Scientific Report 2003-2004

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Message from the Managing Director

The Leibniz Institute of Plant Biochemistry (IPB) is a research center that is rich in contemporary German history. Founded as the Institute for Biochemistry of Plants in 1958 by Kurt Mothes, this former research institute of the East German Academy of Sciences was unique in emphasizing a multidisciplinary approach to studying plant natural products and hormone-regulated processes. Scientists from around the globe were regularly invited to visit and the institute enjoyed international recognition. The IPB, as we know the institute today, was founded in 1992 and has developed into a thriving center of modern plant research. The multidisciplinary style of research introduced by Kurt Mothes continues and has been expanded upon. Within four research departments, more than 170 scientific staff, doctoral and diploma students and guests from the world-over contribute to the exciting bustle of state-of-the-art research.

The fundamental research programs at the IPB center on, in a broad sense, natural product research and molecular interactions. From natural product isolation and structure determination through to investigations of biosynthesis and regulation, new biologically active molecules are sought and production sources optimized through chemical synthesis and genetic engineering. Cellular function as regulated by molecular interactions are investigated at the level of receptor-ligand, enzyme-substrate and protein-protein interactions using computer modeling and a variety of biochemical and genetic techniques. The years 2003-2004 were very productive for us and intriguing new research developments within the departments at the IPB in these areas are highlighted within this scientific report.

Scientists at the institute are participating in an increasing number of local, national and international research networks. A new Priority Program of the German Research Foundation (DFG, SPP 1152, *Evolution of Metabolic Diversity*) was initiated and is coordinated by scientists at the IPB. In 2004, members of the institute contributed to the successful application to the German Research Foundation for a new Collaborative Research Center (SFB 648, *Molecular mechanisms of information processing in plants*) at the Martin Luther University of Halle-Wittenberg.

We have further improved upon our research facilities during the past two years; the institute dedicated a new 500 square meter shared-use building in Spring 2004. The construction provides space for a mass spectrometry center, nucleotide sequence analysis, fermentation and synthetic organic chemistry laboratories. Our greenhouses are also being expanded; we began in November 2004 with the construction of an additional 346 square meters of floor space that will meet the growing demand of the IPB's research programs with transgenic plants. These new plant growth facilities will be ready for occupancy in Spring 2005.

We hope that in this report, we are able to share our excitement for plant biochemistry and its manifold applications with you. We are proud of how our institute has developed over the years and if you are planning on being in the Halle (Saale) area, please do stop by to learn more about us.

Toni M. Kutchan

Departmental Organization

Research Mission Statement

Four thematically, methodologically and organisationally overlapping research priorities form the basis of the research mission statement of the Institute of Plant Biochemistry - plant natural products, molecular interactions, information technology and metabolic engineering.

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 the 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, the realization of genome sequencing and the growing availability of expressed sequence tags of various species 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 Institute of Plant Biochemistry. Structure analysis, synthesis and derivatization of natural products contribute to an understanding of their function and to an increase in their structural diversity. This also forms the basis for investigation of their biosynthesis and for discovering new biologically active compounds. A qualitative and quantitative analysis of natural products in biological materials requires the development of suitable analytical methods. 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. The use of mutants and transgenic plants ultimately makes possible the analysis of biological function as well as the generation of plants with altered natural product profiles.

Molecular interactions form the basis of cellular function. An interdisciplinary analysis of these interactions is therefore of central importance to the research mission of the Institute of Plant Biochemistry. The optimal adaptation of plants to their habitat depends upon receptor-mediated perception of biotic and abiotic environmental parameters. External 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 molecular basis of these processes, receptor-ligand, enzyme-substrate and protein-protein interactions, 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 and signal transduction pathways. Chemical structures of these interacting components are also 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 characteristics (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.
A nexus of natural product research and the study of molecular interactions is the

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 is being established at the Institute of Plant Biochemistry.

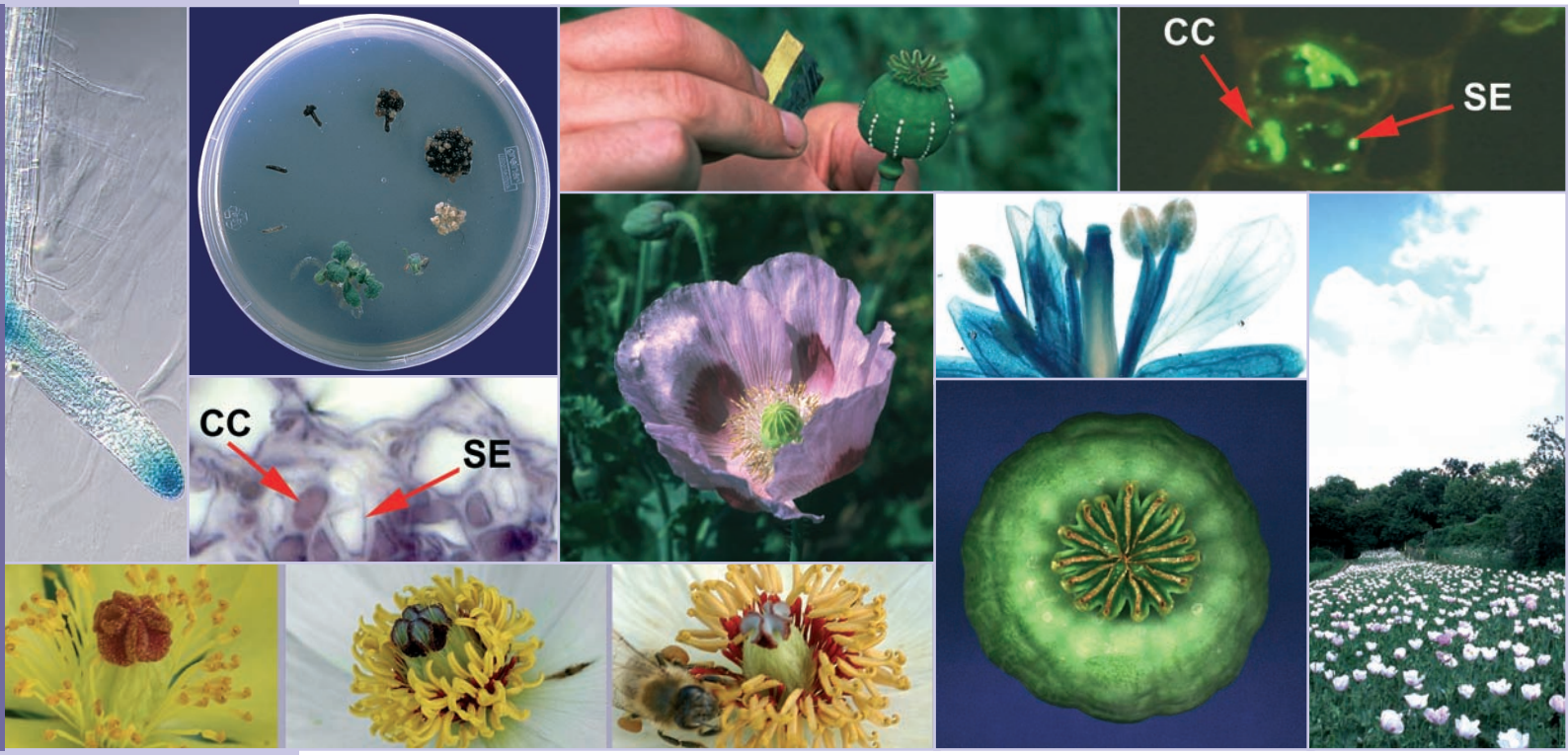
Metabolic engineering is an overlapping priority in three areas of basic research - natural products, molecular interactions and information technology. Model plants are generated that have potential for various types of application. More specifically, designer plants with tailored natural product profiles, containing new health-promoting metabolites or showing improved adaptation to habitat are being developed. Plants with these characteristics could serve for the sustainable production of valuable chemicals, as biological test systems or could be of importance to plant breeders.

Within four departments with distinct, but complementary research directions and state-of-the-art equipment, the Institute of Plant Biochemistry provides optimal conditions with which to execute multidisciplinary research in the areas of chemistry, physiology, cell biology, biochemistry, molecular biology and genetics. The analysis of topics central to modern plant biology and chemistry using this wide array of methodologies enables a meaningful interpretation of the complex interactions in plant development and diversity that would otherwise not be possible. The ultimate transfer of these results to practical applications could make ecologically compatible uses of plant biotechnology a reality.

Department of Natural Product Biotechnology

Head: Toni M. Kutchan, Professor

Secretary: Christine Dietel



Department of

Natural Product Biotechnology

For much of human history, plant extracts have been used as ingredients in potions and poisons. In the eastern Mediterranean, use of opium poppy latex (*Papaver somniferum*) can be traced back at least to 1400 to 1200 B.C. Ancient folks used medicinal plant extracts as purgatives, antitussives, sedatives and treatments for a wide range of ailments from snakebites to fever. Use of medicinal plants then spread westward across Europe. Over the centuries, one of the most important medicinals was opium. Analysis of the individual components of opium led to the identification of morphine. The isolation of morphine in 1806 by the German pharmacist Friedrich Sertürner gave rise to the study of alkaloids. The term "alkaloid" was, in fact, coined by another pharmacist, Carl Meissner, in 1819 in Halle, Germany. Alkaloids were originally defined as pharmacologically active, nitrogen-containing basic compounds of plant origin. Alkaloid-containing plants were mankind's original materia medica. Many alkaloids are still in use today as prescription drugs; one of the best known and widely used is the antitussive codeine from the opium poppy. After 198 years of alkaloid research, this class of natural products still finds significance in the medical field in the treatment of diseases ranging from cancer potentially through to malaria.

The Department of Natural Product Biotechnology emphasizes research on the elucidation of formation of plant natural products at the molecular genetic level. Our research centers on understanding the formation of se - lected natural products at several levels and then exploiting this knowledge using genetic engineering. We are just beginning to metabolically engineer alkaloid metabolism in plants and in *in vitro* culture. Multicellular compartmentation of alkaloid pathways and transport of intermediates must be considered if meaningful metabolic engineering experiments are to be designed. We also need to analyze and use promoters that drive transgene expression in the correct cell types to effect biosynthesis. Regulation of these pathways at the gene and enzyme level is complex and there is still also much to be learned about metabolite levels and pathway interconnection as we systematically over-express and suppress gene transcription. Today, pathway engineering in plants remains highly variable. When we perturb cellular physiology, metabolite homeostasis and intra- and intercellular partitioning can be effected in unpredictable ways. Another aspect that needs attention is the development of efficient transformation and regeneration protocols for alkaloid - producing plant outside of the Sola - naceae. All told, there is still much to be done in this field of research.

Research Group: Alkaloid Biosynthesis

Head: Toni M. Kutchan

The opium poppy *Papaver somniferum* is one of mankind's oldest medicinal plants. Opium poppy today is the commercial source of the narcotic analgesics morphine and codeine. Along with these two morphinans, opium poppy produces approximately 80 alkaloids belonging to various tetrahydrobenzylisoquinoline-derived classes. It has been known for over a century that morphinan alkaloids accumulate in the latex of opium poppy. With identification of many of the enzymes of alkaloid biosynthesis in this plant, biochemical data suggested involvement of multiple cell types in alkaloid biosynthesis in poppy. It has also been suggested, based upon the failure to produce morphine in undifferentiated *P. somniferum* cell cultures, that morphine accumulation is related to cytodifferentiation - noting that laticifers are absent from these cultures, but present in tissue cultures. We now have addressed the question of cell-specific localization of the enzymes of alkaloid biosynthesis and the site of gene transcript accumulation. We have carried through the immunolocalization of five enzymes of alkaloid formation in opium poppy: (*R,S*)-3'-hydroxy-*N*-methylcoclaurine 4'-*O*-methyltransferase central to the biosynthesis of tetrahydroisoquinoline-derived alkaloids, the berberine bridge enzyme of the sanguinarine pathway, (*R,S*)-reticuline 7-*O*-methyltransferase specific to laudanosine formation, and salutaridinol 7-*O*-acetyltransferase and codeinone reductase that lead to morphine.

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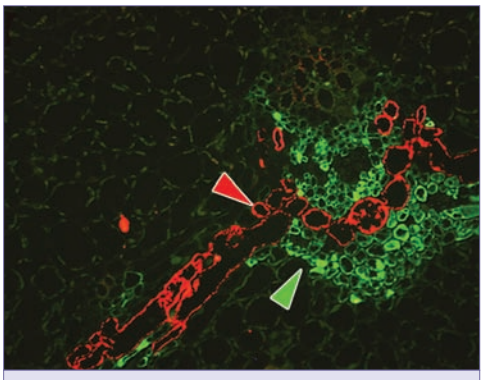
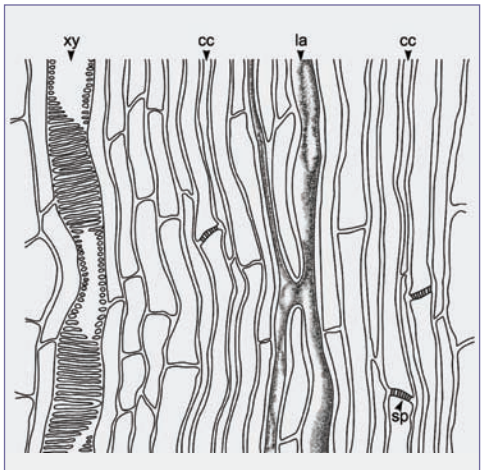
The opium poppy *Papaver somniferum* is the source of the narcotic analgesics codeine and morphine, which accumulate in specialized internal secretory cells called laticifers. In the aerial parts of the plant, the laticifer cells are anastomosed, forming an articulated network. Laticifers are found associated with the vascular bundle in all plant parts. The morphinan alkaloids morphine, codeine and thebaine are found both in roots and in aerial plant parts and specifically accumulate in vesicles within laticifers. The benzo[*c*]phenanthridine alkaloid sanguinarine is found in root tissue. The syntheses of sanguinarine and of the tetrahydrobenzylisoquinoline alkaloid laudanine are completely understood at the enzyme level. Nearly all enzymes of morphine biosynthesis have also been described. In more recent years, cDNAs encoding ten enzymes of alkaloid biosynthesis in *P. somniferum* have been isolated and characterized. The pathway starting from the first tetrahydrobenzylisoquinoline alkaloidal intermediate (*S*)-norcoclau-rine to the central isoquinoline alkaloid biosynthetic intermediate (*S*)-reticuline is understood at both the enzyme and gene level. (*S*)-Norcoclaurine is *O*-methylated by (*R,S*)-norcoclau-rine 6-*O*-methyltransferase. (*S*)-Coclaurine is next *N*-methylated by (*R,S*)-coclaurine *N*-methyltransferase and the cDNA encoding this enzyme has been characterized. (*S*)-*N*-Methylcoclaurine is then hydroxylated by the cytochrome P-450 dependent monooxygenase CYP80B1 [(*S*)-*N*-methylcoclaurine 3'-hydroxlyase]. The cDNA encoding the corresponding cytochrome P-450 reductase has been isolated as well. (*S*)-3'-Hydroxy-*N*-methylcoclaurine is methylated to (*S*)-reticu-line by (*R,S*)-3'-hydroxy-*N*-methylcoclaurine 4'-*O*-methyltransferase (4'OMT). The cDNA *4'omt* has been isolated and



Immunolabeling of of SalAT (salutaridinol 7-*O*-acetyltransferase) of morphine biosynthesis in a cross-section of capsule of *P. somniferum*. The panels show a cross-section through a small vascular bundle of the capsule wall. The green arrows indicate the position of anti-biosynthetic enzyme antibody; the red arrows indicate the position of anti-MLP 15 antibody and is indicative of laticifer cells.

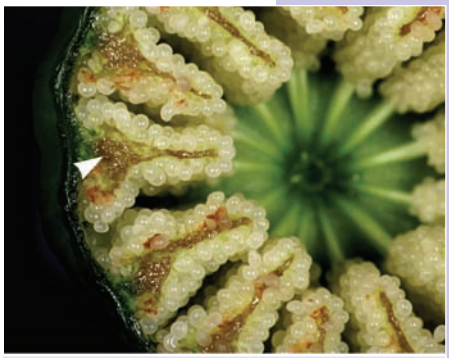
characterized from *P. somniferum*. (*S*)-Reticuline is central intermediate of isoquinoline alkaloid biosynthesis, which leads to a plethora of alkaloidal structures. In *P. somniferum*, (*R,S*)-reticuline can be methylated by (*R,S*)-reticuline 7-*O*-methyltransferase, for which the cDNA *7omt* has been described, to the tetrahydrobenzylisoquinoline laudanine. The *N*-methyl group of (*S*)-reticuline can alternatively be oxidatively cyclized by the berberine bridge enzyme (BBE) to C-8 of (*S*)-scoulerine. (*S*)-Scoulerine is then fur-ther converted in these plants to the antimicrobial benzo[*c*]phenanthridine alkaloids sanguina-rine. Along the pathway on which (*S*)-reticuline is specifically converted to morphine, cDNAs encoding two biosynthetic enzymes have been identified. Salutaridinol 7-*O*-acetyltransferase, encoded by *salat*, transfers an acetyl moiety from acetyl-CoA to the 7-hydroxyl group of salutaridinol. Codeinone reductase is encoded by *cor* and catalyzes the penultimate step in morphine biosynthesis, the NADPH-dependent reduction of the keto moiety of codeinone to the 6-hydroxyl group of codeine.

Fluorescence immunocytological localization was carried out with the 4'OMT (reticuline-), the 7OMT (laudanine-), BBE (sanguinarine-), SalAT and COR (morphine-pathways) with *P. somniferum* capsule, stem and root tissue sections. In this manner, the spatial organization of enzymes occurring before and after a central branch point is analyzed. In addition, *in situ* localization of *7omt* and *cor1* was performed to correlate the site of gene transcription to enzyme accumulation. In capsule and stem, both *O*-methyltransferases and the *O*-acetyltransferase are found predominantly in parenchyma cells within the vascular bundle and codeinone reductase is localized to laticifers, the site of morphinan alkaloid accumulation. In developing root tip, both *O*-methyltransferases and the *O*-acetyltransferase are found in the pericycle of the stele and the berberine bridge enzyme is localized to parenchyma cells of the root cortex. Laticifers are not found in developing root tip and, likewise, codeinone reductase was not detected. These results provide cell-specific localization that gives a coherent picture of the spa-tial distribution of alkaloid biosynthesis in opium poppy.



Schematic drawing of a longitudinal section through the vascular bundle of *P. somniferum* stem. **xy**, xylem; **cc**, companion cell; **la**, laticifer; **ph**, phloem; **sp**, sieve plate.

In summary, two cell types, parenchyma within the vascular bundle and latici - fers, are sites of the biosynthesis of isoquinoline alkaloids in *P. somniferum*. This implies spatial regulation of the biosynthesis of the different classes of *P. somniferum* alkaloids in that the early stages of mor-phine biosynthesis occur in parenchyma cells surrounding laticifers and then at late stages, possibly at the level of either salutaridinol-7-*O*-acetate or thebaine, moves into the laticifer, which is the storage site of the morphinans thebaine, codeine and morphine. The role of intercellular transporters of alkaloidal intermediates as well as intracellular transport into vesicles within laticifers adds an additional potential level of regulation to morphine biosynthesis that still needs to be investigated.



Dissected capsule of *P. somniferum*, white arrow indicates the position of the large vascular bundle; the small vascular bundle is located closer to the capsule wall.

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Research Group: Opium Poppy Biotechnology

Head: Susanne Frick

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 antitumoric agent noscapine and the antimicrobial sanguinarine. We have 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 with tailored alkaloid profiles for industrial and pharmaceutical use.**

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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. A major goal of this project was the development of a stable transformation and regeneration method for opium poppy, which would 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 its residual morphine levels prevents more widespread applications. Because opium is the raw material for the illicit production of heroin, cultivation of poppy is restricted. By completely suppressing morphine biosynthesis, opium poppy could become a "harmless" crop plant. So far there has been no success with breeding programs and mutations to obtain an alkaloid-free poppy. In the best case a reduction of alkaloid biosynthesis has been achieved. The transformation of opium poppy could be an alternative to circumvent these problems.

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 (**Fig. 2, A-E**). Alkaloid-free plants developed in this manner will be used to test the chemical-ecological function of morphinan and benzophenanthridine alkaloids in plants. After the regeneration of plants, the qualitative and quantitative determination of alkaloids is analyzed by HPLC and LC-MS in latex, leaves and roots of opium poppy. Finally the heredity of the alkaloid concentration/pattern is confirmed. With a transgenic cell line expressing the *antisense* construct of berberine bridge enzyme (BBE) we hoped to reduce the metabolic flux through sanguinarine pathway and to enhance the concentration of morphine instead. We are interested whether a transgenic cell line overexpressing codeinone reductase (COR) leads to a poppy plant that contains more morphine or where the concentration of morphine is lowered due to a possible

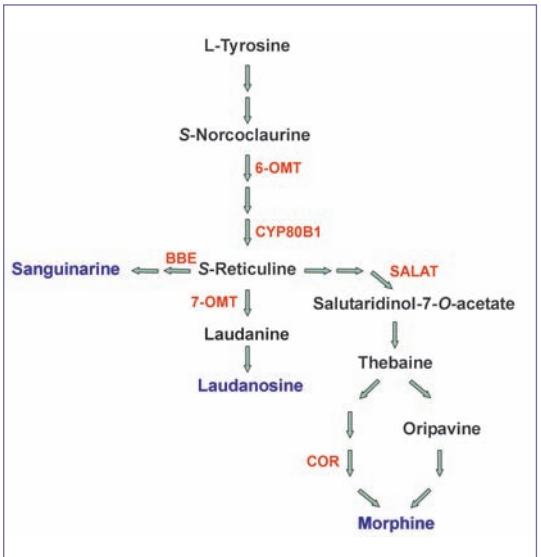

Figure 1: Biosynthetic pathway from *L*-tyrosine to sanguinarine, laudanosine and morphine in *P. sonmniferum*. Enzymes are highlighted in red.

feedback inhibition of this pathway. We have also produced poppy transformants, in which we potentially influenced all cytochrome P450 enzymes of the benzylisoquinoline pathways by introducing a NADPH:cytochrome P450-oxidoreductase (CPR). Finally we are trying to reduce or silence the complete alkaloid biosynthetic pathway with a transgenic cell line containing the *antisense* construct of (*S*)-*N*-methylcoclaurine hydroxylase (CYP80B1). With a cell line overexpressing *cyp80b1* we are trying to stimulate all three pathways together.

With the *antisense* expression of *bbe* cDNA we have been able to produce transgenic opium poppy plants with altered alkaloid profiles. The transformation of these plants was evaluated by PCR, northern and Southern hybridisation. The transgenic plants contained one additional copy of the *bbe* gene. We observed an increased concentration of several pathway intermediates from all biosynthetic branches e.g. reticuline, laudanine, laudanosine, dehydroreticuline, salutaridine and (*S*)-scoulerine. The transformation altered the ratio of morphinan and tetrahydrobenzylisoquinoline alkaloids in latex but not the benzophenanthridine alkaloids in roots. The altered alkaloid profile was heritable at least to the T2 generation.

The overexpression and *antisense* expression of *cyp80b1* cDNA produced poppy plants with altered alkaloid concentrations. The increase or decrease of alkaloid concentration in the transgenic cell lines was heritable at least to the T3 generation. Compared to *cyp80b1* the overexpression of *cpr* cDNA did not give rise to an increased alkaloid concentration in transgenic opium poppy.

Because we have not been able to silence benzylisoquinoline biosynthesis in transgenic poppy plants containing S4S4:*antiBBE* or S4S4:*antiCYP80B1*, we constructed plasmids containing partial sequences that are potentially able to trigger RNA interference in *Papaver somniferum*. Explants of opium poppy were transformed with different constructs. Meanwhile we have been able to produce several T0 plants. The analysis of these plants is in progress.

To study the cell- and tissue specific expression of benzylisoquinoline biosynthetic genes, we started to isolate their endogenous promoters. With promoter/reporter gene constructs we want to analyze how biosynthesis and accumulation of alkaloids are regulated.

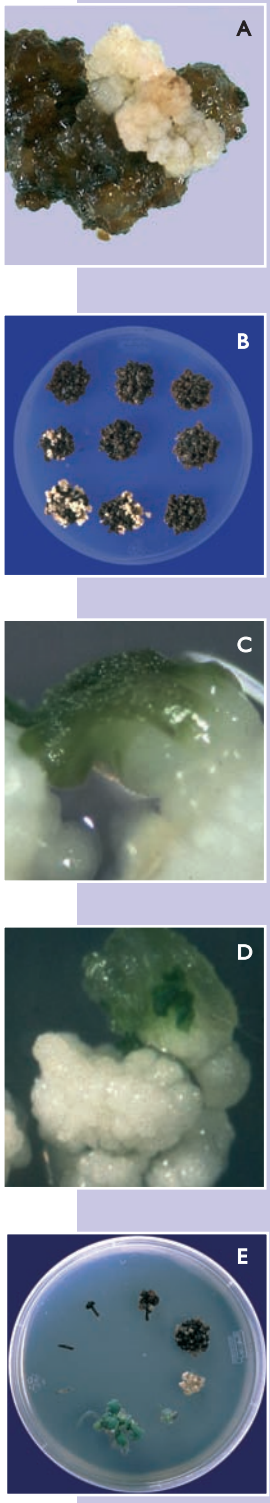

Figure 2: Somatic regeneration of *P. somniferum*. Explants first give rise to a type I callus (**A**), which starts to differentiate after a certain time (**B**). After the transfer to a hormone free medium this type II callus develops small embryos (**C**) and finally little plantlets (**D**). The whole regeneration process is shown in picture **E**.

Research Group: Mode of Action of Jasmonates

Heads: Claus Wasternack & Otto Miersch

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Jasmonates (JA) and their precursors, the octadecanoids, are signals in plant stress responses and in plant development. A mechanistic analysis of the mode of action of jasmonates is performed by a reverse genetic approach using the allene oxide cyclase (AOC)-catalyzed step in jasmonate biosynthesis. "Gain of function" and "Loss of function" studies with transgenic tomato plants revealed modulation of jasmonates and allowed to inspect 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 are 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

The role of JA biosynthesis is analyzed in tomato and Arabidopsis. Preferentially, the AOC-catalyzed step and the profile of jasmonates and other oxylipins are studied to elucidate specific functions in plant stress responses and development.

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. In leaves, the vascular bundle-specific occurrence of AOC attributes to a preferential generation of jasmonates in main veins, which led together with data from various transgenic tomato plants to a proposed amplification model 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 (**Fig. 1**).

Using transgenic tomato plants over-expressing or repressing constitutively the AOC or expressing a reporter gene driven by the *AOC* promoter (coop. T. Roitsch, Wuerzburg, Germany), role of AOC and generation and function of jasmonates could be inspected. Tissue specific expression of *AOC* in seedling development, preferentially in roots (**Fig. 3**) and during flower and embryo development points to new functions of JA and its metabolites. This *AOC* gene activity and JA function differs from that of Arabidopsis suggesting the plasticity of different plant species to use signaling compounds. These differences together with data from the literature point to role of JA in female fertility of tomato, whereas in Arabidopsis male fertility is strictly JA dependent.

