SBIO 2024
Pont-à-Mousson
3rd Plant and Human Sulfur Biology meeting

Pont-à-Mousson, 8th-12th of September 2024

Organizers

Jérémy Couturier, University of Lorraine
Nicolas Rouhier, University of Lorraine

Scientific committee

Stanislav Kopriva, University of Cologne, Germany
Luis Romero, University of Sevilla, Spain
Cecilia Gotor, University of Sevilla, Spain
Guenter Schwarz, University of Cologne, Germany
Jonathan Wolf Mueller, University of Birmingham, UK

arbre
UNIVERSITÉ DE LORRAINE
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Deutsch-Französische Hochschule

Sunday 8th September

16h30-19h Registration

19h30 Welcome Cocktail

Monday 9th September

8h45 Welcome session
Jérémy Couturier and Nicolas Rouhier

S-metabolism session 1 (Chair: Stanislav Kopriva)
9h-9h30 Jutta Papenbrock - Sulfotransferases and their role in glucosinolate biosynthesis analyzed in various stress conditions
9h30-10h Jon Mueller - Sulfation of Steroids in Humans - Conferring Directionality
10h-10h20 Patrick Lehr - Sulfur fertilization enhances drought stress response
10h20-10h40 Anna Wawrzynska - LSU proteins enhance sulfate assimilatory pathway flux in *Arabidopsis thaliana*

10h45 Coffee break

11h10-11h40 Silke Leimkühler - 2-Thiouridylases for tRNA in pro- and eukaryotes
11h40-12h10 Ann Cuypers - How sulfur allocation impacts plant responses to cadmium stress: from signalling to acclimation
12h10-12h30 Daniela Ristova - Interaction of S homeostasis with N signaling in *Arabidopsis thaliana*
12h30-12h50 Takehiro Ito - Elucidation of glutathione degradation pathway in *Arabidopsis thaliana*

13h-14h30 Lunch

S-metabolism session 2 (Chair: Luis Romero)
14h45-15h15 Claus Jacob - Harnessing the power of sulfur: redox catalysis, nanotechnology and biomedical innovations
15h15-15h45 Takaaki Akaike - Metabolism and redox signal regulation by supersulfides
15h45-16h05 Shingo Kasamatsu - Development of mass spectrometry-based supersulfidomics and its potential: alternations in supersulfide production during the germination of broccoli sprouts
16h05-16h25 Suvajit Basu - Exploring uncharted territories: new genes for sulfur starvation responses in plants

16h30 Coffee break

17h Poster session

Plenary Session 1 (Chair: Stanislav Kopriva)
18h Kazuki Saito - A 35-year journey in plant sulfur research: a personal perspective

19h30 Dinner

Tuesday 10th September

Redox regulation session 1 (Chair: Marcel Deponte)

9h-9h30 Stefanie Müller-Schüssele - Class I glutaredoxins in plastid oxidative stress: repair crew or regulators?

9h30-10h Günter Schwarz - Molybdenum in health and disease: Thiol and sulfide signaling in metabolism and synapse function

10h-10h20 Ayaka Kinno - Analysis of pathological progression-dependent changes of supersulfides production in the brain tissues of mouse models of Alzheimer's disease

10h20-10h40 Natacha Donnay - The plant DCC1 are atypical thioredoxins with a holdase activity

10h45 Coffee break

11h10-11h40 Tobias Dick - Site-specific activation of proton pump inhibitors by tetrathiolate zinc centers

11h40-12h10 Jose Ugalde - Decoding the function of Tau Glutathione S-transferases: GSTU24 and GSTU25 in Arabidopsis

12h10-12h30 Ginevra Peppi - Functional insights into the catalytic and redox-based regulatory properties of AKR4Cs from Arabidopsis thaliana

12h30-12h50 Laura Morette - Phylogenetical, biochemical and structural insights into the iota glutathione transferase from the cyanobacteriota Synechocystis sp. pcc 6803, an atypical glutathione transferase exhibiting an unexpected FMN-binding domain

13h-14h30 Lunch

H_2S signalling session (Chair: Cecilia Gotor)

14h45-15h15 Ruma Banerjee - Sulfide signaling and mitochondrial redox metabolism

15h15-15h45 Angeles Aroca - Hydrogen sulfide: a key ally in adapting to climate change

15h45-16h05 Benjamin Selles - The complex interaction network of human sulfurtransferases with H_2S oxidation pathway proteins

16h05-16h25 Tatjana Hildebrandt - Compartmentalization of cysteine metabolism in plants affects stress signaling

16h30 Coffee break

17h Poster session

Plenary Session 2 (Chair: Guenter Schwarz)

18h Milos Filipovic - Protein persulfidation enters a new phase

19h30 Dinner

Wednesday 11th September

Redox regulation session 2 (Chair: Nicolas Rouhier)

9h-9h30 Marcel Deponte - How do glutaredoxins reduce non-glutathione disulfides?

9h30-10h Mirko Zaffagnini - The intricate relationships between glutathione and proteins: interaction and redox modulation

10h-10h20 Sophie Hendrix - Glutathione peroxidase-like 8 (GPXL8): a new player in H_2O_2 signaling in *Arabidopsis thaliana*?

10h20-10h40 Zhichao Liu - Biochemical characterization of thioredoxin-related protein Clot/TRP14 from *Populus trichocarpa*

10h45 Coffee break

Glucosinolates session (Chair: Jutta Papenbrock)

11h10-11h40 Masami Hirai - Plant survival strategies responding to environmental stress by controlling sulfur allocation

11h40-12h10 Luke Bell - The role of sulfur in salad rocket (*Eruca vesicaria subsp. sativa*) nutritional and sensory quality

12h10-12h30 Christian Zörb - Glucosinolates in Kohlrabi after nitrogen fertilization increase as detected by a NIRS method

12h30-12h50 Georgios Stylianidis - Sulfur-based biofortification of wheat with iron: cysteine-based biofortification schemes

13h-14h30 Lunch

Emerging topics session (Chair: Jean-Pierre Jacquot)

14h45-15h15 Andreas Meyer - Lipoylation overkill: boosting lipoylation capacity causes the release of sulfide

15h15-15h45 Stéphane Lemaire - Synthetic and systems biology of carbon fixation in *Chlamydomonas*

15h45-16h05 Uladzimir Barayeu - NOS produces cyclic octasulfur that enables protection against lipid peroxidation in lipid droplets

16h05-16h25 Eve-Lyn Hinckley - Sulfur: a major element undergoing global change

16h30 Closing session
Jérémy Couturier and Nicolas Rouhier

17h Coffee break

17h30 Guided tour of Abbey

20h Conference dinner

Abstracts of talks

S-metabolism session 1

Sulfotransferases and their role in glucosinolate biosynthesis analyzed in various stress conditions

Elia Kletschkus[1], Khushal Borse[1], Johann Hornbacher[1], Ina Horst-Niessen[1] and Jutta Papenbrock[1]

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Sulfotransferases (SOTs) catalyze the final step in glucosinolate (GSL) biosynthesis. They transfer a sulfate group from 3-phosphoadenosine 5-phosphosulfate (PAPS) to desulfoglucosinolates (dsGSLs). In *Arabidopsis thaliana*¸ three SOTS, AtSOT16, AtSOT17 and AtSOT18, are present and differ in their substrate specificity. It was shown that in biotic and abiotic stress conditions concentrations of a number of GLSs are increased [1]. For example, analysis of GSLs in drought-stressed plants revealed higher contents of glucobrassicin in shoots and roots compared to control plants [2,3]. Deuterium incorporation experiments showed the highest turnover of glucobrassicin compared to all other GSLs during drought conditions [3]. To better understand the role of the different AtSOT proteins various single, double and triple mutant lines have been produced. The mutants were grown in standard and under drought and heat stress conditions. Results in phenotyping and GSL concentrations indicate only small differences between the three single *atsot16* to *18* mutants. Also, *atsot16* to *18* double mutants did not show major differences when cultivated in standards conditions in comparison to wild-type plants. When *atsot* double mutants were grown in stress conditions phenotypical differences became obvious and some chemically similar GSLs increased, whereas others decreased in their concentrations. Selected results will be presented and discussed to illustrate the challenging and not yet conclusive observations for the three SOT proteins 16 to 18 in *Arabidopsis thaliana*.

[1] Zamani-Noor, N., Hornbacher, J., Comel, C.J., Papenbrock, J. (2021) Variation in glucosinolate contents in clubroot-resistant and susceptible *Brassica* crops in response to virulence of *Plasmodiophora brassicae. Pathogens,* 10: 563. DOI: 10.3390/pathogens10050563

[2] AbdElgawad, H., Zinta, G., Hornbacher, J., Papenbrock, J., Markakis, M.N., Asard, H., Beemster, G.T.S. (2023) Elevated CO_2 mitigates the impact of drought stress by upregulating glucosinolate metabolism in *Arabidopsis thaliana*. *Plant, Cell & Environment* 46(3): 812-830. DOI: 10.1111/pce.14521

[3] Hornbacher, J., Horst-Niessen, I., Herrfurth, C., Feussner, I., Papenbrock, J. (2022) First experimental evidence suggests use of glucobrassicin as source of auxin in drought-stressed *Arabidopsis thaliana. Frontiers in Plant Science* 13: 1025969. DOI: 10.3389/fpls.2022.1025969

Sulfation of steroids in humans – conferring directionality

Jonathan Wolf Mueller[1]

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Sulfation pathways are the non-reductive part of overall sulfur biology. Sulfation pathways are centered around activation of sulfate, transfer of a sulfuryl group, and cleaving off that sulfate again. For steroid hormones, sulfation represents both, an additional layer of regulation, and a shuttle to transport steroid precursors into otherwise non-steroidogenic compartments. This directionality is achieved by tissue-specific expression of sulfation genes, and by additional levels of regulation.

A number of steroidal compounds get sulfated. A notable group of them are different Vitamin D metabolites. Sulfo-vitamin D seemingly plays a role in shuttling vitamin D into the milk of breastfeeding mothers; where sulfo-vitamin D can be detected at elevated levels.

Sulfation in the liver and the adrenal is supported by high expression of the sulfate-activating enzyme PAPS synthase 2, and the sulfuryl-transferring enzyme SULT2A1. Evidence suggests that, at least in humans, the two enzymes physically interact with each other. Rare disease studies with patients with compound-heterozygous inactivation of different PAPS synthases, suggested non-overlapping gene functions for PAPSS1 and PAPSS2, as one cannot complement for the other. A recent study suggests that these two PAPS synthase enzymes differ in how redox pathways can regulate their enzymatic activity. The PAPSS2 protein lacks a set of four cysteine residues, contrasted to its sister enzyme PAPSS1. This prevents oxidation-induced inhibition of PAPSS2, compared to other PAPS synthase isoforms.

At the “end” of sulfation pathways, there is the steroid sulfotransferase enzyme, that hasn’t been understood in great detail. New insights into the enzymatic machinery of sulfation pathways include a recent structural biology study on the steroid sulfatase enzyme; now suggesting this essential sulfatase as a trimeric complex, adopting a membrane-associated configuration, “swimming” in one half of the double membrane.

[1] Foster PA, and **Mueller JW**. **2023**. New structural insights provide a different angle on steroid sulfatase action. *J Steroid Biochem Mol Biol*. doi: 10.1016/j.jsbmb.2023.106353.

[2] Brylski O, Shrestha P, House PJ, Gnutt P, **Mueller JW**, Ebbinghaus S. **2022**. Disease-Related Protein Variants of the Highly Conserved Enzyme PAPSS2 Show Marginal Stability and Aggregation in Cells. *Front Mol Biosci*. doi: 10.3389/fmolb.2022.860387.

[3] Jenkinson C, Desai R, McLeod MD, **Mueller JW**, Hewison M, Handelsman DJ. **2022**. Circulating Conjugated and Unconjugated Vitamin D Metabolite Measurements by Liquid Chromatography Mass Spectrometry. J Clin Endocrinol Metab. doi: 10.1210/clinem/dgab708.

