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Plenary Lecture

PL1.1

Temporal-spatial interaction between ROS and ABA controls rapid systemic acclimation in plants

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Being sessile organisms, plants evolved sophisticated acclimation mechanisms to cope with abiotic challenges. These are activated at the initial site of exposure to stress, as well as in systemic tissues that have not been subjected to it (termed systemic acquired acclimation; SAA). Although SAA is thought to play a key role in plant survival during stress, little is known about the signalling mechanisms underlying it. We report that SAA in plants requires at least two different signals: an auto-propagating wave of reactive oxygen species (ROS), which rapidly spreads from the initial site of exposure to the entire plant and serves as a priming mechanism, and a stress-specific signal that conveys abiotic stress-specificity. We further demonstrate that SAA is stress-specific, and that ROS and ABA interact to induce SAA in plants. Our findings unravel some of the basic signalling mechanisms underlying SAA in plants.

Lectures

L1.1

Apoplastic ROS sensing and signalling

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Reactive oxygen species (ROS) are formed in plant cells as a response to several stresses in several subcellular compartments and are involved in stress adaptation and acclimation. While production mechanisms for ROS are relatively well understood, the perception of ROS signals has remained unresolved. We have identified proteins that might serve as ROS sensors. Members of a family of receptor-like kinases (RLKs, Cysteine-rich RLKs (CRKs), are involved in mediating the effects of ROS. Mutants for *Arabidopsis* CRKs has revealed individual CRK genes/proteins involved in various ROS-related processes. A small extracellular protein, GRIM REAPER (GRI), which contains protein domains related to DUF26, is also involved in ROS responses and the regulation of cell death. A peptide cleaved from the GRI protein by a metacastpase and perceived by an LRR-type RLK induces superoxide-dependent cell death in *Arabidopsis* leaves. Perception of apoplastic ROS is followed by downstream components involved in gene regulation. Strong evidence has accumulated that ROS play an important role in the signalling through the regulation of nuclear gene expression. Analysis of a large collection of public transcriptome data from the model plant *Arabidopsis thaliana* for biological processes associated with ROS signalling and for the identification of suitable transcriptional indicators has revealed that it is very difficult to identify specific markers or processes downstream of different ROS. However, the use of "ROS signatures", which consists of a set of genes that together can show characteristic and indicative responses, could be used over the use of individual marker genes.

L1.2

Signalling via reactive oxygen species – why and how?

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Although discovered only recently, intra- and intercellular signalling via reactive oxygen species (ROS) appears to represent an evolutionary ancient mode of signalling, its origins dating back to the formation of aerobic atmosphere. Homologies in ROS signalling between microorganisms, animals and plants are thus understandable. Hydrogen peroxide is the main player in the contemporary ROS signalling. In spite of considerable promiscuity of ROS reactions, considerable substrate, spacial and temporal selectivity of ROS signalling is possible. The main signalling targets of H_2O_2 are protein thiol groups, thiols of low K_d being selectively oxidized by H_2O_2 even in the presence of a considerable excess of other thiols. This mechanism is responsible for the selective inhibition of protein tyrosine phosphatases in animals. Site-specific enzymatic generation of ROS by organelles such as chloroplasts or mitochondria or by enzymes such as NADPH oxidases or cell wall peroxidases, in combination with diffusion-dependent formation of concentration gradients and with the ability of some aquaporins to transport hydrogen peroxide contributes to the spacial selectivity of ROS signalling.

Oral presentations

O1.1

A calcium-dependent protein kinase/NADPH oxidase activation circuit in rapid defense signal propagation

