# SCIENTIFIC REPORT 2005-2006

## LEIBNIZ INSTITUTE OF PLANT BIOCHEMISTRY


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## Message from the Managing Director

The Leibniz Institute of Plant Biochemistry (IPB) is a non-university research center of the Leibniz Association. It was originally founded in 1958 by Kurt Mothes as one of the research institutes of the German Academy of Sciences at Berlin. From the beginning, the research focus of the IPB was on plant natural products including hormones. Within four research departments, more than 150 staff scientists, students and guest researchers investigate in a multidisciplinary style structure and function of natural products from plants and fungi, analyze interactions of plants with pathogenic and symbiotic microorganisms and study molecular interactions as part of complex biological processes.

The years 2005 and 2006 were very productive and the new scientific developments are highlighted within the research group reports. Scientists of the IPB have been participating in many regional, national and international research networks. Among the local activities, the Collaborative Research Center, SFB 648 *Molecular Mechanisms of Information Processing in Plants* and the Network of Excellence *Structures and Mechanisms of Biological Information Processing* have been established and are coordinated in cooperation with the Martin Luther University Halle-Wittenberg and the Leibniz Institute of Plant Genetics and Crop Plant Research in Gatersleben. At the national level, the Priority Program, SPP 1152 *Evolution of Metabolic Diversity* of the German Research Foundation has been coordinated by scientists of the IPB. In addition, scientists of the institute have been involved in projects of the German Program GABI (*Genome Analysis of the Plant Biological System*). Beside many bilateral cooperations between research groups at the IPB and groups from different countries worldwide, one trilateral project in the frame of GABI was carried out in cooperation with groups in France and Spain and the institute was involved in two international projects supported by the European Community.

Toni Kutchan, Head of the *Department of Natural Product Biotechnology* accepted the offer of an attractive position of the Donald Danforth Plant Science Center in St. Louis, Missouri, USA, and left the IPB in March 2006.

Stephan Clemens, Head of the Research Group *Metal Homeostasis* of the *Department of Stress and Developmental Biology* accepted the offer of the W3 Professorship of Plant Physiology at the University of Bayreuth and left the institute in fall 2006. We wish both colleagues success at their new positions.

The facilities of the IPB have been further improved by expanding the greenhouse space and adding two new buildings, one of which is accommodating gardeners and craftsmen and the second junior research groups and bioinformaticians.

## RESEARCH MISSION STATEMENT

The large manifold of plant species is reflected in the enormous diversity of their natural products. This content of natural compounds is made more complex by the change in metabolite patterns during development as well as when a plant is responding to its environment. Knowledge of structure and function of natural products is requisite to understanding plant diversity, developmental and adaptation processes. New resources can then become available for innovative application in plant production, plant protection, biotechnology and in the development of biologically active compounds. Furthermore, together with the growing gain in knowledge available from plant genome research, this information is of fundamental importance to functional genome analysis.

The comprehensive analysis of plant and fungal **natural products** is a priority in the research mission of the Leibniz Institute of Plant Biochemistry. An interdepartmental competence group develops and applies suitable analytical methods for a qualitative and quantitative analysis of natural products in biological materials. This forms the basis for investigation of function and biosynthesis of natural products and for discovering new biologically active compounds. Structure analysis, synthesis and derivatization of natural products contribute to an understanding of their function and to an increase in their structural diversity. Subsequent identification and isolation of biosynthetic enzymes can provide access to the encoding genes, which in turn enables study of the regulation of the biosynthesis and of the cellular and organismic organization of its components.

The genetic determination of plant development and its modulation during environmental adaptation rely on receptor-mediated perception of endogenous signals or biotic and abiotic environmental factors. The different signals are evaluated, compared and converted into physiological responses *via* altered gene expression patterns that are controlled by cellular and systemic signal transduction networks. The physiological responses are usually based on transiently and locally altered profiles of natural products. **Molecular interactions** form the basis of the cellular functions responsible for these processes. An interdisciplinary analysis of these interactions is therefore of central importance to the research mission of the Leibniz Institute of Plant Biochemistry. Receptor-ligand, enzyme-substrate and protein-protein interactions form the molecular principle of these processes and have application in the development of new biologically active agents. From this perspective, the mechanisms of communication between plants and their symbionts and pathogens are investigated as are biosynthetic pathways and signal transduction networks. Interdepartmental cooperation includes transcriptomic and proteomic approaches, but supports also the development of novel cell biological methods for the analysis of the dynamics of molecular interactions in the life organism. Chemical structures of the interacting components are modified using gene technological methods, directed evolution and chemical derivatization. The effects of these changes can be monitored in model systems or with activity screens until a molecule with the desired characteristic (e.g. a drug, signal compound or an enzyme) is achieved. The development of new syntheses, screening tests, assays and analytical methods is supported by visualization of molecular interactions *via* computer modeling.

## Research Mission Statement

The tight combination of natural product chemical, biochemical, molecular and cell biological approaches allows new access to **gene function analysis**, the third research priority of the Leibniz Institute of Plant Biochemistry. In the frame of a comprehensive research concept of functional genome analysis that is based on transcriptome, proteome and metabolome data, genes are identified and characterized, which are essential for plant development and environmental adaptation. The use of mutants and transgenic plants makes the direct analysis of gene functions possible and allows the generation of model plants with altered patterns of natural products, with novel health promoting compounds or plants adapted to specific sites or environmental conditions. Such plants will be beneficial for sustained production of valuable substances and biocatalysts, for use as biological indicators and for plant breeding.

A nexus of natural product research and the study of molecular interactions is storage and evaluation of the large amount of data that is generated. In particular, high throughput processes used in metabolome and proteome analysis and in the production of combinatorial libraries make necessary the development of new methods in **information technology**. To this end, a new junior group in information technology has been established at the Leibniz Institute of Plant Biochemistry. Together with the Research Group *Computational Chemistry* this forms a new research priority that will be extended towards an interdepartmental competence area. This priority aims at the integral linkage and analysis of structurally diverse data sets generated within the other research areas towards a better understanding of the biological system of plants.

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*(Part of the German Plant Genome Project, GABI)*
*Stephan Clemens, Dierk Scheel, Ludger Wessjohann & Jürgen Schmidt*

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## Department of Natural Product Biotechnology

*Head: Professor Toni M. Kutchan until March 2006, afterwards Professor Claus Wasternack (prov.)*

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Plants synthesize and accumulate numerous secondary compounds beside their primary metabolism functioning in growth and development. Among these secondary compounds the benzylisoquinoline alkaloids are of great importance, since many of them are of pharmaceutical interest. That is preferentially the case for morphinane compounds, such as the antitussive codeine and the pain-reducing morphine. Three working groups of the *Department of Natural Product Biotechnology* were studying morphine biosynthesis by molecular genetic techniques in 2005 and 2006. The goal of the work is the establishment of molecular tools to analyze the branched biosynthetic pathways of benzylisoquinolines. Using overexpression and suppression of genes coding for enzymes in morphine biosynthesis, an altered profile of intermediates and products (Metabolic Engineering) is envisaged (Research Group *Alkaloid Biosynthesis,* Toni M. Kutchan, Research Group *Opium Poppy Biotechnology,* Susanne Frick). This could be achieved after establishment of proper transformation protocols for *Papaver somniferum* with cDNA constructs related to the following enzymes: Berberine bridge enzyme, (*S*)-*N*-methylcoclaurin-3'-hydroxylase, (*R,S*)-reticuline-7-*O*-methyl-transferase, salutaridinol-7-*O*-acetyltransferase and codeinone reductase. Additional interest was given on biosynthesis of isoquinoline terpenoid alkaloids and of naphthoquinones (Research Group *Alkaloid Biosynthesis*). Ipecac alkaloid biosynthesis of *Psychotria ipecacuanha* has been studied by cloning enzymes involved. The biosynthesis of the naphthoquinone plumbagin has been analyzed by cloning and characterization of a type III polyketide synthase of *Plumbago indica* L.

New insights in morphine biosynthesis were achieved by analysis of ESTs (Research Group *Papaver Gene Expression Analysis,* Jörg Ziegler). Comparison of gene expression and alkaloid profiles between numerous opium poppy varieties and Papaver species led to the identification of new genes functioning in biosynthesis and regulation of morphine. Among them the salutaridine reductase, an *O*-methyltransferase and an ABC-transporter were studied in detail. Homology modeling of the salutaridine reductase led to the characterization of the substrate binding product.

The research groups of Toni M. Kutchan and Susanne Frick went to the Donald Danforth Plant Science Center in St. Louis (USA) in April 2006, and the research group of Jörg Zieg - ler is working now at the University of Calgary (Canada) since October 2006. Conse - quently, scientific activities of all three working groups are finished at the *Department of Natural Product Biotechnology* of the IPB.

A fourth Research Group (*Mode of Action of Jasmonates*, Claus Wasternack & Otto Miersch) of the department is working for many years on functional analysis of jasmonic acid (JA) and its metabolites, commonly named jasmonates. Their compounds act as sig -

nals in plant stress responses and development.
Using reverse genetic approaches, cell biological techniques, and quantitative analyses of these compounds, the role of JA biosynthesis and jasmonates in stress responses and development of *Arabidopsis thaliana* and tomato were studied. New aspects in regulation were found by transgenic approaches and analyses of knockout mutants of the JA biosynthesis enzyme allene oxide cyclase (AOC) in Arabidopsis and its cell type-specific and organ-specific occurrence in tomato. Among the metabolites increasing interest is given to 12-hydroxyjasmonic acid due to its JA-independent properties and its specific accumulation in distinct developmental processes. Numerous collaborative works in the field of jasmonates were continued in 2005 and 2006.

# Alkaloid Biosynthesis

*Head: Toni M. Kutchan until March 2006*

**The main topic of research in the group is the investigation of natural product biosynthetic pathways. Approximately 25 % of current pharmaceuticals are either isolated directly from medicinal plants or are modified from natural products. Multiple stereocenters present in many of these natural products prohibit commercially feasible total syntheses, thereby making an understanding of the enzymes and genes involved in the biosynthesis of central interest to developing improved sources of these medicinals through either metabolic engineering or biomimetic synthesis. The study of the biosynthesis of isoquinoline terpenoid alkaloids and of naphthoquinones has recently yielded intriguing preliminary results.**

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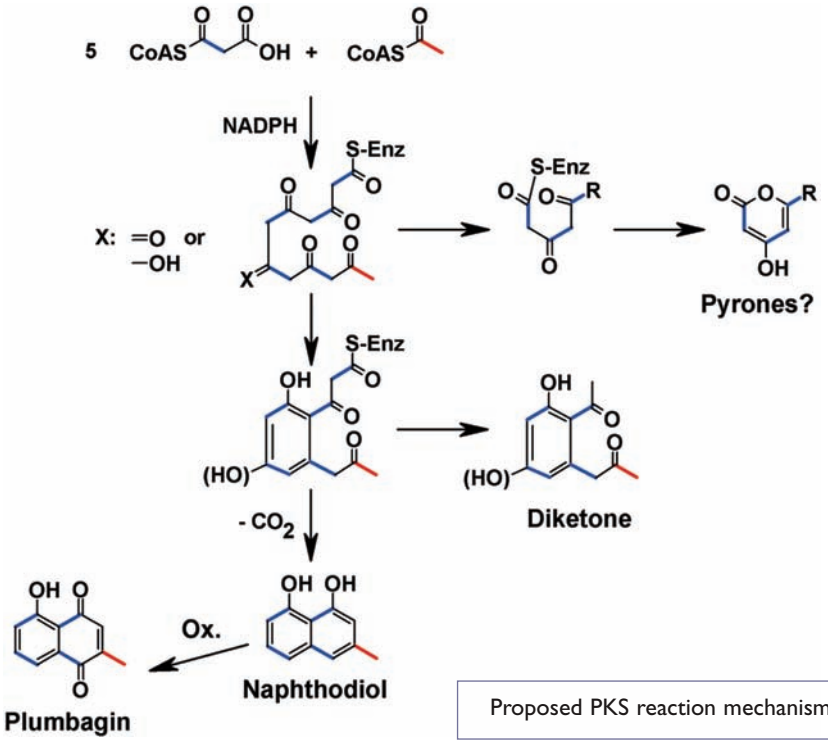
Containing more than 1800 members rich in structural diversity, the monoterpenoid indole alkaloids are a fruitful source of physiologically active molecules. Historically, the use of monoterpenoid indole alkaloid-producing plants, such as *Rauwolfia serpentina*, in traditional medicines can be traced back in India more than 3000 years. Selected examples of pharmaceuticals derived from monoterpenoid indole alkaloid-producing plants are the antineoplastic drugs vinblastine and vincristine and the antihypertensives ajmalicine and ajmaline. The alkaloidal glucoside strictosidine has been recognized as a biosynthetic precursor to the monoterpenoid indole alkaloids. The enzyme that catalyzes the formation of strictosidine, strictosidine synthase, was one of the first indole alkaloid biosynthetic enzymes isolated and the cDNA encoding this enzyme was the first alkaloid biosynthetic gene isolated. Biosynthetically related to the monoterpenoid indole alkaloids are the ipecac alkaloids, such as emetine. Emetine is produced in *Psychotria ipecacuanha*, which grows wild in Central and South America, and is used in medicine as an emetic and amoebicide. Subcutaneous injections of the alkaloid emetine, the chief active principle present in ipecac, produces a rapid cure of amoebic dysentery, while oral administration promotes the emetic properties of the drug.

Based upon catalytic mechanism, we postulate that the first enzyme of ipecac alkaloid biosynthesis is related in primary sequence to strictosidine synthase. Strictosidine synthase catalyzes the Pictet-Spengler condensation of the biogenic amine tryptamine and the iridoid secologanin, whereas the first enzyme of emetine biosynthesis condenses dopamine and secologanin to the alkaloidal glucoside deacetylipecoside. We have attempted to identify the ipecac cDNA by homology to strictosidine synthase, which cDNA has been isolated from the monoterpenoid indole alkaloid-producing species *Rauwolfia serpentina* and *Catharanthus roseus*. Root cultures of *Psychotria ipecacuanha* were developed from rootless *in vitro* plantlets by modification of the hormone components of the tissue culture medium. HPLC-MS analysis of extracted tissues showed that the root cultures accumulate emetine, at approximately 10 % of the level accumulated in *in vitro* plantlet leaves. Cultured roots were chosen for preparation of a cDNA library to be randomly sequenced. Even though less alkaloid is present than in leaf material, the root tissue cultures are not photosynthetic and should be, relatively speaking, "enriched" for alkaloid biosynthetic transcripts. The EST sequences thus obtained were compared to sequences present in the GenBank and in this way a cDNA with homology to the *Rauwolfia serpentina* strictosidine synthase gene, *STR1*, was identified. In addition, cDNAs with homology to *Rauwolfia serpentina* glucosidases involved in monoterpenoid indole alkaloid biosynthesis were also identified. The ipecac homologs were generated as full-length clones using RT-PCR and the conditions for functional heterologous expression are now being developed in *Escherichia coli*. The strictosidine synthase-like enzyme will be tested for activity with secologanin and dopamine as well as with tryptamine and dopamine. For the recombinant glucosidase, we have a series of alkaloidal glucosides that have been tested as substrates. The recombinant glucosidase appears to hydrolyze the glucose moiety of both ipecoside and strictosidine.

*Plumbago indica* L. is a traditional medicinal plant from South East Asia. The roots of *P. indica* accumulate the naphthoquinone plumbagin, which exhibits antimicrobial, insecticidal, antitumor, and antifertility activities. Plumbagin origi-

*Plumbago indica*

nates from six acetate units. This was demonstrated for the first time by tracer experiments with *Drosophyllum lusitanicum* cultures and *Plumbago europaea* plants. In plants, polyketides are synthesized by type III polyketide syntheses (PKS). Type III PKSs are typically homodimeric proteins with two functionally independent active site cavities. They use thioesters of coenzyme A as substrates and catalyze up to seven decarboxylative condensation reactions followed by cyclization of the intermediate polyketide. The prototype of type III PKSs is chalcone synthase (CHS) that synthesizes naringenin chalcone, the precursor of flavonoids, by condensing three acetate units derived from malonyl-CoA with a *p*-coumaroyl-CoA starter unit. To characterize the enzyme potentially catalyzing the first step in the biosynthesis of the naphthoquinone plumbagin, a cDNA encoding a type III PKS was isolated from roots of *P. indica*. The translated polypeptide shared 47 % to 60 % identical residues with PKSs from other plant species. Recombinant *P. indica* PKS expressed in *Escherichia coli* accepted acetyl-CoA as starter and carried out five decarboxylative condensations with malonyl-CoA. The resulting hexaketide was not folded into a naphthalene derivative. Instead, an α-pyrone, 6-(2',4'-dihydroxy-6'-methylphenyl)-4-hydroxy-2-pyrone, was produced. In addition, formation of α-pyrones with linear keto side chains derived from three to six acetate units was observed. Since phenylpyrones could not be detected in *P. indica* roots, it is possible that the novel PKS is involved in the biosynthesis of naphthoquinones, and additional co-factors are probably required for the biosynthesis of these secondary metabolites




Proposed PKS reaction mechanism

## COLLABORATORS

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# Opium Poppy Biotechnology

*Head: Susanne Frick until March 2006*

**Opium poppy (*Papaver somniferum* L.), which contains more than 80 different alkaloids, remains one of the most important industrial medicinal plants. Poppy serves as a renewable resource of a number of medicinally relevant alkaloids. These include the analgesic and narcotic drug morphine, the cough suppressant codeine, as well as the muscle relaxant papaverine, the antineoplastic agent noscapine and the antimicrobial sanguinarine. We developed a transformation system for opium poppy, that will allow us:**

- **to investigate the regulation and ecological function of these alkaloids in plants,**
- **and to alter the alkaloid metabolism in commercial poppy varieties in order to obtain varieties lacking alkaloids or carrying tailored alkaloid profiles for industrial and pharmaceutical use.**

## Group Members

**Kathleen Bräuer**
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During the last years several genes from the biosynthetic pathways for reticuline, sanguinarine and morphine have been cloned (**Fig. 1**). Although the biosynthesis is well understood at the enzyme level, the molecular and biochemical mechanisms that regulate these pathways are not known.

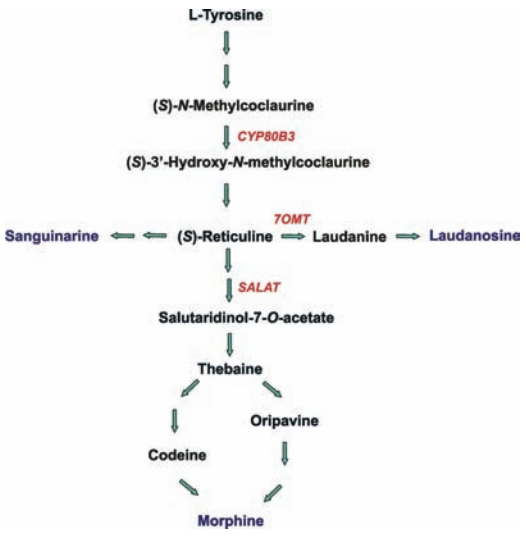


**Figure 1:** Biosynthetic pathway from *L*-tyrosine to sanguinarine, laudanosine and morphine in *P. somniferum*. Enzymes are highlighted in red.

A major goal of this project was the development of a stable transformation and regeneration method for opium poppy, which will make the metabolic engineering of the above mentioned compounds possible. Poppy seed oil finds use in chemical industry for the production of pigments and lacquer, but residual morphine levels prevent a more widespread applications. Because opium is the raw material for the illicit production of heroin, cultivation of poppy is restricted. By completely suppressing morphinane alkaloid biosynthesis, opium poppy could potentially be grown more widely for the food and oil industries.

We have used an *Agrobacterium*-mediated approach to introduce different cDNAs encoding enzymes of reticuline, morphine and sanguinarine biosynthesis as *sense*, *antisense*- or RNA*i*-constructs into explants to attempt to alter the alkaloid profile. Alkaloid-free plants developed in this manner will be used to test the chemical and ecological function of morphinane and benzophenanthridine alkaloids *in planta*. After the regeneration of plants, the qualitative and quantitative determination of alkaloids was determined by HPLC and LC-MS in latex, leaves and roots of opium poppy. Finally the heredity of the alkaloid pattern was confirmed.

Overexpression of the cDNA encoding (*S*)-*N*-methylcoclaurine 3'-hydroxylase (CYP80B3) resulted in an up to 450 % increase in the amount of total alkaloid in latex (**Fig. 2**). This increase occurred either without changing the ratio of the individual alkaloids, or together with an overall increase in the ratio of morphine. Correspondingly, *CYP80B3-antisense* cDNA expressed in opium poppy caused a reduction of total amount of alkaloids in latex up to 84 %, suggesting that the observed phenotypes were dependent on the presence of the transgene. The increase or decrease of the amount of alkaloids in the transgenic cell lines is heritable at least to the $T_3$ generation. The transcript

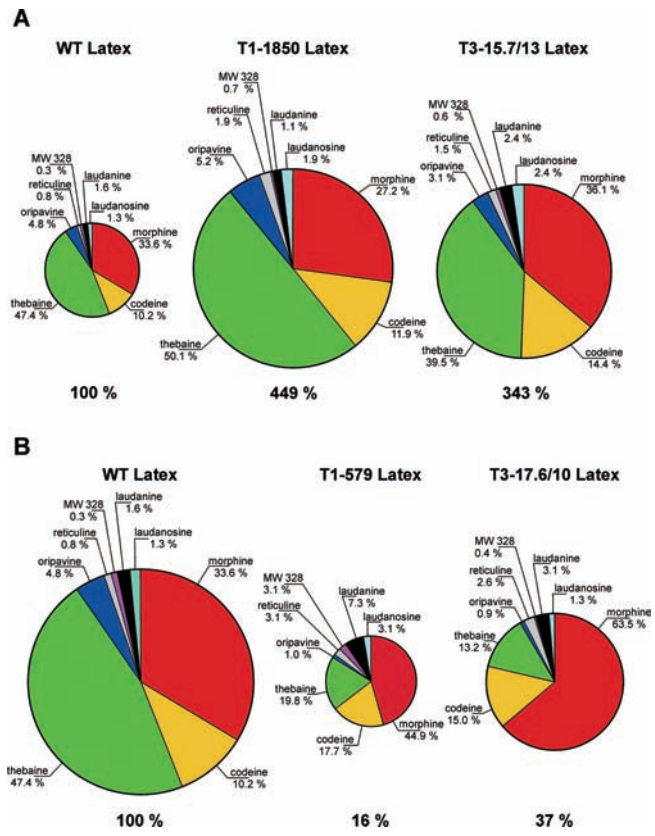


**Figure 2:** Comparative alkaloid analysis of latex from *P. somniferum*. **A-B** wild-type (WT), **A**, T1 plant 1850 and T3 plant 15.7/13, both from transformation with *CYP80B3-sense3*. **B**, T1 plant 579 and T3 plant 17.6/10, both from transformation with *CYP80B3-antisense*. Alkaloids in latex were calculated as µg alkaloid/100 µg soluble protein, then normalized to 100 % to produce the individual divisions within pie graphs, representing relative ratios of the latex alkaloids. Total WT alkaloids were set to 100 % to produce the relative diameters of the pie graphs, representing the relative total alkaloid contents of the transgenic compared to the WT.

levels of *CYP80B3* were determined by a multiplex RT-PCR with 18S rRNA as an endogenous standard. In plants overexpressing *CYP80B3*, higher ratios of *CYP80B3*:18S rRNA in comparison to wild-type was observed. In *antisense-CYP80B3* plants the ratio *CYP80B3*:18S rRNA was reduced. Southern blot analysis revealed patterns that are in agreement with five to six copies of *CYP80B3* in *CYP80B3-sense* plants and three to five copies in *CYP80B3-antisense* plants.

The overexpression of the (*R*,*S*)-reticuline 7-*O*-methyltransferase gene (*7-OMT*) was determined by Northern blot analysis from the $T_0$ to the $T_2$ generation. Southern blot analysis revealed the integration of one to five copies into the plant genome. The amount of reticuline, laudanine and laudanosine was changed in 40 % of the $T_1$ and 75 % of the $T_2$ generation. Some plants of $T_1$ and $T_2$ generation displayed also changed levels of morphinane alkaloids.

The suppression of the salutaridinol 7-*O*-acetyltransferase gene (*SALAT*) with an RNA*i* construct produced transgenic plants, in which pathway intermediates of morphinane biosynthesis accumulated. This alkaloid pattern was heritable to the $T_1$ generation. The suppression of *SALAT* was confirmed by northern blot analysis and real time PCR. With Southern blot analysis, two to four copies could be detected in transgenic plants.

In parallel, we initiated the first crossing experiments. Crossing of the transgenic plant *CYP80B3-sense* with a transgenic plant overexpressing a NADPH:cytochrome P-450 oxidoreductase (*CPR*) produced all four possible phenotypes: wild-type, *CPR* overexpressing plants, *CYP80B3* overexpressing plants and plants overexpressing both transgenes. Plants overexpressing both transgenes inherited five to six copies of *CYP80B3* and one copy of *CPR* from their parental plants. The transcript levels of *CYP80B3* and *CPR* were determined by multiplex RT-PCR with 18S rRNA as an endogenous standard. The mRNA levels of *CYP80B3* and *CPR* were increased in the trait-stacked plants as well as in their parental plants. The new transgenic plants displayed either increased or decreased amount of alkaloids.

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# Mode of Action of Jasmonates

*Heads: Claus Wasternack & Otto Miersch*

**Jasmonates and their precursors, the octadecanoids, are signals in plant stress responses and in plant development. A mechanistic analysis of the mode of action of jasmonic acid (JA) and its metabolite 12-hydroxy-JA was performed by a reverse genetic approach using the allene oxide cyclase (AOC)-catalyzed step in jasmonate biosynthesis and the 12-hydroxy-JA-sulfotransferase. *Gain of function* and *Loss of function* studies with transgenic tomato and *Arabidopsis thaliana* plants revealed modulation of jasmonates and allowed to inspect the role of jasmonates in response to biotic and abiotic stresses as well as flower and seed development. Functional analysis of AOC using genetic approaches is also performed in Arabidopsis to ask on specific versus redundant functions of the four AOCs in this plant. These data were linked to metabolite profiles on jasmonates and octadecanoids under various stress conditions and in different developmental stages. In addition to this analytical work, chemical synthesis of standards and labeled substrates is an essential part of this work.**

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The role of JA biosynthesis (**Fig. 1**) was analyzed in tomato and Arabidopsis. Preferentially, the AOCs, the 12-hydroxy-JA sulfotransferase and the profile of jasmonates and other oxylipins were studied to elucidate specific functions in plant stress responses and development.

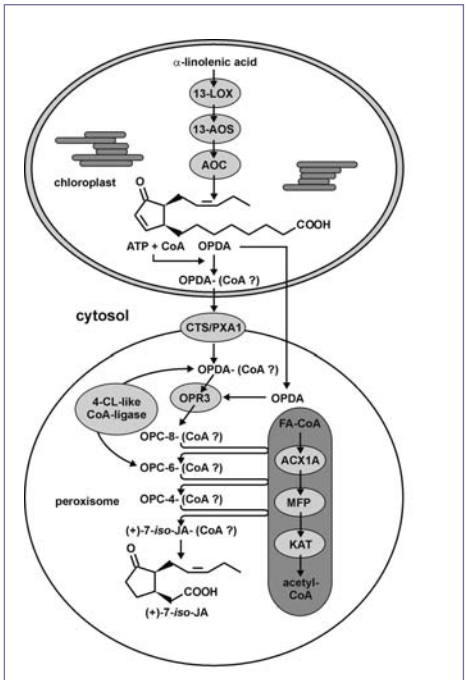


**Figure 1:** Jasmonate biosynthesis takes place in chloroplasts and peroxisomes. The final reactions occur *via* fatty acid β-oxidation steps. In the activation to the corresponding CoA esters 4 CL-like CoA ligase is involved (from Wasternack, C. 2006 Oxylipins–Biosynthesis, Signal Transduction and Action. In *Plant Hormone Signaling* (Hedden, P. & Thomas, S., eds.) *Ann. Plant Reviews*, pp. 185-228. Blackwell, Oxford, UK).

The first AOC was cloned from tomato (Ziegler et al. 2000, patent No. DE 1000 4468.9). This single copy gene is specifically expressed in ovules of young flowers and all vascular bundles, which led together with data from various transgenic tomato plants to a proposed model on amplification in wound signaling with preferential signaling properties of JA. This is supported by detection of JA biosynthetic enzymes including AOC in sieve elements of tomato leaf veins.

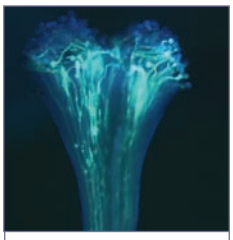

**Figure 2:** Pollen tube growth in tomato flowers 24 hours upon pollination of the stigma. Photo: S. Götz

Abundant accumulation of AOC in ovules of tomato flower buds and detection of a specific oxylipin signature in flower organs of wild-type and *AOC* overexpression lines led to a concept of specific functions of oxylipins in flower development (**Figs. 2-4**). Corresponding to the female sterility of the JA-insensitive mutant *jai1*, a role of oxylipins in early stages of embryo development of tomato was shown. An interesting and important new facet in the role of JA is the identification and characterization of metabolites of JA, such as 12-hydroxy-jasmonate, its sulfated derivative and its glucose ester, both in wounded tomato leaves as well as during flower development. Their content is dramatically altered upon wounding and during flower, embryo and seedling development. In case of 12-hydroxy-JA a sulfotransferase of tomato was cloned and transgenic lines overexpressing or repressing the corresponding gene revealed a flowering phenotype of the day-neutral toma-

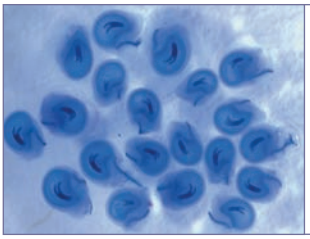

**Figure 3:** Developing embryos in the torpedo stage of tomato flowers (18 hours after pollination). Photo: S. Götz

to plant. In contrast, in the photoperiod-dependent Arabidopsis plants the position of the corresponding sulfotransferase within the various flowering time control pathways is determined due to hints on flowering time control by 12-hydroxy-JA (in cooperation with Luc Varin, Montreal, Canada).