Sulfur fertilization enhances drought stress response

Patrick Pascal Lehr[1], Alexander Erban[2], Joachim Kopka[2] and Christian Zörb[1]

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Sulfur is commonly used as a crop protection agent in viticulture, but its role as a fertilizer has been largely underexplored. Prior research in other plant species suggests that sulfur is crucial for the drought stress response. To explore the potential of sulfur as a protective measure to enhance drought stress tolerance, a greenhouse experiment using both grapevine and maize was conducted. This dual-species approach aimed to determine if sulfur effects are species-specific. The experimental design included additional sulfur fertilization followed by an untargeted metabolite analysis. The results showed a significant increase in sulfate concentrations in the leaves of both grapevine and maize under drought conditions, with this increase only observed with supplemental sulfur fertilization. These findings support the hypothesis that sulfate plays a role in drought response. Enhanced sulfur fertilization led to increased sulfate availability, which in turn improved the plants' drought response. This improvement was marked by a pronounced metabolic adjustment, particularly in amino acids and sugars, under drought stress conditions. The results indicate that additional sulfur fertilization enhances the drought response and stress tolerance of crops. This suggests that sulfur fertilization could be a viable strategy to improve the resilience of crops to drought stress, providing a significant agronomic benefit.

LSU proteins enhance sulfate assimilatory pathway flux in *Arabidopsis thaliana*

Justyna Piotrowska[1], Anna Wawrzynska[1], Michał Krzysztoń[1], Marcin Olszak[1], Anastasia Apodiakou[2], Rainer Hoefgen[2], José María López Ramos[3], Stanislav Kopriva[3] and Agnieszka Sirko[1]

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Unable to move, plants have evolved complex mechanisms to sense and absorb nutrients from the environment, adjusting their growth and development according to the availability of these nutrients. Sulfur is an essential nutrient for plants, therefore, the pathways of its uptake and assimilation have been thoroughly researched. Significant progress has been made in elucidating the individual genes and enzymes, as well as their regulation. However, in comparison to other nutrients, the mechanisms regulating sulfur metabolism and homeostasis remain largely unknown. LSUs (RESPONSE TO LOW SULFUR) are plant-specific proteins with an unknown molecular function that were first identified during transcriptomic studies on the sulfur deficiency response in Arabidopsis. LSU proteins are viewed as essential hubs that integrate signals from environmental cues, playing a key role in responding to various biotic and abiotic stresses. For the first time, we report the direct involvement of LSU proteins in primary sulfur metabolism. We show that transcriptional and metabolic profiles of the quadruple *lsu* mutant, q-lsu-KO, grown in non-limiting sulfate conditions resemble the molecular response of the wild-type plants to sulfur deficiency. That prompted us to check the interaction of LSU proteins with the enzymes of the sulfate reduction pathway. Indeed, we demonstrate that all LSUs interact with ATPS1 and ATPS3 isoforms of ATP sulfurylase, all three isoforms of adenosine 5´phosphosulfate reductase (APR), and sulfite reductase (SiR). Furthermore, we show in the *in vitro* assay that the interaction between SiR and LSU1 serves to stimulate the enzymatic activity of SiR. This result underscores the supportive role that LSU proteins play in facilitating the sulfate reduction.

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2-thiouridylases for tRNA in pro- and eukaryotes

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The wobble nucleoside at position 34 of tRNA ubiquitously carries a thiomodification that enhances codon binding in vivo and is found frequently in all kingdoms of life. The most conserved example is the wobble uridine of tRNA Lys, Gln, Glu in bacteria that encompasses a sulfur-based modification at position 2 (s^2U34). Two important proteins that are involved in this thiolation modification are the L-cysteine desulfurase IscS, the initial sulfur donor, and tRNA-specific 2-thiouridylase MnmA in *Escherichia coli*, that adenylates and finally transfers the sulfur from IscS to the tRNA. While the pathway of mnm^5 s^2 U34 formation was believed to be iron–sulfur Fe-S cluster-independent in *E. coli*, the MnmA homologous proteins NCS2 (yeast) and CTU1 (humans) are Fe-S-cluster-dependent proteins. *E. coli* MnmA was believed to be an Fe-S cluster-independent protein based on the fact that no Fe-S cluster was identified in the crystal structure of the protein. Recently, a report describing the purification of a [4Fe-4S] cluster containing the MnmA protein isolated under anaerobic conditions from *E. coli* reported that this [4Fe-4S] cluster is essential for its activity. However, this report contradicts previous published data, where it was shown that by using an *E. coli* ΔiscS mutant strains complemented with a *sufS*-expressing plasmid, the Fe-S cluster biosynthesis was rescued, but this strain was unable to produce mnm^5s^2U34 modified tRNAs. To clarify this contradiction, we aimed to investigate the activity of the Fe-S cluster containing MnmA under our conditions. Furthermore, we tried to complement the *E. coli* ΔmnmA mutant strain with the MnmA homologs from humans, CTU1 and MTU1. While MTU1 is also Fe-S cluster-independent like MnmA, CTU1 was shown previously to carry a [3Fe-4S] cluster. Nevertheless, both proteins were not able to reconstitute the *E. coli* ΔmnmA mutant strain based on their inability to bind and transfer sulfur to *E. coli* tRNA. We show that *E. coli* tRNA appears not to be bound to MTU1. The reason why some thiouridylases are Fe-S cluster-dependent while others are not, still remains elusive.

How sulfur allocation impacts plant responses to cadmium stress: from signalling to acclimation

Ann Cuypers[1], Isabeau Vanbuel[1] and Sophie Hendrix[1]

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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. Cadmium uptake from polluted soils inhibits plant growth and disturbs physiological processes, at least partly due to disturbances in the cellular redox environment. In this regard, glutathione (GSH) is an important sulphur-containing antioxidant, essential to maintain the redox homeostasis, but it also serves as a precursor for the Cd-chelating phytochelatins (PCs). This duality of GSH is key to in the Cd-induced ecophysiological plant stress response that is characterised by an initial alarm phase, succeeded by a new steady state to induce plant acclimation. The rapid decrease of root GSH concentrations to produce the chelating phytochelatins is proposed to serve as an alert response in *A. thaliana*. This alert response initiates a cascade of signaling events, with the phytohormone ethylene playing a crucial role in subsequent acclimation processes, including the restoration of glutathione levels. Additionally, the interaction with organellar stress responses and downstream acclimation reactions, such as autophagy, is significantly influenced by Cd exposure. Therefore, the dual role of GSH in Cd-induced responses, as an antioxidant and Cd-chelating molecule, is discussed using Arabidopsis mutants defective in GSH production (*cad2*) or PC production (*cad1-3*). These mutants help to clarify the mechanisms involved in short-term cellular responses in both roots and leaves, as well as the overall long-term acclimation of the plant.

Interaction of S homeostasis with N signaling in *Arabidopsis thaliana*

Varsa Shukla[1], Suvajit Basu[1], Stanislav Kopriva[1] and Daniela Ristova[1]

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In natural environments and agricultural production, plants are rarely exposed to isolated signals, and often simultaneously exposed to multiple stresses, including fluctuation of nutrients. Recent investigations have emphasized the strong interconnection between nutrient signaling, response, and metabolism, and demonstrated that plant phenotypes cannot be predicted by studying each signal separately.

Research on sulfur (S) signaling and interplay with other nutrients is lagging, compared to other macronutrients. However, the importance of S is becoming evident in recent literature, as S homeostasis is interconnected with other important nutrients, such as phosphate (Pi), and nitrogen (N), but the combined effects are not well understood at molecular level. For instance, replacement of sulfolipids by phospholipids under S starvation, and vice versa, is a known metabolic switch between S and Pi. N deficiency has negative impact on sulfate assimilation, and vice versa, but molecular mechanisms are missing.

Here, we show the first molecular link of N-S crosstalk. One of the key transcription factors (TFs) of primary nitrate response (PNR), acts as a negative regulator of sulfate transport, uptake and assimilation. Additionally, we identify different clades of the same TF family to regulate sulfur metabolism and glucosinolates (GSLs) synthesis.

Elucidation of glutathione degradation pathway in *Arabidopsis thaliana*

Takehiro Ito[1,2], Shunsuke Miyaji[3], Minori Umahashi[3], Taisuke Kitaiwa[3], Kosuke Nishizono[3], Kana Kuwahara[4], Hiroki Harata[4], Haruna Aoyama[4], Shin-ichiro Agake[5], Ryosuke Sugiyama6, Muneo Sato[2], Jun Inaba[2], Shinya Fushinobu[7,8], Tadashi Yokoyama[9,10], Akiko Maruyama-Nakashita[11], Masami Yokota Hirai[2,12] and Naoko Ohkama-Ohtsu[5,9]

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Glutathione (GSH; ɣ-Glu-Cys-Gly) plays numerous essential roles in plants: redox regulation, signaling, detoxification of toxic compounds, and transport and storage of assimilated sulfur. Despite these functions, GSH is rapidly turned over in plants, suggesting the importance of GSH degradation. The plant GSH degradation pathway is expected to differ from that of animals. Although ɣ-glutamyl cyclotransferase (GGCT: GGCT2;1, GGCT2;2, and GGCT2;3) has recently been identified as a cytosolic GSH degradation enzyme, the complete picture of the GSH degradation pathway remains elusive. In this study, we searched for other GSH-degrading enzymes by combining yeast complementation assay, activity assay using recombinant proteins, metabolite analysis, expression analysis, and protein structure prediction. As a result, ɣ-glutamyl peptidase (GGP: GGP1 and GGP3) was identified as a GSH-degrading enzyme. The *ggp1* mutant exhibited a sign of more severe sulfur deficiency than the wild-type under sulfur-limited conditions, indicating that GSH degradation by GGP functions as an important sulfur reallocation pathway. Furthermore, GGP reportedly processes GSH conjugates in the glucosinolate and camalexin biosynthesis pathways, which also agrees with our data. Therefore, GGP functions in primary and secondary sulfur metabolism, possibly balancing them. Additionally, both GGCT and GGP detach the ɣ-glutamyl bond of GSH to produce cysteinylglycine (Cys–Gly), suggesting the presence of Cys–Gly dipeptidases. We addressed this issue using a similar strategy and identified At4g17830 as a Cys–Gly dipeptidase, namely, cysteinylglycine peptidase 1 (CGP1). Similar to *ggp1*, *cgp1* showed a sign of more severe sulfur starvation compared to the wild-type under sulfur-deficient conditions, indicating its importance in sulfur reallocation. Notably, CGP1 was originally reported as *N2*-acetylornithine deacetylase (NAOD), which functions in the ornithine biosynthesis pathway; therefore, CGP1 may have dual functions in GSH and ornithine metabolism.

[1] Miyaji, S., et al. (2024) *Plant J.*, in press. http://dx.doi.org/10.1111/tpj.16700
[2] Ito, T. and Ohkama-Ohtsu, N. (2023) *J. Exp. Bot.*, 74, 3313–3327
[3] Ito, T., et al. (2022) *Plant J.*, 111, 1626–1642

Abstracts of talks

S-metabolism session 2

Harnessing the power of sulfur: redox catalysis, nanotechnology and biomedical innovations

M. J. Nasim[1], S. Safinazlou[1], E. Tiganescu[1] and Claus Jacob[1]

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The realm of redox catalysis would be significantly limited without acknowledging the contributions of Group 16 elements, particularly sulfur. Reactive Sulfur Species (RSS) have garnered the attention of scientists due to their extensive applications in medicine and agriculture. RSS effectively and selectively modulate intracellular redox states by a variety of mechanisms, including the reversible modification of cysteine residues in proteins and enzymes involved in the cellular thiolstat, the generation of reactive oxygen species (ROS), and the modulation of cellular GSH levels [1]. Consequently, these species hold significant potential as selective cytotoxic agents [2,3]. Moreover, volatile RSS, such as allicin from garlic and its synthetic analogues, have shown significant antimicrobial properties *via* the gas phase [4]. Similarly, selenium enrichment of nutraceuticals improves their biological profile as exemplified by Se-enriched garlic extract triggering growth inhibition and cell cycle arrest in a mammary epithelial cell culture model [5].

