weeks. Tocopherols were determined in plasma and buccal mucosal cells (BMC), along with 5-NGT, high-sensitive C-reactive protein (hsCRP) and interleukin-6 in plasma, peripheral blood mononuclear leukocytes were isolated and activated nuclear factor kappa B (NF-κB) determined in the nuclear fraction.
Plasma and BMC α-tocopherol concentrations increased 1.5-fold in the AT group, with 3-fold reduction in γ-tocopherol (GT); β-tocopherol did not change. MT groups showed significant increases in α-, β-, and γ-tocopherol. Baseline NF-κB activation, 5-NGT and hsCRP did not differ between smokers and non-smokers. In smokers (but not in non-smokers), both MT1 ($P = 0.025$) and MT2 ($P = 0.008$), but not AT, decreased NF-κB activation. In smokers (but not in non-smokers), GT concentrations predicted plasma 5-NGT ($P < 0.001$) and 5-NGT:GT ratios decreased in MT3. In MT groups, hsCRP was reduced in smokers and non-smokers.
This study demonstrates a protective effect of mixed tocopherols on reactive oxygen and nitrogen-induced chronic low-grade inflammation in healthy male smokers.

Vitamin E from an industry perspective: What have we learned in 100 years from human trials about its physiological role as an antioxidant?
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In the past 100 years, Vitamin E has been known as an exceptional antioxidant that impacts physiological functions. In humans, it is recognized for its specific role as chain-breaking antioxidant inhibiting lipid peroxidation in (sub-)cellular membranes, lipoproteins and body fat. Thereby it plays a major role within the body's antioxidant network helping to combat oxidative stress and supporting healthy lipids, proteins and DNA. In recent years, additional indirect antioxidant functions have been suggested that may impact cellular enzyme activities, signaling cascades, gene expression and metabolism. While the mechanisms of vitamin E's action are being elucidated the question remains to what extent the antioxidant effects of vitamin E, mainly inhibiting lipid peroxidation, impact normal physiologic functions and maintenance of health. The requirements of Vitamin E in humans have been defined based on its health impact as an antioxidant, i.e. inhibiting oxidative damage leading to lysis of erythrocytes. However, what else can be recognized after 100 years of research on Vitamin E as an antioxidant?
From an industry perspective, reliable evidence based on high-quality human trials constitutes the foundation of using Vitamin E as a dietary supplement and in functional foods. In this presentation, the state of evidence based on randomized, placebo-controlled, double-blind human trials that used Vitamin E (alpha-Tocopherol) in various population groups will be reviewed. What endpoints related to oxidative stress and physiological functions have been impacted by Vitamin E? How effective is Vitamin E by itself considering that it is part of the antioxidant network? What dosages of Vitamin E have been found adequate? What are new, promising health areas for Vitamin E use?

Cis-Trans Isomerization of Isolated Trisubstituted Olefins Catalyzed by Either Oligothiols or Nitrogen Monoxide
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Isomerization reactions of unfunctionalized olefins into their thermodynamically defined cis/trans-mixture are well known. However, these methods have mostly been limited to conjugated double bonds (e.g. stilbene derivatives, carotenoids). Isomerization has been achieved frequently by palladium- or iodine-catalysis, photoisomerization or simple heat treatment.

In contrast, there is significant less precedence for catalysts that efficiently isomerize compounds containing non-conjugated, unfunctionalized olefins such as those contained in fatty acids and isoprenoids. We are interested in the selective inter-conversion of trisubstituted double bonds of vitamin E precursors by means of separating and recycling the undesired olefin isomer (see Scheme), which simplifies synthetic access to natural (2R,4'R,8'R)-α-tocopherol.[1] To this end, we have developed two isomerization methods for isoprenoids and related olefins, which facilitate the geometric olefin isomerization rapidly and selectively.[2] The methods rely either on oligothiol or nitrogen monoxide catalysis.

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Vitamin E Safety – often revisited and incorrectly communicated

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Few side effects have been noted in adults taking supplements of less than 2,000 mg natural or synthetic vitamin E daily for a few weeks to a few months. The most worrisome possibility is that of impaired blood clotting, which may increase the likelihood of bleeding in some individuals. Side effects occurring as a result of long-term alpha-tocopherol supplementation have not been adequately studied. Since biological mechanisms exist to routinely eliminate potential excess levels of vitamin E, it is unlikely to take a harmful amount. According to the results of multiple meta-analyses, supplementation with vitamin E appears to have no effect on all-cause mortality even at higher doses, and currently there is no consistent mechanistic evidence that vitamin E supplementation causes harm. The latter hypothesis has been based on select, widely-popularized older meta-analyses, which suggest that vitamin E supplements - despite being administered in amounts below the Tolerable Upper Intake Level of 1,000 mg - increase the risk of death from any cause, especially in healthy subjects of the general population. Thus, there is a critical need for evaluation of pharmacological doses of vitamin E for a definition of its safety profile. From a real-life perspective, in patients with pre-existing conditions, an individualized risk-benefit analysis should be considered for each setting and indication.

9) SFRR-E Poster Abstracts – Authors sorted alphabetically

Ex vivo blood culture assessment of anti-oxidative effects of virgin olive oils differing in their bioactive contents.

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In the present study, we have performed an ex vivo blood culture and challenging assay with different inducers or inflammation and oxidative stress conditions, optimized to assess the anti-inflammatory and anti-oxidative response of individuals participants of a trial. The trial encompasses the raw intake of olive oils differing in their composition, in comparison to a standard virgin olive oil. Up to date, additional health benefits based on this trial have been determined and described on inflammatory, metabolic syndrome and endothelial function biomarkers, mainly in vivo. Now, we have determined several oxidative markers including iNOS, presence of Tyr-nitrated proteins and production of NO. This study strongly confirms the first level of evidence already obtained in vivo regarding the health benefits of olive oil components in healthy humans, now in a inflammatory and oxidative scenario induced ex vivo. As discussed, the experimental system also provides an excellent tool to dissect the effects of individual componentes present in the oil in a individual manner. This research was funded by research projects BFU-2016-77243-P, PID2020-113324GB-100 and STED202100X129616SV0 of the Spanish Ministry of Science, Innovation and Universities (MICIIN)/ State Research Agency (AGE)/ European Regional Development Fund (ERDF)/ European Union (EU).

Ganoderma lucidum extract, Lycopene, Sulforaphane, Royal Jelly and Resveratrol as a combination demonstrate antioxidant and anti-inflammatory effects in COVID-19

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COVID-19 is a disease in several stages starting with virus replication to dysregulation in immune system response, organ failure and recovery/death. Our aim was to determine the effect of combination extract of Ganoderma lucidum, lycopene, sulforaphane, royal jelly and resveratrol on the markers of oxidative stress, inflammation (IL-6, IL-8 and TNF-α), routine laboratory analyses and duration of symptoms in patients infected with SARS-CoV-2. The oxidative stress parameters (d-ROM, PAT, OS index) and IL6, IL8, TNF-a were determined in order to estimate the antioxidant and the anti-inflammatory effect of the product using a spectrophotometric and a magnetic bead-based multiplex assay in serum of 35 patients with mild form of COVID-19. Ge132+ Natural™ was used as a product of interest. We have obtained statistically significant differences for all investigated parameters between the treated patients and the control group. Moreover, significantly differences were observed for leukocytes, NLR and iron. The average duration of the symptoms was 9.4±0.487 days versus 13.1±0.483 days in the treatment and the control group, respectively (p=0.0003). The evidence presented herein regarding the anti-inflammatory and the antioxidant effect of the product suggest it could be used as a potent an adjuvant therapy in diseases accompanied by increased oxidative stress and inflammation. Our results demonstrated the promising effect of the product on reducing the oxidative stress and the IL-6, IL-8 and TNF-a levels, and duration of the symptoms in ambulatory COVID-19 patients. The evidence presented herein regarding the anti-inflammatory and the antioxidant effect of Ge132+Natural™ (combination of Ganoderma lucidum extract, lycopene, sulforaphane, royal jelly and resveratrol) suggest it could be used as a potent an adjuvant therapy in diseases accompanied by increased oxidative stress and inflammation.

Correlations between serum vitamin D levels and oxidative stress markers at hospital admission in COVID-19 patients

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Background: Several studies point to the anti-inflammatory, antithrombotic and antioxidant effects of vitamin D, which could have a beneficial impact in the SARS-Cov-2 infection treatment. Aims: This study was performed in order to evaluate the vitamin D status in relation to oxidative stress parameters and disease outcome in hospitalized COVID-19 patients. Methods: Thirty-three hospitalized patients with COVID-19 were included in this study. Serum vitamin D levels, as well as selected laboratory parameters (blood cell count, neutrophil to lymphocyte ratio, C reactive protein, creatine kinase, lactate dehydrogenase and D-dimer) were analyzed at admission. Oxidative stress markers (PAT (total antioxidant power, iron reducing) and d-ROMs (plasma peroxides)) were measured on analytical photometric system. Results: Twenty-four patients had vitamin D levels below 30 ng/mL, whereas the remaining 9 had vitamin D levels above 30 ng/mL. Vitamin D deficient patients had increased oxidative stress, as determined by significantly higher levels of d-ROMs (414.9 ± 15.82 U.Carr vs. 352.4 ± 18.77 U.Carr) and Oxidative Stress Index-OSI (92.25 ± 6.60 vs. 51.89 ± 6.45), but without a difference in the antioxidant capacity between the two study groups (PAT=2665 ± 133.5 U.Carr vs. 2868 ± 160.9 U.Carr). This was accompanied by significantly higher LDH (604.8 ± 76.98 IU/mL vs. 261.57+47.33 IU/mL) and the D-dimer (5978 ± 2028 ng/mL vs. 977.7 ± 172 ng/mL). A significant inverse correlation was detected between vitamin D and d-ROM, OSI and LDH, whereas the correlation between vitamin D and D-dimers was found to be not statistically significant. Regarding the outcome, the group of patients with lower vitamin D levels had a higher mortality rate (37.5%) compared to 22.2% mortality rate in the group with vitamin D >30 ng/mL. Conclusions: Our findings suggest a potential benefit from vitamin D supplementation in the supportive treatment

[YIA] Cytoprotection effect of natural vitamin D in human liver cell lipotoxicity: a transcriptomics approach

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The aim of this study is to assess the cytoprotection function and the corresponding transcriptomics fingerprint of vitamin D (VD; 10 nM) in human hepatocytes (HepaRG) exposed to steatosis and lipotoxicity by means of oleic and palmitic acids (OA-PA) supplementation. A natural source of vitamin D (a Shiitake Mushroom extract) was compared with a synthetic form (DIBASE) and the active metabolite (1,25(OH)2D) of the vitamin in protecting and the presence of lipotoxicity was assessed by the cellular production of the reactive oxygen species (ROS) and induction of specific transcriptomic changes. Cell viability data demonstrated that the in vitro model of steatosis produced conditions of sub-maximal lipotoxicity and cellular damage. Transcriptional modifications indicated the modulation of genes associated with long-chain fatty acid b-oxidation, ROS production, cholesterol synthesis, AMPK activity, and hepatocyte apoptosis, liver fibrosis and damage. the different VD formulations showed similar efficacy in improving both the levels of steatosis and ROS. However, formulation-specific modifications of the cellular transcriptome were observed. Transcriptional fingerprints and biological functions identified by “Ingenuity Pathway Analysis” (IPA) showed higher similarities when the mushroom extract was compared with 1,25(OH)2-D metabolite than DIBASE. In conclusion, VD showed efficient protection against lipotoxicity in HepaRG cells. Comparable cytoprotection activity was observed for the natural and synthetic formulations, but transcriptomics data highlighted some specificities in the mechanism of action.

[YIA] Interaction between Glutathione S-transferase P and Nrf2 transcription factor in mouse hepatocarcinoma: a molecular and physical characterization

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Non-Alcoholic Fatty Liver Disease (NAFLD) is a common chronic liver disease (CLD) highly prevalent in obese patients. This is now becoming the first cause of liver transplantation, and its evolution to steatohepatitis increases the risk of cirrhosis and hepatocellular carcinoma (HCC). The latter is the second cause of cancer deaths worldwide, and the first one in CLD patients. Drug-resistance (DR) limits the efficacy of chemotherapy in HCC. The oncogene Glutathione S-transferase P (GSTP) is involved in DR. Its expression increases in association with cancerogenic transformation of the liver tissue. In human HCC cell lines, we recently observed a possible functional interaction between GSTP and Nrf2, a transcription factor involved in the stress response to cellular electrophiles and in the carcinogenesis process. Such interaction was investigated in the present study using the mouse model of N-nitrosodiethylamine (DEN)-induced HCC.

In this model, the liver expression of GSTP and Nrf2 increased during tumor development in association with progressive desensitization to apoptotic stimuli and DR gene induction. Co-immunoprecipitation experiments showed a physical interaction of these proteins, that in HCC specifically involves the dimeric form of GSTP. Also, a nuclear translocation of GSTP was observed during tumor development in association with β−TrCP protease expression, suggesting a dynamic distribution of GSTP during the feedback response to Nrf2 nuclear translocation. In conclusion, a physical (protein-protein) interaction of the chaperone GSTP with the transcription factors Nrf2 is confirmed in the DEN model of HCC. Its role in carcinogenesis and DR is worth investigating.

[YIA] Melatonin modulates Nrf2/NFkB activity to prevent cadmium-induced H2O2 production and reductive stress in porcine pre-pubertal Sertoli cells.

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Melatonin (MLT) is a cytoprotection agent holding potential to prevent cadmium (Cd) toxicity, a pro-oxidant heavy metal reported to interfere with testicular function and fertility. However, its efficacy in Sertoli cells remains unexplored. Porcine Sertoli cells (SCs) were used in the present study to explore this cytoprotection function of MLT during Cd toxicity; investigated parameters included cellular levels of H2O2, redox-sensitive transcription factors such as Nrf2, c-Jun and NFkB, and downstream detoxification and antioxidant effectors, such as the H2O2-scavenging enzyme catalase (CAT) and some inducible components of the glutathione stress response system. Cd toxicity in SCs resulted in impaired viability and function, that was associated with increased H2O2 generation and induction of reductive stress by the upregulation of Nrf2 expression and activity, cystine uptake, cellular glutathione biosynthesis and efflux, and GSTP expression, whereas cellular protein glutathionylation decreased. MLT produced a potent cytoprotection effect in Cd-exposed SCs, with significant restoration of cell viability and function, and inhibition of H2O2 generation and efflux. Mechanistically, these effects of MLT were associated with increased CAT activity and NFkB phosphorylation, and with marked reduction of Nrf2 activation and GSTP expression to confirm a restoration effect on the cellular redox; at the higher dose of Cd investigated in this study, MLT significantly induced c-Jun, xCT protein expression, and GSH biosynthesis and efflux. In conclusion, MLT is a cytoprotective in SCs exposed to Cd toxicity with efficient activity in modulating Nrf2 and other genes important to control the cellular flux of H2O2 and the reductive stress response.

Modulation of the NLRP3 inflammasome and pyroptosis by the vitamin E derivate trans-13′-carboxy-δ-tocotrienol in murine macrophages

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Introduction: Inflammatory diseases are a global burden of our society. It is well accepted, that the multiprotein complex NLRP3, composed of NLRP3, ASC and caspase-1 subunits, is an important modulator of the cellular inflammatory response and therefore a desirable target in treating inflammatory diseases. The NLRP3 complex is activated by a two-hit process using e.g. lipopolysaccharide (LPS; RNA level, priming) and adenosine triphosphate (ATP; protein level, activation). Natural antioxidants have the potential to affect this system; a derivative of the antioxidant vitamin E, trans-13′-carboxy-δ-tocotrienol, is the focus of our research. Method and Results: We confirmed that the cell line RAW264.7 does not express the ASC subunit – an essential compo-nent for NLRP3 inflammasome complex assembling and following activation. In contrast, the J774A.1 cell line expresses the ASC complex and was therefore used for further investigations. We found that this vitamin E derivate decreases the RNA expression of components of the NLRP3 inflammasome pathway, more precisely NLRP3, interleukin-1β and gasdermin D (GSDMD) in LPS-activated cells. Additionally, it inhibits the protein expression of the marker protein of NLRP3 inflammasome after acti-vation with LPS and ATP - the activated fragment of caspase-1 (p20) in cells. However, the marker protein of inflammasome-dependent pyroptosis is GSDMD. Its N-terminal cleavage product was also significantly downregulated by our vitamin E derivate. Further investigations into caspase-1 activity and LDH release are ongoing. Conclusion: Taken together, our results indicate that the natural vitamin E derivate trans-13′-carboxy-δ-tocotrienol may have positive effects on the inflammatory

response by preventing inflammasome activation and inflammasome-induced pyroptosis. Further studies are required to better understand the molecular modes of action and the physiological relevance of our findings.

[YIA] The influence of skin barrier disruption and melanin content on the formation of DNA lesions and radicals in ex vivo human skin induced by 233 nm far-UVC irradiation from LEDs

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As reported recently, 233 nm radiation emitted by a spectrally pure UVC LED source shows sufficient bactericidal properties at an applied dose between 20 and 80 mJ/cm2. In ex vivo human skin and skin models, the formation of epidermal DNA lesions at bactericidal doses was minor compared to one tenth of the minimal erythema dose of UVB light. This can be attributed to the strong absorption for wavelengths below 240 nm in the upper non-nucleated dermal cell layers. Furthermore, the radical formation was far lower than for a dose equivalent to a stay of 20 min outdoors, which can be compensated by the antioxidant defense system. To assess the influence of skin barrier disruption on radiation-related skin damage, we detached the stratum corneum from the viable epidermis in ex vivo human skin mechanically. After irradiation of the skin with a wavelength of 233 nm, we screened the tissue for the formation of CPD and 6,4-PP positive cells via immunohistochemistry. An increased formation of DNA lesions was determined in barrier-disrupted skin as compared to intact skin after irradiation. However, this effect was absent if artificial wound exudate was applied on the disrupted skin surface before irradiation. Interestingly, intact skin and barrier-disrupted skin showed no significant differences in radical formation as quantified by EPR spectroscopy. We further compared the formation of DNA damage in different skin types using ex vivo human skin. After irradiation with a wavelength of 233 nm, less epidermal DNA lesions were found in dark skin types than in fair skin types. In contrast, irradiation with a wavelength of 222 nm induced no skin type-dependent differences due to the lower penetration depth of photons. In conclusion, 233nm LED irradiation at the studied dose could be suitable for skin antisepsis and indoor air decontamination in the presence of humans.

Mechanisms of NLRP3 inflammasome activation in macrophages by air pollution fine particulate matter (PM2.5)

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Particulate matter (PM2.5) exposure aggravates cardiorespiratory diseases by inflammatory cytokine secretion from alveolar macrophages (AMs). Inhalation of silica particles and asbestos triggers NLRP3 inflammasome activation and IL-1β release. However, the mechanisms involved in NLRP3 engagement by PM2.5 remain unclear. To study this process, THP1-ASC-GFP cells were incubated for 6 or 24 h with 0, 1, 10, or 100 µg/mL of PM2.5 surrogates of variable chemical composition, including ROFA, CAPs, SRM1648a, and SRM2975. NLRP3 priming and specks formation was detected by flow cytometry after incubation with ROFA and SRM1648a, which was confirmed by imaging ROFA-exposed bone marrow derived macrophages (BMDMs) from C57BL/6 ASC-Citrine transgenic mice. No LDH release following PM2.5 stimulation indicated conserved cell viability in our experimental conditions. Increased IL-1β was detected by ELISA in cell culture supernatants of ROFA-exposed BMDMs and AMs from wild type mice, but not from Nlrp3-/- or Casp1-/- mice, or after pre-incubation with the NLRP3-specific inhibitor MCC950. Upregulation of Tnf gene expression and increased TNF-α levels in cell culture supernatants were also observed. Pre-incubation with anti-TNF-α

antibody resulted in decreased IL-1β release from ROFA-exposed AMs. Moreover, increased mitochondrial O2●- production by MitoSOX fluorescence was found in ROFA-exposed BMDMs, together with decreased maximal respiration by the Seahorse MitoStress Test. Accordingly, inhibition of mitochondrial complex I site responsible for O2●- production by S1QEL resulted in decreased IL-1β levels in ROFA-exposed BMDMs. K+ efflux contribution on NLRP3 activation was evident in ROFA-exposed BMDMs incubated with increasing concentrations of KCl. Lysosomal leakage in BMDMs was also observed after ROFA exposure. In conclusion, PM2.5 induces NLRP3 inflammasome priming and activation in macrophages, leading to inflammatory mediator release. TNF-α, mitochondrial O2●- production, K+ efflux, and lysosomal disruption were identified as potential drivers of inflammasome engagement after PM2.5 exposure. These findings unravel the mechanisms by which PM2.5 promotes cardiorespiratory inflammation and disease.

[YIA] The protective effect of carotenoids, polyphenols and sex hormones on skin cells under oxidative stress

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Skin aging is influenced by several factors including environmental exposure and hormonal changes. Reactive oxygen species (ROS), which mediate many of the effects of these factors, can be formed by extrinsic factors, such as sun exposure, or can result from mitochondrial dysfunction which occurs during ageing. ROS activate the nuclear factor-kappa B (NFκB) transcription systems leading to inflammatory processes and increased production of matrix metalloproteinase (MMPs) by skin cells, which leads to collagen degradation. Several studies have shown the protective role of estrogens and of various phytonutrients including carotenoids and polyphenols on skin health. The aim of the current study was to examine the damage caused by ROS that originate in the mitochondria due to its dysfunction, and to examine the protective role of tomato extract containing lycopene, rosemary extract and estradiol. Human dermal fibroblasts and keratinocyte were used to determine ROS levels and their effect on cell viability, MMP1 and pro-collagen secretion as markers of skin damage. Rotenone was used to cause mitochondrial dysfunction and reduction in oxygen consumption, which causes accumulation of mitochondrial and cytosolic ROS, apoptotic cell death by activating caspase 3 activity, upregulation of MMP1 secretion and decreased collagen secretion. This was accompanied by activation of the antioxidant response element/Nrf2 (ARE/Nrf2) and NFκB transcriptional activity. Pretreatment with dietary compounds such as tomato extract and rosemary extract and estradiol reduced ROS level and MMP1 secretion and increased cell viability. These effects can be partially explained by the increased synergistic activity of ARE/Nrf2, and the decreased activity of NFκB transcriptional activities. This study indicates that phytonutrients and sex hormones protect skin cells from damage caused by mitochondria generated ROS and may delay skin aging and improve skin health and appearance.

[YIA] Interference with mitochondrial activity drives the on-set of cardiovascular disease following long-term treatment with SGAs

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The chronic intake of second generation antipsychotics (SGAs) has been related to increased risk of cardiovascular diseases. Taking into account the importance of redox balance in vascular function, we analysed the effect of Ari and Ola in the bioenergetics of primary bovine aortic endothelial vascular cells (BAEC). The results indicate that both Ola and Ari can interfere with mitochondrial function after 3h treatment, increasing the mitochondrial production of O2-. After 24h, we observed a higher recovery capacity in Ola than of Ari treated cells, suggesting a higher mitochondrial toxicity or a blunted capacity to induce compensatory systems in Ari treated cells. The effects of these drugs on mitochondrial respiration were also measured in in peripheral blood mononuclear cells of healthy volunteers treated with Ari or Ola. We found a stronger effect on respiration, ROS production and deficient ATP-linked respiration with Ari treatment than for Ola, consistent with previous results. Next, we analysed whether this SGAs can accumulate in mitochondria. We found that both Ari and Ola accumulated in the mitochondrial membranes, with Ola showing a trend for higher levels but also faster turnover. To evaluate the physiological effects of this inhibition we treated a mouse model of mitochondrial dysfunction (PGC-1-/-) with Ari and Ola. At 5 days treatment, the data seem to indicate a better compensatory response

to Ola's treatment than Ari's in the heart. After 6 months, we observed a reduction in O2 consumption, cardiac fibrosis, left ventricular remodeling and exacerbated cardiac I/R Injury, with all parameters being more evident in Ari than in Ola treated mice. Remarkably, Ola treated mice showed increased mitochondrial content, suggesting that Ola allows a partial compensation by increasing mitochondrial mass. These results suggested that both Ari and Ola interfered with mitochondrial function, and thus lead to increased risk of cardiovascular disease.

