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IPB

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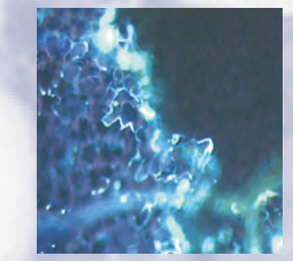
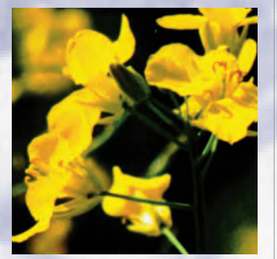
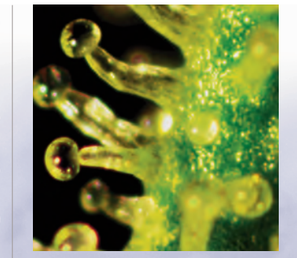
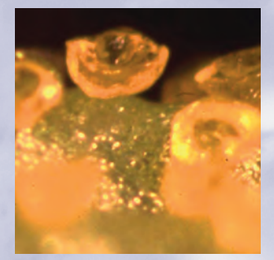
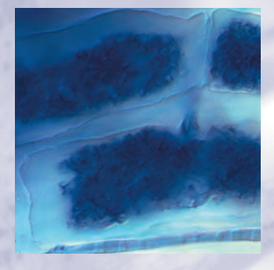
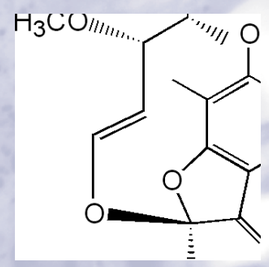












Presentation of the Institute

The Institute of Plant Biochemistry (IPB) in Halle was founded on 1 January 1992 as a **non-university research institute** of the so-called "Blue List". In 1995, the union of the Blue List Institutes formed the Blue List Science Association (Wissenschaftsgemeinschaft Blaue Liste), which was subsequently restructured and renamed the Leibniz Association (Leibniz Gemeinschaft) in October 1997. The IPB belongs to the life sciences section of the **Leibniz Association**. The original institute was founded as "Arbeitsstelle für Biochemie der Pflanzen" on 1 January 1958 by Prof. Dr. Dr. h.c. mult. Kurt Mothes by order of the German Academy of Science in Berlin. In 1960 it was renamed Institute for Biochemistry of Plants.

The IPB consists of four scientific departments and the administration and central services department. Currently 112 employes work at the IPB paid from the regular budget and another 47 funded by third-party funds. The research profile of the institute is unique within the German scientific community. The comprehensive analysis of natural products from plants and fungi, the investigation of the interaction of plants with pathogens, symbionts and abiotic stresses, studies of molecular interactions as part of complex biological processes, and metabolic engineering are at the center of research activities. Excellent basic research is regarded as the indispensable basis for the successful implementation of application-oriented research projects. The institute benefits, in particular, from the fact that the scientific departments of the IPB complement each other in terms of their methodical approaches and the equipment at their disposal. This allows interdisciplinary research using the latest chemical, physiological, cell-biological, biochemical, molecular-biological and genetic methods for comprehensive analysis of complex subjects.

The IPB is located on the Weinberg Campus, which hosts the natural science departments of the Martin Luther University, several non-university institutes and biotechnology companies. Close relationships and cooperations exist between the institute, the university and industries. Beside extensive scientific collaboration with several university departments, the institute's department heads are full professors at the university and, therefore, involved in teaching and supervision of undergraduate and graduate students. Together with the Institute of Plant Genetics and Crop Plant Research (IPK) in Gatersleben and the Max Planck Institutes for Chemical Ecology in Jena and of Molecular Plant Physiology in Golm the IPB forms the Plant Metabolism Network, PlantMetaNet. This network links the plant metabolomics competence that has been developed to an excellent level in these four plant research institutes in Central Germany.

Research mission statement

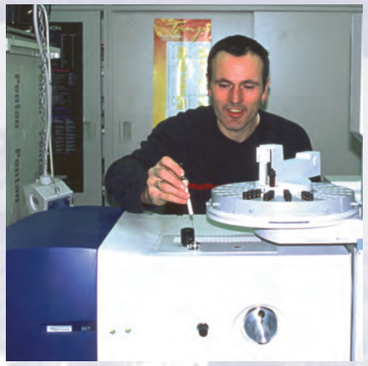
Four thematically, methodologically and organisationally overlapping research priorities form the basis of the research mission statement of the Institute of Plant Biochemistry - plant natural products, information technology and metabolic engineering.

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/ligand 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, a 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 modelling.

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. ■

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[1] in a two-year change
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Department: Natural Product Biotechnology

Head: Prof. Toni M. Kutchan
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Within the Department of Natural Product Biotechnology, the central theme of research is the analysis of the biosynthesis of plant natural products at the molecular genetic level. Of particular interest is the isolation of genes encoding enzymes and regulatory proteins involved in the formation of physiologically active, small molecules derived from L-tyrosine or L-tryptophan (alkaloids) and acetyl Coenzyme A (polyketides).

Alkaloids are pharmacologically active, nitrogen-containing, basic compounds produced in approximately 20 % of flowering plants. Each species accumulates alkaloids in a unique and defined pattern. The role of alkaloids in plants has been a longstanding question, but a picture emerges that supports an ecochemical function for these compounds. Alkaloid-containing plants were also mankind's original *"materia medica"*. Many of these plants are still used today as sources of prescription drugs. These biosynthetic pathways are attractive targets for molecular biology because of their role in plant chemical ecology and the biotechnological potential for the production of commercially important compounds.

The biosynthesis of polyketides present in medicinal plants is also under investigation. The compounds are prominent in tropical traditional medicine and are used to treat a wide variety of ailments, with particular emphasis on parasites. Plant polyketide synthases are encoded by a multi-gene family that has chal-cone synthase as a prototype. Gene family evolution in plants appears to occur by gene duplication followed by nucleotide substitution that can lead to biochemical diversity. Plant polyketide synthases are presu-mably derived from a common ancestor that diverged to perform different reactions. The identification and characterization of polyketide synthases involved in the formation a variety of natural products should lead to a better understanding of the evolution of these secondary metabolites.

The techniques that are used to isolate and identify these genes are wide-ranging, from enzyme purification followed by amino acid sequence determination to EST-sequencing and macro / micro array analysis. Both plant cell cultures and native plant material serve as a source of enzymes and genes. The characterization of the gene products is carried out after over-expression in a heterologous expression system such as bacteria, yeast, insect cells or plants. A part of gene product characterization is the localization of the protein in a plant tissue or cell. To this end, antibodies are raised against the heterologously-expressed biosynthetic proteins and immunolocalization techniques are used to identify the cell type in which the biosynthetic enzymes accumulate. This potentially provides insight into the regulation of natural product biosynthesis and yields information essential to the metabolic engineering of secondary pathways. Ultimately, the biosynthetic genes are transformed back into the na-tive plant as *sense*, *antisense* or RNA*i* vector constructs and the influence of the transgene on metabolic pathways is determined by HPLC-MS. In this manner, plants with tailored natural product profiles can be generated for industrial and research use.

In addition, the signalling properties of jasmonates and octadecanoids in stress-induced and developmental processes con-tinues to be investigated. In particular, the spatial and temporal expression of allene oxide cyclase alleles (a jasmonic acid biosynthetic gene) and the physiological role of jasmonic acid metabolites, such as 12-hydroxy-jasmonate, are being determined. ■

Research Group: Alkaloid Biosynthesis

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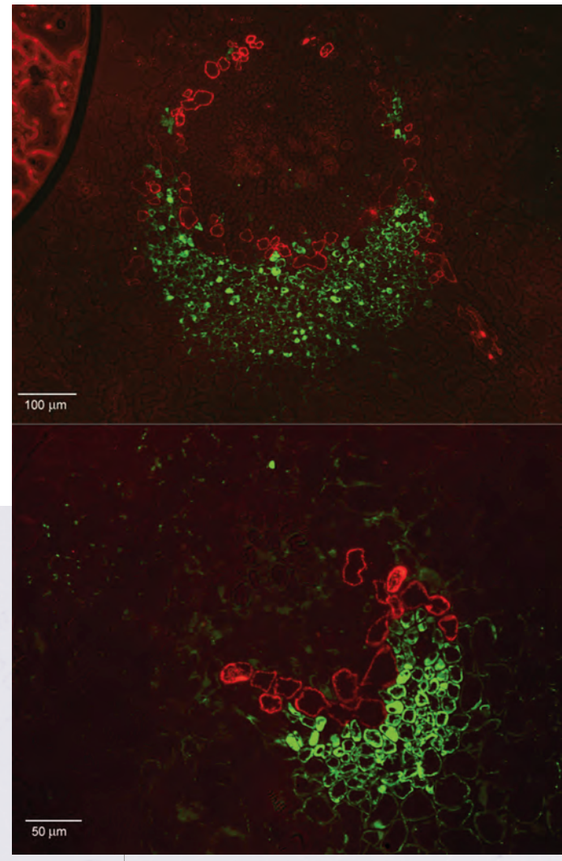
The opium poppy *Papaver somniferum* is still today one of our most important medicinal plants. Among the 80 alkaloids produced by this plant, three are medicinally important. These are the narcotic analgesic morphine, the analgesic and antitussive codeine and the antitussive noscapine. The biosynthesis of codeine and morphine is almost completely elucidated at the enzyme level. Relatively little is understood, however, concerning the biosynthesis of noscapine. We also understand very little of how alkaloid biosynthesis is regulated and of the biological role of these compounds in the plant. We are systematically isolating cDNAs that encode the unique en-zymes of alkaloid biosynthesis in opium poppy. These cDNAs are functionally heterologously expressed in bacterial and insect cell cultures and characterized. The seven cDNAs that we have isolated to date from *P. somniferum* will be used in *in situ* hybridization and the encoded heterologous proteins in immunolocalization studies in order to identify the cellular sites of biosynthesis of some of the various classes of isoquinoline alkaloids (morphinan, benzo[c]phenanthridine, phthalideisoquinoline) produced by this plant. Initial results already provide the first insight as to how biosynthesis and accumulation of the various classes of these alkaloids are regulated in this plant.

In recent years, we have isolated and characterized cDNAs encoding several enzymes of tetrahydrobenzylisoquinoline alkaloid biosynthesis from *P. somniferum*. The first enzyme in the biosynthetic pathway for which we have isolated a cDNA is norcoclaurine 6-*O*-methyltransferase. The next is the cytochrome P-450-dependent monooxygenase (*S*)-*N*-methylcoclaurine 3'-hydroxylase. These enzymes are common to the morphine, noscapine and sanguinarine biosynthetic pathways. Specific to the sanguinarine pathway is the berberine bridge enzyme that oxidatively cyclizes the *N*-methyl moiety of (*S*)-reticuline to the bridge carbon C-8 of (*S*)-scoulerine. Specific to noscapine biosynthesis is reticuline 7-*O*-methyltransferase. Finally, specific to morphine biosynthesis are salutaridinol 7-*O*-acetyltransferase and codeinone reductase, the penultimate enzyme of the morphine pathway that reduces codeinone to codeine.

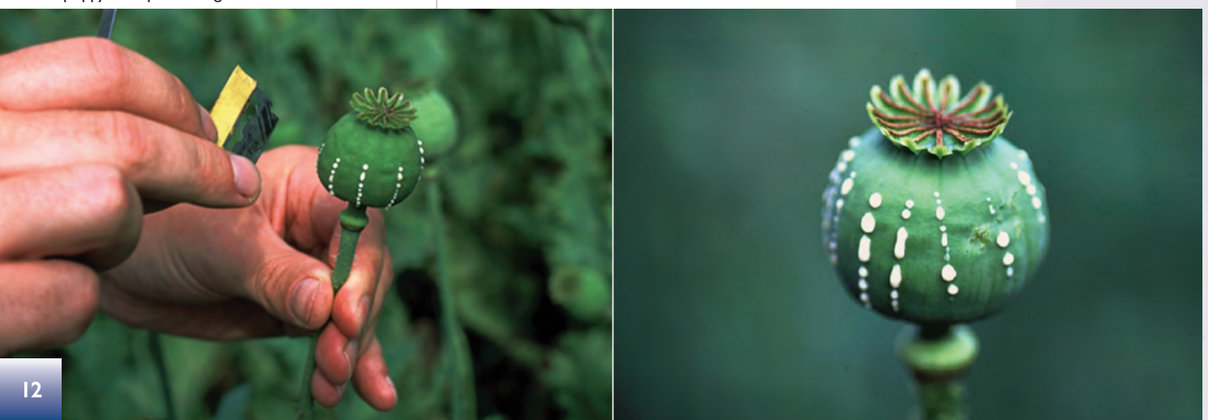
Reticuline 7-*O*-methyltransferase converts reticuline to laudanine in tetrahydrobenzylisoquinoline biosynthesis in *P. somniferum*. This new enzyme of alkaloid biosynthesis was identified during a proteomic analysis of *P. somniferum* latex (Decker *et al. Electrophoresis* **21**, 3500-3516 [2000]). The cDNA was amplified from *P. somniferum* RNA by reverse transcription PCR using primers based on the internal amino acid sequences. The recombinant protein was expressed in *Spodoptera frugiperda* Sf9 cells in a baculovirus expression vector. Steady state kinetic measurements with the heterologously expressed enzyme and mass spectrometric analysis of the enzymic products suggest that the enzyme is capable of carry through multiple *O*-methylations, on the isoquinoline- and on the benzyl moiety of several substrates. The tetrahydrobenzylisoquinolines (*R*)-reticuline (4.20 $s^{-1}mM^{-1}$), (*S*)-reticuline (4.50), (*R*)-protosinomenine (1.67), and (*R,S*)-isoorientaline (1.44) as well as guaiacol (5.87) and isovanillic acid (1.21) are *O*-methylated by the enzyme with the ratio k_{cat}/K_m shown in parentheses. A phylogenetic comparison of the amino acid sequence of this *O*-methyltransferase to those from 16 other plant species suggests that this enzyme groups more closely to isoquinoline biosynthetic *O*-methyltransferases from *Coptis japonica* than to those from *Thalictrum tuberosum*.

It is known that morphine and other alkaloidal biosynthetic intermediates such as dopamine accumulate in smooth vesicles within *P. somniferum* laticifer cells. In the mature plant, laticifers form a reticulated system that extends throughout aerial parts of the plant. Exuded latex is the cytoplasm and vesicles of these reticulated laticifer cells. In order to localize within this complex system the proteins for which we have isolated cDNAs, antibodies have been raised against heterologously expressed reticuline 7-*O*-methyltransferase, salutaridinol 7-*O*-acetyltransferase, codeinone reductase, the berberine bridge enzyme and the cytochrome P-450-dependent monooxygenase (*S*)-*N*-methylcoclaurine 3'-hydroxylase. Immunolocalization studies with each of the antibody preparations is being carried out with sections of *P. somniferum* capsule. Initial results indicate that multiple cell types are involved in alkaloid biosynthesis in this plant. A heterologously expressed major latex protein has been used as a latex marker protein. Of the proteins thus far analyzed, only codeinone reductase can be localized to laticifer cells. These results imply that intercellular transport of either intermediates or enzymes plays a role in isoquinoline alkaloid biosynthesis in *P. somniferum*. ■



The figure shows the immunolocalization of an enzyme of alkaloid biosynthesis in cross-sections of *Papaver somniferum* capsule. The expression of (S)-reticuline 7-*O*-methyltransferase, an enzyme involved in the biosynthesis of tetrahydrobenzylisoquinoline alkaloids in opium poppy, occurs in the phloem of the bundle sheath (green fluorescence). The red fluorescing cells are laticifers, stained by an antibody raised against a major latex protein. Laticifers are the site of alkaloid accumulation in aerial plant parts. These results imply a transport of intermediates from phloem to laticifers during biosynthesis.

The pictures show incised capsules of opium poppy with protruding latex.



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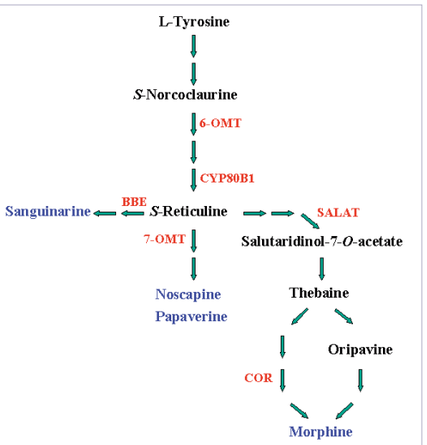
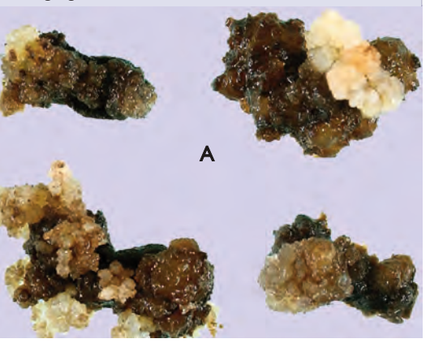


Biosynthetic pathway from L-tyrosine to sanguinarine, papaverine and morphine in *Papaver somniferum*. Enzymes are highlighted in red.



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 are developing transformation systems 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.**

During the last years, several genes from the biosynthetic pathways for reticuline, sanguinarine and morphine have been cloned. Although the biosynthesis is well understood at the enzymatic level, the molecular and biochemical mechanisms that regulate these pathways are not known. The goal of this project is to develop a stable transformation and regeneration method for opium poppy, which will make the metabolic engineering of the above mentioned compounds possible. Poppy seed oil finds use in chemical industry for the production of pigments and lacquer, but its residual morphine levels prevents more widespread applications. As well, 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 a morphine-free poppy. In the best case, a reduction of morphine 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 morphine and sanguinarine biosynthesis in *sense* or *antisense* orientation into explants to attempt to alter their alkaloid profile. Alkaloid-free plants developed in this manner will be used to test the chemical ecological function of morphinan and benzophenanthridine alkaloids in plants.

With a transgenic cell line expressing the antisense construct of berberine bridge enzyme (BBE) we hope to reduce the metabolic flux through sanguinarine pathway and to enhance the concentration of papaverine and / or morphine instead. We are interested if a transgenic cell line overexpressing codeinone reductase (COR) leads to a poppy plant, which contains more morphine or where the concentration of morphine is lowered due to a possible feedback inhibition of this pathway. We have also produced poppy transformants where we influence 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. The last three years we have been able to regenerate 190 F_0 poppy plants *via* somatic embryogenesis from twelve cell lines containing six different cDNA constructs. After the isolation of DNA and RNA, we analyzed these plants from which 150 F_0 have been proven to be transgenic and their seeds have been viable. Seeds from 17 transgenic F_0 plants have not been able to germinate and 23 transgenic F_0 plants did not contain any seeds at all.

The F_0 plants were analyzed by PCR or by dot blots and brought to flower and seed set. The alkaloid pattern of the first generation was determined from leaf extracts by HPLC and showed altered alkaloid concentrations compared to control plants. Molecular and chromatographic analysis of the F_1 generation is underway for all constructs and cell lines. The alkaloid pattern in the second generation is always analyzed in latex and in selected plants, also in leaves and roots.

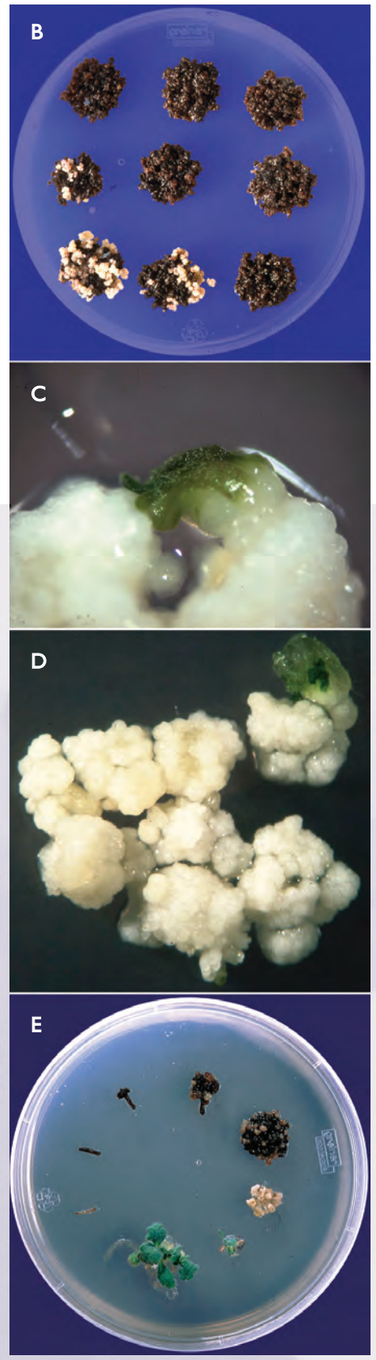
Latex from wild type plants of *P. somniferum* L. inbred parent line showed a high concentration of morphine, thebaine and codeine. Another alkaloid, which is present in this extract, is oripavine. In the roots of the wild type, the major alka-loid is the benzophenanthridine sanguinarine.

From the 150 transgenic F_0 plants, 43 are harboring the S4S4::*antiBBE* construct. We confirmed the presence of the transgene of the T_1 plants with the same methods described for the first generation. Additionally, we examined these plants with Southern- and Northern blotting. The alkaloid pattern was analyzed in latex as well as extracts from roots.

We found ten F_1 plants containing S4S4::*antiBBE* with a different alkaloid pattern compared to the wild type. The major alkaloids in the *bbe* antisense plants are morphine, codeine and thebaine. In contrast, a peak corresponding to oripavine was not always detected in these ten transgenic plants mentioned above. One out of these ten plants showed an increased total amount of reticu - line instead. The pattern of the HPLC chromatogram of the T_1 and T_2 plants is almost identical. These results are a first evidence that an alkaloid pattern in *bbe antisense* plants is an hereditary trait. At the moment, we are working to confirm the heredity of the other alkaloid patterns observed in *bbe antisense* plants and are measuring the HPLC profiles of plants containing the remaining five cDNA constructs (full-length *cor sense*, partial *cor sense*, *cpr sense*, *cyp80b1 sense*, *cyp80b1 antisense*).

Since 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 *P. somniferum*. Explants of opium poppy were transformed with seven different constructs: *bbe* RNA*i*, *cor* RNA*i*, *cpr* RNA*i*, *cyp80b1* RNA*i*, *6-omt* RNA*i*, *7-omt* RNA*i* and *salat* RNA*i*. All the explants have started to develop calli.

Last year three new genes became available from benzylisoquinoline biosynthesis. These encode salutaridinol 7-*O*-acetyltransferase (SALAT), (*S*)-norcoclaurine 6-*O*-methyltransferase (6-OMT) and (*S*)-reticuline 7-*O*-methyltransferase (7-OMT). Both methyltransferases have been cloned in *sense* orientation in our binary vector and have been transformed into opium poppy. All cultures developed calli and have started to differentiate. ■



Somatic regeneration of *P. somniferum*. Explants first give rise to a type I callus (A), which starts to differentiate after a certain time to type II callus (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.

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The main working task of the group is the maintenance of the plant cell culture collection of our department. This collection includes about 250 different plant species of 45 different plant families. About 40 species are cultivated in the form of suspension cultures and all others as callus cultures on various solid media. In *figure 1* a look to a part of our suspension culture collection is to be seen. The culture collection contains a number of plants producing interesting secondary metabolites such as alkaloids and represents the main source of biological material for coworkers and interested colleagues. In addition, we work in the field of alkaloid biosynthesis in the DFG project "Functional genomics in plant cell cultures under use of viral vectors".

Figure 1: A view to one part of the plant cell culture collection of the department "Natural Product Biotechnology": suspension cultures of different plant species.

Figure 2: The development of a suspension culture of *Eschscholzia californica* during one week.

Molecular genetic methods should be used in the investigation of biosynthetic pathways in secondary metabolism of plants. One possibility to bring new genetic information into cells such as plant protoplasts could be the use of viral or bacterial vectors. Such information will be clones of a cDNA library, which should cause gain of function / loss of function effects. Some cultures of our cell culture collection contain colored alkaloids visible under UV and normal light. We selected *Eschscholzia californica* suspension culture because of their production of a red colored mixture of sanguinarine and chelirubine. In ***figure 2***, the changes in the color of this suspension during one growing cycle (seven days) are shown. Vectors containing *antisense* cDNA of biosynthetic enzymes should stop alkaloid production after successful transfection and the cells should show reduced color.

The first steps in the project were the development of a protocol for the formation of protoplasts, a search for cultivation or regeneration methods and a test of transfection methods for protoplasts from suspension culture. The formation of protoplasts from *Eschscholzia californica* was done by use of cellulase and pectolyase as cell wall degrading enzymes and a purification with a Ficoll gradient ***(figure 3)***. We are now able to produce enough protoplasts of good quality and a viability of at least six to nine days.
The next step normally should be the regeneration of transfected protoplasts to a new suspension culture. From a number of methods we tested, the only successful one was the alginate-method. Fresh prepared protoplasts are mixed with an alginate solution (a polyuronic acid from *Macrocystis pyrifera*) and droplets of this mixture are solidified in $CaCl_2$. Alginate clumps are then cultivated in 24-well plates and treated with changing hormone media for cell division and growth. After two weeks first cell divisions are visible and after six to eight weeks, minicalli are produced. The whole process of regeneration takes approximately three months, which is much too long.

During our experiments with suspension cultures of *Eschscholzia californica* we observed that also the protoplasts of this culture can be elicitated with jasmonates or a yeast elicitor ***(table 1)***. This elicitation is visible already after 24 or 48 hours due to increasing amounts of sanguinarine and chelirubine. With help of elicitation, transfected protoplasts can be checked during 48 hours concerning their changes in the alkaloid content. Protoplasts with a reduced alkaloid content (determined spectroscopically or by HPLC analysis) would than be directly the material for localization and characterization of the block in the biosynthetic pathway. For the transfection of protoplasts, we used two different methods - both PEG-mediated transfection and electroporation is possible with protoplast preparations.

	Methyl jasmonate	medium content (µg/ml culture)	cells content (µg/ml culture)	total (µg/ml)	%
1	control	3.18 ± 0.12	6.30 ± 0.18	9.5	100
2	10^{-4} M JM	3.24 ± 0.18	4.92 ± 0.06	8.3	88
3	10^{-5} M JM	7.32 ± 0.12	7.80 ± 0.18	15.1	158
4	10^{-6} M JM	10.14 ± 0.24	13.56 ± 0.30	23.7	249
5	10^{-7} M JM	9.30 ± 0.06	13.02 ± 0.30	22.3	234
6	10^{-8} M JM	8.40 ± 0.36	13.02 ± 0.30	2.3	234

Table 1: Alkaloid content in protoplasts of *Eschscholzia californica* after elicitation with methyl jasmonate after two days.

