Imaging Unit

Nowadays, the elucidation of molecular and biochemical processes requires its combination with investigation of cells – their physiological properties, their structure, the organelles they contain, interactions with their environment, their life cycle, division and death. For centuries, progress in biological research has been connected to the development of tools and equipment that allow new insights into living matter. The invention of and improvements in optical systems were very important because exceeding the limits of the optical resolution of the human eye delivered new insights into tissues, cells and subcellular compartments on the one hand and cellular processes on the other.

The Imaging Unit of the IPB aims to support all research groups in their use of cell biological methods. Currently, at least 13 research groups of the institute are using this unit.

The unit provides:

- Coordinated supervision and maintenance of equipment
- Optimal training of coworkers
- Maximal use of IPB investments
- Updating of equipment according to state-of-the-art and to requirements of actual research

The working principle of this unit is:

- Unit headed by one scientist (Prof. Dr. Bettina Hause) and supported by one technician (Hagen Stellmach)
- Equipment is located decentralized, but its maintenance is carried out centralized
- For all cell biological methods advice, training and help is provided, extensive experiments, however, have to be done by the co-workers themselves

Devices and materials:

Microscopes:
Several stereo microscopes
(Zeiss and Nikon)

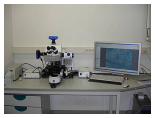
Multipurpose MacroMicroSystem equipped with epi-fluorescence: AZ100 (Nikon) with camera (one in each Dept. MSV and SEB)

Lightsheet Microscope

Lightsheet Z1 (Zeiss)

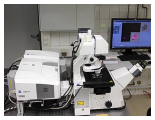
Epi-fluorescence microscopes

Axioplan 2 (Zeiss) with differential interference contrast (DIC) device and ApoTome to obtain optical sections, with two cameras (AxioCam MRm and AxioCam MRc5)



AxioImager (Zeiss) with differential interference contrast (DIC) device and ApoTome to obtain optical sections, with two cameras (AxioCam MRm and AxioCam MRc5)

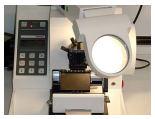
Confocal Laser Scanning Microscope



LSM780 (Zeiss) with Airyscan

LSM700 (Zeiss)

Microtomes

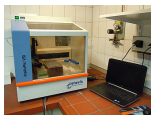


Rotary microtomes to perform semi-thin sections (Microm und Leica)

Vibrating microtome (Vibratome VT1000S, Leica) (Dept. SZB)

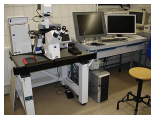
Cryo-Microtom CM1950 (Leica)

Miscellaneous



InsituPro VSi (Intavis) for automated in situ detection (Dept. SZB)

Micromanipulator (Eppendorf)



Laser Capture Microdissection

More devices

- Organelle-marker: Vectors and transgenic lines of Arabidopsis (Nelson et al., 2007)
- Wave-marker: Vectors and transgenic lines of Arabidopsis (Geldner et al., 2009)

Methods established:

- Fixiation, embedding and sectioning of plant materials
- Laser-Micro-Dissection

- Immuno labelling
- in situ-hybridisation
- light microscopy including fluorescence microscopy
- confocal laser scanning microscopy
- Determination of protein interactions via FRET and BiFC (Split-YFP)

Publications by Tag: Cell Biology

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Kopischke, M.; Westphal, L.; Schneeberger, K.; Clark, R.; Ossowski, S.; Wewer, V.; Fuchs, R.; Landtag, J.; Hause, G.; Dörmann, P.; Lipka, V.; Weigel, D.; Schulze-Lefert, P.; Scheel, D.; Rosahl, S. Impaired sterol ester synthesis alters the response of Arabidopsis thaliana to Phytophthora infestans. *Plant J* **73**, 456-468, (2013) DOI: 10.1111/tpj.12046

Abstract

Internet
RIS
BibTeX

Non-host resistance of Arabidopsis thaliana against Phytophthora infestans, the causal agent of late blight disease of potato, depends on efficient extracellular pre- and post-invasive resistance responses. Pre-invasive resistance against P. infestans requires the myrosinase PEN2. To identify additional genes involved in non-host resistance to P. infestans, a genetic screen was performed by re-mutagenesis of pen2 plants. Fourteen independent mutants were isolated that displayed an enhanced response to Phytophthora (erp) phenotype. Upon inoculation with P. infestans, two mutants, pen2-1 erp1-3 and pen2-1 erp1-4, showed an enhanced rate of mesophyll cell death and produced excessive callose deposits in the mesophyll cell layer. ERP1 encodes a phospholipid:sterol acyltransferase (PSAT1) that catalyzes the formation of sterol esters. Consistent with this, the tested T-DNA insertion lines of PSAT1 are phenocopies of erp1 plants. Sterol ester levels are highly reduced in all erp1/psat1 mutants, whereas sterol glycoside levels are increased twofold. Excessive callose deposition occurred independently of PMR4/GSL5 activity, a known pathogen-inducible callose synthase. A similar formation of aberrant callose deposits was triggered by the inoculation of erp1 psat1 plants with powdery mildew. These results suggest a role for sterol conjugates in cell non-autonomous defense responses against invasive filamentous pathogens.