Interestingly, the vascular bundle- and ovule-specific occurrence of AOC protein in flowers is accompanied with a specific profile of octadecanoids and jasmonates as well as JA amino acid conjugates being different in the various flower organs. Constitutive over-expression of AOC led to qualitative and quantitative changes in these profiles (**Fig. 2**) as well as that of the free and esterified fatty acids and their peroxidation products (coop. I. Feussner, University of Goettingen). The data suggest different regulation of JA biosynthesis in flowers and leaves and indicate that the different branches of the lipoxygenase pathway can be altered depending of the terminal products.

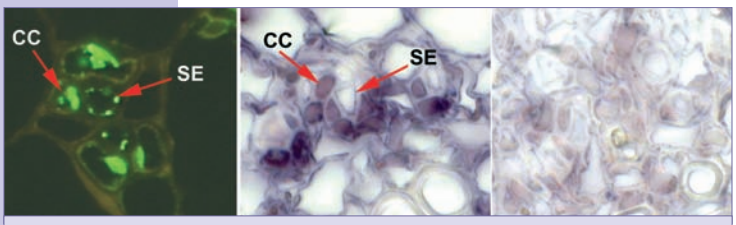

Figure 1: AOC protein occurs in companion cells (CC) and sieve elements (SE), but *AOC* mRNA is exclusively located in companion cells. AOC protein is detected by immunolabeling indicated by green fluorescence (left). *In situ* hybridisation to detect *AOC* mRNA was performed by probing with an *AOCantisense* probe (middle), note that the triangle-shaped sieve elements are free of label. As a control an *AOCsense* probe was used indicating no cell-specific labelling (right) (taken from Hause et al., *Plant Cell Physiol.* **44**, 643-648, 2003).

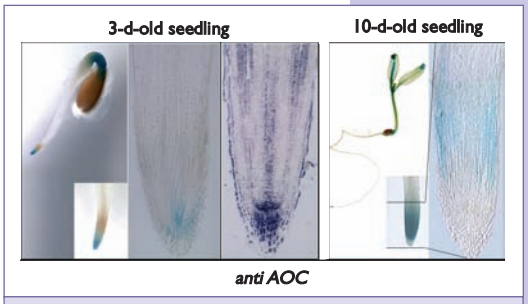

Figure 3: *AOC* promoter activity in tomato seedling development. Seedlings of a transgenic *AOC*(1000 bp)::*uidA* line expressing the β-glucorunidase, whose activity is indicated by blue staining, were inspected after 3 and 10 days, respectively, after sawing (das). Note the *AOC* promoter activity in the root tip and hypocotyl 3 das (left) and the root elongation zone as well as the vascular bundles of cotyledons 10 das (right). *AOC* promoter activity corresponds to location of AOC protein probed with an anti-AOC antibody and visualized by violet staining (left/right) (photos by I. Stenzel and B. Hause).

Data on JA and 12-oxo-phytodienoic acid (OPDA) level in tomato mutants and in our 35S::*AOCantisense* tomato plants following infection with *Xanthomonas campestris* cv. *vesicatori* supported a model of sequential action of jasmonate, ethylene and salicylate in this plant pathogen interaction (coop. H. Klee, Gainesville, USA). Also in collaborative work (M. Köck, Biocenter, University of Halle, Germany), data were found which support a new JA signaling pathway. In tomato the wound-responsive *RNaseLE* gene is expressed only locally and in a JA- and systemin-independent manner.

In Arabidopsis the AOC is encoded by four genes. Inspection of transgenic Arabidopsis plants expressing a reporter gene driven by each *AOC* promoter, revealed non-redundant functions of the AOCs. Preferentially, in root growth and seedling development as well as in flower development (**Fig. 4**) there are tissue and organ specific promoter activities, non-redundantly for *AOC1*, *AOC2*, *AOC3* and *AOC4*. These data and analyses of knockout-lines indicate that Arabidopsis is able to perform a fine-tuning of regulation by spatial and temporal differences in the expression of the four *AOCs*. In contrast, the four recombinant AOCs exhibited similar enzymatic properties and substrate specificities.

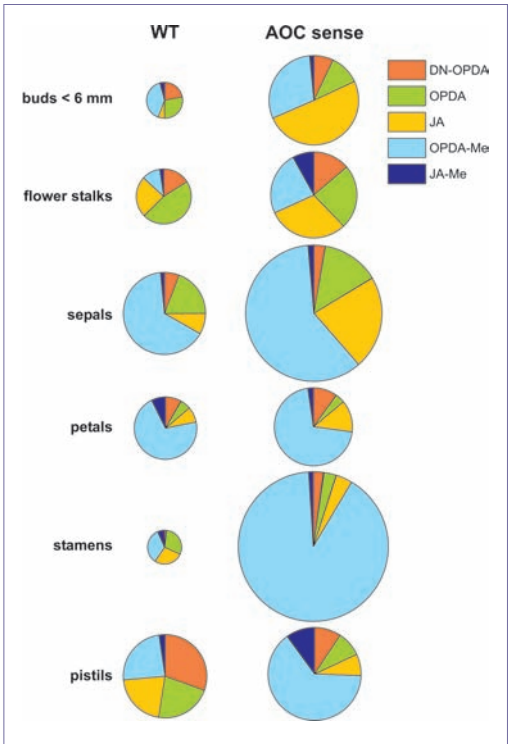

Figure 2: Constitutive over-expression of *AOC* in tomato leads to elevated levels of jasmonates and octadecanoids in flowers but not in leaves. The ratio among the compounds being specific for each flower organ is differentially altered by over-expression of *AOC*. The diameter of each circle is indicative for the total amount of all compounds, the sector within a circle is indicative for a distinct compound given in % of the total amount (taken from Miersch et al., *Phytochem.* **65**, 847-856, 2004).

Previously, 12-hydroxy-JA was only known as tuber-inducing compound in *Solanaceae* species. We could identify 12-hydroxy-JA and its sulfated derivative in *A. thaliana* and tomato. From Arabidopsis a sulfotransferase (AtST2a) was cloned which converts specifically 12-hydroxy-JA (coop. L. Varin, Montreal, Canada). In transgenic plants over-expressing or repressing constitutively *AtST2a* a shift in flowering time depending on the 12-OH-JA level was detected. In tomato 12-OH-JA is a permanent constituent of flower organs. In tomato leaves 12-OH-JA is formed upon wounding in a JA-dependent manner. The role of 12-OH-JA in a photoperiod-dependent plant and a day-neutral plant is in the focus of future work.

National and international cooperation is done in the above-mentioned aspects of tomato and Arabidopsis. 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 AG 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 pyroglutamate formation at the N-terminus. Our effort is to clone plant homologues of the QC as a tool for design of potent QC inhibitors.

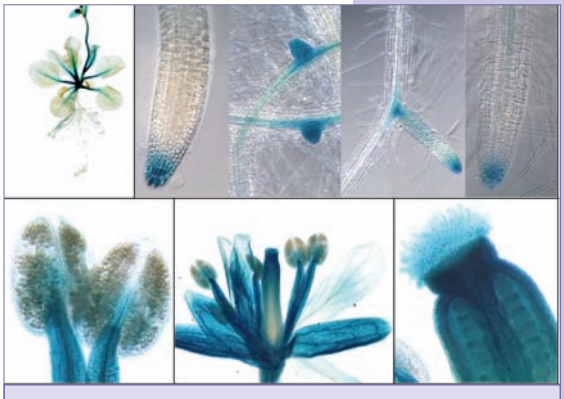

Figure 4: Promoter activity of the *AOC4* gene of *Arabidopsis thaliana*. 14-d-old seedlings (above) and flowers (below) of transgenic lines carrying a *AOC4::uidA* construct were inspected on β-glucuronidase activity visualized by the blue staining. Note *AOC4* promoter activity in root tips, side root primordia and vascular bundles of the seedlings, as well as in sepals, petals, filaments and stigmas of open flowers (photos by I. Stenzel).

Research Group: Papaver-Gene Expression Analysis

Head: Jörg Ziegler

Poppies of the genus *Papaver* produce a large variety of benzylisoquinoline alkaloids. Some of them are of pharmaceutical importance such as the analgesic morphine, the antitussive noscapine or the vasodilator papaverine. The biosynthesis to (*S*)-reticuline, the central intermediate to all monomeric benzylisoquinoline alkaloids is well understood on the molecular level, the knowledge of the later steps, which lead to the diversity of this class of compounds, is still incomplete. Similarly, the regulatory steps leading to the accumulation of these substances, is unknown. To approach cDNA clones coding for these processes we make use of the close genetic relationship but the diversity in the alkaloid profile between *Papaver* species or varieties. We examine and correlate the gene expression profiles on EST-arrays with specific alkaloid profiles. By the combination of many different datasets of alkaloid profile-gene expression correlations, we could re-duce the number of candidate cDNAs with possible functions in benzylisoquinoline accumulation to eleven.

Group Members

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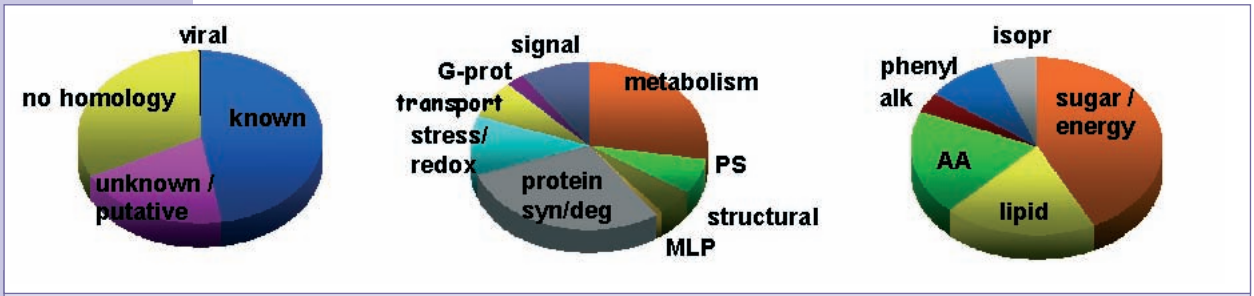
Currently, more than 70 different poppy species belonging to the genus *Papaver* have been described. Roughly, they are able to synthesise about 2,500 different benzylisoquinolines, which can be grouped into nine classes. The profile of benzylisoquinolines produced by the plants are species specific; it is, however also dependent on growth conditions. The same holds true for gene expression. This variability requires sensitive methods to record all needed parameters in one individual plant. HPLC methods were employed to detect the main compounds and LC-MS coupling will be used for the low abundant compounds. For gene expression analysis, a protocol for macroarray production was developed. These methods are sensitive enough to record the alkaloid profile and the gene expression pattern from one individual plant. For ten *Papaver* species and five varieties and mutants of the opium poppy *Papaver somniferum*, the alkaloid profile was recorded by HPLC and HPLC-MS. Some of the major compounds of each plant could be identified by MS-MS. The MS-MS data of all other alkaloids are available now and their interpretation with respect to their identification is in progress.

As probes for the arrays we use PCR fragments derived from two EST-projects of *Papaver somniferum* stems and seedlings. Among all *Papaver* species, this plant synthesizes the largest number of different benzylisoquinolines. Up to now we sequenced about 3,700 ESTs and obtained 2,000 UniGenes. About 50 percent either code for proteins with unknown function or have no homology to entries in the databases. The largest groups of cDNAs coding for proteins with known function are involved in transcriptional and translational control, in responses to stress, and in redox control. Another highly represented group codes for proteins participating in metabolism, mainly primary metabolism. To 20 sequences, a possible role in secondary metabolism could be ascribed. Six sequences code for proteins with known function in the benzylisoquinoline pathway. Up to now, the expression of these cDNAs was examined in nine *Papaver* species and *P. somniferum* varieties, respectively. These expression profiles



Functional classification of ESTs from *P. somniferum* stems and seedlings. Abbreviations: PS: photosynthesis, MLP: major latex proteins, protein syn/deg: protein synthesis and degradation, G-prot: G-proteins, AA: amino acids, alk: alkaloids, phenyl: phenylpropanoids, isopr: isoprenoids.

were compared with the alkaloid profile of each plant. In a first approach, we looked at cDNAs showing differential expression in morphinan alkaloid producing *P. somniferum* plants compared to other *Papaver* species, which do not produce this class of benzylisoquinolines. From 2,000 ESTs examined, only eleven cDNAs showed morphinan specific expression. Mostly, these showed no homology at all or homology to proteins with unknown function or to putative proteins when compared to the NCBI database. One of the differentially expressed cDNAs showed homology to an *O*-methyltransferase. The full-length cDNA was isolated and the protein was overexpressed in bacteria. Functional characterization of the recombinant protein identified this cDNA as 3-hydroxy-*N*-methylcoclaurine 4-*O*-methyltransferase. The enzyme showed a strict substrate specificity in that it only accepted tetrahydrobenzyl-isoquinolines as substrates, and not, as other class II-*O*-methyltransferases, phenolic substances.

A second approach to identify cDNAs implicated in the biosynthesis of benzylisoquinolines and the regulation of alkaloid accumulation implies the functional characterization of ESTs showing homology to proteins, where such a role can be assumed.

The enzyme family of the P450-monooxygenases are known to catalyze many reactions in the structural modification of the benzylisoquinoline backbone. Up to now, 33 ESTs coding for these enzymes could be isolated. One of these showed high homology to a previously characterized P-450 enzyme, which catalyses the formation of a methylenedioxy bridge in benzylisoquinoline biosynthesis. The full-length cDNA of this EST was isolated and its substrate specificity is under investigation. In order to achieve high expression levels of this enzyme, an overexpression system in *Nicotiana benthamiana* using viral vectors is currently established.

Recent localization studies indicated, that the enzymes of benzylisoquinoline biosynthesis are located in different cell types, suggesting that intermediates have to be transported between cells. The large group of ABC-transporters have been shown to transport many secondary metabolites, among them also alkaloids. In our EST database, we found ten sequences coding for ABC-transporters. Currently, we are isolating the full-length cDNAs for three of these transporters. For functional characterization of the transporters, complementation of yeast strains deficient in ABC-transporter will be used. In contrast to the wild-type yeast, the mutants are not able to transport benzylisoquinolines. Overexpression of an ABC-transporter with specificity towards benzylisoquinolines in the mutants should restore wild

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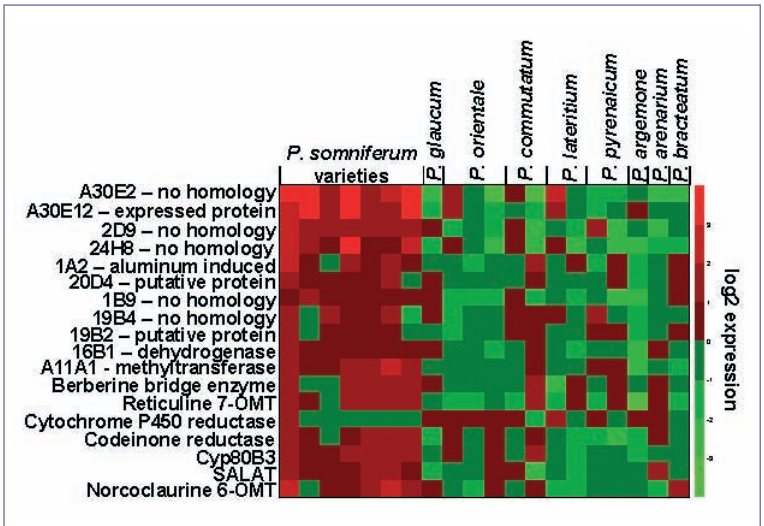

Expression level of selected cDNAs in different *Papaver* species.

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Head: Ludger Wessjohann, Professor

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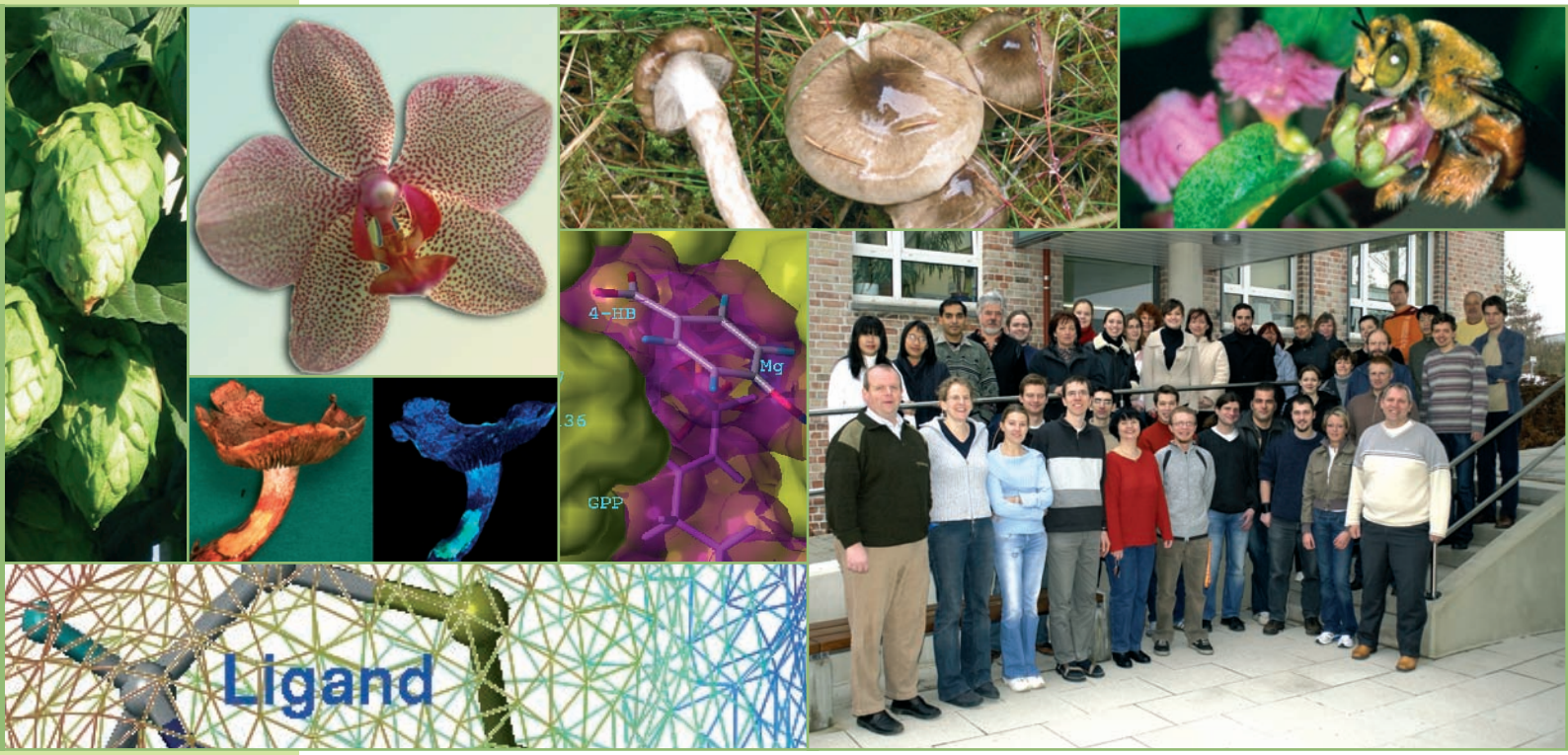

Department of
Bioorganic Chemistry

Natural products play a key role in modern drug discovery. According to a recent survey, more than 40% of all current trade drugs are natural products, or mostly derived or inspired by them, although they constitute less than 1% of the compounds screened for activity. This higher probability of success in essence has evolutionary causes. By our research we try to understand the structural and functional principles underlying this success, theoretically by chemo- and bioinformatic analyses and modelling, and in practice by isolation, characterization, modification, biosynthetic studies, and activity screens of secondary metabolites, and to some extent of proteins involved in their conversion and interaction. The analytical work is backed by an extensive synthesis program, designed to increase compound availability and molecular diversity by combinatorial chemistry, method development, and *de novo* synthesis. The principal compound classes covered are isoprenoids and lipids, phenolics and small cyclopept(o)ides. Applications of this research include the use of metabolites as lead structures for drugs, cosmetics, or as research tools, and the use of enzymes as screening targets, or as catalysts for synthesis.

The department comprises four major research units covering the following topics:

- **Isolation** (Plant and Fungal Metabolites)
- **Isoprenoids** (Biosynthesis and Biocatalysis, Modification)
- **Synthesis** (incl. Method Development, Biocatalysis)
- **Computational Chemistry** (Cheminformatics, Modeling)

These are backed by the analytical units (NMR, MS, Screening, Databases). An excellent intradepartmental cooperation ensures the success of projects, which are not located in one organisational group only, because they require - at different times - the whole range of expertise, from isolation to synthesis and biosynthesis competence, accompanied by analytical and computational methods. Interdepartmental research further broadens the basis of such projects. Thus, with the Department of Stress and Developmental Biology, a group dedicated to the profiling of secondary metabolites (*metabolomics*) and proteomics from Arabidopsis and rape seed (GABI) is continuously successful. Overall, interdepartmental research has increased considerably (Tubulinbinders and Glycosyltransferases with Prof. Strack, Terpencyclases with Prof. Kutchan, Bioinformatics and Metabolomics with Prof. Scheel).

Two highlights of the year 2004 where the successful start of Prof. Bernhard Westermann as successor to Dr. Brunhilde Voigt as new group leader within the synthesis unit, and the inauguration of Building R with state of the art laboratories for combinatorial synthesis, biocatalysis, and specialized rooms such as a night/stink lab, fermentation, GC-lab, DNA-sequencing etc. The laboratory sections dedicated to our department were fully occupied and functional in less than four months.

In the report period, the department hosted researchers of 17 nationalities from four continents, e.g. regularly from Brazil, Vietnam, and Hungary. The international quota of medium term non-permanent researchers (Ph.D.s/postdoctorals) was about 44%. 27% of the co-workers had a scholarship, e.g. from DAAD, CAPES, Daimler-Benz Foundation etc. Apart from diploma theses, six Ph.D.-titles were granted to co-workers, two of them with distinction marks. The department was successful in competitive DFG priority programs with five projects, graduate schools, European Research Council funds, BMBF- and four EU-projects, several DAAD-programs, HWP-program, and with industry financed projects.

The publication record was considerably increased. In the last two years, 69 contributions in reviewed journals and books were published. But not only numbers increased, the publications were also placed in higher valued journals (averaged sum of impact points: 2001: 134, 2002: 152, 2003: 206, 2004: 292). The first patent applications based on IPB-research highlighted extremely potent antibacterial, antifungal and anticancer compounds.

The upcoming period will have to see improvements in the database-, IT- and screening facilities of the department as well as a further concentration and integration of research efforts along the major lines outlined hereafter.

Research Group: Plant and Fungal Metabolites / Microanalytics

Heads: Norbert Arnold & Jürgen Schmidt

The isolation and structure elucidation of plant and fungal metabolites with modern analytical techniques is the basis for investigations of both the native function of natural products as well as for applications. The evolutionary selection undergone by these compounds, favour them as leads for drug candidates as well as for the identification of new targets, which might be derived from understanding the native function. Also, ecological relations or new enzymatic transformations can be deducted from the knowledge of an organism's constituents.

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Floral Oils - Evolution, Analysis and Biological Significance

This project aims at the better understanding of the chemical composition of floral oils, of their biosynthesis and evolutionary origin. The oils serve as attractant for pollinators, for which they have different functions, most prominently nutritional ones. The analysis of floral oils of six plant families (Orchidaceae, Malpighiaceae, Krameriaceae, Iridaceae, Scrophulariaceae and Solanaceae) revealed acylated (3*R*)-hydroxy and dihydroxylated fatty acids, in free form and / or bound as acylglycerols. The hydroxy function is always located at odd numbered carbon atoms. The substitution patterns of the hydroxylated fatty acids are in agreement with recently discovered hydroxylated aldehydes and alkyldiols from epicuticular waxes. This fact suggests a close relationship between the biosynthesis of plant epicuticular waxes possessing a protective function for the plant and floral oils. The microanalytical methods were refined further, based on GC/EI-TOFMS of different derivatives (e.g. methyl esters, trimethylsilyl esters or ethers, pyrrolidides, dimethyldisulfide adducts) and ESI-MS(/MS) of the non-derivatized secretions. This is used to extend the species basis. We could also revise that previous reports on Oncidinae flower oils (Orchidaceae).

Constituents of Higher Fungi

Fungi probably comprise the largest group of higher organisms in our world with estimated 10^6 specimen. Therefore, fungi are an important resource of new metabolites. Our research is mainly focused on the isolation and characterization of fungal metabolites from collected fruit bodies of higher fungi (Basidiomycetes), preferentially mycorrhizal ones. In simple bioassays, the isolated substances are tested for fungicidal and bactericidal activity.

Species in Hygrophorus are rarely attacked by parasitic fungi, insects, or snails. 20 new 5-(hydroxyalkyl)-2-cyclopentenone derivatives (hygrophorones) could be isolated from *Hygrophorus latitabundus*, *H. olivaceoalbus*, *H. persoonii*, and *H. pustulatus*. These hygrophorones have structural similarities to the antibiotic pentenomycin. Chemically, hygrophorones are 2-cyclopentenones with hydroxy or acetoxy substituents at C-4 and/or C-5. An odd-numbered 1'oxidized alkyl chain (C_{11}, C_{13}, C_{15}, or C_{17}) is attached at C-5. In addition, from *H. persoonii* the new γ-butyrolactone derivative (5-(*E*)-2-hydroxytetradexylidene-5*H*-furan-2-one) could be isolated. Some hygrophorones are responsible for the color reaction of the stipes of these fungi upon treatment with potassium hydroxide solution. In our bioassays, we could demonstrate fungicidal activity as well as a remarkable bactericidal activity against gram-positive bacteria. Strains of multiresistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus faecium* (VRE) were inhibited in low concentration. A patent has been filed.

From fruit bodies of the basidiomycete *Hygrophorus eburneus* (Tricholomataceae) eight fatty acids (C_{16}, C_{18}) with γ-oxocrotonate partial structure could be isolated. Initial tests demonstrate their significant bactericidal and fungicidal activity.

Cortinarius bolaris (subgenus Leprocybe) is described in the literature as poisonous. The cap and stem staining yellow when bruised. The underlying principle likely is a new benzofuran glycoside, which can be isola-

Hygrophorus olivaceoalbus, the Sheathed Waxy Cap is growing in the Harz mountains.

ted from freeze-dried samples. This compound turns yellow after exposure to HCl or upon heating. The stereochemistry could not yet be established. Surprisingly, fresh specimen of *C. bolaris* do not contain the benzofuran glycoside. Instead, a new yellow-colored phthalimide derivative exhibiting a bright blue fluorescence appears. Its biosynthesis is assumed to originate from a benzofuran glycoside.

