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Lectures

L7.1

The chloroplast thiol-disulfide redox regulatory network and the sensory roles of 2-cysteine peroxiredoxin and cyclophilin 20-3

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Dynamic environmental acclimation of organisms decisively relies on a redox regulatory signalling network consisting of input elements, transmitters, targets, sensors, buffers and final electron acceptors in the form of reactive oxygen species (ROS) and reactive nitrogen species (RNS). The talk will address the redox network of the chloroplast where the multifunctional and abundant 2-cysteine peroxiredoxin (2-CysPrx) adopts five conformational states in dependence on its redox state. 2-CysPrx serves as peroxidase, chaperone, binding partner, proximity based thiol oxidant and redox sensor. Functional dissection of the 2-CysPrx states will be achieved by use of site directed mutagenized variants. The data show that maximizing peroxidatic or chaperone function was not a driving force in evolution, but instead the functional thiol switch was conserved. The only cyclophilin of the chloroplast stroma Cyp 20-3 interacts with 2-CysPrx. Recent data show that Cyp20-3 mediates stress-induced adjustment of redox homeostasis by modulating cysteine synthesis. Here a new crosstalk between oxylipin signalling, the redox regulatory network and cell redox homeostasis emerges.

L7.2

Ascorbate and photosynthesis: how does *Arabidopsis* adjust leaf ascorbate concentration to light intensity?

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Ascorbate accumulates in *Arabidopsis thaliana* leaves in a light dose-dependent manner reaching concentrations of ~20 mM in full sunlight. This response is consistent with its proposed functions in photoprotection of photosynthesis. Relatively little is known about how this precise relationship between light intensity and ascorbate pool size is controlled and this presentation will review recent results. The first committed step in ascorbate biosynthesis from GDP-mannose is catalysed by GDP-L-galactose phosphorylase, encoded by *VTC2* and *VTC5*. *VTC2* and *VTC5* promoter:luciferase reporters showed that protein expression is controlled by the circadian clock and entrained to light-dark cycles so that expression increases rapidly in the first hour of light and decreased thereafter. VTC2 and VTC5 reporter protein expression was also light intensity dependent and rapidly repressed by exogenous ascorbate and its precursor L-galactose. Results of experiments with photoreceptor mutants, photosynthesis mutants and inhibitors suggest the involvement of blue light and chloroplast-sourced signals. Ascorbate accumulation is therefore controlled by a complex interplay between cryptochromes, photosynthesis and end product repression.

Oral presentations

O7.1

Role of *Sinorhizobium meliloti* glutaredoxins in nitrogen-fixing symbiosis

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Legumes interact symbiotically with bacteria of the *Rhizobiaceae* family to form nitrogen-fixing root nodules. We investigated the contribution of the three glutaredoxin (Grx)-encoding genes present in the *Sinorhizobium meliloti* genome to this symbiosis. SmGRX1 (CGYC active site) and SmGRX3 (CPYG) recombinant proteins displayed deglutathionylation activity, whereas SmGRX2 (CGFS) did not. Mutation of SmGRX3 did not affect *S. meliloti* growth or symbiotic capacities. By contrast, SmGRX1 and SmGRX2 mutations decreased the growth of free-living bacteria and the nitrogen fixation capacity of bacteroids. Mutation of SmGRX1 led to an absence of bacteroid differentiation, whereas SmGRX2 mutation decreased nodule development. The *Smgrx2* mutant strain displayed significantly lower levels of activity than the wild-type for two iron-sulfur containing enzymes, aconitase and succinate dehydrogenase. This lower level of activity could be associated with deregulation of the transcriptional activity of the RirA iron regulator and higher intracellular iron content. Thus, two *S. meliloti* Grx proteins are essential for symbiotic nitrogen fixation, playing roles in bacterial differentiation and the regulation of iron metabolism.