Goetz, S.; Hellwege, A.; Stenzel, I.; Kutter, C.; Hauptmann, V.; Forner, S.; Mc Caig, B.; Hause, G.; Miersch, O.; Wasternack, C.; Hause, B. Role of cis-12-oxo-phytodienoic acid in tomato embryo development. *Plant Physiol* **158 (4)**, 1715-1727, (2012)

Abstract
Internet
RIS
BibTeX

Oxylipins including jasmonates are signaling compounds in plant growth, development, and responses to biotic and abiotic stresses. In Arabidopsis (Arabidopsis thaliana) most mutants affected in jasmonic acid (JA) biosynthesis and signaling are male sterile, whereas the JA-insensitive tomato (Solanum lycopersicum) mutant jai1 is female sterile. The diminished seed formation in jai1 together with the ovule-specific accumulation of the JA biosynthesis enzyme allene oxide cyclase (AOC), which correlates with elevated levels of JAs, suggest a role of oxylipins in tomato flower/seed development. Here, we show that 35S::SlAOC-RNAi lines with strongly reduced AOC in ovules exhibited reduced seed set similarly to the jai1 plants. Investigation of embryo development of wild-type tomato plants showed preferential occurrence of AOC promoter activity and AOC protein accumulation in the developing seed coat and the embryo, whereas 12-oxo-phytodienoic acid (OPDA) was the dominant oxylipin occurring nearly exclusively in the seed coat tissues. The OPDA- and JA-deficient mutant spr2 was delayed in embryo development and showed an increased programmed cell death in the developing seed coat and endosperm. In contrast, the mutant acx1a, which accumulates preferentially OPDA and residual amount of JA, developed embryos similar to the wild type, suggesting a role of OPDA in embryo development. Activity of the residual amount of JA in the acx1a mutant is highly improbable since the known reproductive phenotype of the JA-insensitive mutant jai1 could be rescued by wound-induced formation of OPDA. These data suggest a role of OPDA or an OPDA-related compound for proper embryo development possibly by regulating carbohydrate supply and

detoxification.

Stenzel, I.; Otto, M.; Delker, C.; Kirmse, N.; Schmidt, D.; Miersch, O.; Hause, B.; Wasternack, C. ALLENE OXIDE CYCLASE (AOC) gene family members of Arabidopsis thaliana: tissue- and organ-specific promoter activities and in vivo heteromerization* *J Exp Bot.* **63**, 6125-6138, (2012)

Abstract
RIS
BibTeX

Jasmonates are important signals in plant stress responses and plant development. An essential step in the biosynthesis of jasmonic acid (JA) is catalysed by ALLENE OXIDE CYCLASE (AOC) which establishes the naturally occurring enantiomeric structure of jasmonates. In Arabidopsis thaliana, four genes encode four functional AOC polypeptides (AOC1, AOC2, AOC3, and AOC4) raising the question of functional redundancy or diversification. Analysis of transcript accumulation revealed an organ-specific expression pattern, whereas detailed inspection of transgenic lines expressing the GUS reporter gene under the control of individual AOC promoters showed partially redundant promoter activities during development: (i) In fully developed leaves, promoter activities of AOC1, AOC2, and AOC3 appeared throughout all leaf tissue, but AOC4 promoter activity was vascular bundle-specific; (ii) only AOC3 and AOC4 showed promoter activities in roots; and (iii) partially specific promoter activities were found for AOC1 and AOC4 in flower development. In situ hybridization of flower stalks confirmed the GUS activity data. Characterization of single and double AOC loss-of-function mutants further corroborates the hypothesis of functional redundancies among individual AOCs due to a lack of phenotypes indicative of JA deficiency (e.g. male sterility). To elucidate whether redundant AOC expression might contribute to regulation on AOC activity level, protein interaction studies using bimolecular fluorescence complementation (BiFC) were performed and showed that all AOCs can interact among each other. The data suggest a putative regulatory mechanism of temporal and spatial fine-tuning in JA formation by differential expression and via possible heteromerization of the four AOCs.

Landgraf, R.; Schaarschmidt, S.; Hause, B. Repeated leaf wounding alters the colonization of Medicago truncatula roots by beneficial and pathogenic microorganisms. *Plant Cell & Environment* **35 (7)**, 1344-1357, (2012)

Abstract
RIS
BibTeX

In nature, plants are subject to various stresses that are often accompanied by wounding of the aboveground tissues. As wounding affects plants locally and systemically, we investigated the impact of leaf wounding on interactions of Medicago truncatula with root-colonizing microorganisms, such as the arbuscular mycorrhizal (AM) fungus Glomus intraradices, the pathogenic oomycete Aphanomyces euteiches and the nitrogen-fixing bacterium Sinorhizobium meliloti. To obtain a long-lasting wound response, repeated wounding was performed and resulted in locally and systemically increased jasmonic acid (JA) levels accompanied by the expression of jasmonate-induced genes, among them the genes encoding allene oxide

cyclase 1 (MtAOC1) and a putative cell wall-bound invertase (cwINV). After repeated wounding, colonization with the AM fungus was increased, suggesting a role of jasmonates as positive regulators of mycorrhization, whereas the interaction with the rhizobacterium was not affected. In contrast, wounded plants appeared to be less susceptible to pathogens which might be caused by JA-induced defence mechanisms. The effects of wounding on mycorrhization and pathogen infection could be partially mimicked by foliar application of JA. In addition to JA itself, the positive effect on mycorrhization might be mediated by systemically induced cwINV, which was previously shown to exhibit a regulatory function on interaction with AM fungi.