To start with the search for a viral vector, which is active in our system, we used a TMV-derived viral vector, which should have a broad host specificity. For a simple detection under UV light, we cloned a Green Fluorescent Protein gene into it, but we were not able to detect any green fluorescence. The main task now will be the search for a vector, which can be transfected into *Eschscholzia* protoplasts and expressed. ■

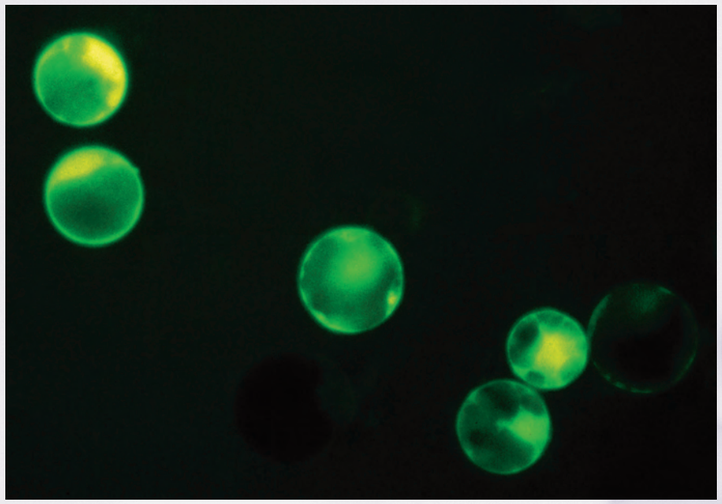

Figure 3: Protoplasts of *Eschscholzia californica*

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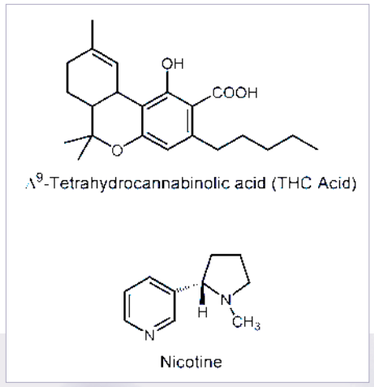
We are tapping the biosynthetic potential of the plant kingdom by studying the metabolic pathways leading to complex natural products. Our group is using functional genomic approaches to dis-cover genes encoding enzymes and transcription factors involved in natural product biosynthesis. This research focuses on biosynthetic processes occurring in tissues or organs, such as glandular trichomes that secrete natural products. We are using virus-induced gene silencing (VIGS) to identify genes involved in alkaloid metabolism and trichome development in *Nicotiana benthamiana*. Biochemical genomics, which combines transcriptome and metabolite analysis, is being applied to uncover enzymes catalyzing the formation of terpenophenolic chemicals in *Cannabis sativa* L. (hemp, marijuana) and *Humulus lupulus* L. (hops), see Research Group "Hops Secondary Metabolism".

Plants respond to virus infection by silencing (turning off) viral genes and thereby blocking viral replication. By cloning plant genes into viruses, plants can be made to direct this antiviral defense against their own genes, leading to a loss-of-function phenotype for the targeted gene. Fast-forward genetic methods using viruses promise to both speed up plant gene discovery and allow for the cloning of novel genes inaccessible to current techniques. The targets of our VIGS efforts are enzymes involved in tropane alkaloid (nicotine) biosynthesis and transcription factors controlling metabolite synthesis and accumulation in glandular trichomes of *Nicotiana benthamiana* Domin. Glandular trichomes are resinous hairs that cover leaves and flowers in many plant species. Their primary function is defensive, although they also play a role in detoxification, and therefore they are a major site of natural product production and storage. In the Solanaceae, they are readily targeted by virus constructs ***(Figure 1)***. We are building a catalog of MYB transcription factors from *N. benthamiana* trichomes and testing the effect that silencing these regulatory proteins has on metabolite content and trichome morphology. Experiments with known enzymes of nicotine biosynthesis, such as putrescine-*N*-methyltransferase (PMT) and quinolate phospho-ribosyltransferase, have shown that gene silencing can reduce nicotine levels to about 30 % of control levels. Based on these results, we are constructing cDNA libraries in viral vectors for use in high-throughput VIGS approaches to alkaloid biosynthesis.

Cannabis is grown worldwide for industrial purposes, yielding fibre and seeds, and for its content of psychoactive cannabinoids (e. g. Δ^9-tetrahydrocannabinol, THC). The biosynthetic pathway leading to cannabinoids is not completely understood at the biochemical or genetic level. Cannabinoid biosynthesis occurs mainly in glandular trichomes ***(Figure 2)*** that cover female cannabis flowers at a high density. Using a high-THC strain of cannabis, we have constructed a trichome-specific cDNA library from purified trichome secretory cell clusters. More than 1.200 ESTs (expressed sequence tags) from this library have been sequenced and assigned putative gene function using bioinformatic comparisons. Through this approach we have identified candidate cDNA clones of type III polyketide synthases, which may participate in cannabinoid biosynthesis, and an oxidocyclase, Δ^9-tetrahydrocannabinolic acid synthase that is a key enzyme in the cannabinoid biosynthetic pathway. Heterologous expression and *in vitro* enzymatic assay are being used to functional characterize these genes.



Stemming from the group's interest in the chalcone synthase superfamily of type III polyketide synthases, the role of these enzymes in forming medicinal plant compounds in *Rheum tataricum* L. (Polygonaceae), and *Cassia alata* L. (Fabaceae) was studied. A new resveratrol-forming stilbene synthase was cloned from the former (Samappito et al, in press), while a series of chalcone synthases was characterized from the latter (Samappito et al, 2002). ■

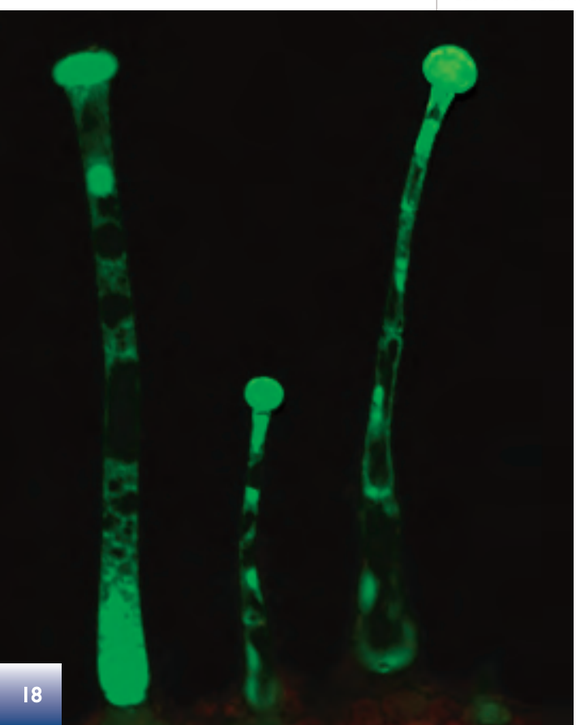


Figure 1: Plant virus targeting of glandular trichomes. *Nicotiana benthamiana* trichomes exhibit GFP expression after infection with a tobacco mosaic virus containing a *GFP* reporter gene. Photo: U.Schäfer

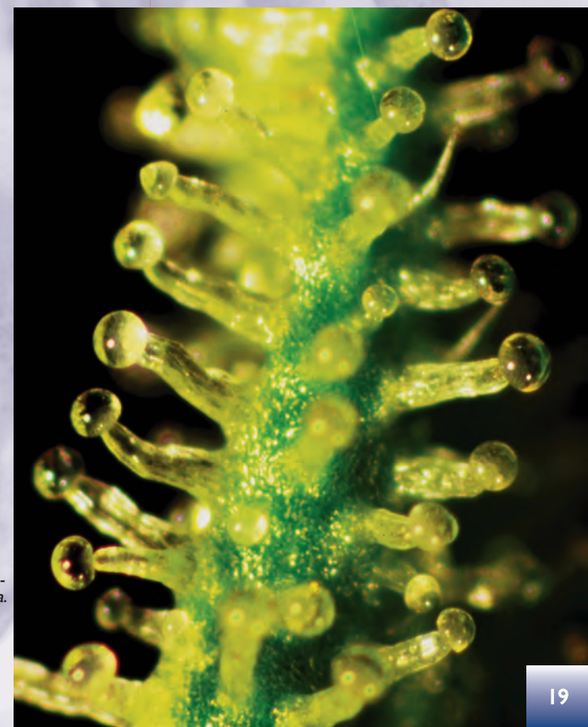


Figure 2: Cannabinoids and terpenoids accumulate in glandular trichomes of *Cannabis sativa*. Photo: J. Page

Research Group: Mode of Action of Jasmonates

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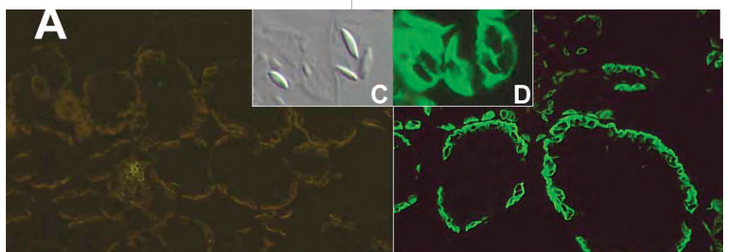
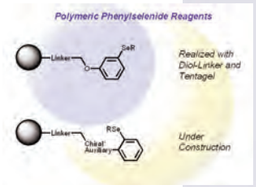
Jasmonates and their precursors, the octadecanoids, are signals in plant stress responses and in plant development. A mechanistic analysis of the mode of action of jasmonates is performed by a reverse genetics approach using the allene oxide cyclase (AOC)-catalyzed step in jasmonate biosynthesis. "Gain of function" and "Loss of function" studies with transgenic tomato plants revealed modulation of jasmonates and allowed to inspect the role of jasmonates in response to biotic and abiotic stresses as well as flower and seed development. In order to use genetic approaches, functional analysis of AOC and jasmonic acid (JA) is also performed in *Arabidopsis thaliana*. Analytics of jasmonates and other oxylipins including chemical synthesis of standards and labeled substrates is an essential part of this work.

Previous work on stress responses and cloning of JA biosynthetic enzymes in barley was finished by analyses of three 13-Lipoxygenases, three allene oxide synthases (AOS's) and one AOC, all of them located in chloroplasts. Since 2000, we are working with tomato, Arabidopsis and tobacco. The first AOC was cloned from tomato. This single copy gene is specifically expressed in ovules of young flowers and all vascular bundles, accompanied by a specific pattern of various jasmonate and octadecanoid compounds (oxylipin signature) in distinct flower organs. In leaves the vascular bundle-specific occurrence of AOC attributes to a preferential generation of jasmonates in main veins. Based on a co-localization of the AOC, the JA-generation, the location of the wound signal systemin in vascular tissues and the data from various transgenic tomato plants, an amplification mo - del on wound signaling is proposed.

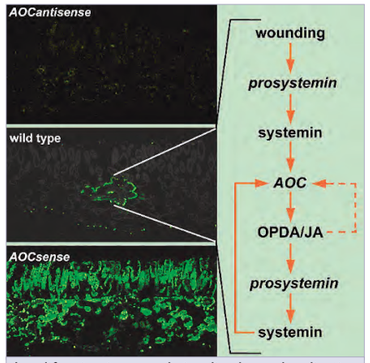
The capacity of the phloem to respond rapidly in wound signaling was further supported by detection of JA biosynthetic enzymes including AOC in sieve elements. The importance of JA in signaling was strengthened by grafting experiments between 35S::*AOCantisense* plants and wild type plants.



Immunocytological localization of AOC protein in leaves of *Arabidopsis thaliana*. (A) preimmune serum,(B) location of AOC in chloroplasts (Stenzel et al. 2003b)



Occurrence of AOC protein in plastids of companion cells (big arrows) and sieve elements (small arrows)of tomato flower stalks (A, B) and petioles (C-E). Longitudinal sections were probed with an anti-AOC-antibody (A, C) or with the pre-immune serum (B). D: differential interference contrast image of C. E: DAPI staining to visualize nuclei. The sieve plate of a sieve element is marked by an asterisks (cf. Hause et al. 2003).

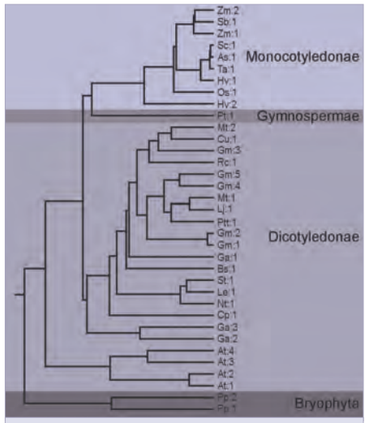


Amplification in wound-signaling by co-localization of AOC, JA generation and systemin formation as well as systemin-dependent *AOC* expression and JA-dependent prosystemin expression. The amplification is compromised in 35S::*AOCsense* plans (cf. Stenzel et al. 2003a).

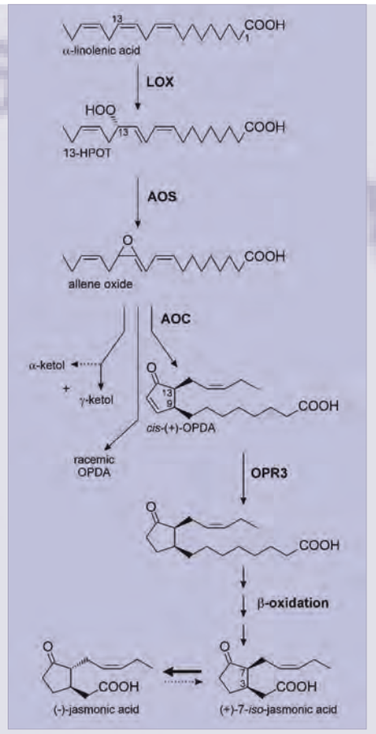
Oxylipin profiling and expression analyses in WT, 35S::*AOCsense* and 35S::*AOCantisense* lines revealed regulation of JA biosynthesis by substrate availability, an activity control of preexisting enzymes and a feed forward regulation. This was substantiated by analysis of Arabidopsis and mutants affected in JA biosynthesis. Here, four different non-redundant AOCs are tissue-specifically active, thus allowing control of the oxylipin signature of different organs.

Using knockout lines of AOC1-AOC4, AOC1-4RNA*i*-lines as well as *antisense* approaches, individual functions of AOC1-AOC4 in stress responses and during development of Arabidopsis is under study and will allow us, to analyze the mode of action of JA.

Previously, 12-hydroxy-JA was only known as tuber-inducing compound in Solanaceae. We could identify 12-hydroxy-JA and its sulfated derivative in *A. thaliana* as a signaling compound in flower development. ■



Phylogenetic tree analysis for AOC's. (from Stenzel et al. 2003b)



Biosynthesis of jasmonic acid catalyzed by a lipoxygenase (LOX), an allene oxide synthase (AOS), an allene oxide cyclase (AOC), an OPDA reductase (OPR3) and β-oxidative steps.

Research Group: Papaver-Gene Expression Analysis

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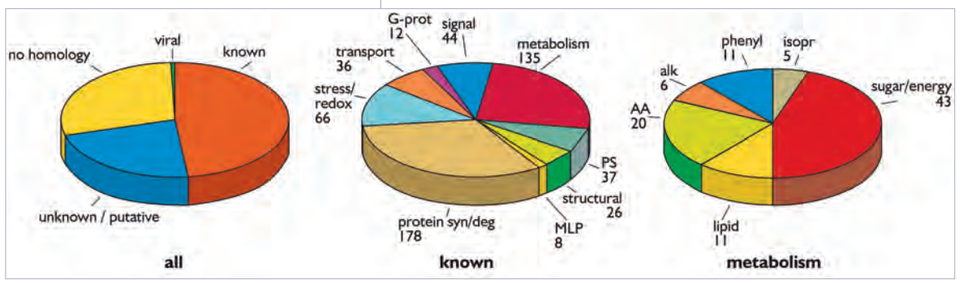
Poppies of the genus *Papaver* produce a large variety of benzylisoquinoline alkaloids. Some of them are of pharmaceutical impor-tance such as the analgesic morphine, the antitussive noscapine or the vasodilator papaverine. The biosynthesis to (*S*)-reticuline, the central intermediate to all monomeric benzylisoquinoline alkaloids is well understood on the molecular level, knowledge on 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 are unknown. To approach cDNA clones coding for the enzymes of these biosynthesis processes, we make use of the close genetic relationship, but the diversity in the alkaloid pro-file, between *Papaver* species or varieties, respectively. We examine and correlate the gene expression profiles on EST-arrays (expressed sequence tag) with specific alkaloid profiles. By the combination of many different datasets of alkaloid profile-gene expression correlations, we want to reduce the number of candidate cDNAs to a manageable number to start their functional characterization.

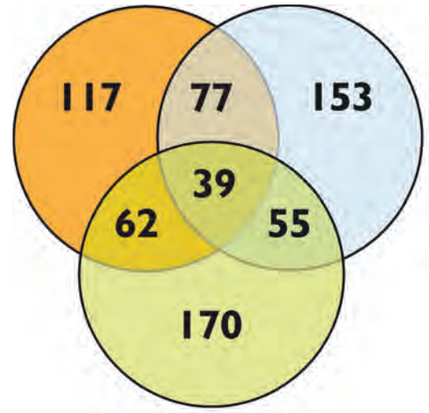
Currently, more than 70 different poppy species belonging to the genus *Papaver* have been described. Roughly, they are able to synthesize about 2.500 different benzylisoquinolines, which can be grouped into nine classes. The profiles of benzylisoquinolines produced by the plants are species-specific, however they are 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 of poppy alkaloids and LC-MS coupling will be used for the low-abundance compounds. For gene expression analysis, a protocol for macroarray production was developed and the establishment of microarray technology has started. These methods are sensitive enough to record the alkaloid profile and the gene expression pattern from one individual plant. For 60 *Papaver* species and ten varieties and mutants of the opium poppy *Papaver somniferum*, the main alkaloids could be identified by HPLC. For low-abundant and not yet identified alkaloids, LC-MS analysis is in progress. As probes for the arrays, we use PCR fragments derived from an EST project of *P. somniferum* stems. Among all *Papaver* species, this plant synthesizes the largest number of different benzylisoquinolines and the stem has been shown to possess the highest biosynthetic activity. Up to now, we sequenced more than 2.000 ESTs and obtained 1.100 unique sequences. About 40 % either code for proteins with unknown function or have no homology to entries in the data-bases. The largest groups of cDNAs coding for proteins with known function are invol-ved 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 role in secondary metabolism could be ascribed. Five sequences code for proteins with known function in the benzylisoquinoline pathway, and for one cDNA, showing high homology to an enzyme involved in another alkaloid pathway that does not occur in *Papaver*, its possible role in the benzylisoquinoline pathway is currently under investigation. The EST-sequencing project still continues, but additionally, to access cDNAs implicated in benzylisoquinoline biosynthesis at a higher frequency, the construction of a P450-monoxygenase specific EST-collection has been initiated. These enzymes play a major role in the modification of the benzylisoquinoline core structures leading to the high structural diversity.



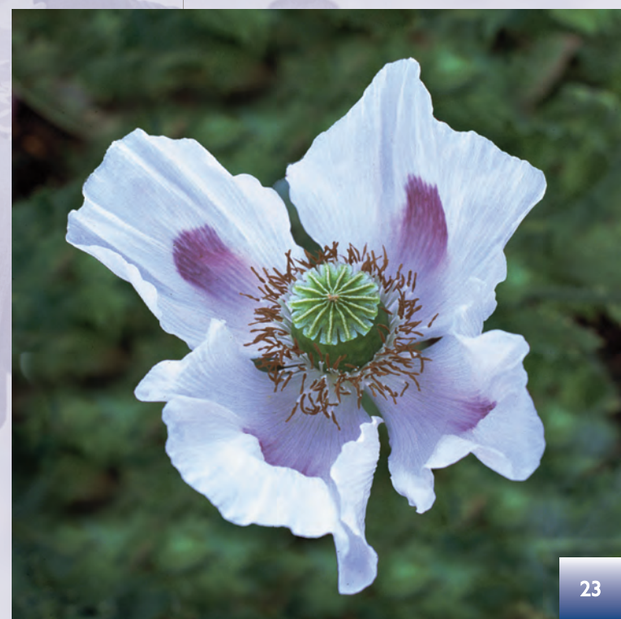
Functional classification of *P. somniferum* stem ESTs. Abbreviations: PS: photosynthesis, MLP: major latex proteins, protein syn / deg: protein synthesis and degradation, G-prot: G-proteins, AA: amino acids, alk: alkaloids, phenyl: phenylpropanoids, isopr: isoprenoids.

The expression of these cDNAs in four *Papaver* species differing in their ability to perform the last steps in the biosynthesis of morphine was examined and correlated with the occurrence of morphine in the respective alkaloid profiles. By combination of all possible datasets, the number of cDNAs possibly responsible for the accumulation of morphine could be reduced to 39 candidates, most of them coding for unidentified proteins. Further comparisons are in progress to decrease the number of cDNAs far enough, that a functional characterization is feasible.

Another project uses the cDNA-AFLP technique to isolate cDNAs differentially expressed dependent on an alkaloid profile. In this approach, we compare a wild type *P. somniferum* plant with a mutant plant accumulating thebaine, which is situated four steps upstream of morphine in the biosynthetic pathway. More than 100 differentially expressed fragments were found. Their specificity for the morphine-free phenotype is currently being examined by macroarray analysis. ■



Interloping diagram of the number of genes differentially expressed between *P. bracteatum* and *P. somniferum* grown in the field (red circle), *P. bracteatum* and *P. somniferum* grown in the greenhouse (blue circle) and *P. bracteatum* and *P. somniferum* Noscapine (green circle). The number in the overlapping areas indicates the number of genes that show differential expression in the combination of the respective comparisons.



Publications, Books and Book Chapters, In press, Patents, Doctoral Theses, Diploma Theses

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Research Group: Hops Secondary Metabolism

Heads: Jonathan Page, Jürgen Schmidt, Frederick Stevens *(until September 2002)*

Group members

Marco Dessoy
(PhD student since May 2002)

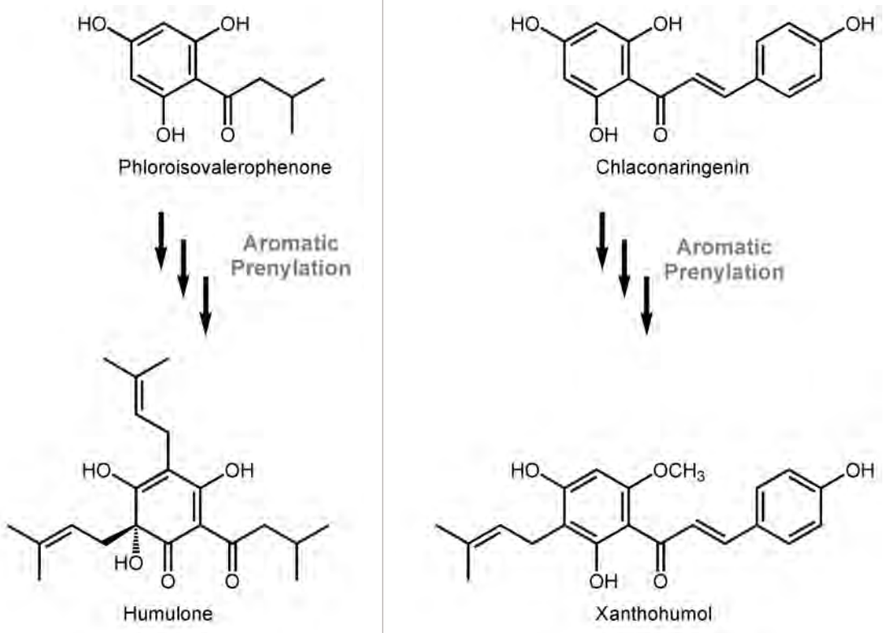
Verona Dietl
(technician)

Martina Lerbs
(technician)

Raik Löser
(research scientist until December 2001)

Vincent Spelbos
(student until March 2002)

Hops (*Humulus lupulus* L., Cannabaceae) are the principal flavor ingredient in beer, contributing phytochemicals with both taste (e.g. the bitter acid humulone) and 'nutraceutical' (e.g. the prenylflavonoid xanthohumol) properties. These terpenophenolic metabolites are of mixed biosynthetic origin, with precursors derived from terpenoid and phenolic (polyketide) pathways. Bitter acids and prenylflavonoids are mainly made and stored in specialized glandular trichomes, termed lupulin glands, found on hop cones ***(s. Figure)***. A key step in the biosynthesis of terpenophenolics is the transfer of isoprenoid unit(s) to the aromatic ring of the phenolic moiety by aromatic prenyltransferase enzymes. A collaborative project between the Departments of Natural Product Biotechnology (Jonathan Page) and Bioorganic Chemistry (J. Frederick Stevens and Jürgen Schmidt) aimed at clarifying the prenyltransferase reactions in hops was initiated in 2002. We developed a sensitive mass spectrometric assay for *in vitro* prenyltransferase activity and were able to detect the enzyme-mediated transfer of dimethylallyl diphosphate (DMAPP) to the aromatic rings of precursor compounds. This industry-supported research will continue with a biochemical genomics project aimed at identifying genes encoding the enzymes of terpenophenolic biosynthesis. At a later stage we hope to characterize the enzymes and utilize them as biocatalysts in chemical transformations. ■