Constituents of traditional medicinal plants

HEATOS - A Vietnamese opiate detoxification symptom medication

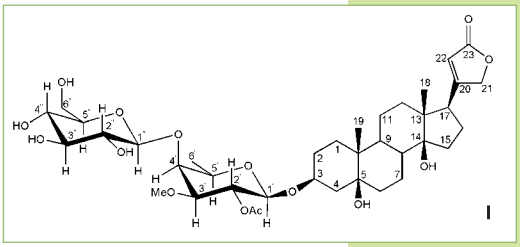
The project HEATOS was originally coordinated by the United Nations (UNESCO and UNOPS). Dr. Dan from Vietnam developed the original herbal aid for opioid detoxification and called it HEATOS (*heat of sun*). HEATOS consists of ca. 13 components and was constantly improved in composition, also with the aid of our findings. In cooperation with Vietnamese partners our research is focused on the isolation and characterization of the constituents of these plants. Most species have been identified. So far some 180 compounds are isolated from eight plants. A substance based overview and extensive literature survey was conducted.

Traditional South East Asian medicinal plants

The aim of this project is to isolate and characterize the phytoconstituents of the medicinal plants *Streptocaulon tomentosum*, *Vitis repens*, *Curcuma comosa*, *Aristolochia tagala* and *Spermacoce hispida* from Myanmar, and to test their bioactivity.

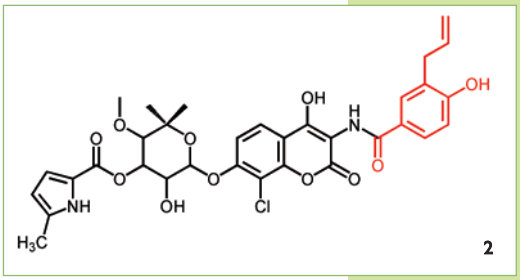
The roots of Streptocaulon species are used medicinally for the treatment of dysentery and stomach - ache, and the leaves are used externally for the treatment of snake poisoning and abscesses. *Curcuma comosa* is a member of the economically important plant family Zingiberaceae. The extract of *C. comosa* is reported to be nematocidal and choleretic in rats. As well as *Aristolochia tagala* this species is used against malaria. Rhizomes of *Vitis repens* are used against different cancers. *Spermacoce hispida* is an effective natural drug by treatment of hypertension.

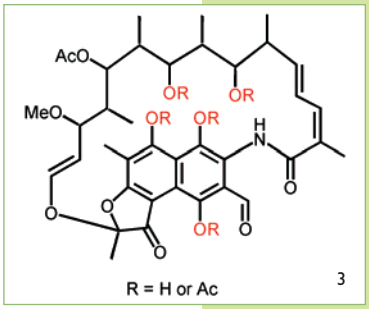
From *S. tomentosum* several triterpenoids, cardenolides, and oleanane-type saponins have been identified. A new cardenolide (**1**) was isolated from the roots. Some cardenolides had remarkable antiprolife - rative activity profiles. Apolar extracts of three plants (*C. comosa*, *A. tagala*, *V. repens*) also show significant antifungal activities.



Microanalytics

In collaboration with the University of Halle the mass spectral fragmentation of morphine and codeine was studied in detail using LC-electrospray MS/MS and high resolution FT-ICR-MS combined with an IRMPD. Labeled codeine was isolated and purified from *Papaver somniferum* seedlings, which were grown in the presence of [ring-13C6]-L-tyrosine, [ring-13C6]-tyramine and [1,2-13C2], [6-*O*-methyl 13C]-(*R,S*)-coclaurine. Using these labeled derivatives it was possible to determine the origin of the carbons in the fragment ions. In collaboration with the Department of Na - tural Product Biotechnology and the Biocenter of the University the LC-ESI-MS/MS investigations of morphinan-type and benzylisoquinoline alkaloids are continued.





Furthermore, a series of new aminocoumarin antibiotics (**2**) produced *in vitro* and *in vivo* by a combined biochemical, genetic and synthetic approach from a cooperation of the NWC-Isoprenoids Group and the University of Tübingen were structurally elucidated by using LC-ESI-SRM both under positive and negative ionization.

The high-resolution FT-ICR-MS was successfully applied for the determination of the exact mass measurements of rifamycin derivatives including their fragments obtained by varying the capillary exit voltage (**3**).

In addition, the group is intensely involved in the metabolic profiling and IT-projects at the IPB.

Research Group:
Structural Analysis & Computational Chemistry

Heads: Wolfgang Brandt & Andrea Porzel

The research group is investigating three-dimensional molecular structures of small molecules proteins and reaction mechanisms (e.g. enzyme catalysis) in the field of bioorganic chemistry by means of molecular modelling techniques, semi-empirical and *ab initio* calculations, bio- and cheminformatics, theoretical chemistry and NMR spectroscopy. The group is also involved in the development of molecular databases. The collected information together with data mining and new data from the other research groups form the basis for cheminformatic analyses which enables new insights into the biological significance of plant and fungal metabolites, and also in drug design.

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Cheminformatics and NMR

Analysis of macrocycles abundant in natural products

A database containing the structures of more than 120,000 compounds was analyzed and data mining was performed for macrocycles with more than 13 atoms in unbridged rings. The resulting data - base was analyzed for molecular weight distribution, the frequency of common substructural motifs, and biosynthetic origin in relation to ring size. The underlying principles of chemical diversity were reviewed in terms nature's strategies for diversity and complexity generation and with respect to biosynthetic origin. Finally, it was suggested that synthetic chemists should not only use nature's molecules, but also its strategies as a source of inspiration. To illustrate this, the biosynthesis of macrocycles by terpene and polyketide cyclases and nonribosomal peptide synthetases, as well as recent advances in employing these strategies in an integrated synthesis/biotechnology approach were briefly reviewed.

In house database, cheminformatic tools and spectroscopy

In collaboration with Volkmar Vill, University of Hamburg, the development of the in house database project *Phytobase* has been continued. Several hundred structural formulas have been added and input/output program options and search tools were im - proved in order to de - sign a user-friendlier surface.

In continuation of our cheminformatic activities, a database of more than 500 experimental as well as calculated $^{1}H/^{13}C$ ^{1}J shift correlated 2D NMR spectra was designed. In collaboration with the Institute of Computer Science, University of Halle, a database management system was developed which provides similarity search and mixture analyses based on the 2D NMR database content.

Modern NMR spectroscopy experiments were used for the structural elucidation of bioactive natural products isolated from plants and higher fungi. Also, the synthetic studies in the department were supported by NMR investigations. 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 and all research groups of the IPB.

Further steps towards an unified ACD-based platform of spectroscopy data were taken and the IT-coordination within the IPB was improved.

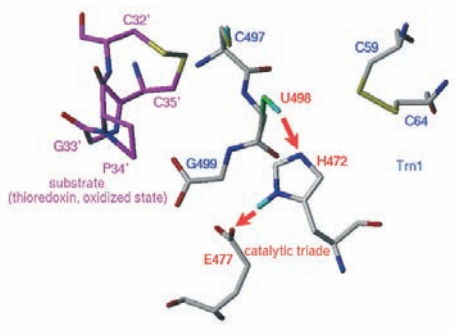

Figure 1: The catalytically active site of thioredoxin reductase in complex with the substrate thioredoxin. The red arrows indicate the proton transfer from Sec^{498} to His^{472} forming the catalytic triad together with Glu^{477}. Carbon atoms of the substrate are colored in magenta.

Modelling and Quantum Mechanical Calculations

The functional role of selenocysteine (Sec) in the catalysis mechanism of large thioredoxin reductases

We investigated the redox mechanism of thioredoxin reductases containing a selenocysteine in the catalytically active site. Thioredoxin reductases catalyze the reduction of thioredoxin disulfide and some other oxidized cell constituents. They are homodimeric proteins containing one FAD and accepting one NADPH per subunit as essential cofactors. Based on the X-ray structure of rat thioredoxin reductase, homology models of human thioredoxin reductase were created, and subsequently docked to thioredoxin to model the active complex.

The formation of a new type of a swapping catalytic triad between selenocysteine (Sec), histidine, and a

glutamate could be detected in the protein structure. By means of DFT calculations we could show that the formation of such a triad is essential to support the proton transfer from selenol to a histidine to stabilize a selenolate anion, which is able to interact with the disulfide of thioredoxin and catalyses the reductive disulfide opening (**Fig. 1**).

Whereas a simple proton transfer from selenocysteine to histidine is thermodynamically disfavored it becomes favored when the carboxylic acid group of a glutamate stabilizes the formed imidazole cation. An identical process with a cysteine instead of selenocysteine will require 4 kcal/mol more energy, which corresponds to a calculated equilibrium shift of ~ 1000:1 or a 10^3 rate acceleration a value close to the experimental one of about 10^2-times.

These results give new insights into the catalysis mechanism of thioredoxin reductase and for the first time explain the advantage of the incorporation of a selenocysteine instead of a cysteine residue in a protein.

The modeled protein structures of both the cytosolic and mitochondrial thioredoxin reductase in complex with thioredoxin have been accepted by and stored in the Protein Data Bank.

Protein 3D-homology modeling of UDP-glucose dependent betanidin 5-O-glucosyl-transferase from Dorotheanthus bellidiformis

This project was based on a close cooperation with the Department of Secondary Metabolism of our institute. The betanidin 5-*O*-glucosyltransferase (UGT73A5) from Livingstone daisy (*Dorotheanthus bellidiformis*) is involved in regiospecific glucosylation of betanidin and various flavonols. A three-dimensional model of this regiospecific betanidin and flavonoid glucosyltransferase has been constructed and the active site has been modeled (**Fig. 2**). To explain the observed inversion in the configuration of the bound sugar, semiempirical calculations favor an S_N1-type reaction as a plausible alternative to the generally proposed S_N2-mechanisms discussed for plant glucosyltransferases. The calculated structural data do not only explain the abstraction of a proton from the acceptor betanidin, but also further imply that the reaction mechanism may involve a catalytic triad with similarities to the serine protease family.

Selected amino acids suggested to be involved in substrate binding and turnover, were substituted *via* site-directed mutagenesis of the functionally expressed protein. Substitution of two highly conserved amino acids, Glu^{378}, located in the proposed UDP-glucose binding site, and His^{22}, located close to the N-terminus, both led to a complete loss of enzyme activity, which considerably supports the principal correctness of the model.

Prenyltransferases

A main project of the department is the investigation of C-C-coupling prenyltransferases, located in the research group Isoprenoids. This work is accompanied by bioinformatic studies, homology modelling, and quantum mechanical calculations. Computational chemistry work on *p*-hydroxybenzoate oligoprenyltransferase (*ubi a*) has been finished for the moment, and results were published. Recently, homology protein modeling of other prenyltransferases of other families was started.

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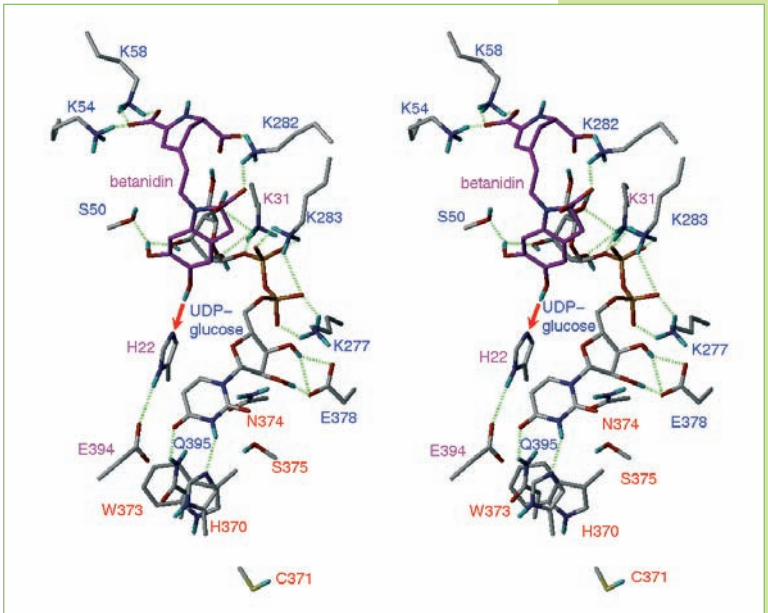

Figure 2: Stereo representation of the active site of the UGT73A5 from *D. bellidiformis* with betanidin (magenta highlighted). Amino acids proposed to be involved in substrate binding and catalytic sugar transfer are displayed. Red-labeled residues belong to the PSPG-box, magenta ones are essential for the catalysis and blue labeled ones for binding of the ligands. Hydrogen bonds are represented by green dotted lines. The red arrow indicates the initial step of the suggested catalysis mechanism.

Research Group: Isoprenoids

Head: Ludger Wessjohann

Isoprenoid units can be detected in some 70,000 natural products, including steroids, terpenoids and conjugates with molecules of other major pathways. The study of isoprenoid compounds and metabolism provides insight into the predominant mechanisms and routes that nature uses to build up carbon skeletons. Understanding these, will provide new enzymes for *in vitro* C-C-coupling reactions as biocatalysts for the production of prenylated and terpenoid compounds, as well as new targets for inhibitors of important metabolic processes in plants, most pathogenic bacteria, and many parasites. Of special interest to us is the transfer of prenyldiphosphates onto sp^2-carbons (aromatic and vinylic substrates). We hope to elucidate mechanistic and structural details, provide better access to probes and substrates, develop mechanism-based inhibitors and finally achieve access to a set of enzymes enabling a multitude of enzymatic C-C-coupling reactions. In addition, synthetic modifications of isoprenoids and diphosphates to increase the compound base are part of the program.

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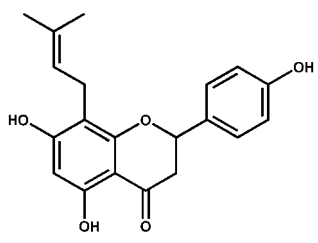
Synthesis of isoprenoid substrates, inhibitors and natural products

A large set of oligoprenyl diphosphates was synthesized as substrates and discovery tools, especially non-natural ones like homo-, nor- or heteroderivatives, or dye labeled ones, to probe substrate specificity. Interme - diates to the non-mevalonate MEP-pathway, e.g. epoxidized and hydroxylated isopentenyl and dimethylallyldiphosphate (DMAPP), were synthesized in isotope labeled form. Further chain-modified derivatives were synthesized as part of a biocatalytic total synthesis program towards prenylated aromatic natural products.

In addition, several farnesyl and geranyl analogues with mimics of the pyrophosphate unit have been prepared for an examination of their ability to inhibit prenyl transfer. 7,11-Dimethyl-3-oxododeca-6,10-dienoic acid, 3-hydroxy-7,11-dimethyldodeca-6,10-dienoic acid, 2-hydroxy-4,8-dimethyl-3,7-nonadienylphosphonic acid, and [[(4*E*)-5,9-dimethyldeca-4,8-dienyl]phos - phinato](difluoro)methylphosphonate were synthesized from geraniol. ω-2,ω-1-dihydroxylated farnesyl diphosphate was prepared from *trans,trans*-farnesol.



8-Prenylnaringenin (8-PN) **I**

8-Prenylnaringenin (8-PN) is the strongest natural phytoestrogen within the flavonoids.

8-Prenylnaringenin (8-PN, **I**) is the strongest natural phytoestrogen within the flavonoids. Recently the compound triggered considerable commercial interest as mild menopause medication. However, the total synthesis is lengthy, complicated and expensive. Also, totally "artificial" synthetic compounds are less acceptable to the anticipated customer base. Based on the abundant natural hop compound xanthohumol, we could devise a simple, protection group free two step synthesis of 8-PN in over 90% yield, in which the natural carbon isotope distribution is retained 100%. The most difficult task was the selective cleavage of a methyl ether in the presence of the acid sensitive prenyl unit, which so far was only achieved with a few percent yield and considerable byproduct formation.

ubi A Prenyltransferase

4-Hydroxybenzoate oligoprenyltransferase of *E. coli*, encoded in the gene *ubiA*, is an important key enzyme in the biosynthetic pathway to ubiquinone, an essential component of aerobic life. It catalyzes the prenylation of 4-hydroxybenzoic acid in the 3-position using an oligoprenyl diphosphate as second substrate. An X-ray structure is not available, not even of a related enzyme. The knowledge of the tertiary structure and possible active sites is, however, essential for the understanding of the catalysis mechanism and the substrate specificity.

The substrate model known for the aromatic part was extended. For the first time a minima - lized model for the prenyl substrate could be defined based on experiments with increasingly truncated GGPP-, FPP-, and GPP-derivatives (G = Geranyl, F = Farnesyl).

With respect to mechanistic studies, through modeling techniques, secondary structure prediction tools, molecular dynamics simulations, and energy optimizations, an acceptable homology model could be created. Multiple alignments of a multitude of related proteins clearly showed 100% conservation of the amino acid residues forming the putative active site. An additional highly conserved region could be detected, which currently is considered to be a rudimentary active site, giving some hints regarding the evolutionary origin of *ubiA*-enzyme. Semi-empiric quantum mechanical PM3 calculations have been performed to investigate the thermodynamics and kinetics of the catalysis mechanism. These results suggest a mechanism closer to S_N1- than to S_N2-type for the cleavage of the diphosphate ion from the isoprenyl unit. Most interesting is the deprotonation behavior of enzyme bound 4-hydroxybenzoic acid, of which formation of the phenolate anion is favored to that of a benzoate anion. This ideally aids the attack of the isoprenyl cation, which appeared to be the rate-limiting step of the whole process according to quantum chemical calculations. Probing this model by site directed mutagenesis failed so far, it proved extremely difficult to obtain functional mutated protein.

Finally, several analogues and mimics of farnesyl and geranyl pyrophosphate were tested for enzyme inhibition in a competitive assay with GPP. The effect of these compounds on *ubiA*-prenyltransferase activity varied substantially, ranging from almost full competitive inhibition to, surprisingly, enhanced enzymatic activity at low concentrations by some compounds. The activity enhancing activity of some diphosphate mimics could be related to their magnesium ion complexing properties and is only effective at high ion/low enzyme concentration. It can be mimicked by EDTA-addition.

Non-mevalonate (dxp- or mep-) Pathway in Plants

Experimental and theoretical investigations were performed concerning the two last steps of the dxp/mep-pathway in isoprenoid biosynthesis in plants. The proposed intrinsic or late intermediates, 4-oxo- and 4-hydroxy-DMAPP were synthesized in deuterium or tritium labeled form according to new protocol especially adapted to work without protection of the diphosphate moiety. In cooperation with Prof. Meinhart Zenk, these as well as the labeled cyclic precursor MEcPP were applied to chromoplast cultures. For the first time, 4-hydroxy-DMAPP was identified as the last intermediate towards DMAPP and IPP in plastids of higher plants: Its isotope labeled form was incorporated into the carotene precursor phytoene *in vitro*.

This finding is in agreement with our mechanistic and structural model of the fragment L_{271} to A_{375} of the enzyme GcpE of *Streptomyces coelicolor* (transforming MEcPP to 4-hydroxy-DMAPP), including NADPH, the Fe_4S_4-cluster and MEcPP as ligand. Based on this homology model, semi-empirical PM3 calculations were used to analyze the likely catalysis mechanism of the reductive ring opening of MEcPP. Our suggested mechanism is characterized by a proton transfer (presumably from the conserved arginine 286) to the substrate, accompanied by a ring opening without high energy barriers, followed by the transfer of two electrons delivered from the Fe_4S_4-cluster and finally proton transfer from a carboxylic acid side chain to the hydroxyl group to be removed from the ligand as water. The proposed mechanism is in agreement with all known experimental findings and the arrangement of the ligand within the putative enzyme model. It differs especially from proposals suggesting epoxide intermediates which, however, are highly unfavorable in energy and could not be supported by theoretical or by experimental data. A principally similar mechanism is also expected for the reductive dehydroxylation of 4-hydroxy-DMAPP in the last step of this basic isoprenoid pathway.

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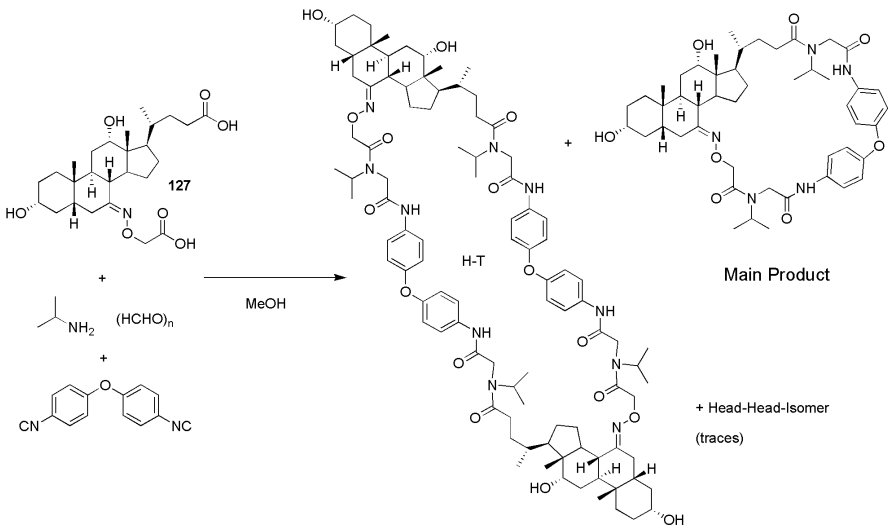
In order to obtain compounds of high biological relevance, the most recent development in organic synthesis favors the combination of (natural product) target-oriented and diversity-oriented synthesis. The synthetic group is ideally placed in this context. Efforts are undertaken to synthesize small natural products (i. a. such as provided by the Plant and Fungal Metabolites Group) and mimics thereof, focussed natural product-like libraries, and designer molecules. The biggest problem with respect to natural product like and functional structures is their complexity, which usually requires complicated multistep syntheses. New synthetic tools, methods and concepts to improve the speed and selectivity of synthetic reactions are the basis to obtain the desired chemical diversity of modified natural products or mimetics with improved or even new biological activity profiles, e. g., by multi component reaction (MCR) strategies. One focus is on macrocyclic compounds, which combine conformational preorganization with flexibility. Combinatorial approaches in liquid as well as on solid phase have been used to obtain higher selectivity, improve reaction conditions, and to form small focussed libraries demonstrating the power of our procedures. Applications in the chemical field include reactions in water, selective separation, and catalysis; in the pharmaceutical field, compounds with anticancer, antibiotic, phytoestrogenic and cosmetic properties have been studied.

Macrocycles

Even functionally very simple molecules like host-molecules already require considerable size and design. Thus, in order to minimize entropy loss upon binding, a reduction of conformational freedom compared to linear molecules is required. At the same time, some flexibility needs to be retained in order to allow adaptive properties or different stages within a functional process, mostly a binding process. This can be achieved by self-organization and folding (as in enzymes), rigidifying elements (e.g. turn inducers), or (macro-)cyclization. In nature, selective hosts or ligands of a MW-range below that of enzymes or medium peptides often are macrocycles with rigidifying and flexible elements placed in the appropriate positions, often of polyketide or peptide origin. Another important element for nature-like selection and interaction properties, recognition, and function is asymmetry. Besides asymmetry, the overall functional group density and variability distinguishes most natural hosts from e.g. crown ethers or calixarenes, which are rather rudimentary hosts from a biomimetic viewpoint and in terms of selectivity.

Thus macrocycles, ideally even with adjustable conformational bias, are good candidates to mimic natural binders in a minimal fashion, e.g. to achieve hosts selective for binding in water, or for the design of protein-protein-interaction tools. Synthetic approaches thus must have the flexibility to introduce a large variety of structural elements of rigidity, flexibility, lipophilicity, and directional functionality.

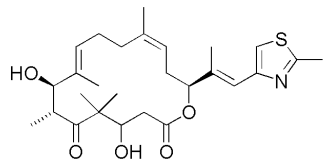
Access to libraries of such macrocycles requires the development of efficient, flexible, diversity-oriented synthesis routes. This still is a big challenge for organic synthesis because of the exceptionally stringent requirements for reaction efficiency, chemoselectivity, functional group tolerance, and stereoselectivity. Thus, diversity-oriented synthesis also provides an important engine for innovation in organic chemistry. Natural product like macrocycles can combine rigidity and flexibility at the same time, contradicting the paradigm that compounds of pharmaceutical interest have to be flat, and nitrogen containing. Therefore, work in the group has been largely focussed on the target- and diversity-oriented synthesis of macrocycles and the development of new macrocyclization reactions.



Scheme 1: Ugi-type MiB combining a steroid and biarylether moiety (MiB = multiple multicomponent macrocyclization including bifunctional building blocks)

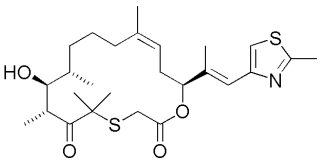
Target-oriented synthesis of macrocycles

Epothilones have pronounced biological profiles, which make them interesting drugs for cancer treatment now explored in phase II/III clinical trials. All four diastereomers of the new epothilone D_5-series, e.g. isomer **1**, were synthesized, and compared to the natural isolate. In this context, the chromium-mediated Reformatsky reaction developed by us proved to be the method of choice for the construction of the southern part.



1

In order to produce third generation epothilones with enhanced accessibility and pharmacological properties, thio-derived congeners of epothilone D lacking one stereocenter, like in compound **2**, have been synthesized. They showed low nanomolar activity against some cancer cell lines and patent applications have been filed.



2

The compounds synthesized proved useful in the study of plant cell cycles in cooperation with Bettina Hause in the Department of Secondary Metabolism.

Diversity-oriented synthesis of macrocycles

For the synthesis of macrocycle libraries we devised three strategies.

Strategy 1 utilizes commercially available macrocycles like rifamycin, tylosin or erythromycin. These are modified by selective reactions, including biocatalytic ones in solution or on solid phase, to produce libraries of rim-modified derivatives, usually without any protective group chemistry. This allows a fast chemical screen for accessibility and biologically importance of rapidly modifiable positions. A new enzyme penetrable resin-linker combination was designed for this purpose.

Research Group: Synthesis and Method Development

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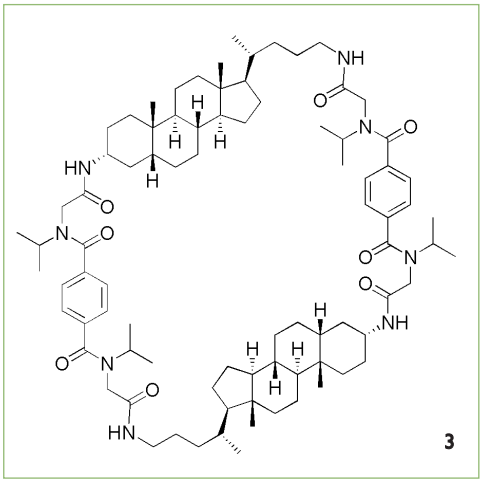
Strategy II utilizes multicomponent reactions (MCRs) like the Ugi-reaction (**Scheme 1**), which is extremely atom-economic and provides rapid access to highly diverse peptoid compounds. Utilizing specially designed bifunctional starting materials, the synthesis of macrocycles can be achieved in a one step multi reaction procedure (MiBs = Multiple Multicomponent Macrocyclization including Bifunc - tional Building Blocks). Initially, we have concentrated on the synthesis of steroid- and (vancomycin inspired) biarylether containing products like compounds **3** and **4**. E.g., some MiBs in a one-pot reaction combine twelfe building blocks assembled in 16 reactions to give a constitutionally defined 60-membered macrocycle with water as the only formal byproduct. For the first time, complex macrocycles with non-identical subunits (cf. the repetitive calixarenes and cyclodextrines) become available in an extremely efficient combinatorial fashion in infinite number and quantity with almost unlimited size, shape, and functionality, thus opening the door to a much improved host-guest chemistry, organocatalysis and artificial enzymes, cyclopept(o)id mimics, and protein-protein interaction studies.