Eschen-Lippold, L.; Landgraf, R.; Smolka, U.; Schulze, S.; Heilmann, M.; Heilmann, I.; Hause, G.; Rosahl, S. Activation of defense against Phytophthora infestans in potato by down regulation of syntaxin gene expression *New Phytologist* **193**, 985-996, (2012)

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Vadassery, J.; Reichelt, M.; Hause, B.; Gershenzon, J.; Boland, W.; Mithöfer, A. CML42-mediated calcium signaling co-ordinates responses to Spodoptera herbivory and abiotic stresses in Arabidopsis. *Plant Physiol* **159**, 1159-1175, (2012)

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Stegmann, M.; Anderson, R.G.; Ichimura, K.; Pecenkova, T.; Reuter ,P.; Arsky, V.; McDowell, J.M.; Shirasu, K.; Trujillo, M. The Ubiquitin Ligase PUB22 Targets a Subunit of the Exocyst Complex Required for PAMP-Triggered Responses in Arabidopsis *Plant Cell* 1-14, (2012)

Abstract
Internet
RIS
BibTeX

Plant pathogens are perceived by pattern recognition receptors, which are activated upon binding to pathogen-associated molecular patterns (PAMPs). Ubiquitination and vesicle trafficking have been linked to the regulation of immune signaling. However, little information exists about components of vesicle trafficking involved in immune signaling and the mechanisms that regulate them. In this study, we identified Arabidopsis thaliana Exo70B2, a subunit of the exocyst complex that mediates vesicle tethering during exocytosis, as a target of the plant U-boxtype ubiquitin ligase 22 (PUB22), which acts in concert with PUB23 and PUB24 as a negative regulator of PAMP-triggered responses. We show that Exo70B2 is required for both

immediate and later responses triggered by all tested PAMPs, suggestive of a role in signaling. Exo70B2 is also necessary for the immune response against different pathogens. Our data demonstrate that PUB22 mediates the ubiquitination and degradation of Exo70B2 via the 26S Proteasome. Furthermore, degradation is regulated by the autocatalytic turnover of PUB22, which is stabilized upon PAMP perception. We therefore propose a mechanism by which PUB22-mediated degradation of Exo70B2 contributes to the attenuation of PAMP-induced signaling.

Fellenberg, C.; van Ohlen, M.; Handrick, V.; Vogt, T. The role of CCoAOMT and COMT in Arabidopsis anthers. *Planta* **236**, 51-61, (2012)

Abstract
RIS
BibTeX

Arabidopsis caffeoyl coenzyme A dependent O-methyltransferase 1 (CCoAOMT1) and caffeic acid O-methyltransferase 1 (COMT1) display a similar substrate profile although with distinct substrate preferences and are considered the key methyltransferases (OMTs) in the biosynthesis of lignin monomers, coniferyl and sinapoylalcohol. Whereas CCoAOMT1 displays a strong preference for caffeoyl coenzyme A, COMT1 preferentially methylates 5-hydroxyferuloyl CoA derivatives and also performs methylation of flavonols with vicinal aromatic dihydroxy groups, such as quercetin. Based on different knockout lines, phenolic profiling, and immunohistochemistry, we present evidence that both enzymes fulfil distinct, yet different tasks in Arabidopsis anthers. CCoAOMT1 besides its role in vascular tissues can be localized to the tapetum of young stamens, contributing to the biosynthesis of spermidine phenylpropanoid conjugates. COMT1, although present in the same organ, is not localized in the tapetum, but in two directly adjacent cells layers, the endothecium and the epidermal layer of stamens. In vivo localization and phenolic profiling of comt1 plants provide evidence that COMT1 neither contributes to the accumulation of spermidine phenylpropanoid conjugates nor to the flavonol glycoside pattern of pollen grains.

Helber, N.; Wippel, K.; Sauer, N.; Schaarschmidt, S.; Hause, B.; Requena, N. A versatile monosaccharide transporter that operates in the arbuscular mycorrhizal fungus Glomus sp is crucial for the symbiotic relationship with plants. *Plant Cell* **23**, 3812-3823, (2011)

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Mielke, K.; Forner, S.; Kramell, R.; Conrad, U.; Hause, B. Cell-specific visualization of jasmonates in wounded tomato and Arabidopsis leaves using jasmonate-specific antibodies *New Phytol* **190**, 1069-1080, (2011)

RIS
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