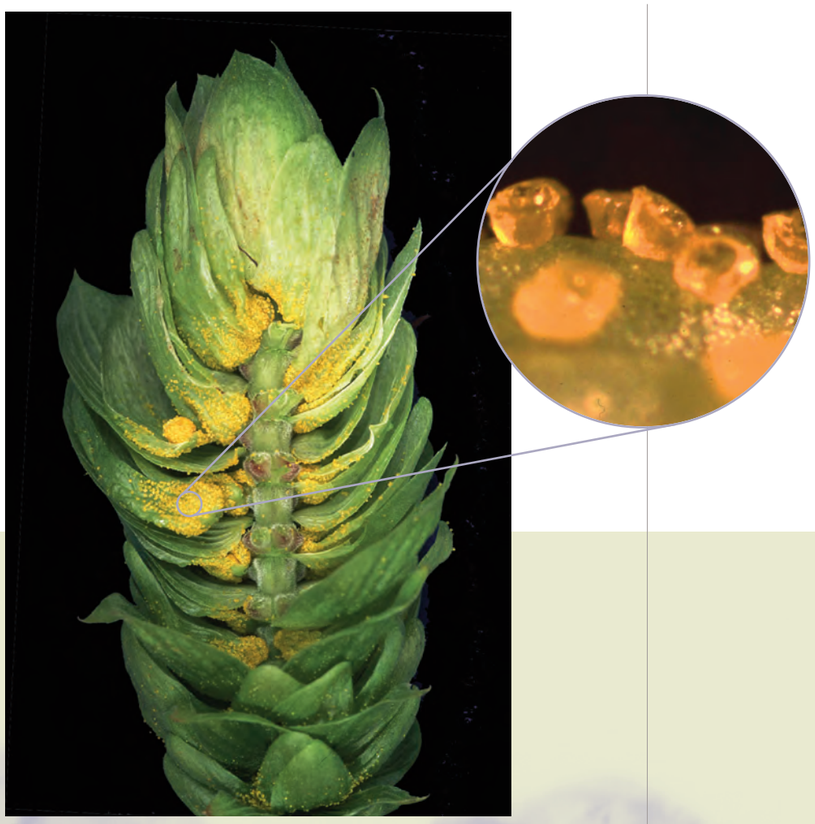
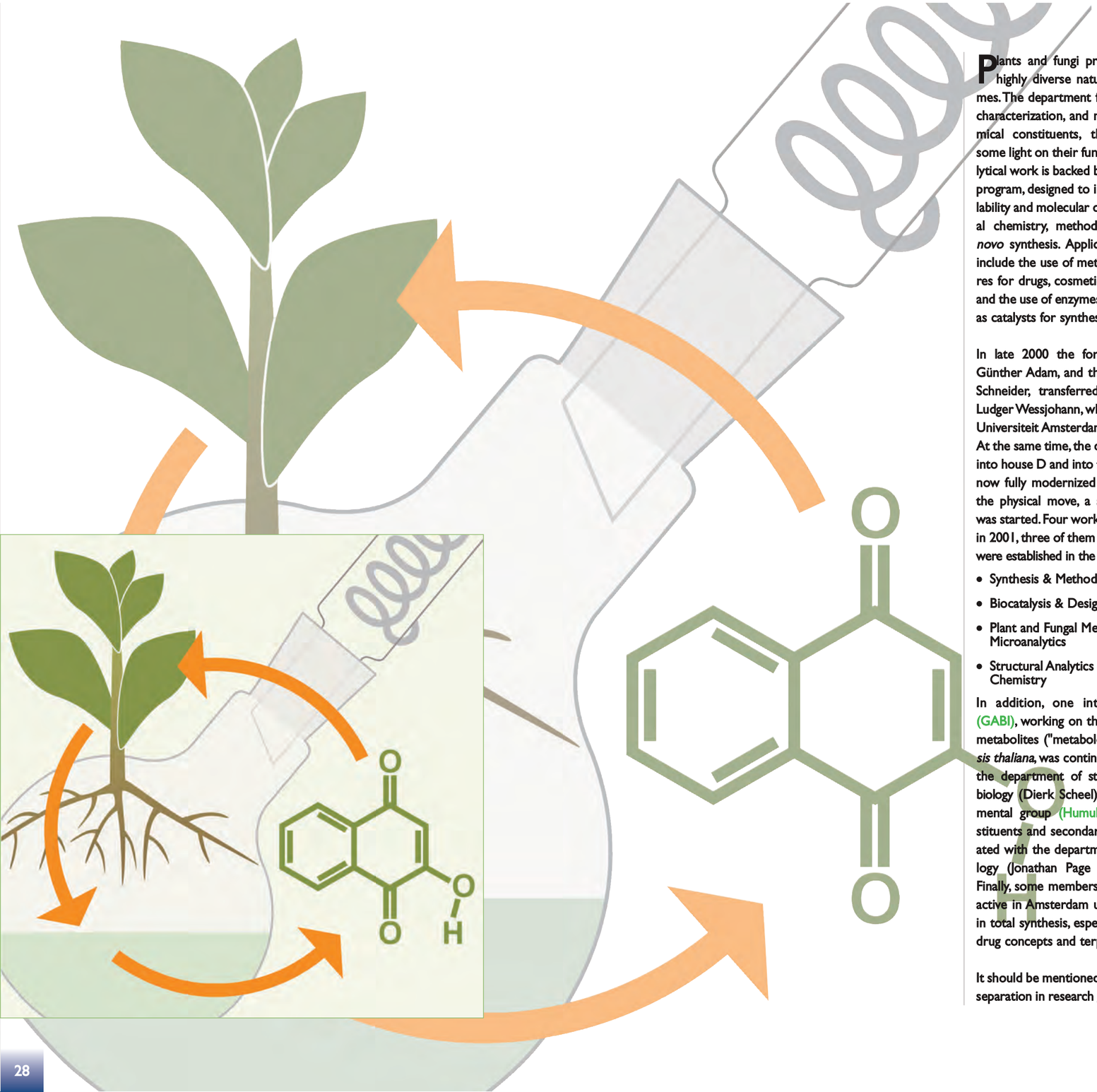


Figure: A mature cone from hops (*Humulus lupulus*) with lupulin glands visible as yellow structures at the base of cone scales. Inset: Magnification of lupulin glands. (Photo: Annett Kohlberg)

Department: Bioorganic Chemistry

Head: Prof. Ludger Wessjohann

Secretary: Elisabeth Kaydamov



Plants and fungi provide a rich source of highly diverse natural products and enzymes. The department focuses on the isolation, characterization, and modification of the chemical constituents, thereby trying to shed some light on their function in nature. 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. 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.

In late 2000 the former department head, Günther Adam, and the interim head, Gernot Schneider, transferred the responsibility to Ludger Wessjohann, who moved from the Vrije Universiteit Amsterdam to Halle in early 2001. At the same time, the department translocated into house D and into the northern part of the now fully modernized house C. In parallel to the physical move, a scientific reorganization was started. Four working groups were formed in 2001, three of them in totally new areas, and were established in the following year:

- Synthesis & Method Development
- Biocatalysis & Design of Ligands
- Plant and Fungal Metabolites & Microanalytics
- Structural Analytics & Computational Chemistry

In addition, one interdepartmental group (GABI), working on the profiling of secondary metabolites ("metabolomics") from *Arabidopsis thaliana*, was continued in cooperation with the department of stress- and developmental biology (Dierk Scheel). A second interdepartmental group (Humulus), studying hop constituents and secondary metabolism, was initiated with the department of plant biotechnology (Jonathan Page and Toni M. Kutchan). Finally, some members of the group remained active in Amsterdam until 2003 with projects in total synthesis, especially towards new prodrug concepts and terpenoid modifications.

It should be mentioned that despite this formal separation in research groups, most projects of the department are integrated, i. e. they span two or even three of these research groups. Thus e. g. the projects on isoprenoids and prenyltransferases involve contributions from the groups Biocatalysis & Computational Chemistry, and to a minor extent from Synthesis & Method Development, Microanalytics and Humulus.

The present heads of research, Brunhilde Voigt, Andrea Porzel and Jürgen Schmidt were enforced by two new head scientists, the mycologist Norbert Arnold (Plant and Fungal Metabolites), who is also the new substitute head of the department, and the biochemist Wolfgang Brandt (Computational Chemistry). During the report period, the group grew from some ten to about 35 members of about ten nationalities. Numerous guest researchers, exchange students and probationers, from around the world as well as from local institutes and schools, visited the department. The closest relationships exist with colleagues in Brazil, Vietnam, Hungary, and The Netherlands.

As part of the reorganization, valuable research equipment was newly installed, or re-installed and totally overhauled, among these three NMR and six mass spectrometer, including one FT-ICR-MS (v.i.), a glovebox, a synthetic robot, a pipetting robot, and a computational chemistry network. A fungal strain collection and a computerized chemical stockroom system were started. Also, the department took considerable action in the planning of the new functional building (house R) in close cooperation with the administration. House R is planned to become the new home of our screening facilities and of the biocatalysis group. In addition, it will contain cross-departmental installations like a night lab, solvent distillation, a fermentation room, and laboratories of other departments. Also, in 2002 Phytobase was initiated as a central information database platform for chemical constituents from plants, fungi, or valuable synthetic compounds. Phytobase is planned to be the cornerstone of our future information integration and partly will be made available within a larger context to all groups dependent on phytochemical data, including e. g. food industry, government and legislation, and research groups in the fields of natural products, nutrition, ecology, bioinformatics, metabolomics or pharmaceutical development.

Despite the scientific and organizational unrest, and increasingly difficult access to outside resources for phytochemical projects, a continuous increase in publication output was achieved from 2000 to 2002. Several diploma/M.Sc.-titles (one in Halle), and six PhDs were granted to group members. Finally, Fred Stevens, who started his habilitation in the department in 2000, in late 2002 accepted a call for a professorship at Oregon State University in Corvallis (USA). ■

Research Group: Synthesis & Method Development

Heads: Ludger Wessjohann & Brunhilde Voigt

Group members

John Bethke
(postdoctoral position since June 2002)

Tran Van Chien
(visiting PhD student since October 2002)

Uwe Eichelberger
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Dirk Michalik
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Lars Ostermann
(postdoctoral position until June 2002)

Eelco Ruijter
(PhD student since March 2001)

Angela Schaks
(technician)

Günther Scheid
(postdoctoral position until June 2002)

Gisela Schmidt
(technician)

Henri Schrekker
(PhD student since January 2001)

Tran Thi Phuong Thao
(PhD student since November 2001)

Mieke Toorneman
(PhD student, based at Vrije Universiteit Amsterdam since February 1999)

Mingzhao Zhu
(PhD student since October 2001)

Friederike Ziethe
(research scientist until December 2002)

Collaborators

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Lucia Gardossi
University of Trieste, Italy

Thomas Hjertberg, Bertil Helgee
Chalmers University of Technology, Sweden

Udo Kragl
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Free University of Amsterdam, The Netherlands

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Polymerlabs UK

Karoly Micskei, Tamas Patonay
University of Debrecen, Hungary

Romano Orru
Free University of Amsterdam, The Netherlands

The targets of our synthetic efforts are natural products, their derivatives, and natural product-like libraries, mostly of polyketide, isoprenoid or small peptoid structure, and to a limited extent designer molecules, e. g. for pro-drug concepts. A crucial prerequisite for an efficient access to such complex molecules is the availability of new methods with improved selectivity. The group developes these, based on our expertise in chromium and selenium reagents, biocatalytic methods (s. also dedicated research group) and multi component reactions. Selective reactions also offer the tools for creating chemical diversity from the modification of natural products. Combinatorial approaches in liquid as well as on solid phase are used to obtain small dedicated libraries, which help to find substances with improved biological activity profiles. However, the access to libraries of structurally complex, natural product like molecules is usually limited because of lengthy multistep procedures. Multicomponent one-pot reactions, multiple catalytic systems, and self-selecting (evolutionary) procedures are possible solutions to improve the accessibility of structurally complex entities. These processes can be applied in chemistry, e. g. for selective separation and catalysis, or for pharmaceutical lead structure development with an emphasis on molecules with anticancer, antibiotic, phytoestrogenic, or cosmetic properties. Especially macrocycles are of interest to us, because their conformational design is poorly understood. They exhibit more flexibility than classical aromatic drugs but have less entropy loss upon binding than open chain forms.

Macrocycles

Total Synthesis of Macrocycles

The most exciting polyketide macrocycles discovered in recent years are the epothilones. They are antimitotic compounds with taxol-like activity, which are also active against multiple drug resistant cancer cell lines. Currently these compounds are in phase II clinical trials. One of the shortest routes to epothilones was developed by us in Amsterdam. This approach is continued towards new analogues, which promise improved properties.

The structural formula of natural epothilone D, a drug lead compound for cancer therapy. The different colors signify structural elements originating from the building blocks used for the total synthesis by Wessjohann et al.

The Fast Track to Macrocycles: Multi Component Reactions (MCRs)

A synthesis of designed macrocycles of high functionality by the traditional total synthesis approach is extremely wasteful in all resources: chemicals, manpower and time. It is only useful for valuable compounds like epothilones. The problem will potentiate if compound libraries will have to be designed, either for quantitative structure-activity relationship (QSAR) or evolutionary adaptation studies. Three problems will have to be solved for a sustainable route to highly functionalized asymmetric macrocycles and are addressed by our MCR-approach:

1. Rapid access to polyfunctional building bloks.
2. The fast and efficient connection of these.
3. Efficient macrocyclization strategies and catalysts not based on the dilution principle.

We concentrated on the synthesis of natural product-like macrocycles inspired by the 14-membered ansa-cyclopeptides from plants and the bis-aryl-ether antibiotics (e. g. vancomycin). Another series is based on bifunctional building blocks derived from plant metabolites, especially terpenoids and steroids. Of the various multi component reactions, the Ugi-four-component-reaction (U-4CR) towards dipeptides and their derivatives proved to be the most successful. The reaction runs in environmentally benign solvent like ethanol, but also reactions in water or without solvent are possible. No waste is produced but one equivalent of water.

In our approach to 14-membered cyclopeptide alkaloids, MCRs were successfully applied for the rapid, atom-economic construction of linear precursors. For even larger, highly functionalized macrocycles, a further extension of "simple" MCRs like U-4CRs towards multiple interlocked MCRs with bifunctional components is necessary (e. g. the 1.5-fold U-5CR, depicted in ***figure 2***). Hereby control of the multiplication factor is crucial in order to avoid polymer formation. Towards this end, new complex components have been developed with two active attachment-points (amine, aldehyde, isonitrile or carboxylic acid).

Starting with bile acids suitable bifunctionalized steroid components were synthesized and two of such steroid units were cyclized *via* peptide bridges by Ugi-multicomponent reactions (U-MCRs) leading to steroid cyclopeptides. Using different reaction components, macrocycle libraries were synthesized with ultimate efficiency, i. e. in one step. These macrocycles are the first members of a class of host compounds, where the rigid backbone and lipophilic surface of two steroid moieties is connected *via* peptide groups allowing variable diameters, conformations and chemical functions. Molecular modeling showed that the cavities of the synthesized macrocycles are large enough to encapsulate small organic substrates ***(figure 3)***.

Modification of Macrocycles

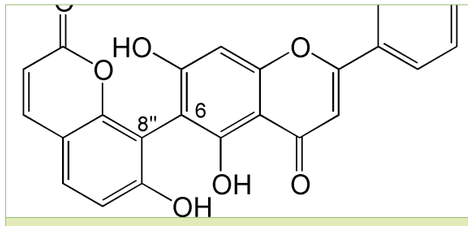


Figure 2: A 6-component macrocyclization reaction constructed from an interlocked 1.5xUgi-4-component-reaction with two bifunctional building blocks with compatible functional groups F (F = e.g. hydroxy-, sugar- or ester-moieties). Pseudodilution is achieved by slow addition of one component. Head-head and head-tail cyclization and the two formed stereocenters will provide a library of eight isomers.

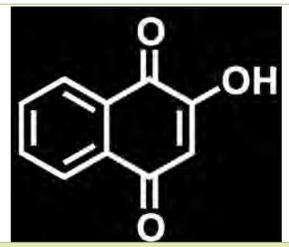


Figure 3: Calculated conformation of macrocyclus XSA 192, $C_{88}H_{152}N_8O_8$, synthesized in one pot, shown with glucose as a guest molecule.

Medicinally active macrocycles are commonly "decorated" with side-chains like lipids, phosphates, and heterocycles like sugars. We are a central partner in the EU-project ComBioCat, which aims at the enzymatic modification of polymer bound natural and natural-product-like macrocycles, their release, "decoration" and screening with self-selecting methods. We could successfully establish a traceless linker for aldehydes on a polymer suitable for enzymatic reactions. This was exemplified with rifamycin, a macrocycle with sensitive enolether, acetal, acetate, and dienoate moieties, which could be modified and released as its antibiotic derivative rifampicin ***(figure 4)***.

Chromium and Selenium Mediated Selective Organic Transformations

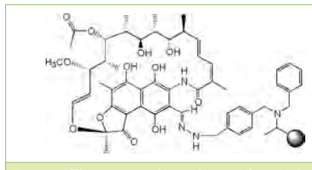
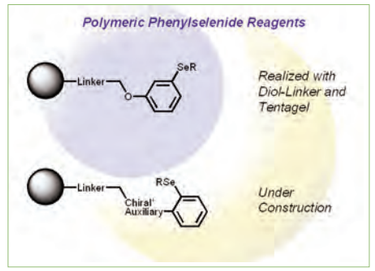


Figure 4: The macrocyclic antibiotic rifamycin bound *via* hydrazon linker to an enzyme penetrable polymeric bakkbone (globe).

Chromium(II) mediated reactions are highly chemoselective and allow transformations in complex molecules without additional protection and deprotection. They also show uncommon selectivity. We could demonstrate that the Hiyama-reaction to homoallylalcohols can give allylketones, depending on the reaction conditions. These findings also suggest solutions to the problem of low enantioselectivity of the reaction with chiral ligands. The results are used to further the development of an iterative process towards polyketide substructures.

Selenium compounds are very versatile reagents in natural product synthesis, unavoidable for some transformations like the ω-oxidation of terpenoids. However, they are toxic, odorous and in some cases not as efficient as desired. We are developing selenium reagents with new characteristics: chiral, selectively removable, or solid phase bound. A fluorous phase selective reagent with improved selenoxide-elimination properties was successfully tested. The synthesis of chiral and solid phase bound versions is under investigation. In a DFG Research Focus Program we are investigating the chemical properties of selenocystein.



Synthesis of Benzopyran Natural Products

Benzopyrans include such common plant secondary metabolites as coumarins, flavonoids, anthocyanins etc. We are developing new synthetic routes towards these compounds, especially to derivatives with new substitution patterns. ■

Research Group: Biocatalysis & Design of Ligands

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The isoprenoid metabolism pathways provide insight into the predominant mechanisms and routes, nature uses to build up carbon skeletons. Understanding these, will provide new enzymes for *in vitro* C-C-coupling reactions, e. g. 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 aromatic substrates, as neither the mechanism nor structural information about these mostly membrane bound enzymes is available. 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.

But also other enzymes, e. g. hydrolyases and oxidoreductases are used for the efficient synthesis of building blocks, especially for enantio- or regioselective transformations. In some cases, enzymatic reactions can also be used for pro-drug like systems, or such systems are discovered in nature.

Prenyltransferases and Isoprenoid Compounds

The synthesis of isotope labeled tentative metabolites of the new non-mevalonate (dxp-) pathway was achieved. In a collaboration, some compounds were utilized to prove for the first time, that 4-hydroxy-dimethylallyldiphosphate (4-OH-DMAPP) is an intermediate of the new pathway in plants. A modified Poulter-procedure for the synthesis of very sensitive aldehyde-pyrophosphates was developed, in addition to our new synthesis of organic diphosphates and cyclic phosphates. Furthermore, we studied the physico-chemical properties of the intermediates of the latter synthesis. *UbiA*-prenyltransferase is a membrane bound enzyme that catalyzes the oligoprenylation of 4-hydroxybenzoic acid (PHB) in 3-position as part of the biosynthesis of ubiquinones ***(figure 1)***. Previously we could demonstrate the use of the *E. coli* - enzyme *in vitro* and elaborate a model of the aromatic substrate. This model was extended and improved, and for the first time also one for the prenyl component was developed. Both substrate models were incorporated in the first protein models of this class of transferases, based on homology calculations (cf. research group "computational chemistry"), which form the basis for future verification by site directed mutagenesis and mechanism-based inhibitors. The synthesis of several such inhibitory compounds was successfully started.

Figure 1: The enzymatic oligoprenylation (n > 1, OPP = diphosphate) of p-hydroxy benzoate (PHB).

For the better production of prenylated hydroxybenzoates, the influence of various parameters and modifiers like cosolvents ***(figure 2)***, additives and metal ions on the reaction was studied. Improved assay-conditions were found,

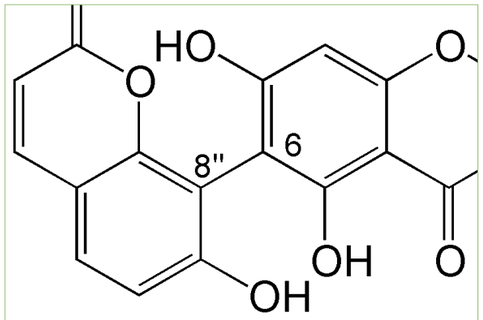

Figure 2: The influence of co-solvents and their concentration on the geranylation yield of 4-hydroxybenzoate catalyzed by *ubiA*-oligoprenyltransferase from *E. coli*.

and a first chromogenic assay was developed. The important factors, which govern the enzyme stability were identified, and consequently the yield of product could be improved to almost 99 % for the natural substrate, reducing at the same time the amount of enzyme required.

Henna

Henna is a powder from dried leaves of the henna-plant *Lawsonia inermis*. Since some 3000 years, henna is used for the temporary dyeing of hair or skin. 2-Hydroxynaphthoquinone (lawsone, ***figure 3***) was considered the main ingredient responsible for the dyeing. However, distinct differences were found in hair colored with henna-paste compared to pure lawsone ***(figure 4)***. Thus, the first severe analysis of the constituents of henna was undertaken. This revealed not only the absence of lawsone in fresh plant material, but also the presence of several new constituents, some of which proved crucial for the dying capability of the plant material. For the first time we could prove that the liberation of dye precursors proceeds enzymatically, and that a complex pro-dye concept is active in the plant powder. Apart from studying the enzymatic transformation, we also started to look at the conjugation of the final dye lawsone to peptides and proteins.

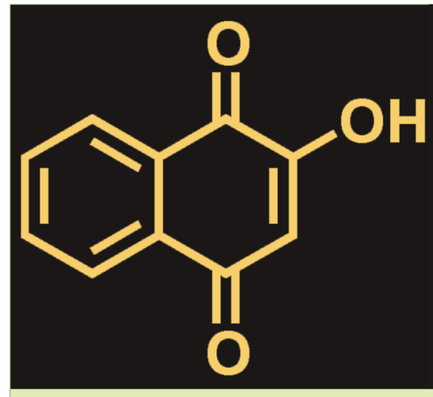

Figure 3: The chemical structure of the henna-dye lawsone

Other

We could achieve the regioselective enzymatic resolution of epothilone acyloin building blocks as part of our on-going effort towards an effective synthesis of epothilone derivatives (cf. research group "synthesis"). The best suitable lipases were identified. We also tested the selective enzymatic acylation / deacylation of macrocyclic ester and hydroxy side-chains in solid phase gels. ■

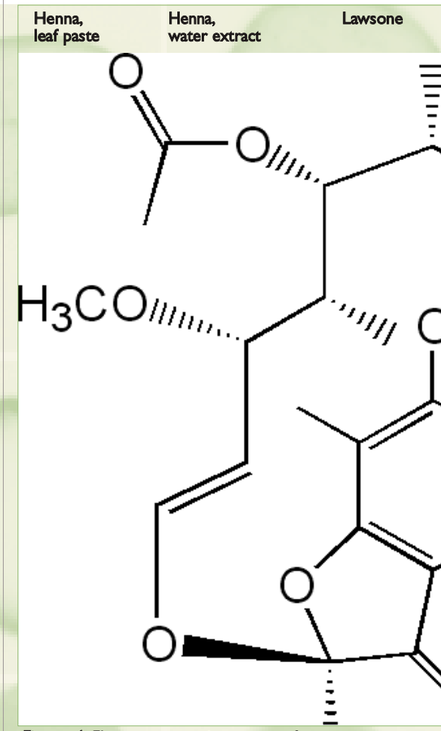

Figure 4: Fluorescence microscopy of cross-sectioned hairs dyed by various preparations. It is clearly visible that henna leaf-paste, still containing active enzyme, dyes hair throughout and fully, whereas a water extract and synthetic lawsone possess greatly reduced almost identical dyeing properties (Photo by Bettina Hause).

Research Group: Plant and Fungal Metabolites / Microanalytics

Heads: Norbert Arnold, Jürgen Schmidt, Ludger Wessjohann & Gernot Schneider
(until June 2001)

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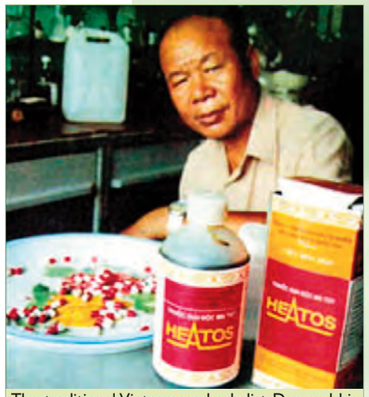
Defined natural substances find numerous applications in society and industry, e.g. as chemical raw materials, food additives, cosmetics, in agriculture, and especially in medicinal chemistry where they form the basis of more than one third of all currently approved drugs. The research group focuses on the isolation and chemical characterization of pure compounds from plants and fungi, which are a rich and diverse source of secondary metabolites. In addition, techniques for the improved analysis and profiling of plant metabolites are developed, especially through the application of mass spectrometry. The biological activities of extracts, fractions and especially pure substances are tested in bioassays. These are designed to elucidate the function of the tested compounds in nature or to screen for medicinally or otherwise useful properties.

Plant metabolites:

Southeast Asia

HEA(N)TOS is a drug used for an effective detoxification treatment of drug addiction. It is based on the traditional herbal medicine of Vietnam. The abbreviation HEA(N)TOS is derived from "heat of the sun", and was originally developed by the Vietnamese herbalist Tran Khuong Dan. It is now produced in an improved formula at the Institute of Chemistry of the National Center for Natural Sciences and Technology in Hanoi. It is composed of 13 medicinal plants and natural products grown in Vietnam. As part of an UNESCO project concerning the international scientific development and standardization of the anti-drug medication HEA(N)TOS, we perform phytochemical studies of the constituents in close cooperation with Sung Tran from the Institute of Chemis - try in Hanoi. The aim of our work is the isolation and structural elucidation of the compounds with potential biological activities and the compilation of literature data on the components already known. These investigations contribute to the botanical identification of the used plant species and provide the necessary prerequisites for further development and a future global use of HEA(N)TOS. Until now, our group investigated the constituents of seven of the 13 components. So far, the phytochemical investigations resulted in the identification of approximately 150 substances. Besides numerous known compounds from many classes, several new compounds were detected.

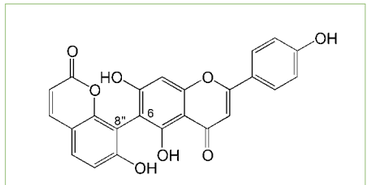
Apart HEA(N)TOS, the investigation of medicinal plants from Southeast Asia, especially Vietnam and Myanmar (Birma), is continued. A PhD-thesis on constituents of *Fissistigma* spec. from Vietnam was concluded and numerous new flavonoid-terpenoid-hybrid compounds could be published.



The traditional Vietnamese herbalist Dan and his Hea(n)tos drug preparation.