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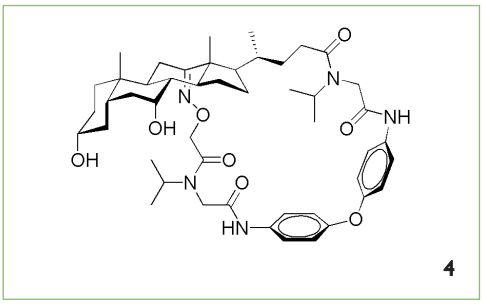
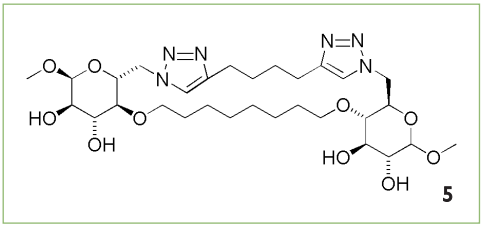


4

Strategy III is a combination of a linear precursor synthesis and a cyclizing reaction like the Grubbs-olefin metathesis and, e.g., the azide/alkyne-"click"-approach to achieve the synthesis of carbohydrate moiety containing macrocycles as in **5**. Such compounds are of particular interest due to their resemblance of aminoglycosides, a class of natural compounds exhibiting antibiotic activity.

Method development



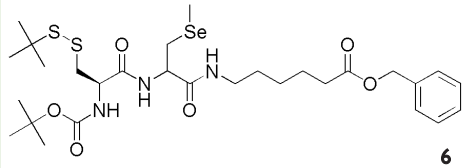
5

In the report period, several selective methods utilizing, e.g., biocatalysis towards asymmetric acyloins and novel hetero-Diels-Alder reactions to form polyketide fragments, Ugi-MCRs, transition metals and transition metal catalysts [Cr(II), Co, Pd] have been developed and utilized in the syntheses discussed above. Selenium-based and organocatalytic processes are a more current development.

Selenium chemistry

Selenium compounds are little studied in chemistry, especially in combination with their catalytic and biological significance. We set out to study the properties of selenium derivatives in comparison to the usually well-studied sulfur analogues, with a special focus on selenocystein (Sec) and Sec-containing peptides (cf. also the Research Group of Computational Chemistry). A reasonable study should be performed under (close to) physiological conditions and not be limited to a single compound. This required a synthetic route

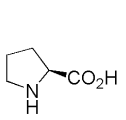
to libraries of (water soluble) Sec-peptide derivatives. In principle, the Ugi-MCR can be utilized for this purpose again, but a difficult adaptation to incorporate the initially problematic selenium containing building blocks had to be made. With the right combination of solvent, catalyst, and building blocks, we are now able to produce libraries of selenopept(o)ids in water, including, e.g., compound **6** with the biologically important Cys-Sec-moiety. Furthermore, new syntheses of seleno- and tellurocystein and related sulfur, selenium and tellurium compounds were devised and uti - lized, e.g., for catalysis.



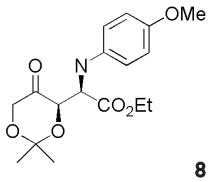
6

Organocatalysis

Organocatalysis with proline **7**, offers the possibility to carry out asymmetric Mannich reactions leading to the appropriate Mannich type bases, like **8**, in high yields and selectivity. While using protected dihydroxy acetone derivatives, this reaction can be seen as a surrogate of aldolase catalyzed reactions found in nature. Organocatalytic processes not only provide the products in high stereoselectivity, they are also "green processes" due to the absence of any metal ion. In addition, a couple of these reactions can be carried out in aqueous solutions decreasing the amount of organic solvents.



7



8

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Michael Fulhorst: Prenyltransferases as potential biocatalysts - substrate and inhibitor studies on *p*-hydroxybenzoate oligoprenyl transferase from *E. coli*. University of Halle-Wittenberg, Department of Chemistry, 22 / 09 / 2003.

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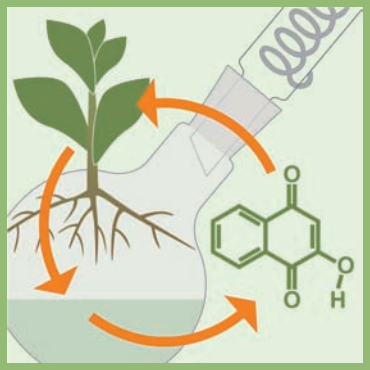

Department of Stress and Developmental Biology

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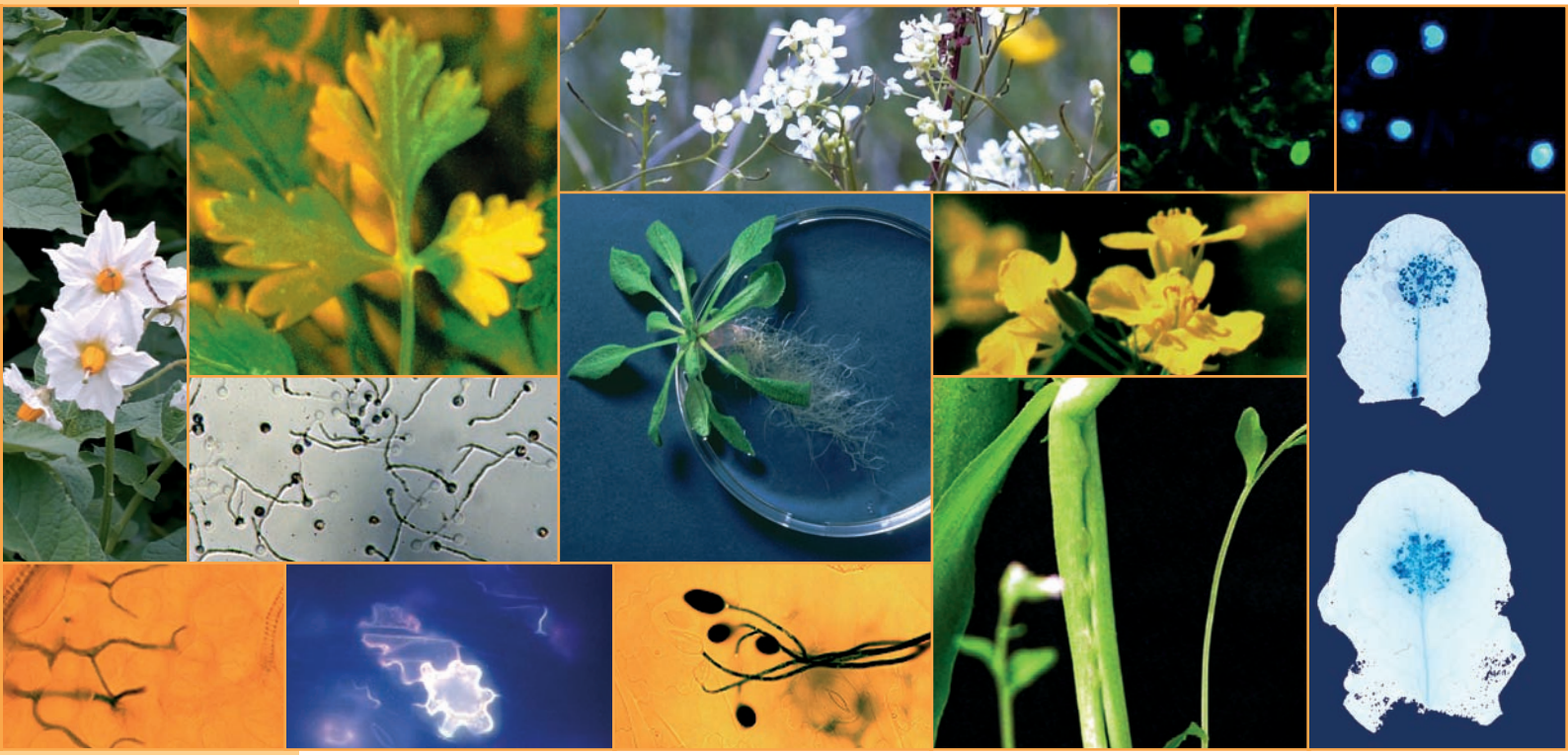
Secretary: Ruth Laue until March 2004, afterwards Susanne Berlin



Department of Stress and Developmental Biology

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. 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. 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 metal homeostasis in plants, using hyperaccumulating model organisms.

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.

Research Group:
Molecular Communication in Plant-Pathogen Interactions

Head: Wolfgang Knogge

Many phytopathogenic microorganisms colonize the intercellular spaces of their host plants. This region is relatively poor in nutrients and in order to optimize their life style pathogens therefore needed to develop strategies that aim at improving the nutrient supply by the host cells. To prevent just this, plants on their part evolved mechanisms that enable the efficient recognition of invaders as the prerequisite for their rejection. These communication processes are crucial for the outcome of an interaction. They are mediated by membrane-localized or intracellular receptors in the plant, which are the targets of pathogen-secreted molecules, frequently of proteinaceous nature. As the consequence of these protein-protein interactions two scenarios can be envisaged. The plant metabolism is redirected in favor of the pathogen and disease ensues. Alternatively, upon recognition of the pathogen through secreted compounds the plant defense is induced leading to the expression of plant resistance. Furthermore, in particular in compatible interactions pathogen 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.

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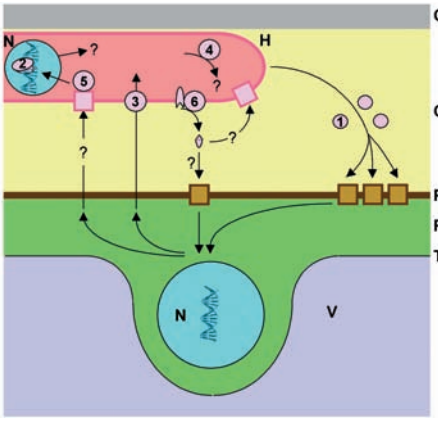
To identify pathogen genes whose products are involved in the communication with the host and, hence, play a role during pathogenesis, mutagenesis strategies can be applied. Loss-of-function mutations in genes whose products contribute to optimal pathogenesis (virulence genes) or that are even essential for microbial penetration and development *in planta* (pathogenicity genes) can be presumed to generate a phenotype that deviates more or less obviously from the wild type situation. Isolation and detailed molecular analysis of the affected genes and their protein products will lead to the unraveling of the processes that are important for the interaction. An alternative strategy aims at identifying the proteins that are secreted by the pathogen ("secretome"). These proteins are either required for the synthesis of microbial extracellular structures or candidates for factors involved in the interaction with the host.

Rhynchosporium secalis, the causal agent of a leaf spot disease on several grasses, is of particular economic importance as a pathogen of barley. After penetration of host leaves the fungus grows in the extracellular region beneath the leaf cuticle and the epidermal cells of susceptible plants. During early stages of pathogenesis epidermal cells collapse, whereas necrotic lesions only become visible during later stages when the mesophyll cells are affected as well. Small proteins that are secreted by the fungus are involved in the expression of disease symptoms, as was shown using fungal knock-out mutants. In the presence of resistance gene *Rrs1* in the host plant, one of these virulence factors, NIP1, becomes a specific recognition signal that serves as the trigger of plant resistance reactions. A specific NIP1-binding site was detected on membranes from resistant and susceptible barley cultivars as well as from other cereals. This indicates that the NIP1 receptor is not encoded by the *Rrs1* gene and that (an) additional component(s) are required for resistance-related signal transduction.

Recently, two different mutagenesis approaches, insertion mutagenesis and promoter trapping, led to the identification of several fungal pathogenicity and virulence genes. The deduced functions of the



Schematic depiction of the communication between *R. secalis* and barley. (1) NIP1, NIP2, NIP3; (2) transcription factor; (3) permease; (4) P450 protein; (5) histidine protein kinase; (6) intramembrane protease. (C) cuticle, (CW) cell wall, (H) fungal hypha, (N) nucleus, (PC) plant cytoplasm, (PM) plasma membrane, (T) tonoplast, (V) vacuole.

gene products, although biochemically not yet confirmed, suggest their role in various pathogenic processes. Thus, a protein with sequence homology to a class of transcription factors is likely to control fungal genes that are only expressed during fungal growth in the plant. A γ-aminobutyric acid / amino acid permease is supposed to mediate fungal uptake of specific plant nutrients, whereas a P450 protein may be involved in the oxidation of substrate with an as yet unknown function. One of the genes encodes a histidine protein kinase. These enzymes are part of intracellular information processing systems that link signals from the environment with specific cellular adaptive processes. Although the immediate function of most of these enzymes is not known, their high number in phytopathogenic fungi is interpreted as indicating an important role in microbial adaptation to their respective host plants. In this context the question arises as to the existence and nature of (a) plant factor(s) that may affect fungal development through these enzyme systems. Finally, a gene was identified that encodes an intramembrane protease of the rhomboid type. These enzymes catalyze the release of extracellular signals from transmembrane precursor proteins. However, neither the identity of the signal generated by *R. secalis* nor its target or the role of "regulated intramembrane proteolysis" in fungus-plant interactions in general are known to date.

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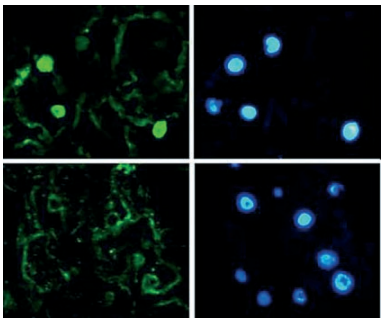
The sessile lifestyle of plants compels the development of a wide spectrum of defense reactions to counteract and deter potential pathogens, insects and parasites. Our research emphasis is on plant defense signal transduction pathways - in non-host plant-pathogen interactions as well as the so-called gene-for-gene type of disease resistance. In addition, infection strategies of *Cuscuta sp.*, a parasitic plant, are also being investigated.

The interaction of parsley with the soybean pathogen, *Phytophthora sojae*, constitutes the main model for studies of plant non-host disease resistance in our group. This form of resistance is comparable to the basal or innate immunity of animal systems. Pep-13, an oligopeptide derived from a *P. sojae* cell wall transglutaminase, serves as a pathogen-associated molecular pattern (PAMP) to trigger defense reactions in parsley cells. Upon recognition of the Pep-13 elicitor by a plasma membrane-localized receptor, a network of cellular signaling events is triggered that culminates in the appropriate defense response. The signaling events elucidated in the parsley-Pep-13 system include ion fluxes at the plasma membrane, activation of mitogen-activated protein kinases (MAPK) and the accumulation of signaling molecules such as reactive oxygen species, phospholipids and jasmonates. In particular, studies with the calcium-sensitive reporter, aqueorin, have shown that calcium influx, with a characteristic signature of sustained cytosolic Ca^{2+} increase, is an early and key step that is essential for all other downstream events studied. Thus, molecular characterization of the calcium channel involved will be pursued.

Parsley genes encoding MAPKs and their upstream MAPK kinases (MKK) have been isolated. The genes designated *PcMPK3a/b*, *PcMPK6* and *PcMKK5* have been identified as being the Pep-13-activated components in the parsley MAPK cascade. Immunofluorescence studies indicate that the activated MAPKs are rapidly translocated into the nucleus while the upstream PcMKK5 is seen to accumulate at the perinuclear region upon elicitation. It appears that MKK might serve as a cytoplasmic anchor for MAPK prior to elicitor stimulus. Phosphorylation of MAPK by MKK leads to its release from a MAPK-containing protein complex, possibly allowing the activated MAPKs to interact with their substrates. The spatial redistribution into the nucleus indicates that some of its substrates are likely to be nuclear proteins, presumably factors that control gene transcription. Transient expression of dominant inactive versions of *PcMPK3a* and/or *PcMPK6* and/or *PcMKK5* interfered with the expression of co-transfected pathogenesis-related (*PR1* or *PR2*) gene promoter-reporter constructs. Experiments with inactive mutants of other, non-Pep-13-activated, MAPK or MKK do not show this effect. In contrast, the inhibitor of the NADPH oxidase, DPI, did not affect expression of this particular subset of defense genes but blocked phytoalexin accumulation in parsley. Hence, the oxidative burst and the MAPK cascade are required in the induction of distinct subsets of defense genes by Pep-13.

A constitutive active version of *PcMKK5* was sufficient to activate PcMPK3/6 and drive *PR*-gene expression in parsley. Heterologous expression of this constitutive active *PcMKK5* in transgenic Arabidopsis, similarly led to activation of the Arabidopsis MPK3/6 orthologs and the mRNA accumulation of many defense-related genes. Binding sites for WRKY transcription factors are over-represented in the promoters of these genes; suggesting that activated MPK3/6 might target WRKY factors directly or



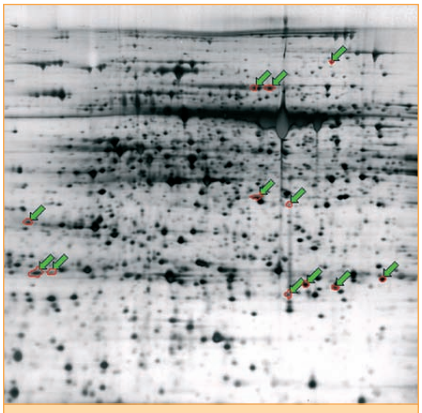
Elicitation of parsley cells leads to translocation of the activated MAPK (PcMPK6, upper panel) into the nucleus while the corresponding upstream MAP kinase kinase (PcMKK5, lower panel) remains cytoplasmic or is localized in perinuclear region despite activation. Right panels are the corresponding DAPI staining (blue) to visualize nuclei position.

indirectly. We are currently attempting to identify MAPK substrates using different biochemical, proteomic and molecular techniques. The completed genome sequence and the extensive post-genomic tools available for the model plant, *Arabidopsis thaliana*, render it the plant of choice for such studies.

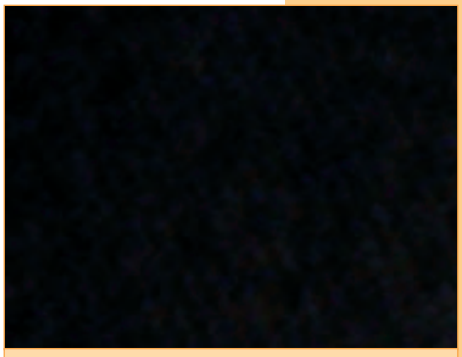
Monospecific antibodies to AtMPK3, AtMPK6 and AtMPK4 have been generated. As observed for the parsley-Pep-13 system, activation of MAPKs, e.g. by ozone treatment, also led to their nuclear translocation. Furthermore, diverse general elicitors and abiotic stresses were shown to activate these kinases. The convergence of various stress factors at the same MAPK activation also introduces the question on how specificity in signaling is maintained. One possibility is the existence of defined protein complexes (signalosomes). Indeed, size-exclusion chromatography showed that MAPKs in protein extracts, albeit only a small portion, migrate as larger molecular complexes. Transgenic plants with tandem-affinity tagged versions of the elicitor-activated MAPKs have been generated to facilitate the isolation of such protein complexes.

We have established high-resolution two-dimensional PAGE analysis of proteins from transgenic Arabidopsis plants with inducible expression of the bacterial avirulence gene, *AvrRPM1*, in the presence or absence of the corresponding resistance *RPM1* gene. Protein spots that are differentially accumulated are identified *via* MALDI-TOF spectrometry. In order to understand the differences/similarities between non-host and the gene-for-gene type of disease resistance, the analysis will be extended to transgenic plants that express a *Phytophthora sojae* Necrosis-Inducing Protein (NIP), which represents a general elicitor of defense in the non-host Arabidopsis plant.

The interaction of the holoparasitic plant, *Cuscuta sp.*, with Arabidopsis was established for studying angiosperm parasites. Not unlike other phytopathological systems, successful invasion requires the transfer of nutrients and water from the host to the parasite; in this case *via* a multicellular haustorial structure. Specific xylem-mobile or phloem-mobile dyes demonstrated a functional connection of the invading haustorium to the vascular system of the host. The high efficiency of nutrient transfer to the parasite can eventually result in death of the host. Incompatibility between *Cuscuta* and its host has occasionally been observed, where the functional haustoria is terminated at various developmental stages. A screen in various ecotypes and defense-related mutants has been initiated to elucidate the components responsible for incompatibility.



Two-dimensional polyacrylamide gel electrophoresis analysis of proteins in transgenic Arabidopsis plants that are induced to express the bacterial *AvrRPM1* gene. The arrows point to protein spots that are more abundant in these plants as compared to isogenic plants lackingg the *RPM1* resistance gene, that is required for perception/transducing the AvrRPM1 signal.



Cuscuta twining around the stem of an *Arabidopsis inflorescence*. The close-up shows an enlarged area where the haustorial region is visible. Inset is a transverse section illustrating the development of a haustorium towards the vascular systems of the host.

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Research Group: Induced Pathogen Defense

Heads: Dierk Scheel & Sabine Rosahl

The oomycete, *Phytophthora infestans,* is the causal agent of late blight disease, one of the most devastating diseases of potato. To characterize defense mechanisms against this pathogen, we are studying the interaction of *P. infestans* with its host plant potato and with the non-host plant *Arabidopsis thaliana*. For potato, our major interest is the analysis of pathogen recognition, signal transduction and characterization of the defense reactions. On the other hand, we are identifying and characterizing mutants of the non-host plant Arabidopsis, which are compromised in non-host resistance to *P. infestans*.

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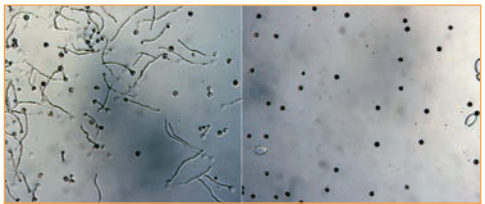
The *Phytophthora sojae*-derived oligopeptide elicitor, Pep-13, originally identified as an inducer of plant defense in parsley and shown to act as a pathogen-associated molecular pattern (PAMP) in evoking innate immune responses, triggers defense responses in potato, such as the oxidative burst, defense gene activation and the accumulation of the signaling compound salicylic acid. Interestingly, derivatives of Pep-13 show similar elicitor activity in parsley and potato, suggesting a receptor-mediated induction of defense responses in potato similar to that observed in parsley. However, unlike in parsley, infiltration of Pep-13 into leaves leads to hypersensitive-like lesions in potato, which show characteristics of programmed cell death. Analyses of transgenic potato plants, which are unable to accumulate salicylic acid, due to the expression of a salicylate hydroxylase gene, suggest that a subset of the Pep-13-induced defense responses in potato leaves is salicylate-dependent.

In addition to the local accumulation of salicylic acid, infiltration of Pep-13 or the phytopathogenic bacteria, *Pseudomonas syringae pv. maculicola*, induces increases in the levels of the signaling compounds, jasmonic acid, as well as its biosynthetic precursor, 12-oxophytodienoic acid. Interestingly, 12-oxophytodienoic acid, but not jasmonic acid, accumulates also systemically, which correlates with the expression of systemic acquired resistance. To analyze the role of jasmonic and 12-oxophytodienoic acid for both local and systemic defense responses, transgenic plants were generated, which express RNA interference constructs targeted at several genes encoding enzymes of the jasmonic acid biosynthetic pathway. Significant reduction in jasmonic and 12-oxophytodienoic acid levels were achieved in plants with reduced expression of genes encoding allene oxide cyclase and 12-oxophytodienoic acid reductase.

Oxylipins synthesized *via* the lipoxygenase pathway possess antimicrobial activity and can act as signaling molecules. We tested 49 different oxylipins for their inhibitory effects against *P. infestans* in an *in vitro* assay. 18 were able to significantly reduce mycelial growth of the oomycete or inhibit spore germination. Among those were products of the 9-lipoxygenase pathway, which also accumulate in potato leaves in response to Pep-13, as well as after pathogen infection. To analyze the role of 9-lipoxygenase-derived oxylipins for the response to pathogens, transgenic potato plants expressing RNA interference constructs targeted at the pathogen-induced 9-lipoxygenase and the 9-divinyl ether synthase were generated. Significantly reduced levels of 9-lipoxygenase products and of the divinyl ethers colneleic and colnelenic acids were observed in several transgenic plants. Lipid peroxidation during the hypersensitive cell death was not significantly altered in the absence of 9-lipoxygenase activity. Real time PCR assays to determine pathogen biomass also did not reveal significant differences in the growth of *P. infestans* on a susceptible host plant with reduced levels of 9-lipoxygenase activity. Whether oxylipins from solanaceous plants like potato can



Oxylipins inhibit germination of *Phytophthora infestans in vitro*. When added to a zoospore suspension of *P. infestans*, oxygenated polyunsaturated fatty acids are able to efficiently inhibit germination (right panel) compared to untreated controls (left panel).

Elicitation of the oxidative burst by Pep-13 is dependent on salicylic acid. The oligopeptide elicitor Pep-13 induces the accumulation of hydrogen peroxide in potato plants as visualized by staining with diaminobenzidine (left). However, in plants unable to accumulate the signaling compound salicylic acid, accumulation of reactive oxygen species is impaired (right).

also be effective against pathogens in other plants is being tested by transferring the respective genes from potato into *A. thaliana*.

Arabidopsis is not a host plant for *P. infestans*. Therefore, the identification of genes involved in this non-host resistance should elucidate mechanisms of defense against the infectious agent of late blight disease. *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. Analysis of more than 40 Arabidopsis ecotypes revealed that, despite differences in their hypersensitive response, none allows significant growth or multiplication of the oomycete. The Arabidopsis mutant, *pen2*, which was isolated by Volker Lipka and Paul Schulze-Lefert (MPI Köln) as allowing enhanced penetration of the non-host pathogen barley powdery mildew (*Blumeria graminis f. sp. hordei*), shows a higher number of penetrated cells and increased cell death in response to infection by *P. infestans*. Seeds of the *pen2* mutant were mutagenized and approximately 70,000 lines were screened for alterations in their response to infection by *P. infestans*. Several mutants were isolated, which show increased hypersensitive cell death, or which allow enhanced penetration of epidermal cells as well as enhanced growth of *P. infestans*. Mapping of the position of the affected genes is in progress.