O7.2

MPK4 is involved in growth and photosynthesis regulation

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Mitogen activated protein kinase (MAPK) pathways regulate signals transduction from different cellular compartments and from the extracellular environment to the nucleus in all eukaryotes. One of the best characterized MAPKs in *Arabidopsis thaliana* is MPK4 which was shown to be a negative regulator of the systemic acquired resistance. *mpk4* mutant accumulates salicylic acid (SA), possesses constitutive expression of pathogenesis related (PR) genes and has an extremely dwarf phenotype. We present that suppression of SA accumulation by knocking down ICS1 gene (by crossing with *sid2* mutant) in *mpk4* mutant background did not revert *mpk4* impaired growth. Furthermore it caused changes in photosynthetic apparatus and severely impaired quantum yield of photosystem II. Transmission microscopy analysis revealed that chloroplasts' structure was altered in *mpk4* and *mpk4*/*sid2* double mutant. Analysis of expression and activity of ROS scavenging enzymes showed that suppression of SA synthesis in *mpk4* mutant caused imbalances in ROS homeostasis which were more pronounced than in *mpk4* single mutant. Taken together it seems that MPK4 regulates growth in SA-independent way and is required for optimal photosynthesis in *Arabidopsis*.

Posters

P7.1

Mitochondrial protease AtFtsH4 affects the level of oxidatively modified proteins in seeds and mitochondria of *A. thaliana*

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Protein carbonylation is an irreversible oxidative modification that has been identified in all stages of plant life cycle. It is now believed that carbonylation is not simply a random process but it may play a significant role in the control of some biological processes. Here we show that the lack of mitochondrial AtFtsH4 protease results in accumulation of carbonylated proteins. In our studies we combined 2D-PAGE with immunodetection of carbonyl groups in DNP-derivatized protein to better understand the connection between oxidative stress and AtFtsH4 in Arabidopsis mitochondria. Comparative studies of protein carbonylation in mitochondria of 3-weeks-old seedlings growing in a long day and moderately elevated temperature (30 °C) show that the loss of AtFtsH4 mainly affects OXPHOS complexes, photorespiration and TCA cycle enzymes. Notably, germination of *ftsh4* seeds at 30 °C was accompanied by changes in carbonylation profile of 12S-cruciferin seed storage proteins, especially a-cruciferin subunits that were oxidized to a lesser extent than in wild type. Our findings indicate that *Arabidopsis* plants lacking AtFtsH4 protease suffer from broad oxidative stress that does not only affect mitochondria but the entire cell.

P7.2

Action mechanisms of antioxidant and prooxidants properties of flavonoids as model for understanding the oxygen metabolism

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Due to its low redox potential ($0.23\,V < E_7 < 0.75\,V$), the flavonoids can reduce free radicals highly oxidized with redox potentials from 2.13 to 1.0 V ($O_2^{\bullet -}$, $^{\bullet}OH$, $NO^{\bullet}$, $RO^{\bullet}$, $ROO^{\bullet}$). In the antioxidant action the flavonoids trap free radicals through the transference of hydrogen depending on flavonoid structure, heat formation ΔH_f, BDE, and free energy. These mechanisms are proper of flavonols. Simple electron transfer/proton transfer depends on IP and is flavones characteristic such as apigenin and diosmetin; the ion metal quelation Fe^{2+}, Cu^{2+} depend on relation between the flavonoid structure and its ability to inhibit the peroxidation of lipids. The mechanism of prooxidants enzymes inhibition depends on flavonoid hydrophobicity. Prooxidant activity of flavonoids depends mainly on the quantity of hydroxyl groups and the metal ions present in the flavonoid molecule. Studies by pulse radiolysis indicates that the reaction constant for superoxide radical $O_2^{\bullet -}$ production was $2.0 \times 109\ M^{-1}s^{-1}$, meanwhile for semiquinone radical was $8.0 \times 107\ M^{-1}s^{-1}$. The study of antioxidant and prooxidant mechanisms of flavonoids is a useful tool for understanding the behavior of free radicals, its formation and metabolism in biological systems.

