Black Forest Retreat
on Molecular Plant Science

September 10 - 13th 2012
http://plantco.de/BFR2012

Abstract Book

Edited by Stefan A. Rensing
Freiburg, Germany, Aug 2012

Venue: Leistungszentrum Herzogenhorn
~1,300 mtrs above sea level

Acknowledgements

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Thank you very much!

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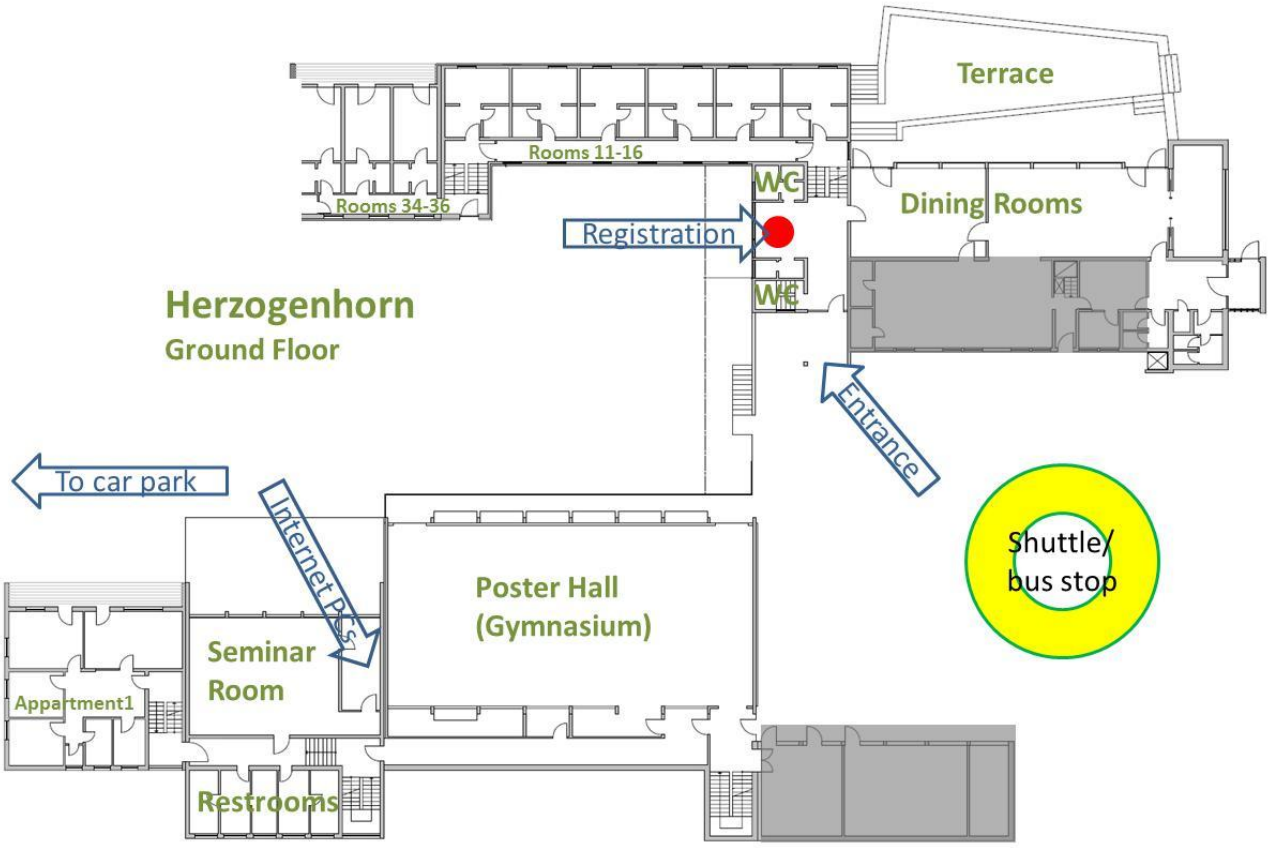
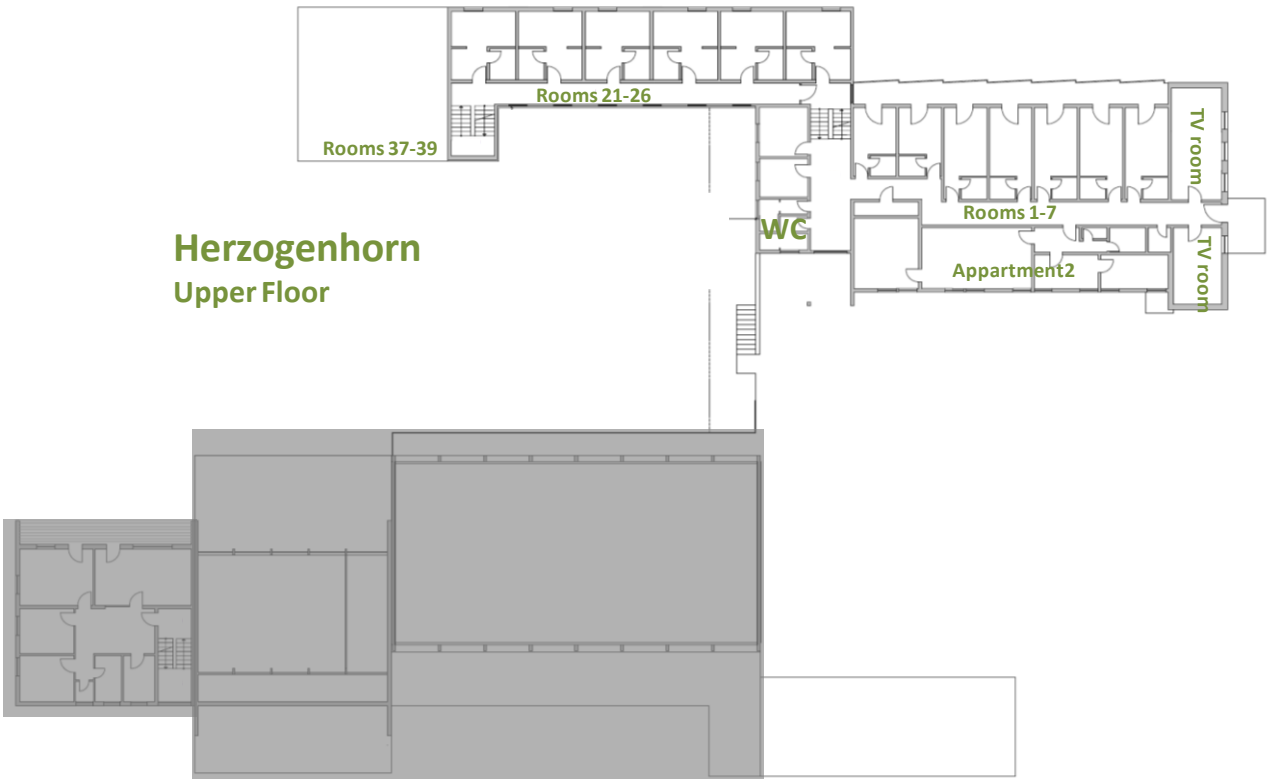
Venue maps