Intriguingly, elemental sulfur also finds widespread applications in nanoscience [6]. Sulfur nanoparticles (SNPs) can be produced through various methods - physically by mechanical grinding, chemically and biologically using thiophilic bacteria such as *Thiobacillus thioparus* able to transform sodium sulfide (Na_2S) into sulfur nanomaterials [7-9]. The ability of *Thiobacillus* to utilize CO_2 as a carbon source and to oxidize sulfur compounds makes this and related species important players in the sulfur cycle and potential candidates for biotechnological applications, including bioremediation and CO_2 sequestration [10]. Sulfur nanoparticles (SNPs) find a broad spectrum of applications in food, agriculture, and biomedical fields. The applications of sulfur-based redox modulating agents in medicine and agriculture represent promising areas of research. This interdisciplinary theme bridges nutrition, nanotechnology, medicine, pharmacy, synthetic chemistry and agricultural sciences, offering a rich field for innovative projects and scientific exploration.

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[2] B. Czepukojc, U. M. Viswanathan, A. Raza, S. Ali, T. Burkholz, C. Jacob, Phosphorus, Sulfur, and Silicon and the Related Elements. 2013, 188 (4), 446–453.
[3] E. Yagdi Efe, A. Mazumder, J.-Y. Lee, A. Gaigneaux, F. Radogna, M. J. Nasim, C. Christov, C. Jacob, K.-W. Kim, M. Dicato, et al., Cancer Letters. 2017, 410, 139–157.
[4] R. Leontiev, N. Hohaus, C.Jacob, M.C.H. Gruhlke, A.J. Slusarenko. Sci Rep. 2018 8(1):6763.
[5] J. Lu, H. Pei, C. Ip, D.J. Lisk, H. Ganther, H.J. Thompson. Carcinogenesis. 1996;17(9):1903-7.
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[7] S.Griffin, M. Sarfraz, S.F. Hartmann, S.R; Pinnapireddy, M.J. Nasim, U. Bakowsky, C.M. Keck, C. Jacob. Antioxidants. 2018 27;7(2):23.
[8] S. Shankar, R. Pangeni, J.W. Park, J.W. Rhim. Materials Science & engineering. C, Materials for Biological Applications. 2018, 92:508-517.
[9] F.P. van den Ende, H. van Gemerden, Sulfide Oxidation under Oxygen Limitation by a Thiobacillus Thioparus Isolated from a Marine Microbial Mat. *FEMS Microbiol Ecol* **1993**, *13* (1), 69–77.
[10] R.C. Perez, A. Matin. J Bacteriol. 1982,150(1):46-51.

Metabolism and redox signal regulation by supersulfides

Takaaki Akaike[1]

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For decades, the major focus of redox biology has been on molecular oxygen, the most abundant element of the planet. The oxygen molecule accepts electrons from the respiratory chain in the mitochondria and is responsible for energy production in aerobic organisms. In addition, oxygen-derived reactive oxygen species that include hydrogen peroxide and oxygen- and nitrogen-related free radicals, such as superoxide, hydroxyl radical and nitric oxide, undergo a complicated way of electron transfer reactions through their interaction with other biological substances, leading to alteration of their physiological functions, and cause diverse biological and pathophysiological consequences (e.g., oxidative stress). Discovery of supersulfides helped us to realize that the oxygen molecule itself accounts only partly for the redox reaction in many organisms, even under aerobic or hypoxic conditions, however. My talk will deal with a brand-new venue of redox biology, which is governed by the redox-active supermolecules that are mostly consisted of supersulfides, i.e., sulfur-catenated molecular species. They are now found abundantly in all organisms but remain largely unexplored in view of the redox biology and life science research. In fact, accumulating evidence show that supersulfides are electron rich and thereby readily ionized or radicalized, so that they can actively participate in the energy metabolism, redox dependent signaling, and oxidative stress responses in the cellular and in vivo context. Moreover, the pharmacological intervention and medicinal manipulation of supersulfides has been shown to be beneficial in prevention as well as regulation of disease pathogenesis. Thus, the supersulfide biology now open up a new era of disease control that includes its potential application to clinical diagnosis, prevention, and therapeutics for various diseases.

Development of mass spectrometry-based supersulfidomics and its potential: alternations in supersulfide production during the germination of broccoli sprouts

Shingo Kasamatsu[1], Ayaka Kinno[1], Somei Komae[1], Chiharu Miura[1], Tomoaki Ida[2], Hozumi Motohashi[3], Takaaki Akaike[4] and Hideshi Ihara[1]

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Sulfur is essential for life and human health, primarily obtained from proteins to synthesize sulfur-containing biomolecules. Recent studies have highlighted the biological significance of endogenous supersulfides, defined as hydropersulfide (RSSH) and polymeric sulfur species with sulfur catenation (RSSnR, n > 1, R = hydrogen or alkyl, or cyclized polysulfides) [1]. Ingesting exogenous sulfur compounds is crucial for producing these supersulfides, however their content in foods and detailed biosynthesis mechanisms are not well understood. In this study, we developed mass spectrometry (MS)-based technologies for supersulfidomics to investigate supersulfide profiles in biological samples including animal cells and tissues, and foods such as fresh vegetables, edible animal meat, and beans [2,3,4]. Quantitative analysis of supersulfide profile revealed that supersulfides are relatively more abundant in fresh vegetables of *Allium* and *Brassica*, such as onions, broccoli, Chinese chive, and garlic [2,3]. Notably, broccoli sprouts had the highest supersulfide content, which increased during germination and growth [3]. Untargeted polysulfide omics analysis showed that supersulfide composition changed significantly over cultivation time. Predominant organic supersulfide metabolites identified included cysteine hydropersulfide (CysS2H) and cysteine hydrotrisulfide. Additionally, novel sulforaphane (SFN) derivatives conjugated with supersulfides were found in broccoli sprouts. An *in vitro* radical scavenging assay using 2,2-diphenyl-1-picrylhydrazyl revealed that the SFN conjugate with CysS2H had greater radical scavenging capacity than SFN and cysteine, suggesting that the abundant supersulfide content in broccoli sprouts may contribute to their human health benefits. These results suggest that the new MS-based supersulfidomics techniques could be useful tools for evaluating the biological significance of endogenous and exogenous supersulfides in redox biology and medicine.

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Exploring uncharted territories: new genes for sulfur starvation responses in plants

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Plants have evolved various adaptive mechanisms to cope with nutrient deficiency stress. These adaptations occur at the morphological, and biochemical levels to enhance nutrient acquisition and utilization efficiency. Optimizing nutrient use efficiency (NUE) is crucial for the primary macronutrients – nitrogen, phosphorus, potassium, and sulfur. Sulfur, in particular, plays a pivotal role in plant growth, development, metabolism, and the ability to withstand environmental stresses. As an essential macronutrient, sulfur is indispensable not only for plant health but also for human nutritional needs. However, plants' molecular machinery controlling sulfur starvation responses is poorly understood. *ETHYLENE-INSENSITIVE3-LIKE3 (EIL3)* or *SULFUR LIMITATION1 (SLIM1)*, emerged as the first transcriptional factor to orchestrate multiple transcriptional changes under sulfur deficiency. It is involved in upregulating sulfate acquisition while concomitantly inducing glucosinolate degradation, enabling sulfur recycling. However, the mechanistic details of *SLIM1*'s regulation of sulfur starvation responses remain incompletely elucidated. These investigations into sulfur metabolism and regulation have predominantly centered on Arabidopsis. Consequently, gaining a more comprehensive understanding of sulfur-related processes in major crop plants is imperative. To contribute to these endeavours, in this work, we have performed RNA-seq analysis in rice and setaria plants under sulfur-starved conditions and compared our dataset with other sulfur starvation RNA-seq datasets from Arabidopsis and tomato. The intersection between four species has resulted in 7 common DEGs. We have characterized 4 of the 7 intersect genes and dissected their involvement in mitigating sulfur starvation responses.

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Abstract of talk

Plenary session 1

A 35-year journey in plant sulfur research: a personal perspective

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My journey in plant sulfur metabolism research began with the pioneering molecular cloning of cysteine synthase (*O*-acetylserine(thiol)lyase) from spinach, as reported in [Saito *et al*., *PNAS*, **89**, 8078 (1992)]. Since then, my group has expanded this research to encompass the molecular mechanisms of sulfate transport, assimilation, and the conversion of sulfur metabolites in the model plant *Arabidopsis thaliana*. This work has included not only the biochemical study of pathway enzymes but also the identification of regulatory genes and proteins, facilitated by advancements in genomics and metabolomics research. In recent years, our focus has shifted a bit towards exploring the health-promoting properties of sulfur metabolites. These achievements have been made possible through collaborations with my former colleagues, who have since established their own independent research careers.

In this plenary lecture, before retiring from my lab research activity, I will share both retrospective insights and forward-looking perspectives on the evolving field of plant sulfur metabolism.

Abstracts of talks

Redox regulation session 1

Class I glutaredoxins in plastid oxidative stress: repair crew or regulators?

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Oxidative stress in chloroplasts is variable, depending on environmental conditions and capacities of local ROS scavenging systems. The tripeptide glutathione can serve as a reductant in enzymatically catalysed alleviation of oxidative stress, as well as modify cysteine residues in proteins via *S*-glutathionylation. Class I glutaredoxins (GRX) mediate protein (de)glutathionylation, but their biological relevance in chloroplast physiology is unclear. Using the model plants *Physcomitrium patens* and *Arabidopsis thaliana*, we investigate consequences of stromal glutathione redox potential dynamics and the GRX-catalysed coupling to protein *S*-glutathionylation and disulfide formation. Absence of class I GRX in the stroma of *P. patens* results in increased steady state oxidation of target proteins as well as slower recovery after an oxidative challenge, as revealed by the biosensor redox-sensitive GFP (roGFP2). Thus, class I GRXs are vital for fast reduction rates of cysteinyl thiol groups. This talk will discuss evidence for resulting biological functions ranging from basic thiol protection to redox regulation and signaling triggered by oxidative shifts of the glutathione redox potential.

Molybdenum in health and disease: Thiol and sulfide signaling in metabolism and synapse function

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Molybdenum is an essential trace element for humans and found in four enzymes involved in amino acid, nucleotide, lipid and drug metabolism. To gain biological activity, molybdenum is inserted into the molybdenum cofactor by a highly conserved biosynthetic pathway. Defects in any step of the pathway lead to a severe inborn error in metabolism with rapidly progressing neurodegeneration. The majority of symptoms trace back to a loss in sulfite oxidase activity catalyzing the last step in cysteine catabolism. Various metabolic changes are causative for brain damage, mitochondrial dysfunction and multi-organ failure impacting H_2S and persulfide signaling. In addition, one of the proteins involved in molybdenum cofactor biosynthesis serves an additional function in synapse formation, which again relies on multiple cysteine-based posttranslational modifications impacting liquid-liquid phase separation at the synapse. Together with the non-canonical function of sulfite oxidase in nitrite reduction, the presentation will discuss the intimate link between thiol-, sulfide- and NO signaling.

Analysis of pathological progression-dependent changes of supersulfides production in the brain tissues of mouse models of Alzheimer's disease

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder causing severe cognitive dysfunction. With the increasing number of AD patients in an aging society, the development of preventive and therapeutic strategies is crucial. Supersulfides, defined as hydropersulfide (RSSH) and polymeric sulfur species (RSSnR, n > 1), are produced enzymatically *in vivo* and regulate antioxidative stress responses and redox signaling. Previous studies showed that endogenous supersulfide production might change in the brain tissue of AD model mice (5xFAD) with severe cognitive dysfunctions. However, the detailed relationship between supersulfides and AD remains unclear. In this study, we performed supersulfide omics analysis on the brain tissue of 5xFAD mice of different ages to investigate the changes in supersulfide production during AD progression. We confirmed AD progression by evaluating insoluble Aβ peptides, neuronal inflammation, and cognitive dysfunction. Quantitative analysis of total polysulfide content revealed a significant decrease in 8-month-old 5xFAD mice compared to wild-type mice. Notably, significant decreases in protein polysulfides were detected in 4- and 8-month-old 5xFAD mice using a novel alkylating agent, *N*-iodoacetyl L-tyrosine methyl ester. These results suggest that endogenous supersulfide production changes during AD onset and progression, with specific forms of supersulfides affected by AD severity. Further investigation is needed to identify AD-related supersulfidated proteins and analyze the forms of supersulfides. These findings highlight the importance of supersulfide-regulated redox signaling in developing preventive and therapeutic strategies against AD.