P7.3

Respiration and oxygen status during bud burst of grapevine

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Bud dormancy is an important strategy in the phenology of temperate perennial trees, including important crops such as grapevine. Temperature and photoperiod play key roles in regulating dormancy, particularly accumulation of chilling hours. However, dormancy is a quantitative state and in many cases facultative. Where chilling is insufficient, bud burst is poorly coordinated; industry commonly apply chemicals such as hydrogen cyanamide to enforce a more even bud burst. Descriptive studies of transcriptional regulation during grapevine bud burst suggest an hypoxic state during the early stages of this transition, coincident with fermentation and an oxidative burst. To date there has been no physiological data to justify these conclusions. We investigated the internal oxygen status and respiration during a 72 h time course of bud burst. Buds were stored at 4 °C to ensure complete satisfaction of endodormancy, before growing single bud explants in the dark or a 12/12 h photoperiod (150 $\mu mol \cdot m^{-1} \cdot s^{-1}$, 22 °C). Transects of internal oxygen concentration showed a hypoxic state at the core of the bud (< 20 mM O_2 at c. 2000 μm depth) at 3 h and 24 h in dark or dark/light ($n \geq 3$). The influence of light, however was pronounced after 72 h; dark-exposed buds had 80-100 mM O_2, while dark/light-exposed buds were near oxygen saturation (200-260 mM O_2). Hypotheses were further explored via respiratory, fermentation and qRT-PCR data.

P7.4

Chemical regulation of photorespiratory hydrogen peroxide-induced cell death in catalase deficient (*cat2*) *Arabidopsis* plants

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Photorespiration operates as a high-flux pathway fueled by the oxigenase activity of RuBisCO. Being deeply intertwined in plant metabolism, the exhaustive list of functions served by photorespiration are still a matter of debate. To extend our understanding of photorespiration in a systems perspective, we performed a chemical screen designed to identify molecular structures that are able to alleviate the negative effects of photorespiration in *cat2* mutant plants. Having only residual peroxisomal catalase activity these plants can not efficiently remove photorespiratory hydrogen peroxide ultimately leading to cell death under conditions favoring photorespiration. From a screening library comprising 10 000 small molecules, more than 20 unique structures were retained as hits following a primary and secondary screen. Currently, we are exploring the mode of action of a selected number of chemicals using a wide range of approaches such as microarrays, mutant screens, metabolite profiling and chemical proteomics. Crucially, this has the potential to identify previously overlooked photorespiratory components and add new layers of complexity to the regulation of the photorespiratory network.

P7.5

Photoproduction of organic peroxides on the donor side of photosystem II after destruction of the water-oxidizing complex

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The removal of manganese from the water-oxidizing complex (WOC) of photosystem II (PSII) by high pH treatment leads to an increase of O_2 photoconsumption. The light-induced O_2 consumption is associated, at least partially, with the generation of a positive charge(s) on the donor side of PSII because it is inhibited by diuron and suppressed by artificial electron donors of PSII. It has also been shown that removal of Mn from the WOC leads to flash-induced O_2 uptake with maximum on the first flash and the O_2 uptake is activated by low concentrations of divalent Mn ions. It is suggested that the light-induced O_2 uptake on the donor side of PSII is related to formation of organic peroxides via a radical chain reaction that starts with the formation of organic radicals produced by photooxidation of organic molecules. Using a lipophilic fluorescence probe Spy-HP specific to peroxides, we confirmed the light-dependent formation of peroxides in Mn-depleted PSII membranes. At least two types of ROOH, one lipophilic and the other hydrophilic, are distinguished, and both are insensitive to exogenous catalase. The formation of these potentially reactive species should be significant important in the photoinhibition mechanisms.

P7.6

The effect of LED lighting on some antioxidants parameters in lambs lettuce

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Plant cells are rich in antioxidant system which controls the level of ROS. One of the factors influencing ROS generation is light. The aim of the study was to evaluate the effect of LED lamps lighting on some antioxidant parameters in lambs lettuce leaves. Plants were illuminated with LED lamps containing red (660 nm) and blue (440 nm) diodes in different ratio. Control plants were illuminated with sodium lamps (HPS). In the all cases PPFD measured at plant height was 200 $\mu mol\ m^{-2} s^{-1}$. After 60 days of cultivation leaves were analyzed. Activity of catalase and peroxidase, concentration of phenolic compounds and ascorbic acid as well as radical scavenging activity with DPPH radical were performed. The activity of POD was lower in plants treated with 100% red LED light and sodium lamp light. Addition of blue light increased activity of POD. Contrary, the highest activity of CAT was detected in plants irradiated with 100% red LED light. The lowest concentration of ascorbic acid was observed in the case of plants treated with sodium lamps. However, sodium lamps increased phenols content as compared with LED lamps. The highest radical scavenging activity was observed in the case of plants illuminated with sodium lamps.