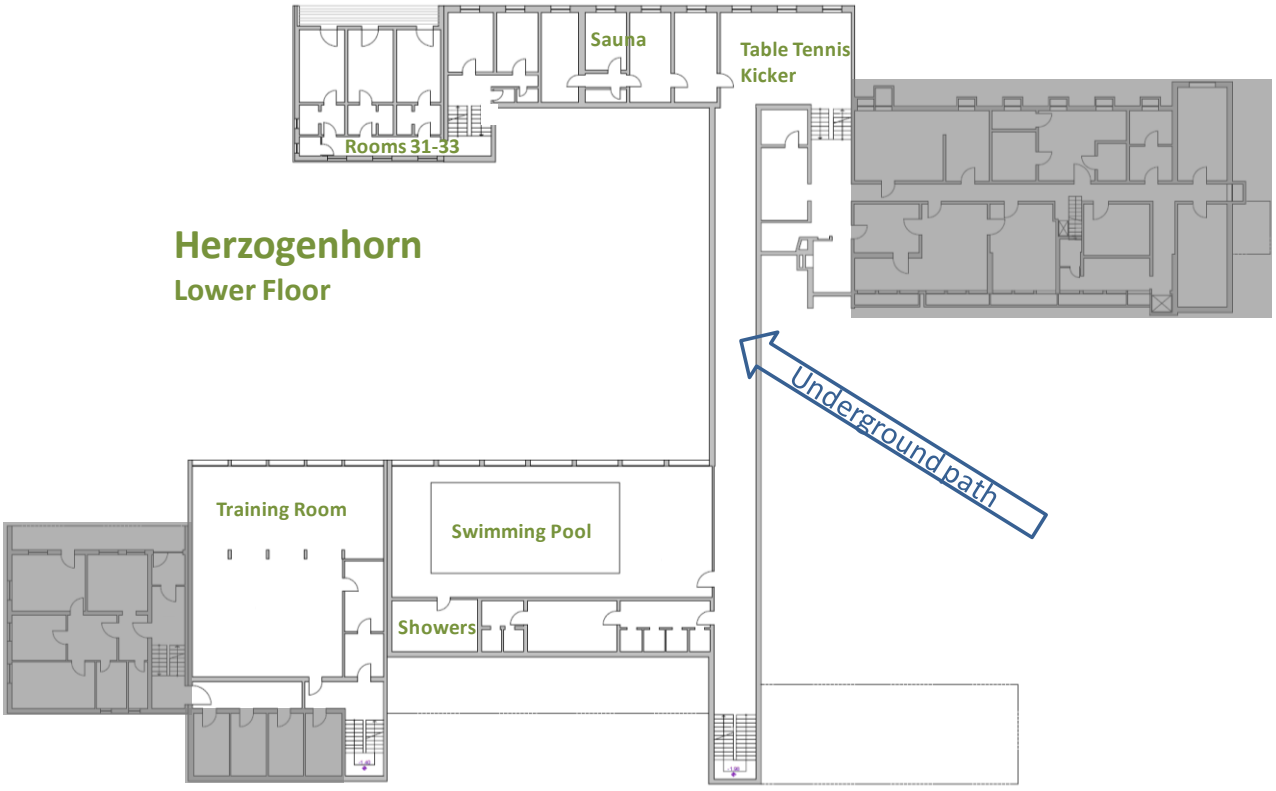
Leistungszentrum Herzogenhorn



All talks take place in the seminar room. Poster sessions take place in the gymnasium. Breakfast, lunch, supper, coffee breaks and evening entertainment are located in the dining rooms.



Herzogenhorn
Upper Floor



Herzogenhorn
Lower Floor

Program

Monday Sep 10th

15:15, 16:15, 17:15	Bus shuttles from Bärental train station to venue
15:30 - 19:00	Registration desk open (also open during the conference at all breaks)
19:00 - 20:00	**Reception with food and beverages**
20:00	**Welcome, organizational remarks**
20:15	**Keynote talk 1: *Ralph Bock*** **Genes gone wild: experimental genome evolution in plants**
later	*"moss cocktail workshop"*

Tuesday Sep 11th

7:45 - 8:45 Breakfast

9:00 – 10:30 Oral session I

9:00 Keynote talk 2: *Yves van de Peer*
Plant genome evolution
10:00 T1 *Rena Isemer*
The dual localized protein WHIRLY1 has functions in plastids and the nucleus
10:15 T2 *Daniel Alvarez*
Investigation of a translation-inhibitory function mediated by the 5'UTR of phytoene synthase from *Arabidopsis*

10:30 Coffee break

11:00 – 12:00 Workshop 1: *Stefan Rensing*
Things you always wanted to know about BLAST and never dared to ask

12:00 Lunch

13:00 – 14:00 Poster session I with coffee

P1 *Josefine Nestler*, Functional characterization of roothairless mutants of maize (*Zea mays*)
P2 *Yvonne Ludwig*, Genetic dissection of the maize (*Zea mays* L.) Aux/IAA gene family
P3 *Kai Gräber*, A guideline to family-wide comparative qRT-PCR analysis exemplified with a Brassicaceae cross-species seed germination case study
P4 *Sandra Scholz*, Role of Calcium Sensor CML37 in *Arabidopsis* response to insect herbivory
P5 *Florian Wernet*, Measuring the effect of mechanical stress on cells
P6 *Eva Zwick*, *Eid3* - an *Arabidopsis* mutant in light signaling