In Arabidopsis the AOC is encoded by four genes. Inspection of transgenic Arabidopsis plants expressing a reporter gene driven by each *AOC* promoters, revealed redundant and non-redundant functions of the AOCs. Prefe - rentially, in root growth and seedling development (**Fig. 5**) as well as in flower development there are tissue and organ specific promoter activities. These data and analyses of knock-out lines indicate that Arabidopsis is able to perform a fine-tuning of regulation by spatial and temporal differences in the expression of the four *AOC*s. In contrast, the four recombinant AOCs exhibited similar enzymatic properties and substrate specificities. Using Arabidopsis mutants affected in the multifunctional protein of fatty acid β-oxidation (*aim1*) or exhibiting a defect in protein import during peroxisome biogenesis (*pex6*) the role of fatty acid β-oxidation in final steps of JA biosynthesis was shown. Quantitative analysis of octadecanoids, jasmonate and its intermediate in biosynthesis upon feeding of deuterated 12-oxophytodienoic acid followed by wounding of leaves revealed that mutants were able to perform β-oxidation of the carboxylic acid side chain of JA precursors. These data correspond to recently published results of other labs.

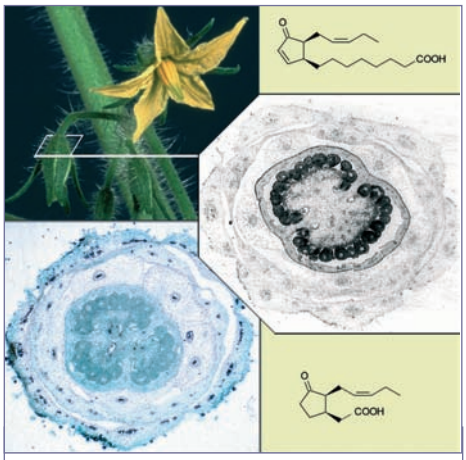


**Figure 4:** The allene oxide cyclase (AOC) establishing the correct enantiomeric structure of the cyclopentanone ring of jasmonate is encoded by a single copy gene in tomato, which is specifically expressed in ovules of young flower buds and in early embryo development as revealed by *AOC* promoter activity tests (blue staining, left bottom) and immunocytological analysis (right) of a cross section. The structure of jasmonic acid (bottom) and its precursor 12-oxophytodienoic acid (top) is given.
Photo: B. Hause

Collaborative work was done with groups in Germany and abroad and could attribute to the following published working areas:

(1) Role of NO in alternative oxidase activity of ozone-treated tobacco plants. (Ederli et al. 2006)
(2) Interaction of salicylic acid and JA signaling. (Mur et al. 2006)
(3) Hormones and gene expression pattern during barley seed development. (Sreenivasulu et al. 2006)
(4) Singlet oxygen and cell death in *A. thaliana.* (Danon et al. 2005)
(5) AOC of hop and its role in organogenesis. (Fortes et al. 2005)
(6) Lipoxygenase-mediated metabolism of storage lipids in germinating sunflower cotyledons. (Gerhart et al. 2005)
(7) 4-CL-like enzymes of Arabidopsis and their role in JA biosynthesis. (Schneider et al. 2005).
(8) Ethylene and JA in CDPK and MAPK signaling of stress responses. (Ludwig et al. 2005).
(9) Modified oxylipin signature of barley by overexpression of a 13-lipoxygenase. (Sharma et al. 2005).

The know-how of the group on JA and oxylipin analytics is used and requested by many groups. A minor activity is linked to collaboration with the Probiodrug GmbH in Halle. The company is working on role of glutaminylcyclases (QCs) of human, animal and plant sources. QCs attribute in mammalian systems to a functionalization of peptide hormones and proteins by py - roglutamate formation at the N-terminus. Our effort is to clone plant homologes of the QC as a tool for design of potent QC inhibitors. So far the potato QC and a QC of Arabidopsis were cloned, overexpressed as recombinant protein and characterized in terms of enzymatic properties (Schilling et al. 2006).

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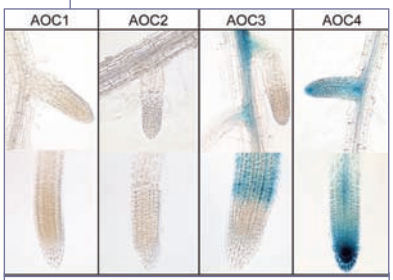


**Figure 5:** Non-redundant promoter activity of *AOC3* and *AOC4* in roots of seven-day-old *Arabidopsis thaliana* plants. Each promoter of the four *AOC* genes of *A. thaliana* was fused to the *uidA* gene coding for β-glucuronidase (GUS). Promoter activity was recorded by blue staining, which appeared in tissues carrying GUS activity.
Photo: I. Stenzel

# Papaver Gene Expression Analysis

*Head: Jörg Ziegler*

**The benzylisoquinoline alkaloids comprise a group of roughly 2,500 compounds identified so far. Among these are compounds of high pharmaceutical value, such as the analgesic morphine, the antitussive codeine, and the antimicrobial sanguinarine. The benzylisoquinolines mainly occur in the Ranunculales within the Papaver species accumulating the most extensive diversity of structures. The biosynthesis of all monomeric benzylisoquinolines is conserved up to the central intermediate (*S*)-reticuline, and biochemical and molecular data are available for every step. After that, the biosynthetic pathways diverge leading to the extensive structural diversity of this group of alkaloids. However, most of these steps are unknown yet. Similarly, factors regulating the accumulation of benzylisoquinolines, as well as components facilitating the transport of intermediates between cell types are not characterized yet. In order to address these issues, we took advantage of the species-specific accumulation of distinct benzylisoquinoline profiles in Papaver species. Correlation of gene expression with the alkaloid profile of each Papaver species was successfully used to detect cDNAs involved in the establishing a distinct benzylisoquinoline profile in individual plants.**

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As a first step, an alkaloid profiling method was developed using FT-ICR/MS as analytical method followed by the development of a program to visualize the data. The high mass accuracy of the FT-ICR/MS allows the correct assignment of empirical formulas to the masses. Although many benzylisoquinolines possess the same mass, the FT-ICR/MS approach gave a fast and, by inclusion of a standard, quantitative overview over the distribution of alkaloids among different Papaver species. The most noticeable masses were further analyzed by LC-MS/MS for structure elucidation. As already known from literature, it was shown that only varieties of *Papaver somniferum* and *P. bracteatum* accumulate high amounts of alkaloids of the morphinane type. Unexpectedly, a morphinane derivative was also found at very low levels in *P. arenarium*.

Based on the profiling results, the gene expression analysis of 2,000 ESTs was performed in order to detect cDNAs whose expression could be related to the accumulation of morphinane alkaloids. The analysis of 16 different Papaver species yielded 69 cDNAs showing higher expression in plants containing morphinanes. Interestingly, eight of these cDNAs code for proteins shown to be involved in benzylisoquinoline biosynthesis (**Fig. 1**). 43 cDNAs showed no homology to any known protein in the data - base, whereas nine code for house-keeping genes. The remaining cDNAs could be classified

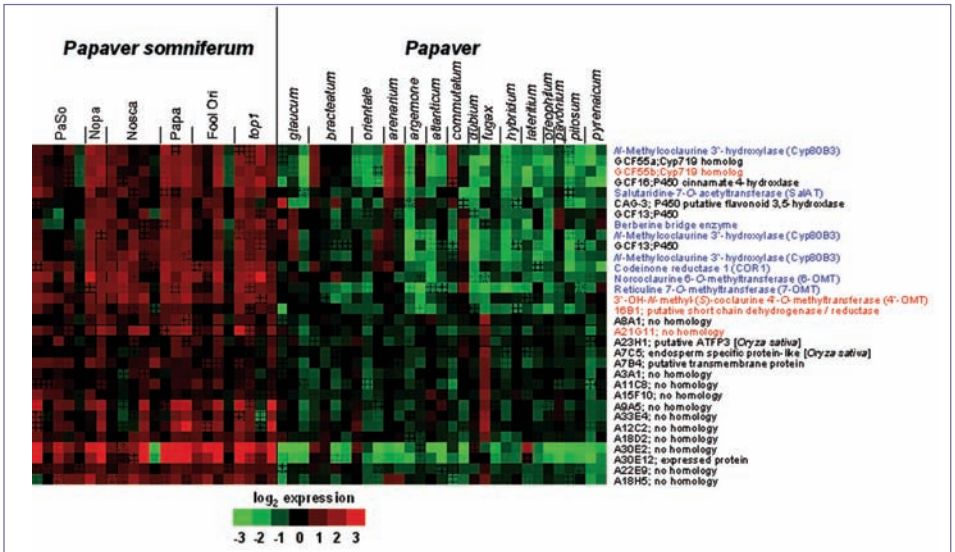


**Figure 1:** Clustered display of gene expression analysis of Papaver species. One cluster showing increased expression in *P. somniferum* varieties is shown. Indicated in blue: cDNAs coding for enzymes in benzylisoquinoline biosynthesis; red: cDNAs currently characterized.

as ABC transporter (1), reductases (2), and P450-monooxygenases (6). With the exception of the house-keeping genes, these cDNAs could putatively be involved in the accumulation of alkaloids in *P. somniferum* and their characterization has been initiated.

Biochemical evidence showed the involvement of ABC transporter in the translocation of intermediates of morphine biosynthesis in *P. somniferum*. The full-length sequence of an ABC transporter was obtained and its characterization is in progress.

Among the six P450 monooxygenases showing higher expression levels in morphinane alkaloid containing plants, three full-length sequences were isolated and successfully expressed in insect cells. One of these was able to convert an intermediate in morphine biosynthesis, but LC-MS/MS analysis showed ambiguous spectra, which could not be assigned to a distinct compound. This was due to the low quantity of products formed in the first experiments, and repetition should result in the structure elucidation of this compound, so that this P450 monooxygenase can finally be characterized with respect to its role in benzylisoquinoline metabolism.

After isolation of their full-length sequence, two cDNAs showing tight clustering to the cDNAs involved in benzylisoquinoline biosynthesis exhibited homology to class 2 *O*-methyltransferases. One cDNA could easily be identified as 3'-hydroxy-*N*-methylcoclaurine 4'-*O* methyltransferase based on its high homology to previously isolated sequences coding for the same enzyme. Biochemical characterization of the bacterial expressed protein, which catalyzes the last step in the basic benzylisoquinoline pathway to (S)-reticuline, showed to possess the highest substrate specificity compared with other *O*-methyltransferases isolated from *P. somniferum*. The other *O*-methyltransferase showed homology to several methyltransferases in benzylisoquinoline biosynthesis, but enzyme assays performed with the overexpressed protein suggested a new function. Homology modeling of the tertiary structure of this methyltransferase, and subsequent docking studies of many different benzylisoquinolines into the active site, suggests a methylating activity of the 3'-hydroxy group of simple benzylisoquinolines. Currently, work is in progress to substantiate these suggestions experimentally.

One reductase showing higher expression levels in morphine containing plants showed homology to the large family of the short chain dehydrogenases/reductases (SDRs), with the highest similarity to the menthone: neomenthol reductase (MNR) involved in menthol biosynthesis. After overexpression, the recombinant protein could be identified as salutaridine reductase (SalR), catalyzing the stereospecific reduction of salutaridine to 7-(*S*)-salutaridinol, an intermediate step in the biosynthesis of morphine. This previously unknown cDNA showed high substrate specificity. Neither other benzylisoquinolines of similar structure, nor substrates of other SDRs involved in plant secondary metabolism, such as tropinone or menthone, are accepted as substrates. Similarly, the overexpression of the MNR from *Mentha piperita* also showed exclusive substrate specificity toward the natural substrate, showing the high selectivity of the SDRs involved in plant secondary metabolism. In order to elucidate the molecular basis for the high substrate specificity of SalR, a model of the tertiary structure was created and the substrate salutaridine was docked into the active site (**Fig. 2**). Careful examination of the model proposed several amino acids to be involved in the selective binding of salutaridine and in the active site of the enzyme. Substitution of the active site residues Asn, Ser, Tyr and Lys resulted in a strong decrease in the catalytic efficiency or the complete loss of enzyme activity, confirming the catalytic mechanism of SDRs. The catalytic efficiency was also decreased by substitutions of amino acids in the proposed substrate-binding site, which was mainly due to the increases in the $K_m$-values. With these results, the SalR model could be substantiated experimentally, and the first clues about the mode of binding of benzylisoquinoline substrates to enzymes were obtained.

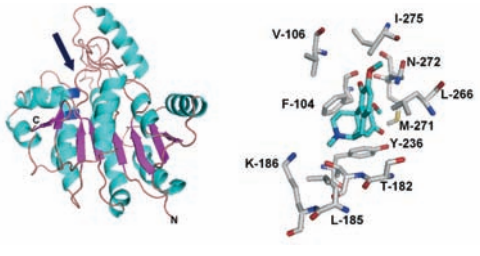


**Figure 2 left:** Model of the tertiary structure of salutaridine reductase. The active site residues are highlighted in blue; the substrate-binding site is indicated by the arrow. **right:** Amino acid residues forming the binding site for salutaridine (in turquoise).

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Sabarna, Khaled: Approach of isolating thebaine synthase from *Papaver somniferum*. University of Halle-Wittenberg, Department of Biochemistry/Biotechnology, 16/11/2006.

# Department of Bioorganic Chemistry

*Head: Professor Ludger Wessjohann*

*Secretary: Elisabeth Kaydamov / Ines Stein*

## Molecules in (inter)action

Research of the department is based on three main topics:

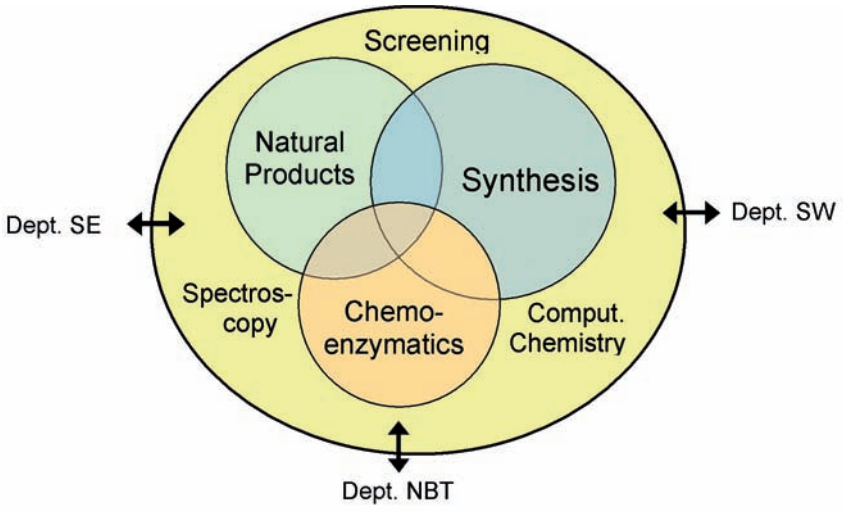
(1.) Learning from nature's chemistry through both elucidation of natural structures as well as understanding basic principals of natures application of chemistry in a biological context.

(2.) Total and diversity oriented synthesis of natural products and derivatives for applications, e.g., in biology or medicine.

(3.) Understanding molecular interactions and develop new tools for recognition compounds and their application.

Many biological questions to be answered are developed in cooperation with the other departments at the IPB. Most efforts directed towards the profiling of secondary metabolites (metabolomics) are done in an interdepartmental collaboration, e.g. together with the *Department of Stress and Developmental Biology*. Common projects also exist with the *Department of Secondary Metabolism* in the areas of glucosidases and glucosyl and methyl transferases and in the application of our microtubuli-active compounds on plant cells.

The isolation, characterization, and modification of secondary metabolites and enzymes from plants and fungi is the basis of our efforts to understand the properties of these compounds or even to disclose their function in nature, and finally to explore their use in chemistry and biology. Applications are driven by the discovered properties and include such diverse areas as nutriceuticals, lead structures in medicinal chemistry or cosmetics, biological research tools, or the utilization of enzymes as biocatalysts in synthesis or as screening targets. This is backed by an extensive synthesis program to increase compound availability and molecular diversity by combinatorial chemistry, method development, and *de novo* synthesis. Advanced projects often proceed in collaboration with industrial partners.



A project in biological chemistry usually will require the competences of several research groups, e.g., research on an active principle of a plant initially requires expertise in natural product isolation, then spectroscopic characterization, in parallel screening is required, at some point synthesis, then synthetic derivatization, again analytics and screening, perhaps metabolic studies and modeling. The demands and expertise needed at any given project time point shift with the progress and discoveries made. At the *Department of Bioorganic Chemistry* we believe that projects as well as the individual groups strongly profit from the excellent horizontal as well as vertical integration that is reflected in the organisational presentation (see figure), in which the three major "wet" chemistry based competence areas (natural products, synthesis and chemoenzymatics) are complemented by the three predominantly analytical ones (spectroscopy, computational chemistry, and screening). The latter also comprise a major service unit for our department and the institute in general.

The department maintained a high publication frequence with some 30 research papers per year, including top chemistry journals such as *Angewandte Chemie*, *Journal of the American Chemical Society*, *Organic Letters*, or *Chemical Communications*. It offers research opportunities in a highly international and competitive environment to students wishing to attend advanced practical courses, visiting scientists and postdoctoral fellows, but primarily to students who wish to pursue a master or Ph.D. in all areas connected to bioorganic chemistry such as synthetic, analytical and physical chemistry, pharmacy, biochemistry, food/ nutritional chemistry, as well as for candidates of related areas like informatics inclusive bio- and cheminformatics, mathematics, or biology.

# Natural Products

*Heads: Norbert Arnold & Jürgen Schmidt*

**The group is engaged in the production, isolation, and structural elucidation of plant and fungal metabolites. The evolutionary selection undergone by these compounds provides a bias for bioactivity and thus can be used for the discovery of novel lead structures for drug candidates or other applications. The investigation of the native function of natural products in natural systems can aid the identification of new targets, the understanding of ecological interactions, or the discovery of new biocatalysts or enzymatic transformations.**

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## Constituents of Fungi

Mushrooms are among the largest groups of higher organisms on our globe with an estimated $10^6$ species. At present only some 7% (75.000) of the estimated total are described. During their evolution fungi developed a large variety of strategies to utilize different substrates for their nutrition. They frequently live in close symbiosis with plants (mycorrhiza). Some genera use dead organisms or even other toadstools as a source for their nutrition. The species diversity of the latter, so-called mycophilic fungi is tremendously rich, also in their chemical arsenal.

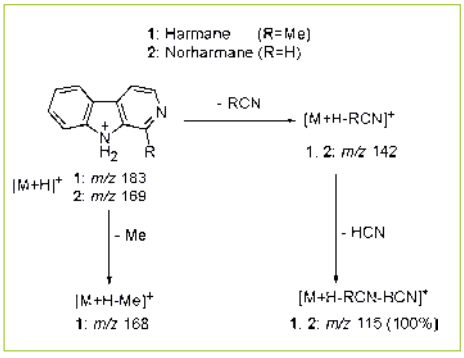
### *Constituents of Mycophylic Fungi*

Species of the genus *Sepedonium* are parasites on fruit bodies of genera in Boletales. They represent the asexual synanamorph of ascomycetes of the teleomorph genus *Hypomyces*, whose perithezia can only be observed rarely. Species of the perthophytic genus *Sepedonium* are first settling on the living host, and in a later stage they kill the host and use the organic material of the host for their own nutrition. Remarkably, Boletales fruit bodies, which are attacked by *Sepedonium* in their enviroment are rarely attacked by other parasitic fungi, whose spores are pervasive. This let us assume that secondary metabolites produced by *Sepedonium* are responsible for such phenomena. The production of fungitoxic substances can be interpreted as preformed defense in the "fight" over the nutritional substrate. To clearify this hypothesis, crude extracts of different *Sepedonium* species were tested for their fungicidal and bactericidal properties, e.g., *S. ampullosporum* Damon proved to possess high antifungal activity. The future aim is the isolation and characterization of the responsible substances.

*Sepedonium spec.* growing on fruit bodies of Bolets

### *Constituents of Higher Fungi*

Fruit bodies of the mycorrizal genus *Hygrophorus* are rarely attacked by parasitic fungi or insects. In continuation of our research on their secondary metabolites, the alkaloids harmane




(**1**) and norharmane (**2**) could be isolated from *Hygrophorus eburneus* (Bull.) Fr. So far, 35 selected *Hygrophorus* species were investigated for these alkaloids by LC/ESI-SRM (selected reaction monitoring), using the reaction of the $[M+H]^+$ ion to the base peak ion at *m/z* 115. Both alkaloids are widely distributed in the genus *Hygrophorus*, independent of the geographic origin, and the occurrence also appears to be quite constant within a species. Therefore, the compounds might be useful chemotaxonomic markers for the genus *Hygrophorus*.

## Constituents of Plants

### *HEANTOS - a traditionalistic*

***vietnamese opiate detoxification remedy***

The project HEANTOS was originally coordinated by the United Nations (UNESCO and UNOPS). Dr. Dan (Peoples Republic of Vietnam) developed the original herbal remedy to aid opioid detoxification and called it HEANTOS ("heat of sun"). The formulation was subsequently improved with the Vietnamese Academy of Science and Technology (Institute of Chemistry, Prof. Sung). HEANTOS consists of at least 13 components and was constantly improved in composition, also with the aid of our findings. In cooperation with Vietnamese partners our research is focused on the isolation and characterization of the constituents of these plants. So far, more than 200 compounds have been isolated from twelve plants. A substance based overview and extensive literature survey was conducted. Simultaneously the very complex coextracted Heantos mixture (capsules) as produced for applications was examined by means of coupled HPLC and mass spectrometric methods to determine the lead substances for the standardization of the medicine. Through this the first standardization protocol could be worked out and was utilized to allow a clinical study to be performed in Germany. It was also the first one to be applied to such a complex traditional Asian medicine.

***Traditional medicinal plants from Myanmar***

The aim of the project was the isolation, structural characterization and study of selected pharmacological activities of phytoconstituents from the Myanmar (Burmese) medicinal plants *Streptocaulon tomentosum* Ellis & Arthur (Asclepiadaceae), *Curcuma comosa* Roxb. (Zingiberaceae) and *Vitis repens* Wight & Arm (Vitaceae). About 20 constituents belonging to triterpenoids, cardenolides, lignanes, and steroidal saponines including three new substances were isolated from the roots of *S. tomentosum*. About 27 constituents belonging to curcuminoids and sesquiterpenoids including nine new sesquiterpenes could be characterized from the rhizome of *C. comosa*. Polyphenols, fatty acids, lignanes were obtained from the rhizome of *V. repens*. Extracts of each plant were tested for their antifungal properties against *Cladosporium cucumerinum*. Six cardenolides isolated from *S. tomentosum* were tested for their antiproliferative activity *in vitro* against a human breast cancer cell line and a mouse fibroblast cell line. Six cardenolides exhibit noteworthy antiproliferative activity ($IC_{50}$ values < 1mM), and four cardenolides show the induction of apoptosis in a human leukemic cell line. The project is finished.

***Medicinal plants from Socotra Island (Republic of Yemen)***

Socotra Island is the largest and most easterly island of a small archipelago in the Indian Ocean. Access is restricted to few researchers. The island is rich in unique and distinguished species of plants: nearly half of them are considered to be endemic. So far, extracts from Socotraous medicinal plants belonging to twelve plant families showing, e.g. antifungal and antioxidant activities, are under investigation.

***Floral Oils***

Flowers produce a variety of constituents, often specific to this part of the plant, e. g., volatiles, floral oils, resins, or waxes. Floral oils are secreted by specialized glands, so-called elaiophores. The lipid profiles of floral oils and resins of various species and in other cases of flowers in various stages of development are conducted by GC/MS and ESI-FT-ICR-MS.

Thus, several *Diascia* species (Scrophulariaceae) occurring in Southern Africa are pollinated by oil-collecting bees of the genus *Rediviva* (Melittidae). β-Hydroxy fatty acids, mono-, di- and triacylglycerols could be identified as main constituents of their floral oils by GC-MS. While the monoacylglycerols possess a long-chain β-acetoxy fatty acid ($C_{14}$, $C_{16}$ and $C_{18}$), the di- and triacylglycerols additionally contain one or two short-chain β-acetoxy fatty acids, respectively.

Similarily, the "chemical" relationship of the European *Lysimachia punctata* flowers with *Macropis fulvipes* bees was studied. Here the col-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# Chemoenzymatics

*Heads: Ludger Wessjohann & Wolfgang Brandt*

**Enzymes are the molecular machines of organisms catalyzing chemical conversions. As such they can be utilized in two different ways: either as targets to modify biological processes within its producing organism, or as biocatalyst to perform its task either in a different organism or *ex vivo* in synthetic chemistry. Of special interest to us is the latter point, complemented by the desire to understand the mechanisms of biological catalysis.**

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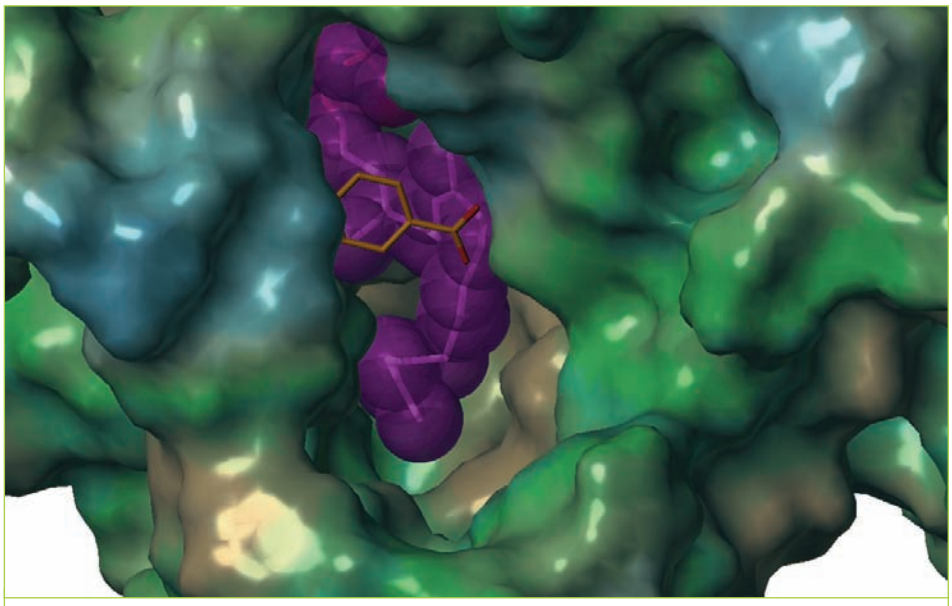
## Isoprenoid Converting Enzymes

The synthesis of the complex natural isoprenoids is based on prenyl group transfering enzymes, which include e.g. oligoprenyl synthases, terpene cyclases or aromatic prenyltransferases.

The ubiA-Enzyme of *E. coli* belongs to the latter group. The enzyme and its relatives are crucial for the production of the essential electron carrier ubiquinone in pro- and eucaryotic organisms. In order to study its active site, an intense bio- and cheminformatic analysis was performed and combined with site-directed mutagenesis studies. Based on previous modeling analyses, amino acids identified as important for the catalytic mechanism were selectively replaced to obtain five new mutants. Their involvement in the catalytic mechanism was strongly supported but at the same time a remodeling of the previously proposed enzyme structure appeared necessary to combine two active sites, which had to be placed into close proximity in the new model. Based on these experimental results and structural classification of prenyl enzymes, a new highly relevant 3D-model could be developed. This model is able to explain a wide range of substrate specificities and is in complete agreement with the results of site-directed mutagenesis.

At the same time, ubi A could be used for synthetic purposes with artificial substrates. It catalyzed a crucial step in a total synthesis of kuhistanols, the natural meroterpenoids.

Using comparative homology modeling techniques reasonable 3D-structure models of two terpene synthases from *Cannabis sativa* could be developed. To prove the correctness of the models and to clarify the catalytic mechanism of these two enzymes both site-directed mutagenesis and quantum mechanical calculations were performed. Combined *ab initio* (catalytic active site) and force field (the whole enzyme except the active site) calculations, the so-called QM/MM method, is applied to study the catalysis mechanism in realistic surroundings.



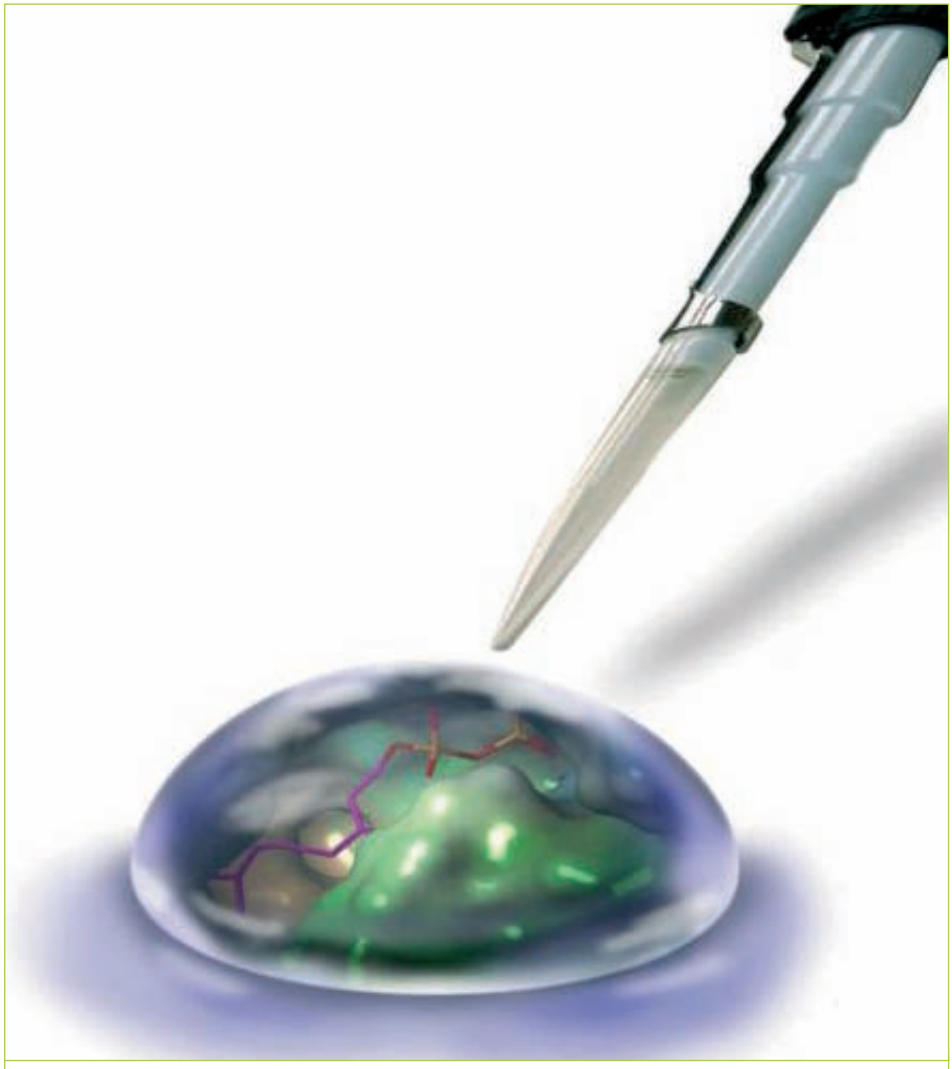
Space filling model of the active site of ubiA-prenyltransferase with the prenyldiphosphate buried (in magenta) and the aromatic benzoate on top (in orange).