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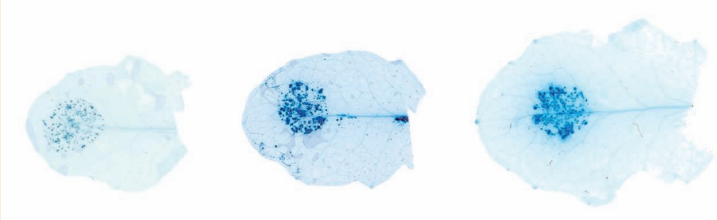

Arabidopsis mutants impaired in non-host resistance to *Phytophthora infestans*. In contrast to wild type Arabidopsis (left leaf), two mutants react to infection with *P. infestans* with enhanced cell death, indicated by stronger staining with trypan blue (middle and right leaves).

Research Group: Metal Homeostasis

Head: Stephan Clemens

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.

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Comparative transcript profiling with *A. thaliana* and *A. halleri* has led to important insights into the molecular mechanisms of metal hyperaccumulation. *A. halleri* is a hyperaccumulator growing on metal-contaminated sites in Central Europe. We found (previous cDNA-AFLP results) that the similarity of coding sequences of *A. thaliana* and *A. halleri* is high enough to use *A. thaliana* GeneChips also for *A. halleri*. We performed a number of microarray experiments to find constitutive differences in the root transcriptomes of the two species and to identify transcriptional changes in response to various doses of both essential and non-essential metal ions. The constitutive comparison revealed several genes that are expressed at a much higher level in *A. halleri*. Interestingly, some of these encode known metal homeostasis factors. The two genes showing highest expression in *A. halleri* roots relative to *A. thaliana* roots encode a nicotianamine synthase (NAS) and a putative Zn^{2+} uptake system (ZIP). The significantly higher activity of these and other genes involved in metal homeostasis could be confirmed. *A. halleri* roots also show higher NAS protein levels. Furthermore, we developed a CapLC-ESI-QTOF-MS-based nicotianamine (NA) analysis procedure and found higher NA levels in roots of *A. halleri*. Expression of a NAS in a Zn^{2+}-hypersensitive *Schizosaccharomyces pombe* mutant demonstrated that formation of NA can confer Zn^{2+} tolerance. Taken together, these observations implicate NA in plant Zn homeostasis and in the hyperaccumulation of Zn by *A. halleri*.

The identified genes are still responsive to elevated metal levels in *A. halleri* albeit at concentrations orders of magnitude higher than in *A. thaliana*. This led us to hypothesize that one molecular mechanism behind metal hyperaccumulation might be a de-regulation of metal deficiency responses. Additional microarray experiments with the "full-genome" ATH1 chip confirmed this pattern. It is an emerging picture that plant adaptation to extreme environments is mostly a regulatory phenomenon and our microarray data lend further support to this. It is now of paramount importance to elucidate the molecular basis for this altered regulation. We therefore cloned the promoters of several metal homeostasis genes from both Arabidopsis species and generated various reporter lines. Preliminary results indicate that the *A. halleri* promoters retain most of their activity in *A. thaliana*. The immediate goal of these experiments is the identification of *cis* elements responsible for the de-regulated expression in *A. halleri*.

The results of our GeneChip studies are also of general importance. They demonstrate the feasibility of comparative microarray analysis of closely related species and their potential to become an extremely valuable tool for the molecular elucidation of biodiversity.

The involvement of nicotianamine in plant metal homeostasis and Zn hyperaccumulation is studied in more detail. All known isoforms from *A. halleri* were cloned and functionally characterized. Regulation of the genes in response to varying supplies of essential micronutrients is being analyzed by real-time PCR. In addition, field samples from sites in the Harz mountains that differ in degree of metal contamination are under investigation. Transgenic approaches are aiming at modulating NA levels in both Arabidopsis species.

Microarray data on the metal responses 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. A collection of T-DNA insertion lines is being established for putative signal transduction components that, based on their metal responsiveness, are hypothesized to be involved in the transcriptional regulation of genes upon changes in external metal concentrations. Homozygous lines are being characterized with respect to tolerance, accumulation and transcriptome changes.

The synthesis of phytochelatins (PCs), small metal-binding peptides derived from glutathione, is essential for the detoxification of Cd and As ions in plants, some fungi and certain animals. A number of questions concerning the function of the ubiquitously present PC synthase encoding genes (*PCS* genes) remain unanswered. Foremost, it is not clear, how the sporadic need to detoxify non-essential metal ions could have provided the selective pressure to account for the presence of *PCS* genes throughout the plant kingdom (with the exception of some mosses). We found two lines of evidence that might help to solve this mystery: (i) studies on two PC-deficient *A. thaliana* mutants clearly showed a key role of PC formation also in buffering excess essential metal ions; (ii) characterization of one of the bacterial proteins with similarity to the PCS Pfam domain, alr0975 from *Nostoc spec.*, revealed that this protein catalyzes only the first formal step in PC synthesis, the cleavage of the C-terminal glycine from glutathione. This finding provides a clue as to the evolutionary origin of PC synthases and points to possible additional roles in glutathione metabolism, e.g. the catabolism of GS-conjugates.

With respect to additional PCS functions, we are also interested in the physiological role of the second *PCS* gene in Arabidopsis, *AtPCS2*. We showed partial complementation of the PC-deficient *cad1-3* mutant with *AtPCS2-HA*. A homozygous insertion line for *AtPCS2* is being studied. Comparative studies both *in vitro* and *in vivo* on the activities of *PCS1* and *2* from *A. thaliana* and *A. halleri* were initiated.

A second major question we are addressing concerning PC synthases is their mode of activation. They are constitutively expressed yet PC synthesis occurs only in the presence of elevated metal ion levels. Several metal ions are known to elicit PC synthesis both *in vivo* and *in vitro*. Peptide scanning experiments helped to identify putative metal binding sites. Also, we have identified differences in the response range among various PC synthases and are now trying to establish the structure-function relationships underlying these differences. This might help to gain insight into the interaction of metal ions and metal-ligand complexes with proteins - a research topic that has come into focus with the realization that aberrant metal-binding might be involved in the pathogenesis of various human disorders, including Alzheimer s and prion diseases.

S. pombe cells are being used as tools for the functional characterization of plant proteins and in order to learn more about the mechanisms of cellular metal homeostasis in an easily workable system, that can serve as a model for PC-forming cells. The analysis of putative metal homeostasis factors in *S. pombe* was extended. We constructed and characterized additional *S. pombe* knock-out mutants - especially for the transporters of the ZIP family. In order to help understand the homeostatic function of the sole metallothionein in *S. pombe* and to develop an additional tool for the analysis of the "metal status" of *S. pombe* cells, a "metal array" was established that covers known metal homeostasis genes as well as general stress response and sulphur metabolism genes.

Collaborators

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Interdepartmental Research Group: Metabolite Profiling in Arabidopsis and Crop Plants

Heads: Stephan Clemens, Jürgen Schmidt, Ludger Wessjohann & Dierk Scheel

Our project is aiming at contributing to the "post-genomic" analysis of the model organism *Arabidopsis thaliana* and of crop plants by establishing an extensive profiling of metabolites and, in addition, of proteins and peptides. These profiles are used for the detection and identification of early stress responses. Also, they are valuable tools for the analysis of various developmental and stress-induced changes as well as for the biochemical phenotyping of mutants and the exploration of natural diversity. Exemplary biotic and abiotic stresses under investigation are pathogen attack and toxic metal exposure, respectively. The metabolite profiling is to be used also for crop plant biotechnology.

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The main emphasis of the group has been on developing a novel and competitive metabolite profiling platform exploiting the potential of hybrid mass spectrometers developed in the past few years. The chemical diversity of the metabolome necessitates the use of different analytical techniques to cover the wide range of polarities found among the metabolites occurring in a cell. Thus, we decided to complement existing GC-MS approaches with a profiling scheme using liquid chromatography (LC). We established an experimental system based on capillary LC coupled to electrospray ionization quadrupole time-of-flight mass spectrometry (CapLC-ESI-Qq-TOF). Major question marks concerning this approach were the robustness of the analysis, its reproducibility, and its suitability for high-throughput and quantitative analysis. We performed an extensive evaluation of the procedures we had developed earlier. Our results demonstrated, that CapLC-ESI-Qq-TOF-MS principally meets all the necessary criteria for powerful metabolic analysis. Retention times of compounds eluting from the CapLC column were found to show only little variation. Evaluation of the mass accuracy showed that the target value in the range of five ppm error can be reached for many signals even in a complex mixture of compounds allowing, as discussed below, the calculation of elemental compositions and thereby the tentative identification of compounds. Robustness of the analysis was illustrated by the fact that the average deviation for a range of mass signals was no higher than eleven percent when the same extract was run several times. Reproducibility of retention time and mass accuracy provided a sufficient basis for the unequivocal detection and identification of mass signals. Quantification is possible because a satisfactory linear relationship between compound abundance and signal strength of the CapLC-ESI-Qq-TOF-MS analysis was found for most of the signals tested.

The raw data generated by CapLC-ESI-Qq-TOF-MS are very complex and require deconvolution. Major efforts therefore had to be devoted to developing and optimizing tools for the automatic data deconvolution and data processing. These achievements greatly increased the depth of analysis and the throughput. Reliability of data was found to be comparable to manual data processing. On average about 1,400 signals are detected in leaf extracts and about 800 signals in root extracts. Given the limited overlap between root and leaf metabolites a total of about 2,000 different signals can be detected in extracts from Arabidopsis plants grown under control conditions. Most likely the numbers will be significantly higher once the analysis is extended to other tissues such as flowers, to different developmental stages and to plants exposed to environmental stimuli.

Thus, this technique is suitable for detecting subtle metabolic changes in response to environmental stimuli or metabolic differences dependent on genetic background. This ability was proven already in pilot studies on Arabidopsis mutants affected in metabolic enzymes and on Arabidopsis stress responses. Furthermore, identification of metabolites is feasible. Electrospray as a soft ionization method combined with the enhanced resolution and mass accuracy of the TOF instrument and tandem MS allow to determine the elemental composition and to detect characteristic in-source fragments. Structural information can also be obtained in a targeted way by performing tandem MS analysis of mass signals showing interesting changes in abundance. In conclusion, the principles of separation and mass analysis of this technique together with its sensitivity and resolution greatly expand the range of me-

tabolic profiling. The development of tools for the automatic data extraction and processing make it an invaluable tool for addressing a myriad of biological questions.

To date, the profiling in its current form has been used mostly for studying metabolic changes in response to excess metals and for elucidating pathogen resistance by analyzing an Arabidopsis mutant with partial loss of non-host resistance. Extensive analysis of different alleles has allowed to identify metabolic changes caused by loss-of-function of the gene in question.

Besides trying to answer biological questions considerable effort is still invested in further improving the profiling platform with regard to scope, the establishment of data bases and the ease and speed of data analysis. Also, the focus of the metabolite profiling has recently been extended to rapeseed biotechnology within the frame of the GABI 2 program.

The profiling of Arabidopsis proteins is based on large-format high-resolution two-dimensional gel electrophoresis (2D-PAGE). Following the improvement of image analysis this technique has mostly been applied to identifying proteins that are showing changes in abundance upon expression of bacterial avirulence genes in Arabidopsis leaves.

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Publications and Other Scientific Activities 2003

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Department of Stress and Developmental Biology

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Publications

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Müller, Claudia: Struktur- und Funktionsanalyse einer mikrobiellen Transglutaminase aus *Phytophthora sojae*. University of Halle-Wittenberg, Department of Biochemistry/ Biotechnology, 22/01/2004.

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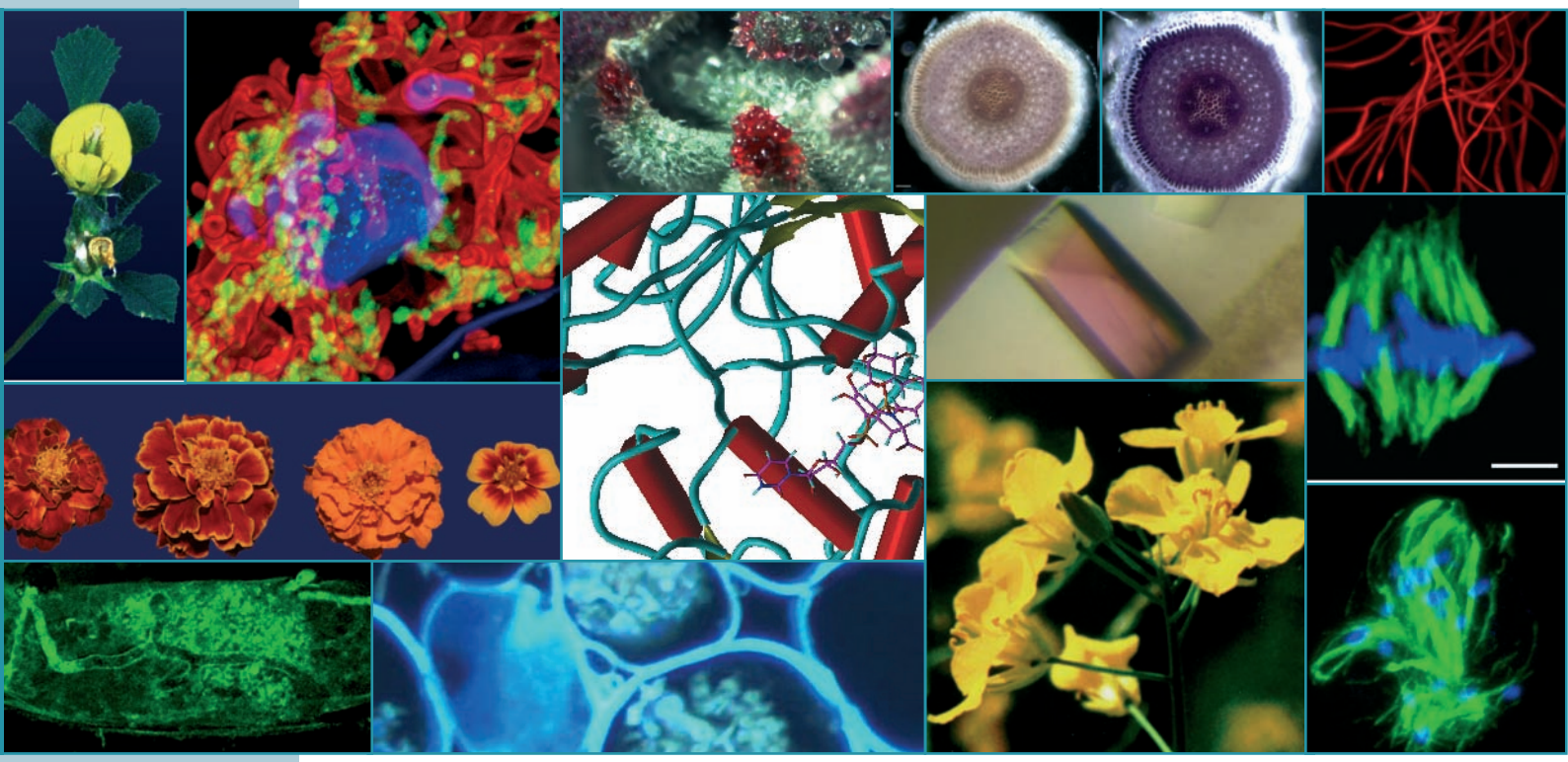
Simm, Claudia: Das Metallothionein Zym1 aus *Schizosaccharamyces pombe* - Untersuchungen zur Funktion des Metallothioneins in einem phytochelatin-bildenden Organismus. University of Halle-

Department of Secondary Metabolism

Head: Dieter Strack, Professor

Secretary: Heidemarie Stolz until October 2004, afterwards Ildikó Birkás



Department of
Secondary Metabolism

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 currently investigate 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*).

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 enzyme involved in the formation of sinapoylmalate in Arabidopsis. This plant harbors a gene family encoding 53 SCPL proteins, of which 21 cluster to a distinct group of SCPL acyltransferases. The existence of such acyltransferases proves a new concept in the regulation of plant secondary metabolism.

Special emphasis is also placed on programs focusing on the molecular interactions of plants with arbuscular mycorrhizal fungi. The work of two groups is concerned with fungus-induced alterations in plant isoprenoid metabolism, in particular carotenoid biosynthesis and degradation, 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.

Research Group: Molecular Physiology of Mycorrhiza

Head: Michael H. Walter

Arbuscular mycorrhizas are mutualistic symbiotic associations between a small number of specialized fungi and the roots of most terrestrial plants. The fungi deliver mineral nutrients to the plant and receive carbohydrates in return. The focus of the group is plant isoprenoid biosynthesis and its molecular regulation in mycorrhizal roots. Starting from an analysis of mycorrhiza-specific apocarotenoids specific gene targets in the plastidial non-mevalonate methylerythritol phosphate (MEP) pathway and in carotenoid cleavage reactions have been selected. In the first step of the MEP pathway, catalyzed by 1-deoxy-D-xylulose 5-phosphate synthase (DXS), an ancient gene duplication has evolved into specialized *DXS* genes for the generation of primary and secondary isoprenoids. The mycorrhiza-regulated *DXS2* gene has duplicated again recently and occurs as a tandem repeat in the genome of the model legume *Medicago truncatula*.

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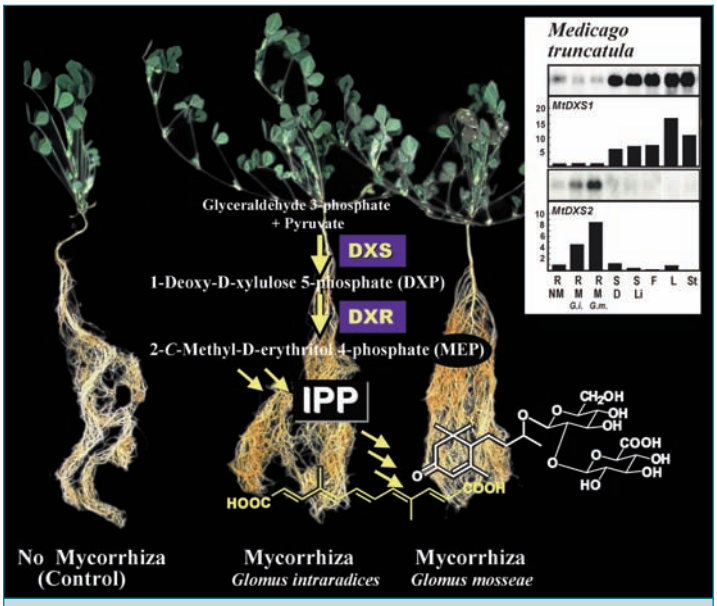
Two classes of apocarotenoids (carotenoid cleavage products) accumulate in the roots of various plants upon colonization by mycorrhizal fungi. These are linear C_{14} mycorradicin and glycosylated C_{13} cyclohexenone derivatives, which are predicted to originate from a common C_{40}-carotenoid or xanthophyll precursor. The first step of plastidial carotenoid precursor biosynthesis in the MEP pathway is catalyzed by 1-deoxy-D-xylulose 5-phosphate synthase (DXS). We have discovered duplicate genes for DXS in *Medicago truncatula*, which are distantly related and differentially regulated. The general concept of dedicated roles of DXS1 in the biosynthesis of primary isoprenoids (e.g. leaf carotenoids) and of DXS2 in secondary isoprenoid formation holds for all plants investigated so far. The only exception is *Arabidopsis thaliana*, which has two *DXS1* copies but no *DXS2* copy. Examples for *DXS2*-correlated biosynthesis of secondary isoprenoids are the monoterpenes of peppermint leaf trichomes, the steviol diterpenes of *Stevia rebaudiana* leaves and the terpenoid indole alkaloids of *Catharanthus roseus* cells. The gene duplication and subfunctionalization leading to this diversification must be very old, since we have shown that duplicate *DXS* genes occur in gymnosperms by data base mining of ESTs and by isolating a spruce *DXS1* cDNA clone. Future work will try to trace back this gene duplication even further in evolution.

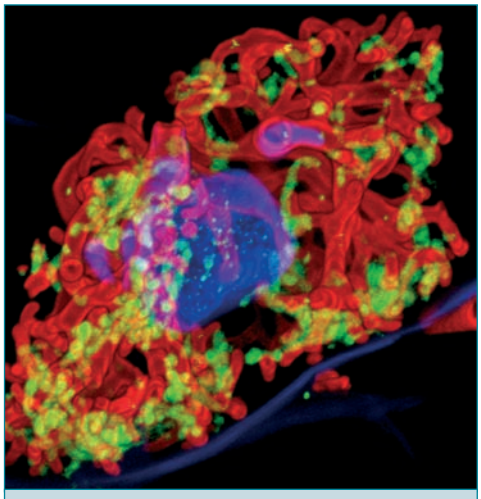
Current experiments on the angiosperm *Medicago truncatula* have identified an additional gene duplication of *DXS2* in this system. The duplicate paralogous genes *MtDXS2-1* and *MtDXS2-2* are almost identical in coding regions, similar in intron and proximal promoter regions and occur as a tandem repeat. These characteristics argue for a recent duplication event. Both genes are activated during mycorrhization. Possible early subfunctionalization features (alterations in expression) of *MtDXS2-2* are currently being investigated. An RNA*i* strategy has been devised to down-regulate both genes concomitantly and



Changes in root color of *Medicago truncatula* plants upon colonization by mycorrhizal fungi due to the accumulation of apocarotenoids, one of them being the "yellow pigment" (mycorradicin derivatives). The inset shows an RNA blot indicating elevated transcript levels of the biosynthetic enzyme MtDXS2 exclusively in mycorrhizal roots (R, M).

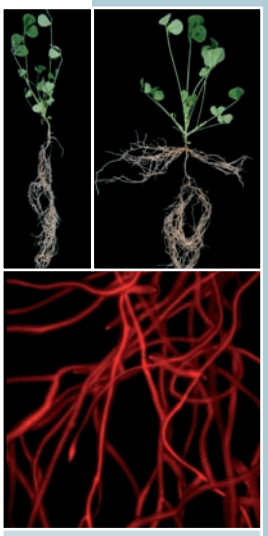
create loss-of-function mutants devoid of apocarotenoid accumulation without interfering with biosynthesis of primary carotenoids in leaves. The RNA*i*-constructs are expressed in roots of *Medicago truncatula* after transformation *via Agrobacterium rhizogenes*. Transformed roots spreading from the injection site of bacteria are recognized by the fluorescent marker DsRed and can thus be further propagated, while non-transgenic root parts are discarded. Preliminary results indicate a significantly lower concentration of mycorradicin derivatives in mycorrhizal roots transgenic for the RNA*i* construct as compared to control mycorrhizal roots. In addition, *MtDXS2-1* promoter-GUS fusions and promoter deletion-GUS fusions are being created to eventually identify promoter elements relevant to mycorrhizal regulation of plant genes.

A recent gene duplication of *DXS2* has occurred also in the genome of African marigold (*Tagetes erecta*). Amplification of genomic fragments from *T. erecta DXS2* genes indicates very strong similarity of two *TeDXS2* genes similar to the *MtDXS2* duplicates. Comparative analyses have identified the *DXS2* duplicates in six out of seven *Tagetes* species and cultivars. Only *T. tenuifolia* appears to lack the second gene copy. By lowering the stringency in PCR amplification experiments a distantly related *DXS1* fragment could be amplified from this species but never a second *DXS2* copy.



Highly branched fungal arbuscule in a mycorrhizal maize root cell visualized by WGA-TRITC staining (red). Immunolocalization of DXR (green) reveals strong proliferation of plastids around the arbuscule. DNA in plant nucleus and fungal hyphae is shown in blue by DAPI staining.

The second step of the MEP pathway catalyzed by 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR) is organized in a more simple fashion than DXS. At least in maize, rice, tomato, peppermint and *Catharanthus roseus* only single genes have been identified for DXR. A single maize *DXR* gene exhibits elevated transcript levels in leaves as well as in mycorrhizal roots. The generation of antibodies created from recombinant maize DXR has allowed for detailed immunolocalization studies on maize DXR in arbuscule-containing mycorrhizal roots. The results demonstrate an extensive proliferation of DXR-containing plastids during mycorrhization, which form a network of interconnected plastids (stromules) around the arbuscules in colonized maize root cells. The DXR accumulation reaches its maximum at or slightly after the peak of arbuscule maturation and declines thereafter indicating a close correlation of (apo)carotenoid formation with the arbuscular life cycle.



M. truncatula plantlets injected with *Agrobacterium rhizogenes* below the hypocotyl develop new transgenic hairy roots at the injection site (above, plant to the right). Transgenic roots of *M. truncatula* fluoresce in red due to expressing the fluorescent marker DsRed

Carotenoid cleavage constitutes a late step in apocarotenoid biosynthesis requiring regiospecific dioxygenases. A carotenoid cleavage enzyme (CCD) clone with specificity for the 9,10 (9',10') double bond creating C_{13} and C_{14} products has been isolated from maize. Again, a single gene appears to be responsible for elevated transcript levels in leaves and in mycorrhizal roots. Expression of the maize *CCD* in *E. coli* strains engineered to produce carotenoids leads to a discoloration of bacterial colonies. However, product identification after incubation of recombinant CCD with carotenoid substrates has not yet been accomplished.

Research Group: Cell Biology of Mycorrhiza

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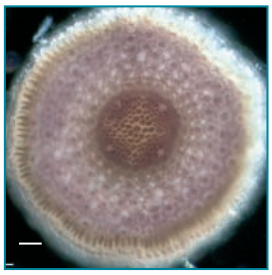
Chimeric *M. truncatula* plant after root transformation with *35S::uidA*. The plant was processed for GUS staining showing the transgenic part of the root in blue.

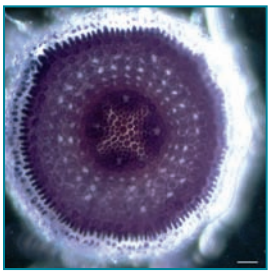
Plant hormones are believed to play a role in the establishment and development of symbiotic interactions between plants and arbuscular mycorrhizal (AM) fungi. Jasmonates, known as regulators in plant response to biotic or abiotic stress, are good candidates for such a role. Therefore, the functional analysis of jasmonates during the interaction between *Glomus intraradices* and barrel medic (*Medicago truncatula*) is the main objective of our group. In a second project, the massive proliferation of plastids in AM colonized root cortical cells is studied. Metabolic and cytological changes as well as respective molecular mechanisms will be elucidated. In a third project, we are analyzing the influence of epothilones on the structure of plant microtubules.

A possible role of jasmonates in the mycorrhizal interaction is indicated by the following data: Jasmonic acid (JA), applied exogenously, promotes colonization and development of mycorrhizal structures, and the endogenous JA level of mycorrhizal roots is remarkably higher than that of non-mycorrhizal roots. The increase of JA content in barley roots upon mycorrhization is accompanied by the expression of genes coding for enzymes of JA biosynthesis and for jasmonate-induced proteins.