14:00 – 15:00 Workshop 2: Stefan Rensing
Phylogenetic reconstruction and *P. patens* as a new model

Excursion

15:00, 15:20	*Departure*
15:30	*Uphill drive with cable car*
16:00	*Reception on 11th floor of Feldberg tower*
16:45	*Glacial relict excursion (Michael Scherer-Lorenzen) and walk down*
18:00, 18:20	*Return*

18:30 Supper

Wednesday Sep 12th

7:45 - 8:45 Breakfast

9:00 – 10:30 Oral session II

9:00 Keynote talk 3: *Klaus Palme*
Towards understanding hormone action in plants
10:00 T3 *Ahmed Ismail*
The interplay among plant hormones orchestrates salt tolerance in grapevines
10:15 T4 *Xin Guan*
Cytoskeleton and stress responses of crop plants: grapevine as model

10:30 Coffee break & industry exhibits (poster hall)

11:00 – 12:00 Workshop 3: *Regina Saum* (Thermo Fisher)
Optimize your cloning!
12:00 Lunch (& industry exhibits)

13:00 – 14:00 Poster session II with coffee & industry exhibits

P7 *Yadira Olvera-Carrillo*, Programmed cell death in unfertilized pistils of *Arabidopsis thaliana*
P8 *Kai Pfannebecker*, The development of a basic carpel toolkit
P9 *Wei-Chun Kao*, Proton Transfer and Reactive Oxygen Species in the Cytochrome bc1 Complex
P10 *Kerstin Redegeld*, Examinations on endogenous degradation of glucosinolates in *Capsella rubella*
P11 *Christopher Lentes*, Cloning, Production and Characterization of the Na+/H+ antiporter NhaA from *Salmonella enterica* serovar Typhimurium LT2
P12 *Antje Hellmuth*, Functional Characterization of Auxin Co-Receptors
P13 *K. G. Srikanta Dani*, Physiology and evolution of isoprene emission in plants

14:00 – 15:00 Poster session III

Discussion of all posters

15:00 – 17:30 Workshops

15:00 *Bernhard Busch* (GATC)
4: Multiplatform-based sequencing: combining libraries, sequencing technologies and software tools
15:50 *Regina Saum* (Thermo Fisher)
5: Optimize your qPCR
16:40 *Martin Kiesel* (Nikon)
6: Fluorescent Proteins - Important Tools in Live Cell Imaging

18:00 Supper

later *Farewell party*

Thursday Sep 13th

7:45 - 8:45 Breakfast

9:00 – 9:45 Oral session III

9:00 T5 *Maria Bucsenez*
Cis-regulatory elements mediating sieve element-specific gene expression
9:15 T6 *Mohammed Khalil*
Molecular analysis of biphenyl biosynthesis
9:30 T7 *Alzahraa Radwan*
The impact of drought stress on relevant natural products in sage (*Salvia officinalis*)

9:45 – 10:30 Workshop 7: *Andreas Schaaf* (greenovation)
Molecular Farming: Plant-based production of biopharmaceuticals

10:30 Coffee break

11:00 – 11:45 Workshop 8: *Wolfgang Jost* (Innovation office)
On ribosomal entry and patent protection

11:45 Concluding remarks, best poster and best talk reward

12:00 Lunch, end of retreat

13:30, 14:30 Bus shuttles to Bärental train station

Keynote Talk Abstracts

K1 Genes gone wild: experimental genome evolution in plants

Ralph Bock
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Plastids (chloroplasts) and mitochondria have evolved from free-living eubacterial ancestors through endosymbiosis. The uptake of the two eubacterial endosymbionts was followed by massive restructuring of the genomes of both the host cell and the symbionts. This process involved the loss of dispensable and redundant genetic information and, most importantly, the large-scale translocation of genes from the endosymbiont's genome to the host cell's nuclear genome.
In my talk, I will describe experimental approaches to study genome evolution in real time. I will discuss three fundamental processes in eukaryotic genome evolution:
- the transfer of organellar DNA to the nuclear genome,
- the conversion of transferred organellar genes into functional nuclear genes,
- the movement of DNA between plants by horizontal gene transfer.

Using transgenic tools and stringent selection schemes, we have reconstructed these processes in the laboratory and analyzed the underlying molecular mechanisms. The implications for our understanding of genome integrity and evolution will be discussed.

K2 Plant genome evolution

Yves van de Peer
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Flowering plants contain many genes, most of which were created during the past 200 or so million years through small- and large-scale duplications. Paleo-polyploidy events, in particular, have been the subject of much recent research. There is a growing consensus that one or more genome doubling or merging events occurred early during the evolution of the flowering plants, and that many lineages have since undergone additional, independent and more recent duplication events. I will review the difficulties in determining the number of genome duplications and discuss how the completion of some additional genome sequences of species occupying key phylogenetic positions has led to a better understanding of the timing of certain duplication events. This is important if we want to demonstrate the significance of genome duplications for the evolution and radiation of (different groups of) flowering plants, of which I will also give some examples.

K3 Towards understanding hormone action in plants

Klaus Palme
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The regulation of plant growth responds to many stimuli. These responses allow environmental adaptation, thereby increasing fitness. In many cases, the relay of information about a plant's environment is through plant hormones. These messengers integrate environmental information into developmental pathways to determine plant shape. This overview will use, as an example, auxin in the root of *Arabidopsis thaliana* to illustrate the complex nature of hormonal signal processing and transduction. It will then make the case that the application of a systems-biology approach is necessary, if the relationship between a plant's environment and its growth/developmental responses is to be properly understood. Specifically we will discuss pathways of auxin transport, perception and signal transduction defining growth control and patterning. Particular emphasis will be given to the temporal and spatial dynamics of auxin-regulated gene expression to aim for a systems understanding of hormone action.

Workshop Abstracts

W1 Things you always wanted to know about BLAST and never dared to ask

Stefan Rensing
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Everybody knows BLAST, everybody uses it. But how do you define homology from a BLAST result? By E-value? By Score? By alignment length? Or by % identity?
In this workshop you will learn how to interpret BLAST results correctly in order to infer homology. You will also learn which parameters should be tuned in which event, why big databases are a problem, and what the twilight zone of protein alignments is.