In practice, the applicability of these enzymes for chemoenzymatic syntheses was explored with the aim to alter product specificity towards non-native products through rational protein design.

### Other enzymes

The selective synthesis of plant benzopyranes (flavonoids, isoflavonoids etc.) could be improved considerably by utilizing plant *O*-methyl transferases.

The enantioselective *O*-acylation and deacylation of acyloins with lipases or esterases so far was only possible by kinetic resolution and thus stopped below the theoretical value of 50% yield. In order to generate a dynamic kinetic resolution scheme, base- or acid-catalyzed racemization in principal can be used. However, both substrate and product are prone to this, and additionally can undergo acyloin shift and aldol reaction under such conditions. A cooperative study elucidated the best conditions for a differential racemization of either species, which proved to run best under acid catalysis in a compartimentalized reactor, separating racemization and resolution spatially.



Rational protein design for biocatalysis: geranylpyrophosphate in the pocket of a terpenoid cyclase.

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# Synthesis

*Head: Ludger Wessjohann & Bernhard Westermann*

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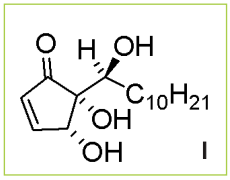
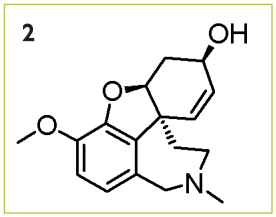
**Chemical genomics aims to use small molecules to probe phenotypic and genetic variations. Specifically, experimental design in chemical genomic screening takes into account genetic and genomic differences in identifying cellular reactions to small molecules. Furthermore, this approach provides a temporary control over cellular states not achieved normally by molecular biology methods. Very recently we have started and expanded successfully programs to evaluate target- and diversity-driven synthetic approaches to provide small molecules. This focus furnishes vehicles to study biological interactions, which we envision as one of the main goals of our scientific work.**

In the context of target-oriented total synthesis we could realize the synthesis of Hygrophoron (**1**), Galanthamin (**2**) and Tubulysin (**3**).

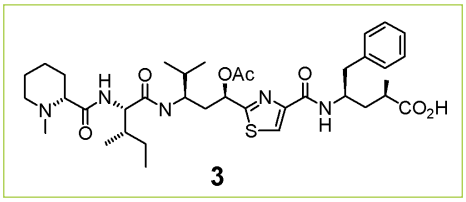


**1**

Hygrophorones have been isolated in our department and have been shown to exhibit a broad spectrum of very interesting biological and pharmaceutical profiles, e. g. activity against Vancomycin-resistant strains. The synthesis providing the enantiomerically pure natural product is started with a Sharpless dihydroxylation reaction, which is providing the chiral, non-racemic intermediates. Further reactions are highly diastereoselective and afford the hygrophorones in eight steps. Variations in order to commit these products in chemical genomics studies can be carried out easily by using different starting materials employing the same strategy.



Galanthamin, isolated some 50 years ago from Caucasian snow drops, is used for the treatment of Alzheimer disease. In addition, dimeric derivatives of this natural product are intensively discussed to exhibit inhibitory activities within the context of fibrill extension of β-amyloid plaques. The total synthesis has been achieved as well as the synthesis of dimeric structures employing the "click" approach.

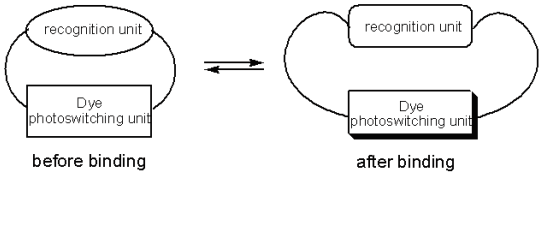


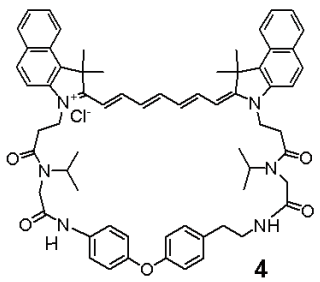
**3**

Tubulysin, which at this moment is the most active natural product for Tubulin stabilization, has been the synthetic goal of a couple of groups worldwide. We have been able to achive the total synthesis first and reported it in September 2006. Up to the end of 2006, two additional total syntheses have been reported. The key reaction of the synthetic route at our department is a multicomponent reaction, which is most atom efficient and provides efficiently the central heterocycle in one step.

The aspects of atom- and eco-efficiency are one of the key factors in our synthetic plans and have been considered largely for the synthesis of **1** and **2**, too. This topic is also one of the main aspects in our diversity driven multicomponent reaction approach towards macrocycles.

For the evaluation of molecular interactions, macrocycles are well suited due to conforma-



**4**

tional preorganization and the incorporation of binding units as well as monitoring units. In our approach towards macrocycles of type **4**, we have been able to tune these products by integrating a sensing unit, which is monitoring binding events through changes in the absorption bands. This facilitates the determination of productive binding through molecular interactions. Parallel to these attempts, optical switches have been incorporated in order to alter the conformation of the macrocycle and the interacting unit itself. This may regulate the binding from an on to off status.

The inherent complexity of natural products could be generated by expanding the MiB (multiple multicomponent macrocyclizations including bifunctional building blocks) approach, which has been established by us recently. By variation of the starting compounds products exhibiting a high degree of functional, topological and skeletal diversity could be provided. The peptoidic framework, which is due to the Ugi multicomponent reaction, offers the advantage of high metabolic lability compared to their natural peptidic counterparts. The high degree of diversity can be generated by a single MiB reaction (**5**), consecutive MiB reaction led to even higher diversified products (**6, 7**).

Concomitantly, the synthetic progress to these highly complex natural and natural-like products, respectively, is enabled by the development of new synthetic methodologies. In this context, the main focus has been directed towards organocatalysis. Organocatalysis has gained its impact due to the non-utilization of hazardous metals and resource-saving procedures. In our group the main emphasis has been drawn towards Mannich reactions and hydroxylations. The arylation of aromatic aldehydes, which inevitable needs Zinc as a metal, could be extended into a catalytic version reducing the amount of metals to only a minimal amount. In addition, the chiral, non-racemic ligands for the asymmetric version has been developed further, they now rely mostly on renewable resources such as amino acids. Moreover, the applicability is strengthened by the short synthesis to obtain these ligands.

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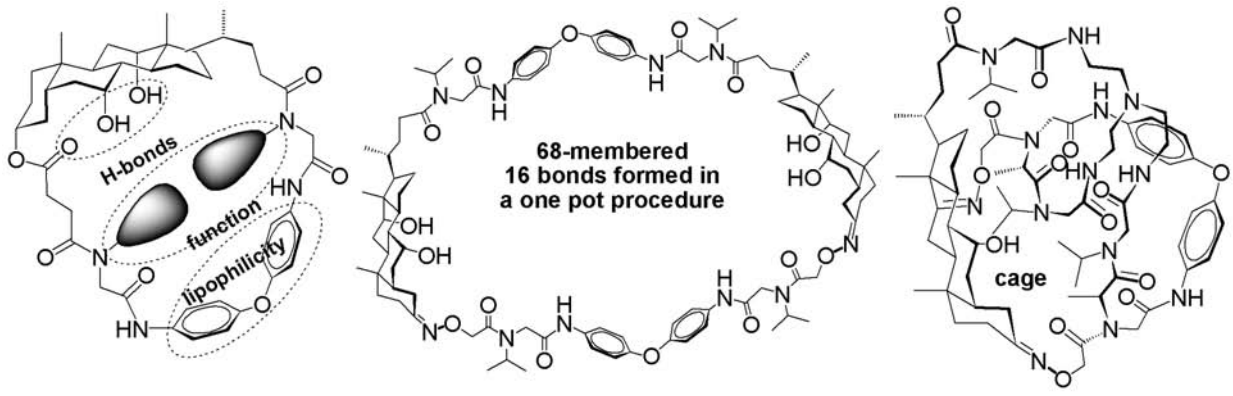


functional diversity **5** | skeletal diversity **6** | topological diversity **7**

# Spectroscopy

*Heads: Andrea Porzel & Jürgen Schmidt*

**The spectroscopy group is engaged in the identification and structural elucidation of small molecules with modern analytical techniques, including questions in the area of metabolomics and physicochemical screening. Modern mass spectrometric methods, NMR-spectroscopic experiments, and methods of the optical spectroscopy (IR, UV, CD) are used and developed for solving structural problems and for the study of noncovalent interactions of molecules. Other departments of the IPB and external research groups are supported in solving structural and analytical problems.**

## Group Members

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## Mass Spectrometry

The mass spectrometry group has carried out a series of mass spectrometric investigations, both with respect to structural investigations of plant and fungal metabolites and the mass spectral service for all groups of the IPB and external cooperation. The electrospray Fourier-transform-ion cyclotron resonance (ESI-FT-ICR) mass spectrometer was successfully applied to a series of analytical problems including profiling experiments of alkaloids, floral oils, and triacylglycerols. Furthermore, a series of dendrimers and macrocycles as well as other synthetic compounds were investigated.

Phenolic compounds from lichens belonging to the family *Physciaceae* were investigated in collaboration with Siegfried Huneck. In that case the depsides atranorin and chloroatranorin could be identified as main components.

In collaboration with the *Department of Natural Product Biotechnology* (Susanne Frick, Robert Kramell, Jörg Ziegler, Toni Kutchan) and the Biocenter Halle a series of LC-ESI-MS/MS investigations of *Papaver* alkaloids and benzylisoquinoline alkaloids were performed. Thus, the alkaloid pattern after feeding with [ring-$^{13}C_6$]-tyramine as a biogenetic precursor of these alkaloids to *Papaver somniferum* seedlings was elucidated both by direct infusion high-resolution ESI-FT-ICR mass spectrometry and liquid chromatography/electrospray tandem mass spectrometry. Based on this procedure, the structure of about 20 alkaloids displaying an incorporation of the labeled tyramine could be elucidated. These alkaloids belong to different classes (morphinane, benzylisoquinoline, protoberberine, benzo[c]phenanthridine, phthalideisoquinoline and protopine type). The valuable information gained from the alkaloid profile demonstrates that the combination of these two spectrometric methods represents a powerful tool for evaluating biochemical pathways and facilitates the study of the flux of distant precursors into these natural products.

## NMR and Optical Spectroscopy

Modern NMR spectroscopy experiments were used for the structural elucidation of bioactive natural products isolated from plants and higher fungi and for synthetic compounds. Routine spectra ($^{1}H/^{13}C/^{19}F/^{31}P$ NMR, IR, CD, ORD, UV) were recorded as a service for the *Department of Bioorganic Chemistry* as well as other research groups of the IPB. The NMR laboratory took successfully part in a Round Robin test on the quantitative analysis of an active pharmaceutical ingredient (rutin) by $^{1}H$ NMR spectroscopy.

In collaboration with the Institute of Organic Chemistry, University of Darmstadt, we started testing of residual dipolar couplings (RDCs) as a tool for determination of the relative stereochemistry of small organic molecules. For these purpose, $^{1}J(^{1}H,^{13}C)$ coupling constants of a hygrophorone derivative were investigated in the chiral liquid crystal system poly-γ-benzyl-L-glutamate/$CDCl_3$.

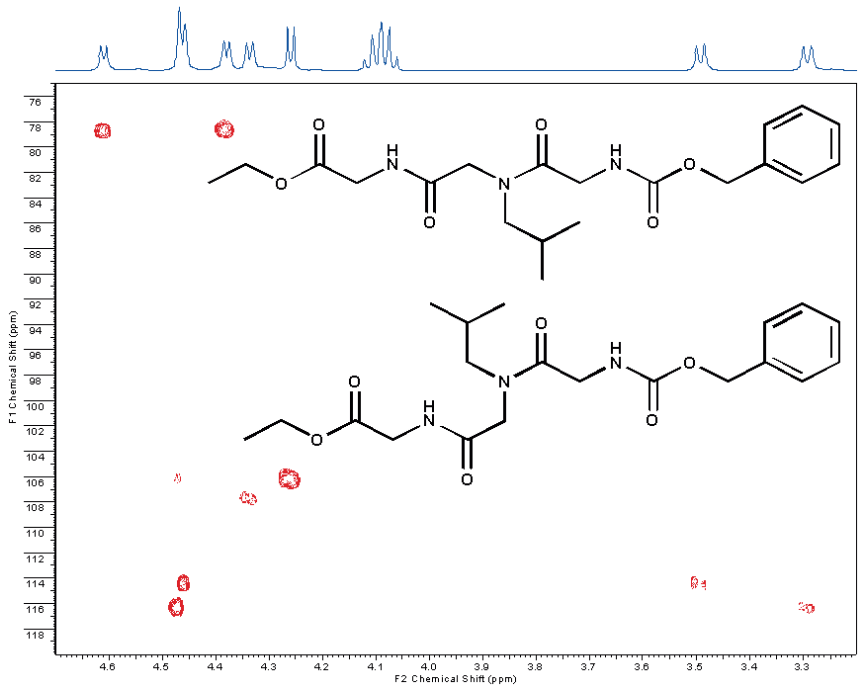
$^{1}H,^{15}N$ heteronuclear 2D NMR correlation experiments were established in order to characterize peptoids with repetitive structural elements. The method was used to determine the number of N-CO *cis*/*trans* isomers.

Gentianae radix (dried rhizome and roots of *Gentiana lutea* L.) extracts were investigated as example for the usability of evaporative light scattering (ELS) and online-$^{1}H$-NMR as HPLC detection methods for investigations of crude plant extracts. ELSD was proven to be a valuable tool for detection of UV active as well inactive constituents in a virtually quantitative manner. Online $^{1}H$-NMR detection suffered

from the low sensitivity (regarding the instrumentation of the department), however, direct investigation of the crude extracts in protonated solvents with sophisticated (WET) solvent suppression technique yielded in promising results.

Using one- and two-dimensional NMR experiments the structures of a series of carboline alkaloids isolated from the fungus *Cortiarius brunneus* were elucidated. From the same source, *N*-glucosidic indole alkaloids were isolated. Also for these compounds, available in sub-milligram amounts only, structural elucidation by means of NMR spectroscopy was successful.

The attenuated total reflection (ATR) FT-IR




Expansion of the $^{1}H$-$^{15}N$ HMBC NMR spectrums of *cis* and *trans* peptoid isomers

## Collaborators

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# Screening

*Heads: Norbert Arnold & Bernhard Westermann*

**The new competence group was established in January 2006, opening the door for the investigation of properties of small molecules by biological, chemical, and virtual screening methods. Thereby it is not only limited to the Department of Bioorganic Chemistry, but is open to all other departments of the IPB and cooperation partners wishing to engage in chemical biology driven by the impact of small molecule on.**

## Group Members

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**Fredy Leon-Reyes**
*PhD Student since May 2005*

## Biological screening

Secondary metabolites isolated from fungal fruit bodies or plants as well as synthetic compounds are tested in simple cell or organism-based assays for their biocidal activity profile, e.g. for fungicidal, bactericidal, herbicidal, or algaecidal properties. These bioassays are complemented by cytotoxicity and more detailed enzymatic and receptor assays in cases of verified positive results or of special interest. Thus in depth tests of steroid-like activities have been established during the first year of this group.

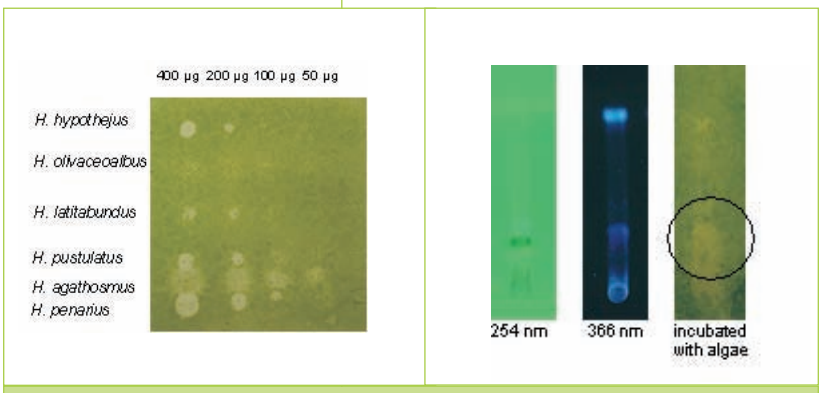
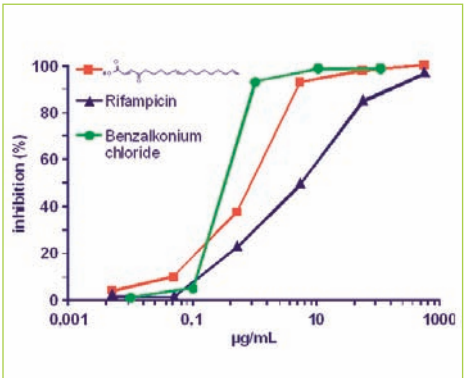



**Figure left:** Algaecidal assay (*Spirulina laxissima*) of crude fungal extracts. **right:** Assay on developed TCL plates.




Results of an antibacterial assay of a fungal fatty acid on the Gram-negative bacterium *Vibrio fischeri.*

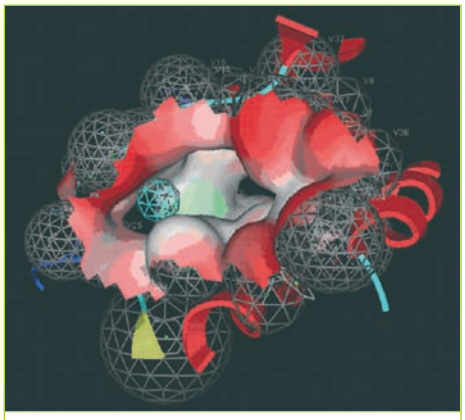
## Chemical Screening

The chemical screening projects are embedded in the research topics related to molecular interactions. Chemical reactions or interactions can be screened by physicochemical methods such as MS, NMR, or optical spectroscopy. E.g., macrocycles, cryptands, and cages were made accessible by the MiB-strategy in unprecedented diversity (see Research Group *Synthesis*). The high degree of complexity and the prospect of suitable binding properties should render them amenable for the selective binding of metal ions. This fact has been proven by the template synthesis of macrocycles (**Scheme 1**). Addition of various earth alkali ions clearly changed the product spectrum as shown by mass spectrometry. In continuation of these studies we will determine whether cationic and anionic guests can be selectively bound and how macrocycles selective for certain species are best generated and selected. Structure-activity driven screening will help to identify those functional groups and conformational preferences most suited for selective host-guest interactions.

## Virtual Screening

Many compounds are too scarce or valuable to be tested randomly on various targets. In other cases, e.g. if a compound acting on a defined target is to be sought, random screening will require high throughput set-ups not available at the IPB or too costly.

In cases of a (bio-)chemically defined target, a solution can provided by the fast pre-selection of candidates out of databases with millions of compounds by pharmacophore search techniques. Subsequently, docking of identified potential ligands to the specific hosts (receptors, enzymes etc.) leads to the prediction of compounds worthwhile for experimental testing or/and synthesis, offering a higher probability of success compared to a random selection. Also other parameters such as calculated cell permeability can be used for the preselection of useful candidates.



Pharmacophor model of a receptor with a ligand fragment (turquoise polyeder).

Especially for effective screening for host-guest interactions of conformationally flexible molecules, new software tools have been developed with the Research Group *Computational Chemistry* to automate the entire process of pharmacophore screening, docking, energy optimization of the host-guest complexes and identification of most favoured guests for the specific host.

## Collaborators

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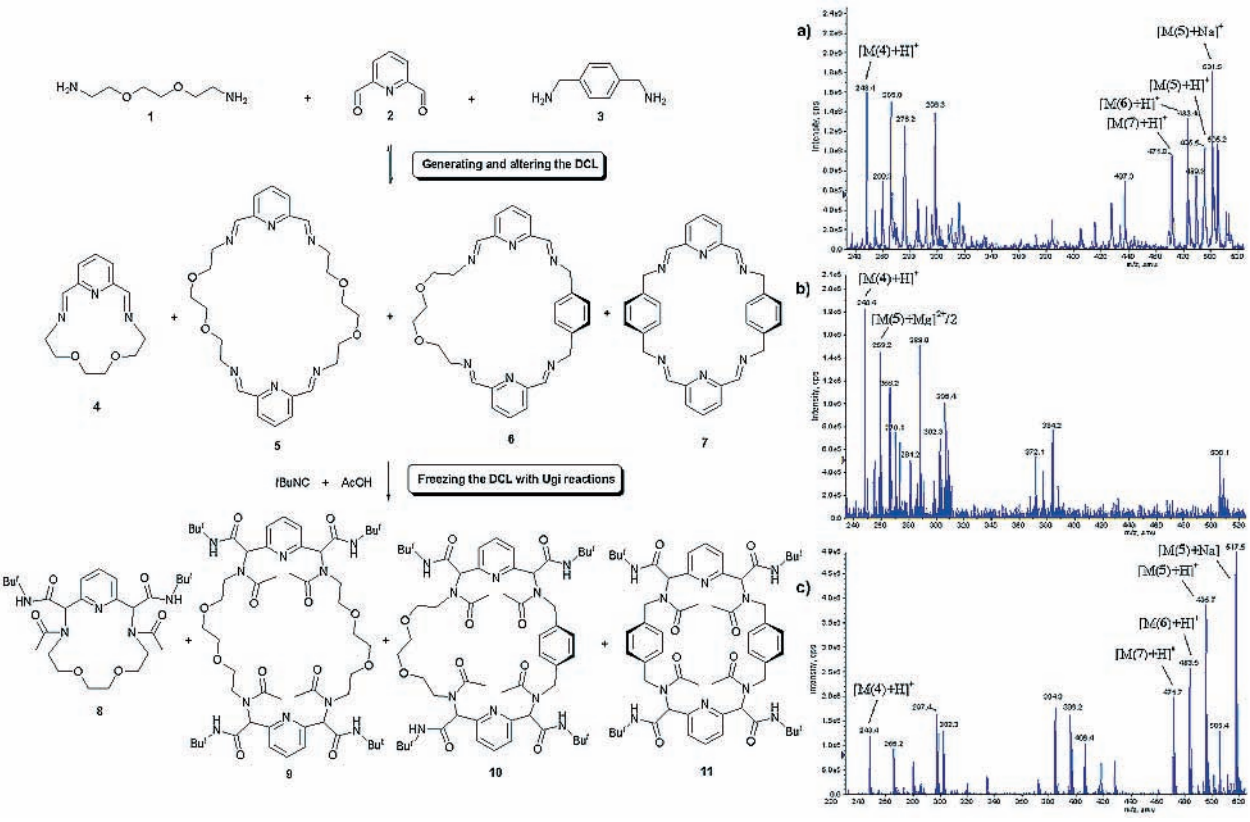


**Scheme 1:** ***Left:*** generation of the dynamic and frozen imine macrocycle library with selected structures shown.
***Right:*** MS-spectra of the libraries under various challanges: a) without any template, b) presence of $Mg^{2+}$, shift to smaller rings like 4, c) presence of $Ba^{2+}$, shift to larger rings like 5-7.

# Computational Chemistry

*Heads: Wolfgang Brandt & Andrea Porzel*

**The computational chemistry activities comprise cheminformatics, molecular modeling, and to some extent theoretical chemistry and quantum mechanical calculations. With these methods, the research group investigates molecular structures (natural products; 3D protein structures), their interactions and reaction mechanisms (e.g. of enzyme catalysis), and physicochemical properties. New bio- and cheminformatics tools for the evaluation of spectroscopic, structural and biological data are developed in cooperation with other departments and external partners.**

## Group Members

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## Protein Homology Modeling

Thioredoxin reductase is a selenocysteine containing homodimeric NADPH-dependent flavoenzyme and a key player of the cellular redox milieu. Based on our recently developed model we suggested that a highly conserved glutamate$^{477}$ in the neighborhood of His$^{472}$ could essentially participate in the catalytic mechanism of this enzyme, acting as proton sink in a proton charge relay mechanism (**Fig. 1**). It thus can favor the formation of the selenolate required for substrate reduction. Based on our theoretical calculations we postulated a new, swapping catalytic triad between Glu$^{477}$, His$^{472}$, and Cys$^{59}$ followed by Glu$^{477}$, His$^{472}$, and Sec$^{498}$ which reacts with Cys$^{32'}$ (or Cys$^{35'}$) of the substrate thioredoxin. Site-directed mutagenesis of Glu$^{477}$ to glutamine, alanine, or lysine led to a significant drop in enzymatic activity as predicted. This provided not only a quantitative limit for the energetic contribution of glutamate$^{477}$ in the catalytic mechanism of thioredoxin reductase and related enzymes but furthermore validates *in silico* DFT-calculations as a useful tool for quantitative considerations in enzymology. The results aided the design of experimental studies of the pH- and neighboring group dependency of selenocysteine redox properties studied by electrochemical analysis of peptide fragments of thioredoxin reductases of various organisms.

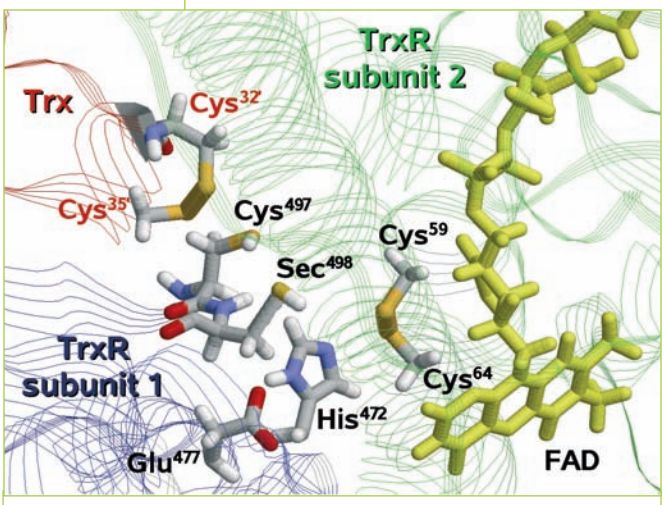


**Fig. 1:** Active site of human thioredoxin reductase showing the suggest and now proven catalytic triad Sec$^{498}$ – His$^{472}$ and Glu$^{477}$.

As part of our isoprenoid research, in cooperation with the *Department of Natural Product Biotechnology*, two homology models of terpene cyclases were created and extensively investigated by site-directed mutagenesis (for more see report of the Research Group *Chemoenzymatics*).

Recently, the NADPH-dependent short chain dehydrogenase/reductase "salutaridine reductase" involved in morphine biosynthesis was cloned from *Papaver somniferum* by Ziegler et al. (IPB). A model of the *Papaver bracteatum* homologe was created based on the x-ray structure of human carbonyl reductase 1. The homology model shows the typical $\alpha/\beta$ folding pattern of such enzymes. Docking of salutaridine into the active site revealed the first data on the molecular interaction of benzylisoquinoline alkaloid with enzymes. Site-directed mutagenesis clarified the involvement of residues relevant in substrate binding and for the proton transfer system.

In close cooperation with the *Department of Secondary Metabolism*, structures of the serine carboxypeptidase-like (SCPL) enzymes 1-*O*-sinapoyl-β-glucose:L-malate sinapoyltransferase (SMT) and 1-*O*-sinapoyl-β-glucose:choline sinapoyltransferase (SCT) were modeled to gain insight into determinants of specificity and substrate recognition. The structures reveal the $\alpha/\beta$-hydrolase fold as scaffold for the catalytic triad Ser-His-Asp. The binding sites of the substrates could be characterized and explain the substrate specificity and aspects of the catalytic mechanism. Several mutations in the active site of these enzymes confirmed the principal cor-

rectness of the models.
In cooperation with Birgit Dräger (University of Halle) a protein model of putrescine *N*-methyltransferase was developed. In comparison to related enzymes, the substrate putrescine and the cosubstrates *S*-adenosylmethionine and decarboxylated *S*-adenosylmethionine appear to bind differently. The modeling studies helped to identify amino acid residues in the active site, which will be subject to mutation to evaluate both the model and possibility to alter the substrate specificity of the enzyme.

In cooperation with Volker Lipka (University of Tübingen) a model of an Arabidopsis PEN2 glycosyl hydrolase has been developed.

Furthermore, in cooperation with Ernst Reiss (Aschersleben) eight different barley PR-5 proteins (TLPs 1 to 8, TLP for thaumatin-like protein) were modeled. The models highlighted the effects of sequence differences between the TLP isoforms in terms of their secondary structures and their molecular electrostatic potentials. We did propose that these sequence differences have implications for the target preferences of the different isomers.

## Protein-Ligand Interaction, Small Molecule Modeling and QSAR*

As a contribution to the investigation of the evolution of isoprenoids, the binding sites of diphosphates in 771 proteins with known 3D-structure have been analyzed, characterized, and correlated to data of prenyltransferases. This analysis led not only to classifications into metal cation mediated and non-cation mediated recognition sites of diphosphates but also to characteristic binding motives with different types of amino acid residues.

Another important group of plant isoprenoids are steroids and steroid mimics, e.g. isoflavonoids. The QSAR of their binding to common steroid receptors and steroid converting enzymes was studied by theoretical methods.

Artificial ligands and hosts are created by synthesis, e.g. macrocyclic compounds by very efficient Ugi four-components reactions. The latter, despite of the formation of a cyclic structure, exhibit rather high conformational flexibility. An automatic procedure has been developed to identify the most likely stable conformers. A subsequent *in silico* screening including large compounds libraries can be used to find matching interactions. *In silico* screening is performed by calculation of so-called pharmacophore queries (**Fig. 2**), e.g. hydrophobic regions, hydrogen bonding donor or acceptor positions, and searching fast in databases to find good matching guests for the host enzyme or macrocycle, or *vice versa*.