To analyze the function of JA during the development of the AM symbiosis, we intend to perform functional analyses by modulating the JA content in mycorrhizal roots of *M. truncatula*. Two cDNAs coding for the allene oxide cyclase (AOC), the enzyme performing the crucial step in JA biosynthesis, were isolated from *M. truncatula*. The AOC protein was localized to plastids and found to occur constitutively in all vascular tissues of *M. truncatula*. In leaves and roots, *MtAOC1* is expressed upon JA application. Enhanced expression accompanied by elevated JA levels was also observed during mycorrhization with *G. intraradices*. A partial suppression of *MtAOC1* expression was achieved in roots following transformation with *Agrobacterium rhizogenes* harboring the *MtAOC1* cDNA in *antisense* direction under control of the CaMV35S promoter leading to chimeric plants with wild-type shoots and transformed roots. In comparison to samples transformed with a control vector (*35S::uidA*), 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 rate, quantified by fungal rRNA, and the formation of arbuscules, analyzed by the expression level of the AM-specific gene *MtPT4*, were affected. In the future work, analyses of transcript and metabolite patterns by cDNA microarrays (collaboration with H. Küster, University of Bielefeld) and metabolite profiling (collaboration with W. Schliemann, IPB Halle), both in wild-type and transgenic roots, will provide answers to the question, which processes of mycorrhization are mediated by JA.

Because higher levels of JA within mycorrhizal roots could be caused by the higher sink function of such roots, transgenic tobacco (*Nicotiana tabacum*) plants specifically modulated in root sugar metabolism were studied as well. For this, tobacco plants expressing a yeast-derived apoplast-directed invertase under the control of an alcohol-inducible promoter (*alc*) were used. Soil-drenching with aqueous acetaldehyde solutions resulted in a rapid and temporary induction of the *alc* gene expression system exclusively in roots. In addition, the split-root system allowed an activation restricted to the treated part of the root. Induction of the invertase upon treatment with acetaldehyde led to a change in the ratio of sucrose to hexoses specifically within the roots. To include invertases targeted to the cytoplasm and vacuole in our analyses, suitable vectors were used for root transformation of tobacco. All the change described, however, did not lead to alterations in AM colonization according to

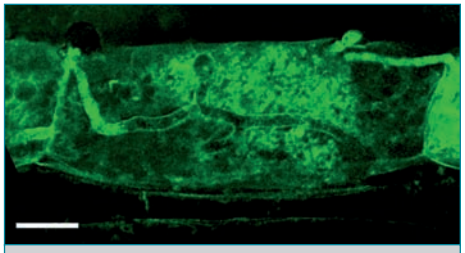




Invertase *in situ* staining of cross sections of *alc::cwINV*-tobacco root tips indicates a high invertase activity in all root cells after induction of the yeast-derived invertase by soil-drenching with 0.05 percent acetaldehyde (right side). Water-treated control plants show only a weak purpur staining (left side). Bars represent 25 µm.

microscopical, metabolite and transcript analyses.

Plastids in colonized root cortical cells are characterized by an active carotenoid biosynthesis and by a strong proliferation close to fungal structures. In the current project we have been looking for further changes in plastid metabolism, for a functional reason for the activation of carotenoid biosynthesis and for mechanisms involved in changing plastid number and shape. Transcript levels of the genes referring to the main plastid biosynthetic pathways have been analyzed by "Electronic Northern" and real-time RT-PCR. Increased transcript levels have been observed in particular for genes involved in fatty acid and asparagine biosynthesis. These findings correlate with data from the research group *Biochemistry of Mycorrhiza*, indicating increased levels of metabolites of the two respec-tive pathways. Regarding carotenoid biosynthesis, we are currently studying a possible connection of the activation of this pathway to the accumulation of reactive oxygen species (ROS) in AM roots. AM roots from transgenic tobacco plants containing reduced levels of catalase (cooperation with Frank van Breusegem, University of Gent) accumulate increased amounts of apocarotenoids. A link between carotenoid biosynthesis and the presence of ROS is further suggested by the finding that both processes occur predominantly in the phase of arbuscule degradation. Regarding the analysis of plastid number and shape, we have analyzed *M. truncatula* after *A. rhizogenes*-mediated root transformation using a construct targeting the green fluorescent protein to plastids. A significant proliferation of this compartment was observed in colonized root cortical cells. The extent of network formation, however, seemed to be less pronounced when compared to tobacco. Further analyses regard the characterization, localization and functional analysis of molecular markers involved in processes like plastid division or plastid deformation. In the case of plastid division, we have chosen the plastid division protein FtsZ for such an analysis. First results from immunolocalization of this protein suggest that the division of plastids is connected to a specific phase of arbuscular development. An *antisense* ap - proach regarding this protein will reveal the functional significance of plastid division for the AM symbiosis in general and for plastid biosynthetic metabolism in particular. Regarding a similar analysis of plastid deformation, we are currently searching for suitable proteins involved in this process.



Confocal laser scanning micrograph of an arbuscule (*Nicotiana tabacum* colonized by *Glomus intraradices*) after staining for reactive oxygen species using dihydrorhodamine-123. Bar represents 20 µm.

Our group is further interested in studying the influence of epothilones, macrocyclic lactones from culture filtrates of the myxobacterium *Sorangium cellulosum*, on the structure of plant microtubules (MTs). Stability and dynamics of MTs are influenced by natural compounds like taxol, the most prominent example for inhibitors of MT depolymerization. Epothilone and its derivatives are known as taxol-like microtubular drugs in human medicine, but their effects on plants are still unknown. We have analyzed the effect of Epothilone D on the plant cell-cycle in cultivated cells. The treatment of tomato cell suspension cultures resulted in a continuous increase of the mitotic index, visible already after two hours. As shown by immunocytological methods, abnormal spindles are formed during metaphase leading to a random distribution of chromosomes in the whole cell without formation of a metaphase plate. The effects of Epothilone D seem to be irreversible, because cells with an abnormal spindle could not be recovered after removal of the drug.

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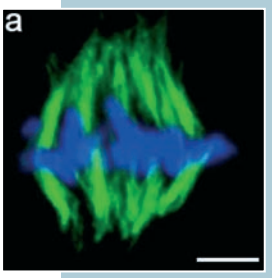

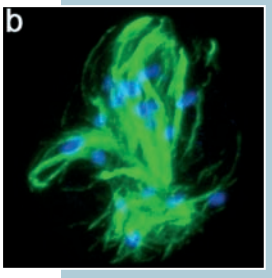

Immunofluorescence images of tomato cells in metaphase showing the microtubular pattern labelled with anti-a-tubulin antibody (green) and the distribution of chromosomes stained with DAPI (blue) in non-treated cells (a) and cells treated with 1.5 µM Epothilone D for 8 h (b). Bar represents 5 µm for both micrographs.

Research Group: Biochemistry of Mycorrhiza

Head: Willibald Schliemann

Besides transcriptomics and proteomics, metabolomics as a further functional genomic approach characterizes a biological system at a given time. Our research focus is arbuscular mycorrhiza originating from the interaction of fungi (Glomeromycota) with the roots of the majority of plants. In a project of the DFG focus program 1084 *Molecular Basics of Mycorrhizal Symbioses* the pattern of primary and secondary metabolites of developing arbuscular mycorrhizal roots of *Medicago truncatula* colonized with *Glomus intraradices* are analyzed by metabolite profiling. The ultimate aim is the characterization of causal relationships between mycorrhiza-specific gene expression and metabolite profiles to assign processes relevant for mycorrhiza development and symbiotic functioning.

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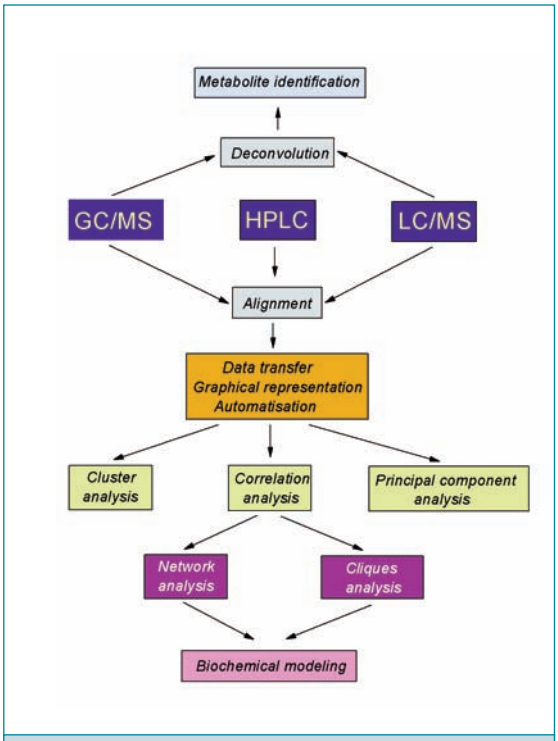
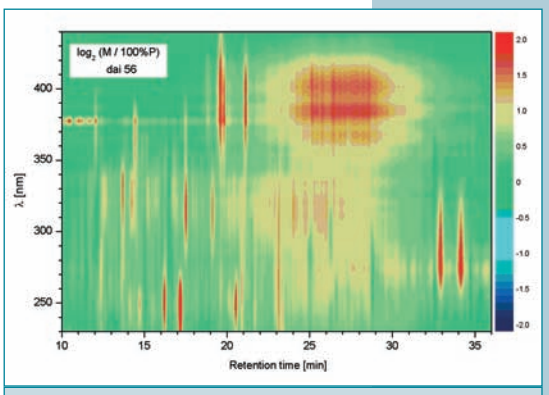
On the basis of metabolite profiling results of preliminary mycorrhization kinetics an extended mycorrhization experiment was performed including additional controls with plants fully provided with phosphate. This should allow to differentiate between metabolite alterations induced by phosphate only from those caused by mycorrhizal interaction. Furthermore, additional harvest time points and parallels enable a more reliable statistical evaluation of the metabolite data obtained by GC/MS, LC/ESI-MS and DAD-RP-HPLC. For a more substantial metabolite identification, the NIST MS-database was supplemented by the plant-specific MSRI database (collaboration with Joachim Kopka, MPI of Molecular Plant Physiology, Golm). The large data sets are subjected to a detailed quantitative analysis requiring self-programmed tools for automated data processing and visualization. The utilized analytical techniques and bioinformational tools are the key elements of our metabolite profiling platform. In the course of preprocessing the raw data are aligned, smoothed, averaged and graphically summarized for recognizing general trends. Qualitative and quantitative differences in the chromatograms are revealed in calculated differential plots. The quantitative (integrated) metabolite data are similarly preprocessed as the raw data. In subsequent steps the data are subjected to various statistical methods to reveal correlations and interactions between the metabolites at different inoculation stages during mycorrhization (Pearson correlation and network analysis, cluster, principal component and regression analysis). The results obtained up to now refer to a 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 real-time RT-PCR analyses - collaboration with Thomas Fester, IPB Halle). Furthermore, higher Pearson correlation coefficients of metabolite pairs in mycorrhizal root extracts (M) indicate a closer metabolic relationship among them-

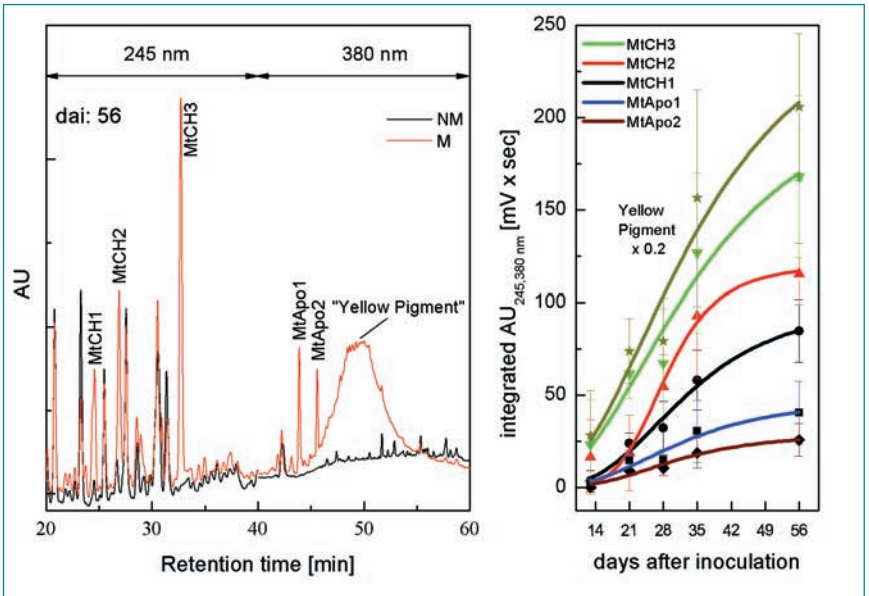


Metabolite profiling platform: analytical methods and bioinformational tools

selves than in non-mycorrhizal controls (NM). The accumulation of the fungus-specific palmitvaccenic acid (C16:1Δ^{11}) can be detected as early as 14 days after inoculation (dai), whereas the fungal trehalose and the mycorrhiza-induced apocarotenoids (cyclohexenones, mycorradicin-derived compounds and the complex "yellow pigment") are accumulating continuously during the complete analysis period. The developed ratio plot for comparison of metabolite data from averaged HPLC runs of different sample sets allows to detect wavelength-dependent differences between them immediately. The controls supplied with increased concentration of phosphate did not show any of these mycorrhiza-characteristic metabolite alterations. However, they showed strong reduction or disappearance of at least two components. The levels of saponins and especially isoflavonoids (malonylononin as the main component) are slightly higher in mycorrhizal than in non-mycorrhizal roots. The metabolite profiling approach is complemented by preparative isolation of secondary compounds and their structural elucidation by MS and NMR.



Retention time-wavelength ratio plot of averaged root HPLC data of mycorrhizal plants (M)/100 % phosphate controls (100 % P)



HPLC profiles and accumulation of mycorrhiza-induced apocarotenoids
MtCH1,2,3 - *M. truncatula* cyclohexenone derivative 1,2,3;
MtApo1,2 - *M. truncatula* mycorradicin-derived compound 1,2

Research Group: Glycosyl- and Methyltransferases

Head: Thomas Vogt

Modifications of plant natural products are performed by a variety of transferases with such prominent families like glucosyltransferases (UGTs) and methyltransferases (OMTs), which can share the same substrates and lead to the observed diversity of plant secondary metabolites. These enzymes may both solubilize and deactivate bioactive low-molecular weight compounds. Sequence identities cluster the UGTs as well as the OMTs according to regiospecificities rather than strict substrate preference. Predictions imply that both superfamilies of proteins have evolved oligophyletically as one of the primary adaptive mechanisms of plants to meet the changing environmental conditions in a timely and developmentally controlled manner. Recent developments have focused to elucidate the structural properties of both classes of enzymes with specific emphasis on substrate and position specificities as well as the reaction mechanisms.

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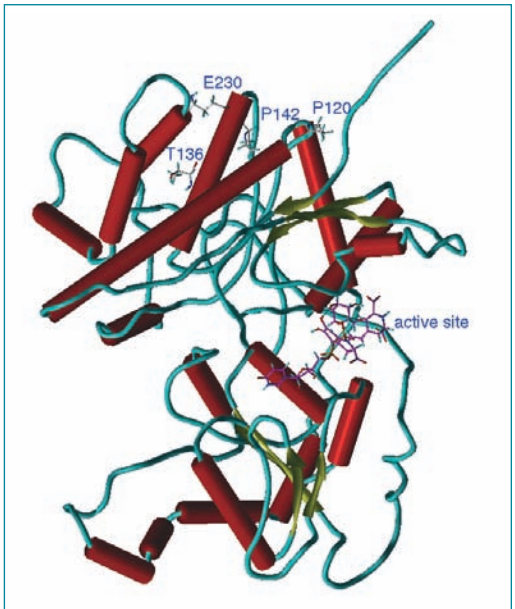
Based on earlier work, our group started on the molecular physiology of betalains, but lately as a result of the ongoing work, the major focus has shifted towards two classes of modifying enzymes involved in the modification of the two classes of plant natural products under investigation, the betacyanins and the flavonoids. By detailed sequence analysis and substrate specificity studies of two UGTs, the betanidin UGT73A5 and UGT71F2 from *Dorotheanthus bellidiformis* (*Dbs*), we were able to demonstrate an oligophyletic origin of the corresponding glucosyltransferase genes from different clusters of flavonoid UGTs. Several lines of evidence, including cloning of highly homologous UGT73A5 and UGT71F1 sequences from red beet (*Beta vulgaris*), suggest that these position specific enzymes involved in betanidin glucosylation have derived from common ancestors, flavonoid and other hydroxylated phenylpropanoid-modifying enzymes. Site-directed mutagenesis, performed with the heterologously expressed UGT73A5 indicated the importance of a highly conserved N-terminal histidine and a glutamate of the conserved UDP-glucose binding site to be catalytically essential. Based on a crystal structure of the microbial UGT from *Amycolatopsis orientalis*, the first 3D-structure of UGT73A5 was modelled, and a new S_N-1-like reaction mechanism proposed, which confirms the essential role of the N-terminal histidine as the catalytic base. Docking studies also demonstrated the substrate preference of UGT73A5 for betanidin.

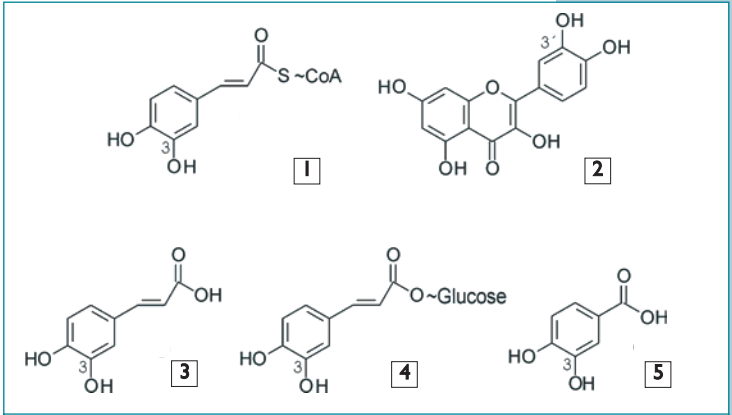
Our second model system, the ice plant (*Mesembryanthemum crystallinum*), adapts to extreme light intensities by the rapid accumulation of glycosylated and methylated flavonol and betacyanin conjugates in leaf epidermal layers. The flavonoid-methylating activity (PFOMT) is involved in the accumulation of a variety of conjugated quercetagetin 6-3 -diOMe derivatives upon light induction. Based on sequence identities and substrate specificities, it defines a new subgroup of cation-dependent OMTs, characterized by a broad substrate specificity, previously assumed to be involved only in the methylation of the lignin precursor caffeoyl coenzyme A. Light-inducible transcript accumulation and localization of the



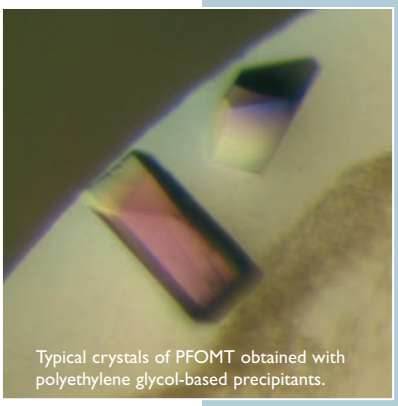
Predicted tertiary structure of UDP-glucose: betanidin 5-*O*-glucosyltransferase (UGT73A5) from *Dorotheanthus bellidiformis* with the docked ligands betanidin and UDP-glucose.

enzyme in epidermal tissues correlates with its important role in phenylpropanoid accumulation.

Optimized heterologous expression resulted in high yields of recombinant PFOMT from *E. coli*, after purification used for subsequent crystallization. Crystals were obtained under various precipitant conditions and several data sets recorded with resolutions down to 1.4 Å, in case of the recombinant PFOMT and 1.8 Å for its selenomethionine-derivative, respectively. Both datasets were sufficient to result in a preliminary 3D-crystal structure of this cation-dependent enzyme. Like in case of the UGT, the N-terminal part of the enzyme is important for position specificity. Compared to the (N-terminally truncated) native plant protein, with eleven amino acids missing, the full length sequence showed an altered product profile, when assayed with the endogenous substrate quercetagetin. The profile of the recombinant PFOMT-isoform, lacking those eleven amino acids is consistent with the observed product profile *in vivo*, whereas the full length recombinant enzyme catalyzes the formation of additional fluorescent flavonol 5-OMe derivatives, never observed in the plant. Tests with related cation-dependent OMTs revealed that not only the amino acid sequence but also that the nature of bivalent cation within the active site is crucial for the position and substrate specificity of this class of enzymes.



Potential substrates of cation-dependent OMTs. 1, caffeoyl CoA; 2, quercetin; 3, caffeic acid; 4, caffeoylglucose; 5, 3,4-dihydroxybenzoic acid;



Typical crystals of PFOMT obtained with polyethylene glycol-based precipitants.

Research Group: Hydroxycinnamic Acids

Head: Dieter Strack

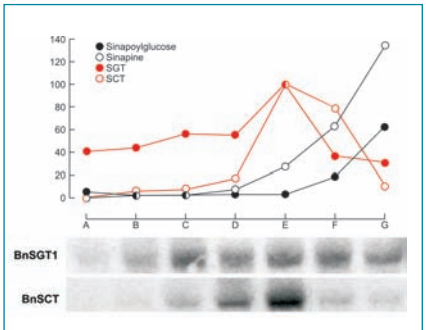
The work of our group is focused on biochemistry, genetics and molecular regulation of sinapate ester biosynthesis in oilseed rape (*Brassica napus*) and the model plant Arabidopsis with the aim to improve rapeseed quality by metabolic engineering. The sinapate esters sinapoylcholine (sinapine) and sinapoylglucose (SinGlc) are abundant compounds, known to accumulate in seeds of *Brassicaceae* plants. To produce the highly diverse pattern of metabolites, plants have evolved a wide array of enzymes to fulfill the need for driving plant secondary metabolism. SinGlc-dependent acyltransferases like SCT belong to this group of enzymes. They are homologous to hydrolases of the serine carboxypeptidase type. Our research interest is aimed at elucidating the molecular changes necessary for conversion of peptidases to acyltransferases by analysis of structure-function relationships. Sinapate esters are regarded as antinutritive factors. In *B. napus* they prevent the valuable seed protein from being used as human food supplement, a significant reduction of sinapine content is therefore a key requirement for establishing rape as a protein crop.

Based on the isolation of cDNAs encoding the enzymes for sinapine biosynthesis, UDPglucose:sinapate glucosyltransferase (BnSGT1) and sinapoylglucose:choline sinapoyltransferase (BnSCT) we analyzed the regulation of sinapate ester metabolism in *B. napus*. BnSGT1 and BnSCT activities were found to be subject to a pronounced transcriptional regulation. *BnSGT1* transcript level increases throughout early stages of seed development until the early cotelydonary stage and stays constant in later stages. In two-day old seedlings the *BnSGT1* transcripts reach their highest abundance followed by a dramatic decrease. Expression of *BnSCT* is restricted to seeds. Enzyme activities in crude extracts and metabolite concentrations reflect the RNA abundance of *BnSGT1* and *BnSCT* indicating that transcriptional regulation of these genes is of major importance for regulation of sinapine biosynthesis. Southern-blot analyses revealed that the amphidiploid genome of *B. napus* contains at least two genes for SGT and SCT, each derived from its progenitors *B. rapa* and *B. oleracea*. As the related genome of Arabidopsis was shown to encode besides SGT three less-specific ester-forming hydroxycinnamic acid glucosyltransferases (HCA-GTs) we assume for *B. napus* the presence of HCA-GT homologues potentially involved in sinapine biosynthesis.

By homology-based cloning techniques two full-length cDNAs with strong sequence identity to HCA-GTs could be isolated from *B. napus*. When expressed in *E. coli*, one of these enzymes displays HCA-GT activity. From seeds various secondary compounds were isolated and identified by MS and NMR as sinapic acid esters of glucose, gentiobiose and kaempferol glycosides, besides sinapine, sinapoylmalate and an unusual cyclic spermidine amide.

We started a transgenic approach to suppress sinapine accumulation. A dsRNA*i* construct designed to silence the *BnSGT1* gene was used to transform *B. napus* (cv. Drakkar). Transgenic plants homozygous for the insertion of the *BnSGT1* suppression cassette showed a severe reduction of sinapine content to about 20 percent of the untransformed plants accompanied by a strong decrease in total sinapate ester content.

HPLC analysis of seed extracts of the transformed plants revealed that the concentration of sinapoylglucose and most of the the minor sinapate esters was under the detection level. This indicates that 1-*O*-sinapoylglucose is a central precursor not only for sinapine biosynthesis but also for transacylation reactions leading to all the other sinapate esters in *B. napus* seeds. As the sinapoyltransferases



Transcript levels of *BnSGT1* and *BnSCT* genes, enzyme activities and metabolite accumulation in developing rape seeds. Maximal enzyme activity was set to 100% (SGT=127 pkat/mg protein; SCT= 185 pkat/mg Protein); metabolite concentration is given in nmol/seed. A-G = stages of seed development

known so far, such as SCT and SMT, have been shown to display high acceptor specificity we hypothesize that each of the sinapate esters should be produced by a distinguished acyltransferase of the SCPL-type protein giving rise to a large protein family of SCPL acyltransferases in *B. napus* as it has been shown for Arabidopsis. To optimize suppression of sinapine synthesis in rape, additional vectors have been constructed making use of the CaMV 35S promoter for constitutive silencing of *SCT* sequences for *BnSCT* to be suppressed or of a double inverted repeat including *BnSGT1* and *BnSCT* sequences for simultaneous silencing of both genes.

Energy-rich β-acetal esters like 1-*O*-sinapoylglucose have been characterized as acyl donors in transacylation reactions of plant secondary metabolism. In Arabidopsis, four related genes (*AtSGT* and *AtHCAGT1-3*) were found to encode glucosyltransferases catalyzing the formation of hydroxycinnamate glucose esters *in vitro*. We have started a systematic approach including modulation of gene expression, mutant analysis and crossing as well as promoter testing to elucidate the function of these genes *in planta*. In wild type plants, it could be shown by real-time RT-PCR that all four genes are expressed during seed development. However, *AtSGT*-mRNA is the most abundant one and expression of *AtSGT1* follows roughly that of *BnSGT1* in *B. napus* with highest expression in two-day-old seedlings. To modulate gene expression in Arabidopsis, overexpression vectors as well as specific dsRNA*i* constructs designed to silence each of the four genes have been constructed and transformed into wild type plants. Several Arabidopsis insertion lines are segregating for isolation of homozygous mutants. For each of the genes promoter-GUS fusions have been constructed and transformed. To elucidate potential links to other metabolic pathways we have included several mutants in our analyses.

Another main interest in our research is to elucidate molecular factors that govern the functional adaptation of hydrolases to perform transacylation reactions. As model protein we investigate the sinapoylglucose:L-malate sinapoyltransferase (SMT) from Arabidopsis. A key requirement for this research aimed at investigation of structure-function relationships is the heterologous overexpression of *SMT*-cDNA to produce appropriate amounts of functional enzyme for crystallization experiments. Since SMT is subject to several post-translational modifications like glycosylation or formation of disulfide bridges functional expression requires an eukaryotic system. By optimization of induction conditions and construct sequences we were able to produce enzymatically active SMT in *Saccharomyces cerevisiae*. However, further work is necessary to increase the yield. As the homologous serine carboxypeptidases belong to the best characterized proteins, a structure modelling approach has been started. Model structures obtained give first evidence for amino acid residues that are critical for transacylation function and substrate recognition. Site-directed mutagenesis will be performed to prove the functional relevance of these amino acid positions. Sequence analyses reveal that SCPL acyltransferases form a distinct group within the large family of SCPL proteins. They are specific for higher plants obviously reflecting the diversity of secondary metabolism.