W2 Phylogenetic reconstruction and *P. patens* as a new model

Stefan Rensing
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Based on the definition of sequence homology by BLAST, you will receive an introduction on how to create phylogenetic trees. The workshop will confer information on multiple sequence alignment generation, visualization and curation (clipping), as well as on the subsequent generation, visualization and interpretation of phylogenetic trees.
The moss *P. patens* has been established as a prime model for plant evo-devo studies in the last decade and has recently been named one out of seven plant falgship genomes by the Joint Genome Institute. In the second part of the workshop you will learn why *P. patens* is a good evo-devo model - and phylogenies play a big part in this.

W3 Optimize your cloning!

Regina Saum
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Molecular cloning is one of the most common methods used in life science research.
Consisting of many steps involving DNA purification, restriction digestion, ligation and transformation, molecular cloning reveals frequently to be a critical key feature in researcher´s everyday life.
In the lecture "Optimize your cloning!" we will present you the most frequent problems and pitfalls around molecular cloning that our technical support team is confronted with in the daily business.
We would like to share our many years experience with you and present you problem solutions and give you ideas about useful control experiments.

W4 Multiplatform-based sequencing: combining libraries, sequencing technologies and software tools

Bernhard Busch
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Highest quality sequencing results can be achieved by combining leading sequencing technologies on the market as ABI 3730xl (Sanger sequencing), Illumina HiSeq 2000 and Roche 454 GS FLX+ System (Next Gen sequencing), as well as Pacific Biosciences PacBio *RS* (Third Gen Generation sequencing) with different library preparation methods and made-to-measure bioinformatic analyses.

The new Pacific Biosciences PacBio *RS* uses a novel technology that enables single molecule, real-time, or SMRT™, detection of biological processes. This feature allows the system to observe certain variation not accessible with other technologies.
The Illumina HiSeq 2000 provides the industry's highest sequencing output and quality at fastest data generation rate and hence can be optimally used to obtain best sequencing results in re-sequencing projects.
Based on projects examples as enrichment, *de novo* sequencing and transcriptome analysis, different approaches for sequencing as well as for bioinformatics will be shown.

W5 Optimize your qPCR

Regina Saum
Thermo Fisher Scientific
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One of the most versatile approaches in molecular and systems biology, the reverse transcription quantitative PCR (RT-qPCR), or simply "real time" PCR, is one of the most helpful tools for the quantification of gene expression.
In this seminar, we give an overview of the work-flow from the entire process of gene quantification via qPCR, starting from sampling, RNA extraction and handling, reverse transcription, reaction setup, controls, qPCR and data analysis.
Additionally, we include the most frequent problems and pitfalls around RT-qPCR that our technical support team is confronted with on a daily basis.

W6 Fluorescent Proteins - Important Tools in Live Cell Imaging

Martin Kiesel
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The discovery of green fluorescent protein (GFP) and its further development to the important tool for live cell imaging was awarded in 2008 with the Nobel price, emphasizing its importance for science. It was discovered by studying bioluminescence already in the 1960ties, but the idea of using it as a reporter for the visualization of gene expression pattern in the 1990ties was the beginning of a rapid development of tools based on the green fluorescent protein and it still continuous to evolve. Today we can select between fluorescent proteins over the entire visual spectral range, enabling us to multi-color live cell imaging and studying protein-protein interactions (FRET, BiFC). Fluorescent proteins have evolved from simple reporter to sophisticated photo-convertible proteins (optical highlighter), facilitating the study of cellular dynamics.

W7 Molecular Farming: Plant-Based Production of Biopharmateuticals

Andreas Schaaf
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Molecular Farming (or *pharming*) is the use of transgenic plants or plant cell cultures for the production of recombinant proteins with high value.
Since its starting point, the first successful expression of a human protein (HSA) in tobacco and potato in 1990, *Molecular Farming* underwent both a tremendous technological development as well as a fundamental ideological refocus. Whereas the initial concept was to exploit existing acreage and agricultural infrastructure for the production of plant-made-pharmaceuticals (PMPs), GMO discussions and pharmaceutical safety issues shifted developmental efforts towards contained and controllable systems.
Aside from basic definitions and a short historical flyover, the workshop will concentrate on carving out the potential advantages, major obstacles and viable economical niches for PMPs.

W8 Workshop on Patenting: On ribosomal entry & patent protection

Wolfgang Jost,
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This introductory seminar will try to interpret a buzzword found not only in many scientific grants of the last decade: “translational research”
We will reveal and discuss some of the fundamental DOs and DON'Ts for promising but yet early inventions to be successfully transferred towards marketable innovations.
As a translational process it by analogy may also be broken down into three phases: initiation, elongation and termination.
Project identification, proper evaluation and launch of solid protective rights typically are crucial but not more than *translational initiations* for all subsequent *elongation* steps (the “D” in R&D) in order to achieve sufficient maturity and finally a flourishing handover to industry (*termination*).
Especially the fast evolving plant sciences increasingly generate prospective applications with relevance and benefits for societies. Beyond your particular scientific interests you are hence invited to take a brief excursion into another world of exciting challenges:
Basic patent filing guidelines for inventors at universities
“Ribosomal entry sites” for up-and-coming academic inventions

Talk Abstracts

T1 The dual localized protein WHIRLY1 has functions in plastids and the nucleus

Rena Isemer, Kirsten Krause, Karin Krupinska

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WHIRLY1 was shown to be targeted to plastids where it is processed by cleavage of its plastid targeting peptide. A WHIRLY1 protein having the same molecular weight as the mature plastid protein was furthermore detected in the nucleus. Recently, we were able to shown that HA-tagged WHIRLY1 synthesized in plastids of transplastomic tobacco plants translocates to the nucleus. Our findings indicate for the first time that nuclear encoded plastid proteins can be released from the organelle and be transported to the nucleus. The translocated WHIRLY1 was shown to activate transcription of target genes in the nucleus. This suggests that WHIRLY1 is stored in plastids before it translocates to the nucleus to adjust gene expression. To distinguish between effects of the plastid and the nuclear form of WHIRLY1, respectively, sequences encoding the complete WHIRLY1 protein or a truncated form lacking the plastid targeting peptide were overexpressed in the mutant background. In plants overexpressing the full-length sequence WHIRLY1 accumulated in plastids and in the nucleus, whereas in plants overexpressing the truncated sequence WHIRLY1 accumulated exclusively in the nucleus. Germinating seedlings containing recombinant WHIRLY1 in both compartments were hypersensitive towards ABA. In contrast, seedlings possessing only the nuclear form of WHIRLY1 showed the same insensitivity towards ABA as the *why1* mutants suggesting that exclusively the plastid form is required for ABA signalling.