## Cheminformatics and Software Developments

### New concepts

Current methods of structural comparison allow substructure searches, but they are not very effective for similarity searches. Especially in huge databases with billons of chemical structures severe limitations appear, and new search concepts and algorithms have to be developed. In cooperation with external partners, a first set of new methods and the corresponding software applications were developed and offer the chance for much faster and more effective tools. Within IPB, these new tools will be applied for the analysis of natural products or bioactive compounds (vs. other compounds vs. medicinal compounds), in order to expand the currently used cheminformatic analysis tools.

### 2D NMR data base system

In continuation of our work on an 2D NMR spectra data base system, we could show (diploma thesis of Karina Wolfram under supervision of Alexander Hinneburg, University of Halle) that probabilistic semantic indexing (PLSI), which is designed for text data created by humans, can effectively handle mapped NMR data originating from HSQC 2D NMR spectra. Additionally, PLSI combined with the new mappings is able to find

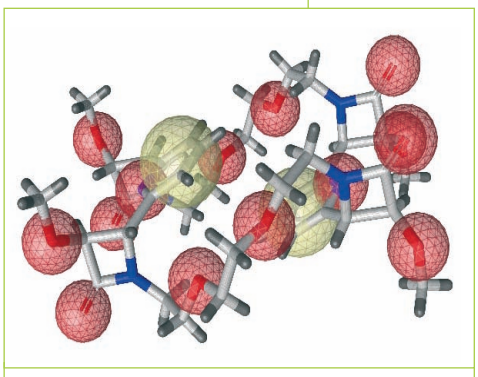

**Fig. 2:** Example structure of a macrocycle with pharmacophore queries (red hydrogen bonding acceptor and yellow hydrophobic sites).

*QSAR = Quantitative Structure Activity Relationship

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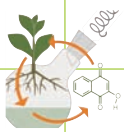

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## Department of Stress and Developmental Biology

*Head: Professor Dierk Scheel*

*Secretary: Susanne Berlin*

Plant development, although genetically determined, is largely modulated by biotic and abiotic environmental factors. In this way, developmental programs are adapted to specific local conditions and protective as well as defense reactions are initiated during stress situations – an advantageous situation for sedentary living plants.

The basis for those processes is the ability of plants to perceive environmental factors and initiate signal transduction networks that modify gene expression patterns. In addition, post-transcriptional reactions play an important role. The investigation of the molecular mechanisms underlying this course of events is the main topic of the *Department of Stress and Developmental Biology*.

Plant pathogens play a major role in biotic stress. Research in this area is focusing on interactions of pathogens with plants that are not their hosts. In these cases plants display a durable resistance (basal or non-host resistance) based on an efficient multi-component defense response. Host plants react with similar if not identical defense responses against infection with avirulent pathogen races expressing avirulence genes complementary to the host's resistance gene. In the absence of such complementary pairs of genes, pathogens suppress the basal defense response and cause disease. The work of several research groups of the department focuses on the analysis of recognition, signal transduction and gene activation processes in plant-pathogen interactions.

The work on abiotic environmental factors centers around studies of the effects of metals on plant development. Using hyper-accumulating model organisms, the Research Group *Metal Homeostasis* analyzed structure and function of genes responsible for the tolerance of plants towards exposure to normally toxic metal concentrations. This work will be continued at the University of Bayreuth, where the head of this research group, Dr. Stephan Clemens, accepted a Professorship for Plant Physiology.

Plant responses to biotic and abiotic environmental factors finally result in altered patterns of proteins and metabolites. In order to be able to directly monitor such alterations, mass spectrometry-based methods have been established allowing the comprehensive profiling of proteins and secondary metabolites. Both methods are also being employed for biochemical phenotyping of mutants. Comprehensive metabolite profiling required the establishment of metabolomics and bioinformatics platforms including the development of appropriate databases and tools for analysis and annotation of primarily mass spectrometry data.

# Molecular Communication in Plant-Pathogen Interactions

*Head: Wolfgang Knogge*

**_Rhynchosporium secalis_, the causal agent of a leaf spot disease on several grasses, is of particular economic importance as a pathogen of barley. The fungus belongs to the group of phytopathogenic microorganisms colonizing the intercellular spaces of their host plants. This region is relatively poor in nutrients and in order to optimize their life style pathogens developed strategies to improve the nutrient supply by the host cells. To prevent just this, plants on their part evolved mechanisms enabling the efficient recognition of invaders as the prerequisite for their rejection. At the molecular level, the interaction between a pathogen and a plant requires therefore exchange and perception of signals. On the pathogen side, receptors may be involved that sense stimuli of plant origin and, hence, allow the adaptation of the pathogen physiology to the specific environment of the host. In the plant, the communication processes are mediated by membrane-localized or intracellular receptors, which are the targets of pathogen-secreted molecules, frequently of proteinaceous nature. The final outcome of an interaction is therefore determined by protein-protein interactions, for which two scenarios can be envisaged. The plant defense is suppressed and the plant metabolism is redirected in favor of the pathogen with disease ensuing. Alternatively, upon recognition of the pathogen through secreted compounds the plant defense is induced leading to the expression of plant resistance.**

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Our research ultimately aims at identifying the processes on the plant side that are involved in, if not pivotal for the development of disease or resistance in barley. To this end, the response of host and non-host plants, respectively, to inoculation with fungal wild-type strains is compared to that upon inoculation with mutant strains, in which single candidate genes were knocked out. Deviations from the wild-type are being followed at the phenotypic level, as well as at the level of gene expression (transcriptome) and protein patterns (proteome). *R. secalis* is characterized by its subcuticular growth in host leaves. After penetration the fungus grows in the extracellular region between the leaf cuticle and the epidermal cells of susceptible plants. During early stages of pathogenesis epidermal cells collapse, whereas necrotic lesions do not become visible until later stages when the mesophyll cells are affected as well. The epidermis is therefore the only tissue in close proximity to fungal hyphae. Consequently, this tissue represents the prime target of investigations aiming at identifying the mechanisms of plant susceptibility or resistance to *R. secalis* and other pathogens such as *Blumeria graminis* (powdery mildew).

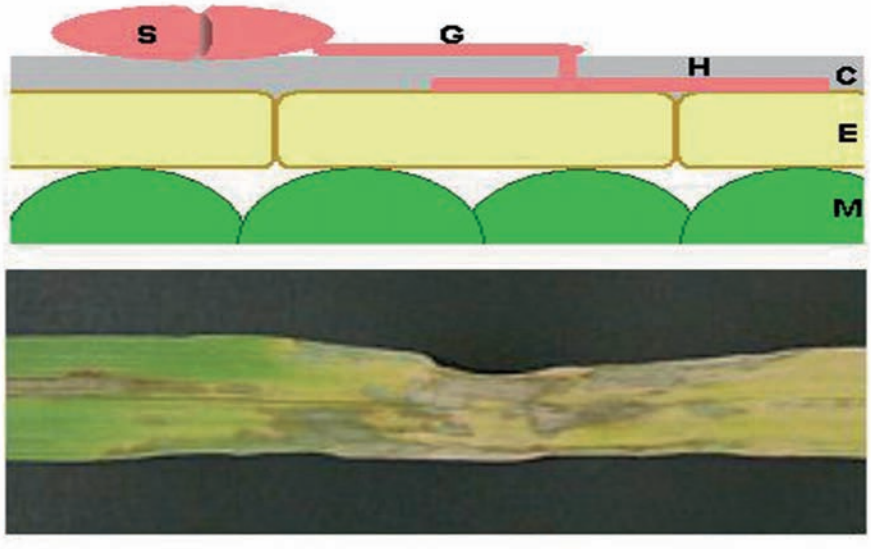
Key point of our strategy is the availability of informative pathogen mutants, suitable for probing the plant response. To identify fungal candidate genes, whose products are involved in the communication with the host and, hence, play a role during pathogenesis, random mutagenesis strategies are being applied. Loss-of-function mutations in genes, whose products contribute to optimal pathogenesis (virulence genes) or that are even essential for microbial penetration and development *in planta* (pathogenicity genes) can be presumed to generate a phenotype that deviates more or less clearly from the wild-type situation. Isolation and detailed molecular analysis of the involved genes and their protein products will lead to detecting those pathogen processes that affect the host physiology. Targeted disruption of the genes will generate the appropriate fungal mutants that are capable of identifying the corresponding plant processes. An alternative molecular genetic approach (signal sequence trap strategy, SST) aims at identifying and isolating fungal genes that encode secreted proteins (secretome). These proteins can be regarded to either be required for the synthesis of fungal extracellular structures or to be candidates for factors involved in the interaction with the host.

Several molecular tools have been established or improved for the use with *R. secalis*. Importantly, targeted disruption of fungal genes can be achieved by homologous recombination following *Agrobacterium tumefaciens*-mediated transformation with specific vectors. These vectors are optimized to facilitate the discrimination between homologous and heterologous

recombinants. In addition, an SST system has been established and shown to function properly with fungal control genes known to encode intracellular or secreted proteins. This system can now be used to screen a fungal cDNA library for genes that encode proteins carrying a secretor signal sequence.

A function in disease development has long been suspected for several small proteins secreted by *R. secalis*. Phenotypic analysis of fungal knockout mutants has now revealed that these proteins are indeed virulence factors required for full expression of the disease symptoms. Furthermore, one of these virulence factors is turned into a specific recognition signal in host plants carrying resistance gene *Rrs1*, i.e., an offensive fungal tool now serves as the trigger of plant resistance reactions. A specific binding site for this fungal protein was detected on membranes from resistant and susceptible barley cultivars as well as from other cereals. This suggests that the receptor is not encoded by the *Rrs1* gene and that (an) additional component(s) are required for resistance-related signal transduction.

Several fungal pathogenicity and virulence genes have resulted from two different random mutagenesis approaches, insertion mutagenesis and promoter trapping. The deduced functions of the gene products, although biochemically not yet confirmed, suggest their role in various pathogenetic processes such as fungal uptake of specific plant nutrients (GABA/amino acid permease), oxidative reactions of as yet unknown nature (P450 protein) and intracellular information processing (histidine protein kinase, MAP kinase). In addition, a protein with sequence similarity to a class of transcription factors may control fungal genes that are only ex - pressed during fungal growth in the plant. Finally, a gene was identified that encodes an intramembrane protease. A subgroup of these enzymes catalyzes the release of extracellular signals from transmembrane precursor proteins. However, neither the identity of the signal generated by the *R. secalis* enzyme nor its target or the role of *regulated intramembrane proteolysis* in fungus-plant interactions in general are known to date. Fungal knockout mutants are now being generated to allow a comparative analysis using a barley cDNA macroarray to unravel the impact of these fungal genes on plant gene expression.




**Pathogenesis of *R. secalis* in barley leaf tissues.** (Top) Schematic presentation (not in scale) of the early stage with two-celled spore (S), germ tube (G) and subcuticular hypha (H); cuticle (C), epidermis (E), mesophyll (M). (Bottom) Disease symptoms on a barley primary leaf; at this late stage necrotic lesions originate from the collapse of epidermis and mesophyll cells beneath fungal hyphae.

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# Cellular Signaling

*Heads: Dierk Scheel & Justin Lee*

**Plants are constantly under the attack of potential pathogenic microbes, invertebrate pests and even parasitic plants. In contrast to vertebrate immune systems where specialized cells are mobilized to sites of infection, plants possess non-circulatory defense mechanisms that are nevertheless equally effective in delimiting the invading organism. Research in our group concentrates on plant defense signal transduction pathways in non-host plant-pathogen interaction and the so-called gene-for-gene type of disease resistance. We also investigate the infection strategies of the parasitic plant *Cuscuta reflexa*.**

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Non-host resistance mechanism was initially studied using the Pep-13-parsley cell suspension culture model system. Pep-13, an oligopeptide derived from a *Phytophthora sojae* cell wall transglutaminase, serves as a pathogen-associated molecular pattern (PAMP) to trigger defense reactions in parsley cells. The concept of PAMP recognition and activation of innate immunity was first characterized in animal systems but is similarly conserved in plants and has been documented in many other plant defense models e.g. the bacterial flagellin-derived flg22 peptide elicitation in Arabidopsis. The first step to defense response activation is the perception of pathogen-derived signals. Peptide and proteinaceous elicitors studied in our laboratory include the above-mentioned flg22 and Pep-13, as well as the elongator factor(EF-Tu)-derived elf18 peptide, harpin from *Pseudomonas syringae* or a *P. sojae* necrosis-inducing protein.

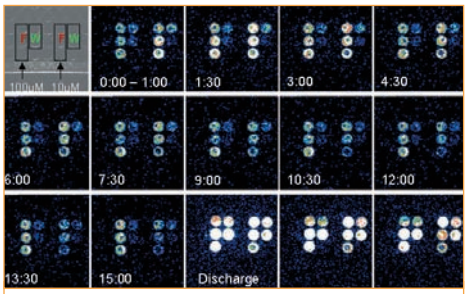


**Fig. 1:** Imaging of cytosolic calcium levels with the luminescent calcium-sensitive protein aequorin. Leaf disks from aequorin transgenic plants were placed in a multi-well plate and time series images taken with a luminescence-recording camera after treatment with flg22 peptide (F) or with water (W) as a control. For eventual calibration of calcium levels, the total luminescence of the remaining aequorin reporter was discharged at the end of the experiment.

A network of cellular signaling events is triggered upon perception of PAMPs by plasma-membrane-localized receptors, and this coordinates the subsequent defense response activation. The signaling events include ion fluxes at the plasma membrane, mitogen-activated protein kinases (MAPK) activation and the accumulation of signaling molecules, such as reactive oxygen species, phospholipids and phytohormones.

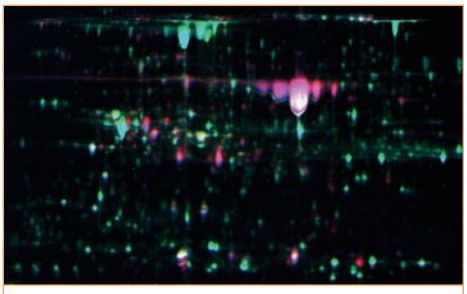

**Fig. 2:** DIGE 2D-PAGE image of two protein samples labeled with different fluorescent dyes. The abundance of Rubisco in green tissues of plants hinders the analysis of low abundant proteins in proteomics studies. A simple separation procedure to remove Rubisco was developed. Here a Rubisco-depleted protein extract (green) from Arabidopsis is compared to a fraction that contains predominantly Rubisco (pink) to show the efficiency of this method.

Studies involving the calcium-sensitive reporter, aqueorin, as well as patch-clamp techniques revealed that changes in cytosolic calcium levels represent an early key step that is essential for all other downstream events studied. The calcium ions may arise from extracellular, as well as intracellular pools. Recent work from other groups suggested that the two-pore-channel, TPC1, might be the elusive channel mediating calcium fluxes during defense responses. A parsley *TPC1* homolog was cloned, but studies, especially those performed using Arabidopsis *tpc1* mutants and TPC1-overexpressing lines, failed to support this concept.

MAPK activation was previously shown in the parsley system as essential signaling event for controling defense gene expression. Yet, the same subset of MAPKs appears to be activated by a plethora of diverse stress signals. The une-

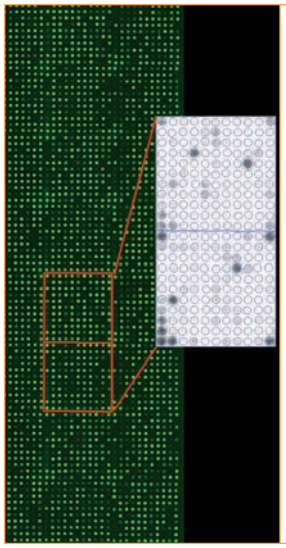

**Fig. 3:** A protein array consisting of 1,700 unique Arabidopsis proteins was used for *in vitro* kinase screening of MAPK substrates. The left image (green spots) is an antibody-based labeling of all proteins on the array and the image on the right is an enlarged section of the chip showing radioactively-labeled spots of phosphorylated substrates.

qual number of genes in the Arabidopsis genome coding for the different MAPK cascade components, namely the MAPKs, MAPK-kinases (MKK) and MKK-kinases (M3K), also indicate that the same protein must, in certain cases, cater to more than one signaling pathway. These observations raise the question of how signal specificity is maintained for MAPK pathways. The specificity may reside at the level of the MAPK substrate diversity as well as the presence of pathway-specific protein complexes that precludes erroneous crosstalk in signaling. We are currently pursuing different strategies to isolate MAPK substrates and scaffold proteins. These include yeast-two-hybrid screens, biochemical isolation of MAPK-interacting proteins by tandem-affinity purification (TAP) and a protein array-based screen for MAPK substrates.

The existence of MAPK protein complexes could be shown by size-exclusion chromatography. Transgenic plants with tandem-affinity tagged versions of the elicitor-activated MAPKs have been generated to facilitate the isolation of such complexes. The functionality of the TAP-tagged MAPKs was verified either by phenotypic complementation of mutant plants or by the ability of elicitors to activate these tagged proteins (as an indication that they have incorporated into the signaling protein complex). Purification of associated proteins is currently in progress. The isolated proteins will be identified through mass-spectrometry-based peptide fingerprinting and sequencing methods.

To verify the putative MAPK-interacting candidates identified in the yeast-two-hybrid screen, we set up fluorescence resonance energy transfer (FRET) technique in Arabidopsis protoplasts. As an example, we could confirm a specific *in vivo* interaction between AtMPK6 and a transcription factor of the ethylene-response factor (ERF) family. This interaction was disrupted upon flg22 elicitation, suggesting some biological relevance of this dynamic interaction during the flg22-signaling. The transcription factor was phosphorylated by active AtMPK6 and may potentially regulate flg22-induced defense gene expression.

Approximately 1700 unique recombinant proteins of Arabidopsis were purified and spotted onto a protein array chip. *In vitro* kinase assays on this chip identified a series of potential substrates of AtMPK3 and/or AtMPK6. One of the AtMPK6 substrates was ACS6 (ACC synthase-6, the rate-limiting enzyme for ethylene biosynthesis), which was previously identified as one of the first plant MAPK substrates. Hence, the *in vitro* screen with this protein array may lead to other authentic MAPK substrates.

The biochemical functioning in a cell is ultimately executed by the diverse regulatory roles of proteins (e.g. as enzymes or structural elements). Hence, proteome studies are increasing in importance in the post-genomic research era. High resolution two-dimensional polyacrylamide gelelectrophoresis (2D-PAGE) is commonly used to study changes in protein levels and post-translational modifications. We have improved current 2D-PAGE methods through a number of technical advances such as Rubisco depletion, economical alternative dyes for DIGE (Fluorescence 2D Difference Gel Electrophoresis) and phosphoprotein analysis. Currently, 2D-PAGE is used to study changes in protein patterns in systemic acquired resistance and the *AvrRPM1* gene-for-gene model system in Arabidopsis.

Not unlike other phytopathological systems, successful invasion of the holoparasitic plant, *Cuscuta reflexa*, requires the directed transfer of nutrients and water from the host into the parasite, in this case *via* a multicellular haustorial structure. Xylem- and phloem-mobile dyes, respectively, demonstrate functional connection of the invading haustorium to the host vascular system. This parasitism can eventually result in death of the host. A screen in various ecotypes and defense-related mutants has been initiated to elucidate the components that may

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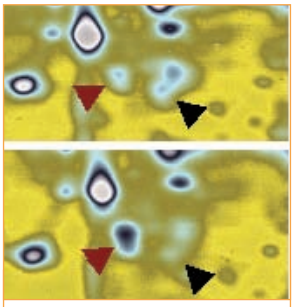

**Fig. 4:** Post-translational modification of proteins can be visualized in 2D-gels. Here a putative lipid raft component protein (black arrowhead) is found to exist in various forms, including a phosphorylated state (red arrowhead), in the *AvrRpm1* gene-for-gene model system.

# Induced Pathogen Defense

*Heads: Dierk Scheel & Sabine Rosahl*

**Late blight is one of the most devastating diseases of potato and is caused by the oomycete *Phytophthora infestans*. Interestingly, the model plant *Arabidopsis thaliana* is able to successfully defend itself against infections by this pathogen. We are interested in studying the interaction of *P. infestans* with both its host plant potato and the non-host plant Arabidopsis. The major focus of our work is the elucidation of the role of the signaling compounds, salicylic acid and jasmonic acid, for pathogen defense in potato and the genetic dissection of non-host resistance of Arabidopsis against *P. infestans*.**

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Basal defense of potato in response to infection with *P. infestans* involves recognition of the pathogen, signal transduction and the activation of defense gene expression. Both salicylic acid and jasmonic acid have been identified as signaling molecules in plant pathogen interactions. Our studies aim at the elucidation of the role of these compounds for pathogen defense responses in potato. To address this issue, transgenic plants were generated that express the bacterial *NahG* gene encoding salicylate hydroxylase. As a consequence, these plants are unable to accumulate salicylic acid. On the other hand, RNA interference constructs against jasmonic acid biosynthetic enzymes were expressed in transgenic potato plants resulting in significantly decreased levels of jasmonic acid. In a compatible interaction of the *NahG* plants with a virulent isolate of *P. infestans*, significantly higher growth of the oomycete was observed, suggesting that salicylic acid is required for basal defense in potato.

The oligopeptide elicitor, Pep-13, a pathogen-associated molecular pattern (PAMP) from oomycetes, induces an oxidative burst, accumulation of salicylic acid and jasmonic acid, defense gene expression and hypersensitive-like cell death in potato. None of these defense reactions are observed in transgenic plants unable to accumulate salicylic acid. In plants with reduced levels of jasmonic acid, salicylic acid still accumulates while the oxidative burst, hypersensitive cell death and defense gene expression are reduced. These data suggest that Pep-13 activates defense responses *via* a signal transduction chain which requires both salicylic acid and jasmonic acid.

In addition to jasmonic acid, which is synthesized *via* the lipoxygenase pathway, other oxylipins are assumed to play a role in pathogen defense responses. In particular, products of the 9-lipoxygenase pathway, such as hydroxylinole(n)ic acid and the divinyl ether containing polyunsaturated fatty acids colnele(n)ic acid, were shown to act as antimicrobial compounds. The role of these oxylipins for defense against *P. infestans* is being analyzed by generating potato plants expressing RNA interference constructs targeted against the pathogen-induced 9-lipoxygenase and 9-divinyl ether synthase. Basal resistance of potato against virulent isolates of *P. infestans* is not affected by the reduction of 9-lipoxygenase-derived compounds. Presently, potato plants carrying the resistance gene *R1*, against *P. infestans* are transformed with the RNA*i* constructs in order to analyze the role of oxylipins for race/cultivar-specific resistance.

**Fig. 1:** Infection of the host plant potato leads to disease (upper panel). In contrast, Arabidopsis can successfully prevent pathogen entry and stays healthy (lower panel).

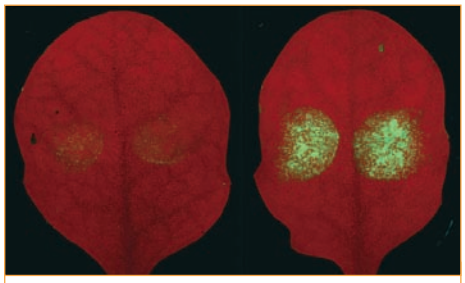

**Fig. 2:** Defense reactions in Arabidopsis leaves in response to drop inoculation with *P. infestans* can be visualized under UV light. Left: wild-type, right: *pen2*, a mutant impaired in penetration resistance.

Since Arabidopsis is not a host plant for *P. infestans*, we are interested in elucidating the mechanisms responsible for successful defense against this pathogen. For this purpose, genetic approaches are applied in order to identify genes involved in non-host resistance. *P. infestans* spores germinate on Arabidopsis leaves and attempt to penetrate cells, which react with the deposition of callose, accumulation of autofluorescent material and hypersensitive cell death. The Arabidopsis mutant, *pen2*, which was isolated by Volker Lipka and Paul Schulze-Lefert (Max Planck Institute for Plant Breeding Research, Cologne) as allowing enhanced penetration of the non-host pathogen, barley powdery mildew (*Blumeria graminis* f. sp. *hordei*), shows a higher number of penetrated epidermal cells and increased cell death in response to infection by *P. infestans*. Seeds of the *pen2* mutant were mutagenized and approximately 70,000 M2-plants were screened for alterations in their response to infection by *P. infestans*. Fourteen independent mutants were isolated, which show increased hypersensitive cell death and in some cases enhanced growth of *P. infestans*. Mapping of the position of the affected genes is in progress.

The protein, NPR1, is a central regulator in the signal transduction leading to systemic acquired resistance (SAR) in Arabidopsis, which confers immunity against secondary infections. NPR1 serves as a mediator between the signaling compound salicylic acid and the salicylic acid-induced expression of pathogenesis-related (*PR*) genes by interacting with a group of basic leucine zipper transcription (TGA) factors. These in turn bind to promoter elements of *PR* genes in a salicylate-dependent manner. *PR* gene expression generally correlates with the establishment of SAR.

Additional interaction partners of NPR1, the NIMIN proteins, were identified. In a yeast-one-hybrid system, NIMIN1 can establish a ternary complex with NPR1 and TGA factors, which bind to a *cis* element of the *PR1*-promoter *via* the TGA factors. Analyses of *nimin1* overexpressing and *nimin1* knock-out plants revealed that NIMIN1 is a negative regulator of NPR1-mediated gene expression. These data suggest that activation of the *PR1* promoter coincides with the binding of the negative regulator NIMIN1, thereby ensuring a fine tuning of *PR* gene expression.

Activation of the salicylic acid pathway in Arabidopsis leads to inhibition of jasmonic acid-mediated defense responses. *Nimin1* knock-out plants exhibit increased *PR* gene expression, however, possibly due to redundance, no enhanced resistance to biotrophic pathogens is observed. Therefore, multiple knock-out mutants were generated and analyzed. The *nimin1-1;nimin2-1* double mutant and the *nimin1-1; nimin2-1;nimin1b-1* triple mutant show enhanced *PR* gene expression as well and are hypersensitive to chemical inducers of the salicylic acid signal transduction pathway. This hypersensitivity leads to chlorosis and eventually to necrosis of the treated plants, rendering them more susceptible to necrotrophic pathogens.

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# Metal Homeostasis

*Head: Stephan Clemens until September 2006*

**Plants – like all other organisms – are able to tightly regulate the intracellular concentration and the distribution of essential metals such as zinc and copper. Also, the cytosolic concentrations of non-essential toxic metals (e.g. cadmium, lead) have to be minimized. Some plant species (so-called metallophytes) can tolerate otherwise toxic concentrations and grow on metal contaminated soil. The projects of our group are aiming at elucidating the molecular mechanisms underlying plant metal homeostasis, metal tolerance and metal hyperaccumulation. Plants under investigation are *Arabidopsis thaliana* and its close relative *Arabidopsis halleri,* which grows, for instance, on medieval mining sites in the Harz mountains. In addition, we are working with *Schizosaccharomyces pombe* as a cellular model for metal homeostasis. Our main emphasis in the past two years has been on (i) comparative root transcriptome studies of *A. thaliana* and *A. halleri*; (ii) screening of genetic diversity in *A. thaliana* for metal homeostasis phenotypes; (iii) functional analysis of two low-molecular weight metal cation ligands: phytochelatins and nicotianamine.**

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Comparative transcript profiling of *A. thaliana* and *A. halleri via* cross-species hybridization of Affymetrix GeneChips led to potentially important insights into the molecular mechanisms of metal hyperaccumulation. Previously, we had seen that several metal homeostasis genes show strong overexpression in *A. halleri* roots relative to *A. thaliana* roots. Nicotianamine synthases (NAS) and transporters of the ZIP family were identified as hyperaccumulation/hypertolerance candidate genes. More recently, we have been studying transcriptome responses to Fe deficiency, because it is not clear how *A. halleri* manages to acquire enough Fe even in the presence of vast excess of Zn (at several *A. halleri* sites in the Harz mountains, for instance, we measured soil Zn content exceeding 1.5 mg/g). Zn and Fe are often competing for the same uptake and translocation pathways.

Microarray data on excess metal responses in *A. halleri* and *A. thaliana* roots provided clues as to the modes of toxicity and helped identifying common as well as species-specific transcriptional changes that will guide functional studies. In particular, "core" response genes, genes responsive specifically to excess of one particular metal and putative signal transduction components were identified. Several of the responsive genes appear to be specific for $Cd^{2+}$ stress.

To date, the mining of microarray data for *A. halleri* is still limited by the fact that the probe sets are designed for the *A. thaliana* genome. In order to identify reliable probes and to establish a way of better normalizing the data, we are in a collaboration with Ute Krämer (Golm) and Roland Eils (Heidelberg) trying to develop respective bioinformatic tools. The data sets this work is based upon were obtained through genomic hybridizations of three different Arabidopsis species.

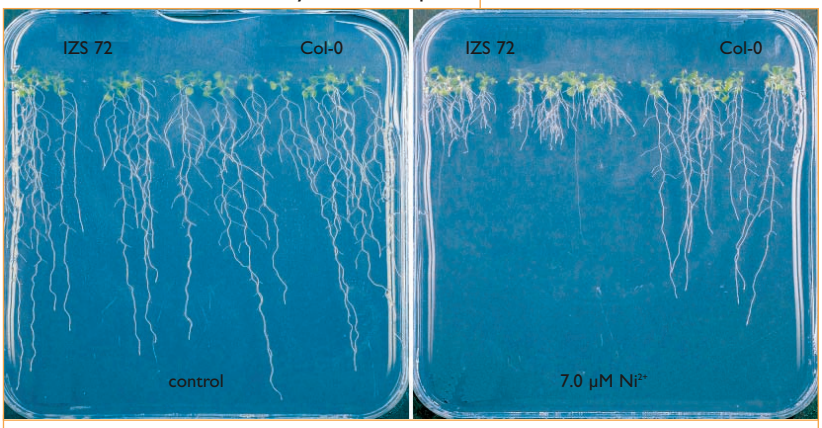
The constitutive strong expression of several metal homeostasis genes in *A. halleri* relative to *A. thaliana* raised the question as to what the molecular mechanisms behind this altered regulation are. We have therefore embarked on comparative promoter studies for various *NAS* and *ZIP* genes using reporter lines. For *NAS2* and *ZIP4* we found evidence that the *A. halleri* promoter is sufficient to drive strong expression in *A. thaliana*. Therefore we initiated the generation of various transgenic lines carrying truncated versions of the *A. halleri* promoters. These will hopefully lead to the identification of *cis* elements required for the activity.