Diamond magnetometry to understand male reproductive decay during aging

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Delaying fatherhood is a global trend. Aging affects the male germline decreasing the reproductive fitness and increasing the frequency of congenital anomalies in the progeny. Oxidative stress (OS) may play a leading role in the mechanisms behind age-related male reproductive problems. Understanding how OS affects the precursor cells of male gametes is critical to connect aging with inheritable genomic alterations that may affect the next generation. Nevertheless, most available methods to measure OS show several drawbacks that hinder the completion of such an ambitious goal. Diamond magnetometry (DM) is a promising technique that allows the measurement of free radical levels in real-time with subcellular resolution. DM uses fluorescent nanodiamonds (FNDs) with NV centers to translate the magnetic noise generated by free radicals into easily detectable fluorescent signals. Performing DM requires cell immobilization on a suitable coating and the optimization of FNDs concentration and incubation times to achieve the cellular internalization of the particles. Using a mouse model of aging we studied representative stages of spermatogenesis. We tested different coating conditions (0.4% gelatin, 0.01% poly-L-lysine, and 1 mg/ml hyaluronic acid) and concentrations of FNDs (2, 4, and 8 µg/ml) at three incubation times (2h, 4h, and 6h). Finally, we performed diamond magnetometry for the first time in male germ cells. Our results show that 0.4% gelatin, 4 µg/ml FNDs, and 6 hours of incubation are the optimal conditions to achieve more than 80% of attachment and at least 50% of cells with 3-5 internalized FNDs for all populations. Free radicals measurements allowed us to differentiate between germ cells from aged and control animals and to compare between different populations from the same group. In conclusion, we optimized the utilization of a novel tool to study OS in the male germline during aging, opening a myriad of potential applications in Reproductive Medicine.

Effects of early life protein restriction on mitochondrial biogenesis and antioxidant defence capacity in skeletal muscle in mice

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There is a strong relation between early life environment and age-related diseases in later life, including sarcopenia. Skeletal muscle development is especially prone to nutritional deficiency. With age, skeletal muscle has higher generation of reactive oxygen species that can cause oxidative stress and can potentially cause muscle atrophy. Also, mitochondrial dysfunction is among the most frequently reported mechanisms of aging muscle atrophy. Hence, we aimed to investigate the association between suboptimal early life nutrition and oxidative stress and mitochondrial turnover in skeletal muscle. Female mice were fed either a normal (20%) or a low-protein (8%) diet prior to mating and during pregnancy. New-born pups were cross-fostered to different lactating dams (maintained on a 20% or 8% diet) within 24h after birth. At 21 days, they moved on to either a 20% or 8% protein diet, creating 3 groups: control (NNN), Normal-Low-Normal (NLN), and Normal-Low-Low (NLL). Mice were maintained for up to 3 months. Antioxidant defence capacity and mitochondrial biogenesis-related targets were measured by Western blotting and qPCR. Citrate synthase activity also measured. NLL mice were smaller than control mice. PGC-1α content ($P < 0.0001$) and mRNA expression ($P=0.0074$) and NRF1 mRNA expression ($P=0.0030$) were significantly decreased in NLL and NLN mice compared to controls, suggesting decreased mitochondrial biogenesis. Muscles from these mice also had reduced SOD2 content ($P=0.0002$) and mRNA expression ($P=0.0079$) and Prx3 content ($P=0.0080$) and mRNA expression ($P=0.0031$). Cat mRNA expression was only reduced in NLL mice ($P=0.0007$). These mice also had a significantly decreased ($P=0.0065$) citrate synthase activity suggesting a reduction in mitochondrial number. These observations suggest that early life protein restriction can affect mitochondrial biogenesis and antioxidant defence capacity at a young age which may explain the impact of maternal diet on skeletal muscle aging.

[YIA] Mitochondrial ROS modulate neuronal calcium signalling through redox-regulation of glutamate receptor trafficking

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Calcium influx through glutamate receptors upon neuronal activity plays an essential role in neurotransmission, but an excessive activation of the pathway leads to calcium overload and neuronal death in a process termed excitotoxity. Together with calcium imbalance, mitochondrial dysfunction and oxidative stress are often involved in the mechanism of neurodegeneration in disorders such as tau-related frontotemporal dementia (FTD). We have employed iPSC-derived neurons of healthy controls and patients of FTD to understand the interaction between these pathways in physiology and pathology. We have found that reactive oxygen species (ROS) produced at the mitochondria (mROS) play an essential signalling role in neurotransmission. mROS, via oxidation of proteins involved in their trafficking, control the surface expression of specific glutamate receptor subunits in neurons, therefore modulating the calcium entry through them. In FTD, tau-induced mitochondrial dysfunction leads to an overproduction of mROS, which in turns upregulates the surface expression of glutamate receptors, leading to excessive calcium influx and promoting excitotoxic neuronal death. Importantly, mitochondrial antioxidants are neuroprotective and prevent the glutamate-induced calcium overload and mPTP opening, caspase-3 activation and neuronal death triggered by mitochondrial calcium overload. These results highlight a) the role of mitochondria in cellular signalling in physiology and pathology, by modulating calcium entry through glutamate receptors via mitochondrial ROS; b) the reversible redox regulation of glutamatergic signalling, which might serve as a target for indirect pharmacological strategies to modulate calcium entry through glutamate receptors in neurodegenerative disorders.

A mechanistic dissection of HyPer7, an ultrasensitive pH-independent biosensor

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HyPer7 is a fluorescent protein-based biosensor for hydrogen peroxide that has distinguished itself from its predecessors in the HyPer family by displaying nanomolar sensitivity, ratiometric pH-independence and increased brightness. HyPer7 has already contributed to the elucidation of H2O2 signalling in a wide variety of organisms. Nevertheless, many mechanistic details about the functioning of the biosensor remain unexplored. These include the kinetics of its reaction with H2O2, as well as of its reduction by the cellular reducing systems such as the thioredoxin and glutaredoxin systems. Further, it is not understood how the conformational coupling between the sensing moiety (OxyR), and the SYG chromophore of cpYFP occurs. Finally, the basis of the pH-independence compared to other HyPer biosensors remains unclear. Using a combination of artificial intelligence predictions and various biophysical and biochemical techniques here we address these questions and provide a mechanistic dissection of HyPer7. Our results will on the one hand contribute to a more informed interpretation and planning of experiments on HyPer7 and on the other hand serve as a basis for the development of further biosensors.

Potential of L-ergothioneine in the treatment of mitochondrial toxicity

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There is increasing evidence that mitochondrial dysfunction is associated with many diseases, including Alzheimer's Disease and vascular disorders, as well as toxicities of common drugs such as fluoroquinolones. Unlike the mixed results from trials involving the use of non-specific antioxidants in alleviating mitochondrial toxicity, mitochondrial-targeted antioxidant therapies such as MitoQ have shown some promise. L-ergothioneine (ET) is a naturally occurring thiol-thione that is found in humans wholly via dietary absorption using a highly specific and almost ubiquitously expressed transporter, OCTN1. ET accumulates at high concentrations in humans with a slow excretion rate. While numerous studies have evaluated its antioxidant abilities in vitro and in vivo, its physiological function remains unknown. Preliminary

evidence suggests that L-ergothioneine may be accumulated within the mitochondria, although this has yet to be conclusively established. In this study, we verified the intracellular localisation of L-ergothioneine within the mitochondria in both in vitro cell and in vivo mouse models using LC-MS, identifying a dose-dependent increase in L-ergothioneine levels within the mitochondria. Subsequently, we explored the relationship between L-ergothioneine and complexes of the electron transport chain using isolated mitochondrial activity assays, identifying L-ergothioneine's potential role in preserving mitochondrial complex I function. As a compound demonstrating antioxidant properties with likely mitochondrial localisation, L-ergothioneine presents as a potential mitochondrial-targeted antioxidant therapy without need for any further structural modifications. In addition, its excellent safety profile combined with slow excretion further illustrates L-ergothioneine's potential as a pharmacological intervention for mitochondrial toxicity. Our present findings suggest a role for L-ergothioneine within the mitochondria, although more work is necessary to elucidate specific therapeutic areas where its pharmacological potential can be explored in relation to mitochondrial toxicity.

Role of oxidative stress in the cytotoxic action of extracts of vegetables and medicinal herbs on human ovary cancer cells

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Natural products often contain compounds, which may be cytotoxic for cancer cells. The aim of this study was to compare the cytotoxic action of 1:10 (w/v) phosphate-buffered saline extracts of several vegetables, teas and medicinal plants on PEO1 and SKOV3 cancer ovary cells and to examine whether oxidative stress contributes to their effects. We found that the extracts of green tea, garlic, and black tea were the most cytotoxic to PEO1 cells (IC50 of 1.66, 2.01 and 3.25 vol%, respectively), while extracts of green tea, horseradish and black tea were the most cytotoxic to SKOV3 cells (IC50 of 1.66, 4.34 and 10.56 vol% when estimated with Neutral Red). The presence of catalase (10 μg/ml) in the cell medium partly protected against the cytotoxicity of the extracts of teas, garlic, curly kale, Cistus incanus, Gingko biloba, and Betula pendula, evidencing the contribution of hydrogen peroxide formed by the extracts to their cytotoxic effects. Tea and horseradish but not garlic extracts increased the intracellular ROS levels detected with d5hydroethidine. These results indicate that oxidative stress, due mainly to the generation of hydrogen peroxide, participated in the cytotoxic effects of the extracts but its contribution depended on the kind of the extract.

Peri/epicellular (pec) protein disulphide isomerase A1 (PDI) regulates platelet-endothelium interaction in cells exposed to high glucose levels

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Background: Diabetes leads to endothelial dysfunction and thrombus formation in a context of oxidative stress. Our group has shown that peri/epicellular (pec) protein disulphide isomerase A1 (pecPDI), a thiol isomerase protein, influences vascular cell adhesion and that platelet PDI is positively correlated with glycaemia. Therefore, we investigated the impact of pecPDI in platelet-endothelium interactions upon the exposure to high glucose levels. Methods: Human umbilical vein endothelial cells (HUVECs) were cultured in a normo- (5.5 mM) or high-glucose (25 mM) medium for 48 hours in the presence or absence of PDI inhibitors while platelets were isolated from healthy donors. Hydrogen peroxide was measured through amplex red and cell adhesion was assessed through immunofluorescence. Results: Platelets adhered more onto HUVECs exposed to high glucose levels, which also presented thicker actin fibres when compared to those of normo-glucose HUVECs. High-glucose HUVECs produced more hydrogen peroxide and were more adhesive onto a collagen matrix. High-glucose cells also showed an augmented fractal dimension, suggestive of increased spreading. Interestingly, there was an increased co-localization between PDIA1 and collagen receptor integrin beta 1 in high-glucose cells when compared to normal HUVECs. Rac1 and RhoA, which are a key GTPases downstream of integrin activation, were markedly translocated to the cell membrane when HUVECs were exposed to 25 mM glucose. PecPDI inhibitors impaired all of the abovementioned processes. Conclusion: Platelets adhere more onto HUVECs exposed to high glucose levels, in a process largely mediated by pecPDI. Mechanistically, pecPDI was shown to regulate oxidant production, cell adhesion, cytoskeleton organization and activation of RhoGTPases. We propose that pecPDI is a master regulator of platelet-endothelium interactions in high-glucose conditions, thus fostering the development of improved targets to treat cardiovascular diseases of diabetic individuals.

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Defense mechanisms neutralizing circulating oxidized phospholipids

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Animal and clinical association studies have shown that phospholipids containing oxidized PUFA residues (oxidized phospholipids, OxPLs) play an important causative role in different pathologies. The data justify the need for deciphering protective mechanisms that destroy pathogenic OxPLs or prevent their interaction with cellular targets. We have developed an immune assay for quantification of total OxPL-inactivating activity of blood plasma. The method, which we call a “masking assay”, is based on two monoclonal antibodies (mAbs) that detect OxPLs that are present in OxLDL particles. We found that preincubation of OxLDL with diluted human plasma significantly inhibited binding of anti-OxPL mAbs, thus suggesting that OxPLs were either cleaved or physically masked by plasma components. In support of this possibility, treatment with serum reduced pro-inflammatory effects of OxPLs on endothelial cells. A pilot clinical study demonstrated reduced anti-OxPL masking activity in patients with coronary artery disease, hypertension and diabetes. We hypothesize that inadequate protection against OxPLs may contribute to the pathogenesis of cardiovascular disease. Furthermore, quantification of protective activity potentially may become a biomarker for risk stratification. We further started characterization of individual plasma proteins and enzymes responsible for the masking effect. Using a PAF-acetylhydrolase inhibitor darapladib, as well as a recombinant PAF-acetylhydrolase, we found that this enzyme, which is known to cleave OxPLs, constitutes only a small part of masking activity present in plasma. We further established a pull-down assay using liposomes containing oxidized palmitoyl-arachidonoyl-phosphatidylcholine (OxPAPC) or non-oxidized PAPC. Bound proteins were identified by mass spectrometry. Several plasma proteins that selectively bound OxPAPC as compared to PAPC were SAA1, SHBG, SFTPB, and CETP. Two purified proteins (SHBG and SFTPB) demonstrated masking activity comparable with that of physiological levels of PAF-AH. The data suggest that the masking effect can be explained by simultaneous action of multiple plasma proteins that cleave or bind OxPLs.

Role of the single cysteine residue of vimentin in interplay with actin under electrophilic stress

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Vimentin is a type III intermediate filament protein with multiple functions in the maintenance of cell homeostasis, cytoskeletal crosstalk and signaling, but also in the extracellular milieu. We have previously shown that vimentin single cysteine residue, C328, is the target of diverse posttranslational modifications that impact the organization of the vimentin network in a structure-dependent manner, thus acting as a regulatory element of vimentin assembly (1,2). Here we have explored the downstream cellular consequences of C328 modifications by subjecting cells expressing vimentin wild type (wt) or various C328 mutants to electrophilic stress. We have observed that treatment with the electrophiles 4-hydroxynonenal (HNE) or dinitro-imidazole (DNI) elicits a marked reorganization of vimentin wt, with disruption of filamentous structures. In parallel, both electrophiles potently induce actin remodeling with the formation of robust stress fibers. Remarkably, expression of a C328H vimentin mutant, which can assemble into filaments but is resistant to electrophiles, blunts actin remodeling. Both, full length and GFP-fusion constructs of vimentin C328H prevent electrophile-induced stress fiber formation. In contrast, two GFP-fusion constructs which cannot form filamentous structures, namely, GFP-vimentin C328D and GFP-vimentin C328S, do not preclude the formation of stress fibers in response to DNI. Our results indicate that disruption of vimentin filamentous structures through modification of C328 may play a permissive role in actin remodeling in response to certain electrophiles. Therefore, vimentin could play a key role as a sensor and effector of electrophilic stress through the modulation of the actin cytoskeleton. (1) Mónico et al., 2019, Redox Biol. 23:101098. Doi: 0.1016/j.redox.2019.101098 (2) González-Jiménez et al., 2021, Free Rad Biol Med 165, Suppl. 1: 26. Doi: 10.1016/j.freeradbiomed.2020.12.319 Funding: Micinn/ERDF RTI2018-097624-B-I00 and PRE2019-088194; RETIC Aradyal ISCIII/EDRF RD16/0006/0021

The Effects of Proportional Oxidative Stress Parameters on the In-hospital Prognosis of ST-Segment Elevation Myocardial Infarction Patients

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Background: Oxidative stress is closely associated with atherosclerosis and acute coronary syndromes. Aims: In this study, we aimed to evaluate proportional oxidative stress parameters in ST segment elevation myocardial infarction (STEMI) patients and also to investigate their effects on in-hospital prognosis. Methods: This is a single centre, prospective, and cross-sectional case-control study. Total oxidative status (TOS), total antioxidant status (TAS), oxidative stress index (OSI), ischemia modified albumin (IMA), myeloperoxidase (MPO), paraoxonase-1 (PON-1) and arylesterase (ARES) enzyme activities as well as MPO/PON-1, MPO/ARES and MPO/HDL ratios were studied in 107 patients. Short-term in-hospital prognosis markers used in this study were; in-hospital mortality, early systolic dysfunction and spontaneous complete revascularization which is Thrombolysis in Myocardial Infarction (TIMI) grade 3 flow. Results: Both patient (n=63) and control (n=44) groups were similar in terms of age and gender. TOS, OSI, IMA and MPO values, as well as, MPO/PON-1 and MPO/HDL ratios were significantly higher and PON-1 and ARES values were significantly lower in STEMI patients compared to the control group. In terms of in-hospital short-term prognostic markers, a significant relationship was found only between OSI value and spontaneous complete revascularization status. The OSI value was higher in the group with TIMI grade 3 flow than in the group with TIMI grade 0-2 flow (2.42 [0.81-4.49] vs 1.63 [0.33-6.07], p = 0.016). Conclusions: As a result of this study, MPO/PON-1 and MPO/HDL ratios are considered as new oxidative stress parameters that are found to be elevated in STEMI patients. Among in-hospital short term prognostic markers, only an association between spontaneous complete revascularization and OSI values was found.

Lon protease knockdown induces mitochondrial DNA damage

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Well known for its important involvement in oxidized protein elimination within the mitochondrial matrix, Lon protease also intervenes with the mitochondrial DNA. In addition to a maintenance action on this genome, Lon contributes to the

regulation of its replication and transcription. Therefore, a Lon depletion can be expected to induce effects on the mitochondrial DNA and, from there, on the mitochondrial function. This is why we decided to deepen the missions provided by Lon by looking for possible damage to mitochondrial DNA associated with its underexpression. We have therefore investigated whether or not the integrity of mitochondrial DNA is preserved in a HeLa cell line stably transfected with an inducible shRNA directed against Lon.
To this end, we carried out qPCR experiments based on the principle that DNA lesions (abasic sites, strand breaks, thymine nucleic acids oxidation,…) can slow down or even block the progression of DNA polymerase. Therefore, the amplification of a damaged sequence will be less efficient than that of an undamaged sequence. To localize the sites of possible lesions, the mitochondrial DNA was divided into nine consecutive sequences and nine pairs of primers were used to amplify these sequences separately.
According to our results, Lon deficiency results in less amplification of the mitochondrial genome with differences between the nine sequences. Thus, Lon depletion results in mitochondrial DNA alterations. What is the nature of these damages ? Due to the oxidative stress (ROS and protein carbonylation) observed in our previous works on the effects of a Lon knockdown, we speculated that nucleotide oxidation could be one of these mtDNA damages. Regarding the guanine tendency to oxidation and the fact that Lon protease binds mt DNA on guanine-rich sequences, we are now working on the evaluation of 8-hydroxy-2'-deoxyguanosine levels with and without Lon.

Oxidative modification of histones in "NETs" – a new driver of vascular dysfunction?
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The release of neutrophil extracellular traps (NETs) is a key innate immune defense to combat infection. NETs consist of a mesh of DNA and histones, and contain other neutrophil proteins, including myeloperoxidase (MPO). Although NETs have important anti-bactericidal properties, they are also implicated in the development of numerous inflammatory diseases, particularly atherosclerosis. However, the mechanisms involved in the pathological effects of NETs are not well understood. Enzymatically active MPO is present on the NET backbone and produces the potent oxidant hypochlorous acid (HOCl). In this study, we examine how the modification of NET-associated histones by HOCl influences vascular smooth muscle cell function. Experiments were performed with a preparation of histones containing both linker (H1) and core (H2A, H2B, H3 and H4) histones incubated in the absence or presence of HOCl for 24 h, to allow decomposition of unstable N-chloramines. Exposure of primary human coronary artery smooth muscle cells to native and HOCl-modified histones resulted in a dose-dependent loss of viability, consistent with the known toxicity of extracellular histones. Interestingly, less cell death was apparent on pre-treatment of the histones with HOCl compared to the non-modified histones. In addition, the change in cell viability observed with the HOCl-modified histones was dependent on the extent of oxidative modification. The HOCl-modified histones altered the redox balance in the cells, as reflected by a decrease in reduced thiol concentration, to a greater extent than that seen with the native histones. Similarly, only the HOCl-modified histones increased the expression of different pro-inflammatory mediators, including vascular cell adhesion molecule 1. Together, these results suggest that HOCl-induced modification could perturb the extracellular reactivity of histones to favour activation of inflammatory signaling. This could have implications for the development of diseases linked to aberrant NET release, particularly atherosclerosis.

UCP1 - a lever of the redox-metabolic seesaw in the regulation of lipid-buffering function of white adipose tissue
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Uncoupling protein 1 (UCP1) is a molecular hallmark of thermogenic adipocytes. It can also reside in the unilocular adipocytes of white adipose tissue (WAT) that do not possess almost any oxidation and thermogenesis capacity. Therefore, the significance of UCP1 in WAT is vague. To clarify the physiological role of UCP1 in white adipocytes we aimed to investigate the relation of UCP1 expression with the components of redox-adaptive homeostasis and metabolic function in WAT. Toward this, we investigated the expression pattern of UCP1 with Nrf2 and its downstream targets, glutathione (GSH), and lipid peroxidation levels during extensive lipolysis induced by long-term cold exposure and during reversal, when lipid deposits in adipocytes recover, upon re-acclimation from cold to room temperature (RT). To this end,

stated molecular targets were investigated at different time points during 45 days of cold acclimation and re-acclimation in the rat retroperitoneal WAT (rpWAT) and compared to respective RT and cold-acclimated controls. The results have shown that in response to cold-induced lipid mobilization transient induction of UCP1 precedes Nrf2 expression and upregulation of downstream antioxidant enzymes (such as MnSOD and GST). The reverse sequence of molecular events was observed during the early (1-12. days) and late (12-45. days) periods of re-acclimation from cold to RT. Namely, in the initial days of re-acclimation high lipogenesis and redox threshold (GSH, and expression of CuZnSOD and MnSOD) correspond to lower UCP1 levels. From the moment of restitution of lipid reserves (revealed by rpWAT mass) and on, UCP1, GSH, and most antioxidant enzymes return to their RT control values. The results emphasize that UCP1 and Nrf2 represent levers of redox-metabolic seesaw fine-tuning of redox homeostasis for optimal regulation of lipid mobilization and deposition in white adipocytes. Research is supported by the Science Fund of the Republic of Serbia, PROMIS, #6066747-WARMED.