T2 Investigation of a translation-inhibitory function mediated by the 5'UTR of phytoene synthase from *Arabidopsis*

Daniel Alvarez, Peter Beyer, Ralf Welsch

Daniel Alvarez
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Phytoene synthase (PSY) represents the rate-limiting step for the carotenoid biosynthetic pathway in higher plants. Overexpression of the *Arabidopsis* PSY ORF (AtPSY) increases carotenoid amounts in *Arabidopsis* roots or callus. However, carotenoid increases were not observed when the AtPSY ORF including its 5'UTR was overexpressed which correlated with only slight increases in PSY protein levels. These results suggest a translation-regulatory function mediated by the AtPSY 5'UTR. To facilitate further investigations, we generated transgenic *Arabidopsis* plants constitutively overexpressing the AtPSY 5'UTR followed by GUS as a reporter gene. First results suggest an inhibition of GUS translation efficiency in presence of the 5'UTR which is attenuated in lines containing 5'UTR truncations. Furthermore, transient expression of 5'UTR-GUS reporter gene constructs in tobacco leaves was chosen as an additional system. Normalization of expression levels was achieved by coexpressing luciferase as a second reporter gene under control of an internal ribosome entry site (IRES). Moreover, in RNA electrophoretic mobility shift assays (REMSAs) with protein extracts from *Arabidopsis* leaves, an RNA/protein complex was observed which specifically occurs with the full-length AtPSY 5'UTR. Currently, different protein enrichment techniques are applied in order to enrich and identify the RNA-binding proteins involved.

T3 The interplay among plant hormones orchestrates salt tolerance in grapevines

Ahmed Ismail, Michael Riemann, Peter Nick

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Salt stress is a major constraint for many crop plants, such as the moderately salt-sensitive economically important fruit crop grapevine. Plants have evolved different strategies of protection against salinity and drought. Jasmonate signaling and abscisic acid are central elements of both biotic and abiotic stress responses. To discriminate stress quality, there must be crosstalk with parallel signal chains. Using two grapevine cell lines differing in salt tolerance, we analysed the response of jasmonate ZIM/tify-domain (JAZ/TIFY) genes (negative regulators of jasmonate signaling), markers for salt adaptation Na+/H+ EXCHANGER (NHX1), and STILBENE SYNTHASE (StSy) as a marker for biotic defence. In addition, the plant hormones ABA, JA and its bioactive form JA-Il, and Auxin were analysed under salinity at different time points. We show that salt-stress signaling shares several events with biotic defence including activity of a gadolinium-sensitive calcium influx channel and transient induction of JAZ/TIFY transcripts. The rapid induction of RS and StSy characteristic for biotic defence in grapevine is strongly delayed in response to salt stress. In the salt-tolerant line, NHX1 is induced and the formation of reactive oxygen species, monitored as stress markers in the sensitive cell line, is suppressed. The data are discussed by a model.

T4 Cytoskeleton and stress responses of crop plants: grapevine as model

Xin Guan, Günther Buchholz, Peter Nick

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The plant cytoskeleton, composed of microtubules (MTs) and actin microfilaments (AFs), mediates the plant response to numerous biotic or abiotic stress factors. Stress resistance is a central topic for sustainable agriculture, for instance in grapevine, one of the field crops with the highest economic output per area. We have therefore generated a transgenic grapevine plant line of *Vitis vinifera* "Chardonnay" transformed with pK7WGF2-FABD2, and a grapevine suspension cell line *V. rupestris* transformed with pCambia1300(CAA)n 2XT-gfp-Tub6. These provide useful tools to understand the role of the cytoskeleton in defence and stress tolerance. *Plasmopara viticola* the cause of grapevine downy mildew is the most dangerous disease in Germany. In this study, we investigate the AFs - *P. viticola* interaction, and MTs biotic/abiotic elicitor responses. Our results based on the first grapevine cytoskeleton-marker line revealed the disassembling of AFs in guard cells and the appearance of a AFs perinuclear basket in lower epidermal cells; slightly bundled of AFs occurred after 5 dpi of bacterial infection, but no obvious AFs rearrangement occurred at the sites of red-*Agrobacteria* attachment. Using the grapevine MT marker cell line we could follow for the first time in vivo the formation of MTs-bundles (so called macrotubules) in response to drought and salt stress within around 30 min.

T5 *Cis*-regulatory elements mediating sieve element-specific gene expression

Maria Bucsenez, Boris Rüping, Gundula Noll, Dirk Prüfer

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The phloem of dicotyledonous and several monocotyledonous plants contains structural phloem proteins (P-proteins), which aggregate within developing sieve elements (SEs) and exhibit a parietal localization in mature SEs. Wounding causes the detachment and subsequent accumulation of P-proteins on the sieve plates, thereby preventing the loss of photoassimilates. Special spindle-shaped P-proteins, called forisomes, evolved exclusively in Fabaceae and are able to undergo an ATP-independent but calcium-triggered, reversible conformational change, thereby sealing the sieve tubes as well. Forisome subunits and conventional P-proteins are encoded by genes of the sieve element occlusion (SEO) family, wherein they comprise distinct subgroups. Numerous SEO genes of various plant species have been shown to be expressed in immature SEs, a pattern coinciding with the site of P-protein synthesis. The *in silico* investigation of the corresponding SE-specific SEO promoter sequences and subsequent in planta mutation analyses revealed conserved cis-regulatory elements that are specific for forisome and conventional P-protein promoters, respectively. Furthermore, a detailed promoter study of the forisome subunit-encoding MtSEO-F1 gene from Medicago truncatula disclosed a complex promoter organization of at least four different, partly redundant SE-activating cis-regulatory elements and surprisingly, also CC-specific activating and repressing elements were identified.