Collaborators

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Victor Wray
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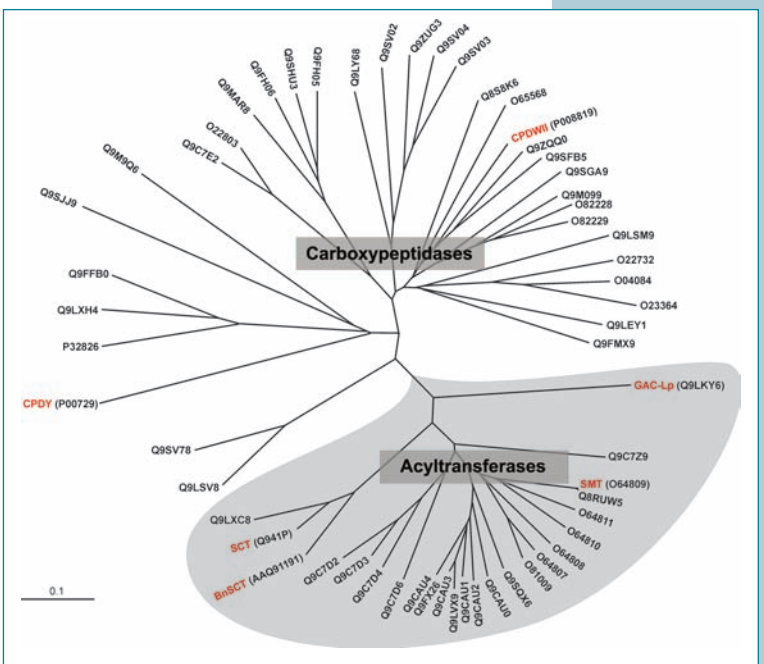

Similarity tree of SCPL proteins from Arabidopsis and functionally proven enzymes, the carboxypeptidases CPDY from yeast and CPDWII from wheat as well as the acyltransferases from Arabidopsis (SCT, SMT), rape (BnSCT) and wild tomato (GAC-Lp).

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Department of
Secondary Metabolism

Publications and Other Scientific Activities 2004

Publications

Bücking, H., Förster, H., Stenzel, I., Miersch, O. & Hause, B. Applied jasmonates accumulate extracellularly in tomato, but intracellularly in barley. *FEBS Lett.* **562**, 45-50.

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Maucher, H., Stenzel, I., Miersch, O., Stein, N., Prasa, M., Zierold, U., Schweizer, P., Dorer, C., Hause B. & Wasternack, C. The allene oxide cyclase of barley (*Hordeum vulgare* L.) - Cloning and organ-specific expression. *Phytochemistry* **65**, 801-811.

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Vogt, T. Regiospecificity and kinetic properties of a plant natural product *O*-methyltransferase are determined by its N-terminal domain. *FEBS Lett.* **561**, 159-162.

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Günther, C., Hause, B., Heinz, D., Krauzewicz, N., Rudolph, R. & Lilie, H. Combination of listeriolysin O and a tumor-specific antibody facilitate efficient cell-type specific gene delivery of conjugated DNA. *Cancer Gene Ther.*

Hause, B. & Fester, T. Molecular and cell biology of arbuscular mycorrhizal symbiosis. *Planta.*

Doctoral Theses

Hans, Joachim: Isoprenoidbiosynthese in mykorrhizierten Maiswurzeln: Klonierung, Charakterisierung und Lokalisierung einer 1-Desoxy-D-xylulose-5-phosphat-Reduktoisomerase (DXR). University of Halle-Wittenberg, Department of Biology, 24/02/2004.

Hans, Judith: Klonierung und Charakterisierung von Flavonoidglucosyltransferasen aus *Beta vulgaris* L. University of Halle-Wittenberg, Department of Biochemistry/Biotechnology, 23/06/2004.

Habilitation

Hause, Bettina: Biosynthese und Funktionen von Jasmonaten - eine zellbiologische Analyse. University of Halle-Wittenberg, Faculty of Mathematics, Natural Sciences and Technology 02/12/2004.

Professor Dieter Strack organized a DFG supported symposium in Japan entitled "Germany-Japan Seminar on Molecular Regulation of Plant Secondary Metabolism". The Japanese organizer was Kazuki Saito of the Chiba University. The symposium took place from September 20-23, 2004 at the Kazusa Akademia Center, Kisarazu, Chiba, Japan and was meant to serve as a starting point for more cooperation and exchange between Japanese and German scientists in the area of plant natural product research. Ten German and 19 Japanese groups presented their work and discussed future plants for possible collaborations.

Administration and Technical Services

Head: Lothar Franzen

Secretary: Heide Pietsch until April 2004, afterwards Cindy Maksimo

Administration and

Technical Services

During the past two years, two construction projects have dominated the activities of the IPB administration. The construction of a new shared-use building started in January 2003 and was completed in March 2004. In June 2004, construction of additional fully climate-controlled greenhouses was initiated.

With a total area of 500 square meters, the shared-use building provides space for 15 researchers and technicians from the Department of Bio - organic Chemistry. In addition to the two wide-span laboratories, there are many rooms for special uses such as fermentation, distillation, liquid chromatography and robotics for combinatorial syntheses. With an inert gas extinguishing system, experiments can also be carried out in a special "night lab" without constant supervision. Laboratories for gas chromatography, mass spectrometry, and DNA sequencing are used by scientists from all departments. The total cost of about three million Euros 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. Nearly half the building outlay is required for the technical facilities for ventilation, special gases, heating and a closed-cycle cooling system.

The new greenhouses, with an additional 350 square meters of floor space, will meet the growing demand of the departmental research programs involving transgenic plants. The nine air-conditioned chambers will be opened in the near future for cultivation of tomato, poppy, tobacco, rape and plants from the mycorrhization projects. In addition, there will be two tropical chambers and incubators for Arabidopsis. The technical equipment for the fine control of climatic conditions is state-of-the-art and costs about 1.1 million Euros. The total construction cost of 2.5 million Euros was also provided by the Federal Ministry of Education and Research and by the Ministry of Education and Cultural Affairs of the State of Saxony Anhalt. These new plant growth facilities will be ready for occupancy in May 2005.

The building that currently houses employees of facilities and construction management, graphic arts and horticulture will be replaced by two new buildings in the near future. Within this new complex, an additional 200 square meters of laboratory space for biological and chemical research for guest professors is planned as well as new offices for the Information Technology specialists from the Department of Stress and Developmental Biology.

Administration Employees

Head of the Administration
Lothar Franzen

Accounting
Head: Barbara Wolf
Gudrun Schildberg
Burgunde Seidl, *until September 2004*
Kerstin Wittenberg

Chauffeur
Jürgen Gaul

Computer Support
Head: Hans-Günter König
Holger Bartz
Kevin Begrow, *trainee since August 2003*
Ronald Scheller

Construction Management
Head: Heike Böhm
Catrin Timpel

Facilities Management
Head: Michael Kräge
Detlef Dieckmeyer, *until February 2004*
Carsten Koth
Michael Kräge
Jörg Lemnitzer
Klaus-Peter Schneider
Eberhard Warkus

General Administration
Head: Rosemarie Straßner
Christel Düfer, *until June 2004*
Heide Pietsch, *since April 2004*
Elviera Schotte
Trainees
Maike Hildebrandt, *since August 2003*
Antje Olschewski, *until July 2003*
Oliver Prudyus, *since August 2003*
Mandy Schatkowski, *since August 2003*
Clemens Schinke, *until July 2003*

Graphic Arts
Head: Christine Kaufmann
Annett Kohlberg

Horticulture
Head: Iris Rudisch
Martina Allstädt
Annett Grün, *Trainee since August 2004*
Nicole Mühlwald, *Trainee until September 2003*
Christian Müller
Philipp Plato, *Trainee since August 2004*
Kristina Rejall
Steffen Rudisch
Katja Scheming, *Trainee*
Andrea Voigt, *Trainee*

Human Resources
Head: Kerstin Balkenhohl
Alexandra Burwig
Cindy Maksimo, *until April 2004*
Antje Olschewski, *until September 2004*
Rita Stelzer, *until August 2004*
Kathleen Weckerle

Library Services
Head: Andrea Piskol
Jessica Ackermann, *Trainee until February 2003*
Anja Gärtner, *Trainee since August 2004*
Antje Werner, *Trainee until July 2004*

Press and Public Relations
Head: Sylvia Pieplow
Jana Krupik, *until September 2004*

Employee Statistics

	2003	2004
Average number of employees	172	172
Full-time employees in %	73	67
Part-time employees in %	27	33
Permanent positions	92	92
Temporary positions	26	23
Employees externally funded	41	31
Employees remunerated by "University Science Funds Program" (Hochschulwissenschaftsprogramm/HWP)	5	6
Proportion of female employees in %	61	59
Personnel turnover in %	20	16
Averade age of employees in years	39	37
Scholarship/fellowship holders	25	26
Vocational training		
general administration	3	3
horticulture	2	4
library sciences	1	1
computer support	1	1
research technical assistant	-	2
Succesfully completed vocational training	3	1
Average number of apprentices	7	11

Involvement of the IPB in National and International Scientific Networks

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

COMBIOCAT
Evolutionary Discovery of Novel Drugs by Orchestration of Polymer-Supported Combinatorial Bio-/Chemistry
Fifth Framwork Programme of the European Communitiy

EPILA
Opioid Treatment of Chronic Pain and Inflammation of the Locomotor System
Fifth Framwork Programme of the European Communitiy

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

HEATOS
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 (DFG)

MOLECULAR CELL BIOLOGY OF PLANT SYSTEMS
Collaborative Research Center SFB 363 of the German Research Foundation

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

NAPUS 2000
Rapeseed breeding
for improved human nutrition
German Federal Ministry for Education, Research and Technology (BMBF)

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

SELENOPROTEINS
Priority Programm SPP 1087 of the German Research Foundation

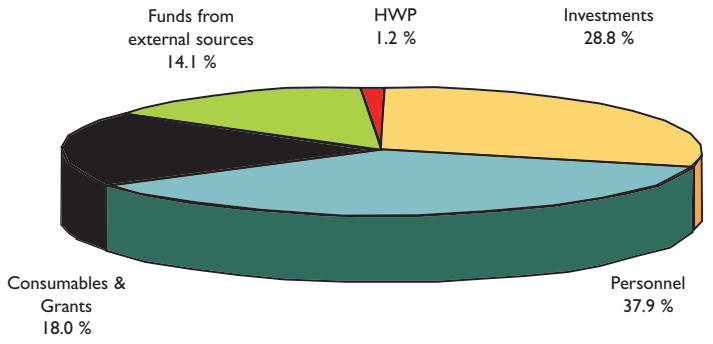
Resources and Investments

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

AFGN	*Arabidopsis Functional Genomics Network (DFG)*
BPS	BASF Plant Science
BMBF	Bundesministerium für Bildung und Forschung - *Federal Minsitry for Education and Research*
DAAD	Deutscher Akademischer Austauschdienst - *German Academic Exchange Service*
D-B Foundation	*Gottlieb Daimler and Karl Benz Foundation*
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	Hochschulsonderprogramm - *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*
PPP Hungary	Projektbezogener Personenaustausch (DAAD)- *Project Based Personnel Ex - change Program*
Probiodrug	Probiodrug AG
VW Foundation	*Volkswagen Foundation*
Wella	*Wella AG*

	in Mio. Euro	in %
Basic financing funds		
Personnel	9.7	37.9
Consumables	4.4	17.2
Grants/Subsidies	0.2	0.8
Investments	7.4	28.8
"University Science Funds Program" (HWP)	0.3	1.2
Subtotal	**22.0**	**85.9**
Funds from external sources		
BMBF	0.8	3.1
MK-LSA	0.1	0.4
DFG	1.5	5.9
Industry	0.5	2.0
EU	0.5	2.0
other	0.2	0.7
Subtotal	**3.6**	**14.1**
Total	**25.6**	**100**

Investments	**in Million Euro**
Equipment	2.3
Construction	5.1
Total	**7.4**



Funds from External Sources

Title & Head of Project	Duration	Financial Source	Amount in Euro (2003-2004)	Personnel Posts Funded
Department of Natural Product Biotechnology				
Jasmonate biosynthesis regulation *Prof. C. Wasternack & O. Miersch*	02/04 04/05	DFG DFG	49,300 16,000	1 1
12-Hydroxyjasmonic acid *Prof. C. Wasternack & O. Miersch*	03/05	DFG	30,200	1
Glutamat cyclase *Prof. C. Wasternack*	01/05	Probiodrug	24,400	0
Allenoxidcyclase *Prof. C. Wasternack*	01/03	Firmenich	6,500	0
Analysis of genes *Prof. T. Kutchan*	00/04	Icon Genetics	57,200	1
Molecular genetics of isoquinoline alk. biosynth. *Prof. T. Kutchan*	01/04 04/05	DFG DFG	93,700 41,000	2 2
Molecular genetics in *Liana Triphyoph. Pellatum* *Prof. T. Kutchan*	03/05	DFG	82,800	1
Cellular signaling *Prof. T. Kutchan*	02/04	DFG/MLU	46,400	1
Transformation and regeneration of *P. somniferum* *S. Frick*	02/04	DFG	115,300	2
Metabolic engeneering *S. Frick*	04/06	DFG	15,000	2
Modulation of jasmonates by transgenic plants *Prof. C. Wasternack & O. Miersch*	02/04	DFG/SFB 363	126,100	1
Salvia fragrances *Prof. T. Kutchan*	02/03	DBU	31,200	0
HUM-NEU *J. Page*	03/05	Hopsteiner	54,200	1
Subtotal:			**789,300**	**16**
Department of Bioorganic Chemistry				
HEATOS *Prof. L. Wessjohann*	00/03	BMBF	90,300	2
COMBIOCAT *Prof. L. Wessjohann*	01/04	EU	185,600	2
EPILA *W. Brandt*	01/04	EU	3,700	2

Title & Head of Project	Duration	Financial Source	Amount in Euro (2003-2004)	Personnel Posts Funded
MCR ligand synthesis *Prof. L. Wessjohann*	02/04	DAAD/Probral	11,900	0
Chrom-(II)-mediated reactions *Prof. L. Wessjohann*	02/03	DAAD/ PPP Hungary	5,200	0
Daimler Benz fellowship *Prof. L. Wessjohann*	02/03	D-B Foundation	-1,100	1
Reactivity of selenopeptids *Prof. L. Wessjohann & W. Brandt*	03/05 04/06	DFG DFG	100,400 15,800	1 1
CERC-3 *Prof. L. Wessjohann*	04/06	DFG	23,000	0
MUSKARIN *W. Brandt*	03/04	MLU	31,900	1
HUMULUS *Prof. L. Wessjohann*	03/04	Hopsteiner	20,000	0
Fungi metabolites *N. Arnold & J. Schmidt*	04/06	DFG	21,000	1
Screening *Prof. L. Wessjohann*	04/06	Wella	42,000	1
Subtotal:			**549,700**	**13**
Department of Stress and Developmental Biology				
Heavy metal tolerance *D. Neumann & S. Clemens*	02/04	DFG/SFB 363	70,600	1
CRISP *Prof. D. Scheel*	01/04	EU	39,000	1
Heavy metal tolerance and silicon *U. zur Nieden & S. Clemens*	00/04	MK-LSA	28,500	1
Resistence in potatoes *Prof. D. Scheel & S. Rosahl*	04/05	DFG	34,500	1
The role of jasmonates in pathogene defense *Prof. D. Scheel*	01/03	DFG	22,900	1
Jasmonate-insensitive mutant *Prof. D. Scheel*	99/03	MK-LSA	13,400	1
Arabidopsis halleri *S. Clemens*	00/03	DFG	7,600	1
Metallophytes *S. Clemens*	01/04	EU	101,800	1

Funds from External Sources

Title & Head of Project	Duration	Financial Source	Amount in Euro (2003-2004)	Personnel Posts Funded
Metalhome *S. Clemens*	03/06	EU	64,700	1
Biomineralisation *D. Neumann*	01/04	DFG	23,700	1
Cooperation with South Africa *Prof. T. Nürnberger*	01/03	VW Foundation	28,100	0
NODO *S. Rosahl*	02/04	EU	147,800	1
Receptor kinases *Prof. T. Nürnberger*	02/04	DFG/AFGN	95,000	2
Bioinformatics and mass spectronomy *Prof. D. Scheel*	02/03	BMBF	152,100	6
GABI-NONHOST *Prof. D. Scheel*	02/06	BPS BMBF	304,700	4
Metal homeostasis *S. Clemens*	04/06	DFG	20,200	1
GABI-GENOPLANTE *S. Clemens*	04/06	BMBF	10,000	1
SARA *Prof. D. Scheel*	04/07	BMBF	11,200	1
Subtotal:			**1,175,800**	**26**
Department of Secondary Metabolism				
Betanidin-Glucosyltransferases *T. Vogt & Prof. D. Strack*	01/03	DFG	22,800	2
Metabolism of isoprenoids *M.H. Walter & T. Fester* *M.H. Walter*	00/03 03/04 04/06	DFG DFG DFG	6,800 49,700 20,000	1 1 1
NAPUS 2000 *Prof. D. Strack*	99/04	BMBF	186,200	2
The role of jasmonates during the establishment of mycorrhiza *B. Hause & Prof. D. Strack*	00/04 04/06	DFG DFG	62,400 16,700	1 1
Carotenoid biosynthesis in arbuscular mycorrhizal roots *T. Fester*	00/04 04/06	DFG DFG	42,800 24,000	1 1
Metabolite profiling *W. Schliemann*	02/04 04/06	DFG DFG	115,200 23,000	1 1
Phytochemistry *Prof. D. Strack*	02/04	Elsevier	53,200	1

Title & Head of Project	Duration	Financial Source	Amount in Euro (2003-2004)	Personnel Posts Funded
HCA-Glucosyltransferases *C. Milkowski & A. Baumert*	03/05	DFG	58,200	1
SCPL-Ayltransferases *C. Milkowski & Prof. D. Strack*	03/05	DFG	48,500	1
PFOMT *T. Vogt*	04/05	DFG	3,200	0
Subtotal:			**732,700**	**16**
Joint projects				
Metabolite profiling in Arabidopsis and crop plants, GABI *Department of Stress and Developmental Biology* *Department of Bioorganic Chemistry* *S. Clemens*	00/04	BMBF	228,300	4
Metabolite profiling in Arabidopsis and crop plants, GABI-2 *Department of Stress and Developmental Biology* *Department of Bioorganic Chemistry* *Prof. D. Scheel, S. Clemens, Prof. L. Wessjohann, J. Schmidt*	04/07	BMBF	87,800	3
Subtotal:			**316,100**	**7**
Total projects granted:			**3,563,600**	**78**
General overview				
DFG			1,497,400	38
BMBF			765,900	19
EU			542,600	8
Industry			534,400	8
Other sources			181,400	3
MK-LSA			41,900	2
Total:			**3,563,600**	**78**
HWP			350,900	4
Total:			**3,914,500**	**82**

Seminars, Workshops and Colloquia 2003

January 14
Luc Varin, Professor,
Concordia University Montreal, Canada
Functional analysis of sulfotransferases in Arabidopsis thaliana.

January 23
Ekkehard Neuhaus, Professor
University of Kaiserslautern, Germany
Physiology and evolution of nucleoside transporters in plastids and bacteria.

Yves Marco, Dr.
Laboratoire de Biologie Moleculaire des Relations Plantes-Micro-Organism CNRS, France
Resistance and disease development in Arabidopsis thaliana in response to the bacterial pathogen Ralstonia solanacearum.

January 30-31
First colloquium of Priority Program 1152,
Evolution of Metabolic Diversity

February 4
Athanassios Giannis, Professor
University of Leipzig, Germany
Synthese und biologische Untersuchungen von Inhibitoren der Angiogenese.

February 6
Zsuzsanna Schwarz-Sommer, Dr.
Max Planck Institute for
Plant Breeding Research, Cologne, Germany
Homeotic control of floral organ identity in snapdragon: The problem of restricting gene expression domains.

February 18
Guy R. Cornelis, Professor
Biocenter of the
University of Basel, Switzerland
How to switch off the innate immunity: The Yersinia lesson.

February 20
Mugio Nishizawa, Professor
Bunri University, Tokushima, Japan
Synthetic approach into sterol biosynthesis by developing mercuric triflate.

February 25
Klaus Naumann, Dr.
Bayer AG, Germany
Chlor - ein wichtiger Substituent in biologisch aktiven Substanzen aus Natur und Synthese.

March 3
Yang Ye, Professor
Institute of Materia Medica, Shanghai
Chemical studies on traditional chinese medicine.

March 5
Wolfram Weckwerth, Dr.
Max Planck Institute of
Molecular Plant Physiology, Golm, Germany
Multidimensionale LC-MS für die Pflanzen - proteomanalyse.

March 12
Christine Schimek, Dr.
University of Jena, Germany
Trisporoids, apocarotenoid signal molecules in zygomycetes.

March 25
Mike Toorneman, Dr.
University of Amsterdam, The Netherlands
A (-)- Myrtenol based trigger system for the controlled formation of enediynes.

April 3
Jörg Hacker, Professor
University of Würzburg, Germany
Escherichia coli as a model to study microbial pathogenicity.

April 10
Patrick Covello, Dr.
NRC Plant Biotechnology Institute
Saskatoon, Kanada
Genomics approaches to bioactive lignan biosynthesis in Podophyllum.

April 15
Ralph Panstruga, Dr.
Max Planck Institute for
Plant Breeding Research, Cologne, Germany
Corruption of host seven-transmembrane proteins by pathogenic microbes: A common theme in animals and plants?

April 30
Bernhard Westermann, Dr.
University of Paderborn, Germany
Synthesen zu Peptid- und Glycopeptidmimetika.

May 20
Helmut Duddeck, Professor
University of Hannover, Germany
Chirale Erkennung von Phosphorderivaten mit einem Dirhodiumkomplex als NMR-Auxiliar.

May 21
Gernot Frenking, Professor
University of Marburg, Germany
Die Natur der chemischen Bindung - alte Fragen, neue Antworten.

May 26
Bernadette Lippok, Dr.
University of Freiburg, Germany
Pythium and Arabidopsis - an interaction that is complex and full of surprises.

June 4
Robert A. Dietrich, Dr.
Syngenta Biotechnology, North Carolina, USA
The Arabidopsis Bos mutants affect tolerance to both biotic and abiotic stresses.

Franziska Krajinski, Dr.
University of Hannover, Germany
Transcriptional changes in the model plant Medicago truncatula in response to symbiotic and pathogenic microorganisms.

June 5
Burkhard Schulz, Dr.
University of Tübingen, Germany
The Arabidopsis immunophilin AtFKBP42 (TWISTED DWARF) integrates hormonal signaling pathways in plants.

June 6
Elisabeth Fuss, Dr.
University of Düsseldorf, Germany
Biosynthesis of lignans in Linum spp.

June 12
Bruno Lemaitre, Dr.
Centre de Genetique Moleculaire,
CNRS, Gif-sur-Yvette, France
Genomic and genetic analysis of the Drosophila immune response.

Károly Micskei, Dr.
University of Debrecen, Hungary
Assymmetric synthesis induced by natural amino acids in aqueous medium.

Tamás Patonay, Professor
University of Debrecen, Hungary
Alpha-azido ketones: Useful tools in the synthesis of polyfunctionalized synthons and heterocycles.

June 19
Regine Kahmann, Professor
Max Planck Institute for
Terrestrial Microbiology, Marburg, Germany
The Ustilago maydis/maize pathosystem: Mating and beyond.

June 24
Naohiro Kato, Professor
University of New Brunswick, USA
Live from plant cells: Analysis of plant nuclear functions using fluorescent proteins.

June 25
Peter Bäuerle, Professor
University of Ulm, Germany
Selbstorganisierende Oligo-, Poly- und Cyclothiophene.

June 26
Peter Bäuerle, Professor
University of Ulm, Germany
Combinatorial Organic Materials Research (COMR) A faster route to organic materials.

Ralf Oelmüller, Professor
University of Jena, Germany
Regulatory proteins and novel processes in

photosynthesis.

June 30
Volkmar Vill, Professor
University of Hamburg, Germany
Naturstoff-Datenbank Phytobase - aktueller Stand der Arbeiten.

July 2
R. Madsen, Professor
Technical University of Denmark, Lyngby
Synthesis of natural products from carbohydrates by the use of ring-closing olefin metathesis.

July 3
Axel Mithöfer, Dr.
University of Munich, Germany
Aspects of friend and foe discrimination in legume-microbe interactions.

July 8
Thomas Weimar, Dr.
University of Lübeck, Germany
Untersuchung biomolekularer Wechselwirkungen mit Hilfe der NMR-Spektroskopie und der Oberflächen-Plasmonen-Resonanz.

Ahlert Schmidt, Professor
University of Hannover
Thioredoxine steuern die assimilatorische Sulfatreduktion und die assimilatorische Nitratreduktion.

July 15
Franz Nussbaum, Dr.
Bayer AG, Germany
Warum - und vor allem wie - sollte man bioaktive Naturstoffe derivatisieren? Anregungen aus der Chemie des -(-)Galiellalactons.

July 16
Werner Engewald, Professor
University of Leipzig, Germany
Die Kopplung GC-MS/MS - wieviel Chromatographie brauchen wir noch?

August 2003
Wolfgang Knogge, Dr.
University of Adelaide, Australia
NIPping grass leaves - a favorite fungal pastime and its consequences.

October 2
Ulrich Zähringer, Professor
Leibniz Center for
Medicine and Biosciences, Borstel, Germany
Peptidoglycan - Toll or Nod? Pathogenic pattern recognition in innate immunity.

October 6
David Mackey, Dr.
The Ohio State University, Columbus, USA
The interface between bacterial pathogens and plants: Virulence functions and resistance responses.

October 15
Harald Schmidt, Professor
University of Linz, Austria
Neues vom Holz.

October 16
Hugo Kubinyi, Professor
BASF AG Ludwigshafen, Germany
Wirkstoffe: Vom Zufall zum gezielten Entwurf.

Murray Grant, Dr.
Imperial College London, UK
Local and systemic signalling networks mediated by the plant disease resistance protein RPMI.

October 21
Reinhard Agerer, Professor
University of Munich, Germany
Ökologische und systematische Aspekte der Ektomykkorrhiza.

October 22
Teun Nunnik, Dr.
University of Amsterdam, The Netherlands
Phosphatidic acid - an emerging plant lipid second messenger.

October 23-24
Second colloquium of Priority Program 1152,
Evolution of Metabolic Diversity

October 23
Birgit Piechulla, Professor
University of Rostock, Germany
Control of time-specific scent emission in plants.