Role of glutathione in the synergistic induction of differentiation of AML cells by a vitamin D analog and activators of the transcription factor Nrf2

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Acute myeloid leukemia (AML) is an aggressive hematologic malignancy, mainly in older adults, characterized by uncontrolled growth of immature myeloid blasts. Despite initial responses to standard chemotherapy, prognosis remains grim for most patients. Differentiation therapy of AML is an alternative to cytotoxic chemotherapy. Natural and synthetic vitamin D derivatives (VDDs) are powerful inducers of monocytic differentiation of AML cells in culture; however, their differentiation-inducing concentrations can be lethal in vivo due to severe hypercalcemia. We have previously shown that fumaric acid esters (FAEs), such as the clinically approved drug dimethyl fumarate and its in-vivo metabolite monomethyl fumarate (MMF), can synergistically enhance the prodifferentiation effects of near-physiologic concentrations of different VDDs [PMID: 30508646]. Since FAEs are known activators of the transcription factor Nrf2, we hypothesized that Nrf2 may mediate the enhancing effects of these agents on the differentiation of AML cells induced by 19-nor-1,25-(OH)2-vitamin D2 (paricalcitol). Here, we demonstrate that in non-transfected and empty vector-transfected HL60 human AML cells, the differentiation-inducing effect of paricalcitol was markedly potentiated by MMF, monoethyl fumarate (MEF) or the Nrf2-activating phenolic diterpene carnosic acid (CA). This potentiation was associated with a marked upregulation of the vitamin D receptor (VDR) protein levels and mRNA expression of VDR target genes, e.g. CAMP and CYP24A1. However, these enhancing effects of the Nrf2 activators were dramatically reduced in HL60 cells stably expressing a dominant-negative Nrf2 (dnNrf2) mutant that lacks the transactivation domain. Notably, co-treatment of dnNrf2-expressing cells with the glutathione precursor N-acetyl cysteine or cell-permeant glutathione ethyl ester partially restored the synergy between paricalcitol and Nrf2 activators. These data suggest that the differentiation-enhancing effects of FAEs and CA are mediated by the transcriptionally active Nrf2, possibly through the Nrf2-dependent elevation of cellular glutathione levels.

Redox mosaic in breast cancer: At the intersection of cancer and adipose tissue

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Altered redox homeostasis is recognized as a hallmark of neoplastic transformation. However, data from various in vitro and in vivo studies often show increased or decreased transcriptional and translational levels of antioxidant defense (AD) enzymes. One of the underlying causes for such conflicting reports is cell heterogeneity within the complex tumor microenvironment, especially in breast cancer. To overcome barriers associated with bulk tissue gene and protein expression analysis, we choose an immunohistochemical approach. We cross-examined serial tissue sections of tumor and adipose tissue from premenopausal women with malignant invasive ductal carcinoma and benign fibroadenoma to gain a comprehensive overview of cell-specific AD enzymes expression and localization patterns. At the level of overall tissue architecture, malignant tumor tissue shows significantly higher immunopositivity for copper, zinc- and manganese-superoxide dismutase, catalase, and glutathione peroxidase compared to benign tumor tissue. Generally, AD enzymes are

specifically localized in the cytoplasm (copper, zinc superoxide dismutase, catalase, glutathione peroxidase) and mitochondria (manganese superoxide dismutase, glutathione peroxidase) of cancer cells and cancer-associated adipocytes. Detailed analysis of different regions of the tumor tissue revealed significant heterogeneity in the degree of immunopositivity along the axis of tumor center–invasive front–adipose tissue. Clusters of cancer cells at the invasive front of the tumor often show a higher degree of immunopositivity for AD enzymes compared to cancer cells in the center of the tumor mass. Similarly, cancer-associated adipocytes that are in close proximity to cancer cells at the invasive front of the tumor show a higher degree of immunopositivity for AD enzymes compared to adipocytes from distant peritumoral adipose tissue. In conclusion, immunohistochemical approach confirms high AD enzymes expression in breast cancer and further reveals distinct regional mosaicism consistent with cell heterogeneity within the tumor microenvironment. This research was supported by the Science Fund of the Republic of Serbia, #7750238-REFRAME.

Fatty acid amide hydrolase deficiency shows harmful effects on ischemic cardiomyopathy

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Introduction: Ischemic cardiomyopathy leads to inflammation and left ventricular (LV) dysfunction. Animal studies provided evidence for cardioprotective effects of the endocannabinoid system, including cardiomyocyte adaptation, inflammation and remodeling. Since endocannabinoid receptor CB2-deficiency led to increased apoptosis and infarct size with worsened LV-function, we investigated the impact of elevated level of the endocannabinoid anandamide in fatty acid amide hydrolase (FAAH)-/--mice undergoing repetitive I/R. Methods: Repetitive daily 15 min. left anterior descending artery occlusion over 1, 3 and 7d in C57/Bl6 (WT)- and FAAH-/--mice (n≥8). Possible PPAR-α mediated effects of anandamide in FAAH-/--mice were eliminated with selective PPAR-α antagonist GW6471 i.v. LV-function was assessed using M-mode echocardiography. Immunohistochemical analysis revealed collagen deposition, macrophage accumulation and remodeling. Hypertrophy was determined by cardiomyocyte area and heart weight/tibia length. Molecular analyses involved Taqman® RT-qPCR and ELISA. Results: FAAH-/--mice showed cardiomyocyte loss after 7dI/R, accompanied by scar formation with persistent LV-dysfunction 60d after discontinuation of I/R, while WT-mice recovered after 60d. Collagen deposition was reduced to WT-levels when FAAH-/--mice were treated with GW6471. CCL2-expression was significantly higher in FAAH-/--mice, followed by higher macrophage infiltration in infarcted areas, which was reversed by GW6471. Further, abolished HMOX-1 induction in FAAH-/--mice, as well as enhanced hypertrophy and adverse remodeling, were normalized by PPAR-α antagonism. Finally, FAAH-/- -mice showed stronger downregulation of PPAR-α when compared to WT, suggesting a compensatory mechanism as endocannabinoids are also ligands for PPAR-α, and its activation causes lipotoxicity leading to cardiomyocyte apoptosis. Discussion: Our study gives novel insights into the role of endocannabinoids acting via PPAR-α. We hypothesize that increase in endocannabinoids may have partially detrimental effects on cardiomyocyte survival due to PPAR-α activation.

In vivo evaluation of the healing effect of silkworm Bombyx mori products on second degree burns: assays of different doses and combinations

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The silkworm and its main product, the cocoon, contain proteins, such as sericin and fibroin, along with enzymes, such as serrapeptase, which can help reduce inflammation and promote skin healing. Previous relative studies have indicated a possible positive in vivo effect of the silkworm products on the healing of 2nd degree burns. The first aim of this study was to examine the action of three different silkworm products (silkworm body, glands, and cocoon) in higher doses than those having already been used. Hairless, female mice, type SKH-hr2, brown and black were used, to which 2nd degree burns were induced. The animals were separated into 9 groups with two control groups. For the treatment groups, gels with silkworm body extract and silkworm gland extract were used, while silkworm cocoons were applied as patches. Histopathological and clinical evaluations were performed, along with measurements of biophysical parameters,

thickness, and surface of the burn area. The cocoon and the higher dose of silkworm body showed the best results, regarding the clinical observations and the histopathological findings. Based on these results a second study was conducted to examine the synergistic action of the cocoon and the higher dose of the silkworm body extract, compared with the effect of each treatment separately. The same type of materials was used. The animals were separated into 5 groups with two control groups. For the treatment groups, gel with silkworm body extract was used while silkworm cocoons were applied as patches, either along with the silkworm body-extract gel or without it. After the evaluation of skin parameters, a slightly better action of the combination group was observed according to the histopathological results. The incorporation of drug substances into the cocoon is proposed as a next step to examine the effect of such product on the healing of burn injuries.

[YIA] S-nitrosation governs the appearance and disposal of cytoplasmic DNA in endothelial cells

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In mammals, DNA is generally confined to the cell nucleus and mitochondria. Its presence in the cytosol is abnormal and its efficient detection is fundamental to control cellular homeostasis. One of pivotal post-translational modifications governing cellular fate is S-nitrosation, which protects against oxidative stress endothelial cells, however predisposes them to premature aging and promotes protein aggregation. Moreover, our preliminary studies indicate that increased S-nitrosation results in the appearance of cytosolic DNA inclusions, which fully colocalise with protein aggregates. We aimed to elucidate the molecular mechanisms governing the release and further fate of cytosolic DNA. Therefore, we combined the molecular biology and the biophysical methods to address this issue. We observed that the cytosolic inclusions are composed of dsDNA, which is not related to the mitochondria content and does not activate canonical pathways related to the recognition of cytosolic DNA. Mechanistically, S-nitrosation causes DNA damage and alters nuclear envelope architecture and permeability. Concomitantly, atomic force microscopy revealed very dense actin cytoskeleton over the nucleus. Live cell actin staining revealed rapid changes in actin cytoskeleton which correlated with the DNA expulsion from the cell. The instantaneous release of dsDNA from cells can be further intensified by induction of autophagy. The secretion is executed by large spheric SNO-positive components and the accumulation of protein aggregates presumably serves as a signal for autophagy activation to enable the disposal of cytoplasmic DNA. To sum up, here we propose a S-nitrosation related mechanistic model of nuclear DNA release, which relies on concomitant occurrence of DNA damage, increased permeability and actin confinement of the nucleus. Interplay between autophagy and S-nitrosated proteins orchestrate the disposal of DNA. Finally, this observations can give valuable insights into understanding the basis of tumorigenicity and autoimmune diseases.

[YIA] A genome wide approach to identify intracellular 'hotspots' of H2O2 action

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Hydrogen peroxide (H2O2) signaling is a process by which particular thiols on particular proteins are reversibly oxidized to modulate protein function in a dynamic manner. H2O2 can be generated from various intracellular sources, but their identities and relative contributions are often unknown. To identify endogenous 'hotspots' of H2O2 generation on the scale of individual proteins and protein complexes, we generated a yeast library in which the H2O2 sensor HyPer7 was fused to the C-terminus of all protein-coding open reading frames (ORFs). We also generated a control library in which a redox-insensitive mutant of HyPer7 (SypHer7) was fused to all ORFs. Both libraries were screened side-by-side to identify proteins located within H2O2-generating environments. Screening under a variety of different metabolic conditions revealed dynamic changes in H2O2 availability which were highly specific to individual proteins and protein complexes. For example, amino acid deficiencies cause H2O2 to increase in proximity to certain proteins, but to decrease in proximity to certain other proteins, even if they are in the same cellular compartment. These findings suggest that H2O2 generation and impact is much more localized and differentiated than previously recognized.

Reduction of the ABTS radical: a modification of and an insight into the method

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Since its introduction over 20 years ago (1), the method of assay of total antioxidant capacity based on the reduction of pre-formed radical of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•) has become one of the most often used method of estimation of total antioxidant activity. According to Google Scholar, the paper presenting this method1 has been cited 23913 times. The aim of this study was to study this reaction in a more detail and to propose a convenient adaptation of the method for a plate reader. We diluted stock ABTS• solution with 10 mM phosphate, pH 7.4, to the final concentration providing absorbance of a 200-µl sample in a well of a Greiner 96-well plate (fluid layer of 6.25 mm) of 1.0; it corresponds to ABTS• concentration of 106.7 µM. ABTS• reactivity with Trolox did not change significantly with pH in the range of 2.0-7.4; the reactivity with Trolox and blood plasma was lowered with increasing ionic strength. Various antioxidants differed in the rates of reaction with ABTS•. In the concentration range not exceeding the total reduction capacity of ABTS•, reactions of some antioxidants (e.g. Trolox) were completed within seconds ("fast antioxidants") while the reactions other compounds ("slow antioxidants") and complex materials like blood plasma or mushroom extracts lasted for minutes and could be not completed after 6 and even 30 min. The reasonable time for the measurement of total ABTS• reduction capacity in such cases was found to be 60 min. The Area Under Curve approach was found to be equivalent to the measurement of final absorbance decrease. For calculation of the Trolox Equivalent Antioxidant Capacity, comparison of the slopes of dependence of ABTS• reduction capacity on the concentration were found superior to the comparison of decreases in absorbance. (1) Re et al., Free Radic. Biol. Med. 1999; 26:1231.

Regulation of murine macrophage viability by the long-chain metabolite of vitamin E through targeting intracellular lipid composition via SREBP1/SCD1.

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Scope Recent studies suggest that lipid metabolism has strong associated with many cellular stress responses including cell survival as well as cell death. For this reason, compounds targeting intracellular lipid homeostasis are of high interest. α-13′-carboxychromanol (α-13'-COOH), a long-chain metabolite (LCM) of vitamin E, has emerged as a new regulatory molecule exhibiting more potent or even different effects compared with its metabolic precursor α-tocopherol. Here, we present new insight into the interaction of α-13'-COOH with the cellular lipid homeostasis in murine macrophages RAW264.7 cells. Methods and results Using cell extraction assays and Western Blot analyses the translocation of the transcriptional factor sterol regulatory element-binding protein-1 (SREBP1) we found that the treatment with α-13'-COOH suppressed significantly the formation of SREBP1 active fragment in the cytoplasmic fraction and also decreased its translocation into the nucleus. We also observed that the level of the enzyme stearoyl-CoA desaturase-1 (SCD1), a target gene of SREBP1, was significantly downregulated by the LCM. Considering the function of SCD1 as a desaturase enzyme, we hypothesized that the lipid desaturation ratio could be changed by the LCM. Using UPLC-MS/MS-based lipidomics, we could show that α-13'-COOH modulates the intracellular lipid composition of macrophages: In both, triglycerides and phospholipids, the amount of species with saturated fatty acid was increased, and the amount of species with mono-unsaturated fatty acid decreased. Next, we observed that α-13'-COOH modulated cell proliferation, cell cycle arrest and cell apoptosis/necrosis using photometric and FACS analyses, with effects that are consistent with the SCD1 antagonist, CAY10566. Conclusions Our results provide evidence that the LCM α-13'-COOH can modulate the viability of macrophages through the regulation of cellular lipid desaturation via SREBP1/SCD1.

Induced inflammation modulation by olive tree dietary byproducts in zebrafish.

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Functional molecules from natural extracts may possess specific properties capable of improving health and reducing disease. Incorporation of such molecules into foods is a promising strategy for the food industry. The olive growing sector, widely spread in Mediterranean countries, generates large amounts of byproducts which include seeds with a high agri-food potential due to their high nutritional value and the presence of bioactive compounds. Zebrafish is a well-established

model, widely used in various fields, including the study of human pathologies and immune responses. Also, oxidative and inflammatory responses can be easily induced in a reproducible manner, and visualized both in early developmental stages and in adult fishes. The main objective of this work is to evaluate the anti-inflammatory capacity of olive seeds, by optimizing commercial diets enriched with this material, in an experimental model of inflammation by LPS treatment in adult zebrafish. A control fish group was fed three times a day (once with Artemia and twice with standard fish chow, while the olive seed fish group was fed with standard fish chow enriched with olive seeds at 20%. After 8 weeks, inflammation was induced with lipopolysaccharide (LPS) for 8h. After the inductions, liver was extracted, and expression of inflammation and oxidative biomarkers were analyzed. Results exhibited a drastic drop in the levels of inflammation-related biomarkers in the group of animals fed with the olive seed-enriched diet, compared to the control group. Results point to a possible protective and anti-inflammatory effect of the olive seed-enriched diet. This research was funded by research projects BFU-2016-77243-P, PID2020-113324GB-100 and STED202100X129616SV0 of the Spanish Ministry of Science, Innovation and Universities (MICIIN)/ State Research Agency (AGE)/ European Regional Development Fund (ERDF)/ European Union (EU).

Increased ROS can improve liver cell survival: stress response PACOS

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Excessive or too small amounts of reactive oxygen species (ROS) lead to oxidative imbalance, named oxidative stress or antioxidative/reductive stress, respectively, while moderate concentrations of ROS are required for normal cell function. H2O2 is the main redox signalling and redox regulation molecule. Increased levels of H2O2 are a part of a normal response to moderate stress in primary liver cells (hepatocytes) and trigger a stress response that prevents apoptosis triggering through caspase-9, called preapoptotic cell stress response (PACOS). An increased amount of ROS production and lower apoptosis triggering are reversible in primary hepatocytes and their function is preserved at all times, in both, stress adapted and normal cells. Antioxidants, like N-acetylcysteine (NAC), annul the PACOS stress response and its protective role against apoptosis. A moderate increase of H2O2 can induce a stress response that prevents the cells from unnecessary apoptosis.

Fibroblast growth factor FGF21, a regulator of oxidative stress cell responses, in early metabolic disturbances of health-to-disease transition in non-communicable diseases (NCD)

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Recently, fibroblast growth factor 21 (FGF21) has been recognized as a stress-responsive hormone that plays a key role in glucose and lipid metabolism and in the control of energy balance. It is also considered as a key regulator of the oxidative stress cell responses, due to relations of the FGF21 gene with NRF2, UCP3, SOD2, ERK and others. Its expression is induced by oxidative stress, and it also exerts protective effects, e.g. by inhibiting inflammation in response to oxidative stress. We hypothesized that circulating levels of FGF21 are affected in health-to-disease transition and focused on early stages of metabolic disturbances in NCDs in comparison to full health. Apparently healthy subjects with mildly impaired renal function (eGFR 30-60 ml/min/1.73 m2) (group A), mildly impaired glucose tolerance (HOMA index >2.5 and HbA1c 38.8-44 mmol/mol) (group B) or early stages of arteriosclerotic lesions (carotid intima-media thickness left and right >75th percentile) (group C) were studied and compared to subjects with clinically and biochemically proven full health. Overlaps between groups were excluded to allow for identifying FGF21 behavior in different types of disturbances. Along with FGF21, a comprehensive panel of metabolic and oxidative stress biomarkers was assessed. Both groups A and B showed higher FGF21 concentrations compared to subjects with full health and group C; they also showed higher levels of branched-chain amino acid-derived C5-acylcarnitine (suggesting incomplete fatty acid oxidation), leptin, triglycerides and fatty liver index, BMI and waist circumference, and lower HDL-cholesterol, vitamin C and β-cryptoxanthin; human mercaptalbumin (reduced:oxidized form) was impaired in group A (all $P < 0.001$). Glutathione peroxidase, myeloperoxidase and malondialdehyde did not differ. These results demonstrate that circulating FGF21 levels are elevated in early stages of metabolic disturbances in insulin resistance and impaired kidney function, but not in early vascular changes in the absence of such disturbances.

Fluorescent nanodiamonds as a biosensor for detecting free radicals during stress condition in Saccharomyces cerevisiae

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Background. Free radicals play a significant role in physiological processes and pathological conditions such as degenerative diseases and the aging process. We have developed a new technology which allows nanoscale MRI measurements for quantifying these with subcellular resolution. We make use of diamond nanoparticles, which change their optical properties based on magnetic surrounding. While the method have already been proven in physics we demonstrate measurements in life cells for the first time. In this study we could measure radical production after provoking Saccharomyces cerevisiae . We were also able to clearly differentiate production rates between young and old as well as wild type and knock out strains. Additionally, we are able to follow how an antioxidant protects the cells from radical damage. Methods. Saccharomyces cerevisiae BY4741 were grown in synthetic defined (SD) complete medium supplemented with 2% glucose and treated with zymolyase for creating spheroplasts which facilitated FNDs uptake. The cells were placed in 96-well plates and 4 compartments cell culture dishes then triggered with 1, 3, and 10% of hydrogen peroxide. They were analyzed by using MTT assay for evaluating metabolic activity and H2DCFDA for measuring ROS production. 0,1 M of L-ascorbic acid had been used for antioxidant during the measurements. Free radicals level during stress condition has been monitored by using diamond magnetometry technique with 50 µwatt laser power. All the measurements were performed triplicates. Results. With our newly developed technique we are able to measure free radical generation with nanoscale spatial resolution.

[YIA] Can physical activity counteract changes in redox-status biomarkers during chemotheraphy of breast cancer patients?

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Breast cancer (BC) is one of the most commonly diagnosed types of cancer in women. Oxidative stress may contribute to cancer aetiology through several mechanisms involving damage to DNA, proteins and lipids leading to genetic mutations and genomic instability. The literature indicates that physical activity (PA) has positive effects on every aspect of breast cancer evolution, including negative effects from treatment. Specifically, how the beneficial association between PA and BC survival are partially related to its influence on antioxidant status of the body. Fifteen newly diagnosed BC patients (40-60 years), who underwent the same surgery and before beginning cancer-related treatments, were recruited and divided randomly into a control group (CG,n=5) undergoing usual care, and an exercise group (EG,n=10), which additionally participated in a PA program. With the aim to verify the ability of PA to counteract the negative effects on systemic redox-homeostasis induced by the BC treatment, we examined the impact of a 4-month exercise program on the modulation of plasma markers of oxidative stress, inflammation and the stress response, such as superoxide dismutase (SOD) and catalase (CAT) activity, total-glutathione (tGSH), lipid-oxidation (TBARs), total-antioxidant-capacity (TAC) and total-free-thiols (tFTH), as well as interleukin-6 (IL6), interleukin-8 (IL8), interleukin-10 (IL10), and tumor-necrosis-factor-alpha (TNFα). Even in the absence of significant changes in CAT activity, TAC, tFTH and TBARs levels ($p > 0.05$), exercise maintained SOD activity and tGSH levels in EG whereas in CG they were significantly decreased ($p < 0.05$). Moreover, we found a significant decrease of IL8 in both groups, whereas only in EG we observed a significant reduction in the pro-inflammatory IL6 and an increase of antiinflammatory IL10 ($p < 0.05$). These results highlighted the importance of PA as a potential coadjuvant therapy, alongside usual care of BC, able to counteract the chemotherapy-induced negative effects on an already compromised redox homeostasis.

Simplified synthesis of oxidized phospholipids on alkyl-amide scaffold demonstrating anti-lipopolysaccharide and endothelial barrier-protective properties

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Oxidized phospholipids (OxPLs) containing enzymatically or non-enzymatically oxidized fatty acids (oxylipins) are increasingly recognized as lipid mediators involved in disease pathogenesis. Further understanding of structure-activity relationship and molecular mechanisms activated by OxPLs is hampered by the complexity of synthesis of individual molecular species. Although dozens of individual free oxylipins are commercially available, their attachment to the phospholipid scaffold requires relatively harsh conditions during activation of carboxyl groups, which may lead to decomposition of unstable oxylipins. Furthermore, additional protection-deprotection steps are required for oxylipins containing hydroxyl groups. In this work we describe synthesis of OxPLs containing oxylipins bound at the sn-2-position via an amide bond that is characteristic of sphingophospholipids. Activation of oxylipins and attachment to the phospholipid scaffold are performed under mild conditions and characterized by high yield. Hydroxyl groups of oxylipins do not interfere with reactions and therefore no protection/deprotection steps are needed. In order to prevent oxylipin migration, the sn-1 residue is bound through an alkyl bond, which is a common bond present in a large proportion of naturally occurring phospholipids. An additional advantage of combining alkyl and amide bonds in a single phospholipid molecule is that both types of bonds are phospholipase A1/A2-resistant, which may be expected to improve biological stability of OxPLs and thus simplify analysis of their effects. As a proof of principle, several alkyl-amide oxidized phosphatidylcholines (OxPCs) containing either linear or prostane cycle oxylipins have been synthesized. Importantly, we show here that alkyl-amide OxPCs demonstrated biological activities similar to those of diacyl OxPCs. Alkyl-amide OxPCs inhibited pro-inflammatory action of LPS and increased endothelial cellular barrier in vitro and in mouse models. The effects of alkyl-amide and diacyl OxPCs developed in a similar range of concentrations. We hypothesize that alkyl-amide OxPLs may become a useful tool for deeper analysis of the structure-activity relationship of OxPLs.

Role of Platanus derived compounds in stress induced premature senescence of human Mesenchymal Stem Cells

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Cellular senescence -a process characterized by the irreversible cell cycle arrest- affects the function of eukaryotic cells, including stem cells, leading to tissue regeneration impairment, and mediating organismal aging and the development of age-related pathologies. Senescence can occur due to telomere shortening (replicative senescence), but can also be the result of damage, induced by internal or external stressors, such as oxidative stress (OS), leading to stress induced premature senescence (SIPS). Several plants and plant derived compounds could serve as potential anti-SIPS agents, possibly due to their antioxidant abilities. Here, we aimed at investigating the effects of Platanus derived compounds on Mesenchymal Stem Cells from the Wharton's Jelly (WJ-MSCs) in the context of cellular senescence. Early passage WJ-MSCs treated or not with H2O2 in order to induce premature senescence were exposed to 3 different concentrations (0,1%, 1%, 10%) of compounds derived from various Platanus parts, extracted with different organic solvents and water. The activity of the senescence associated marker β-galactosidase (SA-β-gal) was then assessed. Our preliminary results showed that while some compounds, at higher concentrations exhibit a pro-senescence or even a cytotoxic function, at lower concentrations have the ability to protect from the establishment of cellular senescence. Compounds derived from Platanus have previously been reported to possess an antioxidative ability and given the well established relationship between oxidative stress and cellular senescence's onset, it would be of utmost importance to analyze the underlying mechanisms of the observed outcome. We have previously reported that caveolin-1 -a component of the caveolae and recently recognized as major regulator of cellular senescence- plays an important role in the cells' ability to repair OS induced DNA damage, and consequently to the development of WJ-MSCs premature senescence. Therefore, the involvement of caveolin-1-depended signaling in these compounds' protective function against SIPS is currently extensively studied.

