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Lectures

L4.1

Ascorbate-glutathione dependent redox homeostasis in stress response and cell cycle control

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Ascorbate (ASC) and glutathione (GSH) are redox pairs playing a pivotal role in controlling redox homeostasis and contrasting adverse developmental conditions. We have recently reported that changes in ASC and GSH metabolism are involved in signalling pathways leading to programmed cell death (PCD). In this context post-translational modification of ascorbate peroxidase, a key enzymes involved in H_2O_2 removal, could play a central role in permitting the oxidative burst needed for PCD induction. Moreover, recent data underlines a relation between ASC and GSH and cell cycle progression. Here we report results on alteration of ASC-GSH metabolism in Tobacco Bright Yellow-2 cells treated with ophiobolin A, a toxin produced by phytopathogens of *Bipolaris* and *Aspergillus* genera. Over a threshold concentration ophiobolin A induces cell death with several molecular and cytological markers of PCD but without ROS production. On the other hand, at lower concentration ophiobolin A blocks cell growth in a reversible manner. Our results show that ophiobolin A perturbs the activity of poly ADP-ribose polymerases, GSH levels and the normal GSH fluxes among different cell compartments that characterize the various phases of cell cycle.

L4.2

Regulatory circuitries of H_2O_2-responsive NAC transcription factors

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Transcription factors (TFs) are central regulators of numerous processes in all organisms. By binding to *cis* -regulatory motifs in target promoters and by interacting with other proteins (including non-DNA-binding regulators) TFs activate or repress their target genes thereby controlling physiological and developmental processes. Although the principle biological functions of a large number of TFs have been uncovered over the last two decades, knowledge about the gene regulatory networks (GRNs) through which they exert their cellular functions are virtually unknown in most cases. Our group studies the involvement of NAC TFs in hydrogen peroxide (H_2O_2)-mediated stress responses and signalling. By combining chemical induction of NAC gene expression in transgenic plants with microarray-based expression profiling shortly after NAC induction we were able to discover transcriptional responses and target genes downstream of ROS-responsive NACs. This work identified previously unknown regulatory connections linking H_2O_2 signalling with phytohormone physiology. Examples will be presented and future directions discussed.

Oral presentations

O4.1

PP2A phosphatase interacts with CPK kinase and regulates pathogenesis responses triggered by intracellular ROS signals

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Plant immunity is governed by converging signalling pathways, which are largely regulated through reversible protein phosphorylation and light-dependent formation of reactive oxygen species (ROS) in organelles. Until now, only a few protein kinase/phosphatase complexes with counteracting effects on stress responses have been identified. We have addressed the role, regulation and interactions of protein phosphatase 2A (PP2A) in plant immunity in *Arabidopsis*. A combination of genetic, proteomic and metabolomic analysis revealed that PP2A regulatory subunit B'γ (PP2A-B'γ) is required to suppress daylength-dependent, salicylic acid mediated pathogenesis responses triggered by intracellular ROS signals. Analysis of protein interactions and molecular modeling further revealed that PP2A-B'γ physically interacts with a Calcium-dependent Protein Kinase (CPK), and in-gel kinase assays demonstrated that PP2A-B'γ negatively regulates CPK activity in *Arabidopsis* leaves. We suggest that formation of CPK/PP2A kinase/phosphatase complexes limits the extent of salicylic acid dependent defense signalling in *Arabidopsis*.

O4.2

Carbonylation-targeted proteomics of NaCl-stressed plants revealing early oxidative events in various cellular compartments

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Lipid peroxide-derived alpha, beta-unsaturated carbonyls (reactive carbonyl species; RCS) mediate oxidative signals in cells. To capture the scene of RCS action in plant environmental stress response, we analyzed the target proteins of RCS in *Arabidopsis thaliana* leaves under salt stress. *A. thaliana* Col-0 plants treated with 0.3 M NaCl for 3 d suffered non-visible but irreversible damage in leaves. 4-Hydroxynonenal (HNE) was increased in 12 h of the treatment, while malondialdehyde rose later. Western blotting with distinct antibodies against various RCS revealed that the protein members prone to RCS-modification (carbonylation) are limited and common among different RCS. RCS-modified proteins were collected via an anti-HNE antiserum affinity capture and aldehyde-reactive biotin labeling/streptavidin column purification. The isobaric-tag-for-relative-and-absolute-quantitation, an LC/MS/MS-based proteomics, identified about 70 proteins that were carbonylated more strongly in the stressed leaves. Sensitive targets were energy-metabolism enzymes in cytosol, antioxidant/detoxification enzymes in peroxisomes, and protein quality control enzymes/proteins. This implies apoptosis-like cell death in NaCl-stressed leaves.