Our comparative transcriptome studies of *A. thaliana* and *A. halleri* had indicated a potential role of nicotianamine synthases (NAS) for Zn hypertolerance/hyperaccumulation of *A. halleri*. Loss-of-function studies for *A. halleri NAS* genes and the other candidate genes we identified require RNA*i* lines of *A. halleri*. The successful transformation of *A. halleri* has been achieved owing to a collaboration with the group of Ute Krämer in Golm. RNA*i* lines for some of the candidate genes are currently being characterized.

A second hypothesis concerning nicotianamine (NA) that emerged from the experiments on *A. halleri* postulates NA function not only as a

ligand for long-distance transport in xylem and phloem, but also as an intracellular ligand for Zn(II). In order to obtain additional evidence supporting this hypothesis we started a series of experiments on *Neurospora crassa*. Genome sequencing had revealed that several filamentous fungi carry NAS homologous sequences. We therefore cloned a putative nicotianamine synthase from *Neurospora crassa* and showed by functional expression in a $Zn^{2+}$-hypersensitive fission yeast mutant that this protein indeed possesses NAS activity. Using capillary liquid chromatography coupled to electrospray ionization quadrupole time-of-flight mass spectrometry (Cap-LC-ESI-QTOF-MS) we unequivocally proved the formation of NA in *N. crassa* mycelium. Expression of the *N. crassa NAS* gene is strongly up-regulated under conditions of Zn deficiency as shown by quantitative real-time PCR. These findings demonstrate that NA is more widespread in nature than previously anticipated. Also, they show occurrence of NA in organisms that do not require extracellular long-distance transport. A hallmark of filamentous fungi is cytoplasmic continuity. The cellular compartments of hyphae are delineated by incomplete septa that allow passage of organelles and cytoplasmic components.

The other low molecular weight ligands we are interested in, phytochelatins (PC), are still raising biologically important questions. PCs are non-translationally derived from glutathione by the enzyme phytochelatin synthase (PCS). It appears unlikely that the proven contribution of PCS genes in Cd and As detoxification alone can explain the almost ubiquitous presence in the plant kingdom, the widespread distribution in other domains of life and their constitutive expression. Thus, we are searching for additional functions of PC synthases and PC synthesis. Two lines of evidence have been obtained by our group and collaborators in Heidelberg for (i) a role of PC synthesis in essential metal homeostasis and (ii) potential other roles of PCS proteins in GSH metabolism: PC-deficient Arabidopsis mutants are less $Zn^{2+}$ tolerant and accumulate less zinc than wildtype. A bacterial ancestor of PC synthases catalyzes only the first step of PC synthesis, the degradation of GSH to γ-glutamylcysteine. Thus, peptidase and transpeptidation activities can be separated by analyzing this protein. Our collaborators in Cadarache, France, have been able to crystallize the bacterial protein (Nostoc PCS) using our *E. coli* clones. They obtained high resolution structure which now serves as our template for the targeted mutagenesis of both the ancestral proteins and plant PC synthases. We are trying to find determinants for the two activities and to understand the evolution of this family of proteins. Also, we generated truncated versions of *bona fide* PC synthases that show GSH degrading activity. One of these is currently being expressed in *E. coli* in order to enable our collaborators to crystallize this protein.




The *izs72* mutant of *A. thaliana* is $Ni^{2+}$ hypersensitive.

A major emphasis of our work in the past two years has been on using genetic diversity in *A. thaliana* to identify new components of the plant metal homeostasis network. Firstly, we established a screening for metal hypersensitive mutants. More than 25 mutants have now been confirmed that differ in their metal specificity. Complementation analysis and mapping experiments are ongoing. Secondly, within the frame of a GABI-Genoplante program we started to explore natural diversity in Arabidopsis for Zn tolerance/accumulation, Cd tolerance/accumulation, Cu tolerance and micronutrient deficiency tolerance. Most of the ecotypes in the Versailles Core24 collection of Arabidopsis accessions have been screened. We identified ecotypes that clearly differ with respect to either Zn tolerance, Cd tolerance or Cu tolerance. Using recombinant inbred lines we are in

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# Bioinformatics & Mass Spectrometry

*Head: Steffen Neumann*

**The research group is the most recently established group in the Bioinformatics Center Gatersleben-Halle, operational since January 1, 2005. Goal of the group is to establish a bioinformatics and metabolomics platform at the IPB by developing databases and tools to support data-driven analysis and annotation, especially for mass spectrometry measurements using advanced pattern recognition methods and database technology.**

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## Mass Spectrometry Databases

The development of databases for mass spectrometry data is the basis of further high-throughput analysis. The modern software development paradigm Model Driven Architecture (MDA) allows to create large portions of the required software and databases from a graphical UML model.

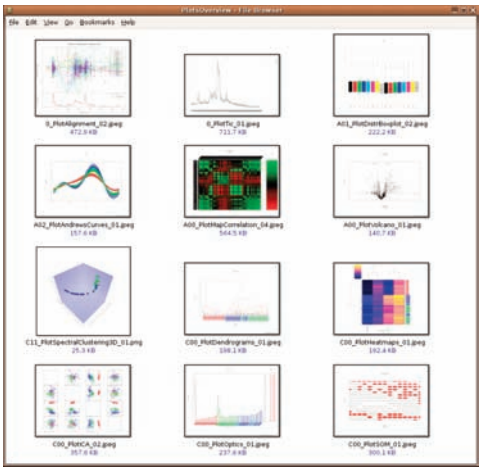

**Fig. 1:** A rich set of clustering and visualization algorithms collected in the metabolomics and datamining toolboxes.

To facilitate data exchange with other laboratories and analysis tools, the mzData and ArMet standards developed in the context of the Proteomics Standards Initiative (PSI) have been chosen as data model. The ***MetArchive*** database has been created as repository for mass spectrometry data generated at the IPB. Several mass spectrometry instruments from different vendors can already submit to this archive. The MetArchive tools include an editor, which allows experimentalists to edit and annotate their mass spectrometry data.

## Signal Processing of Raw Mass Spectrometry Data

The first steps of an analysis pipeline processing mass spectrometry data are signal processing and peak picking tasks. The XCMS software developed at the Scripps Research Institute has been adapted to the specific settings at the IPB. It has an Open-Source license, and in cooperation with the upstream authors, the software has been improved regarding the set of input formats and signal processing. Data import is also possible through a direct database link to the MetArchive.

For the liquid chromatography-coupled mass spectrometry (LC-MS) setup at the IPB the signal processing parameters, such as peak width, S/N and others have been systematically optimized to obtain good results. Different peak models (e.g. the empirically transformed Gaussian model) have been used to fit peaks from actually measured data, and several classes of peaks have been obtained through clustering in the model parameter space.

A set of annotation methods has been implemented, which aim to group together mass signals measured from a single metabolite based on rules for mass differences and peak shape comparison. These annotations greatly reduce the time necessary to interpret mass spectrometry data.

## Data Warehouse for Mass Spectrometry Data

A data warehouse is a database optimized for online analytical processing (OLAP), and allows to retrieve subsets of data based on arbitrary filter criteria. The BioMart framework has been used to establish the ***MetHouse*** for preprocessed peaks obtained e.g. by XCMS.

BioMart provides several frontends, including stand-alone and web interfaces, and a powerful command line and scripting client. The current content of the MetHouse includes ca. 240 mass spectrometry runs with ca. 1.2 million peaks. Possible filters against the warehouse are e.g. plant genotype, growth treatment, model of the mass spectrometry machine, ionization mode or institute.

## Clustering of Metabolite Data

Metabolite data can be interpreted as phenotypic feature and used to distinguish different metabolic states of a system or to identify e.g. ecotypes. Different hierarchical clustering algorithms implemented in the R statistics software have been used with several distance measure-

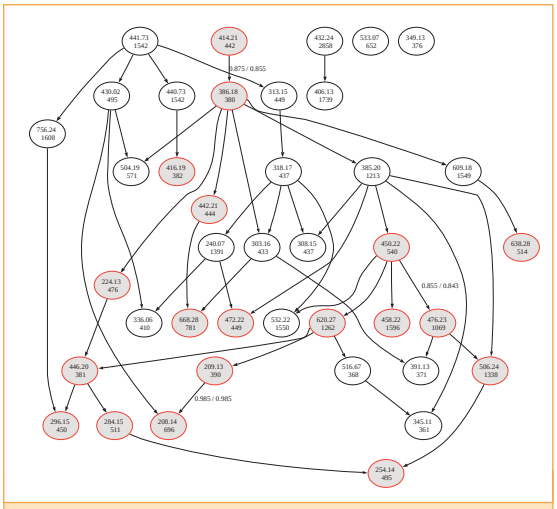

**Fig. 2:** Connections between metabolites (nodes labelled with mass and retention time) for the core (grey) and extended (white) dataset.

ments to create a hierarchical clustering. In several datasets, such as Arabidopsis ecotypes, single gene mutants or stress responses to copper treatment, a clear separation of the groups could be achieved.

Density based clustering algorithms, such as DBScan and Optics, fractal clustering like the dimension induced clustering (DIC) algorithm have been implemented as described in the literature and/or integrated into the pipeline and applied to metabolite data. Advanced visualization methods (see **Fig. 1** for examples), such as parallel coordinate plots or MA plots known from expression analysis have also been integrated. Differential abundance of metabolites between experiments offers insights into the metabolic difference between the samples.

## NETWORK RECONSTRUCTION WITH BAYESIAN NETWORKS

To uncover the causal relationships between metabolites can be considered the ultimate (and ambitious) goal of metabolomics. Training of Bayesian Networks might be one of the tools on the way.

Bayesian Networks are graphical models used to model and visualize biological processes. A Bayesian Network consists of a directed acyclic graph representing metabolites with edges for their relationships and a conditional probability distribution. The Sparse Candidate Algorithm (SCA) has been implemented in Java to efficiently find a network that models the measured data. Interventions in metabolic pathways cause a disturbance of the distribution and can help to identify regulation.

The *tt4* mutant harbors a non-functional chalcone synthase gene causing the disruption of flavonoid, tannin, and anthocyanin biosyntheses resulting in a different colour of the seeds. We selected a subset of 19 mass signals, which are known or believed to be metabolites involved in the phenylpropanoid pathway as core data set. We randomly selected an additional set of 21 mass signals resulting in the extended dataset.

The structure of the Bayesian network learned from the core dataset remains stable even if the 21 randomly chosen mass signals were added to the core dataset.

It is possible to represent extra information, such as the type of the tissue or experiment treatments in additional nodes. Bayesian networks provide a valuable annotation of metabolites and connect influences, such as knock- out or stress conditions to the metabolic state of the system.

## WEB APPLICATION AND INTEGRATION INTO THE PLANT BIOINFORMATICS PORTAL

To achieve an easy integration of R and bioconductor-based applications into web applications Rserve is employed as a communication system between Java clients and an R server. A tag library has been created to couple the JSF pages with the backend compute server, an overview of the architecture is shown in **Figure 3**.

Applications implemented this way include:

- XCMS mass spectrometry signal processing and alignment,
- several normalization and clustering algorithms,
- SiteSeer motif search.

Applications developed by external cooperations have been web-enabled and integrated into the portal, including:

- DISOP Elemental Composition (developed by A. Peruvkin, University of Jena),
- Macroarray overshining correction (developed by D. Müller-Lukas, University of Halle).

The applications are available to in-house users and to the public on the IPB web site. The integration into the distributed BioMoby infrastructure has already started and will enable to access metabolomics resources such as the MetArchive, MetHouse and MetWare remotely.

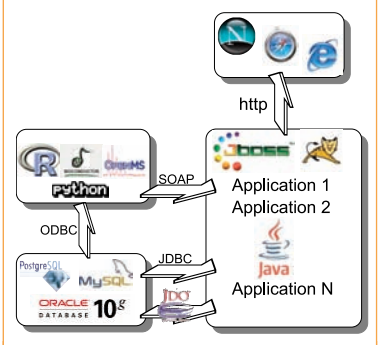


**Fig 3:** Distributed architecture of the MetWare software. Each component could run on a different machine.

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# Metabolite Profiling in Arabidopsis and Crop Plants

*Heads: Stephan Clemens & Dierk Scheel*
*in cooperation with Ludger Wessjohann & Jürgen Schmidt*

**In order to get a better understanding of how plants are using their arsenal of natural products in the daily battle of survival in an ever-changing environment, methods are required which allow sensitive detection, quantification and identification of secondary compounds. Metabolomics approaches are one possible answer to these requirements. Our group has established a reliable liquid chromatography-coupled mass spectrometry (LC-MS) platform for the analysis of various developmental and stress-induced changes, as well as for the biochemical phenotyping of mutants in *Arabidopsis thaliana* and relevant crop plants.**

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Because of the chemical diversity of the metabolome, which for any given multi-cellular species comprises a mixture of thousands of compounds differing in size, polarity and abundance, the need for multi-parallel analytical techniques is obvious and well accepted. LC-MS is a versatile analytical tool. It covers a wide mass range and allows to target many compound classes not detectable by gas chromatography-coupled mass spectrometry (GC-MS). In addition modern LC-MS set-ups offer superior options to structurally elucidate unknown metabolites using accurate mass determination and tandem MS. The main emphasis of this group has been on further developing the already established profiling platform, constantly reevaluating and extending it towards the analysis of crop plants or more specific various rapeseed lines. Doubts about the feasibility and reliability of LC-MS-based metabolite profiling have been raised repeatedly, because LC-MS - especially with electrospray ionization - can be subject to matrix effects. The term matrix effect refers to alterations of ionization efficiency of analytes by the presence of co-eluting substances, which can severely compromise quantification. We evaluated matrix effects for our metabolomics platform with several methodological approaches. Our data demonstrated that there are indeed significant absolute matrix effects when comparing highly divergent samples. However, relative matrix effects are negligible - unless extremely divergent matrices are compared - and do not compromise the relative quantification that is aimed for in non-targeted metabolomics studies.

Data analysis was and is still a major bottleneck of metabolomics. In cooperation with the Research Group *Bioinformatics and Mass Spectrometry*, methods for raw data deconvolution have been developed and improved. The former use of the data deconvolution softwares MetaboliteID and MetAlign was shifted to an open source software called XCMS and tailored to the needs of the LC-MS profiling platform. In order to facilitate the identification and structural elucidation of metabolites, the commercial software package ACD was acquired. The realms of this software were extended to allow - next to managing LC-MS and MS-MS spectra - sophisticated search algorithms within the steadily increasing database. In addition, an LC-MS-Profile database was set up in the institute providing additional data-mining tools. The developed data mining tools for the ACD and XCMS software, as well as the ACD/ESI-MS database will be publicly available at http://msbi.ipb-halle.de/MetWare.

In the frame of the Gabi1 project, the metabolomic profiling was originally based on using *Arabidopsis thaliana* leaves and roots as the source of plant material. Within Gabi2 the focus shifted to crop plants, with a special interest in rapeseed. Extraction and profiling protocols were optimized using as a first step Arabidopsis seed material. Seeds apparently contain in general lower amounts of those classes of secondary metabolites that can be examined with our profiling platform. This was indicated by a reduced number of mass signals (roughly about 800 signals in contrast to 1,500 signals in leaves) reliably detected by LC-MS. In addition, in contrast to roots and leaves, seeds contain high levels of choline esters. To structurally characterize this important compound class, extensive MS-MS analyses were performed resulting in the identification of 25 of these nitrogen containing esters. As initial optimization experiments, seed material from Arabidopsis lines harboring mutations in the well known flavonoid pathway were analyzed. The transparent testa mutants, *tt4* and *tt5*, harbor non-functional chalcone synthase and chalcone isomerase enzymes, respectively (**Fig. 1**). The metabolic

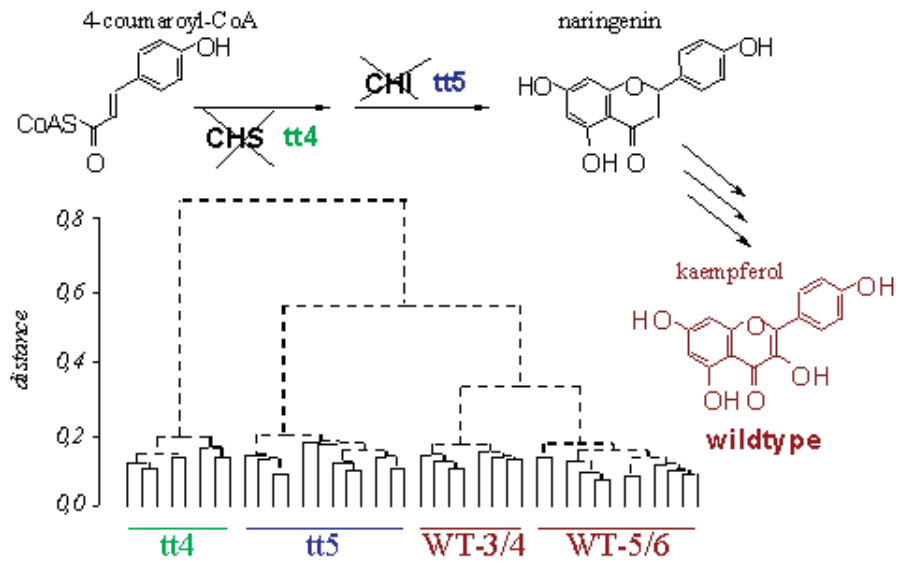


**Fig.1:** In the *tt4* and *tt5* mutants, the flavonoid pathway leading from 4-coumaroyl-CoA to kaempferol is blocked at the stages of chalcone synthase and chalcone isomerase, respectively. The Hierarchical Cluster Analysis showed a clear distinction in the degree of relationship between the two mutant and the wild-type seed materials, as well as between the two wild-type seed batches (due to their different age).

phenotype of these plants results in a more or less complete lack of kaempferol and its derived compounds, hence the light yellow coloring of the seed testa. The non-targeted profiling approach showed not only the expected absence of specific members of the flavonoid pathway. In addition, several flavonoids, which have not been described before, accumulated to high levels in the mutant lines indicating a redirection of metabolic fluxes. Hierarchical cluster analysis (HCA) of this experiment showed not only a clear separation of the different mutant and wild-type materials, but also a clear distinction between seed batches of different age (seed batch WT-3/4 was one year older than WT-5/6, **Fig. 1**). Additional examinations pointed to a changing metabolome during seed storage, emphasizing the need for truly comparable starting materials for profiling experiments.

Having optimized the metabolomics approach for *Arabidopsis thaliana* the system was applied to crop analysis. The comparison of the wild-type rape line, Drakkar, with transformants carrying the thioesterase *ClFatB3* gene revealed that about 10 % of the detected mass signals (the total number of reliably integrated m/z was up to 300) were either increased or reduced in the transformants (**Fig.2**). As the transformant lines showed a reduced long chain fatty acid content (for example oleic acid) and increased shorter chain fatty acid derivatives (for example palmitin) it was not unexpected that quite a number of the variable metabolites appear to be involved in the fatty acid pathway.

To increase the range of metabolites that can be analyzed with the profiling platform, a GC-MS station was established within the last year. It has undergone a thorough evaluation process comparable to the LC-MS system and will soon start broaden the scope of our profiling abilities.


**Fig.2:** LC-MS Profiling of rapeseed. The heatmap shows the comparison of the rape wildtype line, Drakkar, and the FatB3 transformants. The colour red is indicating areas of higher concentration, white is indicating lower concentrations of the roughly 300 reliably detected mass signals.

## COLLABORATORS

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## Department of Secondary Metabolism

*Head: Professor Dieter Strack*

*Secretary: Ildikó Birkás*

Work of the department is concerned with the molecular regulation of plant secondary metabolism, evolution of the enzymes involved and the role of secondary products in interactions of plants with their environment.

The work on metabolic regulation includes isolation and characterization of the enzymes and the encoding genes, focusing on transferases. We investigated malate and choline hydroxycinnamoyltransferases as well as several hydroxycinnamate glucosyltransferases from *Arabidopsis thaliana* and oilseed rape (*Brassica napus*) as well as flavonoid and betanidin glucosyltransferases from betacyanin-accumulating plants or flavonoid methyltransferases from the ice plant (*Mesembryanthemum crystallinum*) and Arabidopsis.

The aim of the work on glucosyl- and hydroxycinnamoyltransferases is to elucidate their evolutionary origin and structure-function relations to predict substrate specificity. Glucosyltransferases involved in betacyanin biosynthesis are considered to be oligophyletic and originate from different clusters of flavonoid glucosyltransferases. A new class of methyltransferases was identified, whose substrate specificities can be modulated by metal cations and N-terminal deletions. Hydroxycinnamoyltransferases, which are dependent on β-acetal esters as acyldonors, are vacuolar serine carboxypeptidase-like (SCPL) proteins as found for the enzymes involved in the formation of sinapoylmalate in Arabidopsis and sinapoylcholine (sinapine) in oilseed rape. Homology modeling of these acyltransferases identified structure determinants and amino acids involved in substrate recognition. The existence of these enzymes proves a new concept in the regulation and evolution of plant secondary metabolism. Additional work on a sinapine esterase from rape and a chlorogenate-specific caffeoyltransferase from tomato (*Solanum lycopersicum*) indicates functional shifts from lipases to a phenylpropanoid-specific esterase and acyltransferase, respectively.

Special emphasis is also placed on programs focusing on the molecular symbiotic interactions of plant roots with soil-borne fungi, i.e. arbuscular mycorrhizal fungi. The work of two groups is concerned with fungus-induced alterations in plant isoprenoid metabolism, in particular carotenoid biosynthesis and degradation leading to massive accumulation of apocarotenoids. This is accompanied by a dramatic reorganization of plastid population in arbuscule-harboring root cells. Another main objective is the analysis of the role of phytohormones, in particular jasmonates, in development and functional maintenance of mycorrhizal symbiosis. It was found that jasmonates obviously play a crucial role in the establishment of arbuscular mycorrhizas. These studies are supported by comprehensive analysis of primary and secondary metabolites (*metabolite profiling*) in wild-type and transgenic mycorrhizal plants. In another project on adventitious root formations of *Petunia hybrida* the possible function of wound-related jasmonates is studied.

## Metabolism of Phenylpropanoids

*Heads: Dieter Strack & Carsten Milkowski*

**The work of our group is focused mainly on metabolism of sinapate esters in oilseed rape (*Brassica napus*) and the model plant *Arabidopsis thaliana* with the aim to improve rapeseed quality by metabolic engineering. Target genes, i.e. UDPglucose:sinapate glucosyltransferase (SGT) and sinapoylglucose:choline sinapoyltransferase (SCT), to lower the content of the antinutritive seed constituent sinapine (sinapoylcholine) were identified. This is a key requirement for establishing rape as a protein crop. With Arabidopsis the basic role of SGT in phenylpropanoid metabolism and some aspects of plant physiology were studied. To study the evolutionary recruitment of enzymes from primary metabolism to produce highly diverse patterns of metabolites for novel functions in plant secondary metabolism we are interested to elucidate the structural changes necessary for conversion of hydrolases (peptidases, lipases) to acyltransferases by analysis of structure-function relationships of these enzymes. This work includes the SCT and the related acyltransferase sinapoylglucose:malate sinapoyltransferase (SMT) as well as the chlorogenate-dependent biosynthesis of caffeoylglucarate (CGT) from tomato.**

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The enzyme UDP-glucose:sinapate glucosyltransferase (SGT) catalyzes the formation of 1-*O*-sinapoylglucose, the principle acyldonor in transacylation reactions leading to a wide array of sinapate esters in species of the Brassicaceae family. Seed-specific suppression of the gene in oilseed rape (*Brassica napus*), *BnSGT1* (*UGT84A9*), led to a significant decrease in total sinapate ester content, establishing *UGT84A9* as key target gene for breeding of low sinapine cultivars. To support TILLING approaches for isolation of low sinapine mutants a genomic BAC library was screened to assess the number of *UGT84A9* alleles and genomic loci in the allotetraploid genome of *B. napus.* Sequence polymorphisms and Southern Blot patterns of isolated BAC clones reveal two alleles and four genomic loci of *UGT84A9* in winter oilseed rape cultivar "Express". For the gene encoding the final enzyme in sinapine biosynthesis, sinapoylglucose:choline sinapoyltransferase (SCT), two alleles were identified in the *B. napus* genome. Since *BnSCT* expression is restricted to developing seeds the promotor was isolated as a potential tool for future transgenic strategies aimed at modification of seed-specific traits. The DNA fragment covering 729 bp upstream of *BnSCT* translation start was sufficient to drive *GUS* expression specifically to the embryo and the aleurone layer of Arabidopsis seeds (**Fig 1**). Approaches to suppress sinapine accumulation were put forward by seed-specific dsRNA*i* knock-down of *BnSGT*/*BnSCT* and PAL (phenylalanine-ammonia lyase), the enzyme catalyzing the entry step into phenylpropanoid metabolism. With the *BnSGT*/*BnSCT* double suppression construct three homozygous single copy insertion lines reduced in seed sinapate ester content were developed. Screening *PAL* suppressing plants ($T_2$ seeds) several low sinapine lines were isolated. In order to identify the sinapine hydrolyzing enzyme, sinapine esterase, the activity was purified from germinating seeds. Based on peptide sequences the full-length cDNA was isolated and shown to encode sinapine esterase by functional expression in *Nicotiana benthamiana*. Sequence analysis revealed that sinapine esterase shares homology with lipases. Related cDNAs from Arabidopsis were cloned and shown to be active towards sinapine.

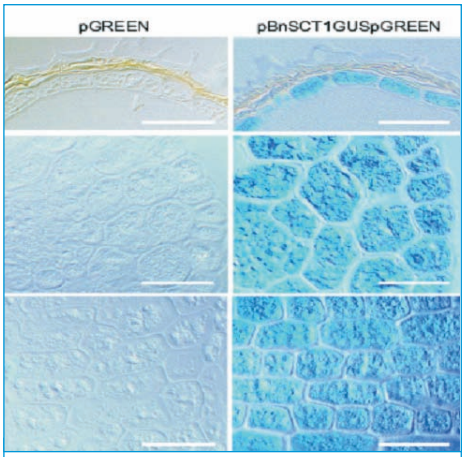


**Fig. 1:** The *BnSCT1* promoter drives seed-specific *GUS* expression in Arabidopsis. The *BnSCT1* promoter-*GUS* fusion cassette was inserted into the vector pGREEN (p*BnSCT1GUS*pGREEN) to transform Arabidopsis. The empty plasmid was used as negative control (pGREEN). The upper images show GUS activities in tissues of the testa and aleurone layer, the middle ones of the hypocotyls and the lower of the cotyledons. Bars represent 20 µm.

Since soluble hydroxycinnamate esters have been ascribed for UV screen function in Arabidopsis, expression of ester forming HCA-GTs (*UGT84A1-4*) was measured under UV-B stress conditions. Treatment of Arabidopsis plants for ten hours under conditions of elevated UV-B radiation resulted in a four-fold increase in *UGT84A2* transcript abundance in rosette leaves accompanied by corresponding increases in enzyme activities towards sinapate. Promoter-GUS fusion experiments corroborated the UV-B stress induction of *UGT84A2* transcription. Under standard conditions, *UGT84A2* expression was highest in cotyledons of three-day-old seedlings whereas UV-B stress clearly induced transcription in rosette leaves.

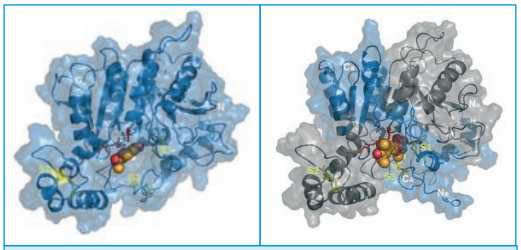

**Fig. 2:** Proposed fold of the ternary structure of SMT (left) and quaternary structure of SCT (right), each with the docked ligand of sinapoylglucose. The catalytic triad (Ser, Asp, His) is colored in red.

To investigate the evolutionary recruitment of enzymes from primary metabolism for novel functions in plant secondary metabolism the hydrolase-related acyltransferases sinapoylglucose:malate sinapoyltransferase (SMT) from Arabidopsis and the tomato-CGT (chlorogenate: glucarate caffeoyltransferase) were chosen. The CGT was purified from tomato leaves. Peptide sequences indicate homology of this acyltransferase with lipases. A full-length cDNA encoding *CGT* was isolated. For Arabidopsis SMT a yeast expression system was optimized. By codon usage adaptation of the *SMT*-cDNA to the requirements of *Saccharomyces* and translational fusion with the signal peptide of a yeast vacuolar protease enzymatically active SMT was expressed from a multicopy vector with a yield of about five milligram per liter. Thus kinetic measurements to study the reaction mechanism of SMT catalysis could be started. In a parallel approach, structure models of the SMT and SCT proteins were generated that reveals functional elements like the hydrogen bond network for substrate recognition, the "oxyanion hole" stabilizing the transition states and the catalytic triad (Ser, His, Asp) for the nucleophilic attack onto the carbonyl carbon of the acyldonor sinapoylglucose. Site-directed mutagenesis of the recombinant SMT approved the functional significance of several amino acid residues.

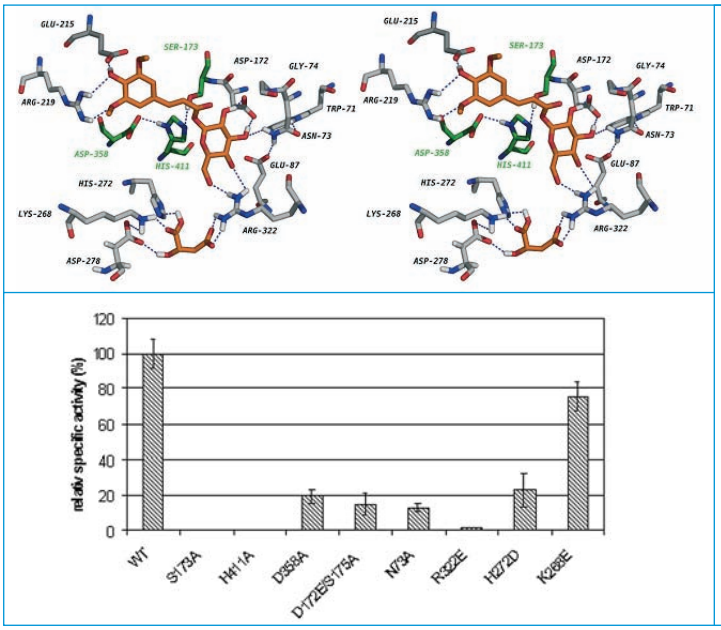


**Fig. 3:** Stereo picture of the structure model of the SMT active side (above). Amino acid residues forming the catalytic triad (Ser-173, Asp-358, His-411) and some of those forming the hydrogen bond network for sinapoylglucose recognition (Asn-73, Asp-172, Lys-268, His-272, Arg-322) were substituted in site-directed mutagenesis of the recombinant enzymes, giving the relative specific activities as shown below.