[YIA] Alternative pre-mRNA processing by the RNA binding protein Sam68 ensures metabolic reprogramming in the skeletal muscle

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Skeletal muscle is one of the most dynamic tissues in the body, inheriting the ability to fine tune its response to environmental and physiological stimuli, including exercise, diet, disuse, and disease. To achieve skeletal muscle adaptations, transcriptional and post-transcriptional programs are carried out by muscle cells. In addition, a complex network of RNA binding proteins is engaged to achieve alternative splicing programs, thus increasing muscle proteome diversity. Exercise activates signaling molecules to promote physiological adaptations, such as fiber type transformation, angiogenesis, and mitochondrial biogenesis. The underlying mechanisms involve a complex interplay of signaling pathways and downstream regulators, sensing the energy state and promoting glucose metabolism and fatty acid utilization. Glucose uptake is a crucial event for energy supply, and GLUT4 protein is the main actor in glucose transport in brain and muscle. GLUT4 pre-mRNA, encoded by Slc25a4 gene, is affected by alternative splicing: in addition to the main full-length isoform, a shorter transcript is produced, which leads to a truncated protein. We found that the RNA binding protein Sam68 modulates GLUT4 pre-mRNA processing. Sam68 expression promotes the full length GLUT4 isoform, whereas its depletion leads to the truncated GLUT4. Mechanistically, we found that Sam68 binds to and inhibits the recognition of an alternative poly-adenylation signal located in the intron 10 of the pre-mRNA. Accordingly, Sam68-/- muscle biopsies, which are enriched in type I fibers, displayed increased level of the truncated Slc25a4 isoform. Cross-linking and immunoprecipitation experiments in mouse myoblasts document that stimulation of the IGF1 signaling promotes Sam68 tyrosine phosphorylation and inhibits Slc25a4 binding. Collectively, our results identify Sam68 as a novel regulator of glucose homeostasis in the skeletal muscle, and highlights an unprecedented link between Sam68, GLUT4 and muscle glycolytic pathway.

Keratinocyte-derived paracrine factors modulates UVB-induced stress response of melanocytes

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Ultraviolet radiation (UVR) plays a role in skin photodamage through triggering various biological responses of skin cells including apoptosis, oxidative stress and melanogenesis. The microenvironment created by epidermal keratinocytes (KC) potentially influences the survival and function of melanocytes (MC) in response to various exogenous insults including UVB. In this study, we identified the candidate paracrine factors derived from UVB-irradiated KC that exerted the regulatory roles in cellular responses of MC to UVB irradiation using in vitro and in vivo models. We observed that conditioned media (CM) from UVB-irradiated KC potentially mitigated apoptosis and oxidative stress as well as stimulated melanogenesis in UVB-irradiated MC. RNA-sequening and functional experiments revealed that, among the upregulated transcripts in UVB-irradiated KC, the GCSF (granulocyte colony stimulating factor) and CCL20 (chemokine (C-C Motif) Ligand 20) mRNA were highly expressed in correlation with the KC's pararince effects on the stress responses of MC to UVB. Then, treatment of MC with the recombinant GCSF and CCL20 revealed the strongest modulatory effects on UVB-induced MC responses including apoptosis, ROS formation and melanogenesis. Exposure of KC to UVB led to a substantial increase in secretion of both GCSF and CCL20. To demonstrate the in vivo relevance of the in vitro findings, immunofluorescence analysis revealed a correlation between protein expressions of the GCSF and CCL20, in KC and tyrosinase in MC in mouse skin exposed to UVB irradiation. In conclusion, GCSF and CCL20 might be the candidate paracrine factors secreted from KC that play a regulatory role in the responses of MC to UVB. Our findings might give novel insight into development of the UVB-responsive genes as epidermal biomarkers for predicting susceptibility of the skin to photodamage.

Identification of compounds derived from the Greek flora with anti-aggregation properties

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Protein homeostasis (proteostasis) refers to the molecular mechanisms that are responsible for the maintenance of the cellular protein network. Proteostatic mechanisms tend to decline with age and this often leads to accumulation of toxic protein aggregates. The Aβ peptide that has been causally related to Alzheimer's disease (AD) onset and progression represents one of these aggregation-prone proteins. Plant secondary metabolites have been shown to be beneficial for proteostasis maintenance and/or restoration. Here, we have searched for natural products with anti-aggregating properties from the Greek flora, using various C. elegans AD models for screening. We have identified a mountain tea extract with anti-aggregation properties derived from the Greek endemic Sideritis clandestina subsp. Peloponnesiaca (SCP). We have further fractionated the extract to identify the specific bioactive compounds that are responsible for these properties. We show that the identified compounds may decelerate: (1) the progression of the AD phenotype in CL4176 nematode strain, a strain expressing the human Aβ1-42 in its body wall muscle cells that undergoes paralysis upon temperature upshift due to Aβ aggregation, as well as, (2) the accumulation of Aβ aggregates in CL2331 nematode strain, a strain expressing the human Aβ3-42 peptide fused to green fluorescent protein (GFP) in its body wall muscle cells where Aβ aggregates can be visualized in vivo. Our study uncovers the need to identify bioactive compounds (that ideally are part of our diet) with anti-aggregation properties.

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Central Nervous System Oxidative Stress interplay with inflammation in a rat model of Type C Hepatic Encephalopathy – brothers in arms?

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BACKGROUND Although oxidative stress (OS) and neuroinflammation are thought to play a role in the etiology of type-C hepatic encephalopathy (C-HE), their involvement and synergistic action is not well understood. Using in-vivo-longitudinal 1H-MRS we have shown an impaired antioxidant system, an indirect presence of OS, together with ex-vivo EPR detected increase of CNS superoxide-anion concentrations in bile-duct-ligated (BDL) rat-model of C-HE. The immunohistochemistry was used to highlight the OS findings as well as to analyze the potential synergistic involvement of OS and neuroinflammation in C-HE. METHODS In-vivo-1H-MRS: Cerebellum/hippocampus of adult rats-scanned before and post-BDL every 2-weeks up to week6 (n=18)(9.4T-MR-Varian/Magnex-Scientific). Ex-vivo-ESR: ESP300E(Bruker-BioSpin)-CMH-spin-probe: intracellular superoxide-anion detection, week 2(BDL n=2, sham n=2), 4(BDL n=2,sham n=2), 6(BDL n=5,sham n=5) and 8(BDL n=2, sham n=2). Immunohistochemistry: BDL/sham-rats (BDL:4-weeks n=3,8-weeks n=3,sham:n=3). Oxidative stress: Oxo-8-dG,GPX1,SOD1/SOD2-relative immunofluorescence quantification. Neuroinflammation: IL-6- accumulation and UV-Vis-spectroscopy- quantitative evaluation(TC5600-microscope-MEIJI-TECHNO) coupled with Ocean-HDX-UV-VIS-spectrometer. RESULTS: Chronic-liver-disease was validated by blood biochemistry(increased bilirubin and ammonium). For sham-rats, ESR revealed differences in redox-state between hippocampus and cerebellum (~31%,p=0.004). However, the relative OS increase in BDL-rats vs. shams at week-6 was similar ~42%. The significant increase of hippocampal/cerebellar OS in BDL(p=0.01,p=0.001) corroborate the 1H-MRS findings of decreased Asc concentrations(Fig1A). BDL-rats exhibited a strong increase in Oxo-8-dG-immunoreactivity in the hippocampus and cerebellum(p=0.001 vs. sham,Fig.1A). The cytoplasmic localization revealed that OS affected the mitochondrial and cytosolic-nucleic-acids. BDL-rats exhibited a significantly increased GPX-1 synthesis(p=0.001) in the hippocampus(Fig.1B). OS-driven regulation of SOD1/SOD2 resulted in enhanced immunoreactivity of both(p=0.001,Fig.1C). SOD1 localization changed from predominantly

cytoplasmic to prominently nuclear(Fig.1C). Sham-rats showed a weak IL-6 immunofluorescence while BDL-rats displayed a significant increase(p=0.001) and upregulation in all brain regions(Fig.1D). CONCLUSIONS This work proves the importance of OS and neuroinflammation in C-HE and highlight a possible vicious-circle between OS and neuroinflammation in C-HE in addition to the well-known contributions of ammonium and brain glutamine.

Metabolomics- and frailty-related signature of lipophilic micronutrients in age-related diseases

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There have been several studies that have tried to build a 'biological age' predictor using metabolomics data. The conclusion from these studies is that metabolomics-based 'biological age' predictors are less capable of predicting chronological age in comparison to, for example, epigenetic-based 'clocks', but they are still able to capture part of an individual's 'biological age'. As healthy nutrition and antioxidant micronutrients are crucial in maintaining health and robustness, we aimed at identifying metabolites and lipophilic micronutrients associated with age-related diseases and their outcomes (mortality, multimorbidity, general health, physical and multidimensional (pre-)frailty and nursing needs). To do so, within a large prospective study with one year follow up in the Emergency Department (ED) (>1,000 participants until time of submission), a blood withdrawal and a deep clinical phenotyping were obtained from a subgroup of 265 patients (140 M, age range 65-96 yeras). High-throughput NMR-spectroscopy metabolomic analyses (Nightingale Health Ltd., Helsinki, Finland) and HPLC measurements of carotenoids, retinol and tocopherols were performed in serum as well as the comprehensive geriatric assessment (CGA) – based Multidimensional Prognostic Index (MPI) as a surrogate marker of biological age. The simultaneous quantification of routine lipids, lipoprotein subclass profiling with lipid concentrations within 14 subclasses, fatty acid composition, and various low-molecular metabolites, including amino acids, ketone bodies, and gluconeogenesis-related metabolites yielded the preliminary results of a differential association of specific carotenoids and tocopherols with lipid classes. Furthermore, selected carotenoids appear to be preferentially associated to the metabolomics mortality score and selected geriatric syndromes and comorbidities. Although analyses are ongoing at the time of submission and the final results will be presented at the Redox Biology Congress, preliminary observations suggest a metabolomics and frailty signature of lipophilic micronutrients which may disclose pathophysiological mechanisms of age-related diseases and new therapeutic options.

Free radicals mediated protein oxidation: Detection and functional relevance in macrophage polarization

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Reactive oxygen species (ROS) regulates cellular homeostasis and acts as a modulator of cellular dysfunction. Exacerbated production of ROS is reported in inflammatory disorders however contribution of free radical-mediated processes in both physiological and pathological conditions has not received much attention. ROS randomly damage lipids, proteins and nucleic acids giving rise to the formation of macromolecule centered radicals. Being highly reactive and with increased abundance within a cellular environment, proteins remain a major target for intermediate radical formation. As macrophage plays a vital role in the progression of metabolic and inflammatory disorders, our study aimed at deciphering the role of ROS in macrophage polarization and subsequent protein centered radicals formation. Our study employs THP-1 as a model system to study and evaluate free radical-mediated protein oxidation in differentiated macrophages. The study utilizes immuno spin trapping techniques in the identification of ROS type, source and its corresponding protein radical intermediate formation along the process of macrophage polarization. Results from this study help in the identification of protein targets undergone modification and their subcellular localization thereby serving as promising biomarkers of free radical-mediated oxidative stress. Herein, we provide a methodological platform for elucidation of redox regulation within a cellular environment and processes involved in metabolic and inflammation-driven tissue dysfunction.

Crude extracts from pepper fruits show anti-proliferative activity against tumor cells altering their catalase and glucose-6-phosphate dehydrogenase profile

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Pepper (Capsicum annuum L.) fruit is characterized by its high amounts of antioxidants (vitamins C and A), polyphenols and capsaicinoids. It has been reported that capsaicin, which is exclusive from pepper fruits, shows anti-inflammatory, antiproliferative and analgesic activities. Recently, by the use of untargeted metabolomic approaches, it has been found that pepper (Capsicum annuum) fruits contain a series of compounds with potential therapeutic properties due to the presence, among others, of quercetin and its derivatives, with their content being modulated by nitric oxide (NO) (Guevara et al., 2021, IJMS 22, 4476). In this work, the anti-proliferative activity of crude extracts from four pepper fruits varieties (Melchor from type California, Padrón, Piquillo and Alegría riojana) against HEPG2 (hepatoma) and MIA Paca-2 (pancreas) cells was investigated. Interestingly, it was was demonstrated that pepper fruit tissues from the four varieties which contained the lower capsaicin content displayed the higher anti-proliferative activity in both cell lines. The antioxidant profile of the two tumor cell lines incubated with pepper fruit extracts from Alegría riojana was then investigated, and the activity profile of catalase, superoxide dismutase, glutathione peroxidase and several NADPH-generating enzyme systems was followed. As the most highlighting results, it has to be remarked that the anti-proliferative pattern of the pepper fruit extracts was linked to an altered profile of the (iso)enzymatic activity of catalase and the glucose-6-phosphate dehydrogenase. This research opens new windows on the pepper fruit's bioactive compounds with nutraceutical and biomedical potentiality, as well as on their posible targets in the tumor cells. [Supported by a European Regional Development Fund cofinanced grants from Junta de Andalucía (P18-FR-1359) and the Ministry of Science and Innovation (PID2019-103924GB-I00), Spain]

Compounds from sweet pepper (Capsicum annuum l.) fruits with potential therapeutic uses are boosted by nitric nitric oxide (NO)

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The treatment of sweet pepper (Capsicum annuum) fruits with nitric oxide (NO) provokes ripening delay and increases in the ascorbate content, linked to a moderate lipid peroxidation and protein nitration, as nitro-oxidative stress markers. This situation was considered as some sort of "phystress" (physiological stress). It has been also demonstrated that the NO treatment triggered changes at both transcriptional and proteomic levels in pepper fruits. Based on these data, a metabolomic study was conducted to investigate the metabolomic profiles in pepper fruits at different ripening stages (ripe red versus green immature) and as a consequence of the NO treatment, with the aim of searching for compounds which may have therapeutic potential, that boosting the nutraceutical value of this vegetable. Thus, high performance liquid chromatography (HPLC) coupled to metabolite identification by high resolution mass spectrometry (HRMS) was achieved, and different platforms and databases were used to characterize the metabolites. Twelve differential bioactive compounds were identified in sweet pepper fruits, including quercetin and its derivatives, L-tryptophan, phytosphingosine, FAD, gingerglycolipid A, tetrahydropentoxylin, blumenol C glucoside, colnelenic acid and capsoside A. The abundance of these metabolites varied depending on the ripening stage and on the presence of NO. Besides, the potential anti-proliferative activity of crude extracts from pericarp and placenta of pepper fruits with different pungency levels (capsaicin content) was investigated against seven tumor cell lines. Results proved that tissues with the highest capsaicin levels did not correlate with the greatest anti-proliferative capacity. Altogether, these data open new research perspectives on the pepper fruit's bioactive compounds with therapeutic potentiality, where biotechnological strategies can be applied for optimizing the level of these beneficial molecules. [Supported by a European Regional Development Fund cofinanced grants from Junta de Andalucía (P18-FR-1359) and the Ministry of Science and Innovation (PID2019-103924GB-I00), Spain]

[YIA] Restructuring of the Redox Proteome in Insulin Resistance

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Recent years have seen the field of redox biology evolve its understanding of free radicals from the toxic by-products of cellular metabolism to hormetic metabolites with a potent second messenger capacity. An emerging area of interest regarding free radical hormesis is that of growth factor stimulus, particularly insulin signalling in relation to both physiology and pathophysiology. This interest is driven by numerous growth factors driving a cellular 'oxidative shift' upon signal transduction and suggests a dynamic role for thiol-based free radical signalling in signalling pathways and their dysregulated, diseased states. Insulin Resistance, a pathophysiological state defined as a loss of insulin sensitivity in several tissues, is defined on the molecular level as the perturbation of glucose transporter GLUT4 trafficking to the plasma membrane by the insulin signal cascade. Indeed, we predict a complex network of cysteine-based thiol groups exists that comes under oxidation and reduction upon insulin stimulus, fine-tuning the activity of target proteins and eliciting the canonical insulin response. Our study utilises a protocol that labels all oxidised cysteines in the proteome with Cysteine-reactive Phosphate Tags (CPTs), phospho-tagging thiols of interest. From here, we utilise the EasyPhos phosphoproteomic workflow developed in house by Dr Sean Humphrey to enrich for never-before-seen coverage of the redox proteome. We apply this technical leap forward to newly optimised models of insulin resistance, designed around chronic exposure of adipocytes to subtle concentrations of perturbations to minimise off-target effects, revealing the hypothesised network of protein thiols responsive to acute insulin stimulus and characterising a reorganisation of this network under insulin resistant conditions. In future, our findings will serve as a launching pad for describing the underlying crossplay between canonical insulin signalling and redox signalling, hopefully contributing a better understanding of insulin resistance that will one day progress to therapeutic applications within a clinical setting.

[YIA] Modulation of H2S production during differentiation of Caco-2 cells to an enterocyte/colonocyte-like phenotype

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By affecting mitochondrial bioenergetics and protein persulfidation, H2S acts as a redox regulator that may modulate the redox metabolome and the thiol proteome. H2S exerts both stimulatory and inhibitory effects, for example on cellular proliferation and differentiation. Particularly large amounts of H2S are produced within the human gut, by commensal bacteria and by intestinal epithelial cells. CBS, CTH and MPST have long been known as human H2S-producing enzymes; recently, selenium-binding protein 1 (SELENBP1) has been reported to generate H2S as well, by means of its methanethiol oxidase activity. Here, we provide the first comparative analysis of the four H2S-producing enzymes and the H2S-catalyzing enzyme SQOR in differentiating Caco-2 human intestinal epithelial cells. Protein and mRNA levels were determined by immunoblotting and qRT-PCR during spontaneous and butyrate-induced differentiation. H2S production deriving from the enzymatic substrates methanethiol, cysteine and/or homocysteine was assessed in an assay based on lead sulfide precipitation. Both spontaneous and butyrate-induced differentiation resulted in pronounced increases in gene expression and enzymatic activity of the differentiation marker ALPI. The levels of the H2S-modulating enzymes were differentially altered during spontaneous differentiation of Caco-2 cells: While SELENBP1 and SQOR were strongly upregulated in differentiated as compared to proliferating cells, CBS was downregulated, and CTH as well as MPST remained largely unaffected. Methanethiol- and homocysteine-derived H2S production was strongly elevated in differentiated cells. Treatment with butyrate also resulted in upregulation of SELENBP1 and SQOR; however, the effects were less pronounced. In contrast, butyrate treatment did not mimic the downregulation of CBS during spontaneous differentiation. In conclusion, CBS and SELENBP1 are reversely regulated during spontaneous differentiation of Caco-2 cells. Moreover, SELENBP1 represents the H2S-producing enzyme whose upregulation was most pronounced during differentiation. Finally, butyrate exposure, while imitating some aspects of spontaneous differentiation, does not elicit the same expression patterns of genes encoding H2S-generating enzymes.

Non-enzymatic S-nitrosation and denitrosation of proteins by variation of superoxide/nitric oxide ratio – implications for prevention of sulfoxidation-dependent enzyme inactivation during ischemia/reperfusion

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Reactive oxygen and nitrogen species play a key role for the development of cardiovascular, metabolic and neurodegenerative disease, but they are also involved in cellular functions via redox signalling. We have previously identified an efficient mechanism of S-nitrosation by low levels of nitric oxide and superoxide (3:1 ratio) with potential formation of N2O3. Here, we elucidated whether S-nitrosation (as observed under hypoxic conditions) could prevent sulfoxidation and thereby oxidative inactivation of enzymes by superoxide/hydrogen peroxide (as observed during reoxygenation). We found that increasing concentrations of xanthine oxidase/hypoxanthine caused conversion of S-nitrosoglutathione (GSNO) to reduced glutathione (GSH) up to a certain concentration of xanthine oxidase, indicating that superoxide can induce denitrosation of GSNO. This finding was unexpected since in the presence of excess superoxide one would not expect regeneration of reduced GSH from GSNO. Also, the observed substantial dihydrorhodamine oxidation that was prevented by uric acid and tyrosine nitration of albumin during denitrosation of GSNO by superoxide, clearly pointed to intermediary formation of peroxynitrite. As a proof-of-concept, we demonstrate that isocitrate dehydrogenase (ICDH2) can be S-nitrosated / inactivated by spermine NONOate and denitrosated / partially reactivated by superoxide formed by xanthine oxidase. In summary, we propose that S-nitrosation of (mitochondrial) proteins during ischemia represents a protective mechanism to prevent irreversible overoxidation of thiols during the reperfusion phase and to re-establish reduced thiol state in (mitochondrial) key enzymes of energy metabolism and cell survival. Wide-spread mitochondrial protein S-nitrosation may represent a central feature of the protective preconditioning effects of nitric oxide.

Spatial and temporal H2O2 production in hepatocytes during non-alcoholic fatty liver disease

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The incidence and prevalence of non-alcoholic fatty liver disease (NAFLD) are rapidly rising worldwide due to the global obesity epidemic. Obesity-induced oxidative stress plays an important role during the progression of NAFLD through different mechanisms, including oxidative modifications of protein thiols, stimulation of transcription pathways, and promoting the recruitment of inflammatory cells. Indeed, it is known that obesogenic environments in NAFLD, characterized by high levels of glucose, fatty acids, insulin, and pro-inflammatory cytokines (i.e., IFN-γ, TNF-α and IL-6), increase the generation of reactive oxygen species like hydrogen peroxide (H2O2). Chronic hepatic oxidative stress in obesity is mainly attributed to palmitate-induced mitochondrial and peroxisomal H2O2 generation, UPR/ER stress, and an increased flux of the DAG-PKC-NOX signaling pathway. However, the contribution of H2O2 originating from different cellular compartments during NAFLD development remains unclear. Likewise, it remains to be elucidated which environmental compounds contribute to H2O2 generation from each of the contributing compartments. To address this, we express the latest ultrasensitive pH-independent H2O2 probe HyPer7 in different cellular compartments of hepatocytes including the cytosol, mitochondrial matrix, peroxisomes, and nucleus. This allows us to monitor H2O2 levels and dynamics live in different cellular compartments with spatial and temporal resolution. By exposing the HyPer7-expressing hepatocytes to obesogenic environments, we attempt to identify the subcellular source(s) of H2O2 responsible for the progression of NAFLD. In the long term, the results of this study can be used to design targeted therapeutic options to curb NAFLD progression.