Africa, Mediterranean, and Middle East

In the course of a phytochemical project with the Sanáa University (Yemen), a series of new natural compounds were found. Thus, from *Dorstenia gigas*



6-(8''-umbelliferyl)-apigenin from *Gnidia socotrana* (Thymelaeceae)

(Moraceae) eleven new furanocoumarins, especially with oxygenated geranyl chains, as well as one benzofuran derivative could be structurally elucidated by high-resolution MS and 2D-NMR-analysis. With a series of new cardanols a potential cancerostatic activity were identified by GC-MS in *Rhus thyrsiflora* (Anacardiaceae). Moreover, besides some known piperidine alkaloids a new chlorinated amide from *Aloe sabaea* (Aloeaceae) as well as a new 5-methylchromone glycoside from *Commiphora socotrana* (Burseraceae) could be isolated. The first finding of two coumarin-flavonoid hybrid compounds from *Gnidia socotrana* (Thymelaeceae), representing a new type of compounds, was an important topic in the report period. A phytochemical investigation of *Eulophia petersii* (Orchidaceae) led to the identification of structurally known phenanthrene derivatives (cooperation with Mohamed Masaoud, Sanáa).

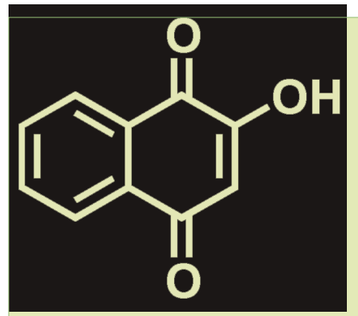
In cooperation with Luay Rashan (Amman) the trail for a chemical basis for the anticancer properties of a regional plant was followed. The work on constituents of African species in *Antidesma*, which led to the discovery of compounds highly active against trypanosomes (Chagas' disease) was concluded. A new project based on plants from Madagascar was initiated. However, as with other projects in this world region, the cooperation was halted for the time being.

We hope to re-establish active projects, when the safety, political and financial situation has stabilized, because the past cooperation was highly successful with respect to our local partners, structures or biological activities found.

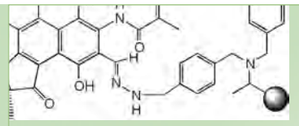
The Americas

Though the political development in Latin America is very favorable, natural product research is increasingly hampered by bureaucracy for both, the local partners as well as the international ones. Together with a veterinary institute in Southern Brazil we looked at pampas plants toxic for cattle. Constituents, which are potentially responsible for the observed bone deformation or even death, have been identified.

Rather than being guided by ethnopharmocological, phytochemical or observative selection of plants, in a different approach we concentrate on the tissue specific profiling of constituents from secretive plant organs. The emphasis is on elaiophores, floral glands that produce oils as rewards for pollinators of predominantly neotropic plants. The oil flower syndrome was not discovered before the seventies. The chemical composition of floral oils is mostly unknown; the analytical methods for their profiling and reliable analyses were not yet established. In cooperation with the Botanical Garden Munich-Nymphenburg (Günter Gerlach) and several international partners (e. g. Beryl B. Simpson), we gained access to various species of oil flower plants. The analytical work included the collection of the floral oils by microscopic techniques, development of micro-derivatization methods, and analysis of the volatile derivatives by GC/MS. The underivatized oils were analyzed by electrospray tandem mass spectrometry. Structures of uncommon chain oxidized lipids could be elucidated, e. g. novel dihydroxylated fatty acids and their glycerides in the floral oil from species of the families Malpighiaceae and Orchidaceae. The investigation of the secretions from seven different, non-related plant families supported the



Oil flower of *Ennelophus euryandrus* (Iridaceae, photo by Günter Gerlach), and formula of a typical floral oil component with a 2-acetoxy fatty acid residue.



Centris bee visiting the oil flower of *Malpighia emarginata*. Photo by Günter Gerlach

Research Group: Plant and Fungal Metabolites / Microanalytics

Heads: Norbert Arnold, Jürgen Schmidt, Ludger Wessjohann & Gernot Schneider

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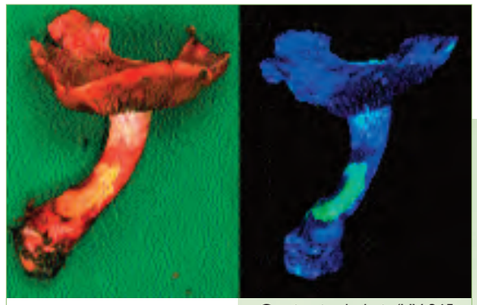
fact, that plant diversity is reflected in different chemical compo-sitions of the floral oils, and that the syndrome evolved independently. In a related project, the floral glands of hops (*Humulus lupulus*) are studied in a cooperative effort with the department of plant biotechnology (see separate chapter).

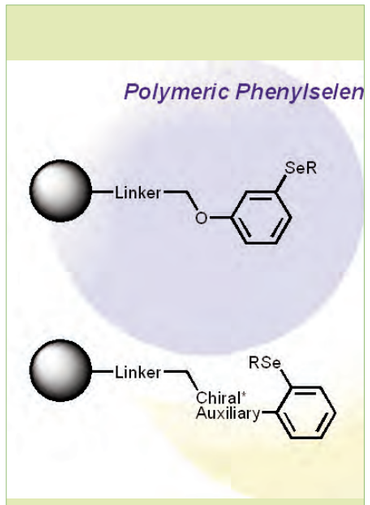
Fungal metabolites:

The kingdom of fungi is composed of an estimated 10^6 specimen and forms on of the biggest group of organisms in our world. Many of them are living in symbiosis with plants (mycorrhizal fungi), others are pathogenic. At this time only 5 % (75.000) of all fungi are well described. In our research on fungal metabolites, we are mainly focused on compounds from fruitbodies of Basidiomycetes (e. g. *Hygrophorus*, *Cortinarius*). Species in the genus *Hygrophorus* Sect. *Olivaceoumbrini* are well characterized by a yellow reaction after treating the stem with base like potassium hydroxide. The responsible constituents could be isolated and their structures elucidated as cyclopentenon derivatives. Information about their biosynthesis is expected from feeding experiments with ^{13}C-labeled precursors. In addition, some compounds show remarkable antifungal activity in our bioassay.

The fruitbodies of *Cortinarius bolaris*, a species described in the literature as poisonous, are staining yellow when bruised or cut. The yellow stained areas show a bright golden fluorescence in UV-light. The chemical principle underlying these phenomena could be isolated and was characterized as a new benzofuran glycoside. Further research is directed at the chemical constituents of *Sepedonium* (Fungi imperfecti), which live as parasites on boletes and bolete relatives (Boletales).



Cortinarius bolaris (day light) *Cortinarius bolaris* (UV 365 nm)



Feeding experiment on *Hygrophorus latitabundus*

Microanalytics:

The coupling of HPLC and electrospray (ES) tandem mass spectrometric methods was successfully applied to the microanalysis of a series of natural compounds in collaboration with all departments of the IPB and external groups. Thus, a LC-ESI-MS/MS method for the determination of 5-methylchromone glycosides was developed and some new compounds of this type from *Aloe* species could be identified. 12-hydroxysulfonyloxyjasmonic acid was identified by selected reaction monitoring in *Arabidopsis thaliana* and *Nicotiana tabacum* (Otto Miersch, Halle; Luc Varin, Montreal). Betalains, resveratrol glucoside, sinapic acid derivatives, flavonoids and other secondary metabolites could be also evaluated by MS/MS techniques (Willibald Schliemann, Alfred Baumert, Thomas Vogt, Dieter Strack, Halle). In collaboration with the group of Lutz Heide (Tübingen) especially the LC-ESI-selected reaction monitoring (SRM) as a sensitive and effective method for trace analysis, has led to the identification of a series of new aminocoumarin antibiotics of the novobiocin-, chlorobiocin - and coumermycin type. Products of the polyketide synthesis were identified by LC-ESI-MS/MS (Toni M. Kutchan, Jonathan Page, Halle). Using both, positive and negative ion electrospray, perlatolic acid derived depsides and depsidones from the lichen *Lecidea inops* were analyzed (Siegfried Huneck). Phytosterols as marker for specific mutations during the embryogenesis of Arabidopsis could be identified by GC-MS (collaboration with Katrin Schrick and Gerd Jürgens, Tübingen).

In 2001, the mass spectrometry facilities were improved by establishing the electrospray Fourier-transform ion cyclotron resonance technique. This new technique allows mass spectral analyses with very high resolution and mass accacy. This allowed the solution of some difficult problems with synthetic compounds (e. g. macrocycles) and for the identification of natural products. ■

7-Tesla-Fourier-transform ion cyclotron resonance mass spectrometer (FT-ICR-MS) with an electrospray (ESI) ion source

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Meinhard H. Zenk
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The research group is investigating three-dimensional molecular structures of small molecules and proteins as well as reaction mechanisms in the field of bioorganic chemistry by means of molecular modeling, semi-empirical calculations, nuclear magnetic resonance (NMR) and optical spectroscopy. The group is also responsible for the development of a database, designed to solve problems involved with phytochemical investigations such as fast dereplication (Phytobase). The collected information together with data mining and new data from the other research groups forms the basis for chemoinformatic analyses, which will enable new insights in the biological significance of plant and fungal metabolites.

In 2000, the DFG supported project "Conformation and structure-activity relationship of brassinosteroids" was finished with investigations of the side-chain conformations of brassinosteroids in aqueous solution with and without the presence of micelle forming agents. Using the sophisticated WET solvent signal suppression technique (**w**ater suppression **e**nhanced through T_1 effects), spectra with sufficient signal-to-noise ratios could be recorded, even if the solubility of brassinosteroids in water is less than 0.2 mmol/l. A highly conserved solution structure of the steroidal side-chain was found in case of brassinolide whereas the less bioactive 24-epi-brassinolide showed different conformations dependent on the medium.

The NMR equipment was largely modernized and expended with the different focus of the department in 2001. The 500 MHz and the 300 MHz NMR spectrometer were equipped with new radio-frequency consoles and new probe heads. *Inter alia*, for the first time high-resolution MAS (**m**agic **a**ngle **s**pinning) proton spectra, deuterium decoupled ^{13}C spectra and DOSY (**d**iffusion **o**rdered **s**pectroscop**y**) spectra could be recorded. A new 400 MHz NMR spectrometer equipped with a four nuclei auto-switchable probe was installed in that year ***(figure 1)***. Since March 2002, this spectrometer is operated as an open-access routine NMR for trained graduate students, postdocs and technicians of the department. In preparation for the open access use, a set of macro programs was developed, which allow the easy set-up of experiments and data processing. This included solvent dependent shim sets and parameter files, which were adjusted at regular intervals. All users were trained in the operation of the NMR spectrometer and had to pass an "NMR driving test" before using the instrument unsupervised.

Figure 1: The new 400 MHz NMR spectrometer used by Dr. Trinh Thi Thuy in open-access mode

Service measurements of NMR spectra as well as opto-analytical (IR, UV, CD and ORD) spectra were carried out for scientists of this and other departments of the IPB. Since 2000, the sample volume increased several times. Currently some 5000 spectra are recorded *per annum*. The NMR laboratory of the IPB participated successfully in a national interlaboratory

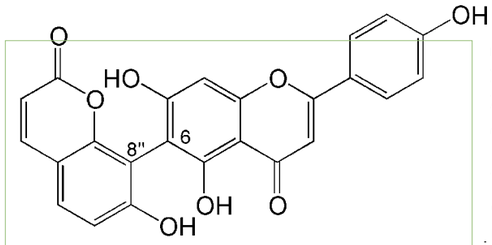

Figure 2: Constitution and configuration of two new aurones

test of the Federal Institute for Materials Research and Testing (BAM, Berlin) for the validation of ^1H NMR spectroscopy as a reliably quantitative analysis method. The main task of the NMR-subgroup was the structural elucidation of natural products and synthetic compounds in collaboration with the other groups of the department. As an example of a successful structural elucidation, ***figure 2*** shows two new aurones (collaboration with Fred Stevens). The constitution as well as the configuration of the double bonds could be elucidated by one- and two-dimensional NMR experiments and NOE investigations.

In late 2001, the subgroup "computational chemistry" was started. A powerful computer-cluster of altogether four UNIX-workstations and six LINUX and WINDOWS-PCs was installed as basic prerequisite for the performance of molecular modeling calculations. For this purpose, software program packages, such as SYBYL, MOE (Molecular Operating Environment) SPARTAN, JAGUAR and GAUSSIAN were installed ***(figure 3)***.

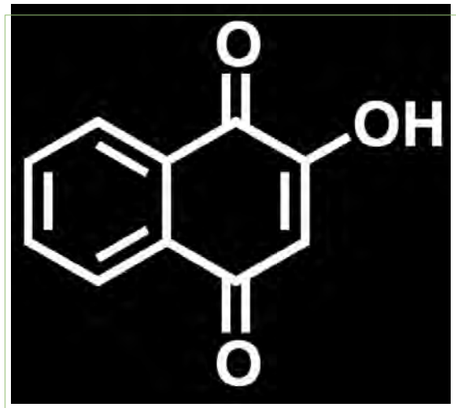

Figure 3: Configuration of the workstations and PC cluster of the modeling group.

The first project aims at the homology modeling of aromatic prenyltransferases, an important group of enzymes of which neither 3-D structural information nor mechanistic details of the catalysis mechanism were known. Most aromatic prenyltransferases are membrane bound, like 4-hydroxybenzoate oligoprenyltransferase (ubiA), a key enzyme in the biosynthesis pathway of ubiquinone. It catalyzes the prenylation of 4-hydroxybenzoate in the 3-position with an oligoprenyldiphosphate and is one of the best-characterized examples, which was also available to us for experimental verification (cf. biocatalysis group). By using homology modeling and multiple alignments, secondary structure prediction, molecular dynamics simulations and energy optimizations, two first models with two possible active sites could be created and refined ***(figure 4)***.

Other investigations concerned the analysis of structure activity relationships of epothilones accompanied by conformational investigations based on NMR data, conformational studies of macrocycles, and calculations on the reaction mechanism of the late enzymes of the non-mevalonate pathway (MEP-pathway) of isoprenoid biosynthesis, especially the conversion of 2-methyl-D-erythritol-2,4-cyclodiphosphates.

Within the EU-project "Opioid Treatment of Chronic Pain and Inflammation of the Locomotor System", 3D-models of the opioid receptors have been developed. Based on these models and corresponding docking studies the structure-activity relationships of a multitude of opioids could be explained and new derivatives with improved properties could be proposed for synthesis. Furthermore, the unusual long duration of action of a new class of kappa selective opioids could be clarified based on mechanistic investigations and *ab inito* DFT-calculations.

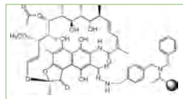

Figure 4: The most likely model of 4-hydroxybenzoate oligoprenyltransferase with docked substrates octaprenyl-diphosphate (OP-PP), 4-hydroxybenzoate (4-HB) and magnesium (Mg) at the expected active site.

In 2002, "Phytobase" was started in collaboration with Volkmar Vill (Hamburg) as a long-term development project. The compound based reference and spectroscopy collection will include biological information and will directly link all available data in an object-oriented database. Phytobase will be the central information system of the department and is a cornerstone for future development of the IPBs natural product research. It will be available to the other departments, and partially to external partners (PlantMetaNet, funding partners) and the public. ■

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Scheid, Günther: A New Synthesis of Epothilone Macrocycles, Free University of Amsterdam (NL), Bioorganic Chemistry, 11/04/2002.

Sinks, Udo Eckard: New Applications of Sulfoxides and Synthesis of Asymmetric Phenylselenide Reagents, Sandwich between Free University of Amsterdam (NL), Bio-organic Chemistry, and Ludwig-Maximilians-Universität München, Dept. of Organic Chemistry, 15/05/2001.

Trinh Thi Thuy: Phytochemische Untersuchungen der vietnameschen Heilpflanzen *Clausena excavata* und *Zanthoxylum avicennae* (Rutaceae), National Center for Natural Scientific and Technology (Vietnam), Institute of Chemistry and Martin-Luther-University of Halle-Wittenberg, 01/11/2001.

Wild, Harry: Neue Anwendungen der Chrom-Reformatsky-Reaktion, Ludwig-Maximilians-Universität München, Dept. of Organic Chemistry, 10/10/2000.

Diploma Theses

Belting, Claudia: Terpene derived enediyes as potential anti-tumor drugs. Hochschule Enschede, The Netherlands, 31/01/2002 (Sandwich).

Bräuer, Lars: Modellierung der 4-Hydroxybenzoat Oligoprenyltransferase und Charakterisierung potentiell aktiver Zentren. Martin-Luther-University of Halle-Wittenberg, 25/06/2002.

Spelbos, Vincent: Prenyltransferasen in *Humulus lupulus.* University of Utrecht, The Netherlands, November 2002.

Schültingkämper, Heike: The combinatorial diastereoselective synthesis of highly functionalized tetrahydropyrans. Hochschule Enschede, The Netherlands, 18/01/2002 (Sandwich).

Wesseling, Claudia: Synthesis of a building block for the natural product cis-gigantrionenine. Hochschule Enschede, The Netherlands, 31/01/2002 (Sandwich).

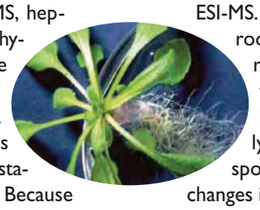
In the report period, another nine students received their diploma-degree (dutch: Drs.) at the Free University of Amsterdam, Bio-organic Chemistry, under the supervision of Prof. Wessjohann. ■

Research Group: Searching for Signals: Stress-Induced Changes in Arabidopsis Secondary Metabolite, Peptide and Protein Patterns (GABI)

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

Our project is aiming at contributing to the "post-genomic" ana-lysis of the model organism *Arabidopsis thaliana* by establishing an extensive profiling of proteins, peptides, and metabolites. These profiles are to be used for the detection and identification of early stress responses and novel signaling molecules. Eventually, they will provide 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 profiling of stress-induced metabolic changes in Arabidopsis plants grown under sterile conditions in a hydroponic system has been established. A standardized extraction procedure for root and leaf (secondary) metabolites is introduced. The methanolic extracts are analyzed by Cap-LC-ESI-Q-TOF-MS, heptane extracts for the more hydrophobic compounds are analyzed by GC-MS. Cap-LC-ESI-Q-TOF-MS represents a new profiling approach that is to complement the more established GC-MS techniques. Because very few tools are available for data deconvolution and data extraction we developed respective procedures for the automatic data analysis. Several samples can now be processed per day. In leaf extracts about 1200 mass signals are resolved and detected, in root signals about 1000 mass signals. Mass data can be directly compared to an Arabidopsis literature database. The exceptional mass accuracy of ESI-Q-TOF-MS together with its tandem MS option allows tentative identification or classification of interesting compounds. An extensive evaluation of the whole Cap-LC-ESI-Q-TOF-MS-based profiling approach is now complete. Changes in response to the stress caused by exposure to elevated heavy metal levels have been analyzed. In the course of collaborations with other groups, several Arabidopsis mutants with defects in, for instance, signal transduction or defense, are being profiled. Similarly, in order to be able to use metabolite profiling for studies on natural diversity, data sets for a number of Arabidopsis ecotypes are being generated.



The profiling of proteins and peptides is based on two-dimensional gel electrophoresis, MALDI-TOF-MS and nano-spray-ESI-MS. Patterns of soluble leaf, root or seed proteins are resolved in large-format two-dimensional gels. Gel images are carefully analyzed. Interesting protein spots showing stress-related changes in abundance are picked, digested and subjected to MALDI-TOF mass spectrometry for identification based on peptide mass fingerprints. Image analysis, which represents the bottleneck of searches for changes within the proteome, has been optimized by adopting new imaging software. A number of Arabidopsis mutants have been analyzed under different stress conditions. For some of the identified proteins that show stress-related changes functional characterization has been initiated by isolating Arabidopsis insertion lines for the respective genes.

The intercellular washing fluid of Arabidopsis leaves is analyzed for peptides and metabolites by nanospray-ESI-Q-TOF-MS. In principle, the detection of molecules in this compartment is possible. Progress, however, has so far been slow due to limitations in MS capacity as the same machine is used for cap-LC-coupled metabolite profiling and nanospray-MS. ■

Group members

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GABI-Arabidopsis-Verbund III

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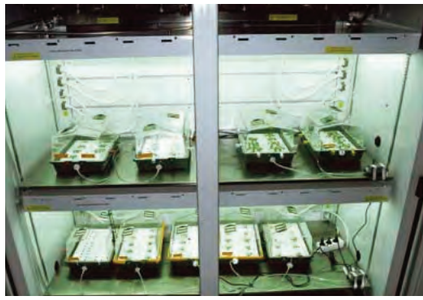
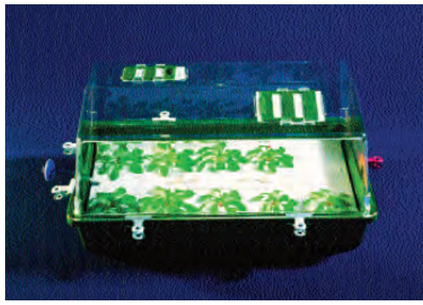




figures: Reproducible plant growth of Arabidopsis

Department: Stress and Developmental Biology

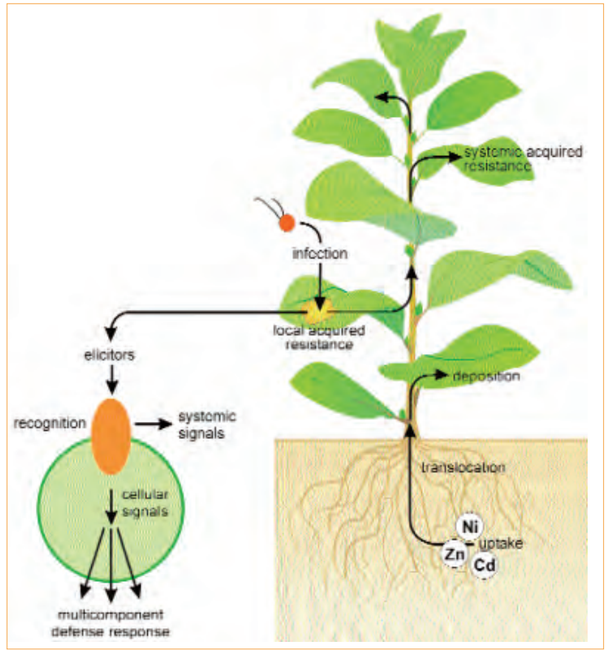
Head: Prof. Dierk Scheel

Secretary: Ruth Laue

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 ini-tiate 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 ana-lysis 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. ■



Research Group:
Signal Perception in Plant-Pathogen Interactions
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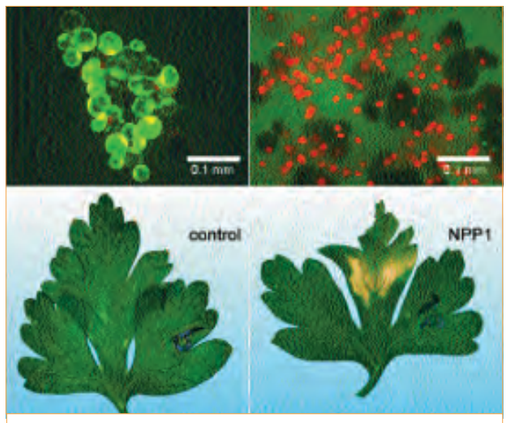
Innate immunity is well described for animals and is also suggested to be important for plants. In vertebrates and insects, microbial pathogen sensing relies on the recognition of pathogen-specific structures, which are not found in hosts and which are indispen-sable for the lifestyle of the microorganism. Receptor-mediated signal perception by the host gives rise to the activation of specific immune responses, such as the synthesis of antimicrobial compounds. We investigate whether pathogen recognition by animals and plants share similar characteristics. Our data suggest that the evolution of pathogen perception systems in plants is likely to be similar to that described for animals. Microbial surfaces constitute complex patterns for the activation of plant pathogen defense. Recognition of microbial pattern by plants appears to result in more sensitive perception of pathogens and synergistically enhanced plant defense. Phytopathogenic bacteria of the genus *Pseudomonas* produce and secrete the effector protein HrpZ during (attempted) infection of plants. HrpZ was shown to insert into lipid bilayer membranes and to form cation-conducting channels. This ion channel-forming activity, however, appears not to be the molecular basis for the activation of defense responses in plants treated with HrpZ.

A calcium-dependent transglutaminase (TGase) present in the cell wall of as many as ten species of the genus *Phytophthora* serves as recognition determinant for the activation of non-cultivar-specific defense responses in parsley and potato. An evolutionarily highly conserved peptide fragment of this protein (Pep-13) was identified within a surface-exposed loop structure of the protein. Pep-13 was shown to be necessary and sufficient for receptor-mediated activation of defense responses in both plants. Mutations within the Pep-13 motif of the *P. sojae* TGase, which reduced or abolished the elicitor activity of the intact protein, similarly affected its enzyme activity. Apparently, during evolution plants have acquired receptors for the recognition of stable and functionally indispensable surface epitopes of microbial pathogens, suggesting that such perception modules may form the molecular basis of durable pathogen resistance in non-host plants.

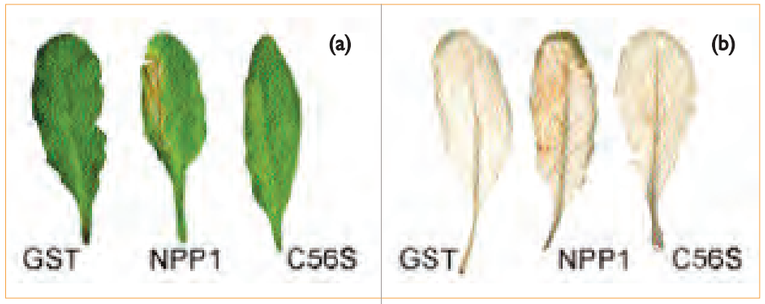


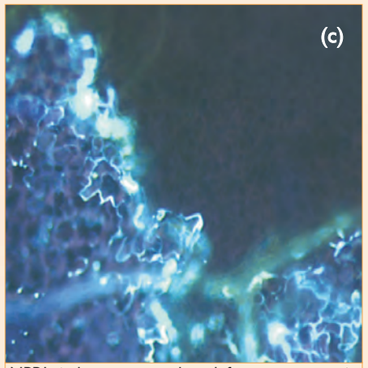
NPP1-induced cell death in parsley
Viability of parsley protoplasts treated with 20 nM NPP1 or water (control) was determined 24 h upon elicitation (upper panel). Viability of parsley protoplasts (5×10^5/ml) was determined by double-staining with 50 µg/ml fluorescein diacetate and 10 µg/ml propidium iodide 24 h after treatment (Jabs et al., 1997).

NPP1 (2.5 µM) or water (control) infiltrated into parsley leaves for 48 h (lower panel).