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# Molecular Physiology of Mycorrhiza

*Head: Michael H. Walter*

**The arbuscular mycorrhizal symbiosis of plants with specialized fungi in the soil provides roots with improved access to mineral nutrients, particularly phosphate, while plant carbohydrates are directed to the fungus in return. A main focus of the group is the elucidation of biosynthesis and function of certain apocarotenoids, which accumulate in later stages of this interaction. An early biosynthetic step in the methylerythritol phosphate (MEP) pathway catalyzed by 1-deoxy-D-xylulose 5-phosphate synthase (DXS), is used as a target for an RNA*i* approach. A specific isogene (*DXS2*) dedicated to apocarotenoid and other secondary isoprenoid biosynthesis allows for specific manipulation of this step without interfering with primary metabolism. Additional functions of *DXS2* independent of mycorrhization are being studied in tomato leaves and their trichomes. Furthermore, the ancestry of diverged *DXS* genes of angiosperms in land plant evolution is investigated by working with the gymnosperm *Picea abies* and the moss *Physcomitrella patens*.**

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Arbuscules are tiny intracellular organs of the fungus with a tree-like morphology, which are the symbiotic transfer sites of phosphate from the fungi into plant cells. Arbuscules are ephemeral structures with only a few days of functionality in nutrient transfer. There is thus a need for constant renewal of arbuscules but the mechanisms of their turnover are unknown. During colonization by arbuscular mycorrhizal (AM) fungi plant roots frequently accumulate two types of apocarotenoids (carotenoid cleavage products). These compounds localize preferentially to cells with degrading arbuscules. Both compounds, yellow linear $C_{14}$ mycorradicin and colorless $C_{13}$ cyclohexenone derivatives, are predicted to originate from a common $C_{40}$ carotenoid precursor. Apocarotenoid biosynthesis can be subdivided into three pathways:

- the general isoprenoid pathway leading to isopentenyl diphosphate / dimethylallyl diphosphate in plastids (MEP pathway),
- the carotenoid biosynthetic pathway, and
- the carotenoid cleavage and subsequent modification steps.

A major recent focus of the group has been the first step of the MEP pathway catalyzed by 1-deoxy-D-xylulose 5-phosphate synthase (DXS), since there are at least two distantly related isogenes, which are differentially regulated and appear to have dedicated roles in primary metabolism (*DXS1*) or secondary metabolism (*DXS2*).

The *MtDXS2-1* gene of the model legume *Medicago truncatula*, one of two closely related *MtDXS2* genes organized in a tandem repeat, exhibits strongly elevated transcript levels in

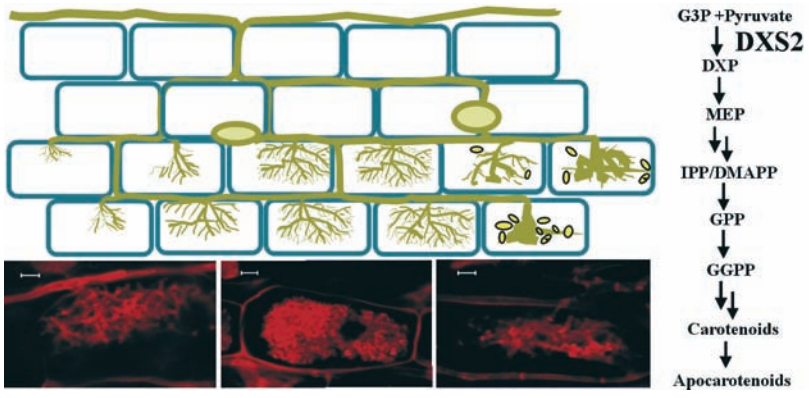


**Fig. 1:** Scheme of arbuscule development, maturation and breakdown in root cells. Arbuscule degradation is accompanied by local apocarotenoid deposition shown as small yellow vesicles (center panel); Confocal images of arbuscule development featuring young, mature and degrading stages from left to right (below); Simplified biosynthetic pathway of apocarotenoids and position of DXS2 (right panel).

**Fig. 2:** Tomato trichomes isolated from leaves. The head of a glandular trichome is visible at the upper left.

mycorrhizal roots, whereas *MtDXS1* is unaffected. RNA*i* constructs to knock down expression of both *MtDXS2* genes, but not *MtDXS1*, were introduced into transgenic hairy roots of *M. truncatula* by transformation using *Agrobacterium rhizogenes* and the pRNA*i*/pRed Root-vector system. The current results indicate a correlation of reduced levels of *MtDXS2* transcripts and a significant reduction in apocarotenoid levels in transgenic roots after nine weeks of root colonization by *Glomus intraradices*. This coincides with fewer stainable mycorrhizal structures, particularly arbuscules, and an even more pronounced drop in transcript levels of *MtPT4*, a mycorrhiza-specific plant phosphate transporter gene. *MtPT4* is used as a molecular marker for the functionality of arbuscules. Additional experiments indicated that this effect is dependent on the growth stage of the plants or growth conditions. However, under the conditions of reduced apocarotenoid levels in transgenic roots there appears to be a general accumulation of older degenerate stages of arbuscules at the expense of young and mature arbuscules as compared to empty vector control plants.

Involvement of *DXS2* genes in non-mycorrhizal isoprenoid pathways is being investigated in tomato (*Solanum lycopersicum*). Glandular trichomes of tomato are known to produce a number of different mono- and diterpenes involved in insect resistance and these compounds are derived from precursors provided by the MEP pathway. The *SlDXS2* gene of tomato is expressed in leaves, roots and flowers, but not in the ripening fruit. In isolated leaf trichomes *SlDXS2* transcript levels are strongly elevated indicating a preferential expression in leaf trichomes. Peptide antibodies specific for SlDXS2 were generated and are currently be - ing used in immunolocalization experiments. Suppression of the biosynthesis of MEP pathway-derived secondary isoprenoids by a knockdown of *SlDXS2* expression in transgenic tomato similar to the apocarotenoid suppression in *M. truncatula* will be performed.

In angiosperms the diversification of *DXS* genes into *DXS1* and *DXS2* is widespread and the only known exception is *Arabidopsis thaliana*, which has two *DXS1* copies, but no *DXS2* copy. Orthologs of both angiosperm *DXS* classes have been identified in the gymnosperm *Picea abies* indicating that an ancient gene duplication has evolved into the present-day divergence of *DXS* genes. The *P. abies DXS* genes are also differentially regulated and appear to have a similar division of labor between primary and secondary metabolism as in the angiosperm case. This is confirmed by a recent report on ginkgolide formation in *Ginkgo biloba* roots linking its biosynthesis to expression of a *DXS* gene belonging to class 2. However, in several gymnosperms the *DXS2* class is further split into two more distantly related *DXS2* genes.

To trace back the separation of *DXS* genes even further in evolution work with the moss *Physcomitrella patens* has been started. This moss produces a tetracyclic diterpene (16-α-hydroxykaurane), which is excreted and can be observed as crystals on leaf tips or on agar medium next to the plants. The function of this compound is unknown, but it has been implicated in microbial defense mechanisms. Since this diterpene can be classified as a secondary compound, it constitutes a suitable target to find out, whether particular subfunctionalized *DXS* isogenes of *P. patens* are involved in its biosynthesis. Starting with EST data four different *DXS* isogenes of the moss have been identified and full-length cDNAs obtained from „Physcobase“ were sequenced. The deduced proteins are all closely related and belong to the *DXS1* class of angiosperms and gymnosperms. Several conditions of diterpene biosynthesis and a number of other stimuli have been studied to find out about potential differential regulation of the four *PpDXS* genes. There are differences in expression levels between the four genes, but at this point a condition, where they are differentially regulated, was not found. In future work individual *PpDXS* genes of *P. patens* will be knocked out and the outcome on general viability and diterpene biosynthesis will be determined. Additional experimental systems located between the mosses and the gymnosperms in land plant evolution will be approached to clarify their *DXS* gene divergence.

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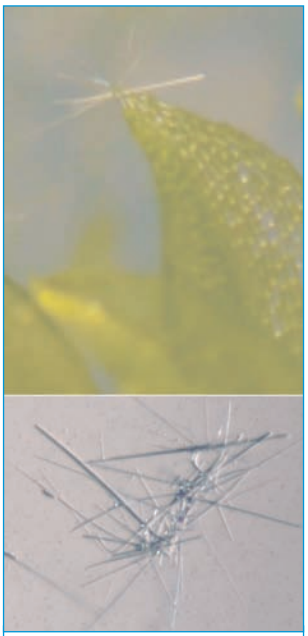

**Fig. 3:** Crystal needles of 16-α-hydroxykaurane appearing at the leaf tip of the moss *Physcomitrella patens* (top) or on the moss culture agar medium (below).

# Cell Biology of Myccorrhiza

*Head: Bettina Hause*

**The mutualistic interaction between plants and arbuscular mycorrhizal (AM) fungi is believed to be regulated in part by the action of phytohormones. Jasmonates, known as regulators in plant response to biotic or abiotic stresses, are good candidates for such a role. Therefore, the functional analysis of jasmonates during the interaction between *Glomus intraradices* and barrel medic (*Medicago truncatula*) is the main objective of our group. Moreover, we are interested in the regulatory role of apoplastic invertases in the supply of the AM fungus with carbohydrates by the plant. In a third project, the massive proliferation of plastids in AM-colonized root cortical cells is studied. Metabolic and cytological changes as well as respective molecular mechanisms are investigated. In a new project the role of jasmonates in the formation of adventitious roots in *Petunia hybrida* will be analyzed.**

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Conclusions for a possible involvement of Jasmonic acid (JA) in mycorrhizal interaction were drawn first from application experiments, which suggested a concentration-dependent effect of JA on mycorrhizal plants. Further results revealed that JA levels are increased in roots of mycorrhizal plants compared to roots of non-mycorrhizal controls. To analyze a possible function of JA during the development of the mycorrhizal symbiosis, roots of *M. truncatula* were transformed with *sense*- and RNA*i*-constructs for the allene oxide cyclase (AOC). AOC is catalyzing the crucial step in JA biosynthesis. All constructs were under the control of the CaMV 35S promoter. In comparison to samples transformed with a control vector (*DsRED*), roots with suppressed expression of *MtAOC1* exhibited lower JA levels and a remarkable delay in the process of colonization with *G. intraradices*. Both the mycorrhization degree, quantified by fungal rRNA, and the formation of arbuscules, analyzed by the expression level of the AM-specific phosphate transporter gene *MtPT4*, were affected. Concluding from this, it appears that jasmonates affect mycorrhization, possibly in multiple ways.

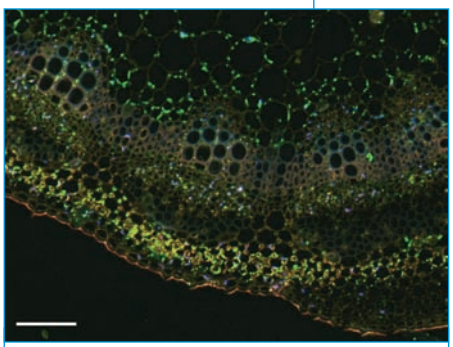

**Fig. 1:** Immunolabelling of the allene oxide cyclase (AOC) in cross section of stem of *Medicago truncatula*. The AOC (green) is located in plastids and occurs mainly in the cortex and the pith as well as in phloem cells of the vascular bundles. Cell nuclei are visualized by DAPI.

Analyses of transcript and metabolite patterns by microarrays and metabolite profiling, both in wild-type and transgenic roots, can help to identify processes mediated by JA during mycorrhization. First analyses of the transcript pattern of transgenic *M. truncatula* roots overexpressing or suppressing *MtAOC1*, both non-mycorrhizal and mycorrhizal, revealed a high number of differentially regulated genes depending on endogenous JA levels (cooperation with Helge Küster, University of Bielefeld). These changes were also reflected by alterations in the patterns of secondary metabolites (cooperation with Willibald Schliemann, IPB).

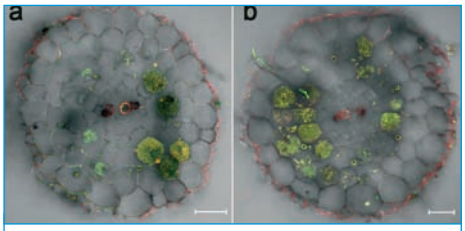


**Fig. 2:** Fungal structures in a wild-type tobacco root (a) and in a root of an invertase-overexpressing plant with moderate invertase activity in leaves (b). Cross sections of *G. intraradices*-colonized roots were stained with fluorescent labelled wheat germ agglutinin (green) and analyzed with a confocal laser scanning microscope. Bars represent 50 µm.

The mutualistic interaction in AM is characterized by an exchange of mineral nutrients and carbon. Data about the regulatory function of carbon availability on AM formation, however, are limited. Therefore, the effect of enhanced root hexose levels, the main form of carbohydrate used by the fungus, on AM formation was analyzed. Modulation of the root carbohydrate status in tobacco was achieved by root-specific expression of a gene encoding a yeast-derived invertase, targeted to the apoplast (plants kindly provided by U. Sonnewald, University of Erlangen). Despite increased root hexose levels we did not detect any effect on the colonization with *G. intraradices* analyzing fungal structures as well as the levels of fungus-specific fatty acids or the plant phosphate contents. Carbohydrate supply for AM fungi, accordingly, cannot be improved by increasing specifically root hexose levels, implying that under normal

conditions carbon supply is not limiting for the AM symbiosis. In contrast, plants with decreased acid invertase activity in roots obtained by root-specific overexpression of an invertase inhibitor (plants kindly provided by Thomas Roitsch, University of Würzburg) exhibited a diminished mycorrhization. Surprisingly, plants with moderately increased invertase activity within the leaves showed a stimulation of mycorrhization. Compared to wild-type, a higher degree of mycorrhization accompanied by a higher density of fungal structures and a higher amount of *G. intraradices*-specific rRNA was detected. The roots of those plants exhibited lower levels of some metabolites (phenolic compounds, amines and amino acids) as well as an increased abscisic acid content compared to wild-type (cooperation with Joachim Kopka, MPI Potsdam/Golm and Jutta Ludwig-Müller, Technical University of Dresden). This points to changes in metabolism and defense status of the plant root due to the increased invertase activity within the shoot.

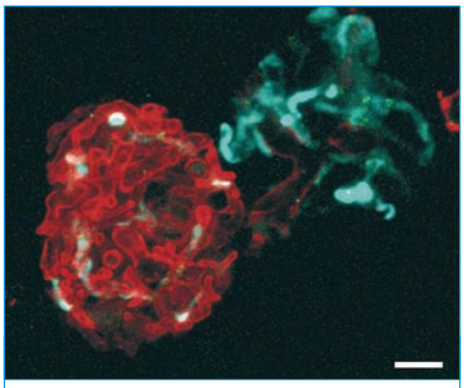

**Fig. 3:** Immunolocalization of the plastid-located protein 1-deoxy-D-xylulose 5-phosphate reductoisomerase (green) in root cortical cells containing an intact and a decomposing arbuscule (red). Tubular plastids containing the enzyme are predominantly observed close to the decomposing arbuscule. Bar represents 5 µm.

Previous work had revealed significant reorganization of plastid shape and metabolism in root cortical cells colonized by AM fungi. Plastids are producing a number of primary metabolites (like fatty acids and amino acids) essential for the establishment of symbiotic structures, in addition they are producing carotenoid cleavage products (apocarotenoids) specific for the AM symbiosis. To obtain a clearer correlation of the development of symbiotic structures and of plastid reorganization the plastid division protein FtsZ, the plastid enzyme DXR (1-deoxy-D-xylulose 5-phosphate reductoisomerase, involved in the formation of apocarotenoids) and a plastid-targeted green fluorescent protein together with fungal structures were colocalized. This analysis allowed a clear distinction of two phases of plastid proliferation in root cortical cells colonized by AM fungi. In the first phase, during the formation of arbuscules, plastids are intensively dividing leading to a large number of lens-shaped organelles between the arbuscular branches. The second phase of proliferation, during arbuscule degradation, is characterized by a smaller number of largely elongated organelles surrounding the decomposing arbuscule. Some rings of the division protein FtsZ are located along these tubular plastids indicating either a fusion/fission mechanism or the beginning of the final degradation of these plastids. The observation of tubular plastids in the second phase corresponds to the formation of apocarotenoids and of reactive oxygen species during arbuscule degradation. One possible functional role of plastids during this phase might be the recycling of metabolites (e.g. ammonia) liberated from decaying arbuscules. Further approaches regarded the examination of arbuscule development in tobacco plants severely disturbed in plastid division and the establishment of an inducible system for the analysis of arbuscule degradation.

The generation and subsequent growth of adventitious roots in stem tissues of excised leafy cuttings is a crucial physiological process in propagation of many ornamental plant species, among them *Petunia hybrida*. Despite intensive control of environmental factors in the modern propagation industry, high economic losses still occur due to insufficient rooting. The formation of adventitious roots is wound-induced and represents a multistage developmental process involving specific gene expression events. To investigate the possible function of the wound-related hormone JA during this process, a cDNA coding for AOC in *P. hybrida* (*PhAOC*) was isolated. Analyses of transcript and protein levels in the time-course of adventitious root formations were done. In future, the *PhAOC*-cDNA will be used for modulating the endogenous JA content in *P. hybrida* by its constitutive overexpression ("gain-of-function") or its suppression *via* RNA*i* ("loss-of-function").

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**Fig. 4:** Flowers of *Petunia hybrida* cv. Mitchell.

# Metabolite Profiling & Biochemistry of Proteins

*Heads: Willibald Schliemann & Thomas Vogt*

**In the framework of the DFG focus program 1084 *Molecular Basics of Mycorrhizal Symbioses* metabolite profiling was used to analyze the pattern of primary and secondary metabolites of developing arbuscular mycorrhizal roots of *Medicago truncatula* colonized with *Glomus intraradices* with the aim to characterize metabolic processes relevant for mycorrhiza development and symbiotic functioning. The generation and application of different biostatistical tools enabled comprehensive data evaluation and presentation. The established metabolite profiling platform will be applied in a running BMBF project with Canadian partners in the development of low sinapine rape. Recent developments in plant natural product transferase research have largely focused on two aspects: first, a detailed molecular study to correlate the observed differences in the structural domains of two distinct clusters of cation-dependent *O*-methyltransferases with observed *in vitro* substrate and position specificities; second, an investigation focused on the natural diversity of the transferase superfamilies to identify and target developmentally and tissue-specific genes and proteins for their specific roles and specificities *in planta*, one of the primary adaptive mechanisms of plants to meet the changing environmental conditions in a timely and developmentally controlled manner.**

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## Metabolite Profiling

In a time course study, the changes of primary and secondary metabolites in roots of barrel medic (*Medicago truncatula*) during the symbiotic interaction with the arbuscular mycorrhizal (AM) fungus *Glomus intraradices* were analyzed by GC-MS, HPLC and LC-MS and compared to nonmycorrhizal controls receiving different phosphate nutrition to discriminate between nutrition- and symbiosis-related effects. The metabolite identification was based on authentic standards as well as the plant-specific Golm metabolome database. Fungus-specific fatty acids from spores and hyphae of *G. intraradices* were used as biomarkers for estimation of the mycorrhization degree. Elevated levels of certain amino acids (Glu, Asp, Asn) and fatty acids indicate an activation of the plastidial metabolism. These results refer to an general activation of mitochondrial (tricarboxylic acid cycle) as well as plastid metabolism (lipid biosynthesis, N-assimilation) during mycorrhization, which correlates with corresponding increased transcript levels (*in silico* and RealTime RT-PCR analyses – T. Fester, IPB Halle, Germany). Additionally, the biosynthesis of some constitutive isoflavonoids, particularly at later developmental stages, was stimulated, which may be related to the mycorrhiza-induced expression of a glucosyltransferase (GT) gene in a similar system. Saponin levels showed only a growth-related increase. Mycorrhiza-specific apocarotenoids (cyclohexenone and mycorradicin derivatives) accumulated parallel to root mycorrhization. Structures of the cyclohexenone derivatives were elucidated as glucosides of blumenol C and 13-hydroxyblumenol C and their corresponding malonyl conjugates. Typical cell wall-bound phenolics were not altered, while tyrosol with reported antifungal properties was found as a major phenolic component only in mycorrhizal roots. Principal component analyses of nonpolar primary metabolites and secondary metabolites clearly separate AM roots from those of controls, an additional grouping in harvest days is given by secondary metabolites. These results are confirmed by hierarchical cluster analysis. Pearson correlation between primary nonpolar metabolite pairs showed stronger and more frequent relation in mycorrhizal roots and those supplied with high levels of phosphate than in the nonmycorrhizal controls indicating a tighter control of the activated root primary metabolism. Network correlation analyses revealed distinct clusters of amino acids and sugars/aliphatic acids with strong metabolic correlations among one another. Mycorrhizal symbiosis reduces the cluster separation and enlarges the sugar cluster size. The amino acid clusters represent cliques (groups of metabolites with strong correlations among one another) differently composed in nonmycorrhizal and mycorrhizal roots. The

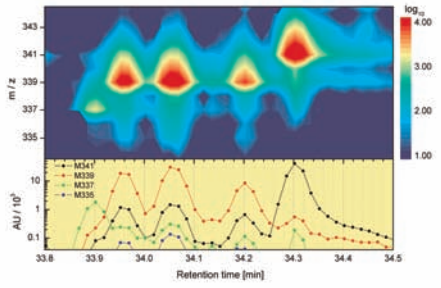


**Fig. 1:** Retention time – m/z plot of GC/TOF-MS of selected fatty acids from *Glomus intraradices*. From left to right: C18:1$\Delta^{9c}$, C18:1 $\Delta^{11c}$, C18:1 $\Delta^{11t}$, C18:0.

results from metabolite profiling provide clear distinction between development-, nutrition (phosphate)- and symbiosis (mycorrhiza)-mediated alterations of root metabolism.

## BIOCHEMISTRY OF PROTEINS

Promiscuous substrate specificities and low amino acid sequence identities to the highly conserved caffeoyl-coenzyme A *O*-methyltransferases (CCoAOMTs) characterize members of a new subcluster of cation-dependent OMTs from various plant species. The conserved position specificity towards aromatic vicinal di- or trihydroxy systems is in contrast to their broad substrate specificity, including aromatics, (iso)flavonoids, and phenylpropanoid conjugates, like caffeoylglucose. Structural information of one the newly discovered OMTs from *Mesembryanthemum crystallinum* (PFOMT), indicates that there are only two domains, which distinguish the 3D-structure of this protein from the 3D-structure of a caffeoyl-CoA-specific enzyme from a recently crystallized CCoAOMT from *Medicago sativa*: the first 15 amino acids of the N-terminus and a C-terminal loop. As in case of the GTs, the N-terminal part of the enzyme is important for the position specificity but not for the substrate specificity of the promiscuous type of enzymes. Various N-terminally truncated versions and some PFOMT/CCoAOMT chimeric proteins were designed to analyze the influence of the corresponding domains on the specificity and efficiency. The lack of several amino acids (as observed also for the native plant enzyme) results in a more position-specific, but also less efficient enzyme. Chimeric PFOMT/CCoAOMT constructs, where the N-terminal parts were derived from the specific CCoAOMT from *M. sativa* resulted in more specific enzymes, but also had a negative impact on efficiency. The turnover rates dropped with all substrates, but the most serious drop was observed with caffeic acid, which is not accepted at all by the chimeric constructs. A remarkable change is observed if both, the loop and the N-terminus of PFOMT, are exchanged simultaneously with the respective CCoAOMT-domains. In this case a further, drastic drop in catalytic efficiency can be detected for all substrates, except for caffeoyl-CoA. The transformation from a promiscuous towards a caffeoyl-CoA-specific enzyme was accomplished. Modification of substrate specificity while maintaining the required efficiency may require further changes on the amino acid level. The drop in specificity by changing the bivalent cations within the active site may add additional complexity to *in vivo* roles and specificities.

The differences in the position specificity of individual enzymes reflect the diversity of the corresponding CCoAOMT-like members on the genomic and proteomic level. Apparently, Arabidopsis requires different CCoAOMT-like proteins of seven transcriptionally active genes, which are developmentally and tissue specifically regulated. The product of at least one of those genes shows highest identity in terms of amino acid sequence as well as substrate specificity to PFOMT, and can clearly be distinguished from the caffeoyl-CoA-specific isoenzyme likely involved in lignin formation. The expression of the promiscuous variety in reproductive organs (flowers, seeds) at the end of plant development and its preference for caffeoylglucose suggests an interesting *in vivo* role of this protein other than lignin formation. A proposed combination of affinity purification, 2-D PAGE, and mass spectrometry should combine and resolve tissue- and developmentally specific arrays of transferases like glucosyl-, acyl- or the (cation-dependent) *O*-methyltransferases rather than focusing on single enzymes. The initial focus aims at characterizing the pattern and abundance of these enzymes in seeds of Arabidopsis and *Brassica napus*. Both tissues deliver the required structurally and developmentally limited systems to correlate transferase patterns with the secondary metabolite patterns and will also enable to identify new transferases with unknown new specificities.

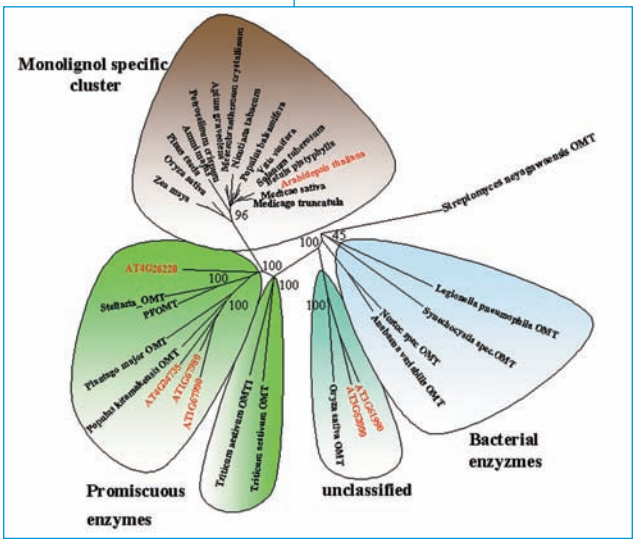


**Fig. 2:** Distribution of the seven cation-dependent enzymes from Arabidopsis (marked in red) within several clusters of promiscuous or specific plant and microbial CCoAOMT-like proteins.

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Tonleu Tonfack, Rostand: Einfluss von Epothilonen und verwandten Substanzen auf das Cytoskelett von *Arabidopsis thaliana* Wurzelspitzen. University of Halle-Wittenberg, Department of Biochemistry/Biotechnology, 20/09/2005.

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Cenzano, A., Abdala, G. & Hause, B. Cytochemical immuno localization of allene oxide cyclase, a jasmonic acid biosynthetic enzyme, in developing potato stolons. *J. Plant Physiol.*

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Hause, B., Frugier, F. & Crespi, M. Immunocytochemistry. In: *The Medicago truncatula handbook.* http://www.noble.org/MedicagoHandbook/

### DIPLOMA THESES 2006

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Knick, Paul: Die systemische Wundantwort zwischen Wurzel und Spross der Tomate (*Lycopersicon esculentum*), University of Halle-Wittenberg, Department of Biochemistry/Biotechnology, 07/09/2006.

### DOCTORAL THESES 2006

Lohse, Swanhild: Untersuchung der Plastidenentwicklung bei der arbuskulären Mykorrhiza an der Modellpflanze *Medicago truncatula*. University of Halle-Wittenberg, Department of Pharmacy, 25/01/2006.
Schmidt, Diana: Die Sinapoylglucose:Cholin-Sinapoyltransferase aus *Brassica napus*. University of Halle-Wittenberg, Department of Biology, 02/11/2006.

### HABILITATIONS 2006

Vogt, Thomas: Plant Natural Product Glycosyl- and Methyltransferases, University of Halle-Wittenberg, Department of Biochemistry/Biotechnology, 08/03/2006.

Fester, Thomas: Struktur und Metabolismus der Plastiden kolonisierter Wurzelzellen bei der arbuskulären Mykorrhiza, University of Halle-Wittenberg, Department of Agricultural and Nutritional Science, 18/12/2006.

## Administration and Technical Services

*Head: Lothar Franzen*

*Secretary: Cindy Maksimo*

During the past two years, two construction projects have dominated the activities of the IPB administration. The construction of additional fully climate-controlled greenhouses was completed in April 2005. In August 2005, construction of a new building (House E) for the employees of facilities, construction management, graphic arts, horticulture and members of the bioinformatic group was initiated. The project was finished in December 2006. The total construction cost of each building was 2.4 million Euros and was provided by the Federal Ministry of Education and Research and by the Ministry of Education and Cultural Affairs of the State of Saxony-Anhalt.

The new greenhouses, with an additional 350 square meters of floor space, met the growing demand of the departmental research programs involving transgenic plants. The nine air-conditioned chambers were opened for cultivation of tomato, poppy, tobacco, rape, and plants from the mycorrhization projects. In addition, there are two tropical chambers and incubators for Arabidopsis. The technical equipment for the fine control of climatic conditions, as light, shadow, temperature and humidity is state-of-the-art and costs about 1.1 million Euros.

Two new buildings replaced the building that accomodated employees of facilities and construction management, graphic arts and horticulture. Within this new complex, an additional 200 square meters of laboratorys space for 15 new working places for biological and chemical research of junior research groups were created, as well as new offices for the bioinformaticians of the *Department of Stress and Developmental Biology*.

Construction of the new green houses was completed in April 2005.

The new building House E.