The plant DCC1 are atypical thioredoxins with a holdase activity

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The thioredoxin superfamily consists of several multigenic families, among which are thioredoxins (TRXs), glutaredoxins (GRXs), protein disulfide isomerases (PDIs). All these proteins share the same structural arrangement, known as thioredoxin fold [1]. They typically use a pair of reactive cysteines in a CXXC motif to perform thiol-disulfide exchange reactions and control the activity or folding of their target proteins. Intriguingly, many proteins possessing one or more CXXC motifs and predicted to adopt a TRX fold remain uncharacterized. Some TRX-like proteins, present in all three domains of life have been referred to as DCC proteins, because of the presence of a DXXCXXC signature at a position similar to the redox active cysteines in TRXs [2]. However, the proteins are extended at both the N- and C-terminal ends. Two to three members are usually found in photosynthetic organisms. In order to biochemically and structurally characterize DCC1 from *Arabidopsis thaliana* and *Populus trichocarpa*, the sequences encoding the mature form of the proteins were expressed as recombinant protein in *E. coli*. Analysis of the redox properties of various full-length and truncated versions, including activity profiling, susceptibility to oxidative modifications and interactions with other oxidoreductases, indicated that although the cysteines are reactive, the proteins are unlikely to be part of the general redox network. Instead, they appeared to form large oligomers with holdase activity. This adds a new layer to the redox chaperone systems, e.g. other TRX or GRX that have been proposed to have such activity.

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Site-specific activation of proton pump inhibitors by tetrathiolate zinc centers

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Proton pump inhibitors (PPIs) represent a class of widely used drugs with global sales. As prodrugs they are activated through protonation in acidic environments, where they effectively inhibit stomach acid secretion by covalently modifying cysteine residues on the gastric proton pump. However, until now, it remained unclear whether PPIs could be activated through alternative mechanisms in non-acidic environments. In this study, we employed a chemoproteomic approach to investigate PPI behavior in living cells. Our findings reveal that PPIs selectively form disulfide conjugates with zinc-binding proteins. We demonstrate that zinc ions coordinated by four cysteines can activate the drug, subsequently facilitating its conjugation to zinc-coordinating cysteines. Remarkably, this activation process occurs independently of low pH, challenging the conventional acid-dependent paradigm. In summary, our results highlight zinc as a Lewis acid capable of promoting PPI activation and conjugation in non-acidic environments. This novel insight not only sheds light on secondary drug effects but also opens avenues for repurposing PPIs beyond their established acid-related applications.

Decoding the function of Tau Glutathione S-transferases GSTU24 and GSTU25 in Arabidopsis

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Glutathione S-transferases (GSTs) are ubiquitous multifunctional enzymes that detoxify xenobiotics and toxic endogenous metabolites. Plant GST families are extensive and commonly arrayed in tandem genes or large gene clusters in the genome due to gene duplication. This increases the chance of functional redundancy and makes their individual characterization highly challenging. Arabidopsis has 53 GSTs grouped into seven classes, of which the plant-specific class tau (GSTU) is the largest. GSTUs have two GSH-dependent enzymatic activities: (1) GSH-binding to the electrophilic center of potentially toxic compounds (GST activity) and (2) a GSH-dependent peroxidase function towards lipid peroxides (GPOX activity). Among all GSTUs, GSTU24 and GSTU25 stand out with the highest GST and GPOX activities, and the genes coding for these are the most highly induced genes under biotic and abiotic stress compared to other stress-related genes, suggesting a potential role in stress defense. Yet, their physiological role under oxidative stress is unknown and no distinct phenotype reported in null mutants for these genes. We have isolated a mutant line for GSTU25, which shows accelerated growth and increased resistance towards methyl viologen (MV)-induced oxidative stress due to reciprocal genetic compensation through upregulation of other GSTUs. This work aims to dissect the critical functions of GSTU24 and GSTU25 under oxidative stress. As an experimental strategy, we compared the structures of multiple GSTs in search for key residues that define the enzymatic activities of GSTU24 and GSTU25, and generated variants of them affected in their GSH-dependent functions. These variants were then evaluated in a yeast strain deficient in GST activity and an Arabidopsis mutant lacking the transcription factors TGA2, TGA5, and TGA6, which is compromised in the expression of 12 GSTUs, including GSTU24 and GSTU25. The respective results provide valuable insight into the function of GSTs in plants and their potential applications in stress defense.

Functional insights into the catalytic and redox-based regulatory properties of AKR4Cs from *Arabidopsis thaliana*

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Protein S-nitrosylation is a redox-based post-translational modification playing an important role in cellular signaling impacting protein function and stability. At physiological level, nitrosoglutathione (GSNO) is considered the main S-nitrosylating agent the concentration of which is mainly controlled by NADH-dependent enzyme S-nitrosoglutathione reductase (GSNOR). However, recent studies show that aldo-keto reductases may be involved in controlling GSNO homeostasis in both humans and plants [1,2]. In this work, we investigate the functional properties of aldo-keto reductases from *Arabidopsis thaliana* (4 isoforms belonging to 4C superfamily, AtAKR4C8-11) which have been shown to catalyze GSNO degradation using NADPH as a source of reducing equivalents[2]. To this aim, we expressed AtAKR4Cs in *E. coli* and purified them to homogeneity through metal-affinity chromatography. Among AtAKR4Cs, AKR4C8 resulted the most catalytically efficient isoform, with affinity towards GSNO like that measured for GSNOR (~30 µM). We then investigated the redox responsivity of AtAKR4Cs by analysing cysteine accessibility in solved or modelled 3D-structures and examining activity modulation following exposure to oxidant molecules (H_2O_2, GSSG, GSNO). While AtAKR4C9-11 were found irresponsive to oxidation, AtAKR4C8 resulted sensitive to both GSSG and H_2O_2, showing a fast and reversible inactivation due to GSNO. To deeply investigate the redox-based regulatory properties of AtAKR4C8, we carried out site-directed mutagenesis to replace the highest solvent accessibility cysteine (Cys287) with alanine (C287A). Catalytic features and possible structural alterations of C287A mutant were analyzed by circular dichroism (CD) analysis and intrinsic fluorescence spectroscopy. Replacing Cys287 with alanine neither specific activity nor native folding were affected but resulted in complete insensitivity to oxidative treatment. This highlights that redox alteration of Cys287 is responsible for redox sensitivity of AtAKR4C8. Overall, our results demonstrate a prominent role of AtAKR4Cs in GSNO catabolism, with AtAKRC8 proving to be the most interesting isoform due to its highest catalytic efficiency towards GSNO and redox sensitivity.

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Phylogenetical, biochemical and structural insights into the iota glutathione transferase from the cyanobacteriota *Synechocystis* SP. PCC 6803, an atypical glutathione transferase exhibiting an unexpected FMN-binding domain

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Glutathione transferases (GST) constitute a widespread superfamily of multifunctional enzymes with essential roles in cellular detoxification and secondary metabolism. In the cyanobacteriota *Synechocystis* sp. PCC 6803, 5 genes coding for GSTs have been identified. Among them, *sll1902* codes for a GST (SynGSTI) belonging to the uncharacterized iota class (GSTI). A phylogenetic analysis revealed that GSTIs were found in cyanobacteriota and in green algae, bryophytes, as well as vascular plants like lycophytes or ferns. GSTIs were distinguished from other GSTs by the presence of an N-terminal extension and additional C-terminal domain without associated function surrounding a central GST domain. Recombinant SynGSTI protein was purified at homogeneity in native conditions after overexpression in *Escherichia coli*. The protein exhibited an unexpected spectrophotometric signature between 300 and 500 nm, reminiscent of flavins. This was attributed to flavin mononucleotide (FMN) using mass spectrometry and subsequently confirmed through X-ray crystallography. SynGSTI also showed reductase activity concomitant to the presence of a catalytic CPYC signature in the GST domain. The analysis of SynGSTI crystal structure revealed a monomeric organization in accordance to analytical gel filtration experiments. The additional C-terminal domain of SynGSTI contained the FMN ligand and showed a similar structure to the α-subunit of phycoerythrins, components of the light-harvesting system of cyanobacteriota. Further experiments are in progress to decipher the role(s) and function(s) of the GST Iota class in cyanobacteriota.

Abstracts of talks

H_2S signalling session

Sulfide signaling and mitochondrial redox metabolism

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Toxic at high levels, H_2S modulates a range of physiological processes. H_2S biogenesis piggybacks on the transsulfuration pathway enzymes; its clearance is catalyzed by a dedicated mitochondrial sulfide oxidation pathway that intersects with the electron transport chain. Hypoxic upregulation of nitric oxide in endothelial cells reprograms the transsulfuration pathway to increase biogenesis of H_2S, a pro-angiogenic metabolite. Decreased H_2S oxidation due to sulfide quinone oxidoreductase deficiency, synergizes with hypoxia, inducing a reductive shift, thereby limiting endothelial cell proliferation [1]. H_2S preconditioning increases the O_2 partial pressure for half maximal cellular respiration, and has pleiotropic metabolic effects that persist for up to two days [2]. Cells prioritize H_2S clearance by using fumarate as a terminal electron acceptor and reversing flow through complex II [3]. Our studies are revealing that H_2S signals by inducing a reductive metabolic shift that fans out to other compartments via metabolite shuttles and redox cofactors. New developments in the H_2S oxidation pathway and signaling via redox remodeling will be discussed.

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Hydrogen sulfide: a key ally in adapting to climate change

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Hydrogen sulfide (H_2S) is increasingly recognized as a crucial signaling molecule in plants, playing a significant role in mitigating the adverse effects of climatic change stresses, including drought and elevated atmospheric CO_2 levels. This gasotransmitter modulates various physiological and biochemical processes, enhancing plant resilience through mechanisms such as stomatal regulation, antioxidant defense, and osmotic adjustment. Under drought conditions, H_2S helps maintain water balance and reduces oxidative damage by upregulating antioxidant enzymes and molecules. In Arabidopsis, H_2S is a promoter of enhanced tolerance to drought stress through protein persulfidation. Bioinformatic analyses of the proteins more persulfidated under drought stress, revealed that the most enriched biological processes were cellular response to oxidative stress, hydrogen peroxide catabolism, protein degradation, and phenylpropanoid pathway, suggesting the importance of persulfidation protecting plants against drought stress [1]. Additionally, in rice crops, we revealed that water transport activity is regulated by sulfide which correlates to an increasing level of persulfidation of aquaporins. Thus, persulfidation role on aquaporin transport activity as an adaptation response in rice differs from the current knowledge in Arabidopsis [2]. In the context of elevated CO_2, sulfide amends the imbalance of carbon/nitrogen in plants ground under suppressed photorespiration using high CO_2 concentration atmosphere, restores ATP levels, balances the high level of ROS and regulates stomatal closure by decreasing ROS burst in stomata under high CO_2 atmosphere [3]. Understanding the multifaceted roles of H_2S in plant stress responses offers potential strategies for developing crops with enhanced tolerance to climatic stresses, ensuring agricultural productivity to face global climate change and growth of world population.

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The complex interaction network of human sulfurtransferases with H_2S oxidation pathway proteins

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Hydrogen sulfide (H_2S) is an emerging gasotransmitter described as a pleiotropic modulator of cell's physiology from mammals, plants to bacteria. H_2S is a Janus faceted molecule with beneficial or deleterious effects depending on its cell type accumulation and concentration [1]. As a consequence, H_2S cellular concentration has to be tightly regulated. Enzymes that are responsible for H_2S biosynthesis are now identified, with cytosolic or mitochondrial protagonists. Regarding H_2S clearance, that occurs in mitochondria for mammals, a tripartite protein complex (Sulfide Oxidation Unit, SOU complex), constituted by the SQOR, the ETHE1 and a sulfurtransferase (STR), is proposed [2,3]. STRs belong to the Rhodanese super family protein and catalyse sulfur transfer from a donor to an acceptor by transient formation of a cysteine persulfide intermediate on their catalytic residue. The human STR equipment is composed of three members, MPST, TST and TSTD1, the two latter being alternatively proposed to be the STR involved in the SOU complex [4]. Hence, the identification of the STR involved in H_2S mitochondrial clearance will be a decisive step forward into the comprehension of the molecular processes responsible for the tight control of H_2S concentration.