October 24
Shin-Ichi Aizawa, Dr.
Core Research for Evolutional Science
and Technology, Tokyo, Japan
Flagella as the type III secretion system.

October 28
Günter Gerlach, Dr.
Botanical Garden Munich, Germany
Blütenöle als Belohnung - Über die Biologie des Ölblumensyndroms.

November 6
Thomas Martin, Dr.
University of Cambridge, UK
Are Arabidopsis T-DNA mutants suitable for the analysis of 14-3-3 specificity?

November 13
David Stern, Dr.
Boyce Thompson Institute
for Plant Research, New York, USA
A medley of ribonucleases: How plastids adjust mRNA levels during plant development.

November 19
Lutz H. Gade
University of Strasbourg, France
Strategien in der Homogenkatalyse: Von der Koordinationssphäre in Einkernkomplexen zu makromolekularen Dendrimerkatalysatoren.

November 20
Georg Frank, Professor
Bayer Bitterfeld GmbH, Germany
Phasen der Arzneimittelforschung am Beispiel Aspirin.

November 27
Klaus Apel, Professor
Swiss Federal Institute
of Technology, Zurich, Switzerland
Singlet oxygen-mediated stress responses of Arabidopsis.

December 1
Karin Groten, Dr.
Rothamsted Research, Harpenden, UK
Mechanisms contributing to the senescence of pea nodules.

December 4
Andreas Freialdenhoven, Dr.
Max Planck Institute for
Plant Breeding Research, Cologne, Germany
Control of race-specific resistance and cell-death in barley.

December 10
Thomas Schrader, Professor
University of Marburg
Self-organization and molecular recognition of biomolecules by artificial receptors.

December 11
Guido van den Ackerveken, Dr.
University of Utecht, The Netherlands
Susceptibility to downy mildew in Arabidopsis: Molecular aspects of hosting an uninvited pathogenic guest.

Workshops

Februrary 2003
Collaborative Research Center *Molecular Cell Biology of Plant Systems* (SFB 363) of the German Research Foundation

June 2003,
GABI Nonhost

Meetings

January 2003

Seminars, Workshops and Colloquia 2004

January 8
Andreas Schaller, Professor
University of Hohenheim, Germany
Jasmonate signaling in plant defense and pollen development.

January 12
Ian W. M. Smith, Professor
University of Birmingham, UK
Chemistry amongst the stars: Reaction kinetics at a new frontier.

January 14
Joachim Stöckigt, Professor
University of Mainz, Germany
Molekulare Analyse der Vinorin Synthase - ein zentrales Enzym der Alkaloidbiosynthese in Rauvolfia.

Manfred Psiorz, Professor
Boehringer Ingelheim
Pharma GmbH & Co KG, Germany
Tropan-Stukturen in Natur- und Wirkstoffen.

January 15
Iwona Adamska, Professor
University of Konstanz, Germany
Protective mechanisms against light stress in the chloroplast of higher plants.

February 12
Georg Coupland, Professor
Max Planck Institute for
Plant Breeding Research, Cologne, Germany
The regulation of plant development by seasonal cues.

February 18
A. Llobet, Professor
University of Girona, Spain
Azomacrocyclic complexes and their application in bioorganic and coordination chemistry.

February 19
Steffen Backert, Dr.
University of Magdeburg, Germany
Function of two type IV secretion systems in Heliobacter pylori: Protein translocation and conjugative chromosomal DNA transfer.

February 26
Helle Ulrich, Dr.
Max Planck Institute for
Terrestrial Microbiology, Marburg, Germany
Control of genome stability by ubiquitin and SUMO.

March 18
Imre Somssich, Dr.
Max Planck Institute for
Plant Breeding Research, Cologne, Germany
Search for in vivo target genes of WRKY transcription factors involved in plant defense and leaf senescence.

March 25
Bonnie Carolyn McCaig, Dr.
Michigan State University, USA
The role of jasmonate perception in tomato reproduction and development.

March 31
Ute Wittstock, Dr.
Max Planck Institute for
Chemical Ecology, Jena, Germany
Special weapons - exceptional countermeasures: How insects cope with an activated plant defense system.

April 7
Jürgen O. Metzger, Professor
University of Oldenburg , Germany
Massenspektrometrische Untersuchung von Reaktionen in Lösung oder wie kann man Carbeniumionen bei SN1-Reaktionen und Radikale bei Radikalkettenreaktionen sehen.

Ruth Niemetz, Dr.
University of Ulm, Germany
Zur Biosynthese komplexer Gallotannine in Rhus typhina und Ellagitannine in Tellima grandiflora.

April 22
Martin Parniske, Dr.
John Innes Centre, Norwich, UK
Plant genetics of root symbiosis with fungi and bacteria.

April 29
Widmar Tanner, Professor
University of Regensburg
Plant membrane transport: From physiology to molecular biology and back.

May 3
Laurent Zimmerli, Dr.
University of Fribourg, Switzerland
Early events in Arabidopsis nonhost resistance.

May 6
Hanjo Hellmann, Professor
Freie Universität Berlin, Germany
Cullins as regulators in the ubiquitin proteasom pathway.

May 10
György Horvath
University of Antwerp, The Netherlands
Biosynthesis of tocotrienols.

May 11
Koop Lammertsma, Professor
University of Amsterdam, The Netherlands
Generating and applying new organophorus reagents.

Randolph J. Alonso-Herrera, Dr.
Venezuelan Institute
for Scientific Research Caracas, Venzuela
Synthetic derivatives of natural products with potential chemotherapeutic applicability.

May 12
Burghard König, Professor
University of Regensburg, Germany
Molecular recognition with coordination compounds.

May 18
Jens Rohloff, Dr.
Norwegian University of
Science and Technology, Trondheim, Norway
Approaches in Arabidopsis research: Space biology and differences of smell.

May 26
Jakob Ley, Dr.
Symrise GmbH & Co KG,
Holzminden, Germany
Geschmack - Physiologie, Moleküle, Modifizierung.

June 3
Herman Spaink, Professor
University of Leiden, The Netherlands
Similarities of microbial recognition by plants and animals.

June 9
Birgit Kersten, Dr.
Max Planck Institute
for Molecular Genetics, Berlin, Germany
Towards plant proteomic studies using protein microarrays.

June 10
Markus Pauly, Dr.
Max Planck Institute of
Molecular Plant Physiology, Golm, Germany
Life on the outside: Why do plant cell walls have to be so complex?

June 18
Cornelia Mrosk, Dr.
University of Jena, Germany
Die ß-Amylase in Turionen von Spirodela polyrhiza: Regulation durch Licht und Nitrat.

June 22
Harro J. Bouwmeester, Dr.
Plant Research International,
Wageningen, The Netherlands
Role of terpenoids in signaling between plants and other organisms.

June 24
Olivier Voinnet, Dr.
Institut de Biologie Moleculaire des Plantes
CNRS, Strasbourg, France

Mechanisms and roles of RNA silencing in plants and animals.

June 29
Pierre Potier, Professor
Institut de Chimie des Substances Naturelles, CNRS, Giv sur Yvette, France
Research and discoveries of new antitumor drugs: NAVELBINE ® & TAXOTERE ®.

July 5
Vincenzo de Luca, Professor
University of Ontario, Kanada
New tools for understanding metabolic pathways in single cells: The case for Catharanthus roseus indole alkaloid biosynthesis.

July 8
Steffen Abel, Professor
University of California, Davis, USA
Glucosinolates: From biosynthesis to pathway regulation.

July 15
Jeff Dangl, Professor
University of North Carolina, Chapel Hill, USA
P. syringae type III effector proteins manipulate host cell biology, and plant disease resistance gene products stop them.

July 23
Gynheung An, Professor
Pohang University
of Science and Technology, South Korea
T-DNA insertional mutagenesis for reproductive development in rice.

September 2
Stefanie Ranf
University of Souht Carolina, Columbia, USA
Virus induced gene silencing of MAP kinases in tomato.

Roger Wise, Professor
Iowa State University, USA
Interplay of gene-specific resistance to barley-powdery mildew and the suppression of host-responses.

September 8
Virinder Parma, Professor
University of Massachusetts, Lowell, USA
Biocatalytic generation of novel materials of importance in drug and gene delivery.

October 4-5
Third colloquium of Priority Program 1152,
Evolution of Metabolic Diversity

October 10
Ko Shimamoto, Dr.
Nara Institute of Science and Technology, Japan
Rac GTPase is a key regulator of defense signaling in rice.

October 18
Bertil Helgee, Professor
University of Technology of Göteborg, Sweden
N-Vinylpyrrolidone co-polymers, possible support materials for chemical reactions.

October 21
Joachim Uhrig, Dr.
Max Planck Institute
for Plant Breeding Research, Köln, Germany
Protein interaction networks: Functional analysis of plant protein families and plant-virus interactions.

October 27
Gerd Jürgens, Professor
University of Tübingen
Apical-basal pattern formation in Arabidopsis embryogenesis.

November 4
Raoul Bino, Professor
University of Wageningen, The Netherlands
Plant Metabolomics for Plant Breeding.

November 11
G. Ungar, Professor
University of Sheffield, UK
Periodic and quasiperiodic patterns in dendrimer nanostructures.

November 16
Christa Kamperdick, Dr.
Institute of
Molecular Biotechnology Jena, Germany
Detection of protein-ligand interactiuons by saturation transfer difference NMR Spectroscopy (STD).

November 18
Beat Keller, Professor
University of Zurich, Switzerland
Diversity and evolution of resistance genes in cultivated and wild wheat: Exploring the resources of a crop plant.

November 24
Klaus Müllen, Professor
Max Planck Institute for Polymer Research
Graphitmoleküle.

November 26
Gopalan Selvaraj, Dr.
National Research Council of Canada
Molecular aspects of reproductive development in cereals and oilseeds.

December 8
Albrecht Berkessel, Professor
University of Cologne, Germany
Biomimetik und Organokatalyse für die Synthese enantiomerenreiner Epoxide, Aldole und Aminosäuren.

December 9
Anne Osbourn, Dr.
John Innes Centre Norwich, UK
Secondary metabolism and plant defense.

Workshop

Guest Researchers and Fellows

Department of Bioorganic Chemistry

Susanne Aust, Dr., Germany
01.03. 2003 - 31.12. 2004

Lilechi Danstone Baraza, Tanzania
NAPRECA-DAAD Fellow
01.06. 2004 - 30.11. 2004

Alessandra Basso, Dr., Italy
EU Fellow
12.01. 2004 - 29.01. 2004

Claudia Bobach, Germany
01.10. 2004 - 30.11. 2004

Christiano Rodrigo Bohn Rhoden, Brazil
DAAD Fellow
01.10. 2004 - 31.12. 2004

Antonio Luiz Braga, Prof., Brazil
DAAD Fellow
16. 09. 2004 - 31.10. 2004

Tran Van Chien, Vietnam
07.10. 2002 - 30.09. 2003

Nguyen Manh Cuong, Vietnam
DAAD Fellow
01.06. 2004 - 31.08. 2004

Katalin Czifrak, Hungary
DAAD Fellow
04.08. 2003 - 30.09. 2003

Myint Myint Khine, Tran Thi Phuong Thao and Kanchana Dumri (from left to right).

Victor Dick, Germany
Fellow Stifterverband für die Deutsche Wissenschaft
01.02. 2004 - 31.12. 2004

Simon Dörner, Germany
Fellow Studienstiftung des Deutschen Volkes
01.03. 2004 - 31.12. 2004

Kanchana Dumri, Thailand
DAAD-Leibniz Fellow
01.03. 2004 - 31.12. 2004

Othilie Vercillo Eichler, Brazil
DAAD Fellow
01.08. 2004 - 31.12. 2004

Gergely Gulyás, Hungary
01.08. 2004 - 30.09. 2004

Zsuzsa Juhasz, Hungary
DAAD Fellow
01.10. 2003 - 31.10. 2003

Christine Kamperdick, Dr., Germany
Humboldt Fellow
22.04. 2003 - 21.10. 2003

Myint Myint Khine, Myanmar
Daimler Benz Fellow
01.01. 2003 - 31.12. 2004

Christine Neuhaus, Germany
01.03. 2004 - 30.09. 2004

Luay Rashan, Prof., Jordan
Humboldt Fellow
12.07. 2004 - 30.07. 2004

Daniel Garcia Rivera, Cuba
Graduiertenkolleg
01.10. 2003 - 31.12. 2003

Juliana Schneider, Brazil
DAAD Fellow
16.04. 2003 - 15.10. 2003

Paulo Henrique Schneider, Brazil
DAAD Fellow
16.04. 2003 - 15.10. 2003

Jasqer Alonso Sehnem, Brazil
03.10. 2004 - 31.12. 2004

Jana Selent, Germany
12.01. 2004 - 30.04. 2004

Katalin Sepreny, Hungary
DAAD Fellow
01.11. 2003 - 30.11. 2003

Tran Van Sung, Prof., Vietnam
February, June and September 2004

Mieke Toorneman, The Netherlands
01.01. 2003 - 30.10. 2003

Larissa Vasilets, Dr., Russia
01.01. 2003 - 31.05.2004

Nguyen Hong Thi Van, Vietnam
01.01. 2003 - 16.04. 2003

Heike Wilhelm, Dr., Germany
Fellow BioService GmbH, EU and the State of Saxony Anhalt
01.01. 2004 - 31.12. 2004

Hasliza Yusof, Malaysia
06.10. - 20.10.2004

Department of Stress and Developmental Biology

Reetta Ahlfors, Finland
DAAD Fellow
08.07. 2002 - 30.04. 2003

Emiko Harada, Dr., Japan
Humboldt Fellow
since 22.02. 2002

Ingo Hofmann, Germany
01.01. 2003 - 31.12. 2003

Thorsten Nürnberger, Prof., Germany
01.08. 2003 - 31.12. 2003

Claudia Simm, Germany
Graduiertenkolleg
since 01.10. 2003

Aleksandra Trampczynska, Dr., Poland
10.11. 2003 - 30.11. 2003

Department of Natural Product Biotechnology

Andrea Andrade, Argentina
DAAD Fellow
05.08. 2003 - 30.09.2003

Andrea Borgogni, Italy
09.04. 2004 - 16.06. 2004

Kum-Boo Choi, Dr., Korea
Humboldt Fellow
07.10. 2002 - 30.09. 2003

Bonnie Carolyn McCaig, Dr., USA
06.01. 2004 - 02.04.2004

Maria Luisa Diaz Chavez, Mexiko
DAAD Fellow
01.10. 2003 - 30.09. 2004

Aphacha Jindaprasert, Thailand
DAAD Fellow
since 18.10. 2004

Kristin Krukenberg, USA
Fulbright Fellow
23.09. 2002 - 15.07. 2003

Natsajee Nualkaew, Thailand
DAAD Fellow
01.11. 2004 - 31.12.2004

Alfonso Lara Quesada, Costa Rica
DAAD Fellow
since 01.04. 2003

Luc Varin, Prof., Canada
01.10. 2002 - 31.01. 2003

Department of Secondary Metabolism

Ma Hnin Hnin Aung, Singapore
13.01. 2003 - 28.02. 2003

Ana Cenzano, Dr., Argentina
01.09. - 30.11. 2004

Diana Schmidt, Germany
Fellow, Bio Service GmbH, EU and the State of Saxony Anhalt
01.08. 2001 - 31.07. 2004


Rostand Tonleu Tonfack

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Technical Services

Press and Public Relations

Head: Sylvia Pieplow

Assistant: Jana Krupik

Press and
Public Relations

National *Year of Chemistry* 2003

The public relations activities in 2003 were effected by the national *Year of Chemistry*. This thematic year, initiated by the Donors Association for the Promotion of Sciences in Germany, has been enriched by our chemists with many projects and ideas.

Special Lectures

In the context of special lectures for the *Year of Chemistry*, Professor Ludger Wessjohann spoke in March 2003 about flavors and pharmaceutically important ingredients from plants. The lecture, named *Pizza Taste and Jungle Drugs - Chemical Substances in Nature*, was addressed to primary school students, high school students and interested laymen.

Very interesting as well was the lecture about the biology of oil flowers in October, given by the curator of the botanical garden in Munich, Günter Gerlach. The talk was organized by the IPB researchers who are investigating the biological role of the poorly understood flower oils in cooperation with the botanical garden.

The Ship of Chemistry 2003

In summer 2003 the, so-called *Ship of Chemistry* started as a floating exhibition its tour on the Rhine. The exhibits about chemistry in every day life could be visited by a large number of people during the stops of the ship in every major harbor. The IPB participated in this exhibition with a multimedia project about the scientific work on the institute. The presentation contained a virtual tour through the IPB and a game, designed to inform people about bioorganic chemist's work. The player has to isolate and analyze active substances from medical plants. At the end of the game, the player knows the structure of the compounds and is crowned with a doctoral hat. The game was planned mainly by our scientists Lars Bräuer, Wolfgang Brandt, Andrea Porzel and Professor Ludger Wessjohann from the Department of Bioorganic Chemistry.

The game and the virtual tour were burned on CD and distributed in August 2004 to many secondary schools, universities, firms, publishing companies and private citizens in Germany, Austria and Switzerland. The response to the game was consistently positive and resulted both in numerous press articles and in an increase in general name recognition of the IPB in Germany.

The Phytolator crowned with a doctoral hat.

Public Events 2003 and 2004

Germany's Day of Reunification

On October 3, 2003, the IPB participated together with other scientific institutes of the state of Saxony Anhalt in the celebration of Germany's Day of Reunification in Magdeburg. Together with the university of Halle, the IPB exhibited the differences between human and plant cell cultures.

Long Night of Science 2003 and 2004

Carsten Milkowski showed electrophoretic experiments in the Long Night of Science 2004.

The second and the third Long Night of Science were both great successes for our institute. On this first Friday in July, the institute's doors were open from 7 pm to midnight to welcome more then 300 members of the public. Visitors enthusiastically participated in guided tours through the labs and greenhouses. In addition, our scientists displayed experiments and exhibits in the foyer. Special lectures were favored by the public. Stephan Clemens spoke about the chances for and risks of green gene technology, whereas Professor Ludger Wessjohann gave an overview about plant natural products and their application as pharmaceuticals.

Guided Tours for Scholars

As in the previous years, the IPB organized many guided tours through the institute for school classes. Teachers and foreign students were also very interested in this possibility to learn more about the institute. In addition, all of the four scientific departments sponsored several periods of practical training, which allowed many high school students to gain insight into lab work and to try out experiments on the bench.

In Biotechnika 2003 in Hannover, the IPB, represented by Dr. Michael H. Walter (left), exhibited topics on mycorrhiza. Here Dr. Walter is discussing with Dr. Horst Rehberger (right), Saxony-Anhalt's Minister for Work and Economy.

Participation in Trade Shows

Biotechnika in 2003

In October 2003, the IPB participated in *Biotechnika*, Germany's most important international biotechnology exhibition. Together with universities and many scientific institutes and firms, our researchers presented their work at the Saxony Anhalt booth in Hannover. In this trade show, Michael H. Walter from the Department of Secondary Metabolism displayed topics on mycorrhiza - a symbiosis between plant roots and fungi.

ABIC in 2004

In a similar Saxony Anhalt booth the, IPB participated in September 2004 in *ABIC* in Cologne. For this Agricultural Biotechnology International Conference, researchers of the Department of Stress and Developmental Biology presented mechanisms of metal hyperaccumulation in plants.

Projects in 2004

In 2004, our institute was highly present in the regional and national press. Some of our scientific work also resulted in interest by public broadcasters. In addition, several high-ranking dignitaries visited the IPB to get an impression about our scientific projects and the conditions of work. With the organization of two art exhibitions, we could revitalize an old custom of the past, which gave many artists the possibility to show their own perspectives about science and other important aspects of life.

Visits of Dignitaries

Members of the German Lower House of Parliament visit the IPB

In April 2004, Dr. Christoph Bergner, member of the German Lower House of Parliament, visited the IPB not only for nostalgic reasons, but also to discuss further promotion of the Leibniz Society institutes. One central point in discussions with the former scientist of this institute was the resolution of the new gene technology law in the near future.

Also in April, Christel Rieman-Hahnewinckel, Ulrich Kasparick, and Dr. Wolfgang Eichler visited the IPB to gain information on the major research projects on the institute. The members of the German Lower House of Parliament were impressed by the excellent research conditions at the IPB. With this visit, the politicians were responding to an initiative of the government to provide more support to the best scientific institutes in the eastern part of Germany. Together with the directors, they discussed the necessary steps of economic fortification by a better presentation of the whole scientific landscape in this region. "In spite of the fact that many of East Germany's research institutes work on an excellent professional level, they have image problems in the western part of the country", Ulrich Kasparick said. These problems could be resolved by visiting the corresponding institutes and by more personal contact between scientists and politicians.

President of the Leibniz Society tours our Labs and Greenhouses

With pleasure, members of the IPB welcomed the president of the Leibniz Society Hans Olaf Henkel in June 2004. Henkel, who is visiting at regular intervals all of the 84 Leibniz Institutes, was impressed by the scientific projects of the institute. After the tour, he discussed with the directors some general questions about the future role of the Leibniz Society within national and international science politics.

Hans Olaf Henkel (right), President of the Leibniz Society, visited the IPB in June 2004. Here, he speaks with Prof. Dierk Scheel, Managing Director of the institute.

European Commission Officer visits important scientific institutes of Saxony Anhalt

In context of the recently started biotechnology offensive by the State of Saxony Anhalt, in October 2004, Dr. Christian Patermann from the European Directorate-General for Research viewed the IPB and other regional scientific establishments with similar orientation in plant biochemistry. In addition to green biotechnology, the implementation strate-

gy of this offensive aims to support the pharmaceutical industry, neurotechnology and red biotechnology as well. After the circuit, Dr. Patermann informed interested persons about current and planned scientific projects of the EU.

New information about Mycorrhiza

Our multimedia presentation about mycorrhiza was in this year completely updated by its creator Thomas Fester. The scientist of the Depart - ment of Secondary Metabolism completed the presentation with the newest scientific results and with references from the literature. The first version of the interactive course about this fascinating biological interaction was produced in 2001 for students and interested nonscientists alike. In September 2004, the renewed program was again burned on CD and sent to many schools, firms, publishing companies and private citizens throughout the German-speaking region in Europe.

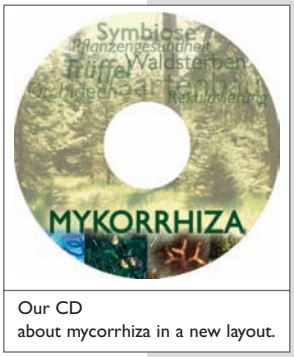
Art Exhibitions 2004

Monotypes from Hanno Lehman

The exhibition *Farbansichten* in July and August 2004 showed pictures of the painter Hanno Lehman. Hanno Lehmann studied chemistry and worked for many years as scientific co-worker at the IPB. His paintings displayed a wide range of themes from landscapes and scientific motifs to dreams and human relationships. All pictures were made as monotype, a special printing technology, which was modified and improved by the painter.

Oil on Canvas from Giacomo Piccoli

The Italian painter and sculptor Giaco - mo Piccoli abducted us into the borderland between science and philosophy. His exhibition named *Perpetuum Mobile - Magic of Biology in Imaginary Worlds* was shown from October 2004 until January 2005 at the IPB. Inspired by plants, fruits and microscopic structures, Giacomo Piccoli displayed amazing spaces of surrealistic perspectives. Giacomo Piccoli, born in Catania (Sicilia) in 1949, he was elected in 1983 National President of the ANTITESI cultural circuits in Rom. Currently he is chancellor of the Italian Bibliographical Center.



Our CD
about mycorrhiza in a new layout.

Temptation
Monotype from Hanno Lehmann.

Hair Cell
Oil in canvas, Giacomo Piccoli

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Die Flora als Forschungsgegenstand: Institut ist spezialisiert auf Pflanzenbiochemie

Wenn das Grünzeug einen Schnupfen hat

INSTITUT FÜR PFLANZENBIOCHEMIE

1958 entstand in Halle ein völlig neuer Wissenschaftszweig: Die Biochemie der Pflanzen. Damals wurde auf dem Weinberg der Vorläufer des Leibnitz-Instituts für Pflanzenbiochemie gegründet. Auch heute noch genießt die Einrichtung internationales Ansehen.

Forschung im Grünen: Das Institut für Pflanzenbiochemie in Halle liegt idyllisch ganz in der Nähe des Saaleufers. In den vergangenen Jahren wurde es umfassend saniert und erweitert. Kurze Wege haben die Forscher auch zu ihren Fachkollegen. Denn ganz in der Nähe befinden sich Uni-Institute des Fachbereichs Biologie sowie das Biozentrum.

Wir möchten gern besser verstehen, wie bei Abwehrreaktionen in den Zellen der Pflanzen passiert.
Thorsten Nürnberger, Biologe

Hier sind wir:

Studiengang zu Stadtumbau

Ausstellung in Studentenwerk

VORTRÄGE ÜBER GENTECHNIK

Praktikum bei gestressten Pflanzen

Entscheidung für Halle als positives Signal

„Der Wissenschaftsstandort Ost ist besser als sein Ruf."
Prof. Dierk Scheel, Leiter des IPB

Er hatte die Qual der Wahl: Institutsdirektor Prof. Dierk Scheel bekam ein Angebot aus Tübingen.

Sylvia Pieplow

Ines Krause

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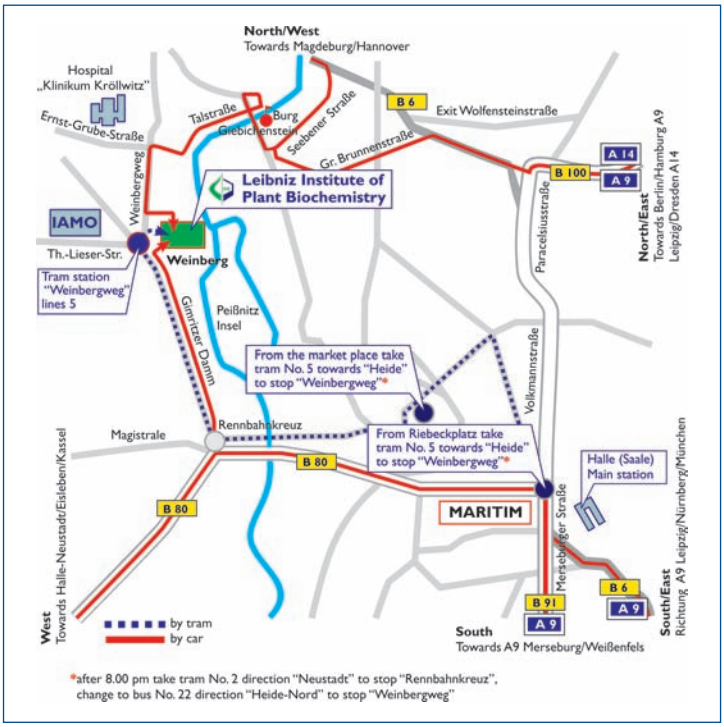

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