P7.7

PsbS protein regulates photon fate in *Arabidopsis thaliana* during high light stress

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In plants evolved capacity of absorption of light energy in excess of that required for photosynthesis. This excess needs to be dissipated as heat and fluorescence. One of the molecular components involved in this process is PsbS protein. We have examined PsbS function in global regulation of light acclimatory responses and fluorescence dynamics in *Arabidopsis thaliana* rosettes partially exposed to excess light. In WT fluorescence dynamics exhibits specific regulation between leaves directly exposed to excess light and leaves undergoing systemic acquired acclimation. In contrast for the *npq4-1* mutant deficient in PsbS protein this behavior is significantly deregulated. Interestingly deregulation of fluorescence decay in *npq4-1* mutant upon partial excess light exposure correlates with foliar temperature instability and with increased photosystem II damage only in local leaves. Amplitude of foliar temperature fluctuations during sequential excess and low light exposure are over two-fold stronger than that observed in WT and in PsbS overexpressor. Our results indicate the crucial role of the PsbS not only in local, but also in efficient systemic and post stress regulation of the absorbed photon fate.

P7.8

RNA-seq reveals differentially expressed genes in cucumber MSC lines possessing mitochondrial DNA rearrangements

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Cucumber is characterized by a large, paternally transmitted mitochondrial genome. Passage of cucumber through cell culture induces rearrangements in mitochondrial DNA associated with a MSC phenotype that is characterized by slower growth and chlorotic mosaic on the leaves. In this study, RNA-seq was applied to compare the transcriptomes of the MSC3, MSC12, and MSC16 lines and wild-type line B. Total RNA was isolated from shoots of 2-week old plants. cDNA libraries were sequenced using 454 and Illumina technologies. Differentially expressed genes (DEGs) shared by the MSC3, MSC12, and MSC16 mutants were identified. A relatively small number of DEGs was shared by all three mutants, whereas many were shared by MSC12 and MSC16, suggesting transcriptional similarity between these lines. Several Gene Ontology terms were found to be enriched in the DEG set including oxidoreductase and electron carrier activities. RT-PCR confirmed that the level of terminal oxidase (*AOX2*) transcription was higher in all three MSC mutants compared to the control line B. This study sheds new light on the biology of cucumber MSC mitochondrial mutants. This work was supported by the Polish National Science Centre NCN grant no. N N310 107740.

P7.9

Aldonolactonase plays not only a component of D-galacturonate pathway but also a negative regulator for ascorbate pool size in the moss *Physcomitrella patens*

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Ascorbate biosynthesis is mainly synthesized via the D-mannose/L-galactose (Man/Gal) pathway in higher plant and the D-galacturonate (D-GalUA) pathway in green alga *Euglena*. The genome information suggests that moss plant *Physcomitrella patens* subsp. *patens* (*P. patens*) possesses dual pathways via D-GalUA and Man/Gal. We investigated the occurrence of D-GalUA pathway in bryophyte *P. patens* by identification of aldonolactonae (ALase), a key enzyme in the pathway by catalyzing the conversion of L-galactonic acid to the final ascorbate precursor L-galactono-1,4-lactone. *P. patens* possessed two ALase paralogous genes (AL-1 and AL-2). Kinetics analyses with the recombinant ALases indicated that AL-1 is the functional enzyme for ascorbate biosynthesis, and also catalyzes dehydroascorbate. In *P. patens* mutant lacking the AL-1 gene by homologous recombination, the level of ascorbate in protonemata was two-fold higher than that in wild type. These results indicated that *P. patens* ALase is involved in the regulation of ascorbate level as a negative regulator, presumably by catalyzing of both L-galactono-1,4-lactone and dehydroascorbate degradation.

P7.10

The lichen molecular bioindicator of CO_2 pollution of air

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The aim of this study is developing of the molecular bioindicator of CO_2 pollution of air. The photosynthetic material – lichen, in combination with so called green chemistry solvent – ionic liquid, was used under the different doses of CO_2. The ionic liquid (1-methyl-3-octyloxymethylimidazolium tetrafluoroborate) was selected for this purpose, after synthesis and careful purification. The electronic absorption and fluorescence emission spectroscopies with the temperature control devices were applied. Our experimental results indicate that system designed is very sensitive to the CO_2 doses and it can be use as molecular bioindicator for CO_2 pollution of air. Acknowledgment: Prof. G. Bialek-Bylka thanks project DS 62-213/2013 for financial support.