[YIA] Harnessing the power of nanodiamond magnetometry for free radical detection in primary cells

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Free radicals play important roles in biological systems. These ubiquitous chemical species are formed by all live eukaryotic cells and are involved both in normal cell metabolism and pathological processes. It is vital to know where, when and in what quantities free radicals are formed. However, commonly used techniques often suffer from low sensitivity or selectivity, making direct in situ detection of free radicals a challenging task. Our group has been developing a new approach for free radical sensing in biological samples – nanodiamond magnetometry. This method is based on using fluorescent diamond nanoparticles (FNDs). As free radicals, by definition, have unpaired electrons, they are paramagnetic. FND fluorescence strongly and reversibly depends on the magnetic properties of the particle's vicinity. Changing concentration of free radicals will affect the fluorescent signal of FNDs, which can be read out with an all-optical setup, based on a simple confocal microscope. This method does not require external magnets, like MRI. The measurements can be done at room temperature, and the cells are preserved in the process. This allows for repeated measurements on the same cell over the course of several days, or using complementary techniques to obtain additional context for the recorded signals. We have used this technology in several biological models. Recently, we have applied nanodiamond magnetometry to detect the initial response of primary bronchial epithelial cells obtained from healthy donors or the donors with pulmonary disease to the cigarette smoke extract. We show that exposure to this stressor results in an altered free radical load, and that the health status of the donor influences this response. This study illustrates the excellent sensitivity of our method and paves the way for the potential applications of nanodiamond magnetometry in the (pre-)clinical setting.

[YIA] Mechanistic insights into inorganic nitrite-mediated vasodilation of isolated aortic rings under oxidative/hypertensive conditions and S-nitros(yl)ation of proteins in germ-free mice

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The prevalence and clinical importance of arterial hypertension is still growing. Inorganic nitrite (NO2-) represents an attractive dietary antihypertensive agent but its metabolism and mode of action are not completely understood, which we

aimed to investigate with the present study. Isolated aortic rings from rats were treated ex vivo with oxidants or rats were infused in vivo with angiotensin-II. Vascular responses to acetylcholine (ACh) and nitrite were assessed by isometric tension recording. The loss of vasodilatory potency in response to oxidants (but not in vivo angiotensin-II) was much more pronounced for ACh as compared to nitrite. This effect may be caused by the redox regulation of conversion to xanthine oxidase (XO). Conventionally-raised and germ-free mice were treated with nitrite by gavage, which did not improve ACh-mediated vasodilation but increased plasma levels of S-nitros(yl)ated proteins in the conventionally-raised but not in the germ-free mice. In conclusion, inorganic nitrite represents a dietary drug option to treat arterial hypertension in addition to already established pharmacological treatment. Short-term oxidative stress did not impair the vasodilatory properties of nitrite, which may be beneficial in cardiovascular disease patients. The gastrointestinal microbiome appears to play a key role in nitrite metabolism and bioactivation.

[YIA] Methods to measure the effect of air pollution in skin

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The amount of air pollutants is increasing globally and causing serious health problems; according to the World Health Organization, more than 7 million people die each year from air pollution. Pollution not only damages the lining of the lungs; it also affects skin health and has been linked to the development of skin diseases. Promotion of the development of oxidative stress can lead to premature skin aging, impaired skin barrier, pigment disturbances, and cellular damage caused by free radicals. Existing symptoms may also worsen. To date, there is no established method to clearly assess the degree of risk of skin contact or the protective effect of the substances used. With the help of electron paramagnetic resonance (EPR) spectroscopy, a new method was established to assess the potential risk of air pollution in the skin and to verify the effectiveness of products designed to protect the skin. Spin-labeled PCA (3-(carboxy)-2,2,5,5-tetramethyl-1-pyrrolidinyloxy) was used to study free radical formation, with cigarette smoke as a model pollutant. To make the stress effect measurable, UVA light was used as an additional external stressor. In addition, the method of confocal Raman microscopy was applied which allows an assessment without the use of an additional marker and external stressor. For subjecting excised pig ear skin samples reproducibly to cigarette smoke, an exposure chamber for ex vivo and in vivo studies was developed. For quantification of the smoke exposure, the deposit of the nicotine concentration next to the investigated area was used as a marker substance. Initial studies have shown that cigarette smoke promotes the production of free radicals in the skin, and there is a positive correlation between nicotine concentration and free radical production. These results will help to establish a new way to measure pollutant effectiveness and preventive measures.

10) POG Poster Abstracts – Authors sorted alphabetically

DPI-dependent production of superoxide in reproductive tissues of the olive tree (Olea europaea L.)

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Reactive Oxygen Species (ROS) are compounds derived from oxygen with important implications in biological processes in plants, some of them related to reproduction. Among ROS, superoxide is the primary oxidant, since an array of other ROS are eventually derived from this anion. There-fore, analysis of the molecular systems able to generate this molecule and the cellular compart-mentalization of these events is of paramount importance. We have used the fluorochrome DCFH2-DA and the chromogenic substrate NBT in association with DPI (a specific inhibitor of Rboh enzymes generating superoxide in plants) in combination with confocal microscopy and stereomicroscopy, respectively to identify cell localization of ROS in general, and superoxide ac-cumulation in olive reproductive tissues. A significant production of both ROS and superoxide has been described, showing a fairly precise spatial and temporal location throughout olive flower development. The reduction of the NBT signal after the addition of DPI suggests that the generation of superoxide is largely due to Rboh or other flavin oxidase activity. At the subcellular level, accumulation of O2.- has been located in the plasma membrane of mature pollen and germi-nated pollen, as well as in the rough endoplasmic reticulum and in mitochondria.

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Data mining of potential carbonylation in the olive tree pollen and its physiological implications.

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The olive tree is an important crop in the Mediterranean area. The study of pollen biology is an essential task for plant improvement and to guarantee efficient fertilization and proper yield. Among the mechanisms modulating gene expression, different posttranslational modifications have been described. Carbonylation represents one of these modifications clearly indicative of the presence of oxidative phenomena derived from the presence of ROS and RNS. The present work shows represents an in silico prediction of the olive pollen carbonylome, obtained after using the iCarPS software tool with an experimentally determined proteome already described in the literature as the target. Bioinformatic prediction shows the presence of a large number of proteins prone to carbonylation, which has been experimentally confirmed in the present work after using an OxyblotTM kit in samples electrophoretically separated. Implications of this PTM on pollen biology have been discussed based in the results obtained.

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Elevated CO2 mitigates the impact of drought stress and oxidative stress by upregulating glucosinolate metabolism in Arabidopsis thaliana

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Elevated CO2 (eCO2) reduces the impact of drought, but the mechanisms underlying this effect remain unclear. We used a multidisciplinary approach to investigate the effect of drought and eCO2 (620 ppm) on growth and oxidative stress of Arabidopsis thaliana leaves. Transcriptome and subsequent metabolite analyses identified a strong induction of the glucosinolate (GL) biosynthesis as a main effect of eCO2 in drought- stressed leaves. Of the 53 genes involved in GL metabolism and regulation, 26, mainly involved in aliphatic GL metabolism, were differentially expressed in response to

eCO2 and/or drought. Transcriptome results highlighted the upregulation of ABI5 and downregulation of WRKY63 transcription factors (TF), known to enhance and inhibit the expression of genes regulating the aliphatic GL biosynthesis (e.g., MYB28 and MYB29 TFs, respectively. In addition, eCO2 positively regulated aliphatic GL biosynthesis by bHLH-mediated signalling via MYB TF and increasing the accumulation of GL precursors, in particular methionine. To prove the role of GL metabolism in the stress mitigating impact of eCO2, we exposed two mutants, deficient in aliphatic GLs (cyp79f2 and cyp79f1f2), and three aliphatic GL overexpressing transgenic lines (35S:MYB76, 35S:MYB29 and 35S:MYB28), to drought and eCO2. Overexpression of MYB TFs improved drought tolerance by inducing stomatal closure and maintaining plant turgor, whereas loss of cyp79f genes reduced the stress mitigating effect of eCO2 and decreased drought tolerance. Thus, we conclude that the GL metabolism plays a role in the stress mitigating effect of eCO2.

Chloroplasts lacking class I glutaredoxins – Unraveling the function of GRXC5 in Physcomitrium patens

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Oxidative stress is known to induce post-translational protein modifications on cysteines such as protein S-glutathionylation. Protein S-glutathionylation is reversible and catalysed by class I glutaredoxins (GRX) belonging to a family of small oxidoreductases. Four classes of GRX are described so far, based on the active site motif. Plastids contain members of class I (GRXC5, GRXS12) and class II glutaredoxins. Class I glutaredoxins are known to be involved in protein (de)-glutathionylation while class II glutaredoxins play a role in iron sulfur cluster coordination. Phylogenetic analysis revealed that GRXC5 is the ancestral isoform and the only plastidial class I glutaredoxin in Physcomitrium patens. However, the exact function and impact of class I glutaredoxins on plastid redox processes in vivo is still unknown. Here we show that P. patens plants lacking plastid class I glutaredoxin are still viable and show alterations in stromal redox dynamics. We generated knock-out lines of GRXC5 in P. patens and introduced plastid-targeted redox-sensitive GFP2 (roGFP2) as model target for protein S-glutathionylation, into WT and mutant background (Δgrxc5). Using plate-reader based fluorometry assays, we found altered light-dependent roGFP2 dynamics compared to WT. Moreover, after oxidative challenge, the ability to deglutathionylate stromal roGFP2 was largely impaired in Δgrxc5 while plant growth under control and tested abiotic stress conditions was not distinguishable from WT. Our results suggest that P. patens Δgrxc5 plants can maintain growth without glutaredoxins catalyzing protein deglutathionylation in plastids under the tested conditions, even though removal of S-glutathionylation is retarded upon stress. Future challenges include to identify GRXC5 target proteins and to further assess S-glutathionylation dynamics in vivo.

Intercropping and sequential cropping between tomato and halophytes: physiological and biochemical responses under saline conditions

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Halophytes are known to accumulate excess salts in tissues, removing them from the immediate environment. This makes them suitable candidates for haloremetiation. In this two-phase experiment, we explored the feasibility of intercropping tomato (Solanum lycopersicum var. Sargento) with the halophyte Arhtrocaulon macrostachyum, native of South East Spain, trying to mitigate the negative effects of saline soil while providing a value-added crop.
Two consecutive greenhouse experiments, from March to July 2021 and from October 2021 to February 2022, were conducted under soil Na+ and Cl- contents of 950 ppm and 1500 ppm, respectively. Plots were arranged in randomised block designs with three replicates. In the first season, three types of plots – halophyte and tomato in monocultures and mix cultivation – were arranged, whereas in the second season tomato was additionally cultivated where halophyte was previously grown (sequential cropping).
Overall, intercropping and sequential cropping affected the activity of antioxidants enzymes in leaf extracts. This was reflected on an enhanced accumulation of reactive oxygen species, which may lead to the establishment of a moderate oxidative stress. In parallel, changes on chlorophyll fluorescence parameters were recorded. In this sense, non-photochemical quenching (NPQ) and electron transport rate (ETR) were higher in intercropping and sequential cropping

than in monoculture. With respect to the halophyte, both leaves and roots showed higher Na and Cl than in monoculture, while in tomato the opposite behaviour occurred. Intercropping did not affect tomato production. However, sequential cropping significantly increased (up to 20%) both tomato number and weight. On the other hand, sequential cropping decreased soluble solids (measured as °Brix) and acidity (as total acidity to citric acid equivalents) for tomato in comparison to both tomato in monoculture and in intercropping. Further experiment replications will be conducted, which will also involve valorization of the halophyte for ulterior uses.

Ascorbate peroxidase 2 of Chlamydomonas reinhardtii is involved in the regulation of the plastocyanin levels

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In the green microalga Chlamydomonas reinhardtii, APX2 is one of the four ascorbate peroxidase isoforms. These H2O2–scavenging enzymes use ascorbate for the reduction of H2O2. APX2 from C. reinhardtii and APX6 from A. thaliana, its orthologous, belong to a new class, named Ascorbate Peroxidase-Related (APX-R). The APX-R enzymes lack the essential amino acids to bind ascorbate and in vitro studies confirmed that AtAPX6 does not bind ascorbate, but several aromatic compounds [1]. In silico analyses showed that APX2 might reside in the lumen of the thylakoid. However, no differences were observed during growth in null apx2 mutants. The photosynthetic activity at increasing light intensities was only impacted when apx2 mutant cells were grown under phototrophic condition in low light. This was accompanied by a faster P700 oxidation upon a sudden increase of light and a slower re-reduction rate, a phenotype observed under all tested growth conditions. Furthermore, no H2O2 increase was detected in the apx2 mutants when they were transferred from low light to high light, suggesting that the lower photosynthetic activity would rather be due to regulation at the intersystem electron carriers than to oxidative stress damage. We then analysed soluble extracts with spectroscopic and mass spectrometry techniques, which showed a reduced levels of PC and the presence of the pre-apo-plastocyanin form in the apx2 mutants. In addition, a functional replacement of PC by cytochrome c6 under Cu2+ deficient conditions restored the wild-type phenotype of the electron transport to Photosystem I, confirming a specific role of APX2 on PC. Additionally, predicted structures with AlphaFold2 indicated a high probability of an interacting complex APX2:PC. Our results suggest that APX2 might be involved in the regulation of the PC levels, which questions the role of APX2 during photosynthesis.

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[1] Lazzarotto et al., (2021). Antioxidants, 10(1):65

Optimizing sulfenylated peptide identification of labelled proteins in the green microalga Chlamydomonas reinhardtii

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In photosynthetic organisms, chloroplast is one of the main sources of reactive oxygen species (ROS). The most common ROS produced during photosynthesis are superoxide anion (O2-), singlet oxygen (1O2) and hydrogen peroxide (H2O2) [1]. In this study we mainly focused on H2O2 which can also act as a signalling molecule by oxidizing cysteine residues of proteins into their sulfenylated form. The aim of the project is to identify these proteins in order to establish their role during oxidative stress in the green microalga C. reinhardtii. We optimized a protocol for trapping sulfenylated proteins from [2] by using a benzothiazine-based chemoselective probe (BTD) [3] specific to recognize -SOH. We succeeded to label sulfenylated proteins after addition of H2O2. However, we are still in the process to optimize the workflow for modified peptides identification by mass spectrometry analyses. Preliminary results on wild type cells have shown that some photosynthetic proteins are susceptible to H2O2 modification, such us the light-harvesting complexes (LHC) of photosysthem II, LHCII3, LHCB3 and LHCII-1.3 important to capture light during photosynthesis. Our analyses will provide information on the Chlamydomonas sulfenome, and will be compared to the other post translational modifications

already identified on the Chlamydomonas proteome, such as glutathionylation and nitrosylation. Funded by FNRS-FWO EOS Project 30829584

Role of Nitric Oxide Synthases from Klebsormidium nitens: first structural characterization and partners identification

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Nitric oxide (NO) is an important cellular signaling molecule regulating various physiological processes, in both animals and plants. In animals, NO synthesis is mainly catalyzed by NO synthase (NOS) enzymes. In plants, NOS-like activities sensitive to mammalian NOS inhibitors have been measured, although no sequences encoding mammalian NOSs have been found in land plants. Interestingly, we identified NOS-like sequences in 20 algae species. These latter include the filamentous charophyte green algae Klebsormidium nitens, a biological model to study the early transition step from aquatic algae to land plants.
In order to understand the mechanisms governing NO synthesis and signaling in green lineage we initiated the functional characterization of K. nitens NOSs (KnNOS) by analyzing their primary sequences as well as their expression levels in response to abiotic stresses. Currently, two NOSs were identified in K. nitens genome: the KnNOS1 which possesses classical mammalian NOS architecture consisting of oxygenase and reductase domains with some specificities as lack of conserved residues in binding domain of BH4 cofactors; and the KnNOS2 displaying a large C-ter extension containing an ANK motif and a globin domain. The two KnNOSs seem to be regulated in different ways. KnNOS1 exhibited constitutive expression during the conditions tested, whereas KnNOS2 appeared to be transcriptionally regulated during stress.
In parallel studies, we also built the in silico protein–protein interaction network of human NOSs using the BioGRID database and human NOS interaction data. Interestingly, genes encoding orthologs of several of these candidates were found in K. nitens genome. Some of these conserved partners are known to be involved in mammalian NOSs regulation and represent interesting candidates for further investigation.
Overall these findings open the way for a deeper characterization of KnNOSs and its protein partners and will facilitate further investigation of NO signaling in green lineage.

PGRL1 redox states alleviate photoinhibition in Arabidopsis during step changes in light intensity

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Non-motile plants have evolved regulatory mechanisms to maintain homeostasis for optimal growth. Responses to environmental changes in light are particularly important not only during the diurnal transition from night to day but also to react to light changes caused by passing clouds or by the wind. Thioredoxins rapidly orchestrate redox control during environmental change by modifying cysteine residues. Here, we assign a function to regulatory cysteines of PGRL1A, a constituent of the ferredoxin-dependent cyclic electron flow (Fd-CEF) pathway and show their role in the regulation of proton motive force (PMF) and nonphotochemical quenching (NPQ). During step increase of low light intensity (10-60 µE*m-2*s-1), the intermolecular disulfide of the PGRL1A 59-kDa complex is reduced transiently within seconds to the 28 kDa form. In contrast, step increases to higher light intensity (60-600 µE*m-2*s-1) stimulated a stable partially reduced redox state in PGRL1A. Measurements of NPQ, PMF and resultant photosynthetic controls Y(ND) and Y(NA) were found to correlate with the redox state of PGRL1A during step increases in light intensity but not in PGRL1mutant plants pgrl1ab or PGRL1A cysteine mutant (PGRL1AC1,2A). Continuous light regimes did not affect mutant growth; however, fluctuating regimes of light intensity showed significant growth reduction in the mutants. Inhibitors of photosynthesis placed control of the PGRL1A redox state as dependent on the penultimate ferredoxin redox state that fuels reducing equivalents to the large set of chloroplasts thioredoxins. Our results showed that redox state changes in PGRL1A are crucial to the optimization of photosynthesis and are regulated by the photosynthetic electron flux.

Exploiting a bacterial cysteine desulfurase-sulfurtransferase fusion to develop a roGFP2-based biosensor for free cysteine

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In recent years, the development of fluorescent probes has revolutionized our experimental access to physiological parameters in live cells. Genetically-encoded-probes based on redox-sensitive yellow fluorescent protein (rxYFP) and green fluorescent proteins (roGFPs), allow real-time monitoring of thiol redox dynamics, combined with the option of precise targeting to specific subcellular locations in any cellular systems or organisms including plants. For instance, the redox-sensitive GFP (roGFP2) has been fused to glutaredoxins and thiol peroxidases to allow dynamic imaging of the glutathione redox potential (EGSH) or H2O2, respectively.
Cysteine is an essential metabolite, that is required for protein synthesis but also for the production of many important sulfur-containing molecules, for the biosynthesis of cofactors such as iron-sulfur centers or molybdenum cofactors but also for the biogenesis of hydrogen sulfide. To broaden our understanding of redox biology in a cellular and physiological context, biosensors able to detect cysteine levels and its degradation/derived products are needed.
In principle, any protein domain with a catalytic cysteine undergoing a specific and reversible oxidative modification may oxidize roGFP2 unless this is sterically/structurally hampered. Cysteine desulfurases (CDs) and sulfurtransferases (STRs) catalyze the transfer of a sulfur atom from sulfur donors to nucleophilic sulfur acceptors by forming an intermediate persulfide on a single reactive catalytic cysteine. Thus, they represent good candidates to establish genetically-encoded probes as they seem perfectly suited to transfer an efficient and selective oxidation of roGFP2. In this work, we first confirmed the ability of a natural CD-STR fusion protein to oxidize the roGFP2 in the presence of cysteine. Then, using in vitro fluorescence assays, we investigated the specificity, sensitivity and reduction (once oxidized) of a CD-STR-roGFP2 fusion. These results prompted us to express the CD-STR-roGFP2 fusion in Escherichia coli, Saccharomyces cerevisiae and Arabidopsis thaliana to perfom in vivo measurements.

The Root-knot nematode effector Mj-NEROSS suppresses plant immunity by interfering with the ROS production in plastids

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Plant-parasitic nematodes produce effectors to overcome plant immunity and finetune plant cellular processes. Molecular mechanisms of how effector proteins co-opt plant processes, especially plant immunity to support nematode survival, have been intensively investigated, but they are still poorly understood. Identifying protein-protein interactions is crucial for understanding this cross-kingdom network.
Using the high throughput screening technique Y2H-seq, we have identified tomato proteins involved in various cellular processes interacting with M. javanica effectors. Among those, Mj-NEROSS (Nematodes effector involved in ROS suppression, previously referred to as 4D01 or Msp3) was found to interact with a Heavy metal transport/detoxification superfamily protein and a Rieske iron-sulphur protein (ISP), one of the putative subunits of the cytochrome b6f complex. Furthermore, we confirmed direct interaction in planta and showed that ISP is a conserved dicot target of Mj-NEROSS.

We showed that the plastid localization of Mj-NEROSS plays a crucial role in its interaction with ISP. Once this interaction has been established, a significant decrease in electron transport rate and subsequently in the host's reactive oxygen species production is observed. Furthermore, we reveal that the presence of the effector in the plastids leads to changes in genes expression. Importantly, we show that the differentially expressed genes (DEGs) are involved in ROS production, protein folding recognition, and upregulation of oxidative phosphorylation.
We hypothesized that DEGs are most likely subsequent consequences of interaction of Mj-NEROSS with ISP and biochemical communication between plastids and the nucleus, leading to suppression of plant basal defense and attenuation of host resistance to the nematode infection.

[YIA] Thiol-based redox regulation of Arabidopsis Class II PABPs

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In eukaryotes, poly(A) binding proteins (PABPs) bind to the poly(A) tail of transcripts and thereby influence the RNA stability, nucleo-cytoplasmic transport and translation. Three members of PABPs (PABP2, 4 and 8) in Arabidopsis, which form the class II PABPs, play various roles in plant growth and development as loss-of-function double mutants (pabp2/4, pabp2/8 and pabp4/8) exhibit phenotypic abnormalities in leaf shape, flowering time and plant height [1]. In previous redox proteomics studies, we charted the sulfenomic landscapes in Arabidopsis. There, we demonstrated that upon H2O2 stimulation of cells, class II PABPs underwent thiol-dependent oxidative post-translational modifications on Cysteine (Cys) residues, which reside in their RNA recognition motif (RRM) domains [2, 3]. Therefore, we hypothesized that Cys oxidation might affect the RNA binding activity of class II PABP. First, by means of Electrophoretic Mobility Shift Assays (EMSA) with recombinantly expressed proteins and biotinylated poly(A), we found that the poly(A) binding capacity of class II PABP proteins decreased under elevated H2O2 concentrations. Mutated isoforms in which the Cys residues were replaced with Serine residues, were insensitive to H2O2 dose-dependent decrease of poly(A) binding activity. In order to functionally characterize the thiol-based redox regulation of Class II PABPs, we are currently generating transgenic plants in which both wildtype and Cys mutant PABPs are ectopically expressed into double mutants (pabp2/4 and pabp2/8) for future phenotypic analysis under normal growth and oxidative stress conditions.

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[YIA] Identification of intramembrane proteases that activate membrane-bound transcription factors during mitochondrial retrograde regulation

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Due to their sessile lifestyle, plants are exposed to ever-changing and often stressful environments, such as drought, heat, and pathogen assaults. To survive these harmful conditions, plants evolved to have complex mechanisms to recognize and counteract these conditions. Besides the plasma membrane, intracellular organelles such as chloroplasts and mitochondria are in a prime position for sensing and reporting stress signals to the nucleus to regulate stress-responsive gene expression. The molecular mechanisms of these organelle-to-nucleus (also referred to as retrograde) signaling networks are not well understood in plants. Our lab identified a novel mitochondrial retrograde signaling pathway, in which transcription factors of the NO APICAL MERISTEM/ ARABIDOPSIS TRANSCRIPTION ACTIVATION FACTOR/ CUPSHAPED COTYLEDON (NAC) family that are anchored to the ER -membranes through their C-terminal transmembrane domain play a key role. Upon mitochondrial perturbation by stresses, the N-terminal part of these transcription factors is released from the ER-membranes and translocated to the nucleus to regulate stress-responsive gene expression. However, the molecular mechanisms that underlie the release of these transcription factors and how mitochondria signal to the ER during plant stress responses remain not well understood. In silico and pharmacological analysis indicate that these ER-anchored NAC transcription factors are cleaved by rhomboid proteases. The aim of my PhD project is to identify the responsible proteases by activity-based-protein profiling and proximity-based labeling approaches and consequently unravel the molecular mechanisms of ER-membrane bound transcription factor activation during mitochondrial retrograde signaling of plant stress responses.