In addition, all *Phytophthora* species tested possess a 24-kDa protein (NPP1) that triggers defense responses in parsley very similar as does Pep-13. NPP1-mediated activation of pathogen defense in parsley does not employ the Pep-13 receptor. However, early-induced cellular responses implicated in elicitor signal transmission (increased levels of cytoplasmic calcium, production of reactive oxygen species, MAP kinase activation) were stimulated by either elicitor, suggesting the existence of converging signaling pathways in parsley. Infiltration of NPP1 into leaves of *Arabidopsis thaliana* resulted in transcript accumulation of pathogenesis-related (*PR*) genes, production of reactive oxygen species and ethylene, callose apposition, and hypersensitive-like cell death. NPP1-mediated induction of the *PR1* gene is salicylic acid-dependent, and, unlike the *P. syringae* pv. *tomato* DC3000(*avrRpm1*)-induced *PR1* gene expression, required both functional NDR1 and PAD4. Importantly, Arabidopsis plants infiltrated with NPP1 constitute an experimental system that is amenable to forward genetic approaches aiming at the dissection of signaling pathways implicated in the activation of non-cultivar-specific plant defense.

The HrpZ gene product from the bean halo-blight pathogen, *Pseudomonas syringae* pv. *phaseolicola* ($HrpZ_{Psph}$), is secreted in an *Hrp*-dependent manner by this bacterium, and exported by the type III secretion system when expressed in the mammalian pathogen *Yersinia enterocolitica*. $HrpZ_{Psph}$ was found to stably associate with liposomes and synthetic bilayer membranes. Under symmetric ionic conditions, addition of 2 nM purified recombinant $HrpZ_{Psph}$ to the *cis*-compartment of planar lipid bilayers provoked an ion current with a large unitary conductivity of 207 pS. $HrpZ_{Psph}$-related proteins from *P. s.* pv. *tomato* or *syringae* triggered ion currents similar to those stimulated by $HrpZ_{Psph}$. The $HrpZ_{Psph}$-mediated ion-conducting pore was permeable for cations but did not mediate fluxes of Cl^-. Such pore-forming activity may allow nutrient release and / or delivery of virulence factors during bacterial colonization of host plants. In addition, HrpZ has been shown to trigger a complex defense response in parsley and tobacco. Ligand / receptor interaction studies revealed the presence of a high-affinity binding site for $HrpZ_{Psph}$ in plasma membranes of both plants. Series of truncated $HrpZ_{Psph}$ proteins were analyzed with respect to their abilities to induce plant defense as well as to form ion-conducting pores in liposomes. The pore-forming activity of $HrpZ_{Psph}$ was found to require the intact protein, while defense responses were stimulated by a C-terminal fraction of the protein in both plants. Thus, pore-forming activity of $HrpZ_{Psph}$ does not determine the activation of plant defense, but may reflect the role of the protein during (attempted) bacterial infection of plants. ■





NPP1 induces a complex defense response in *Arabidopsis thaliana* Col-0. Infiltrations were performed with 2.5 µM of each recombinant NPP1, a mutant derivative of NPP1 with reduced activity, or Glutathione-*S*-Transferase as control. Necrotic lesion formation 48 h upon elicitation (a), production of reactive oxygen species 3 h upon elicitation (b), and callose apposition 24 h upon elicitation (c).

Research Group: Cellular Signaling

Head: Dierk Scheel

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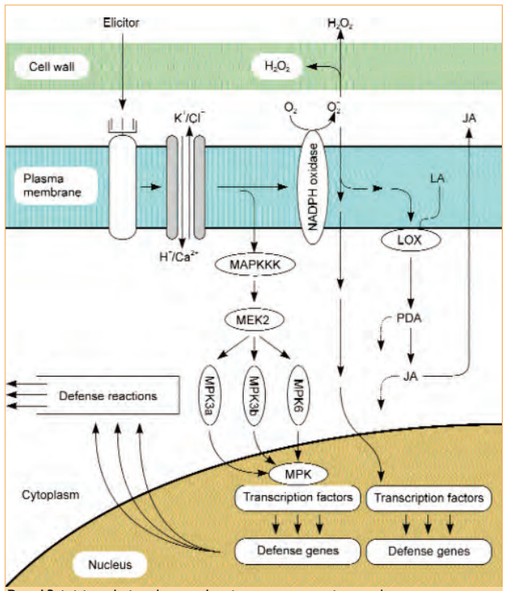
Parsley is not a host plant for the soybean pathogen, *Phytophthora sojae*, but if germinating zoospores of this oomycete try to invade the plant, it responds with a multifaceted defense response that terminates the infection process. The oligopeptide elicitor Pep-13, originating from a hyphal cell wall transglutaminase of *P. sojae*, is one of the pathogen-associated molecular patterns (PAMPs) recognized by the plant cell *via* a plasma membrane-localized receptor (see preceding report). Upon binding of Pep-13, this receptor initiates a cellular signal transduction cascade that causes dramatic alterations of the gene expression pattern, primarily resulting from activation of defense-related genes. The cellular signaling elements linking the Pep-13 receptor to specific activation of defense-related genes include plasma membrane-located ion channels, protein kinases, an NADPH oxidase and jasmonate. Together with additional unknown components, these elements form a modular signaling network tightly regulating the temporal and spatial activation of defense reactions.

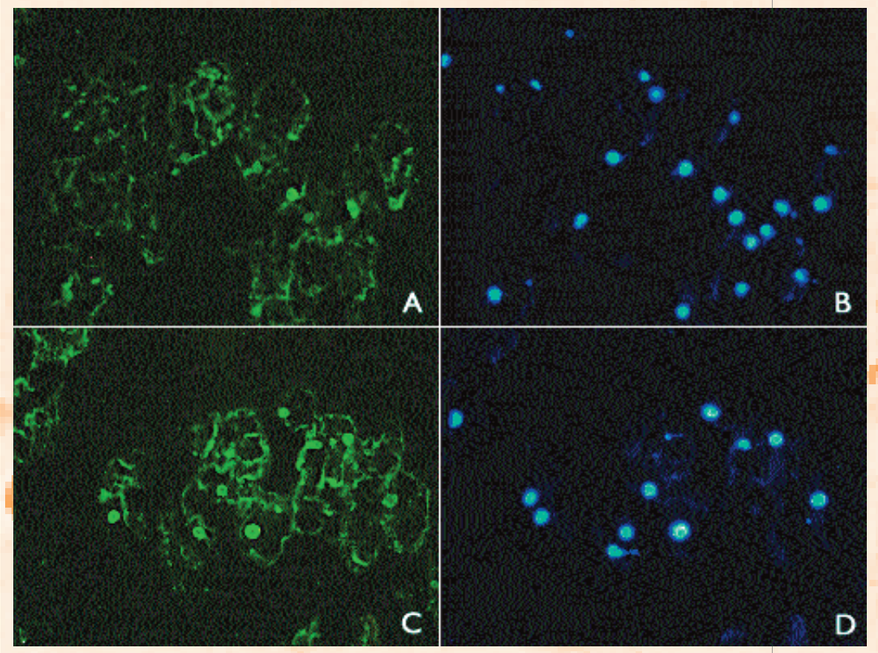
Pep-13 treatment of suspension-cultured parsley cells rapidly stimulates Ca^{2+} influx resulting in a characteristic sustained increase in cytosolic Ca^{2+} levels, which is essential for all the other known elicitor responses. At least four mitogen-activated protein kinase (MAPK) cascades are activated downstream of this Ca^{2+} transient. Four MAPK-encoding genes have been isolated from parsley, designated *PcMPK3a*, *3b*, *4* and *6* according to their sequence similarities to MAPK-encoding genes of *Arabidopsis thaliana*. Upon elicitation PcMPK3a, 3b, 6 and a fourth so far unknown MAPK were found to be activated by phosphorylation of the conserved TEY motif and translocated to the nucleus, whereas PcMPK4 was not affected. Transient co-expression of dominant inactive versions of *PcMPK3a*, *4* and *6* with reporter gene fusions of the *PR1* (pathogenesis-related) and *PR2* promoters has demonstrated that elicitor activation of these genes is regulated by PcMPK3a (and possibly also 3b) and/or PcMPK6, but not by PcMPK4. MAPKs are themselves activated through phosphorylation by MAPK kinases. Two MAPK kinase-encoding genes, *PcMEK1* and *2*, have been isolated from parsley. Only PcMEK2 was found to be activated in Pep-13-treated cells and was then able to phosphorylate PcMPK3a, 3b and 6, not however PcMPK4.



Pep-13-initiated signal transduction processes in parsley.

While the activation of MAPKs and *PR1*, *PR2* and *WRKY* transcription factor genes was found to be independent of the oxydative burst, superoxide anion radicals, the primary reactive oxygen species formed during the Pep-13-stimulated oxidative burst, are necessary and sufficient for phytoalexin production and activation of those genes encoding their biosynthetic and additional phenylpropanoid pathway enzymes. The formation of superoxide anion radicals is catalyzed by NADPH oxidases, which are structurally similar to the catalytic subunit of the mammalian respiratory burst oxidase. Two NADPH oxidase-encoding genes were isolated from parsley. In comparison to the catalytic subunit of the respiratory burst oxidase, these proteins are N-terminally extended by a region harboring two Ca^{2+}-binding EF hands. One of the NADPH oxidase transcripts accumulates rapidly and transiently upon elicitation. In addition, transcripts encoding enzymes with and without EF hands were found to be generated by alternative splicing. Heterologous expression of both type of proteins in yeast resulted in production of active NADPH oxidases embedded in microsomal membranes. Only the larger protein with the EF hands required Ca^{2+} for activity.

The oxidative burst is necessary but not sufficient for Pep-13-stimulated production of the oxylipins, jasmonate and its precursor 12-oxo-phytodienoic acid. Simultaneous treatment of the cells with lipoxygenase inhibitors completely blokked the accumulation of both oxylipins, but did not affect Pep-13-mediated phytoalexin synthesis, suggesting that jasmonate and/or 12-oxo-phytodienoic acid represent the starting point of yet another signal transduction branch. Salicylic acid, a plant defense signaling compound involved in signaling pathways that initiate programmed cell death, does not accumulate in Pep-13-treated parsley cells. Interestingly, parsley cells and leaves do not undergo programmed cell death in response to Pep-13 treatment. ■



Elicitor treatment induces nuclear translocation of MPK3
Cultured parsley cells were treated with Pep13 (100 nM; C, D) or H_2O (A, B) and fixed in 4% paraformaldehyde 15 min. after initiation of treatment. Cells were embedded in paraffin, cut into 6 µm sections and stained with PcMPK3 antiserum (A, C). Goat anti-rabbit secondary antibody conjugated with Alexa 488 was used to visualize the primary antiserum bound to MPK3; nuclei were also counterstained with DAPI (B, D). After treatment with Pep13 most nuclei were stained by PcMPK3 antiserum, whereas no or little nuclear staining was detectable in control cells.

Research Group: Induced Pathogen Defense

Heads: Sabine Rosahl & Dierk Scheel

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To elucidate defense mechanisms against the oomycte *Phytophthora infestans*, the causal agent of late blight disease of potato, we are studying the interaction of *P. infestans* with its host plant potato and with the nonhost plant *Arabidopsis thaliana*. For potato, analysis of the recognition of the pathogen, signal transduction and characterization of the pathogen defense are our major interests.

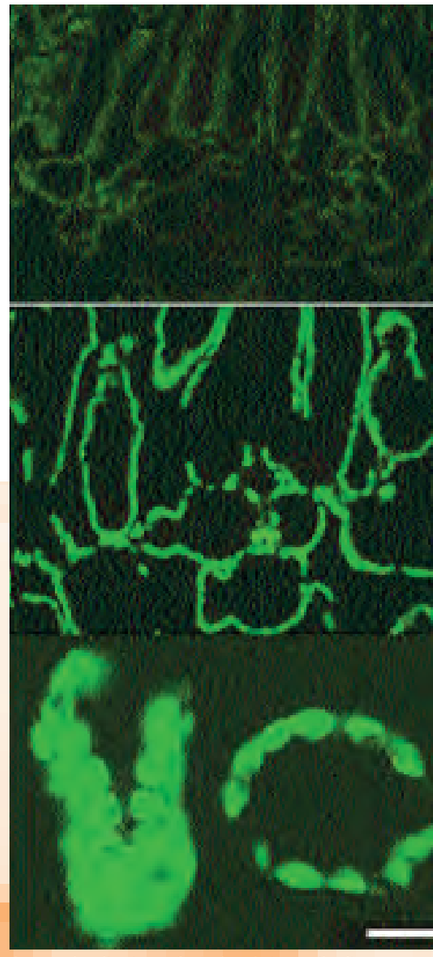
The *Phytophthora sojae*-derived oligopeptide elicitor Pep-13, originally identified as an inducer of plant defense in parsley and shown to act as a pathogen-associated molecular pattern (PAMP) in evoking innate immune responses, also triggers defense responses in potato. In cultured potato cells, Pep-13 treatment results in the formation of hydrogen peroxide, alkalinization of the culture medium, accumulation of 9-lipoxygenase-derived oxylipins and activation of defense genes. Similarly, accumulation of transcripts encoding enzymes of the phenylpro-panoid pathway, lipoxygenases and pathogenesis-related proteins occurs in potato leaves in response to Pep-13 infiltration. Derivatives of Pep-13 show similar elicitor activity in parsley and potato, suggesting a receptor-mediated induction of defense response in potato analogous to that observed in parsley. Interestingly, unlike in parsley, infiltration of Pep-13 into leaves leads to rapid cell death in potato. Using transgenic plants with modulated levels of jasmonic and salicylic acid, the dependence of Pep-13-induced defense reactions on these signaling compounds is being studied.

Oxylipins play an important role in the plant's reaction to pathogen attack. In potato, 9-lipoxygenase-derived oxylipins accumulate in response to Pep-13 and elicitor treatment as well as after pathogen infection. To analyze the role of 9-lipoxygenase-derived oxylipins, transgenic potato plants expressing RNA interference constructs, targeted at the pathogen-induced 9-lipoxygenase of po - tato, were generated and are being analyzed for alterations in their response to pathogen infection. Whether oxylipins from solanaceous plants like potato can also be effective against pathogens in other plants is being tested by transferring the respective genes from potato into *A. thaliana*.

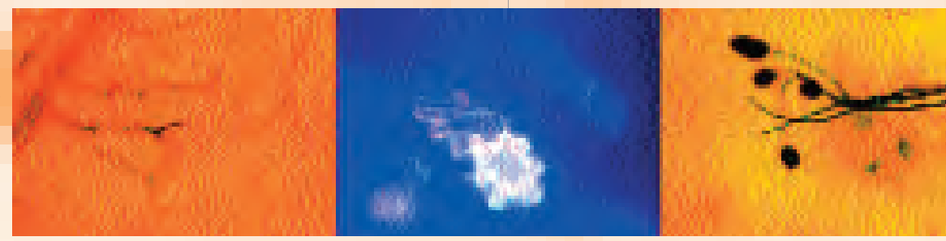
The 13-lipoxygenase products jasmonic acid and its precursor 12-oxo-phytodienoic acid accumulate in potato in response to infiltration of the phytopathogenic bacteria *Pseudomonas syringae* pv. *maculicola*. This nonhost pathogen interaction leads to local and systemic de-fense gene expression and to increased resistance against subsequent pathogen attacks. 12-oxo-phytodienoic acid, but not jasmonic acid accumulates also systemically. To analyze the role of these
13-lipoxygenase products for defense responses, transgenic plants were generated which express single chain antibodies against jasmonic and 12-oxo-phytodienoic acid.
Jasmonate-dependent expression of the proteinase-inhibitor-II-genes is reduced in the transgenic plants indicating that the levels of physiologically active jasmonic acid are reduced due to binding by the antibodies. The effect on defense gene expression and on the response to pathogen infection is presently being analyzed.

Since *P. infestans* is not able to successfully infect *A. thaliana*, the analysis of this nonhost pathogen interaction should elucidate mechanisms of defense against the infectious agent of late blight di-sease. Microscopic analyses revealed that *P. in-festans* spores germinate on Arabidopsis leaves and attempt to penetrate cells. However, successful penetration is only observed in rare cases. The plant cell reacts with the deposition of callose, accumulation of autofluorescent material and localized hypersensitive cell death. The Arabidopsis mutant *pen2* (collaboration with Volker Lipka and Paul Schulze-Lefert, MPI Cologne), identified as allowing enhanced penetration of *Blumeria gramnis* f. sp. *hordei*, reacts similarly to *P. in-festans* infection with higher penetration frequencies and increased cell death. Although the first layer of defense in nonhost resistance appears to be affected in the mutant, *pen2* is still able to contain the pathogen. To identify further components involved in nonhost resistance, *pen2* seeds were mutagenized and are presently being screened for alterations in their response to *P. infestans* infection. ■

Arabidopsis thaliana plants are grown under controlled conditions in a phytochamber. They are subsequently screened for alterations in their response to infection with *P. infestans*, the causal agent of late blight disease of potato. Thousands of plants must be screened to obtain one mutant.



Immunolocalization of single chain antibodies directed against 12-oxo-phytodienoic acid in chloroplasts of transgenic potato plants. In contrast to untransformed control plants (upper panel), transgenic plants express single chain antibodies in chloroplasts as indicated by the green fluorescence (middle and lower panel; B. Hause).



Growth of *P. infestans* in potato.
The oomycete *P. infestans*, the causal agent of late blight disease of potato, spreads intercellularly in susceptible potato leaves (left panel) and is able to sporulate (right panel). Infected cells react with cell death as indicated by the white autofluorescence (middle panel).

Research Group: Metal Homeostasis

Heads: Dieter Neumann & Stephan Clemens

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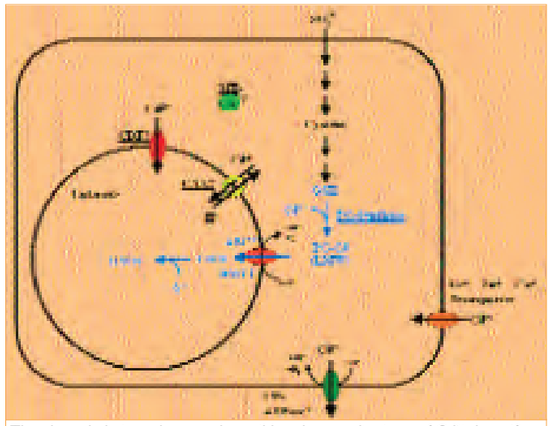
Plants - like all other organisms - are able to tightly regulate the intracellular concentration and the distribution of essential heavy metals such as zinc and copper. Also, the cytosolic concentrations of non-essential toxic heavy metals (e.g. cadmium, lead) have to be minimized. Some plant species (so-called metallophytes) can tolerate otherwise toxic concentrations and grow on heavy metal contaminated soil. Main objective of the group is to elucidate the mechanisms underlying plant metal homeostasis and metal hyperaccumulation. We are using analytical electron microscopy and a range of biochemical and molecular techniques. Plants under investigation are *Arabidopsis thaliana*, its close relative *Arabidopsis halleri*, and other metallophytes (*Silene vulgaris, Minuartia verna* and *Armeria maritima*). In addition, we are working with *Schizosaccharomyces* pombe as a cellular model for metal homeostasis.

The formation of phytochelatins (PCs) is a principle response of plants, many fungi and algae to toxic metal exposure. We showed that invertebrates such as *Caenorhabditis elegans* also express functional PC synthases (PCS) by expressing the respective protein in a PCS-deficient *S. pombe* strain. In a similar way, the existence of a second PCS in *Arabidopsis thaliana* was demonstrated. PCS genes are constitutively expressed. PC synthesis is directly activated by the binding of a heavy metal ion or the corresponding glutathione chelate to the enzyme. In order to further elucidate this activation process and to establish a technique for the characterization of metal-binding sites we used "peptide scans", i.e. spotted peptide libraries representing PCS proteins. Cd^{2+}-binding sites could be localized and functionally characterized by site-directed mutagenesis.

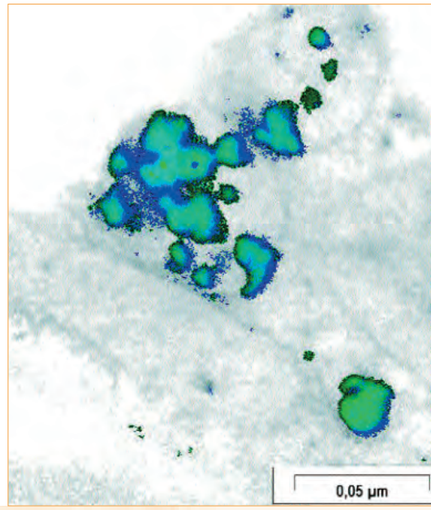


The phytochelatin pathway and possible other mechanisms of Cd^{2+} detoxification. PC synthesis from GSH is activated by several metal and metalloid ions. Cd^{2+} ions enter the cell via Fe^{2+}, Zn^{2+} or Ca^{2+} transporters. Upstream of GSH biosynthesis are sulphate assimilation and cysteine biosynthesis. In *S. pombe*, PC-Cd complexes (LMW) are transported into the vacuole by the ABC-type transporter Hmt1. In plant cells , this transport is hypothesized to be mediated by a protein of the same family. Inside the vacuole HMW complexes are formed by addition of sulphide, which apparently is derived from cysteine. Other mechanisms of Cd^{2+} detoxification discussed for plants and other organisms are vacuolar sequestration dependent on either CDF proteins or Cd^{2+}/H^+ antiporters, binding to metallothioneins or efflux mediated by CPx-type ATPases.

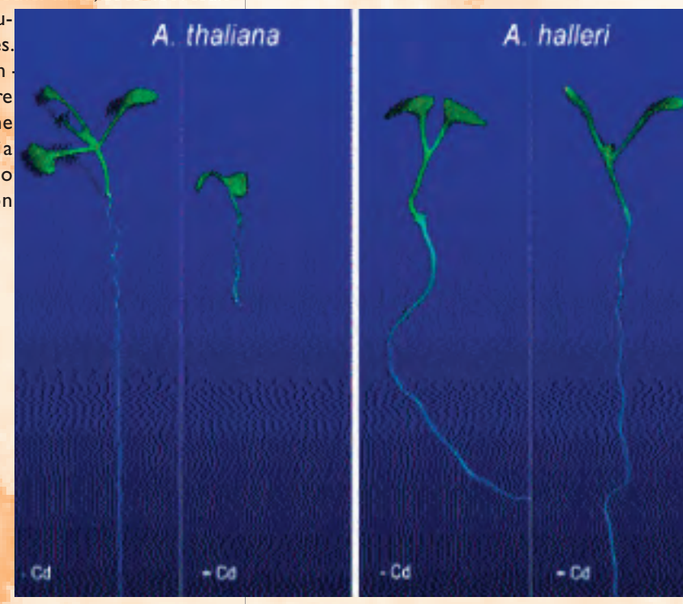
The molecular analysis of plant metal responses and determinants of metal hyperaccumulation is being pursued in the model systems *Arabidopsis thaliana* and *Arabidopsis halleri*. Expression profiling in *A. halleri* by cDNA-AFLP has been continued. The focus is on metal sensing and metal signal transduction since virtually nothing is known about these phenomena. A number of specifically metal-regulated putative signal transduction components have been identified both in *A. halleri* and *A. thaliana*. Five of them are studied in detail. *A. thaliana* knock-out lines have been obtained. Their metal responses and possible metal-related phenotypes are studied using a variety of techniques including microarrays. Since recently it was shown that Zn and Cd hyperaccumulation by *A. halleri* is a constitutive phenomenon found also on soil with normal metal content, the molecular analysis was extended to constitutive differences between the two Arabidopsis species. Gene expression profiles were obtained for roots of hydroponically grown plants by using Affymetrix GeneChips. They revealed a number of about 20 genes which are significantly more active in *A. halleri*. Among them are genes encoding several known metal homeostasis factors such as metal transporters and enzymes involved in metal chelator synthesis. These genes represent prime candidates for determinants of Zn/Cd hyperaccumulation and are therefore studied in detail.

Several putative metal tolerance factors are investigated in *S. pombe*, the model for PC-forming cells. The analysis of a transporter belonging to the Cation Diffusion Facilitators and of the only *S. pombe* metallothionein has led to new insights into mechanisms of Zn homeostasis, Cd toxicity, and intracellular metal distribution.

Plant metal tolerance is an element-specific process. For cell cultures of *Silene jenissiensis* it was shown that Zn and Cu are detoxified by distinct mechanisms. Zn tolerance is mediated by two different Si-dependent processes. Exposure to Zn results in elevated Si content of cells. Zn and Si containing precipitates are detectable in the cytosol and the mitochondria. They were identified as incompletely substituted Zn silicate. Such silicates are unstable and decompose to SiO_2, detectable in the cytosol as an electron transparent structure and identified by EEL spectra. Zn silicate is hypothesized to function as a temporary storage form of Zn, which prevents toxic effects within the cytosol. A second, unusual mechanism may contribute to the Zn tolerance of some plants. Apparently, a large fraction of Zn is directly taken up into the vacuole as Zn silicate without membrane passage. Transport occurs in vesicles formed by plasma membrane and tonoplast. Cu exposure, on the other hand, does not result in ultrastructural changes. Highest Cu concentrations are found in the mitochondria and there is no co-localization with Si. ■



Zn-silicate in mitochondria and cytoplasm of *Silene jenessiensis* (ESI).



Growth of two Arabidopsis species on normal (-Cd) and Cadmium-contaminated (+Cd) soil. *Arabidopsis thaliana* (left) growth is affected, whereas *Arabidopsis halleri* (right) is able to tolerate high Cadmium concentrations.