In order to identify the STR involved in the SOU complex, we have conducted *in vitro* and *in cellulo* protein / protein interactions approaches combining biophysical protein complex characterisation, bimolecular fluorescent complementation and co-immunoprecipitations.

Our results bring *in vitro* and *in cellulo* evidences allowing us to identify the TST protein as the STR member involved in H_2S homeostasis within the mitochondrial SOU complex. In addition, our investigation opens new area of investigation regarding the potential physiological function(s) of TSTD1 and MPST proteins within human cells.

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Compartmentalization of cysteine metabolism in plants affects stress signaling

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Cysteine metabolism in plants is distributed across different intracellular compartments. Several isoforms of cysteine synthesizing enzymes are present in chloroplasts, mitochondria as well as in the cytosol and there are also diverse catabolic pathways. In the mitochondria cysteine is required for the synthesis of proteins and sulfur containing cofactors such as iron-sulfur clusters, lipoate, and biotin. Complete degradation of cysteine including oxidation of the sulfur group can only be achieved by a mitochondrial pathway consisting of three enzymes in combination with the TCA cycle. Transamination of cysteine produces 3-mercaptopyruvate, which is then converted to pyruvate by transfer of the thiol group to GSH catalyzed by a sulfurtransferase. The sulfur is subsequently oxidized by a persulfide dioxygenase. Interestingly, the compartmentalization of cysteine metabolism seems to be critical for signaling functions during abiotic and biotic stress responses. The mitochondrial cysteine synthesis pathway is strongly induced during interaction with pathogens in Arabidopsis leaves leading to an increase in free cysteine concentration. Cysteine accumulation elicits specific metabolic changes in the mitochondria including an induction of alternative respiratory chain components and interferes with photorespiration. We identified cysteine interacting mitochondrial proteins as potential targets of cysteine signaling on the basis of thermal proteome profiling experiments.

Abstract of talk

Plenary session 2

Protein persulfidation enters a new phase

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In order to maintain life, nature relies on a limited number of chemical reactions, one of which is sulfur-based chemistry, primarily utilized for controlling intracellular redox homeostasis and redox-based signaling. Hydrogen sulfide (H_2S), one of the simplest sulfur-containing molecules found in cells, has garnered significant attention since its potential physiological roles were first reported. There is now a burgeoning literature on H_2S signaling. One of the main mechanisms by which H_2S signals in cells is through posttranslational modification of cysteine residues, known as persulfidation. Key questions remain about how protein persulfides are formed in cells and their effects on cellular function, particularly in the context of aging and age-related diseases. This talk will focus on the structural versus functional effects and the controlled versus stochastic formation of persulfides. Specifically, I will introduce liquid-liquid phase separation, a mechanism by which cytoplasmic components (proteins and RNAs) assemble into distinct, membrane-less compartments (biomolecular condensates), as a primary mode through which H_2S-induced protein persulfidation controls cellular function.

Abstracts of talks

Redox regulation session 2

How do glutaredoxins reduce non-glutathione disulfides?

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Class I glutaredoxins are key enzymes for the reduction of low or high molecular weight disulfide substrates with implications for signal transduction and metabolic pathways such as the synthesis of DNA. Disulfide substrates of glutaredoxins include glutathione disulfides and non-glutathione disulfides. While the mechanisms and kinetics for the reduction of glutathione disulfide substrates are well established, a variety of alternative mechanisms have been suggested for non-glutathione disulfide substrates such as ribonucleotide reductase, redox-sensitive fluorescent proteins or the most commonly used model substrate bis(2-hydroxyethyl)disulfide. Here we present data from quantitative analyses using different non-glutathione disulfide substrates, glutaredoxins and mutants thereof to systematically distinguish between the alternative mechanisms, highlight methodological pitfalls, and discuss the implications of our findings for signal transduction, protein-protein interaction studies, and noninvasive redox measurements.

The intricate relationships between glutathione and proteins: interaction and redox modulation

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Glutathione is a tripeptide that plays a major role at the interface between stress conditions and adaptation mechanisms by acting as redox buffer and participating in multiple cysteine-based post-translational modifications. Among redox modifications, S-glutathionylation and S-nitrosylation are widely recognized to actively participate in cellular signaling as they can modulate protein function and conformation. At the physiological level, the interaction of protein cysteines with nitrosoglutathione (GSNO) and hydrogen peroxide (H_2O_2) coupled with reduced glutathione (GSH) constitute the major thiol-switching mechanisms to induce S-nitrosylation and S-glutathionylation, respectively. In this talk I will summarize my lab's work on disentangling the molecular mechanisms and structural determinants underlying the glutathione-related redox modulation of the model enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Enzymatic assays were used to monitor the catalytic response to oxidative treatments and the crystal structures of different GAPDH isoforms were solved and proved instrumental to carry out computational analyses to identify protein residues that determine redox sensitivity and participate in redox-based reaction mechanisms. Finally, we analyzed the contribution of glutathione moieties in the oxidative modulation of GAPDH by testing the capability of truncated forms of glutathione (*i.e.*, γ-glutamate-cysteine and cysteine-glycine) to interact with active site residues. Based on our findings, we provide functional and structural insights into the response of GAPDH to S-nitrosylation and S-glutathionylation, possibly expanding the mechanistic features to other protein cysteines susceptible to be oxidatively modified.

Glutathione peroxidase-like 8 (GPXL8): a new player in H_2O_2 signaling in *Arabidopsis thaliana*?

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Glutathione peroxidases (GPXs) catalyze the reduction of hydrogen peroxide (H_2O_2) and organic peroxides into water and alcohols, respectively. While mammalian GPXs require glutathione (GSH) as an electron donor, their plant counterparts are reduced by thioredoxins (TRXs) and are therefore termed glutathione peroxidase-like proteins (GPXLs). Plant GPXLs contain three conserved cysteines: the peroxidatic cysteine, the resolving cysteine and a middle cysteine of unknown function. Although many GPXLs are transcriptionally induced upon exposure to various stresses, their specific functions in plant stress responses remain largely elusive. It is interesting to speculate that they could function as H_2O_2 sensors, similar to yeast Gpx3 (Orp1), which oxidizes the Yap1 transcription factor.

To test this hypothesis, we investigated the ability of recombinant Arabidopsis GPXL8 to oxidize redox-sensitive GFP (roGFP2) as a proxy for endogenous target proteins. Using in vitro analyses, we first confirmed that GPXL8 displays peroxidase activity and depends on the TRX system for its reduction. Furthermore, we showed that GPXL8 mediates H_2O_2-dependent roGFP2 oxidation. Transfer of the primary oxidation from GPXL8 to roGFP2 likely occurs through a nucleophilic attack of roGFP2 on oxidized GPXL8. Substitution of the peroxidatic cysteine, resolving cysteine or middle cysteine revealed that all conserved cysteine residues of GPXL8 are involved in its thiol oxidase activity. Furthermore, results showed that both TRX and GSH influence GPXL8-mediated roGFP2 oxidation kinetics. Taken together, these results suggest that GPXL8 acts as an integrator of different redox inputs and can function as either a peroxidase or a protein thiol oxidase depending on local redox conditions.

Biochemical characterization of thioredoxin-related protein Clot/TRP14 from *Populus trichocarpa*

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Thioredoxin-related protein Clot possesses an atypical WCPDC motif compared to the classical WCGPC motif. In this study, we observed that Clot isoform from *Populus trichocarpa* is oxidized by cystine, GSSG and GSNO to a smaller extent, resulting in the formation of an intramolecular disulfide bond that is reduced by the GSH system, but not by the NADPH-dependent thioredoxin reductase system. Nevertheless, the reduction by the GSH system appears relatively inefficient which likely prevented a significant cystine reductase activity and caused a low ability of Clot to reduce S-sulfocysteine compared to typical glutaredoxin (GRX) and thioredoxin (TRX) isoforms. On the contrary, PtClot catalyzed both *in vivo* and *in vitro* the oxidation of roGFP2 in the presence of cystine but not GSSG. Introducing the active site of GRX or TRX motif in Clot reduced this cystine-dependent activity, indicating that the WCPDC signature differentiates Clot from regular TRX and GRX.

Abstracts of talks

Glucosinolates session

Plant survival strategies responding to environmental stress by controlling sulfur allocation

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Because plants are sessile, they have adopted a survival strategy of responding to changing environments at the place where they live. Therefore, determining how to allocate the nutrients and energy available is the most important tactic for plants to survive. Sulfur is one of essential macronutrients along with nitrogen etc. Glucosinolates are sulfur-containing specialized (secondary) metabolites produced by Brassicaceae plants and are known as defense compounds against insect herbivores. Our recent study [1] has shown that Arabidopsis plants exposed to sulfur depletion further metabolize glucosinolates to recover sulfur and redistribute it to primary metabolites such as cysteine, although glucosinolates are traditionally considered "end-products" of metabolism. In this presentation, I will introduce the above research and discuss plants' mechanism for control of sulfur allocation.

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The role of sulfur in salad rocket (*Eruca vesicaria* subsp. *sativa*) nutritional and sensory quality

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The genomic and transcriptomic mechanisms regulating sulfur metabolism in *Eruca vesicaria* subsp. *sativa* (rocket) and its impact on glucosinolate (GSL) biosynthesis was investigated as part of efforts to improve nutritional and sensory quality of leaves. This research presents the first de novo reference genome assembly and annotation for *Eruca*, accompanied by ontogenic and postharvest transcriptome data focusing on sulfur assimilation, transport, and utilization.

The study has provided significant insights into the role of sulfur in plant metabolism, particularly in relation to GSL biosynthesis and glutathione production. Diverse gene expression patterns associated with sulfur metabolism were identified among different inbred lines of rocket. Key genes, including multiple copies of *MYB28*, *SLIM1*, *SDI1*, and *ESM1*, were found to have increased and differential expression postharvest, correlating with sulfur content, GSL accumulation and hydrolysis product formation.

Results demonstrate the relationship between sulfur-related gene expression and GSL content. Specifically, two glucosinolate transporter gene copies (*GTR2*) were linked to higher GSL levels in leaves. Significant correlations and co-expression were observed between numerous sulfur metabolism and GSL-related genes, underscoring the interconnectedness of sulfur metabolism and these secondary metabolites, which give rise to potent health benefits and distinctive flavour.

A notable finding was the inverse relationship between the expression of glutathione synthetase genes and those involved in GSL metabolism, suggesting a trade-off between glutathione production and GSL synthesis. In the study, breeding line “B” exhibited increased expression of glutathione synthetase genes and lower GSL content compared to other lines, indicating that enhanced sulfur assimilation into glutathione may reduce GSL accumulation, and thereby reduce potential health benefits of leaves. Co-expression analysis further revealed that genes related to senescence (*SEN1*) and oxidative stress (*OXS3*) were highly expressed in line B, linking postharvest deterioration to lower expression of genes in sulfur metabolic pathways.

Glucosinolates in Kohlrabi after nitrogen fertilization increase as detected by a NIRS method

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The quantity and quality of glucosinolates in Brassica species such as Kohlrabi is an important topic for horticultural production. The bulb yield depends on water availability and nitrogen application. Nitrate is considered janus-faced in horticultural production because it is beneficial for yield and glucosinolate formation but might be harmful to the environment, especially when leaching into groundwater. A pot experiment with Kohlrabi (*Brassica oleracea* var. gongylodes) with increasing nitrogen amounts was carried out in a greenhouse at the University of Hohenheim, Stuttgart, Germany. Bulbs were harvested, and the effect of nitrogen application on the form and amount of different glucosinolates was analyzed. Two fertilization rates, one following common practice and one with 20% reduced nitrogen fertilization, were applied to test whether it would be a good compromise to produce good quality with reduced fertilizer input. Glucosinolates were determined using a fast and convenient Near Infrared method (NIR). Results show promising options for reduced input production of high-quality Kohlrabi bulbs.