[YIA] Different oxidative pattern in sensitive and resistant Amaranthus palmeri populations treated with herbicides inhibiting amino acid biosynthesis

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Glyphosate, the most used herbicide worldwide, inhibits the 5-enolpiruvyl-3-phosphate synthase (EPSPS) in the shikimate pathway. Acetolactate synthase (ALS) inhibitors are a diverse herbicide group that inhibit ALS in the branched-chain amino acid biosynthesis pathway. Besides their different enzymatic target, glyphosate and ALS inhibitors trigger common physiological effects, including oxidative stress. However, the linkage between EPSPS/ALS inhibition and oxidative stress is not completely elucidated. Additionally, the massive usage of these herbicides has led to the development of weed resistant populations, as in Amaranthus palmeri. Physiological effects of herbicides in resistant populations are poorly studied. The objective is to get new insights in the oxidative stress triggered by glyphosate and ALS inhibitors comparing sensitive and resistant plants with resistance mechanisms related to the target enzyme. To this purpose, glyphosate-resistant and ALS inhibitor-resistant populations and their sensitive reference populations were grown and treated with different doses of glyphosate or the ALS inhibitor nicosulfuron, respectively: untreated, field rate and 3 times field rate. Hydrogen peroxide content and gene expression levels of the antioxidant enzymes superoxide dismutase (CuZnSOD) and glutathione reductase (GR1, GR2) were measured as oxidative stress parameters. All herbicide treatments were lethal for sensitive individuals, while resistant individuals survived. Early upon herbicide treatment, H2O2 highly accumulated but only in sensitive plants. Superoxide dismutase gene expression was induced in both sensitive and resistant populations after exposure to both herbicides. The GR1 expression level increased with glyphosate, especially in the sensitive population; while it did not change with nicosulfuron. The GR2 expression was unaltered by herbicide treatments. In conclusion, the increase in antioxidant gene expression induced by glyphosate and ALS inhibitors in sensitive plants was not enough to avoid H2O2 accumulation in contrast to resistant populations. Probably their resistant physiology and mild increase in CuZnSOD and GR1 gene expression is sufficient to avoid oxidative stress.

Thallium induced increases on O2.-, H2O2, NO and H2S production, and morpho-physiological alterations in Ditrichia viscosa plants

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The alterations induced by the toxicity of thallium (Tl) in the roots and leaves of Dittrichia viscosa plants were determined. The plants were grown hydroponically with different concentrations of Tl (0, 10, 50 and 100 µM) during 7 days, a metal which reduces biomass production and growth, and the relative water content decrease. The leaves shown venal chlorosis. Tl is accumulated mostly in the roots, with the concentrations in the leaves being much lower. ICP-MS analysis showed that the K content decreased both roots and leaves. Fe, Cu, Mn concentrations was altered.

Chlorophyll a and b content declined, and the ratio chl a/chl b increased, but the carotenoid content increase (x2.2). The photosynthetic efficiency decrease for higher Tl concentrations (0.779, 0.731, 0.547 and 0.522 for control, 10 µM, 50 and 100 µM Tl, respectively). These results would indicate that Tl causes an alteration of the photosynthetic apparatus, probably by increasing ROS despite the increase in carotenoids.

Increases were observed in the amount of lipid peroxidation in roots, but not in leaves. The O2.-, H2O2, NO increase, specially in roots. The H2S content increases in leaves, but in roots only with the 10 µM Tl. The induced oxidative stress leads to a strong increase in the superoxide dismutase (SOD).

Tl induced alterations in ascorbate homeostasis, the AsA increase and DHA decrease. The ascorbate pool only decrease with 100 µM Tl. However, the glutation pool decrease depending of Tl concentrations. The GSH content decrease and almost all glutathione is GSH.

In summary, in Dittrichia the Tl accumulates more in roots than in leaves. Tl toxicity induces oxidative stress, with redox homeostasis imbalance. These alterations are greater in roots than in leaves, where the antioxidant system is more efficient.

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Phytochrome B regulates reactive oxygen signaling during abiotic and biotic stress in plants

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Plants are essential for life on Earth converting light into chemical energy in the form of sugars. To adjust for changes in light intensity and quality, and to become as efficient as possible in harnessing light, plants utilize multiple light receptors, signaling, and acclimation mechanisms. In addition to altering plant metabolism, development and growth, light cues sensed by some photoreceptors, such as phytochromes, impact many plant responses to biotic and abiotic stresses. Central for plant responses to different stresses are reactive oxygen species (ROS) that function as key signaling molecules. Recent studies demonstrated that respiratory burst oxidase homolog (RBOH) proteins that reside at the plasma membrane and produce ROS at the apoplast play a key role in plant responses to different biotic and abiotic stresses. Here we reveal that phytochrome B (phyB) and RBOHs function as part of a key regulatory module that controls ROS production, transcript expression, and plant acclimation to excess light stress. We further show that phyB can regulate ROS production during stress even if it is restricted to the cytosol, and that phyB, RBOHD and RBOHF co-regulate thousands of transcripts in response to light stress. Surprisingly, we found that phyB is also required for ROS accumulation in response to heat, wounding, cold, and bacterial infection. Taken together, our findings reveal that phyB plays a canonical role in plant responses to biotic and abiotic stresses, regulating apoplastic ROS production, and that phyB and RBOHs function in the same pathway.

Nitrosative stress affects histone deacetylases and acetyltransferases in the plant pathogen Phytophthora infestans

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Phytophthora infestans (Mont.) de Bary is one of the most important plant pathogens in agriculture. The presence of a large number of acetyltransferase and deacetylase orthologs in the genome of Phytophthora species suggests that lysine acetylation of various proteins may be crucial in these fungal-like organisms. The pathogen is also able to synthesize nitric oxide, a signaling molecule that could be engaged in molecular reprogramming via changes in the histone acetylation status.

To gain insight into the acetylation status in the phytopathogen structures we performed the experiments on the avirulent (avr MP946) and virulent (vr MP977) P. infestans isolates in reference to the potato (Solanum tuberosum L.) cv. Sarpo Mira. In order to verify whether and to what extent reactive nitrogen species (RNS) affect nuclear histone deacetylases (HDACs) and acetyltransferases (HATs) at transcript and enzyme activity levels, the pathogen was treated with specific donors to mimic nitrosative stress conditions to which the pathogen is exposed during in planta growth.

The results indicated a RNS-dependent induction of HDACs activity at 2nd h after donors application; however, it reduced starting from 24h after S-nitrosoglutathione and SIN-1 (3-morpholino-sydnonimine) treatments. Among the 5 analyzed genes encoding nuclear HDACs, RNS provoked a significant increase of the transcript accumulation for HDAC1, HDAC3, and HDAC5. Analyses of HATs activity showed the strongest enzyme induction at 48h after donors treatment. Among the 13 analyzed genes encoding HATs, 6 showed altered expression via RNS. Moreover, different patterns of the gene expression were observed in both isolates.

Summing up, nitrosative stress modified HDACs and HATs at transcript and enzyme activity levels in both P. infestans isolates. Thus, the interaction of RNS-HDACs/HATs can be crucial in controlling the expression of a plethora of P. infestans genes.

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Transcriptome and proteome mining of H2S metabolism at the subcellular level of sweet pepper (Capsicum annuum L.) fruits during ripening and under a nitric oxide (NO)-enriched environment

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Omics technologies have become powerful tools to get deeper insights into the mechanism of regulation of the different metabolic pathways. Fruit ripening is a natural physiological process that involves drastic modifications at gene, protein, and metabolite levels. Using the transcriptome and proteome of sweet pepper (Capsicum annuum L.) fruits obtained by RNAseq and iTRAQ approaches, respectively, this study focuses on the identification and modulation of the genes and proteins involved in the metabolism of hydrogen sulfide (H2S) in the main subcellular compartments including cytosol, plastids, and mitochondria at different ripening stages: immature green, ripe red, and fruits subjected to nitric oxide (NO) gas treatment. The obtained data allowed the identification of 26 components distributed in the different subcellular compartments being differentially modulated during ripening and by NO. To our knowledge, this analysis provides the first framework for the metabolism of H2S in this non-climacteric fruit.
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Revealing the nature of RCD1 nuclear bodies

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Plants' nuclei harbour various small membraneless compartments called nuclear bodies (NBs). These allow spatial separation of the nuclear compounds and create local biochemical environments favouring specific processes.
RCD1 is a nuclear protein that can interact with over 35 transcription factors and receive various retrograde stress signals. This makes RCD1 a very capable hub protein for integrating stress signals and orchestrating a balanced response. RCD1 localises to visually distinct NBs whose formation is enhanced by stress. The protein comprises three domains separated by intrinsic disorder regions (IDRs). We recently showed that the N-terminal WWE domain can bind the post-translational modification poly(ADP-ribose)(PAR) and that this binding is responsible for the formation of nuclear bodies. Following the WWE domain is an inactive PAR polymerase (PARP)-like domain, its function has hitherto been unknown. The C-terminal RST domain is responsible for transcription factor binding.
We generated a full-length RCD1-3xVenus line and three deletion lines omitting one domain at a time. By studying these, we observed distinct NB populations, and their size and number are dependent on both stress and on which domains were conserved. To reveal the composition of these NBs we exploited these deletion lines using co-immunoprecipitation followed by mass spectrometry. This approach indicates that the WWE domain, and thus PAR-binding, is associated with pre-mRNA splicing machinery while both the PARP-like domain and RST domain are associated with proteins involved in translation and proteasomal degradation. Therefore, RCD1 may be able to tailor alternative splicing, protein translation and proteasomal degradation to re-establish homeostasis during stress.

Potassium nitrate and hydrogen peroxide as seed germination promoters: modulation of antioxidant metabolism and hormone profile

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Seed germination is the most critical stage in crop establishment, determining crop production. Seed chemical treatment during imbibition has been successfully applied for both fundamental research purposes and the stimulation of seed germination, seedling vigour and seed dormancy alleviation [1]. In the present work, we analysed the effect of H2O2 and of KNO3 treatments during imbibition in the germination and seedling growth of peach and pea seeds, respectively. With regard to peach seeds, both whole and endocarp-less seeds were treated with 10 mM H2O2 following cold stratification. For pea seeds, imbibition in KNO3 was applied at concentrations from 0.25 to 80 mM KNO3.
We observed that a low KNO3 level led to enhanced seedling water uptake and growth, increased activity of some antioxidant enzymes, and decreased levels of reduced ascorbate and glutathione. In addition, the levels of gibberellin GA1 increased whereas abscisic acid (ABA) contents decreased both in the seedlings and in the cotyledons, resulting in a decline in the ABA/GAs ratio [2].
On the other hand, H2O2 imbibition of endocarp-less peach seeds following 8 weeks of stratification, increased germination rate and seedling vigour. The H2O2 imbibition also affected the levels of non-enzymatic antioxidants ascorbate and glutathione, as well as ABA and jasmonic acid content in peach seedlings [3].

Overall, KNO3 and H2O2 were proven to be efficient seed germination promoters, modulating antioxidant defences and hormone profile, which was also reflected in improved seedling growth.

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Cysteine-based redox switches in plant stress response

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The hasher environments resulted by climate change cause oxidative stress to plants, triggering reactive oxygen species (ROS) production in plant cells, which are potent signaling molecules prone to activate defense responses. Relatively stable ROS, such as hydrogen peroxide (H2O2), can provoke reversible and irreversible oxidative post-translational modifications (Ox-PTMs) on protein cysteine (Cys), which might act in diverse signaling pathways in plant stress adaption. Protein Cys thiols (–SH) are very susceptible to H2O2. The initial reaction of H2O2 with –SH forms sulfenic acid (–SOH) that is intrinsically unstable and an intermediary en route to other OxiPTMs, such as the relatively more stable sulfinic (–SO2H) and sulfonic (–SO3H) acids, which are consider as overoxidation. The –SO3H formation is irreversible, whereas –SO2H can be recycled in an ATP-dependent manner by sulfiredoxin (Huang et al. 2018; Willems et al. 2021).
To get insight into Cys-mediated redox switches in plants, we have generated an unprecedented view on the –SOH landscape using variou proteomic approaches that facilitate further functional redox studies (Huang et al. 2019; Wei et al. 2020). To better understand the protection mechanism from Cys overoxidations, our current ongoing work also focuses on –SO2H and its reduction enzyme, sulfiredoxin. We aim to uncover the Cys-based redox switches in plants under oxidative stresses. The identified Cys-based redox mechanisms could be extrapolated and contribute to optimize crops and vegetables to be more resilient against climate change. Here, the general strategy for studying Cys oxidation will be outlined, and several examples for functional studies including ongoing work will be presented.

Evolution and diversification of the NADPH oxidase family in plants

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Reactive oxygen species (ROS) and redox signalling are evolutionary old and likely predated the origin of land plants. To specifically produce superoxide, plants possess enzymes of the NADPH-oxidase family, the respiratory burst oxidase homologs (RBOH). RBOHs are transmembrane enzymes that transfer electrons from NADPH across the plasma membrane to oxygen, producing superoxide in the apoplast. Apoplastic ROS regulate polar growth as well as stress responses. During land plant evolution the RBOH family evolved several isoforms with neo- and sub-functionalisation. However, it is yet unknown which photosynthetic organism were the first to possess a RBOH and which function that ancestral RBOH had. Available transcriptomic and genomic data suggest that RBOHs were already present in streptophyte algae. Using streptophyte algae, the model moss Physcomitrium patens as well as the model flowering plant Arabidopsis thaliana, we aim to unravel the ancestral function of RBOH isoforms. To monitor the balance between ROS generation and detoxification in vivo, we use redox sensitive GFP-based biosensors (roGFP2).

Identification of nitrotryptophan-containing proteins in Arabidopsis WT leaves undergoing dark-induced senescence

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Transfer of nitric oxide message into biological function includes interactions with specific residues of target proteins that undergo NO-dependent posttranslational modifications (PTM) among which S-nitrosation and tyrosine (Tyr) nitration are the most widely recognized. Unlike protein Tyr nitration, there is no information available on protein tryptophan (Trp) nitration in plant senescence or senescence-like phenomena. To identify the potential in vivo targets of Trp nitration in Arabidopsis WT leaves undergoing dark-induced senescence (DILS), a protein immunoprecipitation (IP) with an anti-6-nitroTrp antibody was performed. Day 3 of DILS was selected as the time point corresponding with the most abundant profile of 6-nitroTrp containing proteins. Mass spectrometry analysis of IP eluates revealed 64 characteristic proteins for control leaves (such as dihydrolipoyldehydrogenase 1, 60S ribosomal protein L13a-1, glutamine synthetase cytosolic isozyme 1-3). As many as 6 proteins were detected in both control and undergoing dark-induced senescence leaves. These included: S-adenosylmethionine synthase 2, malate dehydrogenase 1, bifunctional L-3-cyanoalanine synthase/cysteine synthase C1, GF14 protein phi chain, protein EXORDIUM-like 4 and plastid-lipid-associated protein 6. Four proteins were described as exclusively characteristic for DILS - beta-D-xylosidase 1, glyoxylate/succinic semialdehyde reductase 1, dihydrolipoamide dehydrogenase 1 and probable voltage-gated potassium channel subunit beta. The categories containing the highest number of identified protein candidates for Trp nitration in Arabidopsis leaves were related to protein biosynthesis and carbohydrate metabolism. Taken together, the results present the first catalogue of nitroTrp-containing proteins and indicate protein Trp-nitration as a selective and regulatory PTM during optimal development that declines during senescence-like phenomena.

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Dynamics of nitration phenomenon during dark-induced leaf senescence of Arabidopsis WT plants

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The study presents new insights into the role of nitric oxide metabolism in senescing Arabidopsis leaf defined as the dynamics of the nitration phenomenon. Detection of reactive oxygen and nitrogen species in dark-induced leaf senescence (DILS) model allows us to confirm that senescence-like phenomena are accompanied by a gradual decrease in NO emission and indicated a transient wave of peroxynitrite (ONOO–) formation on day 3 of DILS. To assess the metabolic fate of the time-dependent ONOO– bioavailability in individually darkened leaves (leaf 7 of the rosette), nitration levels at protein and nucleic acids level were determined on the following days of DILS (from day 1 to day 7). As evidenced by immunoassay the boosted ONOO– formation did not promote protein tryptophan nitration and progress of senescence depleted the pool of nitrotryptophan containing proteins. On the contrary, nitration of tyrosine containing proteins was intensified twice on day 3 of DILS overlapping with nitrating agent overaccumulation. Also, the abundance of 8-nitroguanine, a marker of nitrative modification of RNA and DNA, increased significantly on days 3 and 7 of DILS, respectively. Taken together ONOO– could be considered as a novel positive regulator of DILS fine-tuning redox environment to selective nitrative modification of biotatrgets such as tyrosine containing proteins and RNA.

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The Arabidopsis mitochondrial protein PPR40 modulates drought tolerance.

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Due to climate change, plant tolerance to environmental stresses is an important objective of breeding efforts. Combination of extreme conditions such as drought and high temperature can affect plants more than individual stresses. Our previous research revealed that the mitochondrial pentatricopeptide (PPR) domain protein 40 (PPR40), connects mitochondrial electron transport and of stress responses by influencing redox balance and ROS signaling in Arabidopsis thaliana (Zsigmond et al., 2008, Plant Physiol. 146:1721-1737). In a subsequent study influence of PPR40 on drought tolerance was characterized with T-DNA insertion mutants ppr40-1, ppr40-2 and PPR40 overexpression plants. Plant growth and survival rates as well as stress-related physiological parameters (chlorophyll fluorescence changes, relative water content, ROS accumulation and oxidative damage and proline levels) were analysed in plants subjected to drought, heat stresses and their combinations. Physiological studies were completed by complex phenotyping of the ppr40 mutants. Our results revealed that T-DNA insertion in the PPR40 gene could enhance drought tolerance by reducing water loss, stabilizing photosynthetic electron transport rates and improving viability of the mutants. Our results suggest that genes implicated in mitochondrial electron transport can be potential candidates for engineering drought tolerance in crop plants.
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MPK4, CIA2 and CIA2-LIKE are required for optimal photosynthesis and oxidative stress responses in plants
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Our functional analysis of MITOGEN-ACTIVATED PROTEIN KINASE 4 (MPK4) for hybrid aspen (Populus tremula × tremuloides) grown under natural field conditions for several seasons provided evidence of the role of MPK4 in the genetic and environmental regulation of stomatal formation, differentiation, signalling, and function; control of the photosynthetic and thermal status of leaves; and growth and acclimation responses. Divergence between absorbed energy and assimilated energy is a bottleneck, and MPK4 can participate in the control of energy dissipation (thermal effects). Furthermore, MPK4 can participate in balancing the photosynthetic energy distribution via its effective use in growth or redirection to acclimation/defence responses. The vast majority of chloroplast proteins are nuclear-encoded, and must be imported into the organelle after synthesis in the cytoplasm. This import is essential for the development of fully functional chloroplasts. On the other hand, functional chloroplasts act as sensors of environmental changes and can trigger acclimatory responses (e.g. SAA and SAR) that influence nuclear gene expression. Signalling via mobile transcription factors (TFs) has been recently recognized as a way of communication between organelles and the nucleus. In this study, we performed a targeted reverse genetic screen to identify dual-localized TFs involved in chloroplast retrograde signalling during stress responses. We found that CHLOROPLAST IMPORT APPARATUS 2 (CIA2) has a functional plastid transit peptide, and can be located both in chloroplasts and the nucleus. Further, we found that CIA2, along with its homologue CIA2-like (CIL) are involved in the regulation of Arabidopsis responses to UV-AB, high light and heat shock. Finally, our results suggest that both CIA2 and CIL are crucial for chloroplast translation. Our results contribute to a deeper understanding of signalling events in the chloroplast-nucleus cross-talk.
1. Witoń et al., Plant Phys. 2021, 186: 2190-2204; 2. Gawroński et al., Plant J. 2021 105: 619–638.

The SAGA complex subunit general control non-repressed protein 5 (GCN5) is redox sensitive
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The evolutionary conserved Spt–Ada–Gcn5–Acetyltransferase (SAGA) complex is a transcriptional coactivator that regulates a myriad of cellular processes through modulation of chromatin structure and transcription. As a part of its histone acetylation module, the histone acetyltransferase GENERAL CONTROL NONDEREPRESSIBLE 5 (GCN5) acetylates lysine 14 of histone 3 (H3K14) in the promoter regions of its target genes. GCN5 has been implicated in stress responses and development processes thus functioning at the crosstalk between environmental and stress programs. Intriguingly, despite its profound impact on gene expression, the molecular mechanisms regulating the acetylation activity of the SAGA complex are still unknown. Here, we report that GCN5 is sulfenylated at an evolutionary conserved cysteine residue and this modification is implicated in mounting an effective stress response. Complementation of the gcn5 mutant

with a GCN5 variant carrying a cysteine to serine mutation reverted the dwarfed gcn5 phenotype under control conditions, whereas it did not impact the sensitivity to oxidative stress observed in gcn5 mutant plants. Our results suggest that GCN5 is an important redox sensitive component of the SAGA complex that might play a role in novel molecular mechanisms integrating redox signaling and chromatin remodeling.

Heat shock may impair non-host resistance of barley to Tobacco mosaic virus by inhibition of an early ROS (superoxide) burst

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Tobacco mosaic virus (TMV) replicates in non-host barley at prolonged high temperatures (30 °C), especially if heat-exposed barley is infected by an adapted virus, e.g. Barley stripe mosaic virus (BSMV) (Dodds and Hamilton, 1972, Virology 59, 418). We have shown recently that non-host resistance to TMV in BSMV-infected barley can be also suppressed by heat shock pre-treatments (Király et al., 2021, FESPB-EPSO Plant Biology Europe Congress). In the present study we aimed at elucidating 1/ How heat shock influences non-host resistance of barley to TMV in absence of an adapted virus like BSMV, 2/ Does an early burst of reactive oxygen species (ROS) like superoxide (O2.-) play a role in the non-host resistance of barley to TMV?
In TMV-inoculated barley (cv. Ingrid), heat shock pre-treatments (30 °C for 3 hours, before TMV inoculation; 49 °C for 20 seconds, at 2 hours before TMV inoculation) resulted in significantly (50-100%) higher TMV accumulation, compared to non-pretreated controls kept at constant 20 °C (assayed by RT-qPCR). It seems that heat shock may impair non-host resistance of barley to TMV even in absence of an adapted virus like BSMV. Expression of a NADPH oxidase gene (HvRBOHF2) governing superoxide production and host disease resistance, and HvSOD1 (superoxide dismutase) and HvBI-1 (BAX-inhibitor), genes encoding for an antioxidant and a cell death regulator, respectively, displayed an inverse correlation with TMV levels, even at relatively late time points (1 to 7 DAI). This implies a pivotal role of these defense-related genes in maintaining non-host resistance to TMV. Furthermore, we found that an early (2 HAI) burst of superoxide is essentially absent in TMV-infected barley exposed to heat shock pre-treatments (assayed by nitro blue tetrazolium chloride /NBT/ staining), pointing to the role of ROS (superoxide) in non-host resistance of barley to TMV.
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Systematic monitoring of 2-Cys peroxiredoxin-derived redox signals unveiled its role in attenuating carbon assimilation rate

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Identifying the intrinsic factors that regulate leaf photosynthetic rate may pave the way toward developing new strategies to enhance carbon assimilation. Transmission of reductive and oxidative cues from the photosynthetic electron transport chain to redox regulatory protein networks plays a crucial role in coordinating photosynthetic activities. The tight balance between these two signals dictates the cellular response to changing light conditions. While the role of reductive signals in activating chloroplast metabolism is well-established, the role of their counterbalanced oxidative signals is still lacking. By developing 2-Cys peroxiredoxin-based genetically encoded biosensors, into Arabidopsis thaliana chloroplasts, we monitored the dynamic changes in photosynthetically-derived oxidative signaling. We showed that chl-roGFP2-PrxΔCR oxidation states reflected the similar oxidation patterns of endogenous 2-Cys peroxiredoxin under varying light conditions. We also demonstrated the induction of 2-Cys peroxiredoxin-dependent oxidative signals, throughout the day, under varying light intensities and their inverse relationship with NADPH levels, unraveling the combined activity of reducing and oxidizing signals. Furthermore, we unraveled the simultaneous activation of reductive and oxidative signals during photosynthesis induction phase and showed that 2-Cys peroxiredoxin activity attenuates carbon assimilation rates, demonstrating the restrictions imposed on photosynthetic performance by oxidative signals.