### Accounting
**Head: Barbara Wolf**
Maike Hildebrandt
Gudrun Schildberg
Kerstin Wittenberg

### Chauffeur
Jürgen Gaul until January 2006
Matthias Barth since February 2006

### Computer Support
**Head: Hans-Günter König**
Holger Bartz
Matthias Franke until December 2006
Ronald Scheller
Kevin Begrow, Trainee until September 2006
Marcel Volkmann, Trainee
Tibor Sari, Trainee since September 2006

### Construction Management
**Head: Heike Böhm**
Catrin Timpel

### Facilities Management
**Head: Michael Kräge**
Carsten Koth
Jörg Lemnitzer
Klaus-Peter Schneider
Eberhard Warkus

### General Administration
**Head: Rosemarie Straßner**
Heide Pietsch until April 2006
Clemens Schinke
Elviera Schotte
***Trainees***
Ines Deák since December 2006
Caroline Stolzenbach since September 2006
Kristin Weinert since December 2006

### Graphic Arts
**Head: Christine Kaufmann**
Annett Kohlberg

### Horticulture
**Head: Iris Rudish**
Martina Allstädt
Annett Grün, Trainee
Christian Müller
Philipp Plato, Trainee
Kristina Rejall,
Steffen Rudisch
Katja Scheming
Andrea Voigt
Sabine Vogt

### Human Resources
**Head: Kerstin Balkenhohl**
Alexandra Burwig
Claudia Haferung-Bornmann since September 2006
Kathleen Weckerle until August 2006

### Library Services
**Head: Andrea Piskol**
Anja Gärtner, Trainee

### Press and Public Relations
**Head: Sylvia Pieplow**
Susanne Kubenz since June 2005

### Stockroom
Hans-Jürgen Steudte

| EMPLOYEE STATISTICS | 2005 | 2006 |
|---|---|---|
| AVERAGE NUMBER OF MMPLOYEES | 188 | 180 |
| Full-time employees in % | 61 | 65 |
| Part-time employees in % | 39 | 35 |
| Permanent positions | 92 | 92 |
| Temporary positions | 26 | 23 |
| Employees externally funded | 43 | 38 |
| Employees remunerated by "University Science Funds Program" (Hochschulwissenschaftsprogramm / HWP) | 5 | 3 |
| Proportion of female employees in % | 58 | 60 |
| Personnel turnover in % | 9 | 29 |
| Averade age of employees in years | 37 | 37 |
| Scholarship / fellowship holders | 27 | 34 |
| VOCATIONAL TRAINING | | |
| general administration | 3 | 3 |
| horticulture | 2 | 2 |
| library services | 1 | 1 |
| computer support | 2 | 2 |
| research technical assistant | 2 | 3 |
| Succesfully completed vocational training | 2 | 4 |
| Average number of apprentices | 10 | 11 |

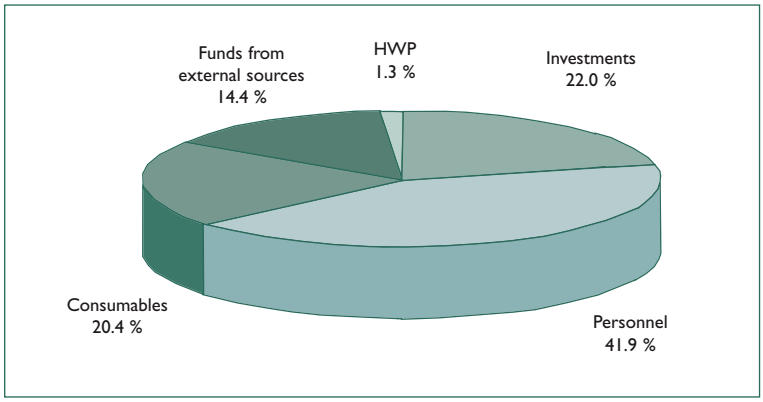
## Resources and Investments & Funds from External Sources in 2005 and 2006

**Research grants listed on this and the following pages were given by**

| | |
|---|---|
| **Bionorica** | Bionorica AG |
| **BMBF** | Bundesministerium für Bildung und Forschung - *Federal Ministry for Education and Research* |
| **BPS** | BASF Plant Science |
| **DAAD** | Deutscher Akademischer Austauschdienst - *German Academic Exchange Service* |
| **DBU** | Deutsche Bundesstiftung Umwelt - *German Environmental Foundation* |
| **DFG** | Deutsche Forschungsgemeinschaft *German Research Foundation* |
| **Elsevier** | Elsevier Science Publisher |
| **EU** | European Union |
| **Firmenich** | Firmenich Company |
| **Hopsteiner** | Hopsteiner Company |
| **HWP** | Hochschulwissenschafts-programm - *University Science Funds Program* |
| **Icon Genetics** | Icon Genetics AG |
| **MK-LSA** | Kultusministerium des Landes Sachsen-Anhalt - *Ministry of Education and Cultural Affairs of the State of Saxony-Anhalt* |
| **MLU** | Martin-Luther-Universität Halle-Wittenberg - *University of Halle* |
| **Probiodrug** | Probiodrug AG |

| | in Mio. Euro | in % |
|---|---|---|
| **Basic Financing Funds (2005 + 2006)** | | |
| Personnel | 9.9 | 41.9 |
| Consumables | 4.6 | 19.6 |
| Grants / Subsidies | 0.2 | 0.8 |
| Investments | 5.2 | 22.0 |
| University Science Funds Program (HWP) | 0.3 | 1.3 |
| Subtotal | 20.2 | |
| **Funds from External Sources (2005 + 2006)** | | |
| BMBF | 0.9 | 3.9 |
| MK-LSA | 0.1 | 0.4 |
| DFG | 1.8 | 7.6 |
| Industry | 0.3 | 1.3 |
| EU | 0.2 | 0.8 |
| other | 0.1 | 0.4 |
| Subtotal | 3.4 | |
| **Total** | **23.6** | **100.0** |

| | in Million Euro |
|---|---|
| **Investments** | |
| Equipment | 2.4 |
| Construction | 2.8 |
| **Total** | **5.2** |



| Title & Head of Project | Duration | Financial Source | Amount in Euro (2005-2006) | Personnel Posts Funded |
|---|---|---|---|---|
| **DEPARTMENT OF NATURAL PRODUCT BIOTECHNOLOGY** | | | | |
| Jasmonate biosynthesis regulation<br>*C. Wasternack & O. Miersch* | 04/05 | DFG | 13,000 | 1 |
| 12-Hydroxyjasmonic acid<br>*C. Wasternack & O. Miersch* | 03/06 | DFG | 26,100 | 1 |
| Glutamatcyclase<br>*C. Wasternack* | 01/05 | Probiodrug | 2,900 | 0 |
| Allene oxide cyclase<br>*C. Wasternack* | 01/05 | Firmenich | 7,600 | 0 |
| Analysis of genes<br>*T. Kutchan* | 00/05 | Icon Genetics | 14,200 | 0 |
| Molecular genetics of isoquinoline alk. biosynth.<br>*T. Kutchan* | 04/05 | DFG | 31,200 | 2 |
| Molecular genetics in *Liana Triphyoph.* Pellatum<br>*T. Kutchan* | 03/05<br>05/06 | DFG<br>DFG | 36,000<br>42,500 | 1 |
| Metabolic engineering<br>*S. Frick* | 04/06 | DFG | 78,500 | 2 |
| 12-Hydroxyjasmonat-Arabidopsis<br>*C. Wasternack & O. Miersch* | 05/08 | DFG / SFB 648 | 176,000 | 2 |
| HUM-NEU<br>*J. Page & T. Kutchan* | 03/05 | Hopsteiner | 19,500 | 1 |
| Genexpressionsanalyse in Papaver-Spezies<br>*J. Ziegler* | 05/07 | DFG | 32,200 | 1 |
| Heterodimerbildung als Regulationsmechanismus der Allenoxidcyclase in Arabidopsis<br>*C. Wasternack & B. Hause* | 2006 | MK-LSA / MLU | 4,500 | 0 |
| **Subtotal** | | | **484,200** | **11** |

| | | | | |
|---|---|---|---|---|
| **DEPARTMENT OF BIOORGANIC CHEMISTRY** | | | | |
| HEANTOS<br>*L. Wessjohann* | 05/06 | BMBF | 96,300 | 2 |
| MCR ligand synthesis<br>*L. Wessjohann* | 04/06 | DAAD / Probral | 11,400 | 0 |
| Reactivity of selenopeptides<br>*L. Wessjohann & W. Brandt* | 03/05<br>04/06 | DFG<br>DFG | 7,500<br>49,600 | 1<br>1 |
| CERC-3<br>*L. Wessjohann* | 04/06<br>06/07 | DFG<br>DFG | 45,700<br>27,800 | 1 |
| HUMULUS<br>*L. Wessjohann* | 03/05 | Hopsteiner | 9,100 | 0 |
| Fungi Metabolites<br>*N. Arnold & J. Schmidt* | 04/06 | DFG | 33,400 | 1 |
| Virtual Screening<br>*L. Wessjohann* | 04/07 | Wella | 108,200 | 1 |
| Benzopyrane<br>*L. Wessjohann* | 05/07 | Bionorica | 69,600 | 1 |

## Funds from External Sources in 2005 and 2006

| Title & Head of Project | Duration | Financial Source | Amount in Euro (2005-2006) | Personnel Posts Funded |
|---|---|---|---|---|
| **Department of Bioorganic Chemistry** | | | | |
| Mannich Diversity<br>*B. Westermann* | 05/07 | DFG | 82,200 | 1 |
| Chalcogen catalysts<br>*L. Wessjohann* | 06/07 | DFG | 61,700 | 1 |
| Prenylierende Enzyme<br>*W. Brandt & L. Wessjohann* | 05/07 | DFG | 29,700 | 1 |
| DBU-Acyloine<br>*L. Wessjohann* | 06/08 | DBU / Uni Greifswald | 4,700 | 0 |
| **Subtotal** | | | **636,900** | **11** |
| **Department of Stress- and Developmental Biology** | | | | |
| Metalhome<br>*S. Clemens* | 03/06 | EU | 130,200 | 1 |
| NODO<br>*S. Rosahl* | 02/05 | EU | 63,300 | 1 |
| Bioinformatics and mass spectrometry<br>*D. Scheel* | 05/07 | BMBF | 272,200 | 2 |
| Resistence in potatoes<br>*D. Scheel & S. Rosahl* | 04/06 | DFG | 28,500 | 1 |
| GABI-NONHOST<br>*D. Scheel* | 02/05 | BPS / BMBF | 78,700 | 2 |
| Metal homeostasis<br>*S. Clemens* | 04/06 | DFG | 125,900 | 1 |
| GABI-GENOPLANTE<br>*S. Clemens* | 04/06 | BMBF | 64,100 | 1 |
| SARA<br>*D. Scheel* | 04/07 | BMBF | 116,700 | 1 |
| Molekulare Kommunikation von *R. secalis*<br>*W. Knogge* | 05/08 | DFG / SFB 648 | 68,900 | 1 |
| Oxylipine bei Pathogenabwehr<br>*S. Rosahl* | 05/08 | DFG / SFB 648 | 103,000 | 2 |
| MAPK-Kaskaden in *A. thaliana*<br>*D. Scheel* | 05/08 | DFG / SFB 648 | 170,000 | 2 |
| Transcriptome and proteome analysis of pathogen attacked barley epidermis<br>*W. Knogge* | 05/06 | MK-LSA MLU | 16,900 | 0 |
| Einfluss der Chromatinstruktur auf die Wechselwirkung von *A. thaliana* mit versch. Pathogenen<br>*D. Scheel* | 2006 | MK-LSA MLU | 9,000 | 0 |
| Role of MAPKs in female gametophyte / embryo development<br>*J. Lee* | 2006 | MK-LSA MLU | 9,000 | 0 |
| **Subtotal** | | | **1,256,400** | **15** |

| Title & Head of Project | Duration | Financial Source | Amount in Euro (2005-2006) | Personnel Posts Funded |
|---|---|---|---|---|
| **DEPARTMENT OF SECONDARY METABOLISM** | | | | |
| Metabolism of isoprenoids<br>*M. H. Walter* | 04/06 | DFG | 48,400 | 1 |
| The role of jasmonates during the establishment of mycorrhiza<br>*B. Hause & D. Strack* | 04/06 | DFG | 59,600 | 1 |
| Carotenoid biosynthesis in arbuscular mycorrhizal roots<br>*T. Fester* | 04/06 | DFG | 50,700 | 1 |
| Metabolite Profiling<br>*W. Schliemann* | 04/06 | DFG | 128,800 | 1 |
| Phytochemistry<br>*D. Strack* | 02/07 | Elsevier | 44,700 | 1 |
| HCA-Glucosyltransferases<br>*C. Milkowski & A. Baumert* | 03/05<br>05/06 | DFG<br>DFG | 2,000<br>31,800 | 1 |
| HCA-Glucosyltransferases<br>*C. Milkowski* | 06/08 | DFG | 17,900 | 1 |
| SCPL-Acyltransferases<br>*C. Milkowski & D. Strack* | 03/05<br>05/07 | DFG<br>DFG | 15,300<br>49,300 | 1 |
| DXS-Isoenzyme<br>*M. H. Walter* | 05/07 | DFG | 59,900 | 1 |
| Struktur und Funktion der Sinapinesterase<br>*D. Strack* | 05/07 | DFG | 65,500 | 1 |
| PFOMT<br>*T. Vogt* | 04/06 | DFG | 9,800 | 0 |
| DXS-Evolution<br>*M. H. Walter* | 06/07 | DFG | 20,100 | 1 |
| Caffeoylglucarat-Synthase<br>*D. Strack & B. Hause* | 06/09 | DFG | 22,100 | 1 |
| YellowSin Rape Seed<br>*D. Strack* | 06/09 | BMBF | 72,000 | 3 |
| Heterodimerbildung als Regulationsmechanismus der Allenoxidcyclase in Arabidopsis<br>*C. Wasternack & B. Hause* | 2006 | MK-LSA MLU | 4,500 | 0 |
| Jasmonat, arb. Mykorrhiza, Cytoskelett<br>*B. Hause* | 2006 | DFG | 9,800 | 0 |
| **Subtotal** | | | **712,200** | **15** |

## Funds from External Sources in 2005 and 2006

| Title & Head of Project | Duration | Financial Source | Amount in Euro (2005-2006) | Personnel Posts Funded |
|---|---|---|---|---|
| **Joint Projects** | | | | |
| GABI 2<br>*D. Scheel, S. Clemens, L. Wessjohann & J.* | 04/07 | BMBF | 296,500 | 3 |
| **Subtotal** | | | **296,500** | **3** |
| **Projects granted total** | | | **3,386,200** | **55** |
| **General Overview** | | | | |
| BMBF | | | 917,800 | 12 |
| MK-LSA | | | 43,900 | 0 |
| DFG | | | 1,860,400 | 35 |
| Industry | | | 302,200 | 5 |
| EU | | | 193,500 | 2 |
| Other Sources | | | 68,400 | 1 |
| **Total** | | | **3,386,200** | **55** |
| HWP | | | 283,100 | 2 |
| **Total** | | | **3,669,300** | **57** |

# INVOLVEMENT OF THE IPB IN NATIONAL AND INTERNATIONAL SCIENTIFIC NETWORKS

### BIOSCIENTIFIC NETWORK OF SAXONY-ANHALT
Structures and Mechanisms of Biological Information Processing.

### CERC 3
Chairmen of the European Research Councils Chemistry Committees
*German Research Foundation*

### EVOMET
Evolution of Metabolic Diversity
*Priority Program 1152 of the German Research Foundation*

### GABI
Genome Analysis
of the Plant Biological System
*German Federal Ministry for Education, Research and Technology (BMBF) and Business Companies*

### GABI-NONHOST
A consortium-based functional genomics initiative on plant non-host disease resistance
*GABI 1b*

### METABOLOMICS PLATFORM
Metabolite Profiling
in Arabidopsis and Crop Plants
*GABI 2*

### SARA
Functional genomics of local and systematic acquired resistance in Arabidopsis
*GABI - trilateral Cooperation, France, Germany, Spain*

### COMPARATIVE GENOMICS
Comparative genomics between Arabidopsis and Brassica for genes directing seed-specific flavonoid biosynthesis
*GABI - Génoplante - bilateral Cooperation, Germany, France*

### HEANTOS
A Vietnamese opiate detoxification symptom medication
*German Federal Ministry for Education, Research and Technology (BMBF)*

### MOLECULAR ANALYSIS OF PHYTOHORMONE ACTION
*Priority Program SPP 1067 of the German Research Foundation*

### MOLECULAR MECHANISMS OF INFORMATION PROCESSING IN PLANTS
*Collaborative Research Center SFB 648 of the German Research Foundation*

### MOLMYK
Molecular Basics of Mycorrhizal Symbioses
*Priority Program 1084 of the German Research Foundation*

### ORGANOCATALYSIS
*Priority Programm 1179 of the German Research Foundation*
*- Mannich Diversity (Prof. Westermann)*
*- Chalcogen Catalysts (Prof. Wessjohann)*

### SELF-ORGANIZATION THROUGH COORDINATION AND NON- COVALENT INTERACTIONS
*Graduate Program 894 of the German Research Foundation*

### SELENOPROTEINS
*Priority Programm SPP 1087 of the German Research Foundation*

### YELLOWSIN
Functional genomic approaches for the development of yellow-seeded, low sinapine oilseed rape (Canola; *Brassica napus*)
*GABI-Kanada, bilateral cooperation*

**JANUARY 13**
**Prof. Udo Johanningmeier,**
*University of Halle, Germany*
Genetic engineering of photosystem II: Potential role of methionine residues as antioxidants.

**FEBRUARY 10**
**Dr. Frantisek Baluska**
*University of Bonn, Germany*
Actin cytoskeleton, myosin VIII and polar transport of auxin: Lessons from maize root apices.

**FEBRUARY 17**
**Prof. Michael Bölker**
*University of Marburg, Germany*
The small GTPases Cdc42 and Rac1 regulate cytokinesis and dimorphism in the phytopathogenic fungus *Ustilago maydis.*

**MARCH 23**
**Prof. Karsten Krohn**
*University of Paderborn, Germany*
Computerunterstützte Synthese von chiralen Bausteinen für Makrolide aus Anhydrozuckern.

**APRIL 6**
**Prof. Karlheinz Drauz**
*Degussa AG Düsseldorf, Germany*
Bedeutung der Katalyse in der industriellen Feinchemie.

**APRIL 13**
**Prof. Uwe Conrath**
*University of Aachen, Germany*
Priming in plants: It is all the world to induced disease resistance

**APRIL 14**
**Prof. Petra Dietrich**
*University of Erlangen, Germany*
Redox regulated calcium channels in Arabidopsis mesophyll cells.

**APRIL 21**
**Prof. Jerzy Paszkowski**
*University of Genf, Switzerland*
Genetics of epigenetic regulation of transcription in Arabidopsis.

**MAY 11**
**Prof. Tim Clark**
*University of Erlangen, Germany*
New concepts for theoretical studies of intermolecular interactions.

**MAY 17**
**Dr. Charl FJ Faul**
*University of Bristol, UK*
Functional nanostructures from ionic interactions - some concepts and general principles.

**MAY 25**
**Prof. Claus E. Schäfer**
*University of Copenhagen, Denmark*
The present day importance of ligand-field theory in a historical perspective.

**MAY 31**
**PD Alexander Dömling**
*R&D Biopharmaceuticals GmbH, Martinsried, Germany*
Recent advancement in multicomponent reaction chemistry.

**JUNE 2**
**Prof. Kurt Eger**
*University of Leipzig, Germany*
Beiträge zur Arzneimittel(weiter)entwicklung an der Hochschule.

**JUNE 7**
**Dr. Günter Hempel**
*University of Halle, Germany*
Verfolgung der Kristallisation von Seitenketten in Polymeren mittels $^{13}C$-CPMAS.

**JUNE 14**
**Dr. Christoph A. Schalley**
*University of Bonn, Germany*
Templateffekte und Selbstorganisation zum Aufbau komplexerer funktionaler Strukturen.

**JUNE 15**
**Prof. Karl Jug**
*University of Hannover, Germany*
Clustermodelle und Molekulardynamik: Neue Methoden für Festkörper, Ober - flächen und Katalyse.

**JUNE 23**
**Prof. Andrzej Guranowski**
*University of Poznan, Poland*
Uncommon dinucleotides - putative sig - naling compounds and enzymes involved in their metabolism.

**JULY 6**
**Prof. Christoph Schneider**
*University of Leipzig, Germany*
Stereoselektive Synthese mit chiralen Auxiliaren und Katalysatoren.

**JULY 12**
**Dr. Jochen Balbach**
*University of Halle, Germany*
Untersuchung der Proteinfaltung mit NMR-Spektroskopie.

**JULY 13**
**Prof. Martin Quack**
*University of Copenhagen, Danmark*
Molekülspektroskopie und kinetische Primärprozesse zwischen Yoctosekunden und Jahrmilliarden.

**AUGUST 4**
**Dr. Siegbert Melzer**
*University of Ghent, Belgium*
Impacts of flowering time genes on plant growth.

**SEPTEMBER 8**
**Prof. Paola Bonfante**
*University of Turin, Italy*
Roots, mycorrhizal fungi and endobacteria: The development of an underground web.

**SEPTEMBER 15**
**Prof. Cathie Martin,**
*John Innes Centre, Norwich, UK*
The molecular genetics underpinning development of specialised pollination syndromes in Solanaceous plants.

**SEPTEMBER 20**
**Stefan Gröger**
*University of Halle, Germany*
NMR-Anwendungsbeispiele aus der pharmazeutischen Industrie.

**SEPTEMBER 22**
**Prof. Peter Meyer**
*University of Leeds, UK*
Natural antisense transcripts in plants.

**SEPTEMBER 29**
**Prof. Klaus Harter**
*University of Tübingen, Germany*
Plant two-component systems: Principles, functions, complexity and cross talk.

**OCTOBER 5**
**Dr. Erich Glawischnig**
*Technical University of Munich, Germany*
Biosynthesis of camalexin.

**OCTOBER 6**
**Pascal Arnoux,**
*Laboratoire de Bioénergétique Cellulaire, CEA, Cadarache, France*
A papain-like enzyme at work: Native and acyl-enzyme intermediate structures in phytochelatin synthesis.

**OCTOBER 12**
**Dr. Deepti Dwivedi**
*University of Basel, Switzerland*
Interactions between arbuscular mycorrhizal fungi and antagonistic bacteria (*Pseudomonas fluorescens* and *Alcaligenes faecalis*) and their effects on *Vigna mungo*.

**OCTOBER 13**
**Prof. Sabeeha Merchant,**
*University of California, Los Angeles, USA*
Between a rock and a hard place: Copper homeostasis in Chlamydomonas.

**OCTOBER 17**
**Prof. Zeev Luz,**
*Weizmann Institute of Science, Rehovot, Israel*
NMR of methyl groups in the thermal and tunneling regimes.

**OCTOBER 20**
**Dr. Christine Oesterhelt**
*University of Potsdam, Germany*
Living on the edge - metabolic versatility of the unicellular red alga *Galdieria sulphuraria*.

**OCTOBER 26**
**Dr. Matthieu Arlat,**
*Institut National de la Recherche Agronomique/INRA Paris, France*
Xanthomonas TonB-dependent receptors: Iron or not iron?

**NOVEMBER 3**
**Prof. Georg G. Gross**
*University of Ulm, Germany*
Biosynthesis of hydrolyzable plant tannins - unraveled by enzyme studies.

**NOVEMBER 10**
**Prof. Maarten DeWaard**
*University of Wageningen, The Netherlands*
Functions of ABC transporters in plant pathogenic fungi.

**NOVEMBER 16**
**Dr. Joachim Kopka**
*Max Planck Institute of Molecular Plant Physiology, Golm, Germany*
Current challenges and developments in metabolite profiling.

**NOVEMBER 21**
**Manfred Knörgen**
*University of Halle, Germany*
*In-vivo* Beobachtung von Arzneiträgersystemen (Tabletten/Pellets) im Magen mittels Suszeptibilitätskontrast-MRT.

**NOVEMBER 23**
**Prof. Janos Szabad**
*University of Szeged, Hungary*
The tubulin-centrosome interplay.

**NOVEMBER 24**
**Dr. Claude Parsot**
*Institut Pasteur, Paris, France*
Control for transcription by the activity of the type III secretion apparatus in *Shigella flexneri*.

**NOVEMBER 29**
**Prof. Rainer Beckert**
*University of Jena, Germany*
Cycloamidine - wertvolle Bausteine in der Synthesechemie

**DECEMBER 5**
**Prof. Frank Glorius**
*University of Marburg, Germany*
Katalysator- und Reaktionsdesign für eine effiziente Synthese.

**DECEMBER 7**
**Prof. Bernhard Eikmanns**
*University of Ulm, Germany*
Die Bedeutung der PEP-Pyruvat-Oxalacetat-Verzweigung im Stoffwechsel des Aminosäureproduzenten *Corynebacterium glutamicum*.

**DECEMBER 8**
**PD Peter Spiteller**
*University of Munich, Germany*
Interaktion zwischen mykoparasitischen Pilzen und ihren Wirtspilzen - neue Ansätze zur Isolierung aktiver Naturstoffe.

**DECEMBER 15**
**Dr. Christian Eckmann,**
*Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany*
Regulating germ cell fate switches at the translational level - a theme of GoLD.

**DECEMBER 19**
**Dr. Thomas Bräuniger**
*University of Halle, Germany*
Exotische Anwendungen der NMR-Spektroskopie.

JANUARY 11
**PD Anke Becker**
*University of Bielefeld, Germany*
Postgenome approaches to *Sinorhizobium meliloti*: a model for nitrogen-fixing endosymbiotic soil bacteria.

JANUARY 17
**Dr. Ulf Mazurek**
*Hebrew University of Jerusalem, Israel*
Das protonierte Serin-Oktamer in der Gasphase.

JANUARY 20
**Prof. Ing. Miroslav Strnad**
*University of Olomouc, Czech Republic*
Biotechnological and medicinal applications of cytokinin analogues.

JANUARY 26
**Dr. Marcel Bucher**
*ETH Zürich, Switzerland*
Mechanisms and interactions in plant phosphate uptake.

FEBRUARY 7
**Hans Wolf Sünnemann**
*University of Göttingen, Germany*
Palladium-catalyzed coupling-sequences as diversity oriented efficient access to steroid-skeletons.

FEBRUARY 23
**Prof. Michael Göttfert**
*University of Dresden, Germany*
Regulation of nodulation and type III secretion genes in *Bradyrhizobium japonicum*.

MARCH 2
**Prof. Kazufumi Yazaki**
*University of Kyoto, Japan*
Plant ABC transporter, PGP4, catalyzes auxin transport in Arabidopsis roots.

MARCH 16
**Prof. Vincent Colot**
*Unité de Recherche en Génomique Végétale, INRA, CNRS, Evry, France*
Trans-generational inheritance of epigenetic variation in Arabidopsis: an epigenomic perspective.

MARCH 23
**Dr. Thomas Müller-Reichert**
*Max Planck Institute of Cell Biology and Genetics, Dresden, Germany*
Electron tomography of centriole assembly in *C. elegans*.

**Dr. Clemens Groepel**
*University of Berlin, Germany*
OpenMS - An open source software platform for mass spectrometry based proteomics.

APRIL 12
**Olaf Schröder**
*Pattern Expert, Borsdorf, Germany*
Detecting biomarkers in mass spectra with pattern recognition methods.

APRIL 25
**Dr. Hanne Schönig**
*University of Halle, Germany*
Ethnobotanisches aus dem Jemen.

APRIL 27
**Prof. Harry Klee**
*University of Florida, Gainesville, USA*
Synthesis and functions of plant volatile compounds. Some unexpected functions.

MAY 5
**Prof. Gregory P. Copenhaver**
*University of North Carolina, Chapel Hill, USA*
The power of four: exploring meiotic recombination in Arabidopsis.

MAY 9
**Prof. Peter Imming**
*University of Halle, Germany*
You can cure by appearances. Similiarity, biomimesis, and the connection between molecular and clinical effects.

MAY 10
**Prof. Hans-Günter Schmalz**
*University of Cologne, Germany*
Übergangsmetalle in der Synthese bioaktiver Verbindungen.

MAY 11
**Prof. John Mansfield**
*Imperial College London, UK*
Evolution of pathogenicity and virulence in *Pseudomonas syringae*: a tale of populations and papillae.

MAY 15
**Dr. Hendrik Metz**
*University of Halle, Germany*
Hallesches NMR-Seminar: EPR in der Pharmazie.

MAY 16
**Prof. Sabine Flitsch**
*University of Manchester, UK*
Biocatalysis on solid phase.

MAY 18
**Prof. Jeff Dangl**
*University of North Carolina, Chapel Hill, USA*
Molecular logic of the plant immune system.

MAY 19
**Prof. Sarah Grant**
*University of North Carolina, Chapel Hill, USA*
The bacterial virulence factor AvrPpiB makes plants more sensitive to ABA.

MAY 24
**Prof. Edward Farmer**
*University of Lausanne, Switzerland*
Genetic approaches to study lipoxygenase regulation and oxylipin biogenesis.

**Prof. Martin Feigel**
*University of Bochum, Germany*
Rings and knots of amino acids in peptide cavitands and steroid peptides.

MAY 29
**Prof. Paul Staswick**
*University of Nebraska, USA*
Plant hormone regulation by amino acid conjugating enzymes; jasmonate, auxins and more!

JUNE 1
**Prof. Gregg A. Howe**
*Michigan State University, USA*
Regulation of plant antiherbivore defense by the jasmonate signaling pathway.

JUNE 7
**Prof. Francois Buscot**
*Helmholtz Centre for Environmental Research Leipzig-Halle, Germany*
Exploring structural and functional diversity of soil and mycorrhizal fungi: why and how?

**Prof. Ute Wittstock**
*University of Braunschweig, Germany*
Metabolic diversity in an activated plant defense system.

JUNE 14
**Prof. Lukas Gooßen**
*University of Kaiserslautern, Germany*
Neue katalytische Methoden von der innovativen Synthesechemie zur "dream reaction".

JULY 13
**Dr. Antonio Molina**
*Centro Biotecnología Genómica de Plantas, Madrid, Spain*
Plant innate immunity and resistance to necrotrophic fungi: sense and defense.

JULY 20
**PD Peter Dörmann**
*Max Planck Institute of Molecular Plant Physiology Potsdam-Golm, Germany*
Biosynthesis and function of galactolipids

and vitamins in chloroplasts.
**Prof. Christiane Gatz**
*University of Göttingen, Germany*
Analysis of transcriptional control mechanisms in plant defense responses.