T6 Molecular analysis of biphenyl biosynthesis

Khalil MNA, Beuerle T, Liu B, Beerhues L

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Species of Pyrinae, such as apple and pear, form biphenyls and dibenzofurans as phytoalexins. Despite these plants high economic value, understanding formation of their inducible defense compounds is poor. Studies were carried out with fire blight-infected apple shoots and elicitor-treated *Sorbus aucuparia* cell cultures [1]. A major biphenyl phytoalexin is aucuparin (3,5-dimethoxy-4-hydroxybiphenyl). The biosynthetic key enzyme is biphenyl synthase (BIS) catalyzing formation of 3,5-dihydroxybiphenyl. The sequence of the downstream steps is O-methylation, 4-hydroxylation, and another O-methylation. Biphenyl 4-hydroxylase is a cytochrome P450 enzyme exhibiting absolute specificity for 3-hydroxy-5-methoxybiphenyl. cDNAs encoding the two O-methyltransferases (OMTs) were isolated using a candidate gene approach based on the recently published apple genome sequence [2]. Functional analysis identified a biphenyl OMT and a multifunctional OMT, which catalyze the first and the second O-methylation reactions, respectively. In a phylogenetic tree, the OMTs grouped together with caffeic acid OMTs and OMT for pinosylvin, a structurally related stilbene. Recombinant BIS was used to produce 14C labeled product for feeding experiments. Biphenyls were found to be precursors of dibenzofurans. These studies may contribute to genetic engineering of phytoalexin biosynthesis in apple and pear.
[1] Chizzali et al. (2012) Plant Physiol 158: 864-875; [2] Velasco et al. (2010) Nat Genet 42: 833-839.

T7 The impact of drought stress on relevant natural products in sage (*Salvia officinalis*)

Alzahraa Radwan, Dirk Selmar

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It is well known that spices derived from plants grown in semi-arid regions are much more aroma intensive than those obtained from equivalent plants, but cultivated in moderate climates. In semi arid regions - due to limited water supply and much higher light intensities - the plants are exposed to a higher level of drought stress than the plants grown in Central Europe. Accordingly, the internal CO2-concentration decreases, leading to an oversupply of reduction equivalents. In consequence, the synthesis of highly reduced secondary metabolites is favoured. Using sage (*Salvia officinalis*) as model plant, the influence of drought stress on secondary metabolism had been analyzed.

Poster Abstracts

P1 Functional characterization of roothairless mutants of maize (*Zea mays*)

Josefine Nestler, Knut Wichterich, Frank Hochholdinger

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Root hairs are unicellular epidermal extensions present in all underground root types. They increase the water and nutrient uptake of the plant by enlarging the root surface. In maize, thus far six roothairless mutants, *rth1* to *rth6* have been identified. All six *rth* mutants show significantly reduced root hair length, but they are also affected in root hair density. While *rth1* and *rth2* mutants show increased, *rth4* and *rth5* exhibit decreased numbers of root hairs. Root hair density is not affected in *rth3* and *rth6*. This indicates that *Rth* genes are not only involved in elongation of root hairs, but also in root hair patterning or initiation. Mapping of the genes and complementation tests of the six rth mutants indicated that in each of the mutants a different gene is affected. To date the *Rth1* and *Rth3* genes are cloned. *Rth1* encodes a SEC3 homologous protein involved in polar exocytosis (Wen et al. 2005). Moreover, *Rth3* encodes a monocot-specific COBRA-like protein related to cell wall biosynthesis and expansion (Hochholdinger et al. 2008). In the present study, *Rth5* was identified to encode a NADPHoxidase producing a tip-high ROS gradient in growing root hairs. A defect in this localized ROS production was found in *rth5* mutants and partial complementation was demonstrated. Mapping & cloning of *rth2*, *rth4* and *rth6* is in progress.

P2 Genetic dissection of the maize (*Zea mays* L.) Aux/IAA gene family

Yvonne Ludwig, Frank Hochholdinger

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In maize 31 Aux/IAA genes are known (Wang et al. 2010). All Aux/IAA proteins contain four characteristic domains and are generally localized in the nucleus. Domain I is a transcriptional repressor while domains III and IV are known to control the homo-/ and heterodimerization with other Aux/IAA proteins and/or Auxin Response Factors (ARFs). Domain II with the conserved degron- sequence GWPPV is important for the stability of Aux/IAA proteins. ssAt present only one Aux/IAA mutant is known in maize. The semidominant gain of function mutant rootless with undetectable meristem 1 (*rum1*) does not show any lateral and seminal roots compared to the wild-type because of a 26 amino acid deletion in domain II (von Behrens et al. 2011). Typically, a point mutation in the degron-sequence is sufficient to stabilize the protein, as demonstrated in several *Arabidopsis thaliana* Aux/IAA mutants like bodenlos (*bdp*) (Hamann et al.1999) or short hypocotyl2 (*shy2*) (Tian et al. 1999). To investigate the function of other Aux/IAA proteins in maize we plan to generate novel maize Aux/IAA mutants. To preselect candidate genes, phylogenetic and syntenic relations were studied and expression analyses of different root and shoot tissues were performed. Candidate genes and their duplicates were chosen for further experiments due to unique expression patterns in specific root-types and tissues.

P3 A guideline to family-wide comparative qRT-PCR analysis exemplified with a Brassicaceae cross-species seed germination case study

Kai Graeber, Ada Linkies, Andy Wood, Gerhard Leubner-Metzger

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Quantitative real-time RT-PCR (qRT-PCR) is a sensitive method to quantify gene expression. Even small differences in transcript abundance can be determined, given that the right experimental and analytical precautions are taken. One major concern is the normalization of transcript data, usually done by reference genes that show stable expression throughout the analyzed samples. It has been shown in plants that many commonly used reference genes are not stable expressed in certain developmental states such as seed germination. This is due to huge transcriptional changes during seed germination rendering most classical reference genes unsuitable here. To address this issue we carried out a cross-species approach with *Arabidopsis thaliana*, *Lepidium sativum* and *Brassica napus* to identify family-wide qRT-PCR reference genes for Brassicaceae seed germination and development. We used germination time course transcriptome data of specific *Lepidium sativum* seed tissues to select candidate reference genes and verified their stability by qRT-PCR in *L. sativum* and *Arabidopsis thaliana*. Further analysis of transcriptome data of *Brassica napus* and *Arabidopsis thaliana* seed development confirmed that the majority of new reference genes are also stable expressed here. This analysis also indicated that reference gene expression stability is higher for a given developmental process between distinct species than for distinct developmental processes within a given single species.

