Session 19: Inside Plant Organelles: Structure, Function and Stress Response

Lectures

L19.1

Exploring organelle-to-nuclear signaling during plant stress responses

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Mitochondria and chloroplasts are crucial for plant viability and stress response. To allow efficient coordination between these organelles and the rest of the cell, a signalling network is in place that mediates feedback communication between mitochondria/chloroplasts and the nucleus. This organelle-to-nuclear communication is termed 'retrograde' signalling and affects nuclear gene expression. We have now identified various transcription factors that modulate retrograde regulation of mitochondrial and chloroplast functions in Arabidopsis thaliana. However, the relative contribution of these regulators and whether they act downstream of separate or overlapping stress signalling cascades is not well-understood. Multiple stress-related signalling pathways, with distinct kinetic signatures, converge on overlapping gene sets involved in energy organelle function. Although mitochondrial retrograde signalling occurs in a wide range of eukaryotic taxa such as yeast, animals and plant, the different taxa appear to have evolved their own specific signalling pathways and target genes. Using next generation sequencing technologies, we have identified a set of novel proteins with an uncharacterised conserved domain, which respond to mitochondrial dysfunction. Surprisingly, phylogenetic analysis revealed that these proteins represent a unique example of *de novo* mitochondrial functionalisation, and have only been incorporated into the retrograde signalling pathway relatively recently. This shows that new stress response strategies are still being created in very recent plant evolutionary history.

L19.2

Structural plasticity of the chloroplast thylakoid network

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Internal plastid membrane network is an intricate spatial structure that develops in chloroplast during plant ontogenesis. At the structural level, the regular network of paracrystalline prolamellar bodies and the flattened porous membranes of prothylakoids develop, upon illumination, into the stroma and grana thylakoids. Thylakoid network is a site of photochemical reactions and through possible rearrangements plays a crucial role in the photosynthesis regulation.

Although 2D ultrastructural studies enable to track detailed membrane rearrangements but the dynamics of the photosynthesis can be better understood when structural relations between thylakoid compartments are described in 3D. Therefore, in our studies, we focus on the detailed spatial analysis of the thylakoid network using electron tomography and confocal laser scanning microscopy.

I will present our studies on membrane transformation during the etioplast-to-chloroplast transition pointing out the importance of particular chlorophyll-protein complex components in the membrane appression during subsequent stages of biogenesis. Moreover, I will show the influence of thylakoid membrane components on the formation of a helical grana shape in fully developed plants and importance of such spatial arrangement on the photosynthetic efficiency. Illumination-induced thylakoid membrane dynamics will be presented as another example of structural plasticity of the thylakoid network.

L19.3

Functional relevance of mitoproteases in plant mitochondria

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Mitochondria are life-essential organelles of eukaryotic cells. The preservation of mitochondrial homeostasis is performed by a coordinated activity of proteases and chaperones. Membrane-anchored metallopeptidases *i*-AAA and Oma1 are of particular importance. These enzymes are highly conserved and are involved in tightly regulated proteolytic reactions affecting mitochondrial biogenesis and dynamics. In this talk, the significance of *i*-AAA and Oma1 proteases in the functionality of mitochondria in yeast, humans and particularly in plants will be discussed. In Arabidopsis plants, *i*-AAA protease, known as FTSH4, as well as OMA1 exhibit both conserved and plant-specific functions and the absence of either protease does not affect plant growth and development under optimal conditions. However, prolonged exposure of plants lacking FTSH4 or OMA1 to moderate heat stress leads to developmental and morphological alterations, which correlate with decreased amount and activity of the OXPHOS complexes and oxidative stress. Mitochondrial dysfunction is especially pronounced in *ftsh4* plants where additionally aberrant morphology and reorganization of the mitochondrial network are evident throughout the entire plant life cycle. More interestingly, our recent data show that a double homozygous mutant of both FTSH4 and OMA1 proteases exhibits growth arrest at the seedling stage under optimal conditions indicating specific need for these metallopeptidases in Arabidopsis.