**Prof. Klaus D. Grasser**
*Aalborg University, Denmark*
Architectural chromosomal proteins and transcript elongation factors.

**Prof. Bernd Weisshaar**
*University of Bielefeld, Germany*
Regulation of flavonol biosynthesis by MYB transcription factors.

JULY 21
**Dr. Jürgen Kroymann**
*Max Planck Institute for Chemical Ecology Jena, Germany*
Functional & evolutionary genomics of Arabidopsis & friends.

**Dr. Arp Schnittger**
*University of Cologne, Germany*
Cell cycle control at the interface between development and environment.

**PD Joachim Uhrig**
*University of Cologne, Germany*
Protein interactions networks: functional plant proteomics and host-virus interactions.

**Dr. Ute Krämer**
*Max Planck Institute of Molecular Plant Phy - siology Potsdam-Golm, Germany*
Metal homeostasis: Extreme natural variation among closely related model plants in a complex functional network that is indispensable for plant biology.

JULY 27
**Prof. Axel Brakhage**
*University of Jena, Germany*
Secondary metabolites as virulence determinants of the human-pathogenic fungus *Aspergillus fumigatus.*

AUGUST 30
**Ronald Peltsch, Andreas Lenz**
*tekom - Gesellschaft für Technische Kom - munikation e.V. Halle, Germany*
Übersichtliche und verständliche Präsenta - tionen und Vorträge.

SEPTEMBER 7
**Dr. Ali Masoudi-Nejad**
*University of Teheran, Iran*
EGENES & EGassembler: new bioinformatics tools for the post-genome era.

**Dr. Mark Helm**
*University of Heidelberg, Germany*
Posttranscriptional modification and structural dynamics in tRNA.

SEPTEMBER 14
**Prof. Steffen Abel**
*University of California, Davis, USA*
Phosphate sensing in the Arabidopsis root meristem.

SEPTEMBER 15
**Prof. Brian Ellis**
*University of British Columbia, Vancouver, Canada*
Map kinases and stress hormone signaling: old and new connections.

SEPTEMBER 25
**Prof. Birger L. Møller**
*Royal Veterinary & Agricultural University, Copenhagen, Denmark*
Metabolic engineering of secondary metabolites for crop improvement: lessons learned from cyanogenic glucosides.

SEPTEMBER 28
**Dr. Melisa Lim**
*Carnegie Institution, Stanford, USA*
A role for phytochelatin synthase in resistance to fungal pathogens in *A. thaliana.*

SEPTEMBER 29
**Prof. Sebastian Böcker**
*University of Jena, Germany*
Decomposing metabolites: Identifying metabolites using high precision mass spectrometry.

OCTOBER 4
**Prof. Maria Harrison**
*Boyce Thompson Institute for Plant Research, Ithaca, USA*
The arbuscule mycorrhizal symbiosis: Deve - lopment and function of the arbuscule/cortical cell interface.

OCTOBER 19
**Dr. Christoph Peterhänsel**
*University of Aachen, Germany*
Signal integration on the chromatin level - a case study in maize.

OCTOBER 24
**Prof. Christian Stark**
*University of Berlin, Germany*
Katalytische Oxidationsreaktionen zur stereoselektiven Synthese von Sauerstoffhete - rocyclen.

OCTOBER 26
**Prof. Paul Tudzynski**
*University of Münster, Germany*
ROS in biotrophic vs. necrotrophic host-pathogen interactions.

NOVEMBER 2
**PD Ralph Hückelhoven**
*University of Gießen, Germany*
Regulation of actin remodelling and cell death in the interaction of barley with powdery mildew fungi.

NOVEMBER 9
**Dr. Giles Oldroyd**
*John Innes Centre Norwich, UK*
Signalling in symbiosis.

NOVEMBER 14
**Dr. Marcel Quint**
*University of Minnesota, USA*
Molecular dissection of auxin response: a 'complex' story of plant development.

NOVEMBER 23
**Prof. Franz Narberhaus**
*University of Bochum, Germany*
Temperature sensing and membrane lipids in plant-interacting bacteria.

NOVEMBER 28
**Dr. Thomas Ott**
*INRA-CNRS Toulouse, France*
Understanding active networks and new systems of cell-to-cell communications in higher plants mediated by proteins harbored on lipid rafts.

**Dr. Verónica Albrecht**
*ETH Zurich, Switzerland*
Snowy cotyledon (sco): a mutant group affected in chloroplast development in seedlings.

NOVEMBER 29
**Ulrich Weininger**
*University of Halle, Germany*
Structure and function of a protein folding catalyzing protein.

DECEMBER 14
**Prof. Johan Memelink**
*University of Leiden, The Netherlands*
Transcription factor ORA47 - a regulator of jasmonate biosynthesis in Arabidopsis.

DECEMBER 21
**Prof. John A. Pickett**
*Rothamsted Research, Harpenden, UK*
Potential for exploiting biotic stress signal-

# Guest Researchers and Fellows

## Department of Natural Product Biotechnology

**Prof. Guillermina Abdala, Argentina**
2005-8-30 – 2005-9-30

**Dr. Chotima Böttcher, Thailand**
2005-3-14 – 2006-4-1

**Maria Luisa Diaz Chávez, Mexico**
Promep Fellow
2003-10-1 – 2007-2-28

**Prof. Andrzej Guranowski, Poland**
2005-6-13 – 2005-7-15

**Aphacha Jindaprasert, Thailand**
The Thailand Research Foundation
2004-10-18 – 2006-1-31

**Jana Kufová, Czech Republic**
2006-2-6 – 2006-4-6

**Dr. Mariko Oka, Japan**
2005-10-4 – 2006-9-30

**Alfonso Lara Quesada, Costa Rica**
DAAD Fellow
2003-4-1 – 2006-12-31

**Khaled Sabarna, Palestine**
2006-9-1 – 2006-12-1

**Anne Schwedt, Germany**
2006-1-2 – 2006-2-28

**Prof. Meinhart Zenk, Germany**
2000-1-1 – 2006-3-31

## Department of Bioorganic Chemistry

**Dr. Nasser Abdullah Ali, Yemen**
DAAD Fellow
2006-7-15 – 2006-10-1

**Muhammad Ayaz, Pakistan**
2006-4-21 – 2006-12-31

**Dr. Susanne Aust, Germany**
Probiodrug AG
2003-3-1 - 2006-12-31

**Cristiano Rodrigo Bohn Rhoden, Brazil**
DAAD Fellow
2004-10-1 - 2006-12-31

**Dr. Carlos Boluda, Spain**
Manuel Morales Fellow
2005-2-1 - 2006-2-28

**Dr. Andriy Buchynskyy, Ukraina**
2006-9-1 - 2006-12-31

**Ivana Correa Ramos Leal, Brazil**
CAPES Fellow
2006-6-2 – 2006-12-22

**Victor Dick, Germany**
Fellow of the Dr. Arnold Hueck Foundation
2004-4-1 - 2006-7-31

**Dr. Alexander Dömling, Germany**
2006-4-25 – 2006-6-23

**Simon Dörner, Germany**
Fellow Studienstiftung des Deutschen Volkes
2004-4-1 - 2006-5-30

**Kanchana Dumri, Thailand**
DAAD Leibniz Fellow
2004-3-1 - 2006-12-31

**Otilie Eichler Vercillo, Brazil**
DAAD Fellow / CNPq
2005-9-12 - 2006-12-31

**Daniel Garcia Rivera, Cuba**
Graduiertenkolleg
2003-10-1 - 2006-12-31

**Gergely Gulyas, Hungary**
2005-4-1 - 2006-12-31

**Alexander Gutsche, Germany**
2006-2-1 – 2006-4-30

**Dr. Nguyen Hoang Anh, Vietnam**
2005-6-1 – 2006-7-31

**Myint Myint Khine, Myanmar**
Daimler Benz Fellow
2002-9-4 - 2006-3-1

**Dr. Tilo Lübken, Germany**
2006-7-1 – 2006-9-1

**Fredy Leon Reyes, Cuba**
Graduiertenkolleg
2005-5-1 – 2006-12-31

**Yulita Mitei, Kenya**
DAAD Fellow
2006-4-1 – 2006-9-30

**Tran Thi Phuong Thao, Vietnam**
2001-11-1 – 2006-8-31

**Prof. Luay Rashan, Jordan**
Humboldt Fellow
2005-7-5 - 2005-10-5

**Jasqer Alonso Sehnem, Brazil**
DAAD Fellow
2004-10-3 - 2005-4-2

**Josef Skopek, Czech Republic**
EMBL Fellow
2005-4-4 - 2005-7-1

**Prof. Tran Van Sung, Vietnam**
July, August and December 2005

**Dr. Trinh Thi Thuy, Vietnam**
2005-6-1 – 2006-7-31

**Marcio Weber Paixao, Brazil**
DAAD Fellow
2005-10-4 - 2006-3-30

**Dr. Heike Wilhelm, Germany**
Bio Service GmbH, EU and the State of Saxony Anhalt
2003 -8-1 - 2006-4-30

### DEPARTMENT OF STRESS AND DEVELOPMENTAL BIOLOGY

**Albor Dobón Alonso, Spain**
2006-9-1 - 2006-11-30

**Dr. Jolly Basak, India**
Humboldt Fellow
2006-8-1 - 2007-7-31

**Annegret Bährecke, Germany**
Graduiertenprogramm
2004-6-1 - 2007-5-31

**Dr. Stephan Clemens, Germany**
2006-9-1 - 2006-11-30

**Dr. Emiko Harada, Japan**
2002-2-22 - 2005-7-22

**Dr. Nicholas Harpham, Germany**
2006-6-23 - 2007-6-30

**Dr. Ingo Hofmann, Germany**
2004-1-11 - 2008-12-31

**Dr. Jens Katzek, Deutschland**
2004-11-1 - 2007-5-31

**Dr. Dirk Schenke, Germany**
2006-2-16 - 2007-12-31

**David Sondermann, Germany**
2006-11-1 - 2007-4-30

**Nicole Staroske, Germany**
Graduiertenprogramm
2006-4-1 - 2007-12-31

**Dr. Aleksandra Trampczynska, Poland**
2006-10-1 - 2006-11-30

**Dr. Stefanie Wetzel, Germany**
Graduiertenprogramm
2006-1-1 - 2006-9-30

**Dr. Michael Weber, Germany**
2006-10-1 - 2006-11-30

**Dr. Esther van der Zalm, The Netherlands**
Graduiertenprogramm
2005-12-16 – 2008-12-31

### DEPARTMENT OF SECONDARY METABOLISM

**Dr. Kirill Demchenko, Russia**
2006-4-24 – 2006-6-30

**Dr. Zakir Hossain, Bangladesh**
Humboldt Fellow
2006-4-1 – 2008-3-31

**Jakub Grzegorz Kopycki, Poland**
since January 2005

# Press and Public Relations

*Head: Sylvia Pieplow*

*Assistant: Susanne Kubenz*

## Public Events

### Long Night of Sciences 2005 and 2006

With about 390 and 300 visitors, respectively, the fourth and fifth Long Nights of Sciences, each on the first Friday in July, proved to be very

Long Night of Sciences 2006

successful for the Leibniz Institute of Plant Biochemistry (IPB). Curious guests enthusiastically participated in guided tours through the laboratories and greenhouses. In addition, our scientists displayed experiments and exhibitions in the foyer. Special lectures were favored by the public. In 2005 Stephan Clemens spoke about chances for and risks of green gene technology, whereas Jürgen Schmidt gave an overview about the history of mass spectrometry. In the next year Andrea Porzel presented interesting aspects of chirality in nature, technology and art. Simultaneously with this public event in 2006, the institute opened as visual highlight an exhibition with interesting pictures and microscopic exposures of researchers own scientific projects.

### Public discussions on plant biotechnology

Within the "6. Jugendumwelttage", a public discussion event concerning political and ecology-minded education of young peoples, in May 2006 in Leipzig, Dierk Scheel gave an insight in new technologies of plant science with his lecture named "Effective genes – how to make new plants".

As supporter of genetic engineering in plants he acted again in December 2006 on a similar public discussion forum, organized by students of Halle's university.

### Day of Chemistry

On September 23, 2006, the IPB participated for the first time in the Open day of Chemistry (Schautag der Chemie), a public performance organized by the German Chemical Industry Association (Verband der Chemischen Industrie) allover Germany. At this event, especially our chemists from the *Department of Bioorganic Chemistry* presented their work by guided tours through the laboratories and lectures on their scientific projects. Approximately 100 guests visited the institute and found out about many possibilities of vocational training in chemical industry.

### Leibniz Association meets Science and Policy

On November 3, 2006, the Leibniz Association organized a panel discussion with specialists from science and policy, who discussed in front of more then 200 members of the public current knowlegde of brain plasticity. Within this event in the Johanniskirche in Magdeburg all five institutes of the Leibniz Association located in the state of Saxony-Anhalt were present with an information booth displaying topics of their own scientific projects. The IPB presented the isolation and characterization of antibiotics from resident fungi, which catched the interest by politicians, press, and the public.

Professor Wolfgang Böhmer, Premier of Saxony-Anhalt (2. from left), Professor Ernst Theodor Rietschel, President of the Leibniz Assocoation (3. from left) and Professor Jan Hendrik Olbertz, Minister of Cultural Affairs of Saxony-Anhalt (right) were very interested in scientific topics of the IPB at the panel discussion, organized by the Leibniz Association in November 2006 in Magdeburg.

### Guided tours for Pupils and Seniors

As in the years before, the IPB organized a couple of guided tours through the institute for school classes, students, and senior groups.

These tours were always coupled with a presentation on genetic engineering in plants.

### VISITORS FROM ECONOMY AND POLICY

Matt Buist, vice president of the Toronto Biotechnology Initiative and member of the Business Development of Toronto visited our institute in November 2005. The aim of the visit in frame of a cooperation agreement between Halle and Toronto was an exchange of information about the scientific topics of and a possible extension of an already existing cooperation between the IPB and Canadian researchers and breeders.

The scientific and economic situation of the state of Iowa displays a strong focus on green biotechnology and renewable primary products, which is similar to the business environment in Saxony-Anhalt. For this reason, members of the Business Development of Iowa and of the Iowa State University visited our and other institutes with comparably research priorities in the whole state of Saxony-Anhalt. The vistit of the IPB in December 2005 was accompanied by lively discussion about basic local parameters in scientific policy and patent law in Germany. The project was organized by the BIO Mitteldeutschland GmbH.

In March 2006 breeders and scientists of the Saaten-Union Resistenzlabor GmbH used the opportunity to gain insight in IPB's scientific topics, especially those of the *Department of Stress and Developmental Biology*. The visits aim was also the discussion of collaboration projects in the future.

About 60 politicians of the German Bundestag and the Parliament of Saxony-Anhalt visited our laboratories and greenhouses in November 2006. Representatives of all political parties discussed with Sylvia Pieplow current problems of plant biotechnology.

### PARTICIPATION IN TRADE SHOW

In October 2005 the IPB participated together with universities, companies, and other scientific institutes of Saxony-Anhalt in Biotechnika, Germany's most important international biotechnology fair. Carsten Milkowski from the *Department of Secondary Metabolism* presented a seminar on transgenic rapeseed within the special congress *Wirtschaftskraft Pflanze – Zukunft durch Innovationen*, whereas Claudia Bobach and Axel Teichert from the *Department of Bioorganic Chemistry* demonstrated the isolation and characterization of powerful antibiotic substances from resident fungi in the exhibition hall.

### NEW INTERNET PRESENCE

During 2005 the Internet presence of the IPB was updated. The new websites are developed in state-of-the art-style with the content management system TYPO3. This system allows fast and simple updating of multiple contents by several responsible persons in an autonomously manner. The websites were provided with databases for staff members and publications, which can also be updated by several authorized persons, avoiding problems of updating in the future. Equipped with a modern layout and more user-friendly sites of the intranet, the new web presence started at the beginning of 2006.

### ART EXHIBITIONS

With four art exhibitions in 2005 and six in 2006 we attracted the public attention to the IPB in another way. At every vernissage there was also the possibility to visit the laboratories and greenhouses, which was enjoyed by our guests. The following art exhibitions could be seen at the institute in the past two years:

**Photos by Ralf Kummer**
January and February 2005

**Die Natur am Brocken**
Photos by Thomas Fester, September and October 2005

**Hortus Botanicus**
Photos by Gudrun Hensling, October and November 2005

Untitled. Painting by Alexandra Fröb.

**Peißnitz – 1 Jahr – 1x täglich**
Photos by Beate Nixdorf, December 2005 and January 2006

**Spuren im Einerlei,**
Paintings by Alexandra Fröb, January and February 2006

**Betrachtungen**
Paintings by Beate Gödecke, March and April 2006

**Impressioni mediterranee**
Paintings by Eckart Haupt, May and June 2006

**IPB in voller Blüte**
Scientific photos, July - September 2006

**Stadtansichten**
Paintings by Iris Band, October and November 2006

**Fernweh nach blauen Welten**
Photos by Gert Edler, December 2006 and January 2007

Marktansichten. Painting by Iris Band.

# Media Presence of the IPB in 2005 and 2006

## Articles and Press Releases 2005

**January 8**
Bank, M. Zusammenarbeit mit Kanada. *Mitteldeutsche Zeitung*, p. 18.

**January 26**
Pieplow, S. Fotoausstellung von Ralf Kummer am Leibniz-Institut für Pflanzenbiochemie, **Press Release.**

**January 27**
Bank, M. Fotoausstellung am IPB, *Mitteldeutsche Zeitung*, p. 8.

**February 5**
Waldpilze liefern Antibiotika, *Die Naturheilkunde* **2/2005**, p. 36.

**March 15**
Aus dem Dschungel in die Apotheke, *Apotheken Umschau*, p. 70-73.

**May 19**
Köhler, M. Auch Pflanzen kommunizieren. *Story Service der Wirtschaftsförderung Halle*, www. wifoe.halle.de .

**May 20**
Hoffmann, R. Schlaflos in Halle. *Scientia Halensis* **5/2005**, p. 3.

**May 23**
Pieplow, S. Neues Domizil für Tabak, Mohn und Raps. Press Release

**May 25**
Pommert, S. Bestes Klima für Tomaten. *Mitteldeutsche Zeitung*, p.9.

**June 10**
Smiljanic, M. Blei fressende Blümchen. *Financial Times Deutschland*, www.ftd.de.

**June 22**
Pieplow, S. Chemie im Grünen Bereich. **Press Release.**

**July 1**
Bank, M. Forschung für tausende Besucher. *Mitteldeutsche Zeitung*, p. 15.

**July 1**
Wiederhold, B. In der Iod-Stärke-Uhr wird es blau. *Mitteldeutsche Zeitung*, Photo, p. 15.

**July 2**
Bank, M. Wie kommt es zum Zelltod? *Mitteldeutsche Zeitung*, p. 18.

**September 6**
Pieplow, S. Egon Stahl Award für Professor Detlef Gröger. **Press Release.**

**September 13**
Pieplow, S. Natur am Brocken auf CD gebrannt. **Press Release.**

**September 13**
Pieplow, S. Vortrag zur Natur am Brocken am IPB. **Press Release.**

**September 14**
Krause, I. Lern-CD zeigt Natur des Brockens. *Mitteldeutsche Zeitung*, p. 10.

**October 7**
Pieplow, S. Hortus botanicus - grüne Oase inmitten der Stadt. **Press Release.**

**October 12**
Krause, I. Fotos im Institut. *Mitteldeutsche Zeitung*, p. 8.

**October 12**
Grüne Oase in der Stadt. *Sonntagsnachrichten.*

**October 18**
Krause, I. Vom Hilfsarbeiter zum Wissenschaftler. *Mitteldeutsche Zeitung*, p. 14.

**October 19**
Hortus botanicus. *AmtsBlatt*, S. 1.

**October 24**
Bank, M. Volles Wachstum nicht nur im Gewächshaus. *Story Service der Wirtschaftsförderung Halle*, www.wifoe.halle.de.

**October 27**
Krause, I. Den Brocken auf CD-Rom gebannt. *Mitteldeutsche Zeitung*, p. 13.

**November 15**
Mäder, A. Analysieren im Akkord. *Berliner Zeitung*, p. 12.

**November 29**
Pieplow, S. Neuer geschäftsführender Direktor am Leibniz-Institut für Pflanzenbiochemie. **Press Release.**

**November 29**
Pieplow, S. Peissnitz - 1Jahr - 1x täglich. **Press Release.**

**November 30**
Neuer Direktor. *Wochenspiegel*

**November 30**
Neuer Direktor am IPB. *Mitteldeutsche Zeitung.*

**November 30**
Pieplow, S. Besuch aus Kanada am Leibniz-Institut für Pflanzenbiochemie. **Press Release.**

**December 1**
Wilhelm, K. Strippenzieher der Unterwelt. *Bild der Wissenschaft* **12/2005**, p. 28-33.

**December 1**
Ausstellung am IPB. *Mitteldeutsche Zeitung*, p. 12.

**December 1**
Bank, M. Kanadier zu Besuch. *Mitteldeutsche Zeitung*, p. 12.

**December 7**
Pieplow, S. Samen ohne Bitterstoffe aus transgenem Raps. *EAST Magazin* **1/12/2005**, p. 10-11.

**December 7**
Wingert, N. Pilze gegen Killer-Bakterien. *EAST Magazin* **1/12/2005**, p. 6-8.

**December 28**
Höhn, T. D. Leibniz-Professor bricht eine Lanze für Gentechnik. *Deutsche Presse-Agentur GmbH Halle.*

Press releases were depending on their topics also published by:
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- www.biomitteldeutschland.de
- www.bista.de
- www.chemlin.de
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- www.planeterde.de
- www.pressrelations.de
- www.uni-protokolle.de
- www.sachsen-anhalt.de
- www.studieren-im-netz.de

## Radio Transmission

**November 30**
Kersten, C. Raps und Rost als Giftkiller - neue Techniken der Altlastensanierung. *Bayerischer Rundfunk*, IQ - Wissenschaft und Forschung.

## Television Transmissions

**January 28**
Simon, S. Gesundmacher wieder entdeckt: Kohl, Rüben, Wirsing & Co. *mdr Fernsehen*, Hauptsache Gesund.

**August 31**
Neue Exzellenzforschung in Sachsen-Anhalt. *mdr Fernsehen*, Nachrichten in Hier ab vier.

**December 15**
Struff, K., Antibiotische Wirkstoffe aus heimischen Pilzen. *mdr Fernsehen,* Hier ab vier.

# Wissenschaftliche Kostprobe

## Leibnitz-Institut für Pflanzenbiochemie präsentiert geschmackvolle Experimente

Tausende Nachtschwärmer flogen gestern zur 5. Langen Nacht der Wissenschaften aus. Von 19 bis 2 Uhr hatten 70 Einrichtungen, darunter Institute, Labors, Kliniken und Bibliotheken zu Experimenten, Führungen und Vorträgen eingeladen.

Von unserem Mitarbeiter MICHAEL DEUTSCH

**Halle/MZ.** Dass Wissenschaft hin und wieder Geschmackssache ist, davon konnten sich Ariane und Andreas Jeschke überzeugen. Die Merseburger waren gestern Abend zur Langen Nacht der Wissenschaften ins Leibnitz-Institut für Pflanzenbiochemie gekommen.

„Kosten sie mal", lautet dort die „süße" Einladung von Norbert Arnold, der am Foyer ein Experiment in Reagenzgläsern serviert. Also nippen die beiden Merseburger und kommen unisono zum Ergebnis, dass die Lösung des Naturstoff-Chemikers süßlich schmecke.

Bettina Hause vom Leibnitz-Institut für Pflanzenbiochemie reist mit den Besuchern am Mikroskop durch den pflanzlichen Zellorganismus.

Doch das Kunststück beginnt erst. Der Wissenschaftler greift zur Pipette, tröpfelt der verbliebenen Zuckerlösung ein geheimes Wässerchen zu und schüttelt das Ganze zur neuen Kostprobe. „Hmm, jetzt schmecke ich nichts mehr", ist Andreas Jeschke über den neutralen Geschmack verwundert.

Die Antwort liefert der Experte gleich nach. „Dem Zuckerwasser wurde ‚Latisol' zugesetzt", eine Verbindung, so Arnold, die erst 1989 entdeckt wurde und aus gerösteten Kaffeebohnen gewonnen wird. Dieser Stoff blockiere einen der zwei unterschiedlich vorhandenen menschlichen Süße-Rezeptoren im Mund. „Unser Geschmack wird quasi ausgetrickst", schmunzelt der Chemiker und verweist zugleich auf die Marmeladenindustrie, die den Süßeblocker zur Herstellung des Fruchtaufstriches äußerst trickreich einsetze.

Bei zu hohem Zuckergehalt der Früchte bremse man den Süße-Geschmack der Marmelade quasi mit „Latisol" aus, weiß der 47-Jährige. Da werden die Probanden schnell hellhörig und sind von der Manipulation doch etwas überrascht.

Der anschließende Rundgang im Leibnitz-Institut, durchgeführt von Pressesprecherin Sylvia Pieplow, hält im Keller mit dem so genannten konfocalen Laser-Scanning-Mikroskop noch einen wissenschaftlichen Leckerbissen bereit. Mit dem Mikroskop könne man die einzelnen Zellschichten von Pflanzen durchforsten, erklärt Pieplow. Ein wichtiger Schritt in der Grundlagenforschung, um beispielsweise Proteinen auf die Spur zu kommen.

## ARTICLES AND PRESS RELEASES 2006

**JANUARY 18**
ACRYL UND PASTELL VON ALEXANDRA FRÖB AM LEIBNIZ-INSTITUT FÜR PFLANZENBIOCHEMIE. **PRESS RELEASE.**

**JANUARY 25**
Blick ins Herz der Blüte. *Mitteldeutsche Zeitung*, photo, p. 13.

**FEBRUARY**
Hupfer, A. Vorhang auf für die kleinen Moleküle. *Laborjournal* **1-2/2006**, p. 20-23.

**FEBRUARY 9**
Blüten in der Kunst. *Mitteldeutsche Zeitung*, photo, p. 14.

**FEBRUARY 23**
Schierholz, A. Eine Stadt muss sich entdecken. *Mitteldeutsche Zeitung*, p. 3.

**MARCH 8**
PROFESSOR TONI M. KUTCHAN FOLGT RUF NACH AMERIKA. **PRESS RELEASE.**

**MARCH 9**
Krause, I. Mohn-Expertin zieht nach Amerika um. *Mitteldeutsche Zeitung*, p. 16.

**MARCH 9**
DER MOHN ZIEHT NACH AMERIKA. **PRESS RELEASE.**

**MARCH 14**
BEATE GÖDECKE STELLT AM LEIBNIZ-INSTITUT FÜR PFLANZENBIOCHEMIE AUS. **PRESS RELEASE.**

**MARCH 16**
Krause I. Kunst von Kunstpädagogin. *Mitteldeutsche Zeitung*, p. 18.

**MARCH 16**
SAMEN OHNE BITTERSTOFFE AUS TRANSGENEM RAPS. **PRESS RELEASE.**

**APRIL**
Bünnagel, D. Ob Weizenmehl, Tofu, Reis. *Leibniz-Journal* **1/2006**, p. 10-11.

**APRIL**
Scheel, D. Nutzen der "Grünen" Gentechnik. *Leibniz-Journal* **1/2006**, p. 14.

**APRIL**
Prof. Toni M. Kutchan verlässt das IPB. *Laborpraxis*, **April 2006**, p. 18., *Process*, **April 2006**, p. 16.

**MAY 11**
MEDITERRANE IMPRESSIONEN AUS DER SICHT DES MUSIKERS. **PRESS RELEASE.**

**MAY 12**
Städter, A. Ein Alleskönner unter den Pflanzen. *Mitteldeutsche Zeitung*, p. 13.

**JULY 4**
Krause, I. Hitzezuschlag für die Bauleute. *Mitteldeutsche Zeitung*, p. 9.

**JULY 7**
BILDER AUS DER FORSCHUNG ZUR LANGEN NACHT DER WISSENSCHAFT. **PRESS RELEASE.**

**JULY 8**
Krause, I. Ein Mord zur Langen Nacht. *Mitteldeutsche Zeitung*, p. 11.

**JULY 14**
Pieplow, S. Virtuellen Doktorhut erwerben. *East Magazin* **2/2006**, p. 38.

**JULY 15**
Deutsch, M. Wissenschaftliche Kostprobe, *Mitteldeutsche Zeitung*, p. 13.

**AUGUST 31**
METALLTOLERANTE PFLANZEN - EINE FRAGE DER GENAKTIVITÄT? **PRESS RELEASE.**

**SEPTEMBER 18**
LEIBNIZ-INSTITUT FÜR PFLANZENBIOCHEMIE ÖFFNET SEINE PFORTEN ZUM SCHAUTAG DER CHEMIE. **PRESS RELEASE.**

**SEPTEMBER 20**
Schautag der Chemie. *BILD-Zeitung.*

**SEPTEMBER 20**
Krause, I. Schautag der Chemie. *Mitteldeutsche Zeitung*, p. 13.

**SEPTEMBER 20**
Institut öffnet seine Pforten zum Tag der Chemie. *Wochenspiegel*, p. 4.

**SEPTEMBER 25**
Möbius, J. Chancen in der Chemie - Leibniz-Institut öffnete Pforten für Besucher. *Mitteldeutsche Zeitung*, p. 8.

**OCTOBER 23**
IRIS BAND ZEIGT STÄDTE AUS DER VOGELPERSPEKTIVE. **PRESS RELEASE.**

**OCTOBER 24**
Iris-Band-Schau. *Mitteldeutsche Zeitung*, p. 14.

**NOVEMBER 3**
Scholtyseck, W. Schau von Iris Band zeigt Stadt-Träume und Traum-Städte. *Mitteldeutsche Zeitung*, photo, p. 17.

**DECEMBER 11**
FERNWEH NACH BLAUEN WELTEN AM LEIBNIZ-INSTITUT FÜR PFLANZENBIOCHEMIE. **PRESS RELEASE.**

**DECEMBER 12**
Pohle, H. Fotograf zeigt Bilder von Kreta. *Mitteldeutsche Zeitung*, p. 11.

**DECEMBER 13**
Schau im Institut. *Mitteldeutsche Zeitung*, p. 12.

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## RADIO TRANSMISSION

**JANUARY 12**
Für stärkere Nutzung der Grünen Gentechnik. Interview with Dierk Scheel. *Deutschlandradio Kultur.*

# Map & Impressum


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