Sulfur-based biofortification of wheat with iron: cysteine-based bioforortification schemes

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In this work we elaborate on the response of durum wheat plants to the biofortification of the spike with iron sulfate alone or in combination with cysteine and the influence of the aforementioned treatments on the biofortification of spike with other micronutrients. Two experiments have been performed within the same cultivation year, in two locations that are under the same climatic profile and present different soil properties (Aliartos vs Ypato, Voitia, Greece). The soil of the experimental plots in Aliartos were characterized by marginally low Fe content, and that of Ypato by medium Fe content, whilst both were marginally low in phosphorus. Foliar applications took place at the beginning of the dough development. Kernel accumulates most of its dry weight during dough development, whilst the transport of nutrients from the leaves, stems, and spike to the developing seed is completed by the end of the stage. Under low content of soil micronutrients, we highlight that the application of $FeSO_4$ biofortified the seeds with Mn keeping Fe, Cu, and Zn within the concentration range of control, cysteine biofortified the seed with Zn, whilst Cu and Fe were kept within the range, and Mn weakened. The combination of $FeSO_4$ and Cys kept Fe within the range and weakened all other micronutrients.

Abstracts of talks

Emerging topics session

Lipoylation overkill: boosting lipoylation capacity causes the release of sulfide

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Lipoic acid (LA) is an essential cofactor for mitochondrial metabolism that is synthesized by lipoyl synthases from octanoic acid, S-adenosylmethionine (SAM) and iron-sulfur clusters [1,2]. The radical SAM enzyme lipoyl synthase extracts two sulfur atoms for synthesis of LA from a [4Fe-4S] cluster [3]. Destruction of a [4Fe-4S] cluster in each catalytic cycle explains why defects in iron-sulfur cluster supply hampers protein lipoylation and results in severe developmental defects in plants and multiple energy metabolism-related diseases of utmost medical interest in human [4,5]. It is, however, unknown whether the cluster residual collapses and releases two sulfide molecules or whether the sacrificed cluster can be repaired. Here, we demonstrate that activity of Arabidopsis lipoyl synthase (LIP1) releases sulfide, which inhibits complex IV in the mitochondrial electron transport chain if it is not counterbalanced by sufficient detoxification capacity. We found that deficiency in cluster supply for LIP1 can be compensated by deleting the highly abundant [4Fe-4S] protein aconitase 3 or by overexpression of LIP1. In wild-type plants, however, overexpression of LIP1 unexpectedly resulted in inhibition of complex IV and retarded growth. This phenotype can be suppressed by sulfide fixation through mitochondrial cysteine synthase. Our results demonstrate that sulfide is released as a byproduct of lipoic acid synthesis and that appropriate local detoxification mechanisms are necessary to avoid toxicity. The results also explain why lipoyl synthase needs to be kept at very low copy numbers in mitochondria. We expect that our approach provides a genetic model to further dissect Fe-S cluster turnover and lipoylation activity. The finding that lipoylation necessarily causes the release of sulfide adds to our understanding of a whole range of Fe-S cluster diseases in plants and human. The inherent release of sulfide also provides a simple explanation for maintaining sulfide detoxification systems in mitochondria of most eukaryotic lineages.

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[3] McCarthy, E. L. & Booker, S. J. Destruction and reformation of an iron-sulfur cluster during catalysis by lipoyl synthase. *Science* **358**, 373-377, doi:10.1126/science.aan4574 (2017).
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Synthetic and systems biology of carbon fixation in Chlamydomonas

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Redox regulation and signaling are crucial in numerous fundamental cell processes and participate in the mechanisms enabling cells to sense environmental changes and trigger adaptive responses. These regulations and signaling pathways involve redox post-translational modifications (PTM) such as disulfide bond formation, glutathionylation or nitrosylation, orchestrated by conserved oxidoreductases like thioredoxins (TRX). Using qualitative and quantitative large-scale proteomic approaches in *Chlamydomonas reinhardtii*, we have unraveled an intricate redox network of more than 1000 proteins regulated by redox PTM. The Calvin-Benson cycle, responsible for photosynthetic carbon fixation, is extensively regulated by TRX and integrates diverse redox signals, with all 11 enzymes of the pathway undergoing multiple redox PTM. The biochemical, structural and synthetic biology approaches we have developed to study carbon fixation and its regulation in Chlamydomonas will be presented including the functional recoding of *Chlamydomonas reinhardtii* thioredoxin type-h into photosynthetic type-f by switching selectivity determinants.

NOS produces cyclic octasulfur that enables protection against lipid peroxidation in lipid droplets

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Cyclic octasulfur (S_8) is known to be one of the end products and substrates in sulfur respiration of sulfur-oxidizing bacteria. According to endosymbiotic theory, mitochondria have evolved from sulfur bacteria. Following this idea, we have developed a novel mass spectrometry-based method to measure S_8 and for the first time, we have detected S_8 in the mitochondria of mammalian cell in concentration comparable with sulfur bacteria. While bacteria store S_8 in sulfur granules, we found large concentrations of lipophilic S_8 in lipid droplets in mouse and human adipocytes. We hypothesized that S_8 could be directly produced by the oxidoreductase associated with lipid droplets. Indeed, we identified eNOS (endothelial NO synthase) associated with lipid droplets in adipocytes. Treatment of recombinant eNOS with a sulfur donor GSSSG and electron donor NADPH resulted in production of S_8 and its accumulation in lipid droplets. We have further found inducible and neuronal NOSs for S_8 production. Treatment of adipocytes with GSSSG has markedly increased levels of S_8 in the lipid droplets. We propose therefore that S_8 in lipid droplets could serve as a reservoir for reactive supersulfides (RSSxH). Supersulfides serve as antioxidants and thus protect cells from lipid peroxidation-driven cell death - ferroptosis. Indeed, depletion of S_8 from adipocytes caused lipid oxidation and ferroptosis. In contrast, supplementation with solubilized S_8 prevented ferroptosis caused by ferroptosis inducers. Also, *in vitro*, S_8-loaded lipid droplets were resistant to lipid peroxidation. The present data indicates that S_8 could serve as an evolutionarily conserved supersulfide reservoir and thus counteract oxidative stress in cells.

Sulfur: a major element undergoing global change

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Sulfur (S) is a key component of life and an element that has been dramatically changed by industrial activities, including mining and fossil fuel combustion. Today, the nature of how humans alter the global S cycle is changing. As atmospheric S deposition declines in response to air quality regulation in the U.S. and Europe, there has been an increase in S fertilizer applications reported in many large, regional crop systems. In addition, intensification of agriculture has driven increased S inputs for other uses: as a pesticide, regulator of soil pH, and soil conditioner. Given that excess S can cause soil acidification and mobilization of heavy metals in ecosystems, it is critical to develop methods to trace its “fingerprint” through complex landscapes, quantify S forms and transformations in soils and surface waters, and determine the consequences of its use. In this talk, I will describe both new analyses of long-term data and process-based studies that provide compelling evidence for how the forms, amounts, flows, and consequences of S have changed from what they were in the 1970s at the peak of acid deposition. I will highlight studies from my research group that show exciting new methodological developments using radio- and stable isotopes of S adapted from the marine literature to trace S applications through large agricultural regions. I will also discuss the collaborative actions that researchers, land managers, and regulators may take to address the consequences of excess S in the environment. Finally, I will touch on the potential extension of these new studies to probe the consequences of excess S in northern latitudes facing rapid climate change and potential rainout from geoengineering applications.

POSTERS

POSTER 1

Sulfur-based schemes for biofortification of maize kernels with zinc

Dimitris Bouranis[1,2], Georgios P. Stylianidis[1,3], Despina Dimitriadi[1,3], Nikoletta Strepkou[1] and Styliani N. Chorianopoulou[1,2]

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In this work we elaborate on the response of maize plants towards potential biofortification of the kernels with zinc applied as sulfate alone, or in combination with ethanolamine borate (FA-Bo: FytoAmino-Bo product of Karvelas AVEE), each treatment coupled with cysteine or methionine. Two areas were selected for field experimentation: Ypato and Aliartos in Viotia, Greece. The soil of the experimental plots in Aliartos was characterized by marginally low micronutrients and phosphorus contents, whilst the soil of the experimental plots in Ypato was characterized by low phosphorous contents and medium micronutrients contents, with the other soil characteristics at comparable levels. The distance between the selected areas is 35 km, and weather dynamics was the same. Foliar applications took place at the beginning of the R2 stage (blister), whilst morphometric measurements and determination of zinc contents took place at the R6 stage (maturity), in the kernels of the harvested ears.

The treatments affected both zinc content and fresh weight of the kernels and there were differences between the crops grown in the selected areas. In Aliartos, the combination of zinc sulfate with cysteine or methionine failed to biofortify the ears with zinc, in contrast to cysteine or methionine alone, or the FA-Bo in combination with cysteine or methionine, which produced ears with kernels biofortified with zinc. In Ypato, all treatments but cysteine alone produced ears with kernels biofortified with zinc. The level of soil zinc differentiated the efficacy of each treatment.

POSTER 2

Sulfur-based schemes for biofortification of maize kernels with zinc: the effects on iron content of kernels

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In this work we elaborate on the response of maize plants to the potential biofortification of the kernels with iron, when zinc was foliarly applied as sulfate alone, or in combination with ethanolamine borate (FA-Bo: FytoAmino-Bo product of Karvelas AVEE), each of them coupled with cysteine or methionine. Iron sulfate alone or combined with cysteine or methionine was tested, too. Two areas were selected for field experimentation: Ypato and Aliartos in Viotia, Greece. The soil of the experimental plots in Aliartos was characterized by marginally low micronutrients and phosphorus contents, whilst the soil of the experimental plots in Ypato was characterized by low phosphorous contents and medium micronutrient contents, with the other soil characteristics at comparable levels. The distance between the selected areas is 35 km, and weather dynamics was the same. Foliar applications took place at the beginning of the R2 stage (blister), while morphometric measurements and determination of zinc contents took place at the R6 stage (maturity), in the kernels of the harvested ears.

The treatments affected both iron content and fresh weight of the kernels and there were differences between the crops grown in the selected areas. In Aliartos, the following treatments increased iron content in the kernels per ear: $FeSO_4$,Cys (25%), FA-Bo (16,3%), FA-Bo,Cys (42,6%), Met (96,9%), FA-Bo,Met (22,8%) and $ZnSO_4$,Met (12,5%), whilst $FeSO_4$ and $FeSO_4$,Met failed to biofortify them. In Ypato, $FeSO_4$, $FeSO_4$,Cys and $FeSO_4$,Met failed to biofortify the kernels per ear with iron. Instead, FA-Bo,Cys and $ZnSO_4$,Met biofortified the kernels be 15,5% and 10% respectively. The level of soil iron differentiated the efficacy of each treatment.

POSTER 3

Characterization of single and multiple *Arabidopsis thaliana* mutants in LSU (RESPONSE TO LOW SULFUR) genes

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Sulfur is an essential macronutrient for plant growth and development. In response to sulfur deficiency plants adjust their transcriptome. Genes from LSU (RESPONSE TO LOW SULFUR) family are strongly induced under sulfur deficiency conditions. In Arabidopsis thaliana, there are four members of the LSU gene family, located tandemly on chromosomes 3 (LSU1 and LSU3) and 5 (LSU2 and LSU4). The LSU proteins consist of about 100 residues and they form coiled-coil structures. In accordance, they are able to form homo- and heterodimers. The molecular functions and importance of LSU proteins are still unknown however there is some evidence that they are involved in plant responses to environmental challenges, such as sulfur deficiency and plant immunity. To better understand the role of LSU genes a set of lsu deletion lines, including the single, double, triple and quadruple mutants were obtained using the CRISPR/Cas9 technology. The series of experiments comparing the morphology of the lsu-KO lines with WT have been conducted. The only observed effect of LSU genes deletion was in the length of seedling roots in response to sulfur deficiency. Further analyses were focused on the quadruple mutant and included measurement of sulfur metabolism-related metabolites as well as monitoring differences in gene expression in sulfur sufficient and deficient conditions in comparison to the wild-type plants. The results suggested the reduced performance of sulfate reduction pathway.