P4 Role of calcium sensor CML37 in *Arabidopsis* response to insect herbivory

Sandra Scholz, Jyothilakshmi Vadassery, Monika Heyer, Michael Reichelt, Wilhelm Boland, Axel Mithöfer

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Insects are the most species rich class of eukaryotes and half of them feed on plants or are herbivores. So during their life, most plants will encounter herbivorous insect attacks. Plants protect against insects by using an array of direct and indirect defense strategies. These include production of anti-herbivore phytohormones, secondary metabolites, volatiles and toxins. Relatively little is known about the early signal transduction pathways that connect insect specific elicitors to the plant defense responses they evoke. The calcium ion (Ca2+) has been implicated as a second messenger in many plant signaling pathways, but its role in herbivory is poorly understood. Calmodulin-like proteins (CMLs) are one class of proteins which decode the calcium signature obtained by release of Ca2+ into the cytosol as response to perception of insect elicitors. We focus on CML37, which is significantly upregulated after insect feeding, mechanical wounding and application of phytohormones. The larvae of the generalist herbivore Spodoptera littoralis show an altered feeding behavior on CML37 knock out plants compared to wildtype. While there are no changes in secondary metabolites like glucosinolates, the production and probably the perception of phytohormones is significantly reduced. According to the bioinformatics prediction CML37 is involved in a number of signaling pathways. To study the role of CML37 in the signaling cascade several stable tagged CML37 knock out lines are generated.

P5 Measuring the effect of mechanical stress on cells

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Mechanostimulation is used to investigate the effects of strain on biological tissue. Here, we describe an approach to analyze the stress-strain-relationship during mechanostimulation. We used a custom-made stimulator in which cells can be mechanically stimulated and their mechanical properties can be analyzed1. Alveolar epithelial cells and fibroblasts were grown adherent on a PDMS-carrier-membrane. This construct was deflected by pressurizing the space below the membranes. Pressure and applied gas volumes were recorded and the compliance of the cell-layers was calculated, using the SLICE-method2. After stimulation, the cells were detached and screened for apoptosis and necrosis. Supernatants were screened for stress-proteins. We were able to stimulate cell monolayers of various cell types in our device. Cells could be analyzed by flow cytometry and supernatants could be screened for stress-respond-proteins. Our mechanostimulator is a potent method for the stimulation of cells and the analysis of their mechanical properties and cellular reactions. It allows to analyze the effects of mechanical stress on biological tissue and their mechanical properties. We used mammalian cells but this stimulation method could easily be applied to plantal cells or tissues, since they only had to be grown/placed on the membrane.
Schumann et al.: J Biomed Mater Res B Appl Biomater. 2008ss2 Schumann et al.: Physiol Meas. 2009

P6 *Eid3* - an *Arabidopsis* mutant in light signaling

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PFT1 (Phytochrome and Flowering Time 1) is a subunit of the mediator complex which induces gene expression. It is involved in the Phytochrome light-signal pathway, responsible for induction of germination and flowering. The *eid3* (empfindlicher im dunkelroten Licht 3) mutant has mutation in the interaction domain of PFT1. It shows a hypersensitive phenotype (short hypocotyls, green cotelydons) under far-red light (FR). *pft1* has the same phenotype as wildtype plants (long hypocotyls, closed hook). In gene expression *eid3* has higher levels of light induced genes than wt and accumulates more anthocyan. To examine the function of *eid3* during light signaling, different interaction studies were performed, revealing an interaction with LAF1 (Long after Far-red 1), a MYB-transcription factor that is involved in far-red light signaling with a hyposensitive phenotype under FR. A double mutant was established and examined in physiological experiments and marker gene expression under different light conditions. The studies showed a dominant phenotype of *eid3* in the double mutant and increased transcript levels of light induced genes. *HY5* is a light induced gene at the end of the signal cascade. Its promoter has a myb-binding motif, so *HY5*-promoter-LUC lines with mutations in several motifs were crossed with *eid3*. The seedlings with a mutation in the myb-binding side had a strongly increased activity of LUC, suggesting a role of *eid3* in *HY5* expression.

P7 Programmed cell death in unfertilized pistils of *Arabidopsis thaliana*

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Plant ovules are the female precursors of the seeds after fertilization and their sensitivity to stress leads to a great decrease in seed set due to ovule abortion. The species-specific life span of unfertilized ovules defines a limited window for successful fertilization and seed set. Thus, the developmentally controlled cell death in unfertilized ovules of *Arabidopsis thaliana* is one of our model systems to investigate the mechanisms orchestrating cell death vs. survival during reproductive development. I will present the molecular toolbox we have developed to study the ovules in a tissue specific way, since the available markers expressed under constitutive promoters are not readily visible in these intricate floral organs. Using confocal laser scanning and transmission electron microscopy we described the morphology and timing of unfertilized ovule abortion, dividing it into discrete stages. We identified from the literature up-regulated genes during ovule abortion and from the study of several mutants of these genes we observed a functional relationship between the activity of at least one of these candidate genes and the onset of cell death during age-dependent ovule abortion. Deep sequencing technology will allow us to analyze the complete transcriptome of decaying ovules to correlate the described morphological changes to the molecular pathways that are differentially regulated during this form of cell death. The results obtained in these subjects will be discussed.

P8 The development of a basic carpel toolkit

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Carpels are crucial structures for any angiosperm flower. We have quite an idea of how a carpel is build in *Arabidopsis thaliana*, but there is significantly less known about other plants. Also the evolutionary origin of the carpel is still unknown. In the following study we are trying to develop a basal carpel toolkit to get information about which genes could have been involved in the building of the first carpels. To achieve that aim we used phylogenetic analysis to search for homologues of genes involved in carpel development in *A. thaliana* up in the plant kingdom. The idea is, that genes needed for a basal carpel development should arise in the same time frame as the early angiosperms. We were able to identify possible homologues of *Arabidopsis thaliana* carpel genes in numerous species. For example it seems that the multiplication of the N-Gatha genes and the Hecate genes in *Arabidopsis* are recent duplications. Also we could confirm, that the CrabsClaw/DroopingLeaf genes are angiosperm specific.