Integrative inference of transcriptional networks in Arabidopsis yields novel ROS signalling regulators

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Gene regulation is a fundamental process during plant growth and development, but is also important for plant responses to external stimuli. Gene regulatory network plays important roles in different physiological responses and pathways to help plant adapt to the environments. However, our global knowledge about the complexity of TF control for different genes and biological processes is incomplete. To enhance our global understanding of regulatory interactions in Arabidopsis thaliana, different regulatory input networks capturing complementary information about DNA motifs, open chromatin, TF binding and expression-based regulatory interactions, were combined using a supervised learning approach, resulting in an integrated gene regulatory network (iGRN) covering 1,491 TFs and 31,393 target genes (1.7 million interactions) in this work. The iGRN correctly inferred known functions for 681 TFs and predicted new gene functions for hundreds of unknown TFs. For regulators predicted to be involved in reactive oxygen species stress regulation, we confirmed in total 75% of TFs with a function in ROS and/or physiological stress responses. This includes 13 novel ROS regulators, previously not connected to any ROS or stress function, that were experimentally validated in our ROS-specific phenotypic assays of loss- or gain-of-function lines (MV, H2O2 or 3-AT). In conclusion, the presented iGRN offers a high-quality starting point to give us a better understanding for gene regulation in plants by integrating different experimental data types at the network level.

Unraveling the interplay between Cu and drought combined action in barley plants – a double trouble?

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The indiscriminate use of copper (Cu) in agriculture results in its accumulation in soils and consequent phytotoxicity, being this problem aggravated by the increasing frequency and intensity of soil drought events. Since the impact of the interaction of these stressors on plant physiology remains unexplored, this study aimed to evaluate the combined action of Cu and drought on barley (Hordeum vulgare L.) plants. Following a bifactorial design, seedlings were grown in a natural soil for 14 d under the following treatments: a) control (CTL) – plants continuously irrigated (14 d) in an uncontaminated soil; b) Cu – plants continuously irrigated (14 d) in a Cu-contaminated soil (115 mg Cu kg-1); c) drought – plants only irrigated during the first 7 d of growth in an uncontaminated soil; d) combined – plants co-exposed to Cu and drought treatments. Results showed that while root length was negatively affected by Cu (individually and combined), drought stress (individually and combined) led to a decreased plant biomass, compared to the CTL. Regarding ROS production, superoxide anion levels significantly decreased in leaves of Cu treatments and increased in roots of plants exposed to the individual treatments, compared to the CTL. Also, leaves of drought-stressed plants (individually and combined) presented higher hydrogen peroxide content than those of the CTL, while in roots, this ROS was enhanced with Cu exposure. Stress combination led to a greater oxidative damage, evaluated through lipid peroxidation, with no differences being detected in individual treatments, compared to the CTL. In terms of antioxidant response, proline levels were boosted by drought (individually and combined) and glutathione content was greater in plants under combined stress, compared to the CTL. Overall, plants subjected to a combination of Cu and drought triggered a differential oxidative response in comparison with each individual stress.

EAL4 methyltransferase plays a specific role in response to oxidative stress

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Hydrogen peroxide (H2O2) is one of the most potent signals that drive changes in gene transcription and therefore plant adaptation to stress and survival. In transcriptomic analyses, we identified EAL4 (EMBRYONIC ABUNDANT PROTEIN-LIKE-RELATED 4) encoding for a methyltransferase, as an early and highly inducible oxidative stress-responsive gene. Molecular and phenotypic characterization of the gain- and loss-of-function mutants suggest that EAL4 has some impact on gene expression and phenotypes in response to a wide range of (a)biotic stresses, suggesting that its

inducibility and function are specific to oxidative stress. However, interestingly, similar to its putative yeast homolog CRG1 (CANTHARIDIN RESISTANCE GENE 1), EAL4 overexpression confers resistance to toxic concentrations of cantharidin, a potent inhibitor of protein serine/threonine phosphatases. Furthermore, interactome and metabolome analyses reveal that EAL4 does not act on non-histone proteins and could rather methylate small molecules. Further analysis of the mechanism of EAL4 upregulation and identification of the in planta substrate are required to shed the light on the function of EAL4.

Analysis of the salt-stress dependent modulation of the redox network in rice

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Global climate change cause various adverse environmental conditions, such as drought, salinity, high temperature, and toxic metal accumulation, which affect plants growth and may affect food security. Rice (Oryza sativa) feeds more than one half of the world's population and is the model system for monocotyledonous. However, rice is the most salt sensitive cereal crop. To ensure rice production and preserve the biodiversity, in salt-affected soils, is crucial to understand the stress-induced metabolic alterations in tolerant and resistant plants, in order to identify interesting traits for improving plant resilience toward unfavorable environmental conditions. We have investigated on the mechanisms underpinning tolerance towards salinity, focusing on root system of two rice varieties showing contrasting resistance to salt, Baldo and Vialone Nano. Plants have evolved several interconnected molecular pathways to defend themselves against different abiotic stresses. A common theme during plants' stress responses and adaptation is the production of reactive oxygen species (ROS) and the redox signaling plays a pivotal role in determining plant tolerance and survival to stress. A detailed analysis of the salt stress-dependent modulation of the redox network is here presented. The different phenotypes observed after salt exposure in the two rice varieties is coherent with a differential regulation of cell cycle progression and cell death patterns observed at root level. Baldo showed a highly responsive antioxidative capacity, and different pattern of H2O2 accumulation compared to Vialone Nano. Moreover, glutathione metabolism was analyzed at transcriptional, post-transcriptional and post-translational level in both varieties. These results contribute to highlight the role of ROS and antioxidative pathways as a part of a complex redox network activated in rice toward salt stress.

Arabidopsis class I glutaredoxins in the cytosol and endoplasmic reticulum have different oxidoreductase activities

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Glutaredoxins (GRXs) constitute a subfamily of the thioredoxin (TRX) superfamily of oxidoreductases. Although they are characterized by a conserved core structure, the so-called TRX-fold, they show different catalytic activities and functions. In Arabidopsis, GRXs form a large family with 31 members and several subclasses. The first class comprises five GRXs termed GRXC1-5 with a CxxC active site motif plus a close isoform of GRXC5, GRXS12 with a CxxS motif. While GRXC5 and GRXS12 are localized in plastids and have been described earlier, the localization and function of the other class I members is less clear. Thus, our aim is to analyze the precise subcellular localization of GRXC1-GRXC4 by using the redox-sensitive GFP2 (roGFP2) as visible tag. In addition, the function of these GRXs is investigated by combining biochemical activity assays with genetics and characterization of null mutants. Stable expression of the roGFP2 fusion proteins in Arabidopsis show a cytosolic localization for GRXC1 and GRXC2 with GRXC1 being attached to membranes through N-terminal myristoylation. Furthermore, we reveal that GRXC3 and GRXC4 are type II membrane proteins with the catalytic domains facing the lumen of the endomembrane system. In accordance to their different localization sites, we found distinct oxidoreductase activities for GRXC1/2 on the one hand and GRXC3/4 on the other. Taking the advantage of roGFP2 as a biosensor that relies of catalytic activity of GRXs for oxidation and reduction, we show that GRXC1/2 have an enhanced capacity to reduce roGFP2, while GRXC3/4 are more efficiently oxidizing roGFP2. In Arabidopsis, the analysis of the respective single mutants and the grxc1 grxc2 or grxc3 grxc4 double mutant does not display any pronounced phenotype under normal growth conditions. We now seek to further elucidate the cause of the different catalytic properties of the GRXs and if they are required under certain stress conditions.

[YIA] Hydrogen sulfide (H2S) in horticultural plants: endogenous detection and its correlation with L-cysteine desulfhydrase (LCD) activity

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H2S has acquired great attention in plant research because it has signaling functions under physiological and stress conditions. However, the direct detection of endogenous H2S and its potential emission is still a challenge in higher plants. Using the fresh extract of different plant species with agronomical interest including pepper fruits, broccoli, ginger, and different members of the genus Allium including garlic, leek, welsh and purple onion, it was determined the endogenous H2S and its emission using an ion-selective microelectrode and a specific gas detector, respectively. The data show that endogenous H2S content range from pmol to μmol H2S · g-1 fresh weight whereas the H2S emission of fresh-cut vegetables was only detected in the different species of the genus Allium with a maximum of 9 ppm in garlic cloves. Additionally, it was characterized the activity and isozymes of the L-cysteine desulfhydrase (LCD), which is one of the main enzymatic sources of H2S, being the different species of the genus Allium which showed the highest activities. Using non-denaturing gel electrophoresis, the data indicated the presence of up to 9 isoenzymes different LCD isozymes from one in ginger to four in onion, leek, and broccoli. In summary, the data indicate a correlation between higher LCD activity with the endogenous H2S content and its emission in the analyzed horticultural species.
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Proline accumulation under glyphosate toxicity - a signal of tolerance or susceptibility?

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Glyphosate (GLY) is the most used herbicide worldwide, and its cumulative use has been resulting in the contamination of agricultural soils, potentially harming non-target organisms, like crops. Despite GLY's herbicidal activity being not directly related to oxidative bursts, research suggests that a disruption in the redox equilibrium is frequently an unintended result of GLY exposure. Thereby, plants must orchestrate a fine regulation of their antioxidant mechanisms to prevent oxidative stress. Once exposed to GLY, plants experience an overaccumulation of proline (Pro), although this does not seem to be accompanied by a tolerance response. Therefore, the primary question driving this research is whether Pro overaccumulation in response to GLY is a tolerance mechanism or a stress signal. Firstly, Pro content was determined in plants growing on a nutritive medium with increasing GLY concentrations (0.5, 1, and 2 mg/L) for 14 days, to prove that GLY leads to a Pro increase. Then, Arabidopsis thaliana T-DNA insertional mutant lines for genes involved in the Pro pathway (P5CS1 and ProDH) were used to unravel the role of this amino acid in GLY-induced stress. After their functional characterization, p5cs1-1, p5cs1-4, and prodh mutants were used, along with wild-type plants, in an in vitro experiment. After 14 days of exposure to GLY (1 mg/L), a decrease in seedlings' biomass upon herbicide exposure was observed in all genotypes, this being followed by an overaccumulation of Pro. The data also showed that prodh seedlings were the most sensitive, reaching growth inhibition values of 80%, and those that accumulated the highest levels of proline in response to GLY. Overall, these findings suggest that Pro overaccumulation under GLY exposure is related to stress sensitivity rather than stress tolerance. To get a wider picture of the compensatory mechanisms involved, the oxidative metabolism of these genotypes under GLY is currently being studied.

Investigating the H2O2 sensitivity of LbAP1, a YAP1-like homolog in the ectomycorrhizal fungus Laccaria bicolor.

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Ectomycorrhizal fungi (ECM) are an important group of organisms that ensure that trees can flourish in all types of environmental conditions. By providing nutrients, water and protection against stress conditions in exchange for sugars, they allow their host trees to grow. The stress response of plants has already been studied in detail. However, stress response signalling remains poorly understood in ECM fungi. In this study, we assess the potential of H2O2 to regulate a putative transcription factor, LbAP1, using a bio-informatic approach, and localisation of heterologously expressed eGFP-fused LbAP1 upon H2O2 treatment. LbAP1 is a homolog of YAP1, a key regulator of ROS responses in Saccharomyces cerevisiae. Oxidation of two cysteines in YAP1, located in the N- and C-terminal CRD, results in nuclear

accumulation through masking of the NES by disulfide bond formation and transcriptional activation of target genes. In contrast to YAP1, results obtained by fluorescence microscopy showed that an LbAP1-eGFP fusion protein did not accumulate in the nucleus upon treatment of transformed Saccharomyces cerevisiae BY4741 with 0.4 mM H2O2. As C-terminal eGFP could be interfering with disulfide bond formation, the H2O2 sensitivity of eGFP-LbAP1 is currently being examined. Upon analysis of both protein sequence and structure of YAP1 and LbAP1, it was revealed that LbAP1 contains 2 Cys less than YAP1. Furthermore, the Cys responsible for nuclear localisation upon oxidation in YAP1 are not conserved in LbAP1. The NLS and NES, however, are partially conserved. Structural predictions of LbAP1 also showed that the helix structures containing these important cysteines are not conserved, possibly indicating that LbAP1 nuclear localisation is not H2O2 dependent. From these results, we can conclude that LbAP1 regulation might be H2O2 independent, and that the cellular mechanisms of ECM fungi should be investigated separately to other fungi.

Polyamines-a novel molecular coordinator of nitro oxidative signaling in barley senescing leaf

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Plant cells sense fluctuations in the levels of cellular polyamines (PAs), namely, putrescine (Put), spermidine (Spd) and spermine (Spm). PA cellular titer regulates biological processes, including senescence. Changes in PA concentration in properly functioning cells are unlikely. Induced senescence disturbs their homeostasis which leads to senescence-dependent metabolic changes. PA catabolism was proposed as process promoting mainly by H2O2 production. Here, we show that darkness-induced plant senescence is associated with changes in the PA levels together with the changes of signaling molecules such as nitric oxide (NO) and hydrogen peroxide (H2O2). Upon blocking Put catabolism, the senescing cells generated lesser amounts of NO and more of H2O2 and, in turn, accelerated senescence. We opine that a possible linkage exists between PAs and signaling molecules such as NO and H2O2. Thus, the balance in the triad network made of NO↔PA↔H2O2 signaling may be involved in re-programming metabolism and regulate the direction in which a plant may be ushered into - growth, senescence or cell death.

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The herbicidal potential of eucalyptus leaves is related to oxidative bursts and subcellular damage – a case study in a model weed (Portulaca oleracea L.)

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Climate change is aggravating the frequency and intensity of diseases and pests, including weeds' proliferation. To counteract this, farmers are dependent on synthetic herbicide application. However, the generalized use of these agrochemicals has been accompanied by significant environmental impacts, inducing non-target toxicity in different biota, including crops. As the development of new eco-friendly solutions to reduce herbicides' footprint while simultaneously controlling weed growth is urgently needed, the targeted use of the bioactivity of eucalyptus (Eucalyptus globulus Labill.) leaves to inhibit the growth of neighbor plants can constitute an effective approach. To test this hypothesis, different percentages (0, 1, 5, and 10% m/m) of dried and fresh leaves of young eucalyptus trees were incorporated in OECD soil, where purslane (Portulaca oleracea L.) seeds were sown. Over 5 weeks, the germination rate and weed growth were monitored. Results revealed that the incorporation of dried leaves at 10% had the highest herbicidal potential, hampering seed germination by 63% and seedling growth by 70%. To unravel the mode-of-action of this biocide in the early germination steps, purslane seeds were placed in Murashige and Skoog medium containing 0 and 250 g/L of an aqueous extract prepared with dried eucalyptus leaves. After 5 days, radicles and cotyledons were processed for ultrastructural analysis and reactive oxygen species (ROS) in vivo detection (superoxide anion and hydrogen peroxide) by confocal microscopy. The presence of the eucalyptus-based biocide impaired seed germination, corroborating soil-based assays, and led to marked subcellular disorganization and deposition of lipid vesicles in both organs. These changes were strictly related to an oxidative burst, with increased ROS accumulation in both purslane radicles and cotyledons.

Overall, these results point to the efficiency of eucalyptus leaves to be used as a natural herbicide, capable of inhibiting weed germination and growth, inducing ROS overproduction and substantial changes in cell ultrastructure.

The role of NADPH oxidases-dependent ROS in regulating leaf gas exchange and ion flux in Arabidopsis plants under Cd stress

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The involvement of NADPH oxidases in the regulation of K and Cd flux and photosynthesis was assessed in cadmium-challenged wild type and three RBOHs (Respiratory Burst Oxidase Homologues) mutants of Arabidopsis plants (AtrbohC, AtrbohD, and AtrbohF) using different approaches. Plants were grown under hydroponic conditions supplemented or not with 50 µM for 24 h. Results showed that Cd differentially affect photosynthesis and trasnpiration in WT and Atrbohs mutants. Electrophysiological experiments revealed that knocking out RBOHs induced a more Cd2+ influx compared to WT with differential trend in mature and elongation zone, being observed in the Atrboh mutants. Under Cd stress the three Atrbohs mutants had a more ability to retain K+ in the elongation root zone compared to WT, while in leaves, a dramatic reduction in K+ influx was observed in Atrbohs mutants and a change from efflux to intensive influx was registered in WT. The analysis of expression of several K+ and Cd2+ genes transporters and transcription factors suggest that RBOH-dependent H2O2 regulates ion homeostasis and Cd in a highly complex process involving multilevel regulation from transpirational water flow to transcriptional and posttranslational modifications of K/metals transporters.

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Exposure to hydrogen peroxide from beverages and food

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It has been reported that hydrogen peroxide is generated in such beverages as tea and coffee, due to autoxidation of polyphenols present in the extracts. As polyphenols are present also in other beverages and food of plant origin, this study was aimed at checking whether H2O2 can be present in other commonly consumed products. We estimated the formation of hydrogen peroxide in wine, herbal infusions and cooked vegetables using the Xylenol Orange method, enhancing its specificity by assaying two parallel samples, one of them preincubated with catalase.

Variable low concentrations of hydrogen peroxide were found to accumulate in freshly opened red wines during 3-h incubation, from 0 to 6.6 µM, depending on the type and batch. Higher concentrations accumulated during 3-day storage of opened wines (up to ca 30 µM and ca 20 µM in a red wine and a white wine studied, respectively). Like in the tea, hydrogen peroxide was generated in infusions of medicinal herbs. From among 16 herbs studied, the highest concentrations of H2O2 were found in fresh 1% (w/v) infusions of the birch Betula pendula leaves (34.3±3.9 µM), of inflorescence of the lime Tilia cordata (25.9±1.2 µM) and of leaves of the plantain Plantago lanceolata (22.3±0.6 µM). Hydrogen peroxide was also found in the homogenates of cooked vegetables. The highest concentration of hydrogen peroxide in 1:2 (w/v) homogenates was found for the broad bean (73.4±9.0 µM) followed by broccoli (18.6±0.3 µM), onion (10.4±1.6 µM) and leek (10.0±0.3 µM) at pH of about 7; H2O2 concentrations were lower at lowered pH.

These results show that our nutritional exposure to hydrogen peroxide may be higher than estimated to date. These small concentrations of H2O2 do not seem to have deleterious health effects and may be even beneficial due to their bactericidal and virucidal action.

How can redox systems explain water stress tolerance in the grain legume Lathyrus sativus – preliminary results

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Given the current Climate Change scenario, a deeper understanding of abiotic stress resistance in crops is a priority towards securing food and feed supplies. Plants have evolved diverse strategies to avoid and/or cope with the increasingly frequent drought and flooding events and the various stresses associated with them, namely oxidative stress.
Studying reactive oxygen species (ROS) homeostasis within the context of water stress in plants is important, not only because antioxidant defense plays a central role in preventing the impacts of oxidative stress, but also, because complex and highly articulate signal transduction networks involving ROS are required for sensing and adapting to new environmental conjectures.
The legume Lathyrus sativus L. (grass pea) is of high economic importance for food and feed in Asian and African developing countries. Interest has been raising also in the Mediterranean region, where this crop is part of cultural heritage of more marginal areas, due to its outstanding robustness under adverse environmental conditions, like salt, temperature, and water stress, compared with other legume species.
The aim of this work is to unravel some of the molecular mechanisms underlying grass pea' water and oxidative stress response by comparing tolerant and susceptible grass pea accessions, previously identified by an extensive phenotyping of a worldwide collection of germplasm of this species, evaluated under three water treatments (well-watered, waterlogging and water deficit).
Some preliminary metabolite quantification results are presented, namely of glutathione and ascorbate oxidized and reduced forms, as an attempt to unravel contrasting redox conjectures among susceptible and tolerant plants, subject to different water treatments.
Future biochemical and transcriptomic studies will deepen the understanding of the potential role of ROS in response mechanisms to water deficit and waterlogging in grass pea.

Shifts in redox balance with senescence trigger NO decay in Arabidopsis WT plants

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To determine if the cellular redox status created by the developmental phase affects nitric oxide (NO) level, we monitored this signal molecule during 7-day-dark-induced leaf senescence (DILS). DILS was used as a model for the perturbation of redox homeostasis and overproduction of reactive oxygen species. Senescence promoted an unfavorable oxidative environment for NO production. Measuring redox parameters including glutathione couple (GSH/GSSG ratio) we found that the senescence-like phenomenon was effective in cellular homeostasis imbalance, and provoked accumulation of hydrogen peroxide starting from day 3 of DILS. Electrodetection in extracts of individually darkened WT Arabidopsis leaves (leaf 7 of the rosette) revealed a significant drop in NO signal from day 1 to day 7. Notably, reversing the DILS program by restoring light access on day 3 recovered the NO pool, increased chlorophyll content, and enhanced GSH/GSSG ratio. Taken together, significant shift in redox balance favoring decrease in NO emission is an important element in reprogramming the overall organization of senescing events.
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Mitigating the effects of climate change in tomato plants – mission (im)possible?

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Currently, climate change is affecting crop production worldwide. For instance, in the Mediterranean basin, comprising the most important European producers and exporters of tomato, productivity has been rapidly declining, mostly due to the increased temperatures and soil salinization, two conditions that frequently occur together in the environment. Indeed, recent studies carried out by our team showed that the combination of these two stressors affects tomato plants more

severely than the sum of the individual effects. In this sense, it becomes highly important to develop and establish sustainable strategies to improve crop growth under this scenario of climate instability. Thus, the goal of this study was to assess how the exogenous application of two phytohormones [brassinosteroids (BRs; 1 µM 24-epibrassinolide) and strigolactones (SLs; 5 µM GR24)] and a beneficial element (silicon – Si; 2 mM) can modulate the response of tomato plants (Solanum lycopersicum L. var. cerasiforme) to heat and salinity. After 7 d of acclimation to growth chamber conditions, salt stress was applied through NaCl irrigation (100 mM) every alternate day during 28 d, with heat stress being applied in the last 21 d, through exposure to 42 ºC for 4 h every other day. SLs, BRs, and Si treatments were given individually as a foliar spray, twice per week, throughout the experiment. After the growth period, the obtained data indicated that regardless of the applied compound, the macroscopic effects observed in the stress situation were maintained, as well as those related to the oxidative (hydrogen peroxide and lipid peroxidation) and photosynthetic (chlorophyll and carotenoids) metabolism. However, it is important to highlight that there was a small tendency for higher biomass in all sprayed plants, as well as increased glutathione and ascorbate, and reduced proline levels in the case of plants treated with SLs, suggesting the activation of protective pathways.