Publications, Books and Bookchapters, Publications in press, Patents, Doctoral Theses, Diploma Theses

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Department: Secondary Metabolism

Head: Prof. Dieter Strack

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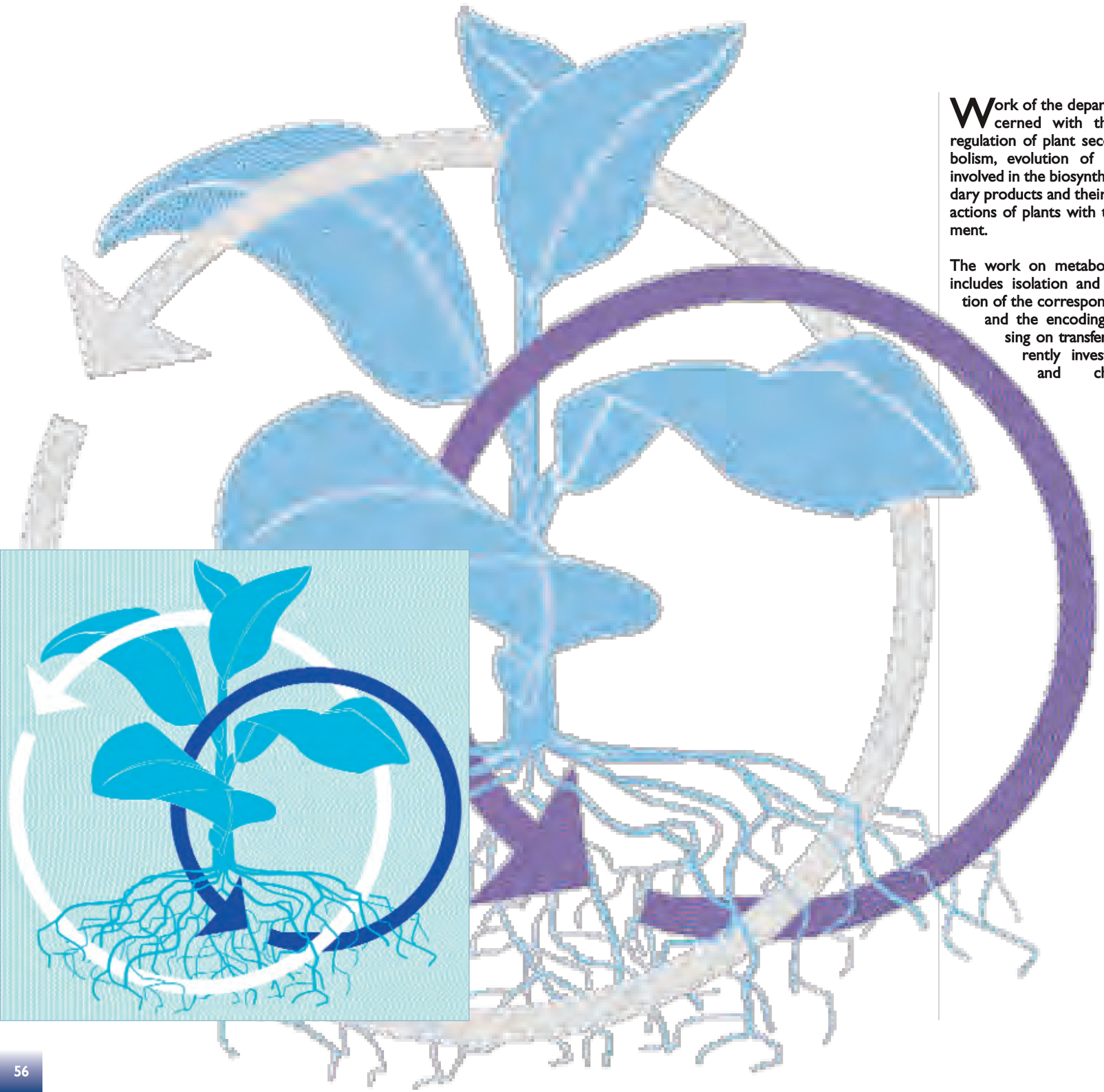

Work of the department is concerned with the molecular regulation of plant secondary metabolism, evolution of the enzymes involved in the biosynthesis of secondary products and their role in interactions of plants with their environment.

The work on metabolic regulation includes isolation and characterization of the corresponding 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 rape (*Brassica napus*). In addition flavonoid and betanidin glucosyltransferases from betacyanin-accumulating plants or flavonoid methyltransferases from the ice plant *(Mesembryanthemum crystallinum*) are investigated.

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. 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. The general existence of vacuolar β-acetal ester-dependent acyltransferases would prove a new concept of cell compartmentation 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-harbouring root cells. Another main objective is the analysis of the role of phytohormones, in particular jasmonates, in development and functional mainte-nance of mycorrhizal symbiosis. These studies are supported by comprehensive analysis of primary and secondary metabo-lites ("metabolite profiling") in wild-type and transgenic mycorrhizal plants. ■

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Head: Michael H. Walter

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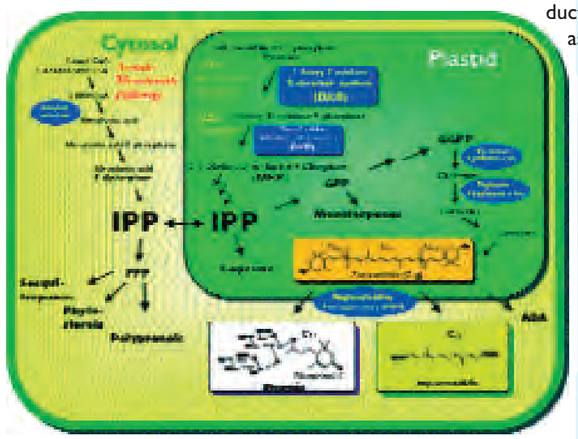
Most herbaceous plants form symbiotic associations with a small number of fungi in the rhizosphere in order to improve their water uptake and acquisition of mineral nutrients. These interactions are called arbuscular mycorrhizas (AM), a term derived from the haustoria-like fungal arbuscules developing in the root cortex. The work of the group focuses on alterations in plant isoprenoid metabolism induced by AM fungi, in particular on reactions located in plastids. Starting from metabolite analyses of various apocarotenoids, a number of fungus-stimulated gene activities from their biosynthetic pathway could be characterized. These include steps from the non-mevalonate methylerythritol phosphate (MEP) and from the carotenoid pathways. For the first reaction of the MEP pathway a diversification and specific expression of a *1-deoxy-D-xylulose 5-phos-phate synthase 2* (*DXS2*) gene in mycorrhizal roots was shown. A new concept of dedicated roles of DXS1 and DXS2 in the biosynthesis of primary and secondary isoprenoids has been introduced.

Metabolite analysis of roots of various plants including cereals, tobacco and legumes colonized by the mycorrhizal fungus *Glomus intraradices* has led to the identification of two classes of apocarotenoids: (i) glycosylated C_{13} cyclohexenone derivatives and (ii) an acyclic C_{14} polyene compound termed mycorradicin. Further biochemical work has now provided a facile and sensitive detection method for mycorradicin. The widespread but not universal occurrence of this compound in mycorrhizal roots of various plant families could be shown. The apocarotenoids are presumably integrated into a complex mixture of es - ters between mycorradicin and glycosylated C_{13} cyclohexenone derivatives. Ac - cumulation of this complex in mycorrhizal roots correlates with degradation of fungal arbuscules.

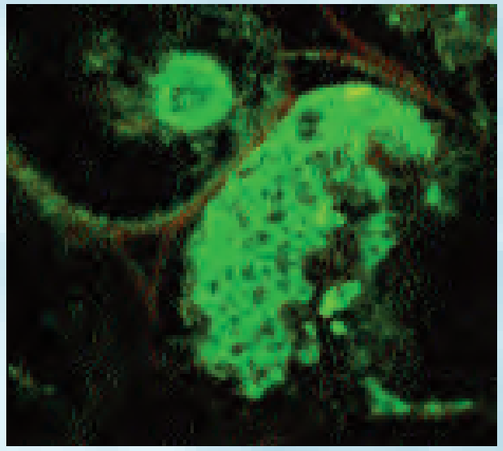
Early steps of apocarotenoid biosynthesis are catalyzed by the enzymes 1-deoxy-*D*-xylulose 5-phosphate synthase (DXS) and 1-deoxy-*D*-xylulose 5-phosphate reductoisomerase (DXR) as part of the recently discovered MEP pathway located to plastids. Strongly elevated transcript levels for both enzymes compared to controls were shown for mycorrhizal roots of several cereals. A more detailed analysis of *DXS* gene regulation and or - ganization was initiated for the model legume *Medicago truncatula* and its interaction with *Glomus mosseae* and *G. intraradices*. For the first time in plants the existence of two distinct, only distantly related classes of *DXS* genes could be deduced from the analysis of *M. truncatula* cDNAs. Only the expression of *DXS2* from *M. truncatula* is regulated by arbuscular mycorrhizal fungi. *DXS1* is expressed in most tissues at a constitutive level and appears to fulfill mainly housekeeping functions. Similar complementary expression profiles and mycorrhiza-regulation of *DXS2* transcript levels were found in maize, tomato and tobacco. Additional data suggest an involvement of *DXS2* in the biosynthesis of many other secondary isoprenoids such as leaf trichome monoterpenes of mint and solanaceous species, petal carotenoids, and terpenoid indole alkaloids. As a result, a new concept of dedicated DXS enzymes for primary and secon-dary isoprenoids can be introduced **(see *figure*)**. Genomic sequences harbouring *DXS2* genes have been isolated. These materials will provide useful tools for gene suppression studies and promoter analyses.



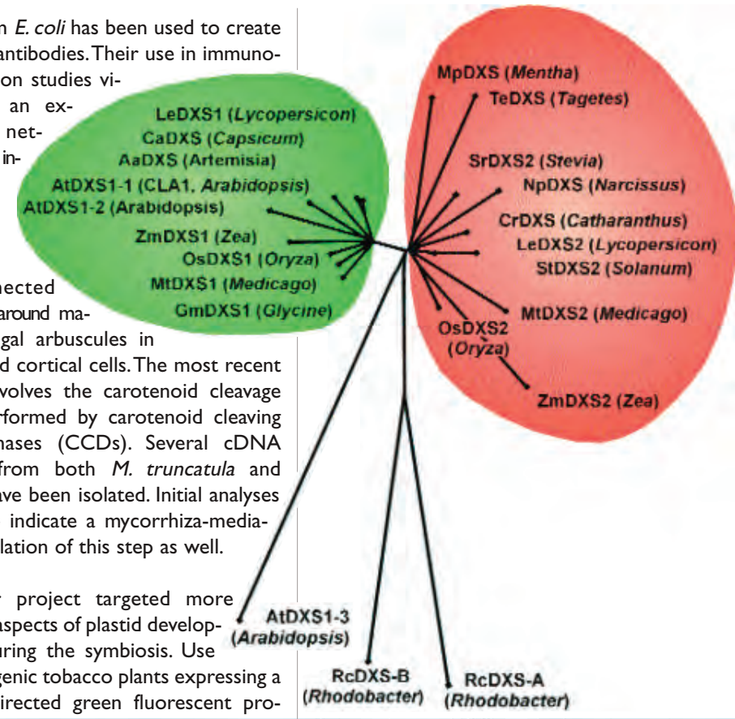
Isoprenoid biosynthesis and its compartmentation. Two separate pathways lead to the key intermediate isopentenyl diphosphate (IPP). The apocarotenoids blumenin (C^{13} cyclohexenone derivative) and mycorradicin accumulating in mycorhizal roots are highlighted.

A single class of cDNAs for *DXR* has been isolated from a maize mycorrhizal root library. DXR transcript levels are elevated in mycorrhizal maize roots but not to the high extent as has been found for *DXS2*. Recombinant maize DXR protein from *E. coli* has been used to create specific antibodies. Their use in immunolocalisation studies visualized an extensive network of interconnected plastids around mature fungal arbuscules in colonized cortical cells. The most recent work involves the carotenoid cleavage step performed by carotenoid cleaving dioxygenases (CCDs). Several cDNA clones from both *M. truncatula* and maize have been isolated. Initial analyses in maize indicate a mycorrhiza-mediated regulation of this step as well.

Another project targeted more general aspects of plastid development during the symbiosis. Use of transgenic tobacco plants expressing a plastid-directed green fluorescent protein has shown dramatic changes in mycorrhizal roots with a similar network of plastids around arbuscules as seen with the DXR antibody. These networks are highly dynamic structures appearing and disappearing concomi-tantly with formation and degradation of arbuscules (see report B. Hause). ■



Visualization of plastid reorganization in a mycorrhized root cell. A fluorescence-labeled antibody specific for DXR reacts with plastids covering a fungal arbuscule (right) or surrounding a nucleus (upper left).



Phylogenetic tree of DXS proteins from plants and the photosynthetic bacterium *Rhodobacter capsulatus*. Branches underlined in green indicate preferential expression in green tissues. Conversely, orange background indicates expression correlated with the biosynthesis of secondary isoprenoids such as apocarotenoids of mycorrhizal roots, petal carotenoids or monoterpenes of leaf trichomes. The data introduce a concept of dedicated roles of DXS1 and DXS2 in primary and secondary functions, respectively.

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Plant hormones are believed to play a role in the establishment and development of symbiotic interactions between plants and arbuscular fungi (arbuscular mycorrhiza, AM). Jasmonates, known as regulators in stress responses of plants against various biotic and abiotic stresses, might be important regulators of this symbiosis. Therefore, the main objective of our group is the analysis of the role of jasmonates during the interaction between *Glomus intraradices* and barley (*Hordeum vulgare*) or barrel medic (*Medicago truncatula*). In a second project, the activation of carotenoid biosynthesis in AM roots is studied. This activation is connected with a massive proliferation of the plastids of colonized root cortical cells. Cell biological phenomena as well as underlying molecular changes of the plastid proliferation will be elucidated.

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 (allene oxide synthase, AOS) and for jasmonate-induced proteins (JIP23). In order to record the kinetics of JA accumulation during development of mycorrhizal structures, a system of "near-synchronous" mycorrhization was established by the use of nurse-pot cultures. Since JA levels increase later than the initial steps of the plant-fungal interaction occur, the development of mycorrhiza rather than the recognition of the interacting partners may cause expression of JA-biosynthetic genes and finally elevate JA levels. In addition to the temporal pattern, the spatial pattern of gene expression ap - pearing during the development of the fungal organs within the root cortex (vesicles, arbuscules) was recorded. By use of *in situ*-techniques (*in situ*-hybridization, immunocytochemistry) accumulation of AOS and JIP23 mRNA and protein, respectively, could be shown to occur in cells harboring arbuscules (see figure). From all data ob-tained, the following hypothetical scenario is suggested: The plant root supplies the fungus with carbohydrates ⇒ the plant root becomes a stronger sink organ upon mycorrhization resulting in an enhanced accumulation of soluble sugars within the apoplast ⇒ expression of genes coding for enzymes of JA biosynthesis occurs ⇒ level of jasmonates increases ⇒ induction of genes involved in response to osmotic stress or in defense against biotic stresses takes place. As a consequence, mycorrhizal roots may be more resistant against secondary infection and/or osmotic stresses.

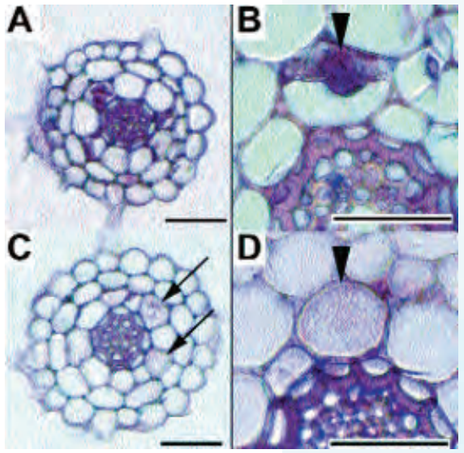
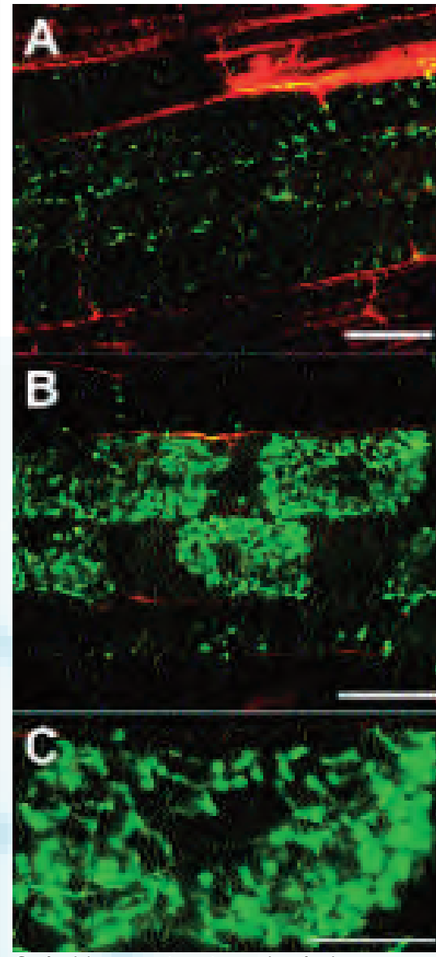


Figure: *In situ*-localization of AOS-transcripts within mycorrhizal barley roots. The detection performed with the *antisense* probe (A, B) exhibits a clear staining of the cytoplasm of root cortex cells containing fungal structures (arrow head in B), whereas the negative control (*sense*, C, D) does not show labeling within arbuscule-containing cells (arrow in C, arrow head in D). Bars represent 50 µm.

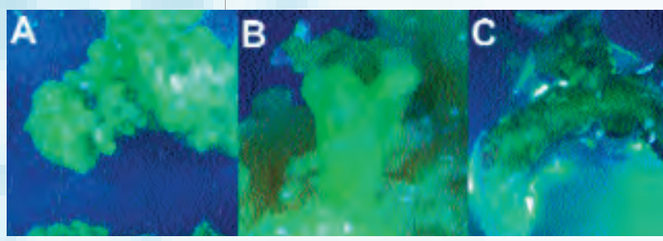
To test this hypothesis we intend to perform functional analyses by modulating jasmonate content in mycorrhizal roots of *M. truncatula*. A cDNA coding for the allene oxide cyclase (AOC), the enzyme performing the crucial step in JA biosynthesis, was isolated from *M. truncatula*. Vectors were constructed containing this cDNA in *sense* or *antisense* orientation or the RNA*i* construct, all of them under control of the CaMV35S promoter. After transformation, plants are expected with increased endogenous levels of JA (AOC*sense*) or decreased levels of JA (AOC*antisense*, AOC-RNA*i*). Assuming that altered JA-levels lead to altered mycorrhizal phenotypes, cell biological and biochemical approaches will be used to analyze these phenotypes. Additionally, gene expression studies will be performed using cDNA microarrays provided by the DFG Research Focus Program 1084 "Molecular Basis of Mycorrhizal Symbioses". In a second approach, transgenic tobacco plants were used, which express a yeast invertase targeted to the apoplast. These plants exhibit altered source-sink relationships and will be analyzed with respect to alterations in the mycorrhizal phenotype expected on biochemical, molecular and cytological level. From both approaches, we hope to get insights into the relationship of mycorrhiza, jasmonate action and the sugar status within the mycorrhizal roots.

Concerning the activation of carotenoid biosynthesis in AM roots, we have shown that this process is virtually ubiquitous in the plant kingdom. The extent of activation, however, is variable regarding the respective plant species. One major characteristic of the phenomenon is the finding that carotenoid intermediates of the pathway are present only in very small amounts, even in plants accumulating large amounts of apocarotenoids (mycorradicin and glycosylated cyclohexenone derivatives). Two hypothetical functional reasons for the activation of carotenoid biosynthesis in AM roots are currently investigated: (i) The possible induction of carotenoid biosynthesis by reactive oxygen species (ROS) produced during establishment of the AM symbiosis and a possible protection of the plant cell against such ROS by carotenoids; and (ii) a possible metabolic role of the chlororespiratory activity involved in carotenoid biosynthesis.

The accumulation of apocarotenoids in AM roots might be part of a complex reorganisation of plastid structure and metabolism in AM roots. Root cortical cell plastids are responsible for a number of biosynthetic processes, which are essential for the formation and functioning of the symbiotic interface. In accordance with this functional importance, massive proliferation of plastids in colonized tobacco root cortical cells leading to network-like structures covering the arbuscules have been observed. Currently, we are analyzing changes in the expression levels of plastid-related genes using DNA-arrays and Real-Time RT-PCR. These analyses will provide first information regarding the molecular and biochemical changes underlying the process



Confocal laser scanning micrographs of tobacco root cortex non-colonized (A) and colonized (B, C) by an AM fungus, respectively. The plastids are visualized by the green fluorescent protein targeted to plastids (transgenic tobacco plants courtesy of M. Hanson, Ithaca, New York, USA). In colonized cells the plastids formed a network-like structure around the arbuscules. Bars represent 50 µm in A, B, and 25 µm in C.



Somatic regeneration of *Medicago truncatula*. Plant explants give rise to embryogenic callus (A), which then develops small embryos (B). After transfer of embryos to "Embryo-Developing-Media" small plantlets are formed (C).

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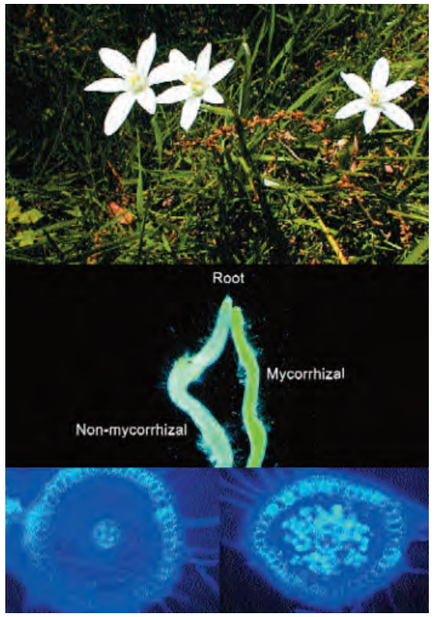
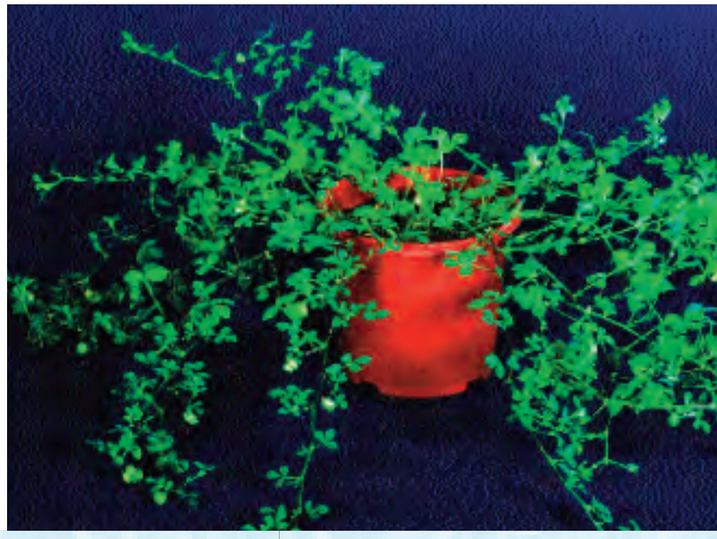
The research is focused on the comprehensive analysis of alte-rations of primary and secondary metabolite patterns during the establishment of the arbuscular mycorrhizal symbiosis in the model system *Medicago truncatula* / *Glomus intraradices* with the aim to characterize the causal relationships between gene expression and metabolite profiles during the symbiosis. Metabolic processes that are essential for the functioning of this root-fungus system have to be elucidated that may be of general importance also in other mycorrhizal systems. Furthermore, metabolite analysis of transgenic *M. truncatula* plants is intended to evaluate the effect of gene transfer or knockouts on the kinetics of mycorrhizal symbiosis and phenotypical changes in plant development (in cooperation with projects of the DFG Research Focus Program 1084 "Molecular Basics of Mycorrhizal Symbioses").

RP-HPLC-PDA, LC-ESI-MS and GC-TOF-MS are the methods used in our metabolite profiling approach. At the beginning databases of reference compound were created using HPLC (flavonoids, isoflavonoids, pterocarpans, coumestans and their glucosides), LC-MS (isoflavonoids, pterocarpans, coumestans) and GC-MS (amino acids, aliphatic acids, phenylpropanoids, in particular isoflavonoids, sugars, sterols) to facilitate subsequently the dereplication of the endogenous compounds. To reduce the chemical complexity of the metabolome, sequential extractions of lyophilized root material with dichloromethane, acetone and 80% aqueous methanol were performed. In these extracts my - corrhiza-specific alterations of more than 300 root metabolites were observed by HPLC. In LC-MS the accumulation of different isoflavonoid glucosides and their corresponding malonates as well as saponins was detected. By GC-MS of the dichloromethane extract of twelve weeks old mycorrhizal roots (*M. truncatula* / *G. intraradices* - 40 % mycorrhization) a dramatic increase of palmitelaidic and oleic acid was detected, whereas other long-chained fatty acids decreased in comparison to non-mycorrhizal controls. In all extracts the levels of some acids of the primary metabolism (lactic, malic, malonic, succinic, citric, γ-amino butyric and trihydroxybu-tyric acids) were higher in non-mycorrhizal than in mycorrhizal roots. Sugars and inositol deriva-tives are the predominating compounds in the acetone and 80 % aqueous methanol extracts, but the levels are different for the individual components. In cooperation with the working group of Thomas Fester (IPB) mycorrhiza-induced cyclohexenone deri - vatives were detected in roots of *Zea mays*, *Medicago truncatula* and *Ornitho - galum umbellatum* by HPLC. In the latter material (***see figure***), besides the "yellow pigment" and cyclohexenones, a group of hitherto unknown apocarotenoids with spectral properties similar to mycorradicin was observed suggesting that they might be precursors of the "yellow pigment". Using lecaton from a leek/*G. intraradices* preculture for *M. truncatula* inoculation a fast and efficient mycorrhization (ca. 90 % after 4 weeks) was observed which will be used in detailed studies of mycorrhization kinetics.

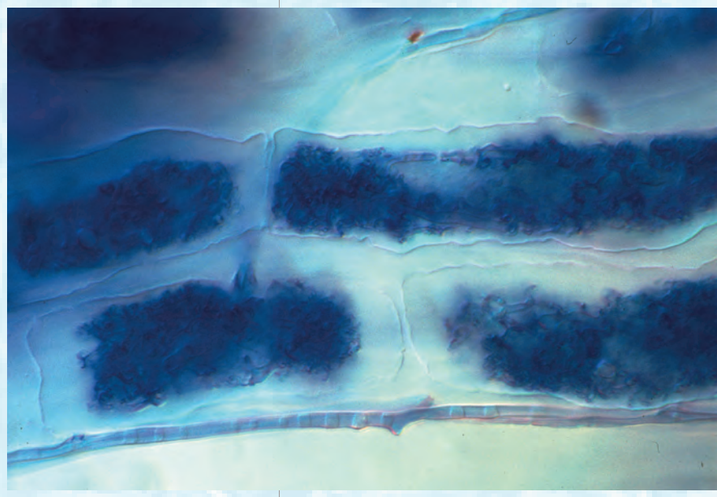


Star-of-Bethlehem (*Ornithogalum umbellatum* L., Hyacinthaceae)
Top: flowering plants; bottom: roots after mycorrhization (6 months) with *Glomus intraradices* in comparison to non-mycorrhized controls (micrographs by courtesy of T. Fester,

To achieve an adequate handling of the quantitative metabolite profiling data, an efficient computing system with statistic software was recently installed. In coordination with other groups of the institute dealing with similar bioinformatic problems, these tools will be used for validation, statistical evaluation and meaningful presentation of the results. In the future material from transgenic plants provided by the collaborating groups will be analyzed to determine the effect of the genetic alterations on the metabolite pattern and to correlate the metabolite profiles with the gene expression profiles in a functional genomic approach. ■



The legume barrel medic (*Medicago truncatula* Gaertn. cv. Jemalong A17), the model plant for functional genomic approaches to arbuscular mycorrhizal symbiosis.



Arbuscules of *Glomus intraradices* in the barrel medic root (trypan blue staining).