O4.3

High-throughput screening of stomatal responses to biotic stresses reveals new components of immune signalling

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Stomata, the pores on the surface of leaves formed by two guard cells, open and close in response to a range of biotic and abiotic stresses. Stomata open to increase carbon dioxide flux and so maximise photosynthetic gain. However, open stomatal pores also provide convenient access to the plant interior for pathogenic microbes. Plants, therefore, have developed sophisticated signalling systems that allow them to close stomatal apertures and prevent infection at the pre-invasive level. We have used a novel high-throughput screening system to identify components involved in pathogen-associated molecular pattern (PAMP) signalling in guard cells. This system allowed us to assess the stomatal responses of a collection of *Arabidopsis* mutants to an assortment of biotic (bacterial flagellin, fungal chitin) and abiotic (ABA, calcium, ROS) stimuli and to pinpoint entire functional categories of interest. We will present recent data from our mutant screening and discuss the relative importance of pathways such as calcium signalling and vesicle trafficking revealing a complex integration between the different signalling pathways. Together, our findings provide a genetic framework of stomatal responses underlying plant immunity.

O4.4

Genetic analyses of cytosolic NADPH-regenerating systems reveal specific functions in oxidative stress responses

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In plants, NADPH is a key molecule involved in numerous cellular processes including ROS metabolism and production. The reduction of NADP+ to NADPH by NADP-dependent dehyrogenases is considered as the most important source of NADPH in the cytosol. The roles of enzymes involved in supplying NADPH to support H_2O_2 metabolizing pathways and to maintain thiol/disulfide status remain an unresolved question (Foyer and Noctor, 2009). To establish the physiological importance of these enzymes we are using *cat2* mutant as a model system allowing easy manipulation of oxidative stress outcomes (Mhamdi et al., 2010a). A combined genetic, biochemical and transcriptomic approach was selected to characterize how NADP-dependent enzyme mutations impact H_2O_2-triggered lesion formation, glutathione homeostasis, gene expression and salicylic acid (SA)-dependent pathogen responses. Our results suggest that different NADP-dependent dehydrogenases play non-overlapping roles in stress responses (Mhamdi et al., 2010b). Furthermore, the analyses suggest that cytosolic glucose 6-phosphate dehydrogenase 5 (G6PD5) is required for SA-dependent pathogen responses triggered by intracellular H_2O_2 signals via NADPH oxidases-independent mechanisms.

O4.5

Whether ferredoxin-dependent cyclic electron transport (CET) limits ROS production in *Thelungiella salsuginea*?

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Among the protective mechanisms against high salinity in halophytic plants, adaptations of chloroplastic metabolisms are relatively less known. In our earlier work we compared the chloroplastic metabolism of closely related glycophytic and halophytic plants (*Arabidopsis thaliana* and *Thelungiella salsuginea*, respectively) and got indication of higher involvement of CEF in the halophytic species. This study was undertaken to evaluate whether CET may modulate ROS generation and scavenging. A much lower level of H_2O_2 and oxidative damage (MDA) was found in leaves of *T. s.*, in comparison to *A. t.*, already in control conditions. This was associated by the lower activities and amounts of several antioxidants (superoxide dismutase, catalase, ascorbate peroxidase, glutathione and ascorbic acid), however, due to salinity they were increased to the higher extent than in *A. t.* In contrast, lipophilic antioxidants (alfa-tocopherol, plastochromanol and hydroxyplastochromanol) were increased due to salinity only in *A. t.*, suggesting the higher excitation pressure at PSII in this species. Obtained data suggest that low generation of ROS during steady state photosynthesis makes a pre-adaptive feature to the high salinity.