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Recognition of pathogen-associated molecular patterns (PAMPs) is a fundamental process of the plant innate immune system, leading to local activation of defense signalling and resulting in systemic responses in the whole plant. We observed rapid biochemical activation of the *Arabidopsis thaliana* calcium-dependent protein kinase 5 (CPK5) in response to recognition of PAMPs. Besides changes in phytohormone levels and defense gene expression, CPK5 signalling resulted in enhanced synthesis of reactive oxygen species (ROS). We were able to identify the NADPH oxidase, respiratory burst oxidase homolog D (RBOHD), as an *in vivo* phosphorylation target of CPK5. This CPK5-dependent phosphorylation occurred in response to both PAMP- and ROS stimulation. Furthermore, rapid CPK5-dependent biochemical and transcriptional activation of defense reactions at distal sites was compromised in *cpk5* and *rbohd* mutants. Our data identify CPK5 as a key regulator of plant innate immune responses and also support a model of ROS-mediated cell-to-cell communication, where a self-propagating mutual activation circuit consisting of the protein kinase and the NADPH oxidase facilitates rapid defense signalling to distal sites within the plant.

O1.2

DYn-2 is an excellent chemical reporter for the *Arabidopsis* sulfenome

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Recent studies suggest that the sulfenylation state (Cys-SOH) of living cells under oxidative stress functions as an intermediate mechanism for transforming redox signals into biological defence responses. Identifying all the sulfenylated proteins (the sulfenome) is a potential way to detect the key redox sensors in *Arabidopsis thaliana*. However, the detection of this highly reactive oxidation state of cellular thiols is quite challenging, since, once formed, it might be subject to overoxidation. We have optimized an *in vivo* sulfenylation method in H_2O_2 stressed *Arabidopsis* cells using the alkyne functionalized chemical reporter DYn-2, which specifically reacts with sulfenic acids. With click chemistry the DYn-2 reporter was biotinylated, and we clearly observe time- and dose-dependent H_2O_2 responses. Moreover, after purification of the DYn-2 tagged proteins, an increased sulfenylation signal was only observed in the stressed cells. The enriched sulfenylated proteins are being identified by mass spectrometry, and they give us a view on potential novel key proteins from several pathways. Our findings open a door to develop oxidative stress tolerance genes in *Arabidopsis* and to understand their roles in stress response.

O1.3

Sensing thiol oxidation in *Arabidopsis thaliana* through a YAP1 genetically encoded probe

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Reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), are key signalling molecules orchestrating plant development and metabolic adaptation to stress conditions. However, plant redox signal perception mechanisms are still poorly understood. To get insights into these processes we focus on proteomic identification of the H_2O_2 dependent-sulfenome. Sulfenome is a set of proteins in which at least one cysteine thiol (–SH) is oxidized to a sulfenic acid (–SOH) under oxidative stress. This reversible post-transcriptional modification functions as a "redox switch" that alters the biochemical properties of sensor proteins which are constantly monitoring the redox status of the cell. By a unique combination of *in vivo* Cys-SOH trapping with a YAP1-based probe and tandem affinity enrichment, we identified a portfolio of ~90 redox active *Arabidopsis thaliana* proteins involved in signal transduction, redox homeostasis and a plethora of other metabolic pathways. We aim to elucidate the redox properties and the mode of action of these newly identified proteins in order to assess their role in oxidative stress signal transduction events.

O1.4

Antagonism of salicylic acid signalling on apoplastic reactive oxygen species signalling

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Interaction between signalling pathways is frequently observed exemplified by the negative interaction between salicylic acid (SA) and jasmonic acid (JA). We explore a novel interaction - the negative effect of SA on apoplastic reactive oxygen species (ROS) signalling. Short pulses of the air pollutant ozone cause the formation of ROS in the apoplastic space of leaves and leads to extensive changes in gene expression. The *dnd1* mutant, encoding a Ca^{2+} channel CNGC2, has reduced responsiveness to ozone. The *dnd1* mutant was initially isolated in a screen for lack of pathogen induced cell death and in addition to this phenotype it also has elevated SA concentration and constitutive activation of defence genes. In selected double or triple mutants where SA or JA biosynthesis was removed from *dnd1*, the responsiveness to ozone was restored when SA signalling was compromised. In addition other mutants with a similar phenotype to *dnd1* i.e. high concentration of SA and constitutive defense gene activation were found to have a reduced response to ozone. Finally plants pretreated with SA were compromised in subsequent ozone responses. We conclude that SA and SA signalling act antagonistic to apoplastic ROS signalling.