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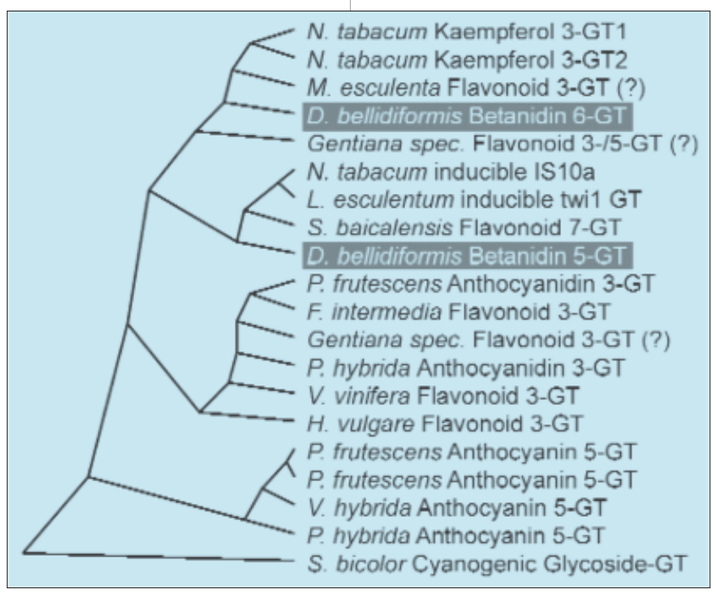
Stabilisation and solubilisation of plant natural products are performed by a wide range of glucosyltransferases (GTs) with often overlapping substrate specificities. These enzymes may also detoxify bioactive low-molecular weight compounds, in particular those from exogenous sources. Sequence identities cluster GTs according to regiospecificities rather than substrate specificities imply that this superfamily of proteins has evolved oligophyletically as one of the primary adaptive mechanisms of plants to meet the changing environmental conditions in a timely and developmentally controlled manner. Similar observations hold true for the superfamily of plant *O*-methyltransferases (OMTs), with a new subclass of enzymes involved in the modification of UV-induced flavonoid conjugates discovered recently.

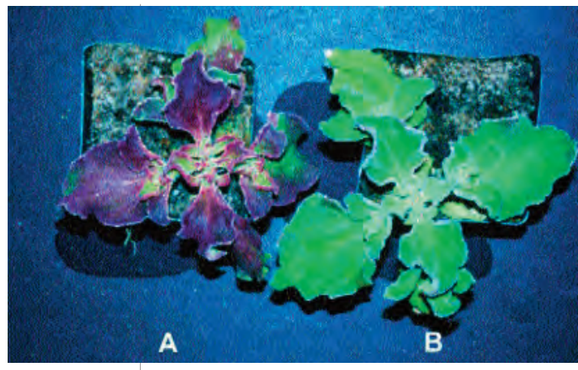
Based on earlier work, our group started on the molecular physiology of betalains, but lately as a result of the ongoing work, the major focus has shifted towards enzymes involved in the modification of the two classes of plant natural products under investigation, the betacyanins and the flavonoids. Primarily, our work has been directed towards a detailed understanding of the biochemistry and molecular evolution of plant natural product glycosyltransferases. By detailed sequence analysis and substrate specificity studies of two GTs, the betanidin 5-GT and 6-GT from *Dorotheanthus bellidiformis (Dbs)*, we were able to demonstrate an oligophyletic origin of the corresponding glucosyltransferase genes from different clusters of flavonoid GTs. From this study, it is concluded that regiospecificity and not substrate specificity appears to be the organizing principle in cluster formation. This may have important consequences for evaluating the structure/function relationship within the rapidly growing genomic databases for a variety of crop and non-crop species, like rice, corn or Arabidopsis, where a total of 110 GT-sequences with largely unknown substrate specificities has al-ready been described. Site-directed mutagenesis, performed with the heterologously expressed 5-GT protein from *Dbs*, among more than 20 conserved residues, indicate the presence of several catalytically essential amino acid residues. They are probably involved in the substrate binding of all GTs of the β-group, catalyzing enzyme reactions, which lead to an inversion of the sugar configuration). Although we were able to modify and reduce the specific activities of this en-zyme, changing one amino acid only, this was apparently not sufficient to alter position specificity or leading to a significant change in substrate specificity.



Cladogram illustrating the distribution of selected glucosyltransferases involved in betacyanin biosynthesis.

Besides our approach to correlate GT function with sequence information, our research also contributes to the investigation of betacyanin biosynthesis in the Caryophyllales. Several lines of evidence, including cloning of highly homologous 5- and 6-GT sequences from red beet (*Beta vulgaris*), suggest a conserved glycosylation of betacyanins at the betanidin level among all families within the Caryophyllales. GTs performing either 5- or 6-glycosylation are phylogenetically derived from two different classes of enzymes, involved in the position-specific glycosylation of flavonoids or other hydroxylated phenylpropanoids. The presence of a GT, glycosylating *cyclo*-dopa, which has been proposed as the glucose acceptor, cannot be ruled out, but is most unlikely.

Our second model system, the ice plant (*Mesembryanthemum crystallinum*), is faced with extreme arid conditions in its na-tural habitat. Besides its well-studied adaptation to drought stress, the plant is capable of tolerating exposure to ex-treme light intensities, combined with a high dose of UV radiation, by a rapid accumulation of glycosylated and methylated flavonol and betacyanin conjugates in leaf epidermal layers (***see figure***). At the molecular level a subtractive cDNA library of a light-in-duced *versus* non-induced leaves revealed the presence of several inducible cDNAs possibly involved in the adap-tive process of light tolerance. No transcripts encoding GTs were found. Among a variety of induced transcripts ranging from catalase to JIP-23 (Jasmonat Induced Protein) or a salt tolerant protein, several *O*-me - thyltransferases (OMTs) were selected as putative candidates to be involved in the methylation of the observed (UV-) light-induced flavonol conjugates. One of the corresponding OMT-proteins showed the required enzyme activities, and its presence was consistent with the occurrence of the conjugated flavonol-6,3 -di-*O*-methylether derivatives in light-induced bladder cells of the ice plant. This protein was purified from leaves of the ice plant. Based on amino acid sequence information, the cDNA was cloned from a cDNA library and expressed in a prokaryotic system. The recombinant enzyme displayed high position-specificity towards methylation of *ortho*-dihydroxyl groups, with the ability to methylate a variety of potential substrates, including flavo-noids, hydroxycinnamic acids and their corresponding CoA-esters. Protein sequence analysis indicates that this flavonoid-methylating activity most likely defines a new subgroup of small, Mg^{2+}-dependent OMTs, previously shown to be involved only in the methylation of the lignin precursor caffeoyl coenzyme A. ■



Red coloration of the ice plant due to epidermal accumulation of betacyanins and flavonol conjugates after five days of exposure to high light (1500 µM/m² x s) irradiation is observed only for plant **A** (UV-A/B radiation, cut-off filter 305 nm), but not for plant **B** (UV-A/B radiation, cut-off filter 360 nm).

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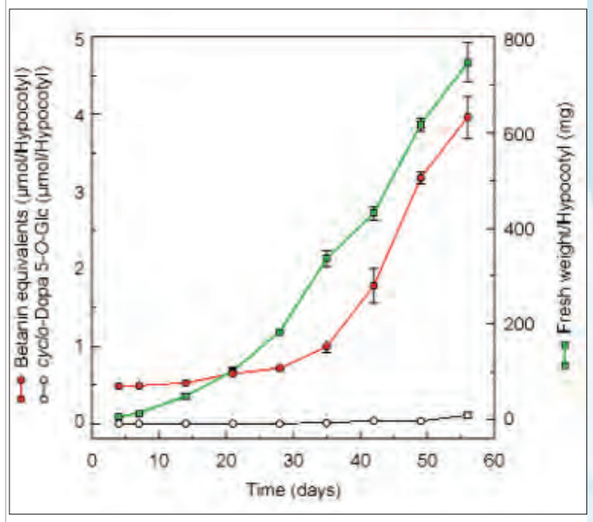
Betalains (red-violet betacyanins and yellow betaxanthins) are chromoalkaloids of chemotaxonomical importance. They functionally replace the anthocyanins in members of most families of the Caryophyllales. Betacyanins are also of commercial interest as food colorants. The main objective of our research is the unravelling of betalain biosynthesis. After the characterization of the bifunctional tyrosinase and spontaneously proceeding steps, experiments to detect the elusive dopa 4,5-dioxygenase are of particular interest as this enzyme forms the chromophore betalamic acid, the key intermediate in betalain biosynthesis.

A definite proof of the detection of dopa 4,5-dioxygenase activity in plants has not be achieved. In various enzyme assays with [^{14}C]dopa, no soluble betalamic acid could be detected. However, radioactivity was released from the assay proteins by alkaline hydrolysis; and by the addition of (*S*)-Phe, labeled (*S*)-Phe-betaxanthin could be identified. This proved the formation of betalamic acid, but in very low amounts. For a molecular approach to identify dopa dioxygenase using particle bombardment, a cell suspension culture of *Tinospora cordifolia* (Menispermaceae) was selected which showed a 4-fold increase in dopamine content after treatment with 30 µM methyl jasmonate. However, feeding of betalamic acid to the induced cells did not lead to the formation of yellow miraxanthin V, the essential prerequisite for the detection of a transient expression of a dopa dioxygenase cDNA.

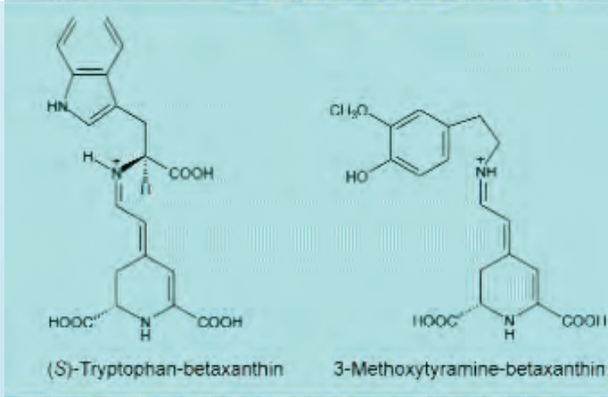
To answer the question whether betanin biosynthesis in red beets proceeds exclusively *via cyclo*-dopa or *via cyclo*-dopa 5-*O*-glucoside, the contents of betanin and *cyclo*-dopa 5-*O*-glucoside in red beet hypocotyls were monitored during eight weeks of plant development. Whereas the betanin content increased in parallel with the fresh weight, the *cyclo*-dopa 5-*O*-glucoside did not accumulate. The low amount of *cyclo*-dopa 5-*O*-glucoside found originates from betanin, which is in equilibrium with *cyclo*-dopa 5-*O*-glucoside and betalamic acid under slightly acidic conditions. This result is in contrast to previous data from Wyler et al. [Helv. Chim. Acta 67, 1348-1355 (1984)], but in accordance with recent studies (Thomas Vogt, IPB) that glucosyltransferases from red beets accept betanidin, but not *cyclo*-dopa as substrate.

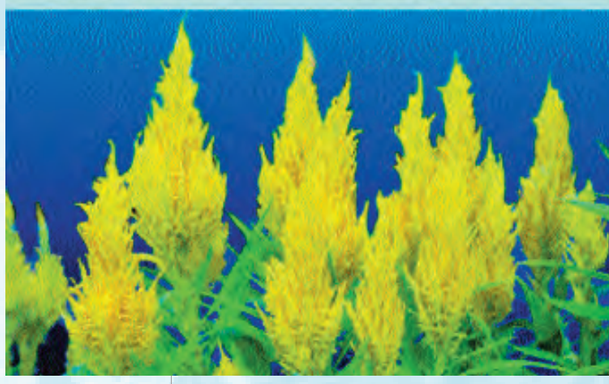


Time course of betanin and *cyclo*-dopa 5-*O*-glucoside accumulation during the development of red beets.

The structures of betalains occurring in inflorescences of two *Celosia* varieties (*Celosia argentea* var. *cristata* and *Celosia argentea* var. *plumosa*) were elucidated in cooperation with partners of the IPB and from China. Three yellow pigments were found to be immonium conjugates of betalamic acid with dopamine, 3-methoxytyramine and (*S*)-tryptophan.

In studies on vacuolar transport of betaxanthins, the inhibition pattern of the MgATP-stimulated vacuolar uptake of the beet-specific miraxanthin V and vulgaxanthin I (by 1 mM vanadate) and of the unnatural (R)-phenylalanine-betaxanthin (by 0.1 µM bafilomycin A1 and 5 mM NH_4Cl) suggests the participation of an ABC-like directly-energized transport mechanism and H^+/antiport system, respectively. Both systems have been described in literature for the vacuolar uptake of endogenous luteolin glucuronides in rye and flavonoid glucosides in barley, respectively. The research on betalains was terminated with the end of 2001. ■





Flowering *Celosia argentea* var. *cristata* (above), structures of two new endogenous betaxanthins and *Celosia argentea* var. *plumosa* (below).

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Higher plants accumulate a wealth of hydroxycinnamate (HCA) conjugates, mostly esters and amides. They are of prime ecological importance for plant survival. They protect plants against DNA-damaging UV light. Acylation of anthocyanin pigments with HCAs results in an (intramolecular) copigmentation effect, protecting these pigments against degradation. Soluble and cell wall-bound HCAs participate in plant defense against microbial attack. With regard to their biosynthesis, HCAs are usually activated as coen-zyme A (CoA) thioesters or 1-*O*-acylglucosides (β-acetal esters), being the substrates of the HCA transferases involved in formation of various conjugates. Our group is interested in structural and functional characterization of the UDP-glucose- and acylglucose-dependent glucosyl- and HCA transferases.

Cloning of the 1-sinapoylglucose:malate sinapoyltransferase (SMT) gene from *Arabidopsis thaliana* and immunolocalization of the SMT protein

SMT catalyzes the formation of sinapoylmalate, one of the major phenylpropanoid secondary metabolites accumulated by some members of the *Brassicaceae, e. g. Arabidopsis thaliana*, rape (*Brassica napus*) or red radish (*Raphanus sativus*). In cooperation with Clint Chapple, we identified previously an Arabidopsis mutant, *sng1* (***s****in*apoyl***g***lucose accumulator 1), which is defective in synthesis of sinapoylmalate. We have cloned the corresponding gene and have found that it encodes a serine carboxypeptidase-like (SCPL) protein. Expression of *SNG1* in *E. coli* demonstrated that it encodes the SMT. This finding suggests that SCPL proteins have acquired novel functions in plant metabolism and provides an insight into the evolution of secondary metabolic pathways in plants.

In an approach to immunolocalize the SMT protein, rabbit polyclonal antibodies were raised against the recombinant SMT expressed in *E. coli* from the corresponding Arabidopsis cDNA. Immunoblot analysis of proteins from different Arabidopsis tissues showed that the SMT is produced in all plant organs, except in the seeds and young seedlings. Immunofluorescent labeling of Arabidopsis leaf sections localized SMT to the central vacuoles of mesophyll and epidermal cells (***see figure***). In accor-dance with characteristics of SCPL proteins, we conclude that Arabidopsis SMT is synthesized as a precursor protein that is targeted to the endoplasmic reticulum. The protein is probably glycosylated in the Golgi apparatus from where it is subsequently routed to the vacuole.

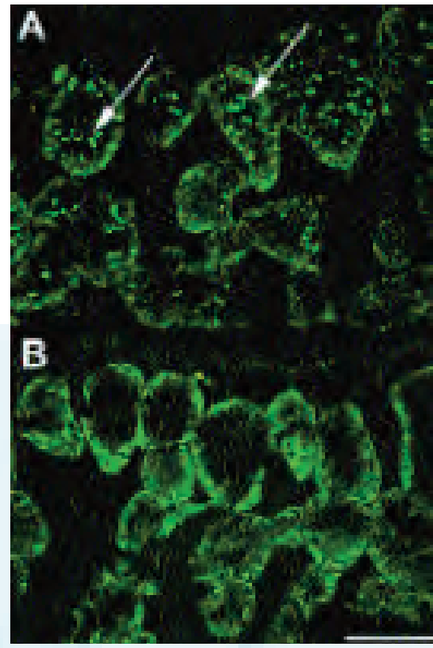
Cloning of the cDNAs encoding UDP-glucose:sinapate glucosyltransferase (SGT) and 1-sinapoylglu-cose:choline sinapoyltransferase (SCT) from *Arabidopsis thaliana* and *Brassica napus*

This work is part of the BMBF project "NAPUS 2000 - healthy food from transgenic rape" and focuses on reduction of the antinutritive sinapine (sinapoylcholine) content in rapeseed. We are following two strategies. A molecular approach (dsRNA*i*) aims to suppress the pivotal enzymatic steps of sinapine synthesis and a physiological one trying to divert one of the sinapine precursors, choline, into a new metabolic sink. The two enzymes in focus are the SGT and the SCT. A cDNA encoding SGT was isolated from cDNA libraries constructed from immature seeds and young seedlings of rape. The deduced SGT amino acid sequence indicated that SGT belongs to a distinct subgroup of glucosyltransferases that catalyze the formation of 1-*O*-acylglucosides. The SGT-cDNA from rape was cloned and functionally expressed in *E. coli*. The recombinant SGT carrying the His-tag at the C-terminus was purified. The enzyme showed a molecular mass of 60 kDa (gel filtration) and 62 kDa (electrophoresis), respectively. It exhibited a broad substrate specificity, accepting cinnamate, 4-coumarate, caffeate, ferulate and sinapate. DNA cassettes for the dsRNA*i*-mediated seed-specific suppression of SGT were constructed and cloned into a binary vector. Plant transformation was performed by collaborators of the University of Goettingen (Ch. Moellers).

As a result of sequence comparison analyses, four homologous genes encoding hydroxycinnamate glucosyltransferases were cloned from Arabidopsis. These genes were functionally expressed in *E. coli*. According to the acceptor specificity, we identified one of them (AtSGT1) with high affinity to sinapate, whereas the remaining three displayed broader acceptor specificity. Based on the cDNA sequence of AtSGT1, DNA cassettes for the dsRNA*i*-mediated suppression of the SGT, using a seed-specific (napine) and a constitutive promoter (CaMV 35S), were constructed. Both suppression constructs were used to transform Arabidopsis. Homozygous transgenic lines are developed that will be used for quantification of sinapine and 1-sinapoylglucose in seeds.

As the SMT, the SCT belongs to the group of SCPL enzymes. By a "homology based cloning strategy", a full-length cDNA could be isolated from rape seeds sharing about 85 % identity with the SCT-cDNA from Arabidopsis. After expression in *E. coli*, the recombinant protein was shown to be in the insoluble fraction. As this is one of the main problems with the class of SCPL proteins, the optimization of heterologous expression has come into the focus of our present work.

In the physiological approach, bacterial genes (*betA* and *betB*) encoding choline oxidase have been introduced in Arabidopsis and rape. It is assumed that the glycine betaine pathway will compete for choline as substrate in sina-pine synthesis. Choline feeding experiments using immature Arabidopsis and rape embryos revealed that the level of free choline is limited. Thus, to provide choline in a non-limiting concentration for glycine betaine synthesis, we will suppress SGT activity in transgenic plants expressing *betA* and *betB*. This strategy will hopefully not only improve rapeseed used as healthy food but will enhance by the accumulated oxidation product of choline, glycinebetain, the tolerance of rape to environmental stresses, such as salt, low temperature or drought that often affect seed germination and plant productivity. ■



Intracellular localization of SMT within Arabidopsis rosette leaves. Cross sections were immunodecorated with polyclonal monospecific antibodies raised against the recombinant SMT protein followed by fluorescence labelled secondary antibody. A green fluorescent label within the vacuoles of mesophyll cells of wild-type leaves (**A**) is indicative of the SMT protein (arrows). In contrast, in the vacuoles of mesophyll cells of the deletion mutant *sng1* (**B**), defective in synthesis of sinapoylmalate, the fluorescent signals are absent (bar = 50 µm).

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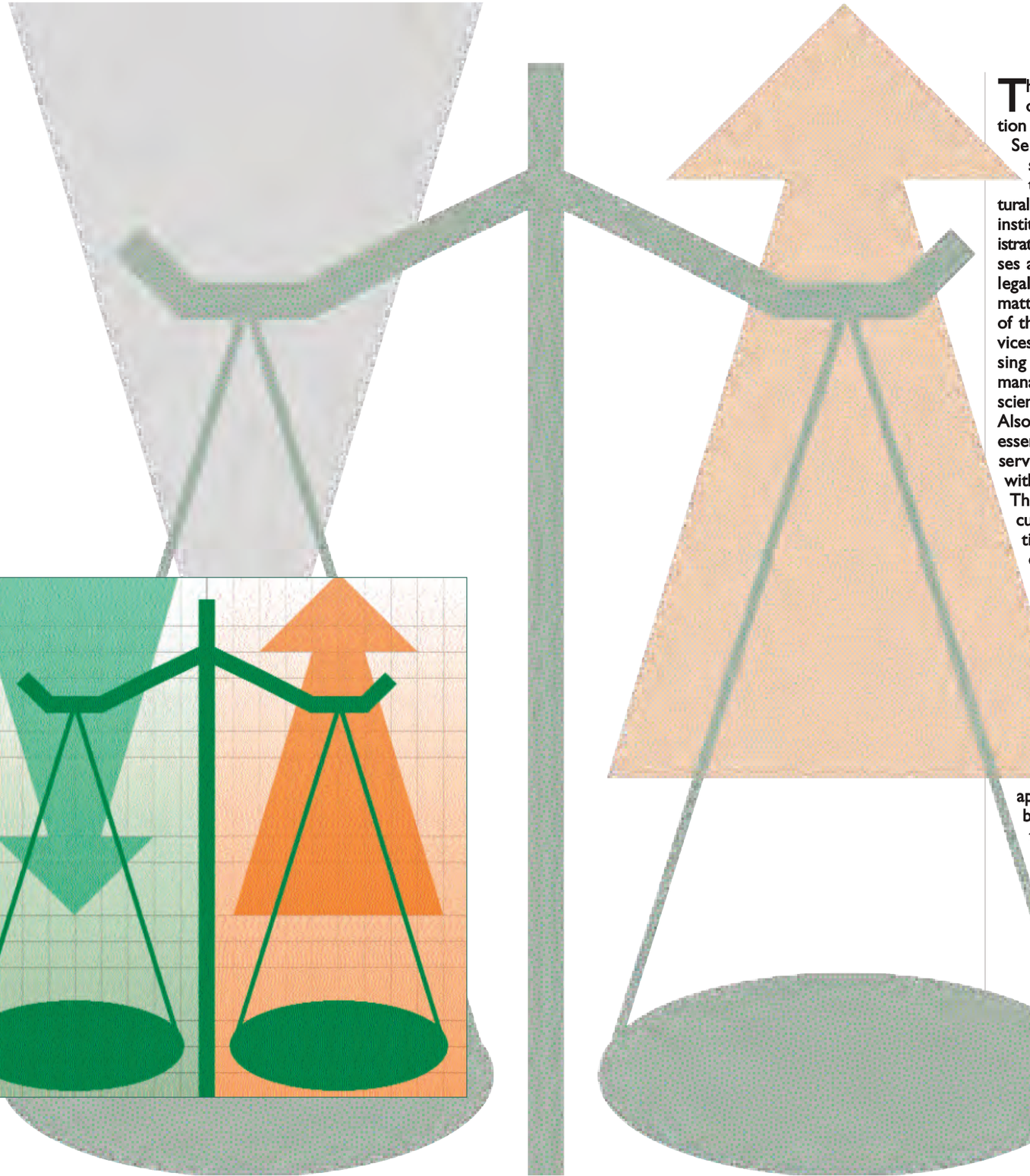
Department: Administration and Technical Services

Head: Lothar Franzen

Secretary: Heide Pietsch



The department of Administration and Technical Services represents the central infrastructural unit within the institute. Administrative main focuses are personnel, legal, and financial matters. Main tasks of the central services are purchasing and account management, and maintenance of the scientific library and chemical store. Also the gardeners represent an essential component of the central services. The Technical Services deal with the buildings and properties. The technical co-workers in particular take care of new constructions, the maintenance of the existing buildings and laboratories, and the technical and scientific equipment of the laboratories.

The scientific library of the institute offers excellent possibilities for literature-based research. The library has subscriptions to 83 of international research journals and stocks approximately 5.000 hardback books. A reading hall with 18 internet-connected computers and five individual rooms are also avialable.

In addition to an experimental field area, a series of fully air-conditioned green houses and phytochambers are available for the research programs. In these areas the gardeners take care of the experimental plant material.

Since the reestablishment of the institute in the year 1992, most main buildings were restored completely. The main focus of the construction works concentrated on the laboratory and technology areas, all of which are now well equipped.

Beside the work the redevelopment of the existing buildings, new constructions were undertaken in the last years. Currently, a new building is under construction that will accomodate new highly sensitive analytical instruments. In the near future, additional greenhouse facilities and a central service building will be constructed.