P9 Proton Transfer and Reactive Oxygen Species in the Cytochrome bc1 Complex

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The mitochondrial cytochrome bc1 complex links electron transfer from ubiquinol to cytochrome c by a protonmotive Q cycle mechanism in which ubiquinol is oxidized at center P and ubiquinone is rereduced at center N. E272 of the conserved PEWY loop of most cytochrome b has been suggested as ligand in the enzyme-substrate complex and as proton acceptor in parallel proton-electron transfer towards heme bL. E272D and E272Q mutations support the importance of the residue for correct ubiquinol oxidation, showing effects such as lowered ubiquinol cytochrome c reductase activity, elevated bypass reactions, and altered KM for ubiquinol oxidation. However, these effects may also be indirect and the role of E272 as direct ligand of ubiquinol is debated. Furthermore, E272 is not fully conserved across all species. We suggested that in Beta- and Gamma-proteobacteria of which the PEWY glutamate is substituted by valine or leucine, a glutamate equivalent to yeast H253 is conserved, which could take over the proton transfer function. To challenge this hypothesis, single and double substitutions of H253 and E272 have been constructed in *Saccharomyces cerevisiae*. Eight variants were produced and the detergent-solubilized and purified complexes were characterized. Inhibitor titration, growth analysis of mutants and x-ray structure of E272Q co-crystallized with UHDBT in 2.7 Å resolution revealed details of the rotational displacement mechanism of the catalytic mechanism of quinol oxidation.

P10 Examinations on endogenous degradation of glucosinolates in *Capsella rubella*

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Glucosinolates are sulphur-rich secondary metabolites that serve as storage components and function in defense against herbivores. During wound-induced degradation, they are hydrolyzed by myrosinases, which belong to a subfamily of β-glucosidases. Products of this degradation are nitriles, epithionitriles, or isothiocyanates, depending on the presence of certain protein factors. Interestingly, glucosinolates are also degraded endogenously in young seedlings, which leads to the release of sulphur. To investigate the mechanism of endogenous catabolism, we have chosen *Capsella rubella* as a model organism because it contains only three different glucosinolates. Two of them occur in seeds and are degraded endogenously in seedlings. To identify β-glucosidases that are involved in this endogenous degradation, *in silico* expression studies using the corresponding *Arabidopsis thaliana* genes were performed. Sequences of *Capsella* candidate genes were identified by genome sequencing data. Four Î²-glucosidases were overexpressed heterologously in *Nicotiana benthamiana* and examined regarding several substrates. Moreover, we established a germ-line transformation method for *Capsella rubella* and reduced the life cycle by cold-treatment. In future, the expression profiles of *Capsella* β-glucosidases will be verified by quantitative real-time PCR. In addition, knock-down mutants will promote the identification of responsible enzymes and protein factors of endogenous glucosinolate catabolism.

P11 Cloning, production and characterization of the Na+/H+ antiporter NhaA from *Salmonella enterica* serovar Typhimurium LT2

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Na+/H+ antiporters control a number of important cellular mechanisms. From bacteria to humans, antiporters are maintaining cell homeostasis by regulating pH and Na+/Li+ tolerance and even influencing cell proliferation and cell cycles. We amplified the gene for the Na+/H+ antiporter from *Salmonella typhimurium* (STNhaA) and expressed it in *Escherichia coli*. Functional production of STNhaA in *E. coli* was shown by growth complementation of a Na+/H+ antiporter deficient *E. coli* strain. The antiporter was over expressed and purified via a one-step IMAC purification strategy. The oligomeric state of the detergent solubilised purified protein was determined via size exclusion chromatography coupled to static light scattering detection. We used solid supported membrane based electrophysiology to characterize the purified STNhaA protein reconstituted in proteoliposoms. It showed a pH dependent activity pattern and an acidic down regulation mechanism similar to NhaA from *E. coli*. STNhaA can be inhibited by 2-Aminoperimidin, a specific inhibitor of bacterial Na+/H+-antiporters.

P12 Functional characterization of auxin co-receptors

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Auxin is an important regulator of plant growth and development. It activates expression of auxin response genes by promoting the degradation of AUX/IAA transcriptional repressors. AUX/IAAs together with the F-box protein TRANSPORT INHIBITOR RESPONSE 1 (TIR1) or one of its paralogs AUXIN SIGNALING F-BOX 1-5 (AFB1-5) constitute the auxin co-receptor. TIR1/AFBs are substrate receptors of SCF-type E3 ligases and bind to the degron domain (DII) of AUX/IAAs, thus targeting them to proteasomal degradation. The diversity of AUX/IAA as well as TIR1/AFB protein families gives rise to numerous possible co-receptor combinations. Since defined levels of auxins trigger specific physiological responses, plants might be able to perceive different auxin levels through co-receptors with specific auxin-sensing properties. We have demonstrated the assembly and auxin-binding capability of selected TIR1-AUX/IAA co-receptors in radioligand binding assays. We have also shown differential auxin-dependent TIR1/AFB-AUX/IAA interaction in a yeast two-hybrid (Y2H) approach. Our results indicate that regions outside the highly conserved DII contribute to complex formation. Currently, we are narrowing down these regions and assessing their role in co-receptor assembly. Additionally, we plan to address co-receptor formation *in vivo* by tracking AUX/IAA stability in tir1/afb mutant backgrounds. Our studies will help understanding hormone perception and its role in triggering distinct downstream responses.

P13 Physiology and evolution of isoprene emission in plants

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Light-dependent de novo volatile isoprenoid emissions, particularly isoprene, by some terrestrial plants (mainly trees) contribute nearly 0.5 Pg C/year to the global carbon cycle. It influences tropospheric ozone chemistry and atmospheric life time of methane. Over decades, isoprenoid emission has been analysed from the biochemical to the biome level, exposing many unanswered questions about the physiology of emission. This is primarily because of: (1) the vastly different rates of isoprenoid emission between plant taxa; (2) the lack of a sound evolutionary basis for predicting which taxa are isoprene emitters; (3) the diversity of biogenic VOCs and their complex atmospheric interactions. In this poster, we propose, with supporting published and primary data on isoprene emission rates (1, above), a novel hypothesis on the evolution of isoprene biosynthesis and emission in plants (2, above), that looks at it as a phenomenon independent of abiotic selective pressures and overcomes the limitations posed by phylogenetic notions of isoprene emission occurrence and distribution. In a step towards making the global emission models more credible (3, above), based on our hypothesis, we propose an informed way to look for representative large-scale emitters that matter amidst a plethora of emitting tropical tree taxa.

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