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POSTER 4

Persulfide metabolism in *Arabidopsis thaliana*

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Communication is the key for plant's survival in its environment. Here amino acids play multifaceted roles in the survival of the plant, serving as both nutrient carriers and precursors for signalling and defence compounds. Yet, how plants sense and translate fluctuations in amino acids into decipherable signals remains unclear. One such way could be through post-translational protein modifications such as persulfidation, emerging as potential mechanisms for amino acid-based signalling. There are several mitochondrial enzymes that could be potentially involved in the production and oxidation of persulfides [1]. A presently unknown aminotransferase converts cysteine to 3-mercaptopyruvate. The sulfurtransferase STR1 transfers the sulfur group of 3-mercaptopyruvate to glutathione (GSH) producing glutathione persulfide (GSSH) and could potentially also catalyze persulfide transfer to protein cysteine residues. Persulfide oxidation in the mitochondrial matrix is catalyzed by the persulfide dioxygenase ETHE1 [2]. However, it is not known whether ETHE1 can use protein persulfides as a substrate and thus could be involved in the regulation of this post-translational modification. Using a novel proteomics-based approach- Thermal Proteome Profiling (TPP), we were able to identify a list of enzymes in the mitochondria, that showcased the ability to bind and interact with cysteine. These candidate enzymes were recombinantly produced in a heterologous host and utilized to reconstruct the potential cysteine based persulfide signaling pathway of *Arabidopsis thaliana in vitro*. By deciphering these intricate molecular mechanisms, we hope to gain insights into the language of amino acid mediated communication, offering new avenues for understanding and manipulating plants.

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POSTER 5

MPST-1: A thiosulfate sulfurtransferase with transpersulfidase activity regulating development in *C. elegans*

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The therapeutic potential of thiosulfate ($S_2O_3^{2-}$) has been emerging in recent years. As an oxidation product of hydrogen sulfide metabolism, thiosulfate paradoxically mimics the effects of sulfide donors, potentially through involvement in protein persulfidation. Our group previously demonstrated that exposing the nematode *C. elegans* to thiosulfate results in lifespan extension and increased protein persulfidation. Thiosulfate is processed by thiosulfate sulfurtransferases (TST), also known as rhodaneses, which are part of a superfamily of proteins that transfer sulfur atoms to various substrates, producing H_2S in reactions catalyzed by enzymes like mercaptopyruvate sulfurtransferase (MPST). Recently, mammalian MPST was proposed to be the first identified protein persulfidase, although the secluded active site and inaccessibility of its target thiol sites challenge this claim. The *C. elegans* UNIPROT database does not list any gene/protein described as a thiosulfate sulfurtransferase but does report seven different mpst genes. To differentiate their roles, we compared the lifespan extension effects of thiosulfate on *mpst-1* and *mpst-3* mutants, finding that only *mpst-1* mutants fail to respond to thiosulfate treatment. These mutants have shorter lifespans, exhibit strong embryonal delay, and reduced fertility. AlphaFold modeling shows that the active site of MPST-1 protein is more surface-exposed compared to any mammalian sulfurtransferase, leading us to hypothesize that MPST-1 uses thiosulfate as a substrate and may function as a direct protein persulfidase. We purified the MPST-1 protein and demonstrated that this enzyme indeed acts as a rhodanese and cannot use mercaptopyruvate as a substrate. Adding purified MPST-1 to native worm lysates increased protein persulfidation, suggesting that MPST-1 also serves as a direct persulfidase. We are currently using proteomic and interactomic analyses to explore the involvement of TST in protein persulfidation, aging, and development.

POSTER 6

Perturbations in thiol redox status control GAPDH LLPS and aggregation in aging brain

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Cellular homeostasis is based on precise regulation of both chemical and physical processes. One of these physical processes, known as Liquid-Liquid Phase Separation (LLPS), is a mechanism through which cytoplasmic components, namely proteins and RNAs, can assemble in to distinct membraneless compartments. In an intracellular environment, LLPS is strictly regulated and failure to control the formation and dissipation of these compartments leads to protein misfolding and aggregation which has been proposed as one of the causes of age-related diseases. With the hypothesis that cysteine posttranslational modifications could affect the creation of these biomolecular condensates, we turned to well-studied redox-regulated protein GAPDH. Surprisingly, we found that GAPDH undergoes phase separation despite being a fully structured protein without intrinsically disordered regions. Phase separation could be induced by both, molecular crowding and interaction with RNA. We found that once phase-separated, GAPDH activity increases but so does its reactivity towards H_2O_2 and ability to become hyperoxidized and inactivated. Inducing persulfidation in pre-existing GAPDH condensates causes their rapid dispersion into a single phase within minutes. Furthermore, age-induced hyperoxidation of C245 drives this protein into aggregation via phase transition. These observation bear patho-physiological significance as CSE knockout mice, which exhibited shorter lifespans, also demonstrate high number of spontaneously formed neurofibrillary tangles and aggregates with aging.

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POSTER 7

Investigation of the antioxidant activity and functional oxidized derivatives of 2-oxo-imidazole-containing dipeptides

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Imidazole containing dipeptides (IDPs) such as carnosine are endogenously produced in various vertebrates and have multiple physiological functions such as antioxidant capacity and anti-fatigue effect. However, several *in vitro* assays revealed that the antioxidant capacity of IDPs was significantly weaker than that of other endogenous antioxidants such as glutathione, and the detailed mechanisms underlying the antioxidant activity of IDPs are not fully understood. Recently, we revealed that novel oxidized derivatives of IDPs, 2-oxo-IDPs such as 2-oxo-carnosine, were endogenously produced in various vertebrate tissues, and the production increased in an oxidative stress-dependent manner. Furthermore, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay showed that 2-oxo-IDPs exhibited a much higher antioxidant capacity than the precursor IDPs did; however, the detailed mechanisms underlying the antioxidant activity of 2-oxo-IDPs remain unclear. In this study, we analyzed the overall antioxidant capacity and chemical properties of 2-oxo-IDPs. Three different *in vitro* antioxidant assays including DPPH radical scavenging assay showed that the antioxidant capacity of 2-oxo-IDPs was greater than that of the precursor IDPs. Mass spectrometric analyses also demonstrated that the reactivity of 2-oxo-IDPs with several endogenous radicals such as peroxynitrite (ONOO–) was more potent than that of the precursor IDPs. Notably, we succeeded in detecting several candidates for oxidized products derived from the reaction of 2-oxo-carnosine with ONOO– by mass spectrometry, and one of these products (Compound X) was found to react with thiol compounds such as glutathione. Further, the endogenous production of glutathione adduct of Compound X was detected in the rat skeletal muscle tissue. The results obtained herein suggest that 2-oxo-IDPs can function as a potent antioxidant and efficiently react with endogenous oxidants to form further oxidized derivatives which have a potential to react with thiol compounds, involving in the regulation of physiological functions of IDPs.

POSTER 8

The use of dimedone switch method to unravel persulfidation mechanisms in health and disease

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Hydrogen sulfide (H_2S) is one of the simplest sulfur-containing molecules found in cells. Since the first report of its potential physiological role, there has been burgeoning literature on the subject of H_2S signaling. One of the main mechanisms by which sulfide signals in cells is via posttranslational modification of cysteine residues called persulfidation. We recently reported the development of a dimedone-switch method to selectively label and monitor protein persulfidation, which allows us to do so even at the proteome level. Using this method, we looked at persulfidation changes in wild-type, CSE, and MPST knockout animals across different tissues and during aging. We also compared the effects of different diseases (such as diabetes) as well as H_2S donors on the changes in the protein persulfidation landscape in various tissues to define the common targets and pathways affected in each condition. Our data suggest that persulfidation predominantly occurs via H_2S produced by either of these enzymes, with proteins being controlled by either or both of them. Most tissues appear to be more affected by CSE, and CSE plays a greater role in aging-induced loss of protein persulfidation. Furthermore, our data argue against the proposed protein transpersulfidation catalyzed by MPST.

POSTER 9

Auxin converted from indole glucosinolate degradation product controls the amplitude of cell enlargement in cotyledon size regulation in *Arabidopsis thaliana*

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Reduced cell number in Arabidopsis leaves often enhances cell expansion, which is known as Compensation. This phenomenon suggests that a leaf can perceive its size, and that decreased cell number as “input” is somehow translated into compensated cell enlargement (CCE) as “output”. This suggests the existence of a molecular network regulating leaf size, and indicates that understanding the molecular mechanism underlying compensation is fundamental towards unveiling the relationship between cell number and size in an organ-wide context. However, the detailed systems are not uncovered.

Recently, we discovered that CCE in *fugu5*, a loss-of-function mutant of vacuolar H+-pyrophosphatase, is triggered by a transient excess of indole-3-acetic acid (IAA). Importantly, the IAA level was significantly increased in *fugu5* via metabolic regulation at 8 days after sowing (DAS). Provided that the reduced cell number in *fugu5* cotyledon has been ascribed to gluconeogenesis disorder during seed storage lipid mobilization (upon imbibition ~ 3 DAS), this relatively early metabolic alteration is likely to affect later IAA homeostasis at 8 DAS and accomplish compensation. Here, multi-platform widely-targeted metabolomics combined with bioinformatics has been adopted to test the above hypothesis.

By clustering the time-dependent metabolome data, we identified a metabolite whose accumulation pattern was similar to that of IAA and named it “Highly Accumulated in *fugu5* at 8-day-old seedlings (HAF8)”. Exogenous HAF8 supply enhanced CCE in *fugu5* in a concentration-dependent manner, indicating that it is indeed a key player in CCE. Moreover, we identified other candidate phytochemicals affecting CCE by correlation network analysis and isotope-labeled HAF8 feeding experiment. Based on RNA-seq analysis and following genetic experiment, we identified that biosynthesis and degradation of indole glucosinolates and reuse of indole skeleton for IAA synthesis occurred at 8 DAS in *fugu5* and were essential for CCE.

POSTER 10

Supersulfides catalyze nitric oxide metabolism via glutathione-coupled electron transfer from formaldehyde

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Abundant formation of endogenous supersulfides, which include reactive persulfide species and sulfur catenated residues in thiols and proteins (supersulfidation), has been observed. We found here that supersulfides catalyze S-nitrosoglutathione (GSNO) metabolism via glutathione-dependent electron transfer from aldehydes by exploiting alcohol dehydrogenase 5 (ADH5). ADH5 is a highly conserved bifunctional enzyme serving as GSNO reductase (GSNOR) that down-regulates NO signaling and formaldehyde dehydrogenase (FDH) that detoxifies formaldehyde in the form of glutathione hemithioacetal. C174S mutation significantly reduced the supersulfidation of ADH5 and almost abolished GSNOR activity but spared FDH activity. Detailed examination of C170, which resides in proximity to C174, indicated that C170 may confer stability to the supersulfidation of C174, thus implying its potential participation in the supersulfide catalytic process inherent to ADH5. Notably, *Adh5*C174S/C174S mice manifested improved cardiac functions possibly because of GSNOR elimination and consequent increased NO bioavailability. Therefore, we successfully separated dual functions (GSNOR and FDH) of ADH5 (mediated by the supersulfide catalysis) through the biochemical analysis for supersulfides in vitro and characterizing in vivo phenotypes of the GSNOR-deficient organisms that we established herein. Supersulfides in ADH5 thus constitute a substantial catalytic center for GSNO metabolism mediating electron transfer from aldehydes.