P7.11

Monitoring photosynthetic electron transport during stress responses in *Arabidopsis*

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The plant cell not only uses its chloroplasts for house-keeping needs, but also employs them in many ROS-related signalling pathways as signal receptors, mediators or amplifiers (Shapiguzov A., Vainonen J.P., Wrzaczek M., Kangasjärvi J. Front. Plant Sci. 2012). Early on during the immune responses provoked by pathogen infections, the changes occur in the operation of photosynthetic electron transfer chain (ETC) located in chloroplasts. Presumably, the controlled destabilization of ETC by a plant serves the purpose of generating specific ROS (Göhre V., Jones A.M., Sklenár J., Robatzek S., Weber A.P. Mol. Plant Microbe Interact. 2012; Nomura H. et al. Nat. Commun. 2012). In turn, the accumulation of ROS in the chloroplast leads to the transcriptional reprogramming with large consequences for plant adaptation and development. The mechanisms of propagation of such signal, particularly of its transfer across the membrane barriers between the organelles, are largely unknown. In our studies we address the role of chloroplasts in plant stress response by inducing defense reactions in *Arabidopsis* and by closely monitoring the state of the chloroplast ETC *in vivo* with the help of chlorophyll fluorescence imaging.

P7.12

GGT-assisted glutathione degradation is functional to apoplastic redox control and seed storage proteins accumulation

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The existence of a gamma-glutamyl cycle consisting of intracellular GSH synthesis, extrusion to the apoplastic space and recovery by gamma-glutamyl transferase (GGT)-assisted degradation into its constituent amino acids, has been demonstrated in plants. To address the significance of this cycle in plant cells, we performed quantitative proteomic analyses in leaves of *Arabidopsis thaliana ggt1* knockout (lacking apoplastic GGT1 isoform) and seeds of iRNA lines where *ggt1* and *ggt2* expression was lowered. The combined iTRAQ and LC-MS/MS based quantitative proteomics approach showed that disruption of gamma-glutamyl cycle in *ggt1* knockout leaves was associated with the induction of genes encoding four GSTs in the *phi* class (*GSTF2*, *GSTF6*, *GSTF9*, and *GSTF10*), GSH peroxidase 1, and glyoxylase II. In seeds, a lower expression of *ggt1* and *ggt2* results also in reduced storage proteins accumulations. Present findings suggest that the disruption of the gamma-glutamyl cycle results in pleiotropic effects related to biotic and abiotic stress response, antioxidant metabolism, senescence, carbohydrate metabolism and photosynthesis, seed storage proteins accumulation, with strong implications for plant's adaptation to environment.

P7.13

Singlet oxygen scavenging by tocopherol and plastochromanol under photooxidative stress in *Arabidopsis thaliana*

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Plant has evolved different types of protective mechanisms against the photooxidative damage; the synthesis of low molecular weight antioxidant in chloroplast is among one of them. Singlet oxygen ($^1O_2$) scavenging activity of tocopherol and plastochromanol was examined in *vte1* mutant of *Arabidopsis thaliana* deficient in both tocopherol and plastochromanol. Synthesis of tocopherol and plastochromanol occur in chloroplast by conversion of intermediates 2,3-dimethyl-5-phytyl-1,4-benzoquinol and plastoquinol catalyzed by tocopherol cyclase, respectively. We observed more $^1O_2$ formation in leaves and isolated chloroplast of *vte1* compared to WT *Arabidopsis* plant by using confocal laser scanning microscopy and electron paramagnetic resonance spin-trapping technique, respectively. Furthermore, the content of malondialdehyde (secondary product of lipid peroxidation) was measured in *vte1*, which was found to be high as compared to WT. The effect was confirmed by the two-dimensional imaging of ultra-weak photon emission (know to reflect oxidation of lipids). Our data reveals that lipid soluble tocopherol and plastochromanol act as singlet oxygen scavenger in *Arabidopsis* plant and protect lipid damage against photooxidative stress.