Altogether, the institute offers a best possible infrastructure for its diverse research projects. ■

Working Groups

Finance
Head: Barbara Wolf
Astrid Ortloff *(until August 2002)*
Gudrun Schildberg
Burgunde Seidl *(since October 2002)*
Kerstin Wittenberg *(since May 2002)*

Personnel
Head: Kerstin Balkenhohl
Alexandra Burwig
Cindy Maksimo *(since April 2002)*
Rita Stelzer
Kathleen Weckerle

General Administration
Head: Rosemarie Straßner
Alexandra Burwig
Cindy Maksimo *(since April 2002)*
Rita Stelzer
Kathleen Weckerle

Trainees
Antje Olschewski
Clemens Schinke

Library
Head: Andrea Piskol
Jessica Ackermann *(Trainee)*
Antje Werner *(Trainee)*

Graphics & Photography
Head: Christine Kaufmann
Annett Kohlberg

Construction and Maintenance
Head: Matthias Böttcher *(until December 2002)*
Detlef Dieckmeyer
Carsten Koth *(since January 2002)*
Michael Kräge
Jörg Lemnitzer
Klaus-Peter Schneider
Catrin Timpel
Eberhard Warkus

Electronics
Holger Bartz
Hans-Günter König
Ronald Scheller

Gardening
Head: Iris Rudisch
Martina Allstädt
Nicole Mühlwald *(Trainee)*
Christian Müller *(since April 2002)*
Kristina Rejall *(since June 2002)*
Steffen Rudisch
Katja Scheming *(Trainee since August 2002)*
Andrea Voigt *(Trainee since August 2002)*

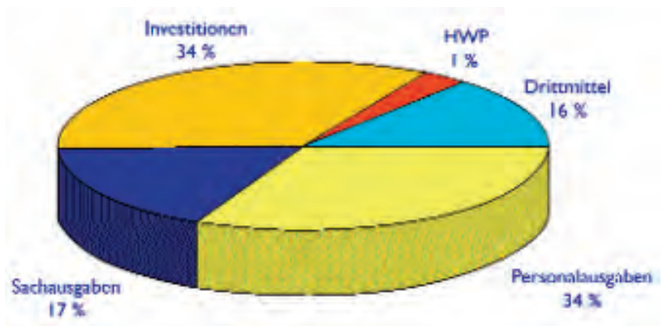
Resources and Investments

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

AFNG	*Arabidopsis Functional Genomics Network (DFG)*
BMBF	Bundesministerium für Bildung und Forschung - *Federal Ministry of Education and Research*
BML	Bundesministerium für Verbraucherschutz, Ernährung und Landwirtschaft - *Federal Ministry of Consumer Protection, Food and Agricultur*
BPS	BASF Plante Science GmbH
D-B Foundation	Gottlieb Daimler and Karl Benz Foundation
DBU	Deutsche Bundesstiftung Umwelt
DFG	Deutsche Forschungsgemeinschaft
DAAD	Deutscher Akademischer Austauschdienst - *German Academic Exchange Service*
Elsevier	Elsevier Science Publisher
EU	European Union
Firmenich	
GABI	Genom Analyse im Biologischen System Pflanze
GTZ	Gesellschaft für Technische Zusammenarbeit
Hopsteiner	
HSP III	Hochschulsonderprogramm III
Humboldt Foundation	Alexander von Humboldt Foundation
HWP	Hochschulwissenschaftsprogramm
Icon genetics	
KWS	KWS SAAT AG
MK-LSA	Kultusministerium des Landes Sachsen-Anhalt - *Ministry of Education and Cultural Affairs of the State of Saxony Anhalt*
PPP	Projektbezogener Personenaustausch (DAAD)
Probiodrug	Probiodrug AG
SFB 363	Sonderforschungsbereich 363 - *Collaborative Research Centres*
VW Foundation	Volkswagen Foundation

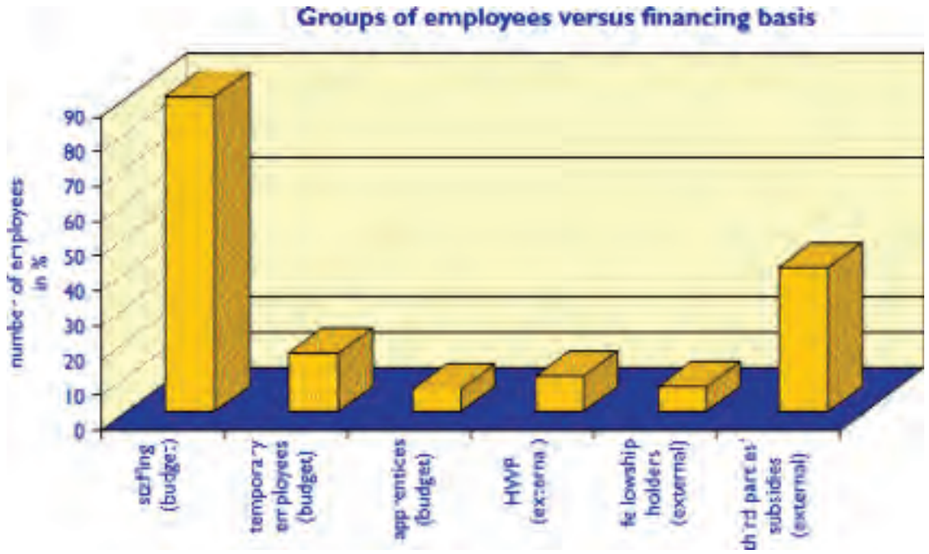
	in Mio. Euro	in %
Basic Financing Funds		
Personnel	13,0	32,4
Consumables	6,2	15,5
Grants / Subsidies	0,3	0,7
Investments	13,8	34,3
"University Science Funds Programme" (HWP)	1,2	3,0
Subtotal	**34,5**	
Funds from external sources		
BMBF	1,3	3,2
MK-LSA	0,8	2,0
DFG	2,4	6,0
Industry	0,6	1,5
EU	0,5	1,2
other	0,1	0,2
Subtotal	5,7	14,1
Total	**40,2**	**100**

Investments	
Equipment	5,8
Building	8,0
Total	**13,8**



Staffing Schedule

Staffing schedule	2000	2001	2002	Total	Average
Number of members on annual average	166	167	169	502	167
Full-time employees in %	77	77	75	229	76
Part-time employees in %	23	23	25	71	24
Number of established posts	89	89	92	270	90
Temporary employees (budget)	19	11	20	50	17
Employess remunerated by third parties subsidies (avarage)	42	44	38	124	41
Employees remunerated by "Universitiy Special Funds Programme III" (Hochschulsonderprogramm III / HSP III)	1	-	-	1	1
Employees remunerated by "University Science Funds Programme" (Hochschulwissenschaftsprogramm / HWP)	-	11	9	20	10
Proportion of female employess in %	60	59	61	180	60
Personnel fluctuation rate in %	16,4	10,4	13	39,8	13
Avarage age of employees	40	39	39	118	39
Scholarship/fellowship holders	10	6	5	21	7
Vocational training					
commercial area	2	2	2	6	2
horticultural area	3	2	3	8	3
library	1	2	2	5	2
Successfully completed vocational training	4	-	-	4	1
Avarage number of apprentices	6	6	7	19	6



Use of Funds from External Sources

Project & Head of Project	Total duration	Financed by	Amount 2000 - 2002 (in Euro)	Personnel posts financed
Department of Natural Product Biotechnology				
Jasmonate biosynthesis regulation (Prof. C. Wasternack & O. Miersch)	99/04	DFG / SPP	111.000	1
Glutamate cyclase (Prof. C. Wasternack)	01/03	Probiodrug	30.700	0
Allenoxidcyclase (Prof. C. Wasternack)	01/02	Firmenich	60.900	1
Papaver somniferum (Prof. T. Kutchan)	00/01	DFG / SFB 363	57.300	1
Papaver somniferum (Prof. T. Kutchan)	01/02	DFG	117.900	1
Functional genomics (G. Herrmann)	00/02	DFG	26.300	0
Analysis of genes (Prof. T. Kutchan)	00/02	Icon Genetics	230.900	1
Molecular genetics of isoquinoline alk.biosynth. (Prof. T. Kutchan)	01/04	DFG	67.600	2
Cellular signalling (Prof. T. Kutchan)	02/04	DFG / MLU	24.100	1
Transformation and regeneration of *Papaver somniferum* (S. Frick)	02/03	DFG	57.300	2
Modulation of jasmonates by transgenic plants (Prof. C. Wasternack & O. Miersch)	02/04	DFG / SFB 363	175.500	1
Salvia fragrances (Prof. T. Kutchan)	02/03	DBU	13.850	1
Subtotal:			**973.350**	**12**
Department of Bioorganic Chemistry				
Conformation of brassinosteroids (A. Porzel, W. Brandt [MLU])	99/00	DFG	6400	1
New bioactive natural products from endemically occuring Yemenian plants (J. Schmidt, G. Adam)	97/02	DFG / GTZ	49.700	0
HEA(N)TOS (Prof. L. Wessjohann, Prof. G. Adam)	00/03	BMBF	264.700	2
Structural elucidation and combinatorial chemistry (Prof. L. Wessjohann)	2000	MK-LSA / HWP	490.800	0
COMBIOCAT (Prof. L. Wessjohann)	01/04	EU	142.100	2
EPILA (W. Brandt)	01/03	EU	29.800	2
Fungi excursion (N. Arnold)	2001	DFG	1.100	0
MCR ligand synthesis (Prof. L. Wessjohann)	02/03	DAAD / Probral	8.800	0
Chrom-(II)-mediated reactions (Prof. L. Wessjohann)	02/03	DAAD / PPP Hungary	5.700	0
Daimler Benz fellowship (Prof. L. Wessjohann)	2002	D-B Foundation	1.700	1
Subtotal:			**1.000.800**	**8**
Department of Stress and Developmental Biology				
Heavy-metal tolerance (D. Neumann & S. Clemens)	02/04	DFG / SFB 363	96.400	1
Pathogen defense-related genes (Prof. D. Scheel)	98/00	DFG	30.000	1
Signal transduction (Prof. D. Scheel)	02/04	DFG / SFB 363	201.100	1
Oxidative burst (Prof. D. Scheel)	99/00	DFG / Innovationskolleg	25.500	1
Plant peptides (Prof. D. Scheel)	2000	DFG	460.200	1
Signal transduction (Prof. D. Scheel)	99/00	DFG	2.900	1
Chromatin and gene regulation (Prof. D. Scheel)	99/01	DFG / SFB 363	68.600	1
Elicitor receptors (T. Nürnberger)	99/01	DFG	54.100	2
CRISP (Prof. D. Scheel)	01/04	EU	154.400	1
Heavy metal tolerance and silicon (U. zur Nieden)	00/04	MK-LSA	67.700	1
Non-host resistance (T. Nürnberger)	98/02	KWS	120.300	1
The role of jasmonates in pathogene defense (Prof. D. Scheel)	01/03	DFG	74.700	1
Jasmonate-insensitive mutant (Prof. D. Scheel, S. Berger)	99/03	MK-LSA	64.700	1
Gene silencing (Prof. D. Scheel)	00/01	MK-LSA	91.400	1
Arabidopsis halleri (S. Clemens)	00/03	DFG	60.300	1
Ozone signaling (Prof. D. Scheel)	2001	DAAD / PPP Finnland	3000	0
Metallophytes (S. Clemens)	01/03	EU	104.100	1
Biomineralisation (D. Neumann)	01/03	DFG	49.100	1
Heat stress proteins (D. Neumann)	99/00	DFG	8.300	1

Use of Funds from External Sources

Project & Head of Project	Total duration	Financed by	Amount 2000 - 2002 (in Euro)	Personnel posts financed
Signals, delivery and response (T. Nürnberger)	97/00	EU	68.600	2
Humboldt fellowship (Prof. D. Scheel)	01/02	Humboldt Foundation	5.600	0
Cooperation wtih South Africa (T. Nürnberger)	01/04	VW Foundation	40.000	0
NODO (S. Rosahl)	02/04	EU	50.700	1
Receptor kinases (T. Nürnberger)	02/04	DFG / AFNG	47.600	2
Arabidopsis thaliana interactions (Prof. D. Scheel)	01/02	BPS	10.900	0
Bioinformatics and Mass Spectronomy (Prof. D. Scheel)	02/07	BMBF	100.000	6
GABI-NONHOST (Prof. D. Scheel)	02/06	BMBF	109.700	4
Pathogene defense in *Arabidopsis thaliana* (S. Rosahl)	2002	MK-LSA	10.800	0
Subtotal:			**2.180.700**	**34**
Department of Secondary Metabolism				
Betalains (W. Schliemann & Prof. D. Strack)	99/01	DFG	61.800	1
Betanidin-Glucosyltransferases (T. Vogt)	01/03	DFG	121.900	2
TIMBER (M. Walter)	98/00	EU	5.600	1
Endomycorrhiza (W. Maier & Prof. D. Strack)	98/01	DFG	24.000	1
Metabolism of isoprenoids (M. Walter & T. Fester)	00/04	DFG	90.500	1
NAPUS 2000 (Prof. D. Strack)	99/04	BMBF	378.100	2
Jasmonates in the development of barley (B. Hause & Prof C. Wasternack)	99/01	DFG	40.600	1
The role of jasmonates during the establishment of mycorrhiza (B. Hause & Prof. D. Strack)	00/04	DFG	83.500	1
Carotenoid biosynthesis in arbuscular mycorrhizal roots (T. Fester)	00/04	DFG	82.000	1
Metabolite profiling (W. Schliemann)	02/04	DFG	24.400	1
Phytochemistry (Prof. D. Strack)	02/04	Elsevier	8.000	1
Stable transformation of *Medicago truncatula* (B. Hause)	2002	MK-LSA	47.300	0
Subtotal:			**967.700**	**13**
Joint projects				
Profiling of metabolites, proteins and peptides Dep. Stress and Developmental Biology and Dep. Bioorganic Chemistry (S. Clemens)	00/04	BMBF / GABI	588.200	4
HUMULUS Dep. Bioorganic Chemistry and Dep. Natural Product Biotechnology (F. Stevens, Prof. L. Wessjohann & J. Page)	01/02	Hopsteiner	9.900	0
"Analytica 2000" (E. Peerenboom)	2000	MK-LSA	11.200	0
"Achema 2000" (E. Peerenboom)	2000	MK-LSA	1.300	0
Public Understanding of Sciences and Humanities (PUSH) - Multimedia project about the mycorrhiza (T. Fester & E. Peerenboom)	2001	Donors Association for the Promotion of Sciences and Humanities in Germany	6.200	0
Subtotal:			**616.800**	**4**
Projects granted, total:			**5.739.350**	**71**

General view

BMBF	**1.331.000**
MK-LSA	**785.200**
DFG	**2.401.700**
Industry	**573.300**
EU	**555.300**
Other sources	**92.850**

Guest Researchers and Fellows

Name	Country	Period
Department of Natural Product Biotechnology		
Prof. Guillermina Abdala	Argentina	08.06.2001 - 16.07.2001
Dr. Maged Abou-Hashem	Egypt	01.07.2002 - 22.10.2002
Arysyak Abrahamian (DAAD Fellow)	Armenia	04.07.2000 - 31.12.2000
Nigel Bailey	UK	19.11.2001 - 14.12.2001
Dr. Davide Berlanda	Italy	05.06.2001 - 21.06.2001
Hubert Chassaigne (Humboldt Fellow)	France	15.01.2000 - 31.12.2000
Dr. Kum-Boo Choi (Humboldt Fellow)	Korea	since 07.10.2002
Predro Salvador de Rocha (FEBS Fellow)	UK	10.04.2000 - 20.04.2000
Satinder Gitta	Canada	05.04.2000 - 04.06.2000
Kristin Krukenberg (Fulbright Fellow)	USA	23.09.2002 - 15.07.2003
Tamara Krupnova (DAAD Fellow)	Kazakhstan	01.10.1999 - 30.04.2000
Anan Onaroon (DAAD Fellow)	Thailand	26.07.1999 - 30.09.2002
Matjaz Oven	Slovenia	03.08.1999 - 31.07.2001
Suppachai Samapitto (DAAD Fellow)	Thailand	04.05.2000 - 03.05.2001
Anastasia Tkatcheva (SFB Fellow)	Canada	01.10.2001 - 28.02.2002
Prof. Gülacti Topku (DAAD Fellow)	Turkey	02.04.2000 - 02.07.2000
Prof. Luc Varin	Canada	01.10.2002 - 31.01.2003
Dr. Ana Vigliocco	Argentina	01.04.2002 - 31.05.2002
Dr. Bathany Zolman (SFB Fellow)	USA	15.08.2002 - 31.10.2002
Department of Bioorganic Chemistry		
Prof. Antonio Luiz Braga (CAPES Fellow)	Brazil	06.04.2002 - 21.04.2002
Tran Van Chien	Vietnam	since 07.10.2002
Marco Aurelio Dessoy (DAAD Fellow)	Brazil	01.02.2001 - 30.06.2002
Csongor Hajdu (Erasmus + DAAD Fellow)	Hungary	29.01.2001 - 31.07.2001 19.08.2002 - 13.12.2002
Dubravko Jelic	Croatia	10.03.2002 - 22.03.2002
Myint Myint Khine (Daimler-Benz Fellow)	Myanmar (Burma)	since 04.09.2002
Lazlo Merczs (DAAD Fellow)	Hungary	10.11.2002 - 11.12.2002
Prof. Károly Micskei (DAAD Fellow)	Hungary	17.06.2002 - 26.06.2002
Nguyen Hoang Anh	Vietnam	01.09.2000 - 31.08.2001
Nguyen Hong Thi Van	Vietnam	since 17.04.2002

Name	Country	Period
Prof. Tamás Patony (DAAD Fellow)	Hungary	13.10.2002 - 22.10.2002
Prof. Luay Rashan (Humboldt Fellow)	Jordan / Iraq	01.07.2002 - 31.08.2002
Dr. Oscar Dorneles Rodriguez (CAPES Fellow)	Brazil	01.04.2002 - 20.09.2002
Lars Seipold	Germany	01.01.2002 - 31.05.2002
Prof. Tran Van Sung	Vietnam	01.07.2002 - 18.12.2002
Trin Thi Thuy	Vietnam	20.11.2001 - 19.11.2002
Larissa Vasilets	Russia	since 28.11.2002
Dr. Svetlana Zakharova	Russia	30.10.2002 - 31.12.2002
Department of Stress and Developmental Biology		
Reetta Ahlfors (DAAD Fellow)	Finland	since 08.07.2002
Dr. Susanne Berger (DFG Fellow)	Germany	01.04.2001 - 31.03.2002
Anne-Claire Cazalé (Humboldt Fellow)	France	01.02.2000 - 31.12.2001
Clarice de Figueiredo	Brazil	01.11.1999 - 30.09.2001
Anna Drobek	Poland	01.09.2001 - 30.09.2001 26.02.2002 - 30.04.2002
Dr. Emiko Harada (Humboldt Fellow)	Japan	since 22.02.2002
Emma Jack	Netherlands	12.03.2001 - 20.04.2001
Dr. Anano Dinakar Karve (Humboldt Fellow)	India	16.11.1999 - 14.02.2000
Dr. Magdalena Krzymowska (Humboldt Fellow)	Poland	01.08.1999 - 30.06.2002
Ma. Shaokang	Singapore	06.05.2001 - 21.06.2001
Srpryia Paranthaman (Humboldt Fellow)	India	25.10.2002 - 20.12.2002
Lizelle Piater	South Africa	01.06.2002 - 29.07.2002
Joe Chou Hung Sim	Singapore	06.05.2001 - 21.06.2001
Claudia Simm (Fellow, Graduierten Kolleg)	Germany	since 01.10.2000
Anne Varet	France	01.01.2002 - 30.04.2002
Department of Secondary Metabolism		
Stijn Jan Freddy Desmyter	Netherlands	10.01.2000 - 05.02.2000
Dr. Shiming Liu	China	13.08.2001 - 13.06.2002
Dr. Nirmal Sahay	India	10.01.2000 - 31.12.1999
Dr. Sudha Sahay	India	24.09.1999 - 31.08.2001
Diana Schmidt (Fellow, Bio Service GmbH, EU and the State of Saxony Anhalt)	Germany	since 01.08.2001

Press and Public Relations

Head: Sylvia Pieplow

Participation in trade shows

In 2000, the institute exhibited several projects at exhibitions and trade shows. These activities were planned and organized by Ellen Peerenboom. In March, the IPB participated in one of the biggest international conventions for biotechnology, "Bio 2000" in Boston.

Together with the universities of Halle and Magdeburg and several other scientific institutes, our re - searchers presented their work at the Saxony Anhalt booth at "Analytika" (in 2000 and 2002) in Munich and at "Achema" (in 2000) in Frankfurt. In addition to the projects on display, Claus Wasternack chaired a workshop to the topic "Plant Biotechnology - Novel Food" at a meeting held together with "Analytika 2000".

Furthermore, in 2001 the IPB participated in "Biotechnika" in Hannover, Germany's most important international biotechnology exhibition. At all the exhibitions and trade shows, visiting scientists and journalists showed keen interest in the institute's work. As a result, several articles were published in different newspapers and journals.

Exhibitions at the frontier between science and art

Hosting the exhibition "Gene world and nutrition" in June 2000, presented by the Alimentarium Food Museum of Vevey, Switzerland, was a great success for the IPB. More than 1000 guests visited the institute to view and critically discuss the interactive exhibits. This exhibition pre-sented the history of plant breeding and the increasing role of gene technology for identifying and creating new kinds of productive and resistant plants.

The exhibition "Life Science Art" in July 2000 examined the theme of the human being that lies behind the scientific researcher and his work. Silvia Stabel, painter and ex-scientist, displayed paintings with scientific themes and objects from the artist's perspective. The paintings' message about the beauty and aesthetic qualities of molecules, cells and scientific motifs was underlined by short explanations and quotations from researchers. Many visitors were very impressed by the exhibits, which were shown under titles like "Alphabet of life", "Hope" or "Orientation".

Public events - a bridge to the people

As in the years before, the IPB participated in 2000 and 2001 in the "Science day" on the market square in Halle. Our researchers presented the work of the institute by displaying posters and small-scale experiments. The event, organized by the municipality and the University of Halle, led to increased contact and discussion with interested citizens.

A similar presentation of scientific institutes on the market square in Halle was celebrated on the University's 500th Anniversary in June 2002. Researchers of the IPB displayed and explained living mo - dels of mycorrhiza - a close partnership between plants and fungi. In addition, visitors had the possibility to see mycorrhized plant cells under the microscope. A display of computer simulations about the characteristics and behavior of proteins was also shown.

In February 2000, members of the institute organized a charity concert for the community kitchen of St. Elizabeth's Hospital in Halle. This community insti-

Group members

Gesine Krüger
(Head until März 2002)

Jana Krupik
(Assistent and Webmaster since November 2000)

Ellen Peerenboom
(Head until Juli 2001)

"Biotechnika" 2001 in Hannover

"Science day" 2001 on the market square in Halle

University's 500th Anniversary in June 2002 on the market square in Halle

"Long Night of Sciences" 2002. Guests were verry interested in the interactive CD about the mycorrhiza.

"Long Night of Sciences" 2002. Prof. Dierk Scheel guided the visitors through the institute.

tution provides about 70 poor people with a warm meal every day. As a result of this classical concert, the IPB colleagues proudly donated the money for 325 meals to the hospital.

Under the motto "Green gene technology - prospects and risks", 24 teachers for chemistry and biology had the possibility to learn more about new methods and molecular techniques of gene transfer into plants in May 2002. The training, held under the auspices of the Central Marketing Organization of German Agricultural Industries, was organized by the IPB.

Instead of the annual "Science day", in 2002 the university and the other research institutes of Halle celebrated a new event - the "Long Night of Sciences". At this day in September, the institute's doors were open from 7 pm to midnight to welcome more than 300 members of the public. Visitors enthusiastically participated in guided tours through the labs and greenhouses of the IPB. In addition, experiments were displayed in the foyer, guests viewed the IPB's collection of colorful cell cultures and had the possibility to learn how a confocal laser-scanning microscope works. Because of the great success of this event, the "Long Night of Sciences" will become an annual event in Halle.

Public projects

The IPB participated in the competition "Public Understanding of Sciences and Humanities" (PUSH) with a multimedia project about mycorrhizal symbiosis. The interactive course about this fascinating biological interaction was produced by Thomas Fester and Ellen Peerenboom and was designed for students and interested nonscientists alike. As one of the 22 final winners selected, the project was sponsored by the "Donors Association for the Promotion of Sciences and Humanities in Germany". Interactive CD's were send away in 2001, at first to all secondary schools of Saxony Anhalt, and afterwards as a result of numerous articles in the regional and national press, to many interested private citizens. This year an update and production of an English version are planned.

As in previous years, the IPB organized many guided tours through the institute for school classes and senior groups in 2000 to 2002. 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.

Since the beginning of 2001, the IPB has had a new corporate design. The internally designed logo was successfully introduced and promptly accepted by people from both within and outside the IPB. Since then, all of the letterheads on business letters, flyers, publicity brochures and business cards display the new logo. In addition, the institute's homepage was completely reorganized and renewed. The new version in German and English has been online since May 2001.

Celebrations

The year 2002 was a time of many celebrations for the IPB. The institute honored two former members, each a celebrity due to his personal qualities and scientific lifework, with a splendid colloquium. In August, Benno Parthier, the institute's former director and president of Germany's biggest and oldest academy, the German Academy of Natural Scientists Leopoldina, celebrated his 70th birthday together with his former colleagues and the entire institute. Günter Adam, former head of the department Natural Product Chemistry, also turned 70 in December.

In May 2002, the institute celebrated its own birthday and foundation ten years ago on a large scale. Representatives of the Federal Ministry of Education and Research, the Ministry of Educationand Cultural Affairs of Saxony Anhalt, the Leibniz Association, the city council of Halle and scientists from all over the world attended the official ceremony and expressed their best wishes for the future. In a ceremonial address, Dierk Scheel, director of the institute, spoke about the successful scientific tradition of the IPB. Three scientific reports and a concert by the chamber orchestra of the university completed the program. Afterwards the institute members had a party together with all invited guests. ■

Publications

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Press releases

Leckere Gene? Gen-Welten Ernährung Sonderausstellung am IPB (E. Peerenboom, 06.06.2000)

Eine Symbiose aus Kunst und Wissenschaft: Sonderausstellung “Life Science Art” am IPB (E. Peerenboom, 03.07.2000).

Prof. Dr. Ludger Wessjohann wird neuer Abteilungsleiter der Abteilung Naturstoffchemie am Leibniz-Institut für Pflanzenbiochemie (E. Peerenboom, 26.10.2000).

PlantMetaNet - neues Forschungsnetzwerk - Vier führende Institute auf dem Gebiet der Pflanzenforschung vereinbaren Kooperation (E. Peerenboom, 06.06.2001).

Wissenschaftler entwickeln Lernmaterial für Schüler - Lern-CD für das Fach Biologie: Mykorrhiza (G. Krüger, 12.12.2001).

IPB feiert sein 10jähriges Gründungsjubiläum (J. Krupik, 21.05.2002)

IPB ehrt langjährigen Direktor Prof. Dr. Benno Parthier (J. Krupik, 27.08.2002)

Lange Nacht der Wissenschaften “Blick ins Innere der Pflanze (S. Pieplow, 18.09.2002)

Festveranstaltung zu Ehren von Professor Adam (S. Pieplow, 09.12.2002)

Pflanzliche “Staubsauger” ziehen Schwermetalle aus dem Boden (S. Pieplow, 09.12.2002).

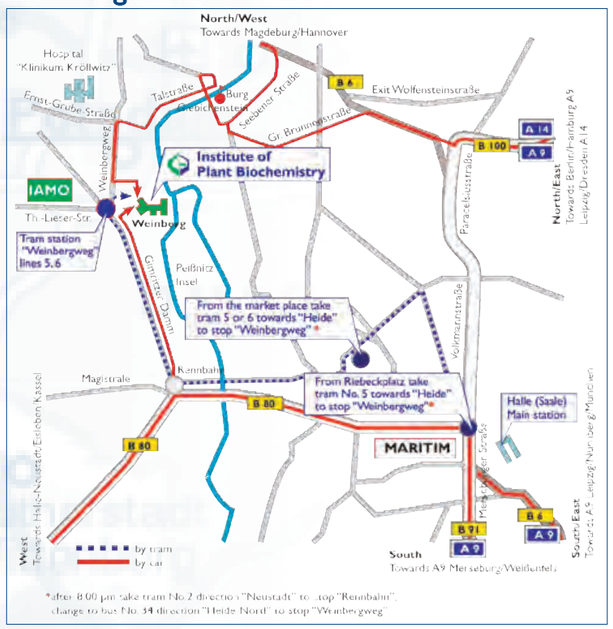
Benno Parthier celebrated his 70th birthday in August 2002

Congratulations for Günter Adam. His 70th birthday was celebrated in December 2002

IPB's 10th birthday in May 2002

Map & Impressum

How to get to the IPB



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