POSTER 11

Biochemical characterization of thioredoxin-like 3, a conserved protein in the green lineage

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Thioredoxins (TRX) are ubiquitous proteins regulating the redox state of numerous proteins, in particular in chloroplasts. These small proteins have a particular fold and a CxxC motif comprising two active-site cysteines. A predicted chloroplastic protein possessing these characteristics has been automatically annotated as TRX-like but its function is unknown and its predicted fold is more related to thiol peroxidases (*i.e.* AhpC_TSA2-like domain). It is present in microalgae and land plants including trees and we referred it to as TRX-like3. The recombinant TRX-like3 proteins from *Arabidopsis thaliana* and *Chlamydomonas reinhardtii* have been expressed in *Escherichia coli* and purified. The structure of *At*TRX-like3 has been solved. Like regular TRXs, it is a monomer and the CxxC motif present in the first □-helix is surface exposed. However, *At*TRX-like3 possesses an elongated N-terminal region and an insertion between the β3 and β4 strands compared to conventional TRXs. While both TRX-like3 are oxidized by H_2O_2, no thiol peroxidase activity has been detected. A reductase activity was not detected either, using insulin as a substrate. Since the biochemical analyses do not give information about a possible function as a reductase, Chlamydomonas mutants have been selected. Preliminary results indicate that *Trx-like3* mutants exhibit a growth retardation on a minimal medium under light which was not visible when grown photo-autotrophically on rich-media. This opens the way for a deeper functional analysis.

POSTER 12

Toward the characterisation of an unexplored class of glutaredoxins in photosynthetic eucaryotes

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Glutaredoxins (GRXs) are oxidoreductases that catalyze the reduction of protein disulfide bonds or of glutathionylated proteins using the reducing power of glutathione. In plants, they help maintaining redox balance notably under stress conditions [1], and participate to the maturation of iron-sulphur cluster-containing proteins [2]. Among the six classes of GRXs, the roles of class IV GRX members (GRX-IVs), which are specific to photosynthetic eucaryotes, remain enigmatic. In addition to the GRX domain, GRX-IV proteins contain two extra domains named DEP **(**Dishevelled, Egl-10 & Pleckstrin domain) and DUF547 [3]. The DEP domain is also present in well-characterized signaling proteins of eucaryotic organisms. In these proteins the DEP domain is described as a membrane anchor[4] and as a cross-linker that acts through a conformational switch from monomer to domain-swapped dimer [5]. The DUF547 domain is surprisingly mainly found in bacteria and archaebacteria, and its function remains totally unknown. In this context, our work aims at laying the first foundations for identifying GRX-IV functions, focusing on members of poplar trees *(Populus trichocarpa).* While all investigated angiosperm genomes contain only two *GRX-IVs*, three members were systematically identified in species of the *Salicaceae* family to which poplars belong. By digging published expression data, we found that poplar *GRX-IV 1* and *GRX-IV 2.2* are mainly expressed in inflorescences, like their orthologs from *Arabidopsis thaliana.* Because of the absence of published information regarding *GRX-IV 2.1,* we analyzed RNAseq data from poplars growing under specific conditions. We found that *GRX-IV 2.1* is expressed in roots, especially when they form associations with mycorrhizal fungi [6]. Thanks to the use of transgenic lines of both *A. thaliana* and *P. trichocarpa* along with the expression and purification of recombinant proteins, our long-term goals are to reveal both biochemical and physiological functions of GRX-IVs.

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POSTER 13

Biochemical characterization of thioredoxin-related protein Clot/TRP14 from *Populus trichocarpa*

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Thioredoxin-related protein Clot possesses an atypical WCPDC motif compared to the classical WCGPC motif. In this study, we observed that Clot isoform from *Populus trichocarpa* is oxidized by cystine, GSSG and GSNO to a smaller extent, resulting in the formation of an intramolecular disulfide bond that is reduced by the GSH system, but not by the NADPH-dependent thioredoxin reductase system. Nevertheless, the reduction by the GSH system appears relatively inefficient which likely prevented a significant cystine reductase activity and caused a low ability of Clot to reduce S-sulfocysteine compared to typical glutaredoxin (GRX) and thioredoxin (TRX) isoforms. On the contrary, PtClot catalyzed both *in vivo* and *in vitro* the oxidation of roGFP2 in the presence of cystine but not GSSG. Introducing the active site of GRX or TRX motif in Clot reduced this cystine-dependent activity, indicating that the WCPDC signature differentiates Clot from regular TRX and GRX.

POSTER 14

Dynamic monitoring of the intracellular cysteine pool with the genetically-encoded biosensor CyReB

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In the last decade, the development of fluorescent probes has revolutionized our experimental access to physiological parameters in living cells, particularly in redox biology. Considering the essential role of cysteine, we aimed to develop a genetically-encoded biosensor to monitor intracellular cysteine dynamics in real-time. In a previous work, we demonstrated the ability of a particular cysteine desulfurase of *Pseudorhodoferax* to promote the oxidation of roGFP2 in the presence of cysteine. We have developed a potential Cysteine Redox Biosensor (CyReB) by coupling this cysteine desulfurase isoform to roGFP2. The specificity, sensitivity, and the oxidation-reduction dynamics of CyReB have been investigated *in vitro*. Finally, the expression of CyReB and its inactive version in both wild-type and mutant strains of *Escherichia coli* demonstrates that CyReB enables the monitoring of intracellular cysteine dynamics. This paves the way for its widespread use in other model organisms, notably eukaryotes.

POSTER 15

Analysis of pathological progression-dependent changes of supersulfides production in the brain tissues of mouse models of Alzheimer's disease

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder causing severe cognitive dysfunction. With the increasing number of AD patients in an aging society, the development of preventive and therapeutic strategies is crucial. Supersulfides, defined as hydropersulfide (RSSH) and polymeric sulfur species (RSSnR, n > 1), are produced enzymatically *in vivo* and regulate antioxidative stress responses and redox signaling. Previous studies showed that endogenous supersulfide production might change in the brain tissue of AD model mice (5xFAD) with severe cognitive dysfunctions. However, the detailed relationship between supersulfides and AD remains unclear. In this study, we performed supersulfide omics analysis on the brain tissue of 5xFAD mice of different ages to investigate the changes in supersulfide production during AD progression. We confirmed AD progression by evaluating insoluble Aβ peptides, neuronal inflammation, and cognitive dysfunction. Quantitative analysis of total polysulfide content revealed a significant decrease in 8-month-old 5xFAD mice compared to wild-type mice. Notably, significant decreases in protein polysulfides were detected in 4- and 8-month-old 5xFAD mice using a novel alkylating agent, *N*-iodoacetyl L-tyrosine methyl ester. These results suggest that endogenous supersulfide production changes during AD onset and progression, with specific forms of supersulfides affected by AD severity. Further investigation is needed to identify AD-related supersulfidated proteins and analyze the forms of supersulfides. These findings highlight the importance of supersulfide-regulated redox signaling in developing preventive and therapeutic strategies against AD.

POSTER 16

Development of mass spectrometry-based supersulfidomics and its potential: alternations in supersulfide production during the germination of broccoli sprouts

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Sulfur is essential for life and human health, primarily obtained from proteins to synthesize sulfur-containing biomolecules. Recent studies have highlighted the biological significance of endogenous supersulfides, defined as hydropersulfide (RSSH) and polymeric sulfur species with sulfur catenation (RSSnR, n > 1, R = hydrogen or alkyl, or cyclized polysulfides) [1]. Ingesting exogenous sulfur compounds is crucial for producing these supersulfides, however their content in foods and detailed biosynthesis mechanisms are not well understood. In this study, we developed mass spectrometry (MS)-based technologies for supersulfidomics to investigate supersulfide profiles in biological samples including animal cells and tissues, and foods such as fresh vegetables, edible animal meat, and beans [2,3,4]. Quantitative analysis of supersulfide profile revealed that supersulfides are relatively more abundant in fresh vegetables of *Allium* and *Brassica*, such as onions, broccoli, Chinese chive, and garlic [2,3]. Notably, broccoli sprouts had the highest supersulfide content, which increased during germination and growth [3]. Untargeted polysulfide omics analysis showed that supersulfide composition changed significantly over cultivation time. Predominant organic supersulfide metabolites identified included cysteine hydropersulfide (CysS2H) and cysteine hydrotrisulfide. Additionally, novel sulforaphane (SFN) derivatives conjugated with supersulfides were found in broccoli sprouts. An *in vitro* radical scavenging assay using 2,2-diphenyl-1-picrylhydrazyl revealed that the SFN conjugate with CysS2H had greater radical scavenging capacity than SFN and cysteine, suggesting that the abundant supersulfide content in broccoli sprouts may contribute to their human health benefits. These results suggest that the new MS-based supersulfidomics techniques could be useful tools for evaluating the biological significance of endogenous and exogenous supersulfides in redox biology and medicine.

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POSTER 17

TRX*o*1 involvement in the persulfidation of Arabidopsis under salinity

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Reactive oxygen, sulfur and nitrogen species generated during cellular metabolism provoke post-translational modifications (PTMs) in sensitive amino acid residues, generating structural and functional changes in proteins. Among them, persulfidation (-SSH) occurs by reaction of H_2S on the oxidized sulfenic form (-SOH) of thiols. Thus, one of the proposed roles for this reversible PTM is the protection of thiols from overoxidation in an oxidative situation as the one generated by biotic and abiotic stresses, which may lead to an irreversible loss of protein activity. In plants, H_2S seems to be essential for the regulation of various environmental stress conditions that nowadays provoke important losses in crops productivity, although the effect of salinity on the persulfidation pattern of proteins needs to be investigated.

Thioredoxins (TRXs) are small oxidoreductases involved in the thiol-disulfide exchange of target proteins, and among them, TRX*o*1 has been located in mitochondria and nucleus, where it regulates specific target proteins. Persulfides are reversible by TRXs in animal systems, but this role has not been studied in plants. In this work we analyzed the possible role of TRXo1 in the protein persulfidation pattern induced by salinity in *Arabidopsis thaliana* L. wild type and two *Attrxo1* mutant lines, to deepen on the involvement of redox regulation of persulfidation during plant acclimation.

Results show that under salinity, mutant lines showed a lower amount of persulfidated proteins. Focusing on mitochondrial antioxidant systems, the results seem to support their possible regulation by persulfidation and the participation of TRXo1. In mutants, the TRXo1 target mercaptopyruvate sulfutransferase, that uses 3-mercaptopyruvate as a sulfur donor, was found more persulfidated. Also, the identification of nuclear proteins such as NRX and PCNA2 as persulfidated in the mutants opens the field of post-translational regulation of persulfidation towards the metabolism of this organelle and the intervention of TRXo1 on it.

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Cdo1/Suox Double Knockout Mice Demonstrate Hydrogen Sulfide Toxicity in Sulfite Oxidase Deficiency

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Sulfite oxidase (SOX, Gene: *SUOX*) is a mitochondrial enzyme that catalyzes the final step of cysteine catabolism oxidizing toxic sulfite to sulfate. Mutations in the *SUOX* gene cause hereditary sulfite oxidase deficiency (SOXD). Hallmarks of SOXD are the accumulation of sulfite, S-sulfocysteine (SSC), and thiosulfate, the terminal product of H_2S catabolism. Neurodegeneration is the major lethal and irreversible phenotype of SOXD patient resulting in premature death. While SSC was shown to contribute to neurotoxicity, the contribution of H_2S-related thiosulfate has not been studied so far. We generated and characterized the whole-body *Suox* knockout mice, and they recapitulate the phenotype of SOXD patients with an average lifespan of 9.6 days. Recently, a knockout (KO) of cysteine dioxygenase (CDO, Gene: *Cdo1*), the first and rate-limiting enzyme in cysteine catabolism was found to reverse SOXD phenotypes in *Caenorhabditis elegans*. Therefore, we aimed to rescue SOXD mice ($Suox^{-/-}$) by an additional *Cdo1* KO by generating $Cdo1^{-/-}$ $Suox^{-/-}$ double knockout (dbKO) mice. In contrast to *C. elegans*, *Cdo1* KO did not reverse *Suox* KO phenotypes in mice. On the contrary, body weight of the dbKO mice was smaller, and the average lifespan was about 30% shorter than that of *Suox* KO mice. As expected, plasma sulfite and SSC levels in the dbKO mice were almost back to wild-type levels demonstrating a drastically reduced formation of sulfite. Surprisingly, H_2S and thiosulfate levels showed even higher accumulation in dbKO mice than in $Suox^{-/-}$ mice suggesting a shift of cysteine catabolism. In addition, cysteine persulfide levels of dbKO mice were drastically increased and methemoglobin was found to be elevated in $Suox^{-/-}$ and dbKO mice too. In conclusion, our data indicate that besides sulfite and SSC, altered H_2S metabolism contributes to the pathology of SOXD.