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Posters

P19.1

Changes in gene expression and identification of potential molecular markers of resistance to clubroot disease (*Plasmodiophora brassicae*) in *Brassica* plants

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Clubroot disease caused by *Plasmodiophora brassicae* inflicts heavy losses in *Brassica* crops. The disease can be controlled chemically and by agronomic measures, but with limited effectiveness. One of the priorities of *Brassica* breeding programmes is resistance to clubroot.
The aim of the study is to elucidate of molecular mechanism of resistance to the disease. Differential gene expression analysis in response to pathogen infection has been done using cDNA-AFLP on seven *Brassica* genotypes differing in the resistance level.
Infection with *P. brassicae* caused significant changes in the transcriptome of the tested plants. Over 60% of ESTs were overexpressed in infected plants. BLAST analysis revealed that they are homologous to known genes encoding plant proteins involved in regulation of host-pathogen interaction (resistance proteins: N-like, Pid3, TAO1; MA3 domain-containing protein; glycoprotein CD1; protease SBT3.3; aldehyde dehydrogenase B7; haloacid hydrolase; PDCD1 protein; ATM kinase; dioxygenase PcbC; snare-domain-containing protein) and also in signal perception and transduction, regulation of transcription, posttranslational protein modification and transmembrane transport.
Basing on the expression pattern, some genes may be sorted out as potential components of clubroot resistance mechanisms in *Brassica* plants.

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P19.2

High zinc in medium affects for changes of content in individual fractions of pectin in tobacco leaves

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It is known that high concentrations of zinc cause remodelling of plants cell wall [1]. Specifically, there is evidence that similar to cadmium, the presence of high zinc levels increases amounts of pectin with a lower degree of methylesterification [1].
The aim of this study was to determine whether there is a difference in the pectin fraction composition between leaves of tobacco plants grown under control conditions compared to leaves from plants grown in the presence of 200 μM Zn.
Microarray based polysaccharide profiling [4] was performed using the following monoclonal antibodies that recognize distinct structural features of pectic polysaccharides: LM7, LM18, LM19, LM20, LM8, LM5, LM9, LM6, LM13, LM16, LM12.
Our data showed that three pectic sub-domains were detected at similar levels both in the control and Zn-exposed leaves (partial methylesterified - LM18; un-esterified -LM19; linear tetrasaccharide in (1-4)-β-D-galactans – LM5 and rhamnogalacturonan I – LM16). Only one epitope of rhamnogalacturonan I, was present exclusively in the Zn-exposed tobacco which one. Interestingly, the level of rhamnogalacturonan I increased with time of exposure to high Zn.
To analyze the differences in pectin localization between control and Zn-treated plants, the immunolocalization of already identified pectin fractions was performed on the leaf cross-sections. Microscopic analysis showed similar levels *per se* of the partial metylesterified, un-esterified, and linear tetrasaccharide in (1-4)-β-D-galactans in the control and Zn-exposed leaves. However, difference were noted in localization patterns. In the Zn-treated plants, pectins were localized preferentially near the veins, while in control plants they were present regularly throughout the leaf tissues.
The research presented showed that exposure of tobacco plants to high zinc changed the composition of individual fractions of pectins in leaves and also their tissue-specific localization.

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P19.3

Spotlight on mitochondrial respiration-dependent activity of transcription in *Arabidopsis* chloroplast

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Our studies allowed on characterization a new type of the relationship between the organelles, mitochondria and chloroplasts, in a plant cell. Comparative transcriptomic analysis of *rps10 Arabidopsis thaliana* mutant (Kwasniak M *et al.,* 2013) with published microarray data of 26 different mitochondrial mutants revealed huge similarity between *rps10* and *aox1a:rpoTmp* plants (Kuhn K *et al.*, 2015). These two types of mutants have restricted both cytochrome and alternative mitochondrial respiration. Transcriptomic data suggested that one of the common responses to restriction of both cytochrome and alternative respiration pathways in *rps10* and *aox1a:rpoTmp* is diminished chloroplast transcription. Similarly, using *in vitro* approaches, we revealed that simultaneous inhibition of the activity of two mitochondrial respiratory pathways induced decreasing level of chloroplast-encoded transcripts. Based on performing analyses of discovered dependency, we speculate that the rate of transcription in chloroplast is closely related with specific activity of respiration pathways in mitochondria.

References:
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