Session 4: Signaling in Plant Cells

Lectures

L4.1

Calcium regulation of plant organellar metabolism

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Calcium is an important second messenger and many abiotic and biotic signals are transduced into a cellular response by temporal and spatial changes in calcium concentration. A large variety of metabolic processes takes place in plant organelles and it is by now clear that they are tightly integrated into the calcium signalling network. My group is analysing the molecular basis of calcium regulation for organellar function with a special focus on calmodulin-like proteins and calcium-dependent phosphorylation. We have shown that calcium affects the import of nuclear-encoded proteins into chloroplasts and mitochondria mediated by calmodulin. The Arabidopsis genome encodes over 50 CaMs and calmodulin-like proteins (CMLs), several of which contain potential targeting sequences. Our systematic approach to analyse their subcellular localization has yielded CMLs targeted to mitochondria, peroxisomes and the endomembrane system. We have also show that several chloroplast proteins undergo calcium-dependent phosphorylation, including transketolase and the calcium signalling receptor CaS. TKL phosphorylation is not the only calcium-dependent process described for the CBB. These results strongly support calcium as a regulatory factor of primary carbon metabolism and photosynthesis.

We have furthermore generated a set of transgenic plant-lines targeting aequorin to the inside of mitochondria and chloroplasts as well as several chloroplast subcompartments. These plant lines can be utilized to analyse organellar calcium changes in response to environmental stress and plant development.

L4.2

Plant calcium signaling in biotic interactions

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Plants react to insect attack employing an array of direct and indirect defense strategies. As a prerequisite for such appropriate reactions, first the perception of insect-derived physical and chemical cues (elicitors in insect oral secretion) followed by several well-coordinated local and systemic signaling processes including $[Ca^{2+}]_{cyt}$ changes and phytohormone signals, in particular jasmonates, are necessary. Relatively little is known about the early signal transduction pathways that connect insect specific elicitors to the plant defense responses they evoke. Ca^{2+}-ions have been implicated as second messenger in many plant signaling pathways, but its specific role in herbivory and jasmonate-dependent pathways is still poorly understood. Moreover, systemic $[Ca^{2+}]_{cyt}$ signals can be detected within the plant, mainly in adjacent leaves with direct vascular connections to the treated leaf. Downstream of $[Ca^{2+}]_{cyt}$ changes various calcium sensor proteins, in particular calmodulin-like proteins (CMLs), decode Ca^{2+} signals and translate them into specific cellular responses. These CMLs include positive and negative regulators of downstream phytohormone signaling and eventually may modulate the appropriate defense reactions. The underlying mechanisms of local and systemic herbivory-induced signaling will be discussed.

L4.3

Ca^{2+} and Mg^{2+} in blue light signaling in the mesophyll cell

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Ca^{2+} ions serve as second messengers in the signal transduction pathway from phototropins. These blue light receptors mediate both short-term movement and long-term growth responses. In Arabidopsis two phototropins share redundant functions at different light intensities. Both control chloroplast accumulation in weak light. In contrast, only phot2 controls avoidance response in strong light. Both types of chloroplast responses depend on Ca^{2+} released from apoplast and/or internal stores. These processes are mediated by phosphoinositides [1]. Many aspects of phototropin signaling pathways are poorly understood. Among the key questions are the origin of Ca^{2+} ions and the control of their release. Glutamate receptor-like receptors are promising candidates for Ca^{2+} regulation in plant cells. GLRs are plant counterparts of animal NMDA/AMPA/kainate channels mostly characterized in the CNS. They control root morphogenesis, hypocotyl elongation, photosynthesis and stress responses. Using two inhibitors specific of NMDA and AMPA receptors, MK-801 and CNQX respectively, we showed that GLRs are involved in chloroplast movements in Arabidopsis. Pharmacological perturbation of calcium homeostasis, phosphoinositide pathway and microfilament organization inhibits chloroplast responses in *Nicotiana tabacum* [2]. This can be reversed by extracellular Ca^{2+} and Mg^{2+}, the latter ions acting more effectively. At least part of this effect can be discussed in the context of GLR channels regulation.

References:
1. Aggarwal C *et al.* (2013) *Plos One* **8**: 1-11.
2. Anielska-Mazur *et al.* (2009) *BMC-Plant Biol* **9**: 1-14.

L4.4

The role of calcium in cellular membrane flow

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In plants vesicle trafficking is necessary for proper signal transmission during growth and development and tolerance different stresses. For example, tolerance relies partially on non-transcriptional responses, such as coordinated endo- or exocytosis of plasma membrane ion channels, water channels or hormone transporters. The role of Ca^{2+} in intracellular membrane trafficking is not well elucidated yet. Experiments with calcium chelators show that transport pathway is a mosaic of calcium-dependent and independent events. The best known example of the former event is the fusion of secretory granules and synaptic vesicles with the plasma membrane in neurons/neuroendocrinal cells. As membrane fusion can occur many times during the secretory pathway Ca^{2+} could potentially affect all these steps. The possible mechanisms involved modulation of activity of transport-related proteins, stabilization of vesicle coat and direct calcium action on membrane fusion. Annexins are able to bind and position the membrane structures in a calcium-dependent manner. They are believed to be engaged in vesicular trafficking, endo- and exocytosis, phagocytosis and autophagy. There are some evidences showing able to influence specific steps in membrane trafficking associated with yeast cell growth, secretion and the PM remodeling. We attempt to characterize annexin function in membrane transport by identification of their interactors within proteins involved in membrane trafficking.

Oral presentations

O4.1

Exogenous amino acids affect the image of actin cytoskeleton in Arabidopsis mesophyll cells

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The actin cytoskeleton is the key element for perception of environmental stimuli, signal transduction and organelle transport in plant cells. The structure and function of actin depends, among others, on intracellular concentration of calcium ions. The *Arabidopsis thaliana* genome contains 20 sequences of GLR channels. These ionotropic, ligand-gated receptors are located in plasmalemma. Upon binding of ligand amino acids they activate calcium influx into the cytosol.

The aim of this work was to analyze the influence of exogenous amino acids on the organization of actin in *Arabidopsis thaliana*. GFP-fabd2 and lifeact Arabidopsis mutants were used as model plants.

The results demonstrate that several amino acids (alanine, histidine, valine, tryptophan, serine, cysteine) cause changes in the actin architecture following 20 minutes of tissue incubation. Except tryptophan, the effect is reversed by CNQX, the antagonist of GLR channels. The most reliable results have been obtained for cysteine and serine, thus these amino acids may be considered as ligands of AtGLRs. The results point to a potential role of amino acids in the cytoskeleton control. A working hypothesis has been proposed that the amino acids change the cytoskeleton *via* regulation of GLR channels.

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