How does climate change affect Castanea sativa oxidative metabolism? – an insight into the combined effects of drought and high temperatures

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Chestnut (Castanea sativa Miller) is an important species throughout Europe, particularly in Portugal. However, it is highly sensitive to episodes of high temperatures and water scarcity scenarios that are increasingly common due to climate change. Given the need and importance of understanding the impact of these stress factors on the physiology of C. sativa, this study focused on the evaluation of the response of young chestnut plants (3 months old) to the combination of drought and heat (4 h/d 42 ºC). The exposure to high temperatures did not substantially affect the physiological performance of these plants, as no major impacts were found on growth and oxidative metabolism. On the other hand, after 21 days of exposure to drought, individually and in combination, this condition was the one that most affected plant growth. This effect was accompanied, in the case of individual stress exposure, by a decrease in the production of reactive oxygen species, namely superoxide anion (O2.-) and hydrogen peroxide (H2O2), as well as by an increase in lipid peroxidation and proline levels. A prevalence of the effect of drought in the co-exposure treatment was observed, although the plants subjected to the combination of stresses showed an increase in carotenoids and O2.- contents. Ongoing studies are focused on the evaluation of the photosynthetic and antioxidant response of C. sativa to these abiotic stresses ensure a holistic view on the impact of climate change on chestnut plants. Additionally, and recognizing the imperative need to develop new effective and eco-friendly approaches to overcome the harmful consequences of these unfavorable conditions, studies are also underway to assess the potential of mycorrhization as a strategy to mitigate the effects of climate change on this species.

Ameliorative effects of ascorbate on increasing tomato tolerance to NiO nanomaterials - an in vitro approach

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While nanomaterials (NMs) offer wide-ranging solutions, their intensified use has been resulting in the contamination of the environment, posing ecotoxicological risks to diverse organisms, including plants. In this sense, it becomes important not only to understand the phytotoxicity of NMs, but also to find efficient and sustainable strategies to enhance plant tolerance to these emergent contaminants. Thus, this study aimed at assessing the potential of ascorbic acid (AsA), a powerful antioxidant (AOX), in enhancing the tolerance of in vitro grown tomato seedlings to nickel oxide NMs (nano-NiO). For this purpose, seeds of Solanum lycopersicum L. cv. Micro-Tom were germinated in half-strength MS medium supplemented with 30 mg L-1 nano-NiO alone or in combination with 150 mg L-1 AsA. A control situation, without nano-NiO nor AsA, was also included. After 28 days of exposure, the growth and development of S. lycopersicum was

severely repressed by nano-NiO, with evident phytotoxicity symptoms (leaf chlorosis and necrosis), that did not translate, however, into severe redox disorders. Even so, proline levels, SOD and DHAR activities were diminished in shoots of nano-NiO-exposed plants, while glutathione, phenols, CAT and GR activities were increased. In response to the co-exposure to AsA, nano-NiO-induced growth inhibition was efficiently counteracted, being accompanied by a more notorious response of the AOX network, especially of glutathione, phenolics, SOD and GR. Surprisingly, the solo AsA administration in the culture medium suppressed growth and development of tomato seedlings, increased the lipid peroxidation of membranes and inhibited key enzymes of the AsA-glutathione cycle, in spite of enhancing the total AOX capacity, with increased levels of proline, phenols and glutathione. Overall, exposure to nano-NiO negatively impacted tomato seedlings' growth, and co-application of exogenous AsA had stress-ameliorative effects by enhancing the AOX response to counteract nano-NiO-induced growth inhibitory effects.

Phytoglobin expression alters the Na+/K+ balance and antioxidant responses in soybean plants exposed to Na2SO4

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Soybean (Glycine max) is an economically important crop which is very susceptible to salt stress. Tolerance to Na2SO4 stress was evaluated in soybean plants over-expressing or suppressing the phytoglobin GmPgb1. Salt stress depressed several gas exchange parameters, including photosynthetic rate, caused leaf damage, and reduced water content and dry weights. Lower expression of Respiratory Burst Oxidase Homologs (RBOHB and D), as well as enhanced antioxidant activity, resulting from GmPgb1 overexpression, limited ROS-induced damage in salt-stressed leaf tissue. The leaves also exhibited higher activities of the H2O2-quenching enzymes, catalase (CAT) and ascorbate peroxidase (APX), as well as enhanced levels of ascorbic acid. Relative to WT and GmPgb1-suppressing plants, over-expression of GmPgb1 attenuated the accumulation of foliar Na+ and exhibited a lower Na+/K+ ratio. These changes were attributed to the induction of the Na+ efflux transporter SALT OVERLY SENSITIVE 1 (SOS1) limiting Na+ intake and transport, and the inward rectifying K+ channel, POTASSIUM TRANSPORTER 1 (AKT1), required for the maintenance of the Na+/K+ balance.

Mj-MSP18 effector suppresses ROS production and plant immunity: Could BSK7 be the explanation?

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Plant-parasitic nematodes produce effectors to overcome plant immunity and finetune plant cellular processes. Many effectors have been shown to influence plant immunity to support nematode survival, but their direct targets and exact mode of action are still poorly understood. Identifying protein-protein interactions and newly formed complexes with effectors is crucial for understanding this cross-kingdom network. On the other side, it has been shown that host-derived ROS (reactive oxygen species) production plays a huge role in plant basal defense against nematodes.
Using the proximity labeling technique (TurboID-MS), we have identified tomato proteins involved in various cellular processes interacting with M. javanica effectors. Among those, the Mj-MSP18 effector, whose homolog has been shown to suppress cell death, was found to interact with tomato Sl-BSK7 potentially, a homolog of arabidopsis BSK7 (BRASSINOSTEROID-SIGNALLING KINASE7), serine/threonine kinase that acts as a positive regulator of brassinosteroid (BR) signaling downstream of the receptor kinase BRI1. To examine the influence of Mj-MSP18 on downstream signaling, we performed RNA-seq upon overexpression of Mj-MSP18 in tomato hairy roots and found downregulation of plant defense and response to hydrogen peroxide-related genes. Moreover, many downregulated genes have been previously reported to play a role in brassinosteroid-mediated resistance against root-knot nematodes. Furthermore, we confirmed a significant decrease in ROS production on a biochemical level upon transient expression of Mj-MSP18 in flagellin22 treated N. benthamiana leaves. Additionally, we depicted its role in suppressing plant cell death and callose deposition, one of plant immunity's main markers.
We hypothesized that differentially expressed genes are a likely consequence of MSP18 interaction with Sl-BSK7 and suppression of downstream signaling of BR receptor and its signaling transduction with FLS or other PAMP receptors leading to suppression of ROS, basal defense, and attenuation of host resistance to the nematode infection.

Small paraquat resistance proteins modulate paraquat and ABA responses and confer drought tolerance to Arabidopsis.

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Several small peptides have recently been described to modulate responses to stress conditions. The Small Paraquat resistance protein (SPQ) of Lepidium crassifolium has previously been identified due to its capacity to confer paraquat resistance to overexpressing transgenic Arabidopsis plants. Here we show, that overexpression of the closely related but previously unknown Arabidopsis SPQ can also enhance resistance to paraquat, while the knockout Arabidopsis mutant is slightly hypersensitive to this herbicide. Overexpression of SPQs enhanced sensitivity to abscisic acid (ABA), while the knockout spq1 mutant was less sensitive to ABA. Both Lepidium and Arabidopsis-derived SPQs could improve drought tolerance by reducing water loss, stabilizing photosynthetic electron transport, reducing oxidative damage and enhancing plant viability in a water-limited environment. Enhanced drought tolerance of SPQ overexpressing plants could be confirmed by characterizing parameters of growth, morphology and photosynthesis using an automatic plant phenotyping platform with RGB and chlorophyll fluorescence imaging. Our results suggest that SPQs can be regulatory small proteins connecting ROS and ABA regulation and through that influence responses to certain stresses.

Bioactivity of rush-specific (Juncus sp.) compounds on antioxidant defence mechanisms of Arabidopsis thaliana

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The monocotyledonous rush species (Juncus) are mainly perennial plants, but some representatives are annual. Several Juncus plants are used in the Traditional Chinese Medicine for the treatment of numerous diseases. Juncaceae species produce different types of secondary metabolites e.g. phenantrenes, flavonoids, triterpenes and steroids, which can be very promising for the modern medicine as well. These natural compounds possess antiproliferative, anticancer, antimicrobial, anti-inflammatory, antioxidant, cellular protective and antialgal effects. However, these compounds have not been tested on antioxidant defense system of higher plants. Here, we showed the first bioactivity tests of phenantrene-like compounds, effusol and juncusol isolated from J. gerardii on Arabidopsis thaliana seedlings. Tested concentrations of phenantrene deterred the growth of A. thaliana. However, plants treated with effusol and juncusol did not show negative effects at these concentrations, influencing the antioxidant defense system of plants. Our results could help us to decipher the role of phenanthrene-like compounds in plant growth and development.

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Carotenoid biosynthesis contributes to the ascorbate-glutathione cycle regulation to protect against ROS accumulation in microalga Euglena gracilis

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The antioxidants, ascorbate and glutathione, which are regenerated by the ascorbate-glutathione cycle, and carotenoids, are required for oxidative stress defense in photosynthetic organisms. The euglenophyte Euglena gracilis is a model microalga, which is used for both biological studies and industrial applications. Previous study has shown that treatment of E. gracilis with carotenoid biosynthesis inhibitor, norflurazon, suppresses photo-induction of ascorbate peroxidase enzyme (APX), suggesting the functional relationship between carotenoids and the ascorbate-glutathione cycle in this alga. In this study, using RNAi-mediated knockdown cells of carotenoid biosynthetic gene lycopene cyclase (EgLCY), we investigated the effects of suppressed carotenoid biosynthesis on the ascorbate-glutathione cycle and oxidative stress status in E. gracilis.

The KD-lcy cells, which were introduced double-stranded RNA, showed colorless appearance and 81% decrease in the total carotenoid contents compared to wild-type cells. The APX and superoxide dismutase (SOD) activities of KD-lcy cells decreased by 52% and increased 7.9 times, respectively, compared to wild-type cells. Among the ascorbate-glutathione cycle, the dehydroascorbate reductase (DHAR) and monodehydroascorbate reductase (MADR) activities of

KD-lcy cells increased by 77 and 45%, respectively, compared to wild-type cells and their glutathione reductase (GR) activity decreased by 29%. Correlating with the altered enzyme activities of the ascorbate-glutathione cycle enzymes, the ascorbate contents of KD-lcy cells increased by 55%, whereas their glutathione contents decreased by 38% compared to wild-type cells. The imaging analysis using H2O2-specific fluorescent reagent demonstrated that KD-lcy cells accumulated significantly higher level of H2O2 than that of wild-type cells. These results reveal that carotenoid biosynthesis contributes to chloroplast development and the ascorbate-glutathione cycle homeostasis, resulting oxidative stress defense in E. gracilis. To our knowledge, this study clearly reports for the first time the possible relationship between major antioxidant systems carotenoid biosynthesis and the ascorbate-glutathione cycle in photosynthetic organism.

An Exploration of Mitochondrial Respiration and ROS in the Green Alga Chlamydomonas reinhardtii

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In this study, we examined the cellular response to ROS-associated oxidative stress in instances of mitochondrial respiratory dysfunction. Our model of study is the green alga Chlamydomonas reinhardtii. As a preliminary assessment of the cellular ROS response, we monitored the growth of a collection of algal respiratory deficient mutants via tenfold dilution series. The cells were challenged with exogenously applied H2O2 and different light intensities that we presume augment internal ROS production. From this study, we uncovered that complex I (CI) mutants, regardless of their degree of defect in holoenzyme assembly and/or activity, do not appear to be more resistant or sensitive when exposed to our ROS treatment. This suggests that mutants impaired for CI function do not produce deleterious amounts of ROS unlike what has been reported in the context of mitochondrial CI dysfunction in humans. One possible explanation that accounts for this discrepancy is the operation of the natural CI bypass in plants, namely alternative NADH dehydrogenases, which are nonexistent in human cells. Interestingly, complex III (CIII) null mutants displayed an enhanced sensitivity when challenged with ROS. CIII mutants that have partially reverted to respiratory proficiency, however, display wildtype-like resistance to ROS treatment. This suggests that CIII deficiency may result in increased internal ROS production within the cell that causes death when additional external oxidative stress is applied. Alternatively, CIII function could be necessary to activate the physiological response necessary to detoxify ROS as evidenced in another model system, the yeast Saccharomyces cerevisiae. Moving forward, we intend to assay the expression and activity of ROS-detoxifying machinery in CIII deficient and wild-type cells.

Aldo keto reductases: New players in nitric oxide homeostasis

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Nitric oxide (NO) is a short-lived gas that acts as a signaling molecule in all higher organisms, including plants. Despite the involvement of NO in multiple plant processes, including germination, root growth and fertility, a basic understanding of the mechanisms by which NO exerts its effects is lacking. NO and its derivatives impact these physiological processes through reversible S-nitrosation of critical protein cysteines. In cells, regulation of NO-levels is predominantly achieved by reaction of reactive nitrogen species (RNS) with glutathione (GSH), thereby forming S-nitrosoglutathione (GSNO), a principal NO reservoir. Mutation of Arabidopsis thaliana S-nitrosoglutathione reductase (GSNOR; hot5-2) leads to higher intracellular concentrations of S-nitrosothiols, confirming that the GSNOR reduction of GSNO is a major route of GSNO catabolism in plants and other eukaryotes. We demonstrate in Arabidopsis that absence of GSNOR results in differential regulation of proteins involved in chlorophyll metabolism, the general stress response and photosynthesis. In addition, our proteomic analysis identified a significant increase in proteins that belong to the aldo-keto reductase (AKR) protein superfamily, AKR4C8 and 9. Since specific AKRs have been linked to NO metabolism in mammals, we expressed and purified
Arabidopsis AKR4C8 and 9 and close homologues AKR4C10 and 11 and determined that they have NADPH-dependent activity in GSNO and S-nitroso-coenzyme A (SNO-CoA) reduction. Plants lacking GSNOR also show increased activity of NADPH-dependent GSNO reduction, consistent with increased AKR activity. Taken together, these data define a new, NADPH-dependent component of NO metabolism that may be integrated with NADH-dependent GSNOR activity to control NO homeostasis in plants and other organisms.

Transcriptomics analysis of the roles of a specific glucose-6-phosphate dehydrogenase in oxidative stress responses

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Catalase-deficient plants such as the Arabidopsis cat2 mutant are useful systems to study responses to intracellular oxidative stress. In certain conditions, cat2 shows lesions on the leaves and accumulation of typical pathogenesis-related factors such as salicylic acid (SA) and SA-dependent gene expression. All these downstream effects of oxidative stress can be prevented by the sid2 mutation, which causes deficiency in the isochorismate synthase 1 (ICS1)-dependent pathway of SA synthesis.

NADPH is a key redox carrier in cells and can play multiple roles in reactive oxygen species (ROS) homeostasis and signaling. NADPH is required both for antioxidant metabolism (e.g., supporting the ascorbate-glutathione pathway) and for pro-oxidant ROS signaling (e.g., NADPH oxidase functions). Several types of enzyme can produce NADPH from the oxidized form, NADP+, and each of these enzymes is encoded by several genes. To explore specific roles of NADPH-producing enzymes in oxidative stress signaling, multiple loss-of-function mutants have been crossed with cat2. One of the double mutants obtained, cat2 g6pd5, in which a cytosolic glucose-6-phosphate dehydrogenase activity is compromised, shows much decreased oxidative stress responses, notably an absence of lesions on the leaves and weakened SA biosynthesis and signaling.

To explore how the g6pd5 mutation produces these effects, several approaches are being used, including transcriptomic comparison of cat2 g6pd5 and cat2 sid2. Mutants for NADPH-dependent pathways (ascorbate-glutathione pathway and NADPH oxidases) have also been crossed with cat2 to establish whether their transcriptomic signatures are similar to that observed in cat2 g6pd5. This will help to decipher the potential roles of a specific NADPH-producing respiratory enzyme in determining the outcome of oxidative stress of intracellular origin.

Ferritin improves aluminium tolerance in chickpea by regulating the formation of reactive oxygen species

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The severity of aluminum (Al) toxicity on agricultural productivity in acidic soil has spurred vast research studying Al tolerance in plants. The efflux of organic acids e. g. citrate, malate, and activation of their transporters have been proved as the key mechanism of Al tolerance. However, like other abiotic stresses, Al toxicity has great potential to generate oxidative stress in the plant cell. But research elucidating the effective management of oxidative stress in the plant during Al toxicity is not available. The present study demonstrates the development of Al tolerance in chickpea by controlling oxidative stress. Two contrasting genotypes of chickpea e. g. RSG 974 (Al tolerant) and RSG 945 (Al sensitive) were used as test plants. Al-tolerant genotype (RSG 974) showed lesser inhibition of root growth as well as lower oxidative damages, measured in terms of the accumulation of H2O2 and lipid peroxidation compared to the Al-sensitive genotype (RSG 945). Subsequently, DDRT-PCR results showed the differential expression of two genes, chitinase and ferritin in chickpea genotypes after the treatment of 1.0 mM Al. Further, Q-PCR analyses confirmed the Al-responsive expression of chitinase in the Al-tolerant chickpea genotype, whereas, the expression of ferritin was found to be constitutively higher in the Al-tolerant genotype compared to Al-sensitive chickpea. The biochemical data showed that the higher expression of ferritin in Al-tolerant chickpea genotypes in the present study is associated with the efficient sequestration of free iron responsible for generating reactive oxygen species. Whereas, aluminium sensitive genotype of chickpea (RSG-945) due to negligible expression of ferritin could not efficiently control the free form of Fe and hence failed to avoid the consequences and thereby became more susceptible to Al toxicity. Further experiments are underway to provide the mechanistic details of managing oxidative stress in chickpea by ferritin to alleviate Al toxicity.

The redox environment differentially regulates autophagy in leaves and roots of Arabidopsis thaliana during cadmium stress

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Pollution of soils with metals such as cadmium (Cd) inhibits plant growth, thereby hindering their economic validation. Cadmium induces an imbalance between reactive oxygen species (ROS) and antioxidants at the cellular level, which can damage macromolecules. Damaged cellular components can be engulfed by vesicles (i.e. autophagosomes) that transport them into the vacuole for nutrient recycling in a process called autophagy. Autophagy is increasingly put forward as a protective mechanism during various stresses that may be intertwined with the redox environment. Therefore, this research aims to investigate autophagy in leaves and roots of Arabidopsis thaliana exposed to Cd and its connection to the Cd-induced oxidative challenge.

One important protein that is involved in autophagosome formation is ATG8, which is encoded by nine isoforms in A. thaliana. At the transcript (RT-qPCR) and protein levels (western blot), A. thaliana plants exposed to 5 µM Cd for 24 h in hydroponics show an increased expression of ATG8. Confocal microscopy analyses are currently performed in Cd-stressed GFP-ATG8 reporter lines to further confirm autophagy induction. To investigate the link between autophagy and the redox environment during Cd stress, the expression of all ATG8 isoforms was compared between wild-type and glutathione (GSH)-deficient cad2 plants. Overall, no clear difference could be observed between both genotypes under control conditions. However, an overall lower induction could be observed for Cd-responsive ATG8 isoforms in the leaves of mutant plants following Cd exposure. In contrast, the overall expression level in the roots was rather higher in Cd-exposed mutants compared to wild-type plants, which was mainly attributed to ATG8E. This indicates that the redox environment differentially affects ATG8 expression and potentially autophagy in both plant organs during Cd stress.

Analysis of the different isoforms of Arabidopsis monodehydroascorbate reductase

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Hydrogen peroxide (H2O2) is a major reactive oxygen species (ROS) whose level must be carefully controlled to allow optimal cellular signaling. One important plant antioxidant enzyme that removes excess H2O2 is ascorbate peroxidase (APX), which reduces this reactive molecule to water. Ascorbate peroxidase requires ascorbate and, in reducing H2O2, produces monodehydroascorbate (MDHA), which must be regenerated to ascorbate by reductases.
One of the regenerating enzymes is MDHA reductase, which converts MDHA directly to ascorbate.
In Arabidopsis, several MDHAR isoforms exist and they are found in different subcellular compartments. While there are two peroxisomal isoforms (MDHAR1 and MDHAR4), MDHAR2 and MDHAR3 are found in the cytosol, and dual targeting of MDHAR5/6 allows expression in mitochondria and chloroplasts, respectively. It is widely thought that MDHAR plays an important role in the response of plants to oxidative stress by maintaining the redox state of intracellular ascorbate, but few specific detailed studies of the different isoforms have been conducted.
We are performing a detailed comparison of the different MDHAR isoforms. To establish how these enzymes work within the cellular redox network, a comparison of their affinities for NADH and NADPH is being conducted using recombinant enzymes while a reverse genetics approach using specific mutants aims at assigning specific biological functions to each gene. To this end, single and double mutant lines are being produced. Questions we aim to answer include whether the different MDHARs show specificity in biochemical properties as well as the importance of each for the functioning of plants under optimal and stress conditions.

Characterization of the Arabidopsis RBPome in oxidative stress conditions

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Cellular redox signaling is triggered by accumulation of various reactive oxygen species (ROS) that integrate with other signaling cascades to enable plants to ultimately respond to (a)biotic stresses. The identification of key regulators underlying redox signaling networks is therefore of high priority. We improved an mRNA interactome capture method that allows to systematically detect oxidative stress responsive regulators in the post-transcriptional gene regulation (PTGR) pathway. The protocol includes Arabidopsis cell cultures, setup of oxidative stress conditions, short-term exposure to UV irradiation, cell lysis, pull-down and purification of crosslinked messenger ribonucleoproteins, their mass spectrometric analyses and identification of the proteome by statistical analyses. As a result, a comprehensive inventory of the functional oxidative stress responsive RBPome (OxRBPome) is generated. Novel candidate proteins with previously unlinked functions to RNA biology, were further explored to pave the way towards new insights into PTGR processes in redox signaling. The obtained comprehensive view of the RBPome under oxidative stress provides us a more detailed insight and understanding of post-transcriptional regulatory processes in redox signaling in plants.

Characterization of mitochondrial electron transport mutants under stress conditions

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Plants responses to adversely altered environmental conditions encompass changes in plant growth and development as well as in photosynthesis, respiration, metabolite assimilation and catabolism. Accumulation of reactive oxygen species (ROS) is a consequence of abiotic stresses when ROS can damage proteins, lipids, and other macromolecules and therefore create additional oxidative stress for the plants. Although chloroplasts are main source of ROS, mitochondria are also important in the maintenance of the cellular redox homeostasis and regulation of cellular ATP supply in adaptive responses to stress conditions. In mitochondria, over-reduction of the electron transport chain is the primary reason for ROS accumulation, which can be reduced by protecting and stabilizing the electron flow.

To reveal the importance of genes encoding the mitochondrial proteins in stress responses, we analyzed insertion mutants of 12 Arabidopsis thaliana genes, encoding the subunits of Complex I and III of mitochondrial electron transport. Phenotypes of the mutants were characterized in osmotic, salt and oxidative stress conditions. Morphological alterations and differences in tolerance to drought and salinity were revealed through germination and growth tests and by complex phenotyping. One mutant was characterized in detail in which the mutation disrupted the NDUSF8.2 gene. The ndusf8.2-1 mutant was hypersensitive to oxidative stress, although had less hydrogen peroxide level and lower lipid peroxidation rates under osmotic stress. Changes in chlorophyll fluorescence under stress treatments suggested that this Complex I mutation can influence photosynthesis as well. Moreover, alteration were found in activities of mitochondrial complexes, in ATP/NAD(P)H levels and activities and expression of the alternative oxidoreductases (altNDs, AOXs). Our data revealed that NDUSF8.2 is important in plants stress responses, and strong correlation exist between mitochondrial functions, photosynthetic activity and energy production.

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